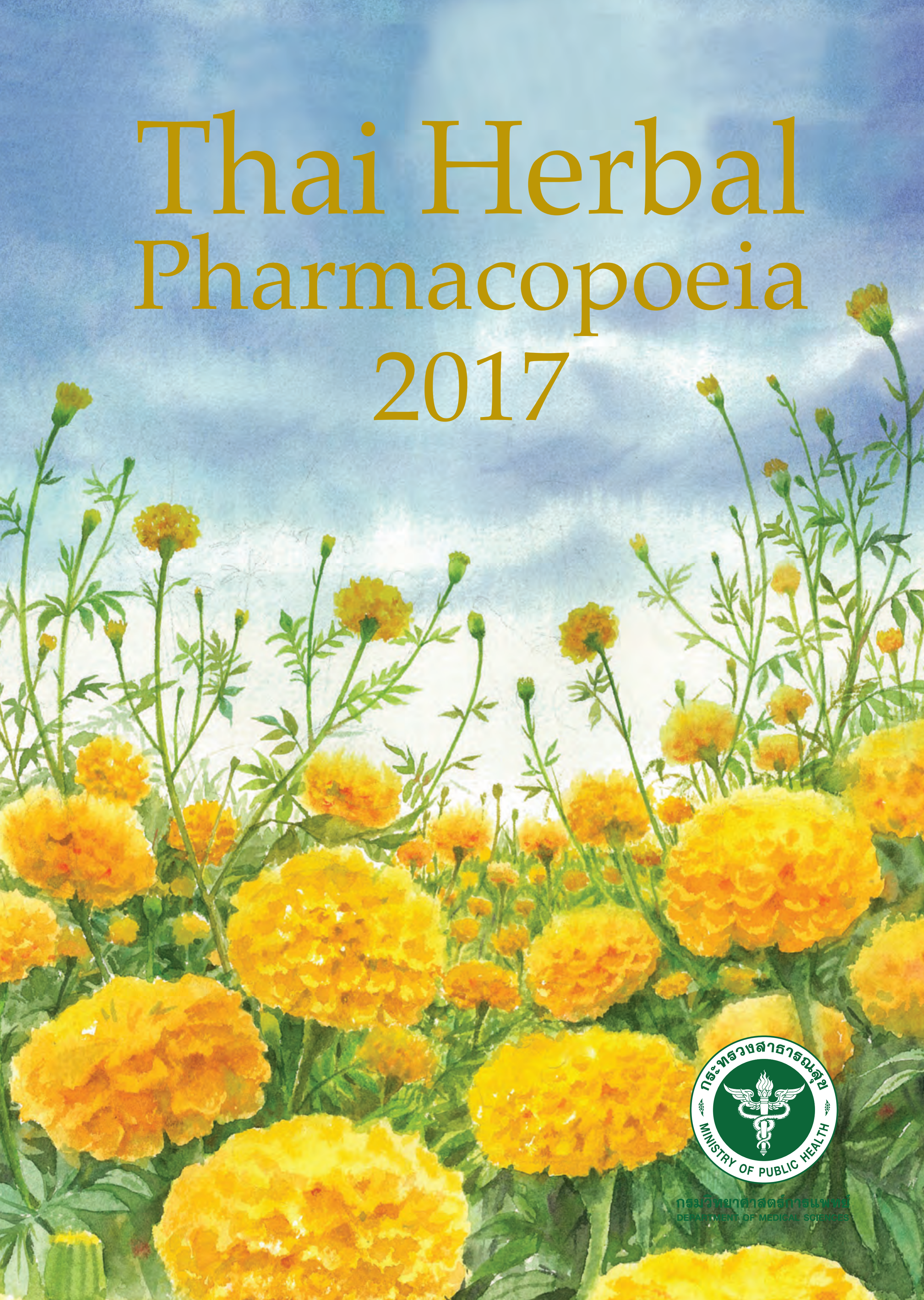

Thai Herbal Pharmacopoeia 2017

กระทรวงสาธารณสุข
MINISTRY OF PUBLIC HEALTH

กรมวิทยาศาสตร์การแพทย์
DEPARTMENT OF MEDICAL SCIENCES

กรมวิทยาศาสตร์การแพทย์
DEPARTMENT OF MEDICAL SCIENCES

THAI HERBAL PHARMACOPOEIA
2017

Department of Medical Sciences, Ministry of Public Health
Nonthaburi 11000, Thailand

Publisher by Department of Medical Sciences
ISBN : 978-616-11-3421-1

Printed by:
The Agricultural Co-operative Federation of Thailand., Ltd.
79 Ngamwongwan Rd., Chatuchak Bangkok, Thailand.
Tel: 0-2561-4590-6 Fax: 0-2561-3327

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PREFACE

This special volume of the Thai Herbal Pharmacopoeia 2017 is being released to commemorate the 70th anniversary of the reign of King Rama IX. His past activities include more than four thousand royal development projects of considerable scope, ranging from irrigation, farming, drought and flood alleviation, crop substitution, public health and distance learning to initiatives to promote employment, as well as, especially, his philosophy of "the sufficiency economy" as a way of life for the Thai people. These have helped improve the lives of Thais, particularly farmers, in all parts of the kingdom. In addition, His Majesty King Bhumibol Adulyadej impressed upon his subjects in all parts of Thailand the importance of the use of Thai herbal medicines as an alternative treatment. The Thai Herbal Pharmacopoeia 2017 was set up by the Department of Medical Sciences, Ministry of Public Health, not only to provide for standardization of herbal medicines and their preparations, but also to follow in the royal footsteps of His Majesty King Rama IX concerning public-health issues for the health and well-being of the Thai people.

The Department of Medical Sciences has developed the Thai Herbal Pharmacopoeia (THP) with the aim of providing standards for herbal drugs and herbal drug preparations since the quality of herbal medicines can directly affect their safety and efficacy. The choice of the plants included in the Pharmacopoeia is limited to commercially available plants and to other plants which appear on the List of Medicines from Herbal Drugs under the Thai National List of Essential Medicines. The selection process is based on the critical evaluation of the documented therapeutic merit and the accessibility of each plant. Up to the present, six publications have been issued (THP Volume I [1995], THP Volume II [2000], Supplement to THP [2004], THP Volume III [2009], Supplement to THP [2011], THP Volume IV [2014], and THP 2016) and officially promulgated by the Ministry of Public Health. These publications comprise 70 monographs of herbal drugs and herbal drug preparations. These 70 monographs consist of 62 monographs from the published monographs of THP 2016 and, additionally, eight new monographs on herbal drugs and herbal drug preparations.

The Thai Herbal Pharmacopoeia is one of the achievements of the Thai Pharmacopoeia Committee. The monographs on both herbal drugs and herbal drug preparations, together with the appendices, as well as other necessary information in the Thai Herbal Pharmacopoeia 2017, were prepared by the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia in affiliation with other Subcommittees, viz. the Subcommittee on the Pharmacognostic and Botanic Specifications for the Thai Herbal Monographs, the Subcommittee on the Physico-chemical Specifications and Safety for the Thai Herbal Monographs and the Subcommittee on Standards for Thai Herbal Drug Preparations under the supervision of the Thai Pharmacopoeia Committee. The generous assistances and technical support from contributors, various government agencies, academic institutions and other organizations, particularly the Botanical Garden Organization, the Department of National Parks, Wildlife and Plant Conservation Forest and Plant Conservation Research Office, the Government Pharmaceutical Organization, Chiang Mai University Faculty of Pharmacy, Chulalongkorn University Faculty of Pharmaceutical Sciences, Kasetsart University Faculty of Sciences, Khon Kaen University Faculty of Pharmaceutical Sciences, Mahasarakham University Faculty of Medicine, and Srinakharinwirot University Faculty of Pharmacy, and those individuals who have provided comments and advice and shared their time and expertise to make this impressive undertaking possible are gratefully acknowledged.

(Clin. Prof. Emeritus Piyasakol Sakolsatayadorn, M.D.)
Minister of Public Health

INTRODUCTION

In 1989, the Thai Pharmacopoeia Committee appointed the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia with the mission of establishing the Thai Herbal Pharmacopoeia, a companion publication to existing Thai Pharmacopoeia. The Subcommittee's responsibilities are:

1. selecting appropriate herbal drugs and herbal drug preparations based on public health and industrial demand for further consideration by the Thai Pharmacopoeia Committee;
2. establishing specifications for herbal drugs and herbal drug preparations selected by the Thai Pharmacopoeia Committee and compiling the corresponding monographs;
3. publishing the Thai Herbal Pharmacopoeia;
4. attending to all matters related to the preparation of the Thai Herbal Pharmacopoeia.

In 2010, the Thai Pharmacopoeia Committee appointed three specialized subcommittees to provide the existing subcommittee with data on specific fields in order to facilitate the work of the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, as well as to accelerate the work process, making it possible to meet the goal of launching new editions of the Thai Herbal Pharmacopoeia regularly. The responsibilities of each of these Subcommittees are described as follows:

1. The Subcommittee on the Pharmacognostic and Botanic Specifications for Thai Herbal Monographs

This Subcommittee is responsible for:

1.1 producing drafts of the pharmacognostic and botanic specifications of the Thai herbal monographs, i.e. nomenclature, definitions, plant descriptions, macroscopical and microscopical descriptions, and other related information;

1.2 submitting the drafts to the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia for approval;

1.3 attending to all matters related to the preparation of pharmacognostic and botanic specifications.

2. The Subcommittee on the Physico-chemical Specifications and Safety for Thai Herbal Monographs

This Subcommittee is responsible for:

2.1 producing drafts of the physico-chemical specifications of the Thai herbal monograph, i.e. constituents, packaging and storage, identification, assay, ashes, extractives, and other related information;

2.2 producing draft information on the safety of the Thai herbal monographs, i.e. categories, contra-indications, warnings, precautions, additional information, dosage, and other related information;

2.3 submitting the drafts to the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia for approval;

2.4 attending to all matters related to the preparation of the physico-chemical and safety specifications.

3. The Subcommittee on Standards for Thai Herbal Drug Preparations

This Subcommittee is responsible for:

3.1 producing draft specifications for Thai herbal drug preparations preselected by the Thai Pharmacopoeia Committee and compiling these specifications in monographs in the Thai Herbal Pharmacopoeia;

3.2 submitting the drafts to the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia for approval;

3.3 attending to all matters related to establishing the specifications for Thai herbal drug preparations.

This publication is included in a series known as the Thai Herbal Pharmacopoeia. Despite the fact that the majority of monographs being on medicines of plant origin, due to their extensive use, the various terms described in the text, such as herbal drugs or materials, refer not only to plants but also to animals and minerals used for medicinal purposes. The pattern of the various monographs is relatively inconsistent due to the fact that they were compiled from various publications of the Thai Herbal Pharmacopoeia. For instance, the illustrations of thin-layer chromatograms may be found as colour drawings or colour photographs.

Any comments or suggestions from readers/users are welcomed by the Subcommittees in order to further refine the information in this publication. Such comments will be considered when the monographs are revised so that the most useful information is provided.

Given the continuing progress of the Thai Herbal Pharmacopoeia, the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia on behalf of the Department of Medical Sciences wishes to express its gratitude to Than Phuying Preeya Kashemsant Na Ayudhya, the former Director General of the Department of Medical Sciences, Ministry of Public Health. She successfully initiated the Thai Pharmacopoeia in 1979 and the Thai Herbal Pharmacopoeia in 1989 and contributed to this valuable task with her great effort, inspiration and leadership.

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[1] Effective from October 2002 (formerly Drug Analysis Division)

[2] Effective from October 2002 (formerly Thai Pharmacopoeia Section)

[3] Effective from April 2005 (formerly Thai Pharmacopoeia and Reference Substances)

[4] Effective from October 2007 (formerly Thai Pharmacopoeia Section)

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    - Nalinphat **Saktiyasunthorn** (deceased), B.Sc. in Pharm., M.Sc. in Pharm.
    - Nuchattra **Chansuvanich**, B.Sc., M.S.
    - Kalaya **Anulukanapakorn**, B.Sc., M.Sc., Dr. rer. nat., *Representative*
    - Jaree **Bansiddhi**, B.Sc., M.Sc., *Representative*
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    - Amporn **Kun-anake**, B.Sc. in Pharm., *Representative*
    - Narumole **Mongkolchaipak**, B. Pharm., M.Sc., *Representative*
    - Pairin **Thongkhoom**, B.Sc., M.Sc. in Pharm., *Representative*
    - Wilawan **Rattanathirakul**, B.Sc., M.Sc., *Representative*
- Director, Bureau of Drug and Narcotic (2014-)
    - Suratchanee **Savetsila**, B.Sc. in Pharm., M.Sc. in Pharm.
    - Supanee **Duangteerapreecha**, B.Sc. in Pharm., M.S., Ph.D., *Representative*
    - Jiranuch **Jamtaweekul**, B.Sc. in Pharm., M.Sc. in Pharm., *Representative*
    - Somsak **Sunthornphanich**, B.Sc. in Pharm., M. Chem., *Representative*
- Chief, Phytochemistry Section, Medicinal Plant Research Institute, Department of Medical Sciences (2000-2015)
    - Thidarat **Boonruad**, B.Sc., M.Sc. in Pharm.

Yenchit **Techadamrongsin**, B.S., B.S. Phar., Post. Cert.
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Chief, Pharmacognosy Section, Medicinal Plant Research Institute, Department of Medical Sciences (2000-2015)
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Chief, Herbal Quality Assurance Center, Medicinal Plant Research Institute, Department of Medical Sciences (2004-)
Thidarat **Boonruad**, B.Sc., M.Sc. in Pharm.
Prapai **Wongsinkongman**, B.Sc. in Pharm., M.Sc. in Pharm., Ph.D.
Jiranuch **Mingmuang**, B. Pharm., M.Sc. in Pharm., *Representative*
Duangpen **Pattamadilok**, B.Sc. in Pharm., M.Sc. in Pharm., Ph.D., *Representative*
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Jaree **Bansiddhi**, B.Sc., M.Sc. (2010-)
Rapepol **Bavovada**, B.Sc. in Pharm., M.Sc. in Pharm., Ph.D. (1993-2006)
Chitra **Chaiyawat**, B. Pharm. (2010-2014)
Kongkanda **Chayamarit**, B.Sc., M.Sc., D.Sc. (2013-)
Thaweephol **Dechatiwongse Na Ayudhya**, B.Sc. in Pharm. (1989-)
Supatra **Im-erb**, B.Sc. in Pharm., M.Sc. (Pharm. Chem.) (1990-)
Panida **Kanchanapee** (deceased), B.Sc. in Pharm. (1986-2000)
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Secretaries: Sasiwan **Aim-ot**, B.Sc. in Pharm. (2003-2004)
Chitra **Chaiyawat**, B. Pharm. (1996-2010)
Buussayamas **Charoensuk**, B.Pharm. (1999-2000)
Kornvika **Charupant**, B.S. Pharm., M.Sc. in Pharm., Ph.D. (1998-1999, 2001-2003, 2008-)
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Jiranuch **Jamtaweekul**, B.Sc. in Pharm., M.Sc. in Pharm. (2010-2015)
Wichuda **Jariyaphun**, B.Sc., M.Sc. (1989-1990)
Sarunyaporn **Kongchira**, B.S. in Pharm., M.S. (1993-1996)
Sarinee **Lenapun**, B.S. Pharm., M.Sc. in Pharm. (2004-2008)
Santi **Nimnoi**, B.S. in Pharm. (2017-)
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Thanita **Patthamajinda**, B.S. in Pharm., M.A. (2009-2015)
Supattra **Phongsri**, B.Sc. (Pharm.) (2000-2001)
Thanyarat **Putta**, B.Sc. in Pharm., M.Sc. in Pharm. (1996-1998)
Nantana **Sittichai**, B.Sc. in Pharm., M.S. (1986-2008)
Panit **Somhom**, B.Sc. in Pharm., M.Sc. in Pharm. (1991-1992)
Prapai **Wongsinkongman**, B.Sc. in Pharm., M.Sc. in Pharm. (1990-1991, 1993-1996)

This subcommittee is responsible for:

1.1 selecting the appropriate herbal drugs and herbal drug preparations based on public health and industrial demands for further consideration by the Thai Pharmacopoeia Committee;

1.2 establishing the specifications of herbal drugs and herbal drug preparations selected by the Thai Pharmacopoeia Committee and compiling the corresponding monographs;

1.3 publishing the Thai Herbal Pharmacopoeia;

1.4 attending to all matters related to the preparation of the Thai Herbal Pharmacopoeia.

2. SUBCOMMITTEE ON THE PHARMACOGNOSTIC AND BOTANIC SPECIFICATIONS FOR THAI HERBAL MONOGRAPHS

Chairperson: Chayan **Picheansoonthon**, B.S. in Pharm., Ph.D., FRI (2010-)

Vice-chairperson: Thatree **Phadungcharoen**, B.Sc. in Pharm., M.Sc. in Pharm. (2010-2015)

Advisors: Chirayupin **Chandraprasong**, B.Sc., M.Sc., Hon. Ph.D. (2010-2015)
Kongkanda **Chayamarit**, B.Sc., M.Sc., D.Sc. (2010-2015)

Members: Chief, Pharmacognosy Section, Medicinal Plant Research Institute, Department of Medical Sciences (2010-)
Pairin **Thongkhoom**, B.Sc., M.Sc. in Pharm.
Wilawan **Rattanathirakul**, B.Sc., M.Sc., *Representative*
Jaree **Bansiddhi**, B.Sc., M.Sc. (2010-)
Bhanubong **Bongcheewin**, B. Pharm., M.Sc., Ph.D. (2014-)
Kongkanda **Chayamarit**, B.Sc., M.Sc. D.Sc. (2015-)
Pranom **Dechwisissakul**, B.Sc., M.Sc. in Pharm. (2010-)
Jiranuch **Jamtaweekul**, B.Sc. in Pharm., M.Sc. in Pharm. (2010-2015, 2017-)
Ornusa **Khamsuk**, B.S., M.S., Ph.D.) (2014-)
Thatree **Phadungcharoen**, B.Sc. in Pharm., M.Sc. in Pharm. (2015-)
Kalaya **Pharadai**, B.Sc. in Pharm., M.Eng. (2010-)
Sawanee **Sathornviriyapong**, B.S., M.S., Ph.D. (2010-)

Secretaries: Sasiphimol **Boontavee**, B.Pharm (2017-)
Kornvika **Charupant**, B.S. Pharm., M.Sc. in Pharm., Ph.D. (2010-)
Jiranuch **Jamtaweekul**, B.Sc. M.Sc. in Pharm, (2015-2017)
Sasiwimon **Patasema**, B.Pharm., M.Sc. in Pharm. (2015-)
Thanita **Patthamajinda**, B.S. in Pharm., M.A. (2010-2015)

This subcommittee is responsible for:
2.1 producing drafts of the pharmacognostic and botanic specifications for Thai herbal monographs, i.e. nomenclature, definitions, plant descriptions, macroscopical and microscopical descriptions, and other related information;
2.2 submitting the drafts to the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia for approval;
2.3 attending to all matters related to the preparation of pharmacognostic and botanic specifications.

3. SUBCOMMITTEE ON THE PHYSICO-CHEMICAL SPECIFICATIONS AND SAFETY FOR THAI HERBAL MONOGRAPHS

Chairperson: Khanit **Suwanborirux**, B.Sc. in Pharm., M.Sc. in Pharm., Ph.D. (2010-)

Vice-chairperson: Nantana **Sittichai**, B.Sc. in Pharm., M.S. (2010-2015)

Advisor: Yupadee **Payakkapan**, B.Sc. in Pharm., M.Sc. in Pharmaceutical Analysis (2010-2015)

Members: Chief, Herbal Quality Assurance Center, Medicinal Plant Research Institute, Department of Medical Sciences (2010-2013, 2015-)
Somchit **Niumsakul**, B.Sc., M.Sc.

Nawarat **Chadchen**, B.Pharm., M.Sc. in Pharm., *Representative*
Apirak **Sakpetch**, B.S. Pharm., *Representative*
Chitra **Chaiyawat**, B.Pharm. (2010-2014)
Veena **Nukoolkarn**, B.S. in Pharm., Ph.D. (2014-)
Yupadee **Payakkapan**, B.Sc. in Pharm., M.Sc. in Pharmaceutical Analysis (2015-)
Chada **Phisalapong**, B.Sc. in Pharm., M.Sc., Ph.D. (2010-)
Nantana **Sittichai**, B.Sc. in Pharm., M.S. (2015-)
Uthai **Sotanaphun**, B.Sc. in Pharm., M.Sc. in Pharm., Ph.D. (2014-)
Taweesak **Suntorntanasat**, B.Sc. in Pharm., M.Sc. in Pharm. (2010-)
Witchuda **Thanakijcharoenpath**, B.Sc. in Pharm., M.Sc. in Pharm., Ph.D. (2014-)
Prapai **Wongsinkongman**, B.Sc. in Pharm., M.Sc. in Pharm., Ph.D. (2011-)

Secretaries: Sasiphimol **Boontavee**, B.Pharm. (2017-)
Kornvika **Charupant**, B.S. Pharm., M.Sc. in Pharm., Ph.D. (2010-2014)
Sirichai **Krabesri**, B.Sc. in Pharm., M. Pharm., LL.B., B.L. (2014-)
Santi **Nimnoi**, B.S. in Pharm. (2015-)

This subcommittee is responsible for:

3.1 producing drafts of the physico-chemical specifications for Thai herbal monograph, i.e. constituents, packaging and storage, identification, assay, ashes, extractives, and other related information;

3.2 producing draft information on the safety for Thai herbal monographs, i.e. categories, contra-indications, warnings, precautions, additional information, dosage, and other related information;

3.3 submitting the drafts to the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia for approval;

3.4 attending to all matters related to the preparation of the physico-chemical and safety specifications.

4. THE SUBCOMMITTEE ON STANDARDS FOR THAI HERBAL DRUG PREPARATIONS (2010-)*

Chairperson: Yupadee **Payakkapan**, B.Sc. in Pharm., M.Sc. in Pharmaceutical Analysis (2010-)

Members: Kornvika **Charupant**, B.S. Pharm., M.Sc. in Pharm., Ph.D. (2015-)
Piyaporn **Prayakprom**, B.Sc. in Pharm., Ph.D., (2017-)
Nidapan **Ruangrittinon**, B.Sc. in Pharm., M.Sc. in Pharm. (2010-)
Churairat **Rakwatin**, B.Sc. in Pharm. (2010-)
Nantana **Sittichai**, B.Sc. in Pharm., M.S. (2010-)
Prapai **Wongsinkongman**, B.Sc. in Pharm., M.Sc. in Pharm., Ph.D. (2010-)
Sasida **Yoosuk**, B.Sc. in Pharm., M.Sc. in Pharm. (2017-)

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*Effective from June 2017 (formerly The Ad Hoc Subcommittee on Standards of the Thai Herbal Drug Preparations)

Secretaries: Sasiphimol **Boontavee**, B.Pharm (2017-)
Sirichai **Krabesri**, B.Sc. in Pharm., M. Pharm., LL. B., B.L. (2010-)
Sarinee **Lenapun**, B.S. Pharm., M.Sc. in Pharm. (2010-2015)
Santi **Nimnoi**, B.S. in Pharm. (2015-)

This subcommittee is responsible for:

4.1 producing draft specifications for Thai herbal drug preparations preselected by the Thai Pharmacopoeia Committee and compiling these specifications in monographs in the Thai Herbal Pharmacopoeia;

4.2 submitting the drafts to the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia for approval;

4.3 attending to all matters related to establishing the specifications for Thai herbal drug preparations.

5. THE SUBCOMMITTEE ON EDITORIAL STYLE

Chairperson: Komol **Pengsritong** (deceased), M.D., M.S., Ph.D., Hon.D.Sc. in Pharm. (1980-1988)
Nadhirat **Sangkawibha** (deceased), M.D., M.P.H. (1989-1997)
Sumana **Vardhanabhuti** (deceased), B.Sc. in Pharm., M.Sc. in Pharm., M.P.H., Cert. in Immunol. (WHO) (1997-2010)
Boonchua **Dhorranintra**, M.D., Dr.med (magna cum laude) (CU)., FRCP(T) (2010-)

Advisors: Prachaksvich **Lebnak**, M.D. (2000-2003)
Komol **Pengsritong** (deceased), M.D., M.S., Ph.D., Hon.D.Sc. in Pharm. (1988-1991)
Rachanee **Pinthaworn**, B.Sc. in Pharm. (2003-2005, 2009-2010)
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Kamphol **Raksrivong**, B.Sc. in Pharm. (2000-2013)
Nadhirat **Sangkawibha** (deceased), M.D., M.P.H. (1997-2009)
Suntana **Sutadarat**, B.Ed. (Hons.), M.A., Ph.D. (1997)
Prakorb **Tuchinda** (deceased), M.D., Hon, D.Sc. in Med. (1988-1991)
Sumana **Vardhanabhuti** (deceased), B.Sc. in Pharm., M.Sc. in Pharm., M.P.H., Cert. in Immunol. (WHO) (2010-2015)
M.L. Pranod **Xumsaeng** (deceased), Ph.G., B.Sc. in Pharm. (1991-1997)

Members: Manas **Attawish**, B.S. (Pharm.) (2013-2015, 2017-)
Supong **Akesiripong**, B.Pharm. (Hons.), Ph.D. (2000-)
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Kornvika **Charupant**, B.S. in Pharm., M.Sc. in Pharm., Ph.D. (2008-)
Boonchua **Dhorranintra**, M.D., Dr. med.(magna cum laude), FRCP(T) (2000-2010)
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Than Phuying Preeya **Kashemsant Na Ayudhya**, B.Sc. in Pharm., M.Sc., Hon.D.Sc. in Pharm.(CU) (1989-1992)
Sirichai **Krabesri**, B.Sc. in Pharm., M. Pharm., LL. B., B.L. (2009-)
Prachaksvich **Lebnak**, M.D. (1997-2000)
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Sunibhond **Pummangura**, B.Sc. in Pharm., M.Sc., M.S.P., Ph.D. (1989-1997)
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Kamphol **Raksrivong**, B.Sc. in Pharm. (1993-1997)
Churairat **Rakwatin**, B.Sc. in Pharm. (2006-)
Chanai **Sambhandharaksa** (deceased), B.S. Phar., Hon. D.Sc. in Pharm. (MU) (1989 - 2008)
M.L. Othong **Sawasdimongkol**, B.Sc. in Pharm. (1993-2015)
Nantana **Sittichai**, B.Sc. in Pharm., M.S. (2005-)
Nongluck **Sookvanichsilp**, B.Sc. in Pharm. (Hons.), M.Sc. in Pharm., Dr.Phm.Sc., LL.B., B.B.A. (2005-)
Suntana **Sutadarat**, B.Ed. (Hons.), M.A., Ph.D. (1989-1996, 1998-)
Parkpoom **Tengamnuay**, B.Pharm., Ph.D. (1993-)
Charurat **Tantraporn**, B.A., M.A. (1997-2000)
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Rewadee **Vongsaroj** (deceased), B.Sc. in Pharm., M.Sc. (1989-1997)
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Secretaries:
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Santi **Nimnoi**, B.S. in Pharm. (2013-)
Sasiwimon **Patasema**, B.Pharm., M.Sc. in Pharm. (2003-)
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Yupadee **Payakkapan**, B.Sc. in Pharm., M.Sc. in Phramceutical Analytical Analysis (1980-1991)
Thomayant **Prueksaritanont**, B.Sc. in Pharm., Ph.D. (1989-1991)
Kamphol **Raksrivong**, B.Sc. in Pharm. (1980-2000)
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Panit **Somhom**, B.Sc. in Pharm., M.Sc. in Pharm. (1991-1993)
Wanida **Suchonwanit**, B.Sc. in Pharm. (1997-2000)

This subcommittee is responsible for:

5.1 designing the format and style for printing;

5.2 editing the text;

5.3 keeping conformity of the molecular formulae, chemical names, molecular weights, and expressions of the symbols of units throughout the text;

5.4 organizing the information compiled by the subcommittees into a pharmacopoeial form and completing the final draft of the Thai Pharmacopoeia;

5.5 attending to all matters related to editing the Pharmacopoeia.

6. WORKING GROUP ON PRINTING THE THAI HERBAL PHARMACOPOEIA (1989-1995)

Chairperson: Director, Drug Analysis Division, Department of Medical Sciences,
Ministry of Public Health
Chongdee **Wongpinairat**, B.Sc. in Pharm., M.Sc., Ph.D.

Members: Chief, Herbal Quality Assurance Center, Medicinal Plant Research
Manas **Attawish**, B.S. (Pharm.)
Jaree **Bansiddhi**, B.Sc., M.Sc.
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Kamphol **Raksrivong**, B.Sc. in Pharm.
Nongluck **Ruangwises**, B.S. (Pharm.), M.S., Ph.D.
Nantana **Sittichai**, B.Sc. in Pharm., M.S.
Wanida **Suchonwanit**, B.Sc. in Pharm.
Prapai **Wongsinkongman**, B.Sc. in Pharm., M.Sc. in Pharm

***Contributors*:**

The following persons were engaged in the production of THP Volume I (1989-1995):

Jaree **Bansiddhi**, B.Sc., M.Sc.
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Pranom **Dechwisissakul**, B.Sc., M.Sc. in Pharm.
Warunee **Jirawattanapong**, B.Sc. in Pharm.
Walapa **Juladusitpornchai**, B.Sc. in Pharm.
Sirichai **Krabesri**, B.Sc. in Pharm.
Amporn **Kun-anake**, B.Sc. in Pharm.
Vitit **Nammoonnoy**, B.A.
Daroon **Pecharaply**, B.Sc., M.S.
Arom **Phichitkanka**, B.Sc.
Chada **Phisalaphong**, B.Sc. in Pharm., M.Sc., Ph.D.
Vansiri **Rimsamut**, Cert.A.
Amporn **Ruangchan**, B.Sc. in Pharm., M.Sc.
Weena **Sathianpokkasap**, B.Sc. in Pharm.

Kamol **Sawasdimongkol**, B.Sc. in Pharm., M.S.
Panit **Somhom**, B.Sc. in Pharm., M.Sc. in Pharm.
Suntana **Sutadarat**, B.Ed. (Hons.), M.A., Ph.D.
Yenchit **Techadamrongsin**, B.S., B.S. Phar., Post. Cert.
Parkpoom **Tengamnuay**, B. Pharm., Ph.D.
hawatchai **Thawonying**, B.Sc. in Pharm.

The following persons were engaged in the production of THP Volume II (1995-2000):

Jaree **Bansiddi**, B.Sc., M.Sc.
Nuchattra **Chansuwanit**, B.Sc., M.S.
Vanida **Chantaratheptawan**, B.Sc. in Pharm., M.Sc. in Pharm.
Pranom **Dechwisissakul**, B.Sc., M.Sc. in Pharm.
Warunee **Jirawattanapong**, B.Sc. in Pharm., M.Sc. in Pharm.
Tathaporn **Maison**, B.Sc.
Narumole **Mongkolchaipak**, B. Pharm., M.Sc.
Arom **Phichitkanka**, B.Sc.
Proramate **Sansongsak**, B.Sc. (Pharm.)
Yenchit **Techadamrongsin**, B.Sc., B.S. Phar., Post. Cert.
Pairin **Thongkhoom**, B.Sc.
Rawat **Tiasakul**, B.Sc. (Pharm.)
Suppachai **Tiyaworanant**, B.Sc. (Pharm.), M.Sc. in Pharm.

The following persons were engaged in the production of Supplement to THP (2000-2004):

Nuchattra **Chansuwanit**, B.Sc., M.S.
Tanwarat **Kajsongkram**, B.S., M.S. (Chemistry)
Pichitra **Maekprayoon**, B.Sc.
Tathaporn **Maison**, B.Sc.
Jiranuch **Mingmuang**, B.Sc. in Pharm.
Piya **Mokkamul**, B.S. (Forestry)
Narumole **Mongkolchaipak**, B. Pharm., M.Sc.
Thanawat **Thongchin**, B.S.
Pairin **Thongkhoom**, B.Sc., M.Sc. in Pharm.
Rawat **Tiasakul**, B.Sc. (Pharm.)

The following persons were engaged in the production of THP Volume III (2004-2009):

Juthamas **Bhura**, B.Sc. in Pharm., M.Sc. in Pharm.
Chalermchoke **Boonchit**, B.F.A.
Nuchattra **Chansuwanit**, B.Sc., M.S.
Supanee **Duangteraprecha**, B.Sc. in Pharm., M.Sc. in Pharm., Ph.D.
Pajaree **Inthachub**, B.Sc., M.Sc.
Jiranuch **Jamtaweekul**, B.Sc. in Pharm., M.Sc. in Pharm.
Tanwarat **Kajsongkram**, B.S., M.S.
Supachai **Koonterm**, B.Sc.

Sirichai **Krabesri**, B.Sc. in Pharm., M. Pharm., LL.B., B.L.
Sumanee **Lertakanokkun**, B.Sc. (Pharm.)
Pichitra **Maekprayoon**, B.Sc.
Duangpen **Pattamadilok**, B.Sc. in Pharm., M.Sc. in Pharm., Ph.D.
Wittaya **Pongamornkul**, B.Sc., M.S.
Sununta **Srisopon**, B. Pharm., M. Pharm.
Thanawat **Thongchin**, B.S.
Pairin **Thongkhoom**, B.Sc., M.Sc. in Pharm.
Niran **Vipunngeun**, B.Sc., M.Sc.
Wimonwan **Warinpramote**, B. Pharm., Pg Dip Sc (Biotech.), M. Sc.
Sopidawan **Wichienkul**, B.S.

The following persons were engaged in the production of Supplement to THP 2011:
Sasima **Juntryam**, B.Sc.
Sirichai **Krabesri**, B.Sc. in Pharm., M. Pharm., LL.B., B.L.
Areerat **Surathanjanya**, B.S., M.S.
Akekmol **Suttisun**, Cert. in Medical Science Technology
Parkpoom **Tengamnuay**, B. Pharm., Ph.D.

The following persons were engaged in the production of THP Volume IV (2011-2013):
Chalermchoke **Boonchit**, B.F.A. (Visual Arts)
Kamthorn **Intharapichai**, B.Sc. (TTM.)
Pornpimon **Wongsuwan**, B.Sc., M.Sc.

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GENERAL NOTICES

GENERAL NOTICES

The information given in the general notices provides the basic guidelines for the interpretation and applications of the standards, tests, assays and other specifications of the Thai Herbal Pharmacopoeia.

In the text of the Thai Herbal Pharmacopoeia the word “Pharmacopoeia” means the Thai Herbal Pharmacopoeia. The official abbreviation for the Thai Herbal Pharmacopoeia is THP. An herbal material is not of the pharmacopoeial quality unless it complies with all the requirements of the relevant monograph. The statements under the headings: Description, Solubility, Constituents, Packaging and storage, Contra-indication, Warning, Precaution, and Additional information are not to be regarded as analytical requirements. However, the macroscopic and microscopic descriptions under each monograph are important means for the identification of the drug and its corresponding origin.

Unless otherwise specified, the rules of the General Notices of the Thai Pharmacopoeia (TP) apply to the Thai Herbal Pharmacopoeia.

Monograph Nomenclature

A Thai name is adopted as the main title of each pharmacopoeial substance. It is transcribed to English following the Royal Institute’s official transliteration system[1] and printed with capital letters. Subsidiary titles, where applicable, are other Thai name(s), Latin genitives of plants, English common name(s), and English synonym(s).

In the text, English common names are usually mentioned in place of the main titles. When the English common names are not available, the English names derived from the Latin genitives of plants will be used instead. All titles (main and/or subsidiary) and names (synonyms as well as botanical names) are listed in the index.

Reference Substances

Where a test or an assay calls for the use of a Reference Substance, the ASEAN Reference Substance or other recognized reference substances may be used. The ASEAN Reference Substances are available from the Bureau of Drug and Narcotic, the Department of Medical Sciences, Nonthaburi, Thailand.

Authenticated Reference Specimens

For the botanical evaluation of the crude drug samples, the herbarium specimen numbers of the corresponding plants provided in the text are taken from the Department of Medical Sciences Herbarium (DMSC), the Department of Medical Sciences, Nonthaburi, Thailand, or other recognized herbaria such as the Bangkok Herbarium (BK), the Department of Agriculture, Bangkok, Thailand; the Forest Herbarium (BKF),

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[1] *Rules for Transcribing Foreign Words to Thai Script*: *English, French, German, Italian, Spanish, Russian, Japanese, Arabic, Malay* (The Royal Institute ed.), Bangkok: the Royal Institute, 1992.

the Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand; the Herbarium of Queen Sirikit Botanic Garden (QSBG), Chiang Mai, Thailand. If not provided, the herbarium specimens could be compared to the existing named specimens at the above-mentioned herbaria.

For some plants non-native and not commercially cultivated in Thailand so that their herbarium specimens are not available at the above-mentioned herbaria, citation of the herbarium specimen numbers will be indicated under the Additional information of such monographs. If not indicated, it is suggested to investigate from other internationally-recognized herbaria.

The crude drug numbers (DMSc) are also cited. The reference crude drug specimens are authenticated by the Medicinal Plant Research Institute, the Department of Medical Sciences, Nonthaburi, Thailand.

Freshly and Recently Prepared

The direction that a preparation must be freshly prepared indicates that it must be made not more than 24 hours before it is issued for use. The direction that a preparation should be recently prepared indicates that deterioration is likely if the preparation is stored for longer than about 4 weeks at 15° to 25°.

Description

In addition to macroscopical and microscopical descriptions of crude drugs, the morphological and anatomical descriptions of plants are provided for the botanical identification of the samples. Colour photographs of the plants and crude drugs are also given.

Macroscopical descriptions in the monographs refer to features which can be seen by the unaided eyes or with the aid of a hand lens. Statements of the characteristic microscopical description of the whole drug are included in the monograph as a means for determining identity, quality, or purity. Most of the transverse sections of the plants are line drawn but some are photomicrographed and inserted to illustrate the authenticity of the cellular structures.

Identification

Thin-layer chromatography is used as one of the principal means of identification of herbal drugs. In some cases where isolated constituents of herbal drugs are available, chromatographically separated constituents are related to the known constituents used as markers[1]. For purposes of evaluation, an hR_f value is used in place of an R_f value in order to preclude the use of decimal fractions. The hR_f value is the R_f value multiplied by the factor 100, resulting in values of 0 to 100.

In the monograph, the hR_f values of known and unknown constituents are listed in the table, accompanied by the corresponding thin-layer chromatograms. The illustrations of thin-layer chromatograms are provided in colour photographs.

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[1] Constituent(s) of a herbal material which is/are chemically defined and of interest for quality control purposes.

In cases where isolated constituents of herbal drugs are not readily available, a fingerprint of the separated constituents is obtained and the positions of major spots or bands in the chromatogram are described in relation to a non-constituent marker, in terms of their relative R_f values (RR_f). RR_f can be determined by the formula:

$$RR_f = a/b$$

where a = R_f value of a constituent of interest, and
b = R_f value of a non-constituent marker.

Due to variations in the levels of constituents in different samples of herbal drug, minor deviations from one chromatogram to another can be observed. A judgement by the analyst is needed as to the extent of deviation allowed before samples are considered incorrect or contaminated with foreign matter. Further investigations should be carried out in case of doubt.

Quantitative Determination

Unless otherwise specified, all quantitative determinations prescribed in the monographs are carried out on materials which have not been specially dried and calculations are made accordingly.

Arsenic and Heavy Metals

With regard to vegetable drugs, the toxic elements which may be present in sufficient quantity to pose potential risk vary from plant to plant. The amount of these elements depends on the location, the quality of the soil, or environmental pollution. Because of their toxic natures, arsenic and heavy metals are of major concern. Although not specifically required in the monograph, it is suggested that the maximum amounts of the toxic elements, based on the acceptable daily intake (ADI) values, in final dosage forms of plant materials be as follows:

Arsenic	4	ppm
Cadmium	0.3	ppm
Lead	10	ppm
Mercury	0.5	ppm

Microbial Contamination

Although not specifically required in the monographs, possible microbial contamination should be controlled to such an extent that the preparations derived from them meet the requirements as described in the “Limits for Microbial Contamination” (Appendix 10.5).

Strength(s) Available

Strength(s) available is provided only as a guide and is not necessarily comprehensive. For Solid dosage forms such as Capsules, the strength is usually given as the amount of herbal drugs, in powder form, in each unit. For herbal drugs intended for oral aqueous preparations such as Herbal Teas, the strength is usually given as the amount of herbal drugs, in powder form, in each unit dose.

Contra-indication

This section specifies those conditions in which the drug should NOT be used.

Warning and Precaution

Under the heading "Warning", the possible risks of certain hazards from the use of a herbal drug are to be observed and taken care of before prescribing or administering it to a patient. Caution and careful consideration on the risk-benefit ratio of the drug should therefore be contemplated on an individual basis prior to the decision to use it.

On the other hand, important notes to be observed and carefully followed during and after the administration of a drug are described under the heading "Precaution".

Where there is a clear risk, the important warnings and precautions are selected and included under the headings "Warning" and "Precaution" in some monographs. However, it should not be assumed that the omission of a warning or a precaution in any particular monograph means that warning or precaution may not be of clinical significance for a specific patient.

Additional Information

Any personal observation of a particular drug and other special relevant information concerned are to be categorized under the heading "Additional information". It is not regarded as analytical requirements.

Category and Dose

The statements given under "Category" are provided only for information on the drug's main pharmacological actions, which are presumably based on its use in traditional medicine. It should not be assumed that the substance has no other actions or uses. Information on doses is also related to its traditional use and is intended only for general guidance. The dose of a drug specified in this Pharmacopoeia is the usual dose for adults; some adjustments may be necessary for individual patients, including children, depending on their conditions. Unless otherwise stated, the information is given for internal use.

Remark It is to be noted that the actions and doses stated in the Pharmacopoeia do not imply any regulatory acceptance for the purpose of licensing.

Packaging and Storage

The substances and preparations described in the Pharmacopoeia are stored in such a way as to prevent contamination and, as far as possible, deterioration. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat, and light are indicated, where appropriate, in the monographs.

CONTAINERS

The container is the device that holds the substance, either in the form of the raw material or of the finished dosage form. The closure of the container, including the stopper, the cap, the attached dropper, etc., is considered as a part of the container.

The *immediate container* is the one which is in direct contact with the substance.

The container should be cleaned before use, and no extraneous matter should be introduced into it or into the substance placed in it. It must, likewise, not interact physically or chemically with the substance which it holds so as to alter the latter's quality, purity, or therapeutic potency to a level below its Pharmacopoeial requirements.

Well-closed container

A well-closed container must protect the contents from extraneous matter or from loss of the substance under ordinary or customary conditions of handling, shipment, storage, or sale.

Tightly closed container

A tightly closed container must protect the contents from contamination by extraneous matter or moisture, from loss of the substance, and from efflorescence, deliquescence, or evaporation under the ordinary or customary conditions of handling, shipment, storage, or sale, and shall be capable of tight reclosure. Where a tightly closed container is specified, it may be replaced by a hermetically closed container for a single-dose of the substance.

STORAGE

The following expressions are used in monographs under Packaging and storage with the meaning shown.

Protected from light means that the product is to be stored either in a light-resistant container or in a container enclosed in an outer cover that provides such protection or stored in a place from which all such light is excluded.

Protected from moisture means that the product is to be stored in a tightly closed container. Care is to be taken when the container is opened in a damp atmosphere. A low moisture content may be maintained, if necessary, by the use of a desiccant in the container provided that direct contact with the product is avoided.

STORAGE TEMPERATURES

When special conditions of storage are necessary, including limits of temperature, they are prescribed in the monograph. Where, in a monograph, the storage conditions are mentioned using the general expressions “at room temperature”, “in a cold place”, and the like, these terms are generally defined as follows.

Very cold temperature Any temperature above –10° but not higher than 8°. A *refrigerator* is a very cold place in which the temperature is maintained thermostatically between 2° and 8°.

Cool temperature Any temperature above 16° but not higher than 23°.

Room temperature Any temperature above 23° but not higher than 35°.

MONOGRAPHS

บอระเพ็ด (BORAPHET)

Tinosporae Crispae Caulis
Tinospora Crispa Stem

Category Antipyretic, bitter tonic, stomachic.

Tinospora Crispa Stem is the dried stem of *Tinospora crispa* (L.) Hook. f. & Thomson [*T. crispa* Diels, *T. rumphii* Boerl., *T. tuberculata* (Lam.) Beumée ex K. Heyne, *T. gibbericaulis* Hand. Mazz., *T. mastersii* Diels, *T. thorelii* Gagnep.] (Family Menispermaceae), Herbarium Specimen Number: DMSC 354, 355.

Constituents Tinospora Crispa Stem contains tinosporine, tinosporidine, picroretin, *N-trans*-feruloyl tyramine, *N-cis*-feruloyl tyramine, tinotuberide, borapetoside A, borapetol A, ceryl alcohol, *β*-sitosterol, stigmasterol, etc.

Description of the plant (Fig. 1) Woody climber with tuberous roots; young stems smooth, older ones very prominently tuberculate with exceedingly bitter sap; aerial roots filiform, very long. Leaves broadly ovate to orbicular, 5 to 14 cm long, 4 to 12 cm wide, apex acuminate, base cordate, palmately 5 to 7 nerved at the base; petioles 5 to 15 cm long. Inflorescences pseudoracemose, not coetaneous with the leaves. Male inflorescences very slender; a few in groups. Male flowers small, on filiform pedicels; sepals pale green, 3 outer ones ovate, 3 inner obovate; petals 3; stamens 6. Female inflorescences similar to male ones but shorter. Female flowers with sepals and petals as in male; staminodes 6; carpels 3. Drupe orange, ellipsoidal, up to 2 cm long.

Description Odour, indistinct; taste, intensely bitter.

Macroscopical (Fig. 1) Tinospora Crispa Stem occurs as cylindrical, transverse or oblique pieces, from 3 to 30 mm long and from 3 to 18 mm in diameter; externally brown, longitudinally wrinkled and numerous warty lenticels, internally pale greyish yellow, exhibiting a bark from 1.5 to 2.5 mm in thickness, surface, radiate and a minute disintegrated pith.

Microscopical (Figs. 2a, 2b) Transverse and longitudinal sections of the stem show cork, several layers of rectangular brownish cells. Cortex, broad zone of parenchyma cells containing starch granules; groups of stone cells (sclereids) containing prismatic crystals, occur beneath cork layers; parenchyma cells containing prismatic crystals occur in the innermost part of cortex adjacent to bast fibres. Stele composed of phloem and xylem separated by cambium, occurring several bands from cortex to pith, with medullary ray between the bands. Phloem composed of thick-walled bast fibres and phloem tissue; cambium, several layers of rectangular cells; xylem composed of large size vessels, xylem fibres and xylem parenchyma containing prismatic crystals; annular, spiral, reticulate, pitted and bordered-pitted vessels, up to 160 µm in diameter; medullary rays, non-lignified parenchyma containing starch granules. Pith, parenchyma cells containing starch granules.

Tinospora Crispa Stem in powder possesses the diagnostic microscopical characters of the unground drug.

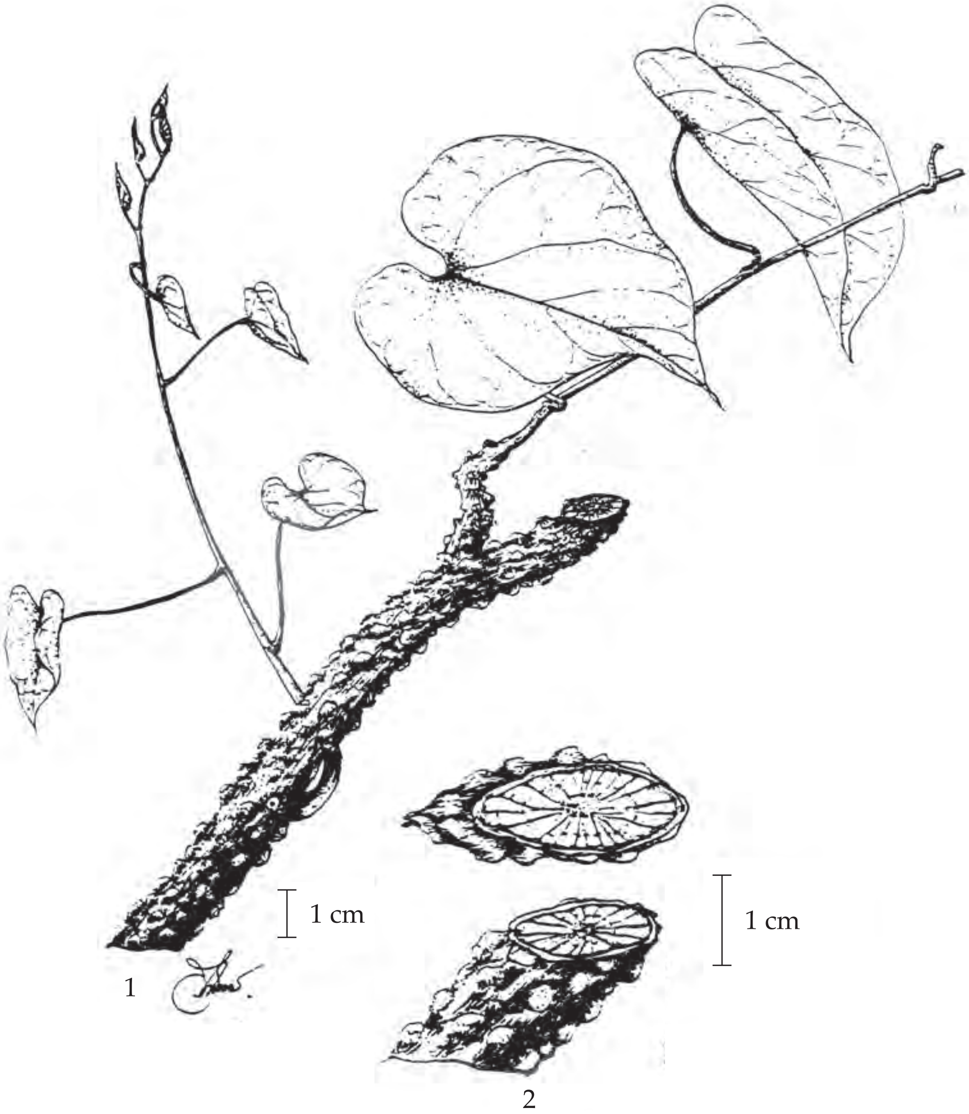


Fig. 1 *Tinospora crispa* (L.) Hook. f. & Thomson
1. branching stem 2. crude drug

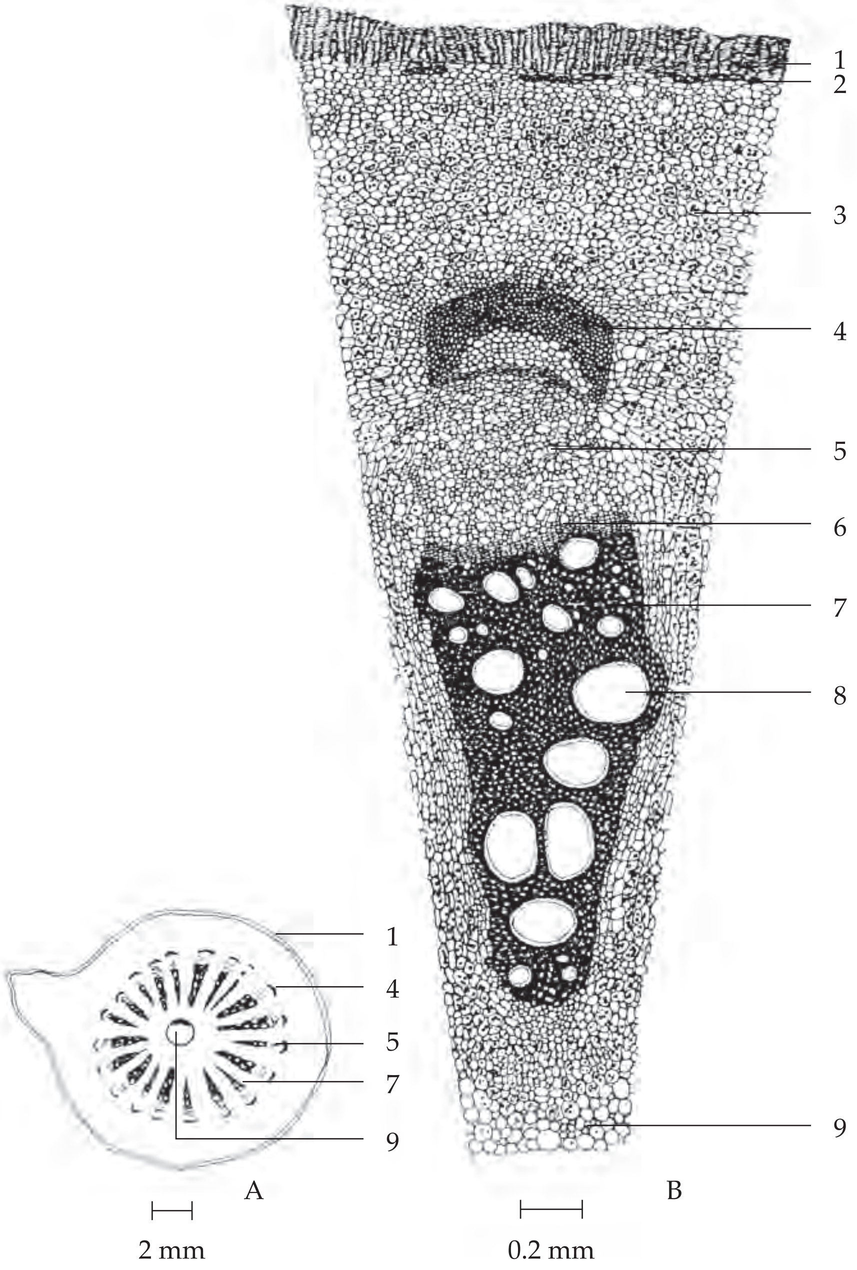


Fig. 2a Transverse Section of the Stem of *Tinospora crispa* (L.) Hook. f. & Thomson

A. Diagram
B. Part of Transverse Section

1. cork
2. sclereid
3. cortical parenchyma
4. fibre
5. phloem
6. cambium
7. xylem fibre
8. vessel
9. pith

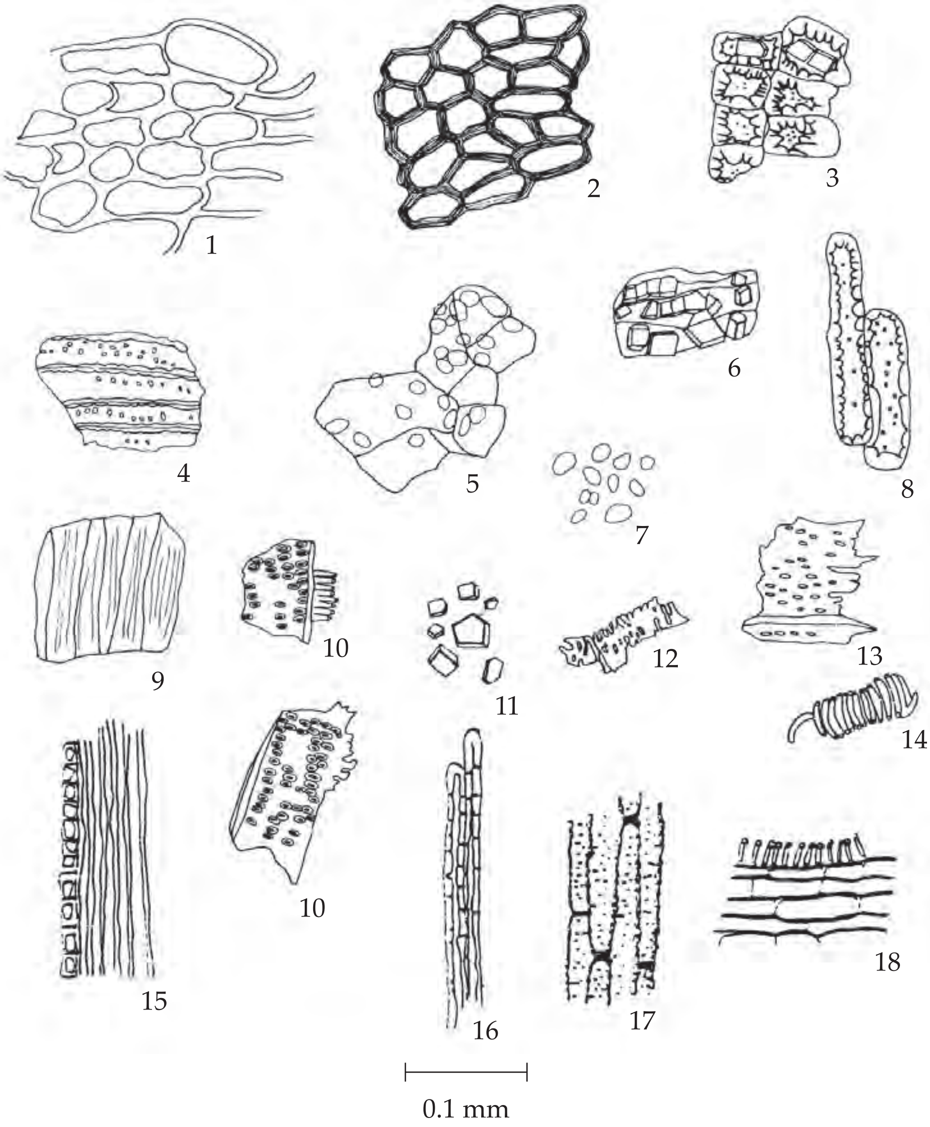


Fig. 2b Powdered Drug of the Stems of *Tinospora crispa* (L.) Hook. f. & Thomson

1. cortical parenchyma
2. cork in surface view
3. stone cells
4. xylem parenchyma
5. parenchyma cells with starch granules
6. parenchyma cells with prismatic cystals
7. starch granules
8. sclereids
9. phloem cells
10. bordered-pitted vessels
11. prismatic crystals
12. reticulate vessel
13. pitted vessel
14. spiral thickening
15. parenchyma cell containing calcium oxalate crystals adjacent to bast fibres
16. fragments of bast fibres with dentated wall
17. fragments of lignified parenchyma
18. fragments of annular vessel with parenchyma cells

Packaging and storage Tinospora Crispa Stem shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. To 500 mg of the sample, in powder, add 2 ml of *acetic anhydride*, warm on a water-bath for 2 minutes and filter. Slowly add 1 ml of *sulfuric acid* to the filtrate to form a layer: a brownish red ring forms at the zone of contact.

B. Shake vigorously 200 mg of the sample, in powder, with 10 ml of *water*: a long lasting foam is produced.

C. Add 10 ml of *methanol* to 1 g of the sample, in powder, warm on a water-bath for 10 minutes, shake intermittently, cool, and filter. To 1 ml of the filtrate, add a few drops of *acetic potassium iodobismuthate TS*: a brown precipitate forms.

D. **Test a** Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 75 volumes of *hexane*, 25 volumes of *ethyl acetate* and 1 volume of *glacial acetic acid* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply to the plate, 20 µl of the test solution prepared by warming 1 g of the sample, in powder, with 10 ml of *methanol* on a water-bath at 60° for 10 minutes, shaking, filtering, and evaporating to 2 ml. After removal of the plate, allow it to dry in air and examine under ultraviolet light (366 nm), marking the spots. Several spots of different colours are observed (Table 1); see also Fig. 3. Spray the plate with a 10 per cent w/v solution of *phosphomolybdic acid* in *ethanol*, and heat at 100° for 5 to 10 minutes. Several blue spots are observed (Table 1); see also Fig. 3.

Test b Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 75 volumes of *chloroform*, 20 volumes of *methanol* and 5 volumes of *strong ammonia solution* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply to the plate, 20 µl of the test solution prepared as described in *Test a*. After removal of the plate, allow it to dry in air and spray with *acetic potassium iodobismuthate TS.* Several spots of different colours are observed (Table 1); see also Fig. 3. Repeat the same procedure on another plate but spray with *iodoplatinate TS.* Several spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Stems of *Tinospora crispa* (L.) Hook. f. & Thomson

Spot	hR_f Value	Detection			
		Mobile Phase I		Mobile Phase II	
		UV 366	*Phosphomolybdic Acid*	*Acetic Potassium Iodobismuthate TS*	*Iodoplatinate TS*
1	1-3	light blue	dark blue	–	–
2	4-7	carmine red	–	–	–
3	9-12	moss green	–	–	–
4	12-17	light blue	–	–	–
5	14-16	–	–	orange	purple
6	20-24	light blue	–	–	–
7	25-29	carmine red	–	–	–
8	31-34	red	–	–	–
9	37-40	–	–	orange	purple
10	42-45	–	dark blue	–	–
11	45-50	red	–	–	–
12	49-51	–	–	orange	purple
13	51-55	carmine red	dark blue	–	–
14	55-58	–	dark blue	–	–
15	59-63	light blue	–	–	–
16	61-64	–	dark blue	–	–
17	86-90	–	dark blue	–	–
18	91-94	–	dark blue	–	–
19	92-96	–	–	–	orange
20	96-98	yellow	dark blue	moss green	moss green
21	98-99	–	–	yellow	yellow

Mobile phase I: 75 volumes of *hexane*, 25 volumes of *ethyl acetate* and 1 volume of *glacial acetic acid*

Mobile phase II: 75 volumes of *chloroform*, 20 volumes of *methanol* and 5 volumes of *strong ammonia solution*

Loss on drying Not more than 11.0 per cent w/w after drying 10 g at 105° for 5 hours (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 0.5 per cent w/w (Appendix 7.6).

Total ash Not more than 7.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 10.0 per cent w/w (Appendix 7.12).

Determination of bitterness Not less than 210 units per g when determined by the following method.

Standard preparation Transfer about 100 mg of *quinine hydrochloride*, accurately weighed, to a 100-ml volumetric flask, dissolve in safe drinking water, dilute to volume with the same solvent, and mix. Dilute this solution quantitatively with safe drinking water to obtain the solution containing 10 µg of *quinine hydrochloride* per ml. This solution is used as the stock solution of quinine hydrochloride (S_Q).

Prepare a serial dilution of S_Q in nine test-tubes according to the following table for the first series of testing.

No. of Tubes	1	2	3	4	5	6	7	8	9
ml of S_Q	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
ml of *water*	5.8	5.6	5.4	5.2	5.0	4.8	4.6	4.4	4.2
µg of *quinine hydrochloride* in 10 ml of the solution (C)	42	44	46	48	50	52	54	56	58

Test preparation Transfer about 200 mg of the sample, in powder, accurately weighed, into a 100-ml conical flask, add 45 ml of safe drinking water, and reflux in a boiling water-bath for 1 hour with frequent shaking. Cool, filter and dilute the filtrate with safe drinking water to 50.0 ml. Pipette 1.0 ml of this solution into a 100-ml volumetric flask and dilute with safe drinking water to volume. This solution is used as the stock solution of the sample (S_T). Calculate its concentration and express it in µg per ml.

Prepare a serial dilution of S_T in 10 test-tubes according to the following table for the second series of testing.

No. of Tubes	1	2	3	4	5	6	7	8	9	10
ml of S_T (b)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
ml of *water*	9.0	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	–

Procedure After rinsing the mouth with safe drinking water, taste 10 ml of the dilution swirling it in the mouth mainly near the base of the tongue for 30 seconds. Unless otherwise specified, always begin with the lowest concentration of the serial dilution. If the bitter sensation is no longer felt, withdraw the solution and wait for 1 minute to ascertain that there is no delayed sensitivity. Then rinse with safe drinking water. The next highest concentration of dilution should not be tasted until at least 10 minutes have passed. The threshold bitter concentration is the lowest concentration of dilution at which a material still provokes a bitter sensation. After the first series of tests, rinse the mouth thoroughly with safe drinking water, until no bitter sensation remains and wait for at least 10 minutes before carrying out the second series of tests. In this series of testing and in order to save time, it is advisable to first ascertain whether the solution in tube no. 5 (containing 5 ml of S_T in 10 ml) gives a bitter sensation. If noted, find the threshold bitter concentration of the material by tasting the dilutions in tubes nos. 1 to 4. If the solution in tube no. 5 does not give a bitter sensation, find the threshold bitter concentration in the dilutions of tubes nos. 6 to 10. All solutions and safe drinking water for mouthwashing should be at 20° to 25°.

Calculation

$$\text{Bitterness} = \frac{2000 \times C}{a \times b} \quad \text{units/g},$$

where a = µg of sample contained in 1 ml of S_T,

b = ml of S_T contained in 10 ml of the solution of threshold bitter concentration, and

C = µg of *quinine hydrochloride* contained in 10 ml of the solution of threshold bitter concentration.

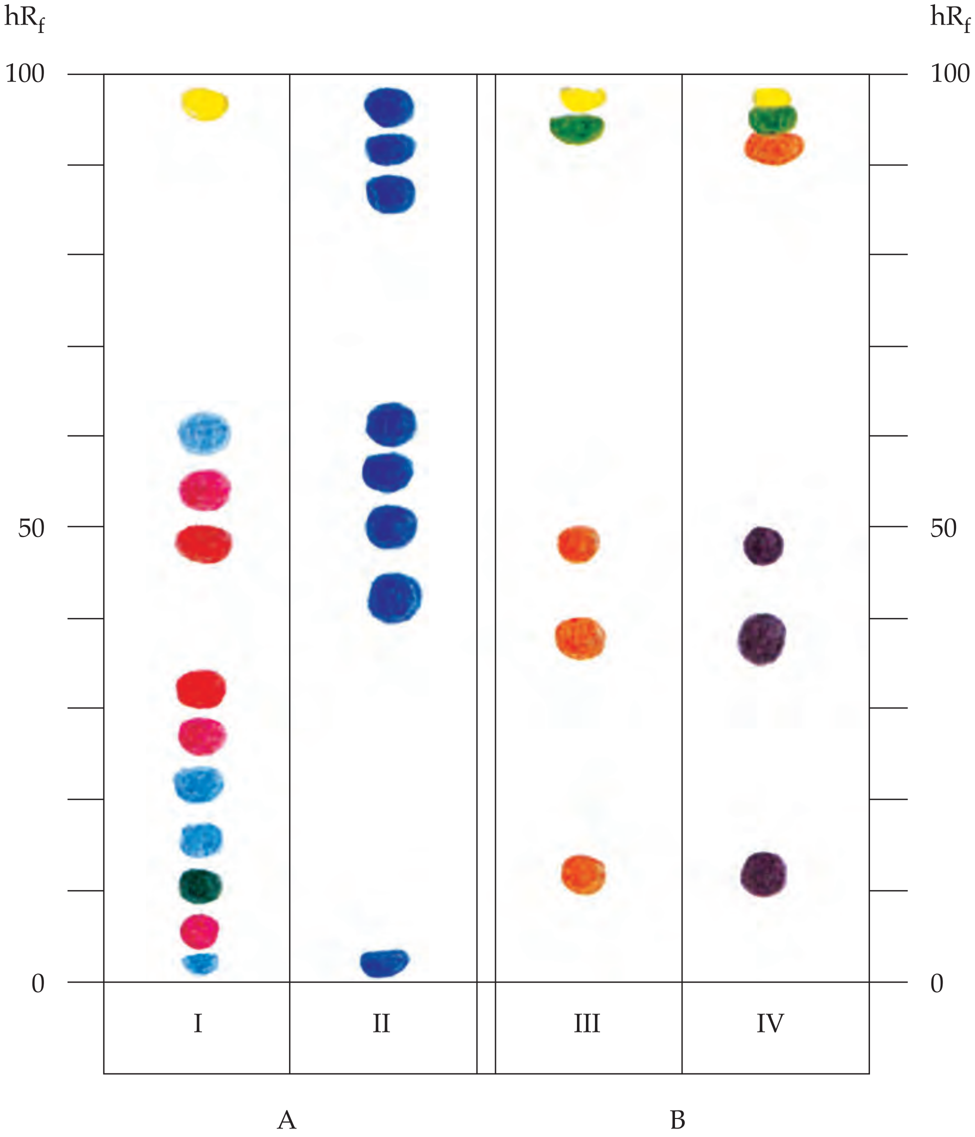


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Stems of *Tinospora crispa* (L.) Hook. f. & Thomson

A = use 75 volumes of *hexane*, 25 volumes of *ethyl acetate* and 1 volume of *glacial acetic acid* as the mobile phase.
B = use 75 volumes of *chloroform*, 20 volumes of *methanol* and 5 volumes of *strong ammonia solution* as the mobile phase.
I = detection under UV light (366 nm)
II = detection with a 10 per cent w/v solution of *phosphomolybdic acid* in *ethanol*
III = detection with *acetic potassium iodobismuthate TS*
IV = detection with *iodoplatinate TS*

บัวบก (BUABOK)

ผักหนอก (PHAK NOK)
Centellae Asiaticae Herba
Centella

Synonyms Asiatic Pennywort, Gotu Kola, Indian Pennywort, Indian Water Navelwort

Category Mild diuretic, anti-inflammatory, wound healing (topical).

Centella is the dried aerial part of *Centella asiatica* (L.) Urb. (*C. coriacea* Nannf., *Hydrocotyle asiatica* L., *H. lunata* Lam., *Trisanthus cochinchinensis* Lour.) (Family Umbelliferae), Herbarium Specimen Number: DMSC 1461, Crude Drug Number: DMSc 1261.

Constituents Centella contains triterpenoid saponins, including asiaticoside and madecassoside and their aglycones which are asiatic acid and madecassic acid, respectively. It also contains volatile oil, pectin, trace of alkaloids, etc.

Description of the plant (Figs. 1a, 1b) Slender trailing herb, rooting at the nodes. Leaves simple, 1 to 6 in rosette at each node, orbicular to reniform, more or less cupped, glabrous and shiny above, paler beneath, 1 to 7 cm in diameter, apex rounded, base cordate, margin entire, crenate, or usually repand-dentate; petioles (1) 4 to 10 (50) cm long. Inflorescence in single umbel, bearing solitary or 2 to 5 together in the axils; peduncles shorter than petioles. Flowers usually 3, middle one sessile, lateral ones pedicellate; involucres 2, ovate; petals 5, minute, white or rose-tinged; ovary laterally flattened, style filiform. Fruit small, compressed, 8 mm long, orbicular to ellipsoid, manifestly ribbed, slightly hairy when young.

Description Odour, characteristic; taste, slightly bitter-sweet.

Macroscopical (Fig. 1a) Aerial part, greenish brown, rough and brittle; stem thin, long, twisted; leaves rennate or cordate, brittle; petiole long.

Microscopical (Figs. 2a, 2b, 2c, 2d) Transverse section of the fresh leaf shows upper epidermis, a layer of rectangular cells, polygonal and straight-walled in surface view; stomata, anisocytic, some paracytic and rarely anomocytic. Palisade cells, a layer of large columnar cells. Spongy cells, parenchymatous, some containing calcium oxalate crystals in the forms of rosette aggregate or prism. Collenchyma, occurring beneath upper and lower epidermis in the midrib. Vascular bundles, xylem in the upper part and phloem in the lower part; vessels, annular, spiral, scalariform, or reticulate. Lower epidermis, a layer of rectangular cells, slightly wavy-walled in surface view; stomata, anisocytic, paracytic or anomocytic. Oil ducts, occurring beneath collenchyma in the middle of midrib.

Transverse sections of the fresh petiole and stolon show epidermal layer with cuticle. Collenchyma, present. Parenchyma containing chloroplastids, oil droplets, spreading circularly beneath collenchyma. Vascular bundles, collateral. The centre of petiole, hollow. Unicellular trichomes may also be found, but rare, in the section near the base of petiole.

Centella in powder possesses the diagnostic microscopical characters of the unground drug.

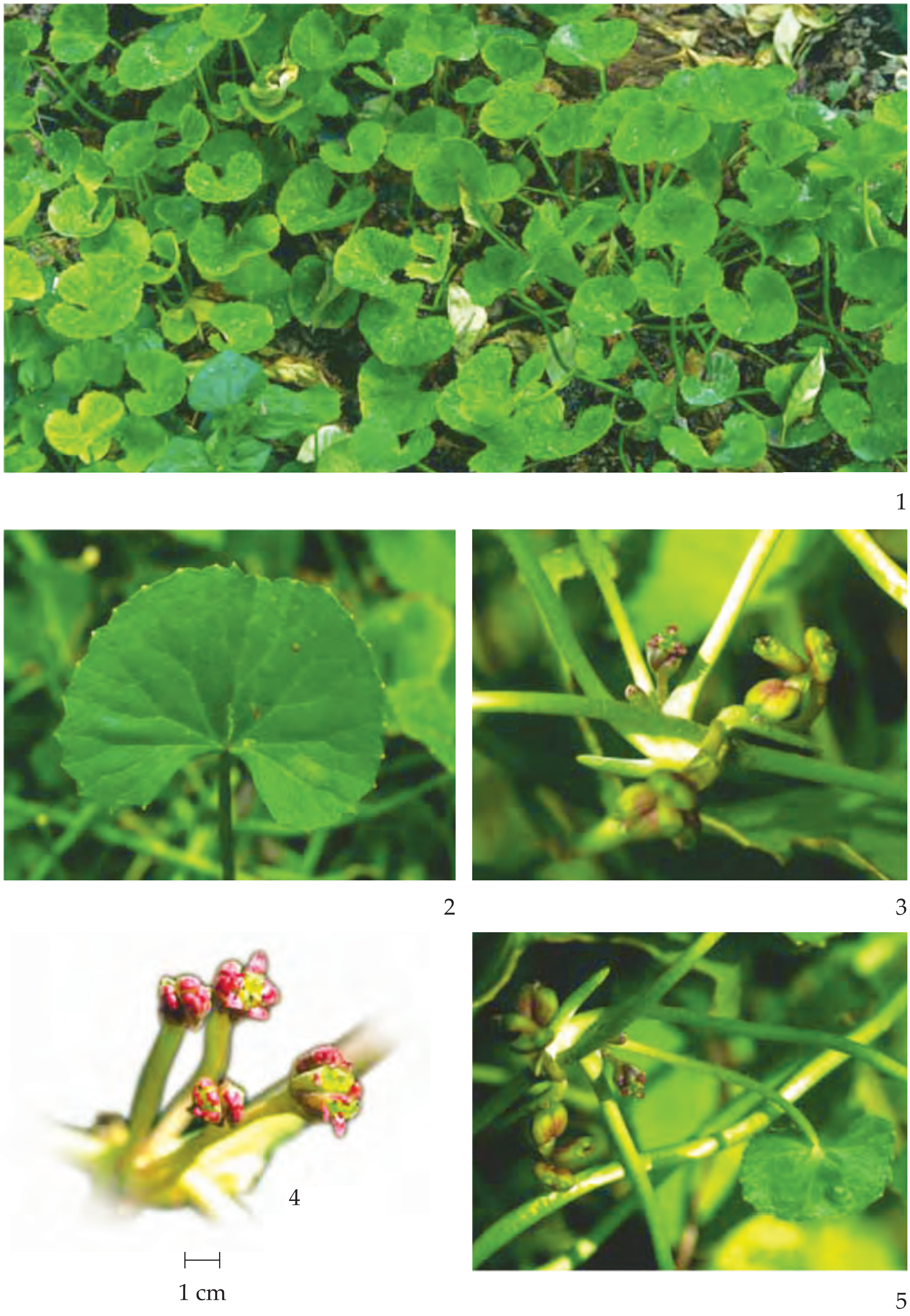


Fig. 1a *Centella asiatica* (L.) Urb.
1. habit 2. leaves 3. flowers and fruits 4. inflorescence 5. leaves, flowers, fruits

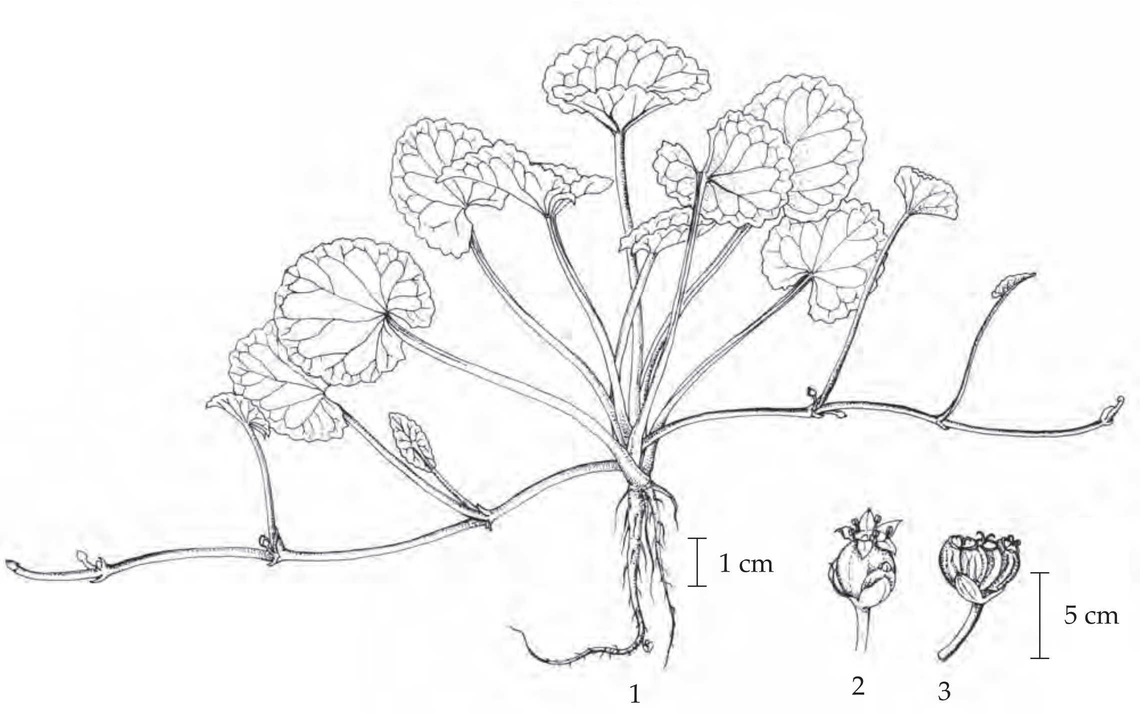
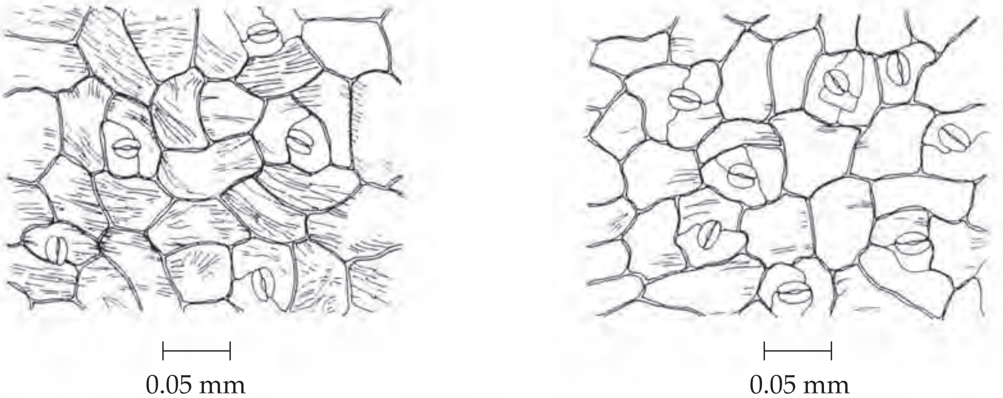


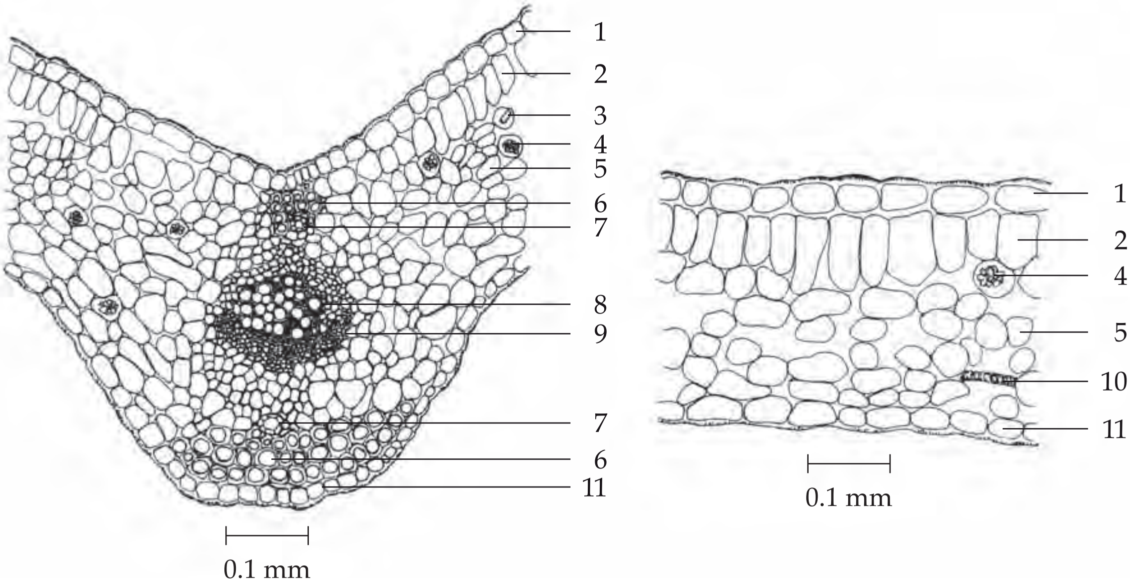
Fig. 1b *Centella asiatica* (L.) Urb.
1. habit 2. inflorescence 3. fruits



Upper Epidermis of the Lamina

Lower Epidermis of the Lamina

Fig. 2a Epidermises of the Fresh Leaf of *Centella asiatica* (L.) Urb.

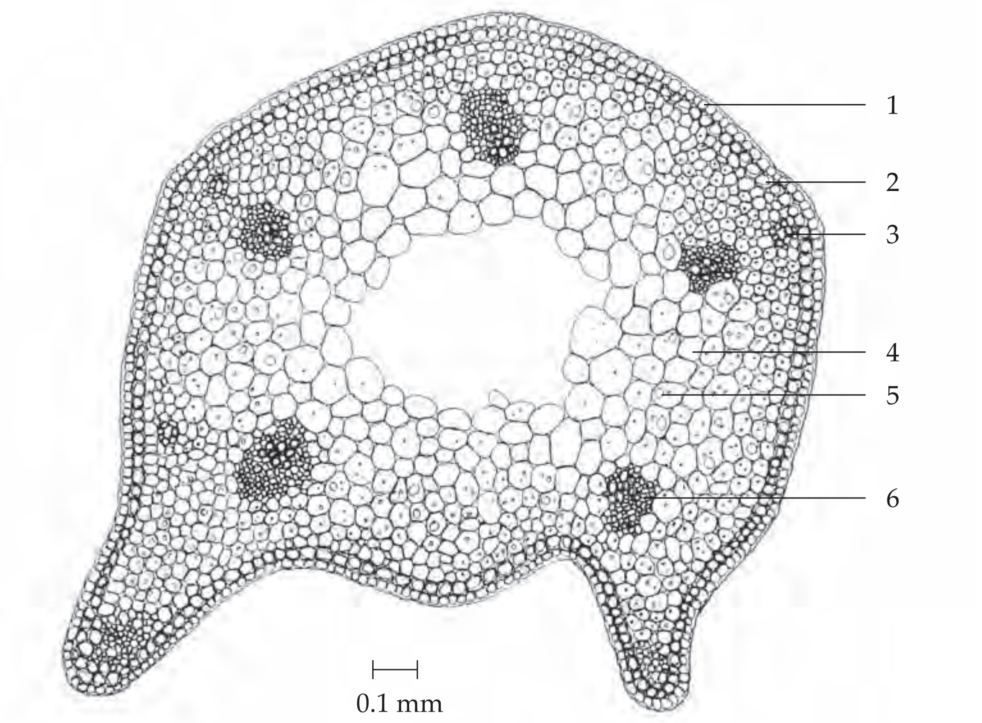


Transverse Section of the Midrib

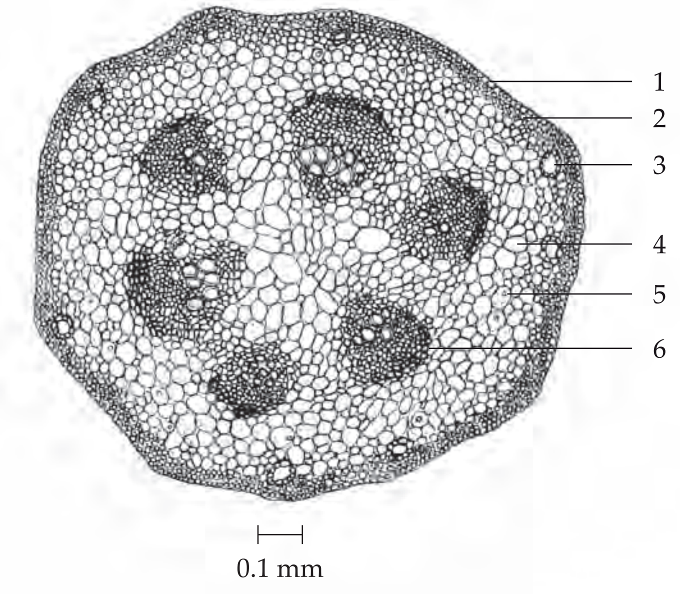
Transverse Section of the Lamina

Fig. 2b Transverse Section of the Fresh Leaf of *Centella asiatica* (L.) Urb.

1. upper epidermis
2. palisade cell
3. prismatic crystal
4. rosette aggregate crystal
5. spongy cell
6. collenchyma
7. oil duct
8. xylem
9. phloem
10. vessel
11. lower epidermis



Transverse Section of the Petiole



Transverse Section of the Stolon

Fig. 2c Transverse Section of the Fresh Petiole and Stolon of *Centella asiatica* (L.) Urb.

1. epidermis	4. parenchyma
2. collenchyma	5. oil droplet
3. oil duct	6. vascular bundle

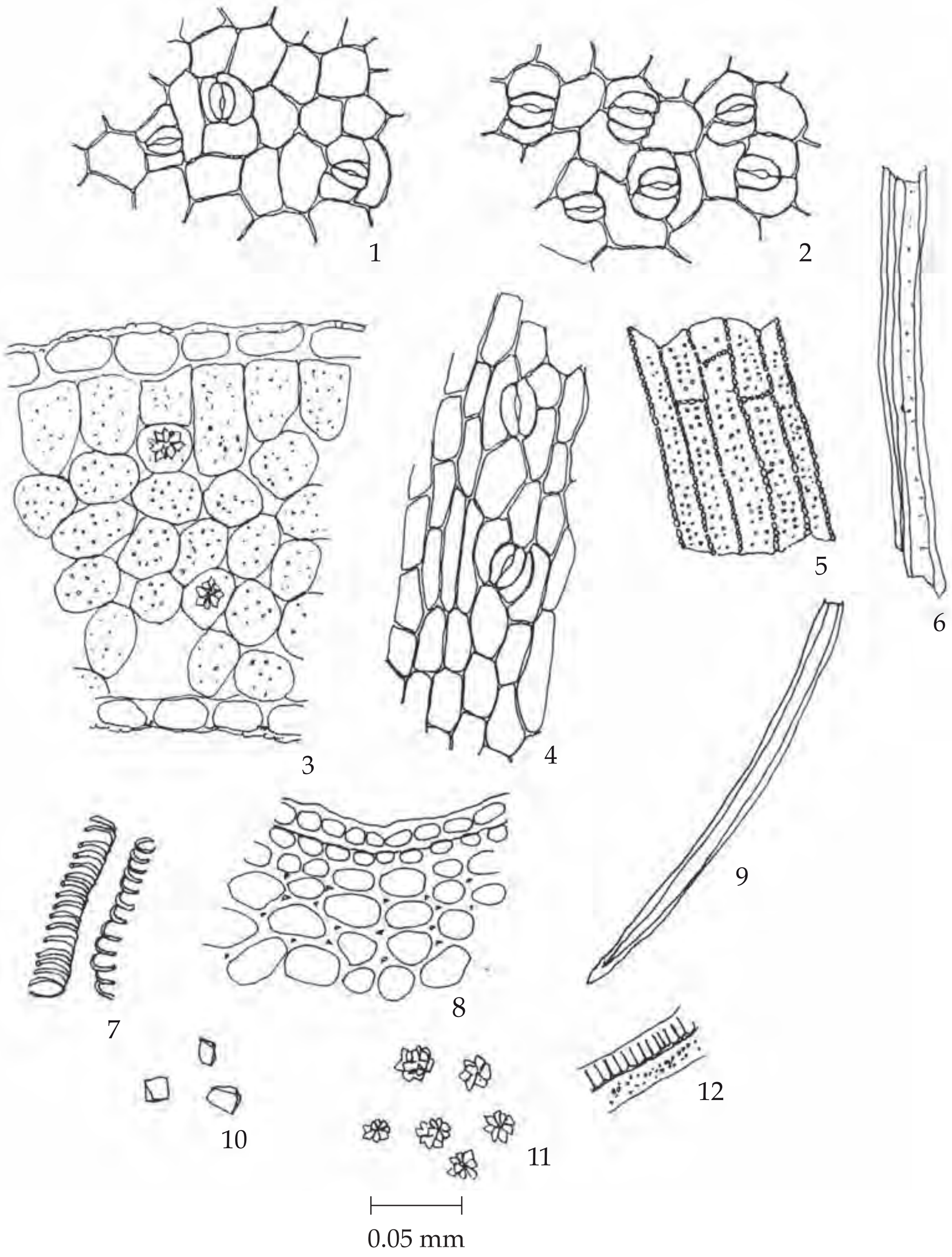


Fig. 2d Powdered Drug of the Aerial Parts of *Centella asiatica* (L.) Urb.

1. upper epidermis
2. lower epidermis
3. lamina in sectional view
4. epidermis with stomata from petiole
5. pitted vessels
6. fibres
7. spiral vessels
8. epidermis and collenchyma in sectional view
9. unicellular trichome
10. prismatic crystals
11. rosette aggregate crystals
12. scalariform and pitted vessels

Warning Excessive oral administration should be avoided during pregnancy and lactation.

Packaging and storage Centella shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Warm 500 mg of the sample, in powder, with 5 ml of *ethanol* for 5 minutes and filter (solution 1). To 2 ml of solution 1, add a few drops of *sulfuric acid*: a green colour develops.

B. Evaporate 2 ml of solution 1 to dryness and dissolve the residue in 2 ml of *acetic anhydride*. Add slowly 1 ml of *sulfuric acid* to form two layers: a green colour develops in the upper layer and a brownish red ring forms at the zone of contact.

C. Shake vigorously 500 mg of the sample, in powder, with 10 ml of *water*: a long lasting foam is produced.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 15 volumes of *chloroform*, 7 volumes of *methanol* and 1 volume of *water* as the mobile phase. Apply separately to the plate, 5 µl each of the following solutions. Prepare solution (A) by refluxing 1 g of the sample, in powder, with 20 ml of *ethanol* for 10 minutes and filtering. Evaporate the filtrate under reduced pressure at 40° until dryness, and dissolve the residue in 4 ml of *ethanol*. For solution (B), dissolve 1 mg of *asiaticoside* in 2 ml of *ethanol*. For solution (C), dissolve 1 mg of *asiatic acid* in 2 ml of *ethanol*. After removal of the plate, allow it to dry in air. Spray the plate with *anisaldehyde TS* and heat at 105° for 5 minutes. The chromatogram obtained from solution (A) shows a purple spot (hR_f value 27 to 28) and a violet spot (hR_f value 72 to 74), corresponding to the asiaticoside and the asiatic acid spots from solutions (B) and (C), respectively. Several other spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Aerial Parts of *Centella asiatica* (L.) Urb.

Spot	hR_f Value	Detection
		Anisaldehyde TS
1	2	green
2	5	green
3	8	green
4	16	green
5*	27-28	purple
6	33-34	violet
7	43	violet
8	63-64	violet
9	67	green
10**	72-74	violet
11	79-80	brown-violet
12	84	brown-green
13	87-88	violet-green
14	91-92	green

*asiaticoside
**asiatic acid

Loss on drying Not more than 14.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 7.0 per cent w/w (Appendix 7.6).

Total ash Not more than 17.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 15.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 24.0 per cent w/w (Appendix 7.12).

Dose 0.6 g three times a day.

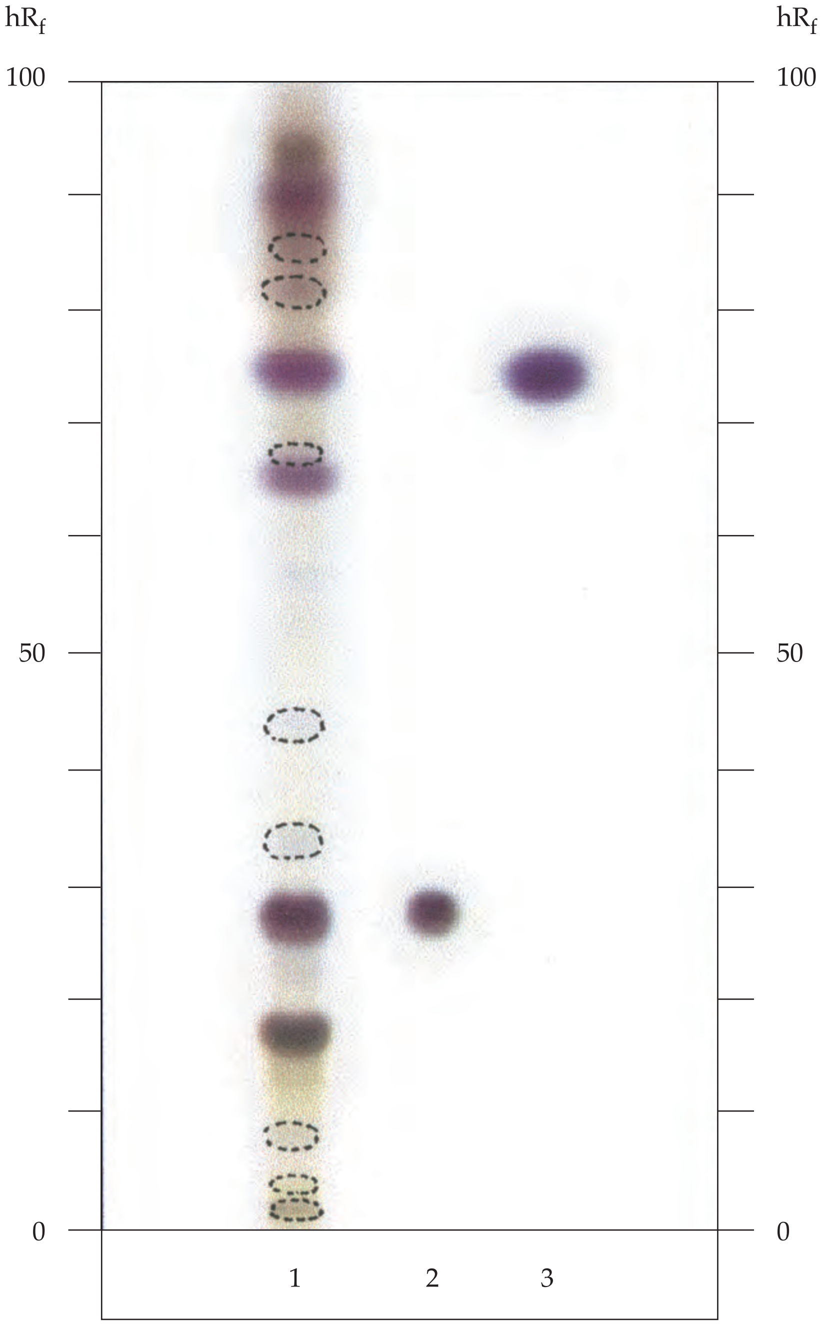


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Aerial Parts of *Centella asiatica* (L.) Urb., Detected with *Anisaldehyde TS*

1 = solution (A)
2 = solution (B)
3 = solution (C)
◌ = spots developed in some samples

สารสกัดแห้งบัวบก (BUABOK DRY EXTRACT)

Centella Dry Extract

Category Anti-inflammatory, wound healing (topical).

Centella Dry Extract is prepared from the powdered Centella by extraction with *ethanol*. It contains not less than 90.0 per cent and not more than 110.0 per cent of the labelled amounts of asiaticoside ($C_{48}H_{78}O_{19}$) and madecassoside ($C_{48}H_{78}O_{20}$); the labelled amounts of asiaticoside and madecassoside are not less than 3.0 per cent and 4.0 per cent, respectively, calculated on the dried basis.

Description Pale greenish yellow to brownish yellow powder; slightly hygroscopic.

Packaging and storage Centella Dry Extract shall be kept in tightly closed containers, protected from light, and stored in a cool and dry place.

Labelling The label on the container states the amounts of asiaticoside and madecassoside.

Identification

A. The chromatogram of the Assay preparation shows several peaks, two of which correspond to those of the Standard preparation, as obtained in the *Assay*.

B. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 15 volumes of *chloroform* and 7 volumes of *methanol* and 1 volume of *water* as the mobile phase and allowing the solvent front to ascend 8 cm above the line of application. Apply separately to the plate as bands of 8 mm, 15 µl each of the following three solutions. For solution (A), dissolve 50 mg of the sample in 4 ml of *methanol*. Solution (B) contains 0.1 mg/ml of *asiaticoside* in *methanol* and solution (C) contains 0.1 mg/ml of *madecassoside* in *methanol*. After removal of the plate, allow it to dry in air. Spray the plate with *anisaldehyde TS* and heat at 105° for 5 minutes. The chromatogram obtained from solution (A) shows a greenish blue band and a violet band, corresponding to the asiaticoside and the madecassoside bands from solutions (B) and (C), respectively. Several other bands of different colours are observed.

Loss on drying Not more than 10.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Assay Carry out the determination as described in the “High-pressure Liquid Chromatography” (Appendix 3.5)

Mobile phase A Use *acetonitrile*, containing 6.0 per cent v/v of *methyl* tert-*butyl ether* and 0.01 per cent v/v of *trifluoroacetic acid*. Make adjustments if necessary.

Mobile phase B Prepare a 0.01 per cent v/v solution of *trifluoroacetic acid.* Make adjustments if necessary.

Standard preparation Dissolve an accurately weighed quantity of *asiaticoside* and *madecassoside* in *methanol* to obtain a solution having known concentrations of about 100 µg of asiaticoside and 100 µg of madecassoside per ml.

Assay preparation Transfer an accurately weighed quantity of Centella Dry Extract, containing about 5 mg of asiaticoside, to a 50-ml volumetric flask. Dissolve and dilute with *methanol* to volume and mix. Centrifuge, if necessary. Pass a portion of the clear solution through a filter having a 0.45 µm or finer porosity.

Chromatographic system The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (4 µm), polar end-capped (Synergi Hydro RP C_{18} or equivalent is suitable.), equipped with a similarly packed guard column, maintained at a temperature of 30°, (b) *Mobile phase* at a flow rate of 1.0 ml per minute (the retention time is about 60 minutes for the main asiaticoside peak.), and (c) an ultraviolet photometer set at 210 nm. The step gradient of mobile phases is as follows:

Time (Minutes)	Mobile Phase A (Per Cent V/V)	Mobile Phase B (Per Cent V/V)
0	17	83
10	17	83
45	19	81
70	19	81

To determine the suitability of the chromatographic system, chromatograph *Standard preparation*, and record the peak response as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0 per cent, the column efficiency determined from the asiaticoside peak is not less than 2000 theoretical plates, the symmetry factors for the madecassoside peak and for the asiaticoside peak are not more than 1.5, the relative retention times are about 0.6 for madecassoside and 1.0 for asiaticoside, and the resolution factors, *R*, between the madecassoside and its adjacent peaks and between the asiaticoside and its adjacent peaks are not less than 1.5.

Procedure Separately inject equal volumes (about 20 µl) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the madecassoside and asiaticoside peaks.

Calculation Calculate the contents of $C_{48}H_{78}O_{19}$ and $C_{48}H_{78}O_{20}$ in the portion of the Extract taken, using the declared contents of $C_{48}H_{78}O_{19}$ and $C_{48}H_{78}O_{20}$ in *asiaticoside* and *madecassoside*, respectively.

Other requirements Complies with the requirements described under "Extracts" (Appendix 1.16H).

ยาครีมบัวบก (BUABOK CREAM)

Centella Cream

Category Wound healing (topical).

Centella Cream is Centella Dry Extract in a suitable hydrophilic cream base. It contains not less than 90.0 per cent and not more than 110.0 per cent of the labelled amounts of asiaticoside ($C_{48}H_{78}O_{19}$) and madecassoside ($C_{48}H_{78}O_{20}$).

Strength available 7 per cent w/w of centella dry extract (about 0.2 per cent w/w of asiaticoside).

Dose *Topical*, to the wound, one to three times a day.

Contra-indication It is contra-indicated in patients hypersensitive to herbal drugs belonging to Family Umbelliferae.

Warning

1. Contact with eyes should be avoided.
2. It may cause allergic contact dermatitis.

Precaution Treatment with Centella Cream should be discontinued if there is no response after two weeks.

Packaging and storage Centella Cream shall be kept in collapsible tubes or in tightly closed container, protected from light, and stored at a temperature not exceeding 30°.

Labelling The label on the container states (1) the amounts of asiaticoside and madecassoside; (2) expiration date.

Identification

A. The chromatogram of the Assay preparation shows several peaks, two of which correspond to those of the Standard preparation as obtained in the *Assay*.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 15 volumes of *chloroform* and 7 volumes of *methanol* and 1 volume of *water* as the mobile phase and allowing the solvent front to ascend 8 cm above the line of application. Apply separately to the plate as bands of 8 mm, 15 µl each of the following three solutions. For solution (A), transfer 6 g of the sample to a 125-ml Erlenmeyer flask. Add 25.0 ml of *methanol*, shake well and sonicate for about 30 minutes. Transfer the solution to a 50-ml volumetric flask, dilute with *methanol* to volume and mix. Centrifuge the resulting solution at 13,684×*g* (12,000 rpm) at –20° for 15 minutes. Pass a portion of the supernatant through a filter having a 0.45 µm or finer porosity. Solution (B) contains 0.1 mg/ml of *asiaticoside* in *methanol* and solution (C) contains 0.1 mg/ml of *madecassoside* in *methanol*. After removal of the plate, allow it to dry in air. Spray the plate with *anisaldehyde TS* and heat at 105° for 5 minutes. The chromatogram obtained from solution (A) shows a greenish blue band and a violet band, corresponding to the asiaticoside and the madecassoside bands from solutions (B) and (C), respectively. Several other bands of different colours are observed.

Microbial limit Complies with the requirements for Category 1B in the "Limits for Microbial Contamination" (Appendix 10.5).

Assay Carry out the determination as described in the "High-pressure Liquid Chromatography" (Appendix 3.5).

Mobile phase A, **Mobile phase B, Standard preparation, Chromatographic system,** and **Procedure** Proceed as directed in the Assay under *Centella Dry Extract*.

Assay preparation Transfer an accurately weighed quantity of Centella Cream, containing about 5 mg of asiaticoside, to a 125-ml Erlenmeyer flask. Add 25.0 ml of *methanol*, shake well and sonicate for about 30 minutes. Quantitatively transfer the solution to a 50-ml volumetric flask, dilute with *methanol* to volume and mix. Centrifuge the resulting solution at 13,684×*g* (12,000 rpm) at –20° for 15 minutes. Pass a portion of the clear solution through a filter having a 0.45 µm or finer porosity.

Calculation Calculate the contents of $C_{48}H_{78}O_{19}$ and $C_{48}H_{78}O_{20}$ in the portion of the Cream taken, using the declared contents of $C_{48}H_{78}O_{19}$ and $C_{48}H_{78}O_{20}$ in *asiaticoside* and *madecassoside*, respectively.

Other requirements Complies with the requirements described under "Topical Preparations" (Appendix 1.16).

บัวหลวง, เกสร (BUA LUANG, KESON)

บัว, เกสร (BUA, KESON)

Nelumbinis Stamen

Sacred Lotus Stamen

Synonyms Indian Lotus Stamen, Lotus Stamen

Category Cardiotonic, antipyretic.

Sacred Lotus Stamen is the dried stamen of *Nelumbo nucifera* Gaertn. (*Nelumbium speciosum* Willd.) (Family Nelumbonaceae), Herbarium Specimen Number: DMSC 5156, Crude Drug Number: DMSc 0891, 0892.

Constituents Sacred Lotus Stamen contains flavonoids and carotenoids as its major components. It also contains arbutin, β-sitosterol glucopyranoside, etc.

Description of the plant (Figs. 1a, 1b) Perennial aquatic herb with milky latex and stout creeping rhizomes. Leaves simple, alternate, arising above the water surface, circular, 10 to 100 cm in diameter, margin entire or slightly undulate, papery, greyish green below, green on the upper, peltate, veins radiate from its centre in all directions, become forked near the margin; petiole terete, stout, up to 1 m or more long, about 1 cm in diameter, smooth or prickly. Flower solitary, large and showy, on thick peduncle rising several centimetres above the leaves; flower bud ovate, acute, 5 to 8 cm long, flower hemispheric as anthesis, 8 to 25 cm in diameter; perianth with 4 or 5 outermost sepals, elliptic to ovate, 1.5 to 5 cm long, 0.8 to 3.5 cm wide, free, green or pinkish green, to white or pink with age, petal-like, incurved; petals 5 to numerous, elliptic, 4 to 15 cm long, 2 to 8 cm wide, obtuse, incurved, white to dark pink or reddish; stamens numerous, 2.2 to 4.5 cm long, anther linear, 1.5 to 2 cm long, up to 2 mm wide, golden yellow, with white clavate connective appendage up to 7 mm long at the apex, incurved; ovary apocarpous, 12 to 30 free carpels; receptacle expanded to cone-shape, flat upper surface, 3 to 5 cm long, about 2.5 cm wide with individual carpels sunken into it; style short. Fruit indehiscent nutlets, embedded in an accrescent spongy conical receptacle, 9 to 13 cm long, 4 to 7 cm wide; nutlet ovoid, 1.5 to 2 cm long; pericarp thick, hard, brownish or greyish black when dried, endocarp thin, whitish pulp. Seed 1.

Description Odour, slightly aromatic; taste, astringent.

Macroscopical (Fig. 1a) Sacred Lotus Stamen consists of anther, anther connective appendage and filament. Anther linear, twisted, longitudinally split, 1.2 to 1.5 cm long, about 1 mm in diameter, pale yellow to brownish yellow. Filament slender, slightly curved, 1.5 to 1.8 cm long, pale yellow to brownish.

Microscopical (Figs. 2a, 2b) Transverse section of the filament shows an epidermal layer with papillae, secretory substances in a parenchyma layer adjacent to the epidermal layer, loose parenchyma cells some of which contain secretory substances, air spaces, and a vascular bundle at the centre.

Transverse section of the anther reveals an epidermal layer with papillae, a fibrous layer, pollen grains in 4 pollen sacs. The contact region between filament and the 4 pollen sacs shows an epidermal layer, parenchyma cells some of which contain secretory substances, and a vascular bundle at the centre.

Fig. 1a *Nelumbo nucifera* Gaertn.
1. habit (Buntharik, บุณฑริก) 2. flower (Buntharik, บุณฑริก) 3. habit (Pathum, ปทุม)
4. flower (Pathum, ปทุม) 5. stamens and receptacle 6. crude drug

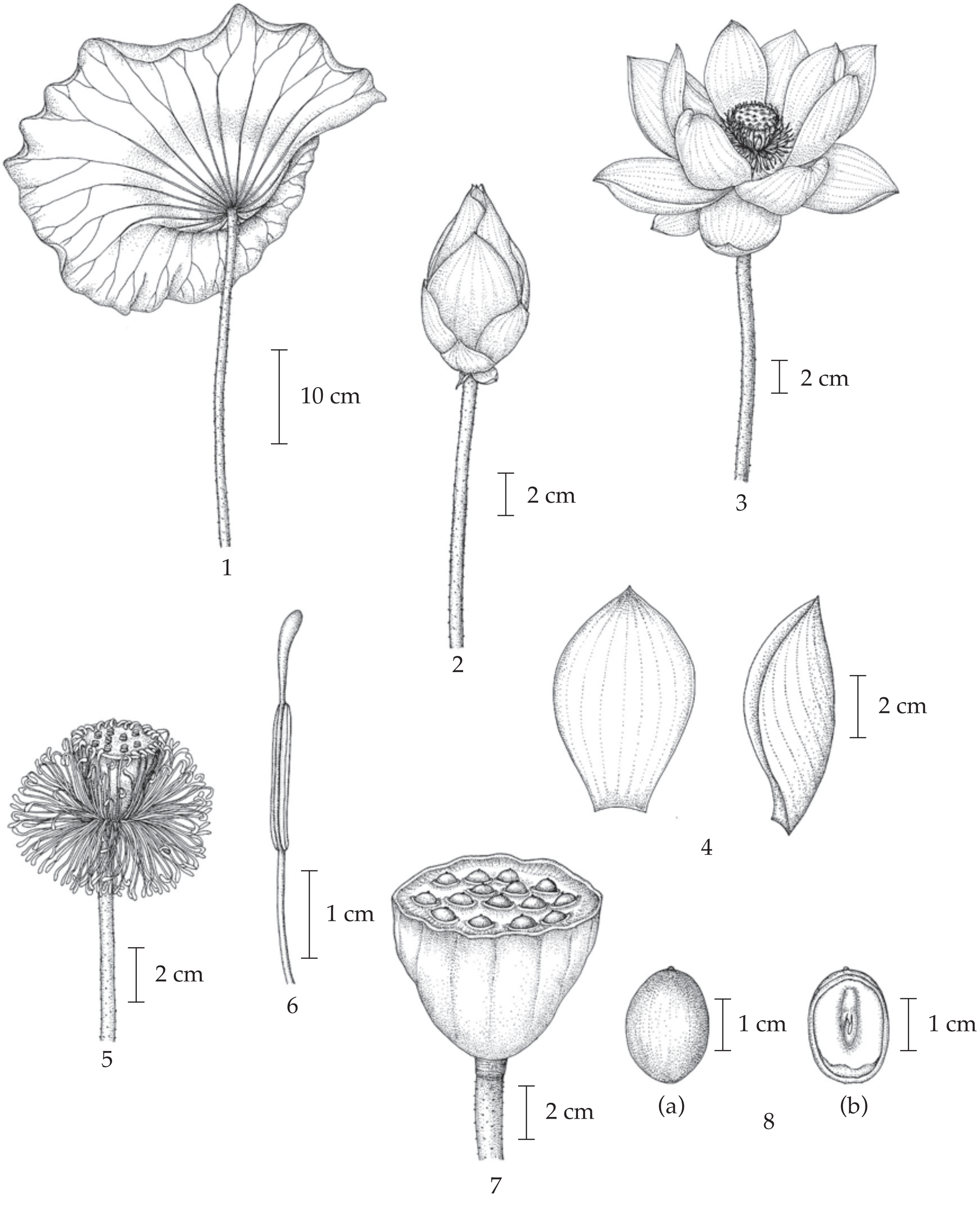


Fig. 1b *Nelumbo nucifera* Gaertn.
1. leaf 2. flower bud 3. flower 4. petal, in front and side views
5. central part of flower showing stamens and receptacle
6. stamen showing connective appendage, anther and part of filament
7. infructescence 8. nutlet (a), longitudinal section of nutlet showing embryo (b)

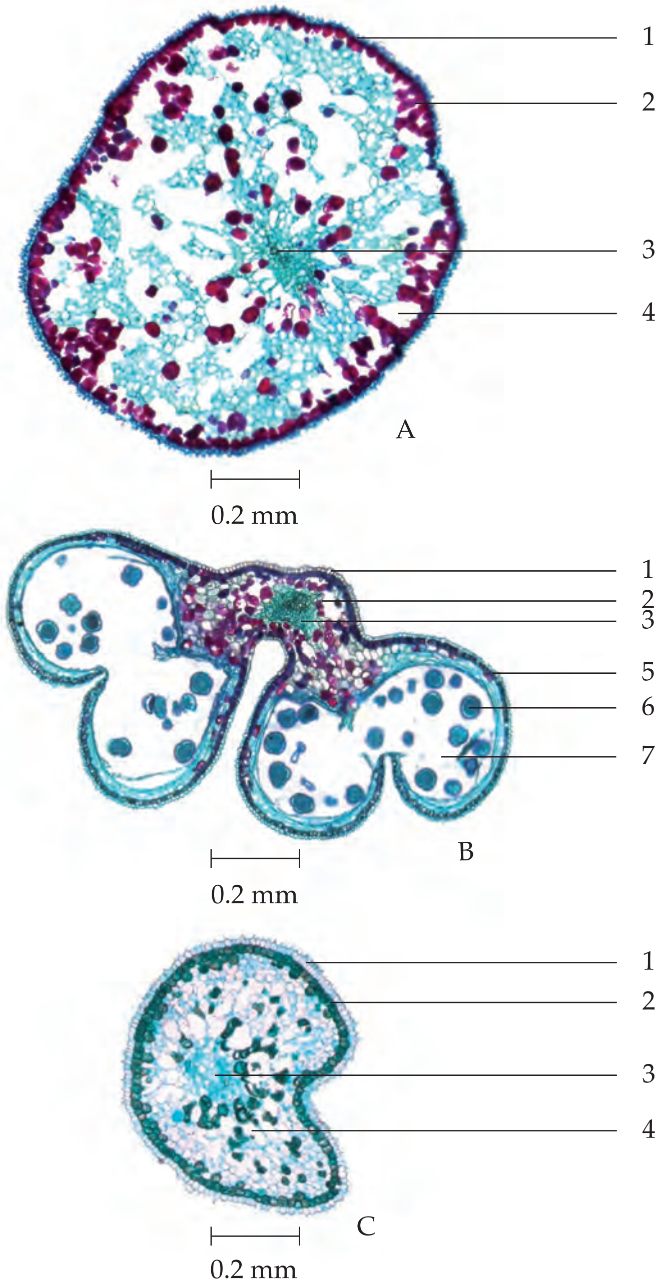


Fig. 2a Photomicrographs of Transverse Section of the Stamen of *Nelumbo nucifera* Gaertn., Stained with Safranin-Fast Green

A. Appendage
B. Anther
C. Filament
1. epidermal layer with papillae
2. parenchyma cell with secretory substances
3. vascular bundle
4. air space
5. fibrous layer of pollen sac
6. pollen grain
7. pollen sac

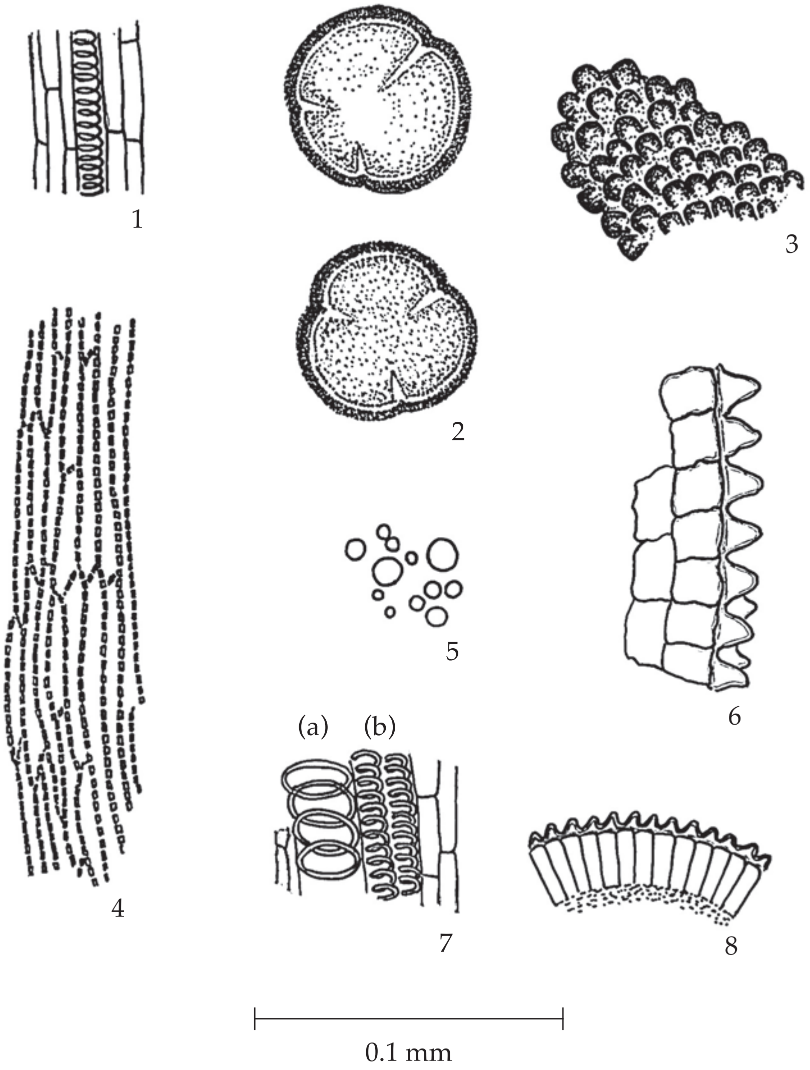


Fig. 2b Powdered Drug of the Stamens of *Nelumbo nucifera* Gaertn.

1. fragment of a filament in longitudinal view
2. pollen grains
3. papillae of epidermal layer in surface view
4. fibrous layer of pollen sac in surface view
5. oil globules
6. filament showing papillae of epidermal cells, and parenchyma cells in sectional view
7. annular vessel (a) and spiral vessels (b) in vascular bundle
8. fragment of pollen sac containing epidermal layer with papillae in sectional view

Transverse section through appendage of the stamen illustrates an epidermal layer with papillae, secretory substances in a parenchyma layer adjacent to the epidermal layer, loose parenchyma cells some of which contain secretory substances, air spaces, and a vascular bundle at the centre.

Sacred Lotus Stamen in powder possesses the diagnostic microscopical characters of the unground drug.

Additional information

1. Sacred lotus cultivated in Thailand is classified into four types based on the form of the flower bud and the colour of the flower: Pathum (ปทุม), Buntharik (บุณฑริก), Sattabongkot (สัตตบงกช), and Sattabut (สัตตบุษย์). Only stamens of the first two types are used in Thai traditional medicine.
2. It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Sacred Lotus Stamen shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Sonicate 2.5 g of the sample, in *fine powder,* with 10 ml of *methanol* for 45 minutes, centrifuge and use a clear supernatant (solution 1). To 1 ml of solution 1, add 2 or 3 pieces of *magnesium ribbon*, shake well and mix with 4 drops of *hydrochloric acid*: a pink colour develops.

B. To 1 ml of solution 1, add 10 mg of *zinc powder* and 4 drops of *hydrochloric acid* and shake well: a reddish orange colour develops.

C. To 1 ml of solution 1, add 4 drops of *sulfuric acid* and shake well: a brown colour develops.

D. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 60 volumes of *ethyl acetate* and 40 volumes of *toluene* as the mobile phase. Apply to the plate as a band of 5 mm, 15 µl of the test solution prepared by sonicating 100 mg of the sample, in *fine powder*, with 10 ml of *methanol* for 15 minutes, filtering, and evaporating the filtrate to dryness. Dissolve the residue in 1.5 ml of *methanol*. After removal of the plate, allow it to dry in air and examine the plate under ultraviolet light (366 nm); two blue bands are observed. Spray the plate with *vanillin-sulfuric acid TS1* and heat at 120° for 5 minutes; several violet bands appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Stamens of *Nelumbo nucifera* Gaertn.

Band	hR_f Value	Detection	
		UV 366	*Vanillin-Sulfuric Acid TS1*
1	52-53	–	pale violet
2	61-62	–	pale violet
3	69-70	blue	violet
4	77-79	–	pale violet
5	84-85	light blue	violet

Loss on drying Not more than 12.0 per cent w/w after drying at 105° for 3 hours (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 6.0 per cent w/w (Appendix 7.7).

Water-soluble extractive Not less than 10.5 per cent w/w (Appendix 7.12).

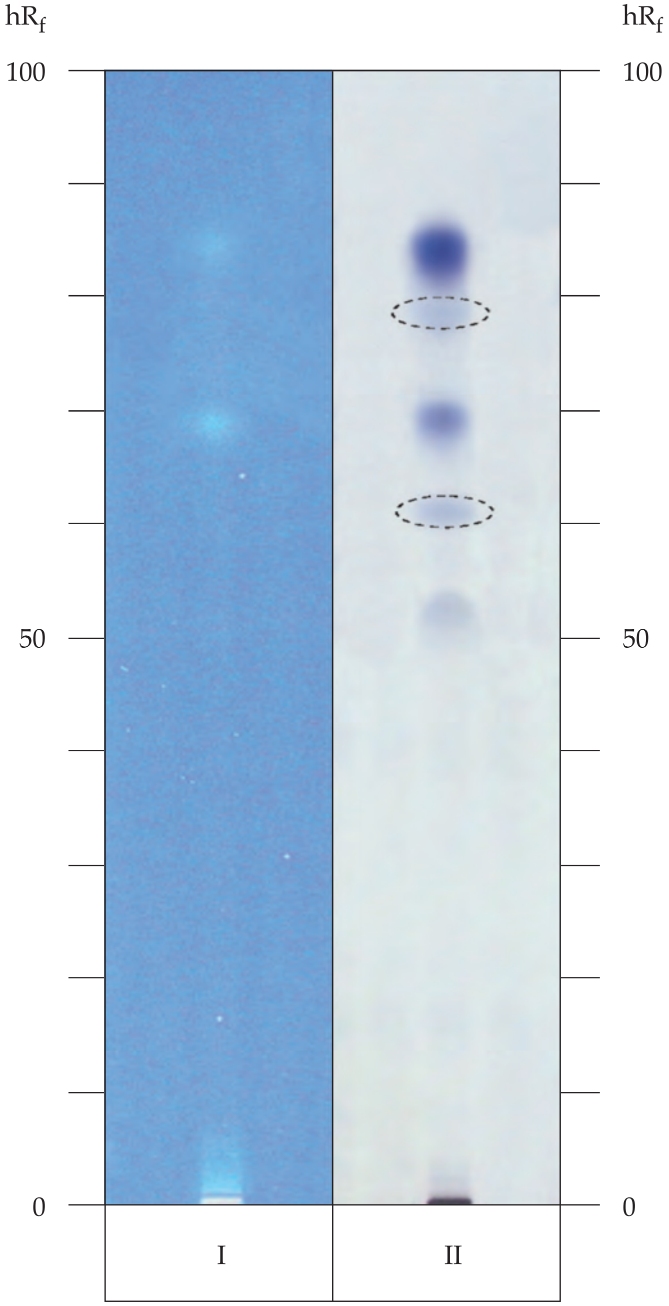


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Stamens of *Nelumbo nucifera* Gaertn.

I = detection under UV light (366 nm)
II = detection with *vanillin-sulfuric acid TS1*
◌ = bands developed in some samples

บุนนาค (BUNNAK)

Mesuae Ferreae Flos
Ironwood Flower

Synonyms Ceylon Ironwood Flower, Cobra's Saffron Flower, Indian Rose Chestnut Flower

Category Cardiotonic, antipyretic.

Ironwood Flower is the dried blooming flower of *Mesua ferrea* L. (Family Clusiaceae), Herbarium Specimen Number: DMSC 5165, BKF 166239, Crude Drug Number: DMSc 0763.

Constituents Ironwood Flower contains 4-phenylcoumarins, biflavonoids, chromones, triterpenes, volatile oil, oleoresin, etc.

Description of the plant (Figs. 1a, 1b) Tree up to 30 m high, trunk upright, cylindrical, often buttressed at base, young twig slender, exuding aromatic white resin when wounded. Leaves simple, opposite, elliptic, oblong or lanceolate, 6 to 13 cm long, 1 to 4 cm wide, apex acute, base acute or obtuse, margin entire, blade leathery, midrib faint and depressed on both surfaces, lateral veins numerous, very fine, almost invisible, lower surface whitish glaucous; young leaves reddish to pinkish; petiole 0.4 to 1.2 cm long. Flower solitary or fascicled, fragrant, axillary; peduncle 0.8 to 2.3 cm long, slender; sepals 4, light green, orbicular, 1 to 1.5 cm long, arranged in 2 rows, outer pair small, inner pair larger, densely velvety puberulous outside, fleshy; petals 4, white or pinkish, obovate or obcordate, 1.5 to 4 cm long, base cuneate, margin curled, brown or purple striations, caducous; stamens numerous, anthers orange to golden yellow, linear, 0.4 to 1 cm long; ovary superior, ovoid, up to 5 mm long, 2-loculed, each locule 2-ovuled, style 1, about 1 cm long, stigma peltate. Fruit ovoid to ellipsoid, 2.5 to 3.5 cm long, with conical apex, striate; sepals enlarged up to 4 cm long, persistent, dark orange or purplish brown; pericarp tough. Seed(s) 1 to 4, up to 2.4 cm long, woody, smooth, glossy, brown, oily.

Description Odour, aromatic; taste, astringent.

Macroscopical (Fig. 1a) Complete dried flowers, broken flowers, fragments of sepals, petals and stamens are commonly found. Sepals dark brown; petals and stamens, light or golden brown.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the sepal shows rectangular cells of upper and lower epidermises, with unicellular trichomes. Mesophyll composed of polygonal parenchyma cells, some containing rosette aggregates of calcium oxalate, sclereids, resin ducts, small air spaces, and vascular bundles.

Transverse section of the petal shows rectangular cells of upper and lower epidermises. Mesophyll composed of loose polygonal parenchyma cells, resin ducts, air spaces, and vascular bundles.

Transverse section of the filament reveals a layer of epidermis with papillae, wavy parenchyma cells and a vascular bundle.

Transverse section of the anther shows 4 pollen sacs containing a layer of epidermis with papillae, a fibrous layer of pollen sac, pollen grains; at the centre comprising epidermis, parenchyma, resin ducts, and a vascular bundle.

1

2

3

4

Fig. 1a *Mesua ferrea* L.
1. habit 2. flower 3. fruiting twig 4. crude drug

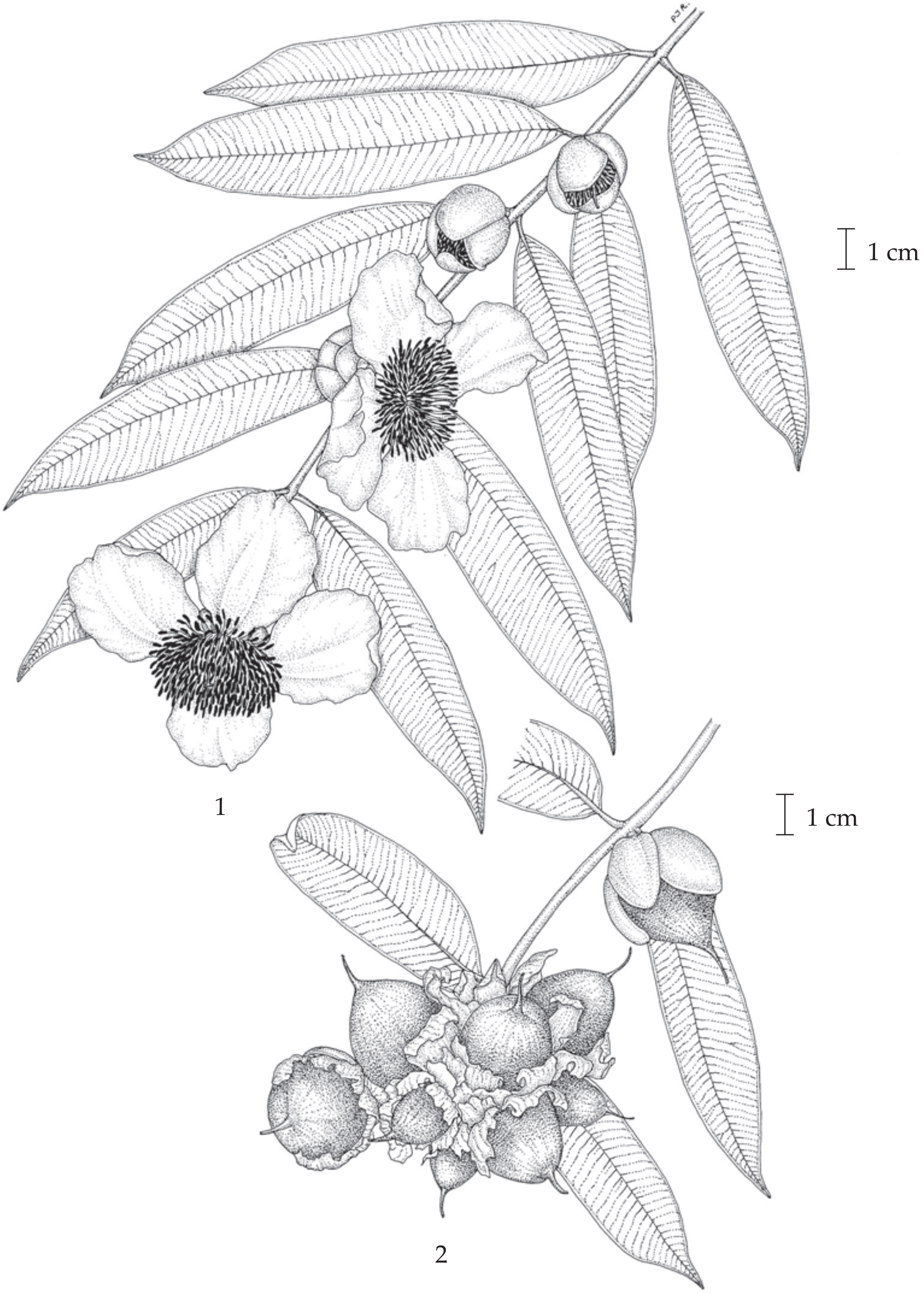


Fig. 1b *Mesua ferrea* L.
1. flowering twig 2. fruiting twig

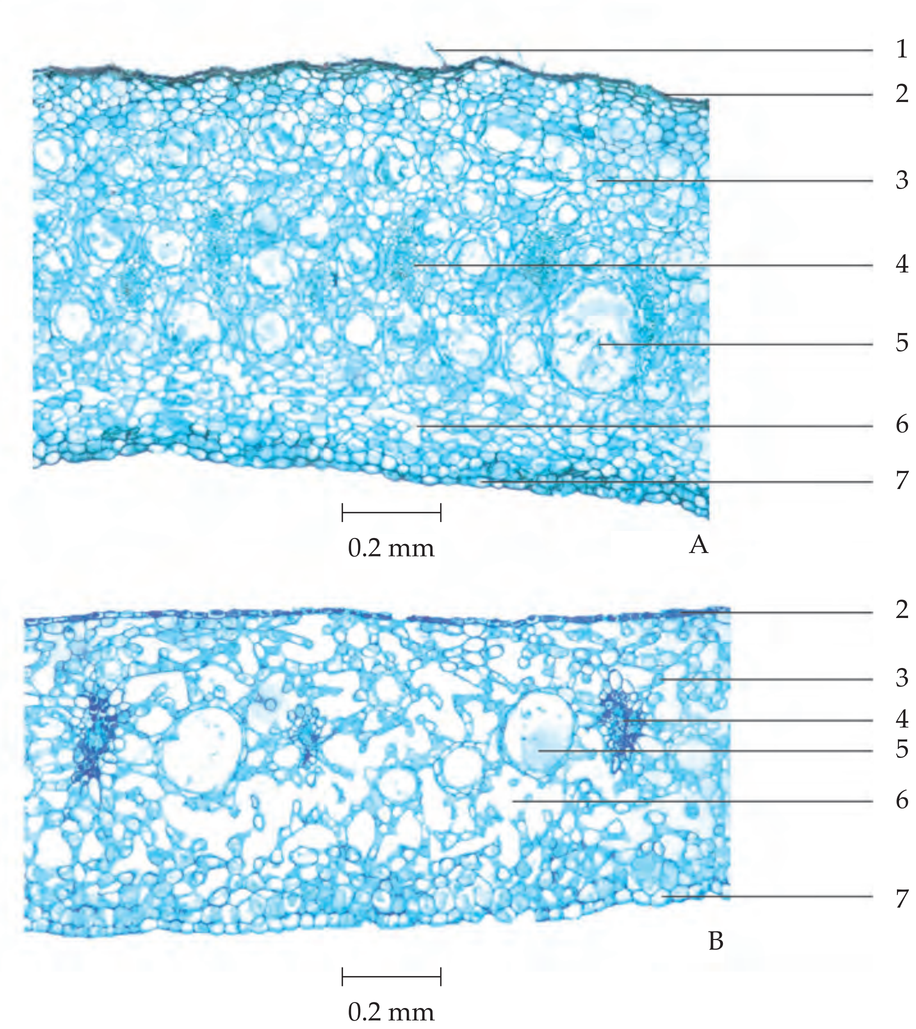


Fig. 2a Photomicrographs of Transverse Section of the Flower of *Mesua ferrea* L., Stained with Safranin-Fast Green

A. Sepal
B. Petal

1. unicellular trichome
2. upper epidermis
3. parenchyma
4. vascular bundle
5. resin duct
6. air space
7. lower epidermis

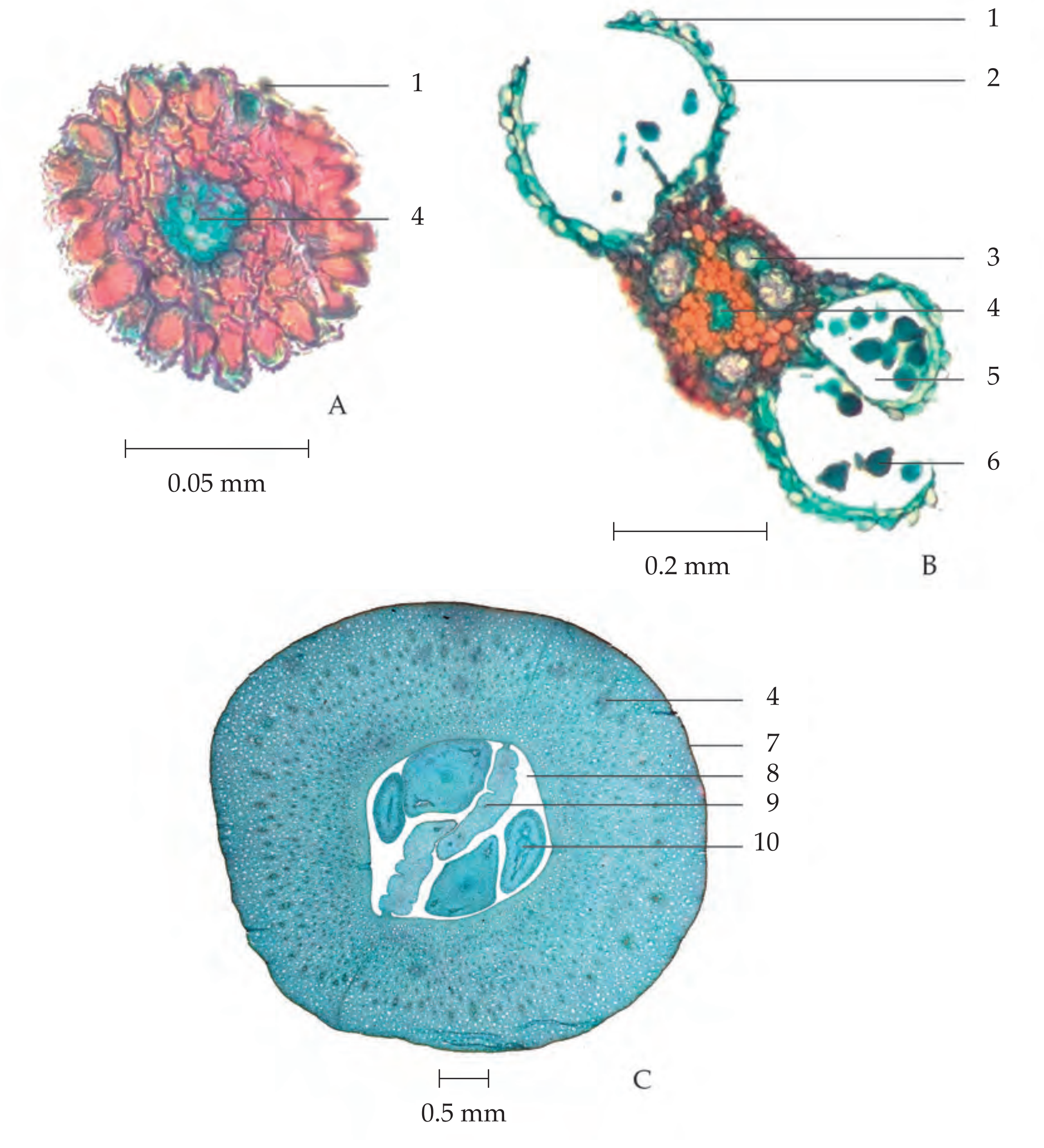


Fig. 2b Photomicrographs of Transverse Section of the Flower of *Mesua ferrea* L., Stained with Safranin-Fast Green

A. Filament
B. Anther
C. Ovary

1. epidermal cell with papilla
2. fibrous layer of pollen sac
3. resin duct
4. vascular bundle
5. pollen sac
6. pollen grain
7. epidermis of ovary wall
8. locule
9. septum
10. ovule

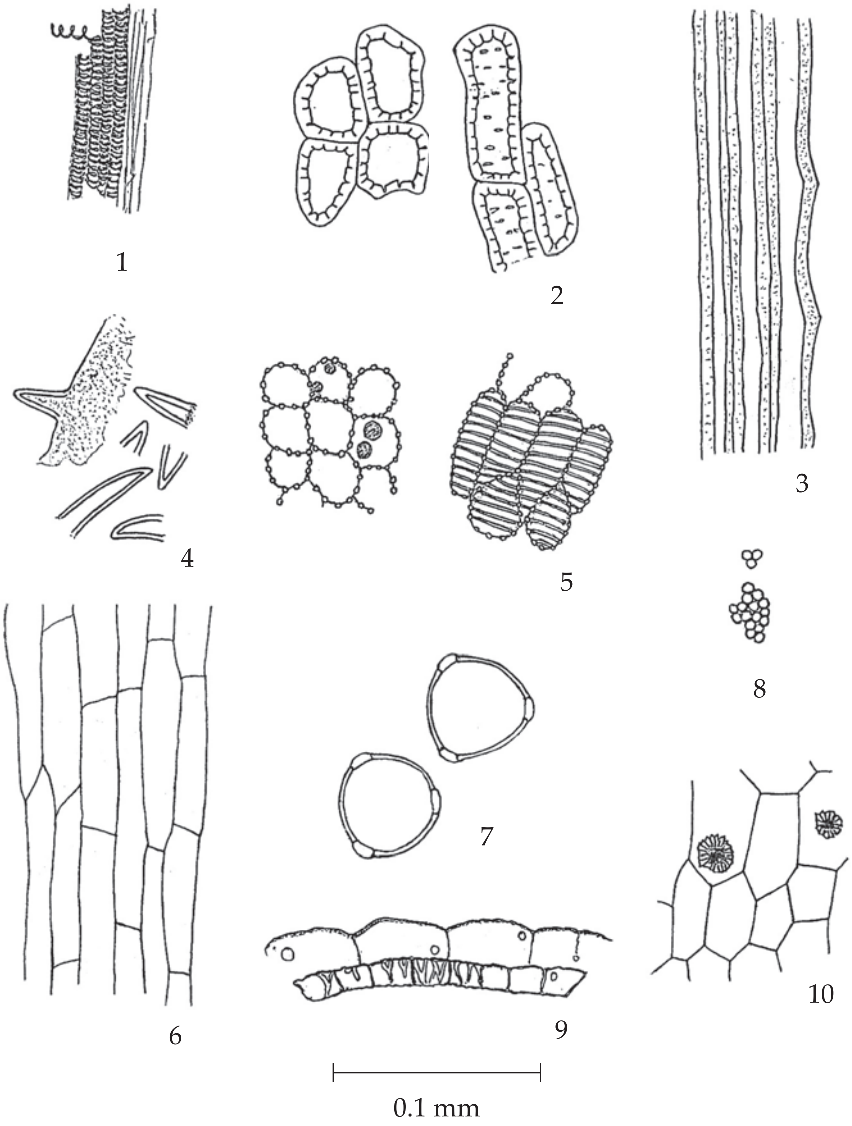


Fig. 2c Powdered Drug of the Flowers of *Mesua ferrea* L.

1. fragment of spiral vessels and fibres
2. sclereids
3. fibres
4. unicellular trichomes of sepal
5. fragments of fibrous layer of pollen sac in different views, some containing oil globules
6. fragment of filament showing elongated epidermal cells
7. pollen grains
8. starch grains
9. part of pollen sac in sectional view with epidermal layer and underlying fibrous layer
10. fragment of sepal showing parenchyma cells and rosette aggregates

Transverse section of the ovary shows ovary wall, 2 locules, septum, and ovules. The ovary wall composed of a layer of small epidermal cells, parenchyma cells and vascular bundles. The locules divided by a septum, each containing 2 ovules.

Ironwood Flower in powder possesses the diagnostic microscopical characters of the unground drug.

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Ironwood Flower shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. To 5 g of the sample, in powder, add 20 ml of *ethanol*, sonicate for 1 hour and filter (solution 1). To 2 ml of solution 1, add 4 or 5 pieces of *magnesium ribbon,* shake well and mix with a few drops of *hydrochloric acid*: a pink colour develops.

B. To 2 ml of solution 1, add a few drops of a 2.5 per cent w/v solution of *iron(III) chloride* and shake well: a blue-green colour develops.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 80 volumes of *hexane* and 20 volumes of *ethyl acetate* as the mobile phase and allowing the solvent front to ascend 14 cm above the line of application. Apply to the plate as a band of 10 mm, 20 µl of the test solution prepared by macerating 1 g of the sample, in *fine powder*, with 10 ml of *hexane* for about 12 hours, shaking frequently, filtering and evaporating the filtrate to dryness. Dissolve the residue in 1 ml of *hexane*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. Spray the plate with a 20 per cent v/v solution of *sulfuric acid* in *ethanol* and heat at 105° for 5 minutes; several bands of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Hexane Extract of the Flowers of *Mesua ferrea* L.

Band	hR_f Value	Detection	
		UV 254	20 Per Cent V/V Solution of *Sulfuric Acid* in *Ethanol*
1	14-16	quenching	pale brown
2	23-25	–	violet
3	27-31	strong quenching	brown
4	37-40	weak quenching	pale brown
5	41-43	–	purple
6	58-61	weak quenching	purple
7	70-71	weak quenching	–
8	74-79	weak quenching	–
9	80-81	–	pale purple
10	83-84	weak quenching	–
11	85-88	–	pale purple
12	89-92	–	purple

Loss on drying Not more than 11.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Total ash Not more than 5.0 per cent w/w (Appendix 7.7).

Ethanol (80 per cent)-soluble extractive Not less than 4.5 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 2.5 per cent w/w (Appendix 7.12).

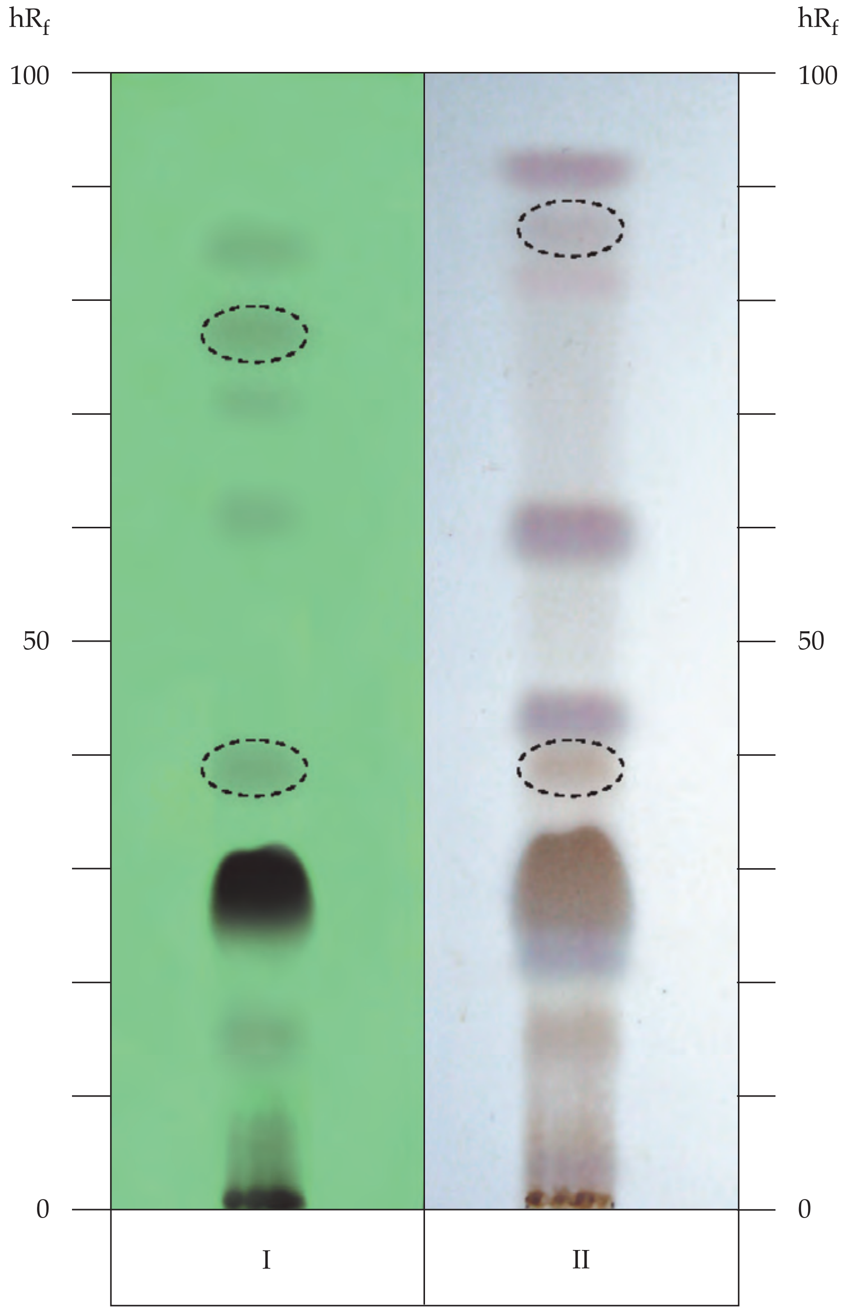


Fig. 3 Thin-layer Chromatogram of Hexane Extract of the Flowers of *Mesua ferrea* L.
I = detection under UV light (254 nm)
II = detection with a 20 per cent v/v solution of *sulfuric acid* in *ethanol*
◌ = bands developed in some samples

จันทน์แดง (CHAN DAENG)

รักตจันทน์ (RAKTACHAN), รัตจันทน์ (RATTACHAN)
Santali Rubri Lignum
Red Sandalwood

Synonyms Red Sanders, Red Saunders, Rubywood, Saunders Wood

Category Antipyretic, anti-inflammatory, cardiotonic.

Red Sandalwood is the dried heartwood of *Pterocarpus santalinus* L. f. (Family Fabaceae), Herbarium Specimen Number: BKF 170463, Crude Drug Number: DMSc 0933.

Constituents Red Sandalwood contains red pigments consisting mainly of santalin A and santalin B. It also contains isoflavones, a small amount of volatile oil, triterpenes, sterols, etc.

Description of the plant (Figs. 1a, 1b) Tree, up to 15 m high, deciduous; bark deeply cracked into vertical and horizontal rectangular plates, blackish brown. Leaves mostly trifoliate, rarely 4 or 5, alternate, 10 to 18 cm long; petiole swollen at base; leaflet broadly elliptic to ovate, 7 to 10 cm long, 3.5 to 5.5 cm wide, apex round or deeply notched, base round or slightly cordate, margin entire, blade leathery, shiny. Inflorescence axillary or terminal, raceme or panicle; bract and bracteole small, often caducous. Flower yellowish, 1.6 to 2 cm long; petals 5, papilionaceous; stamens 10, subdiadelphous; ovary superior, shortly stalked, 2-ovuled. Fruit samara, flat, obliquely rounded, broadly hardened winged around margin, beaked, 3.5 to 5 cm in diameter. Seeds 2, smooth, reddish brown.

Description Odour, aromatic; taste, slightly astringent.

Macroscopical (Fig. 1a) Irregular pieces of woody segment, dense, hard and heavy, but readily split. Dark red to blackish red. Transversely cut surface showing ring zones. Longitudinally cut surface showing alternating dark and light zones.

Microscopical (Figs. 2a, 2b, 2c, 2d, 2e) Transverse section of the heartwood shows vessel, xylem parenchyma, xylem ray, and fibre. Vessel, large, thick-walled, scattered, solitary or small cluster, some containing red resin. Xylem parenchyma, oval, thick-walled, band paratracheal. Xylem ray, mostly 1 row. Fibre, thick-walled.

Tangential and radial longitudinal sections of the heartwood show vessel, xylem parenchyma, xylem ray, and fibre. Vessel, large, with bordered-pitted and simple perforation plate, some of which contain red resin. Axial parenchyma, rectangular, thick-walled, some of which contain prismatic crystals of calcium oxalate. Xylem ray; in tangential longitudinal view, mostly uniseriate, oval; in radial longitudinal view, rectangular, perpendicular to other adjacent cells. Fibre, thick-walled, simple pitted.

Red Sandalwood in powder possesses the diagnostic microscopical characters of the unground drug.

Storage Red Sandalwood shall be stored in a dry place, protected from light.

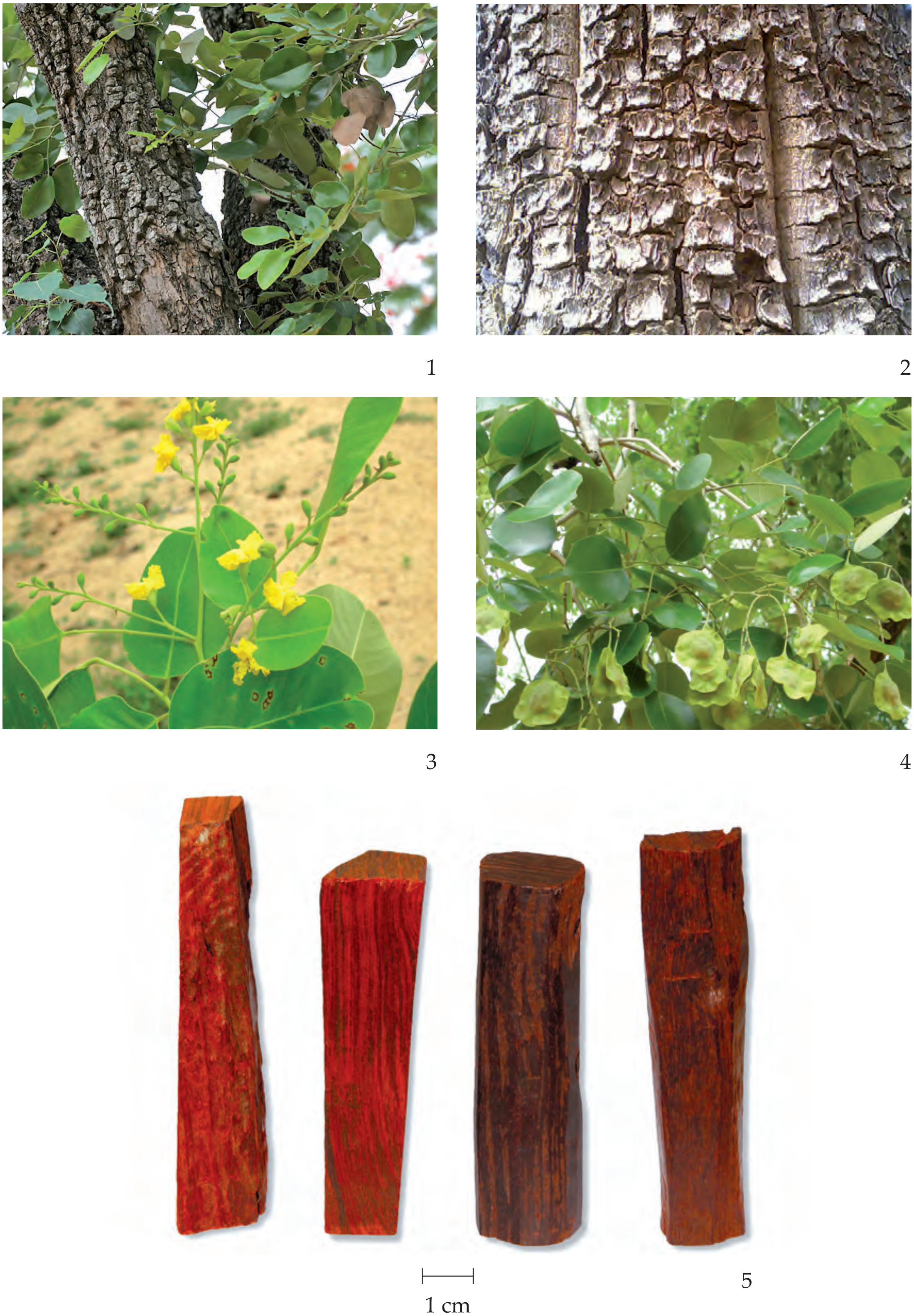


Fig. 1a *Pterocarpus santalinus* L. f.
1. part of stem and twig 2. bark 3. inflorescence 4. infructescence 5. crude drug

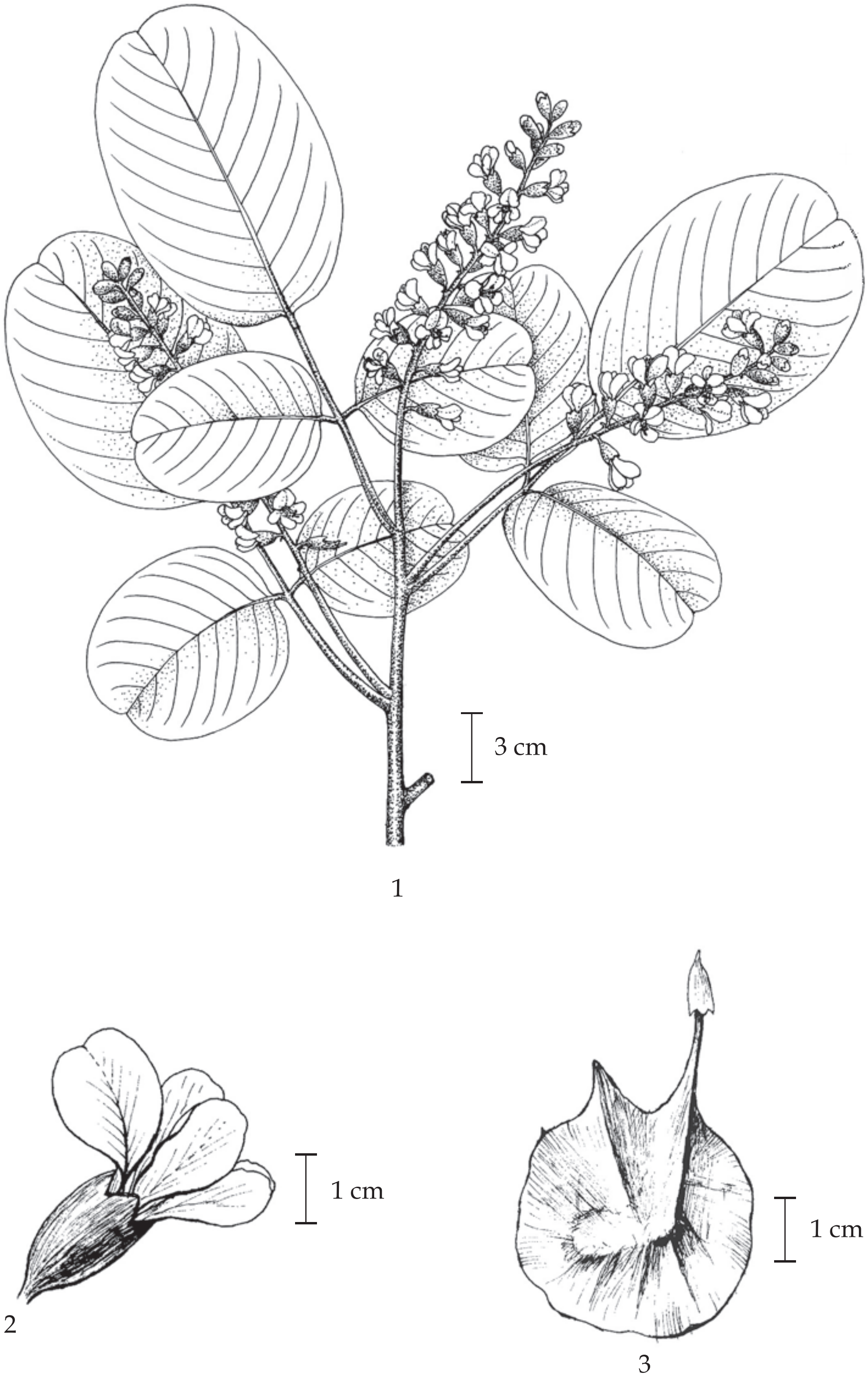


Fig. 1b *Pterocarpus santalinus* L. f.
1. flowering twig 2. flower 3. fruit

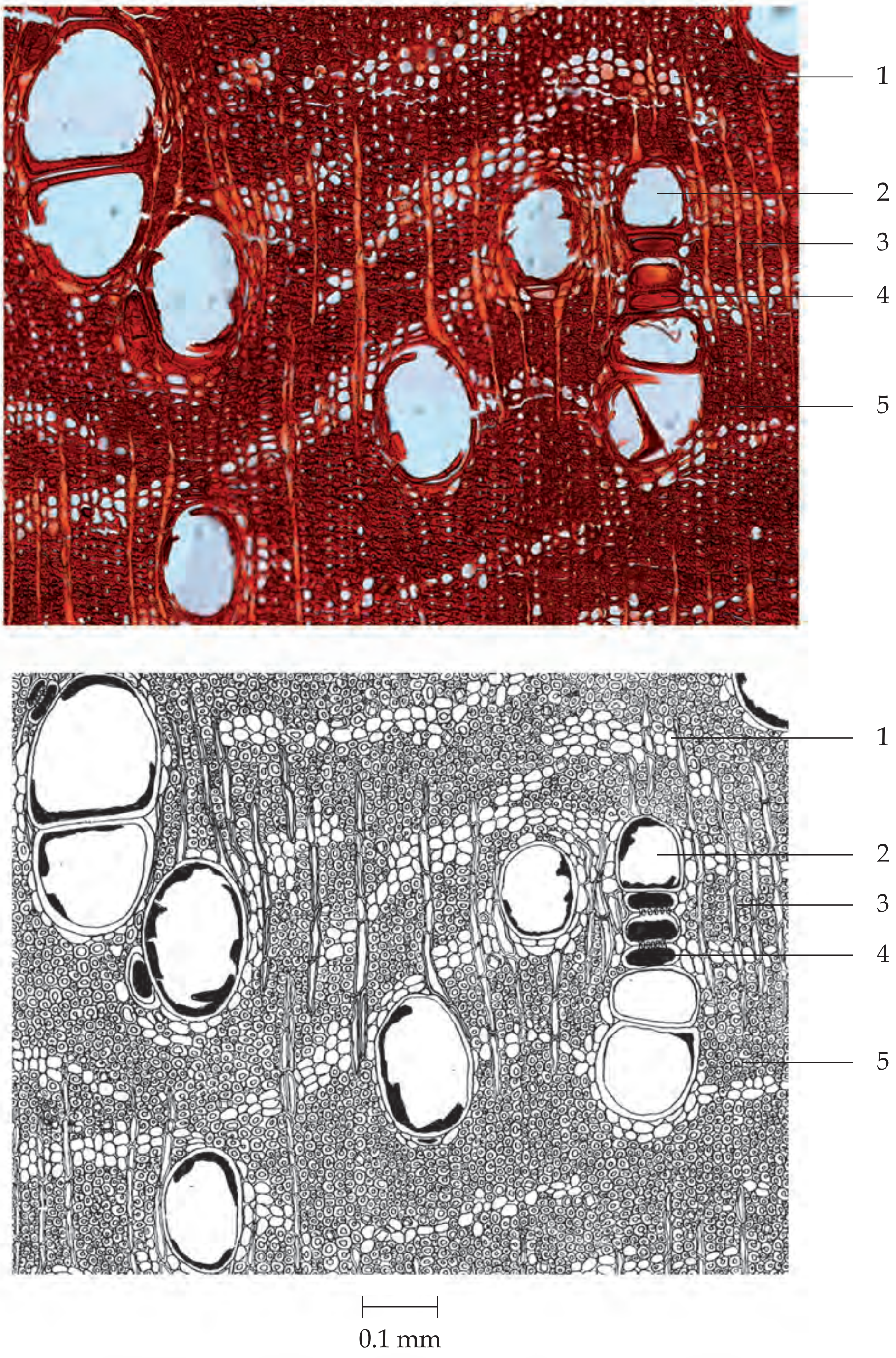


Fig. 2a Photomicrograph and Line Drawing of Transverse Section of the Heartwood of *Pterocarpus santalinus* L. f.

1. xylem parenchyma
2. vessel
3. xylem ray
4. red resin in vessel
5. fibre

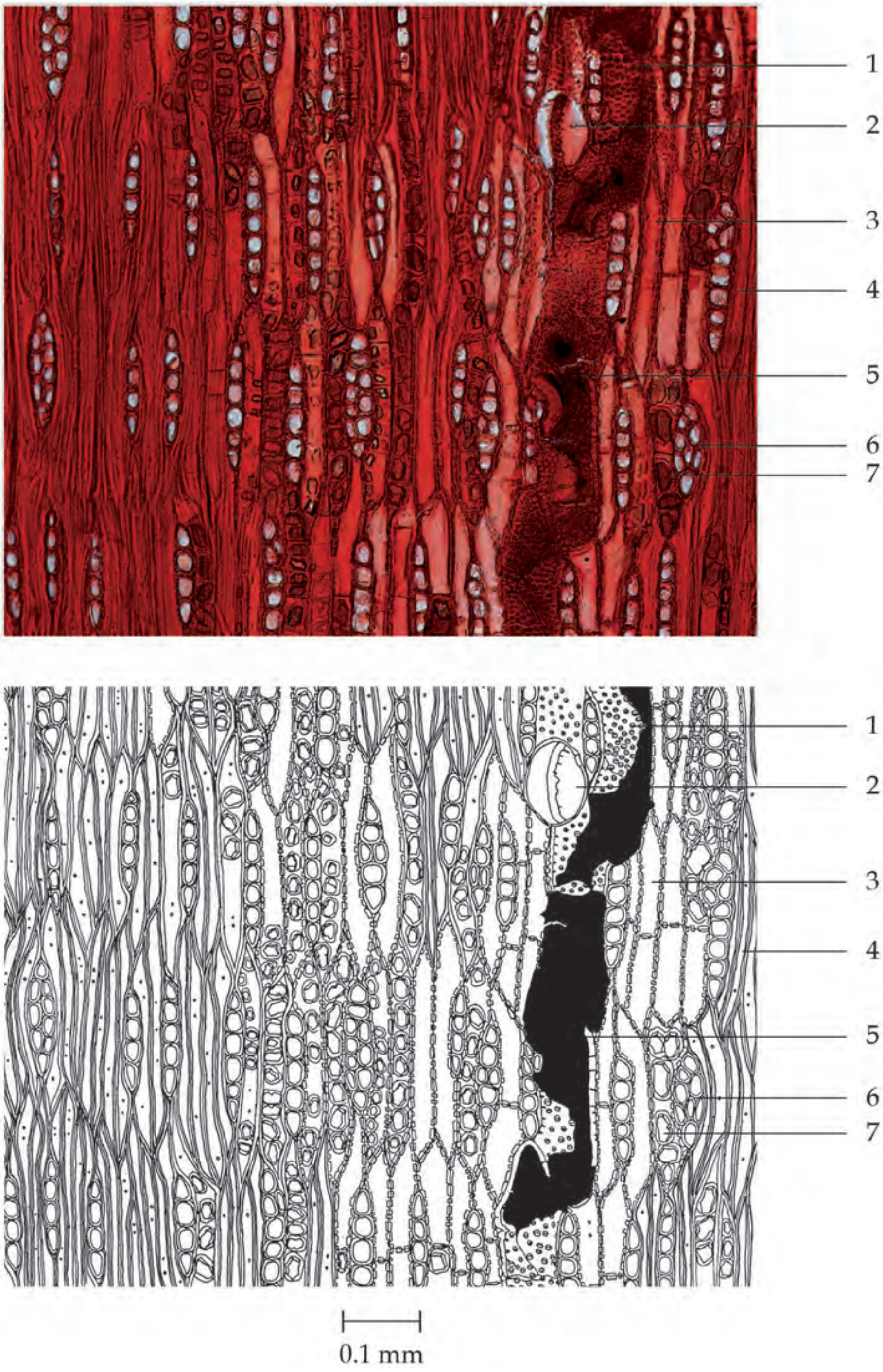


Fig. 2b Photomicrograph and Line Drawing of Tangential Longitudinal Section of the Heartwood of *Pterocarpus santalinus* L. f.

1. bordered-pitted vessel
2. simple perforation
3. xylem parenchyma
4. fibre
5. red resin in vessel
6. xylem ray
7. prismatic crystal of calcium oxalate in xylem parenchyma

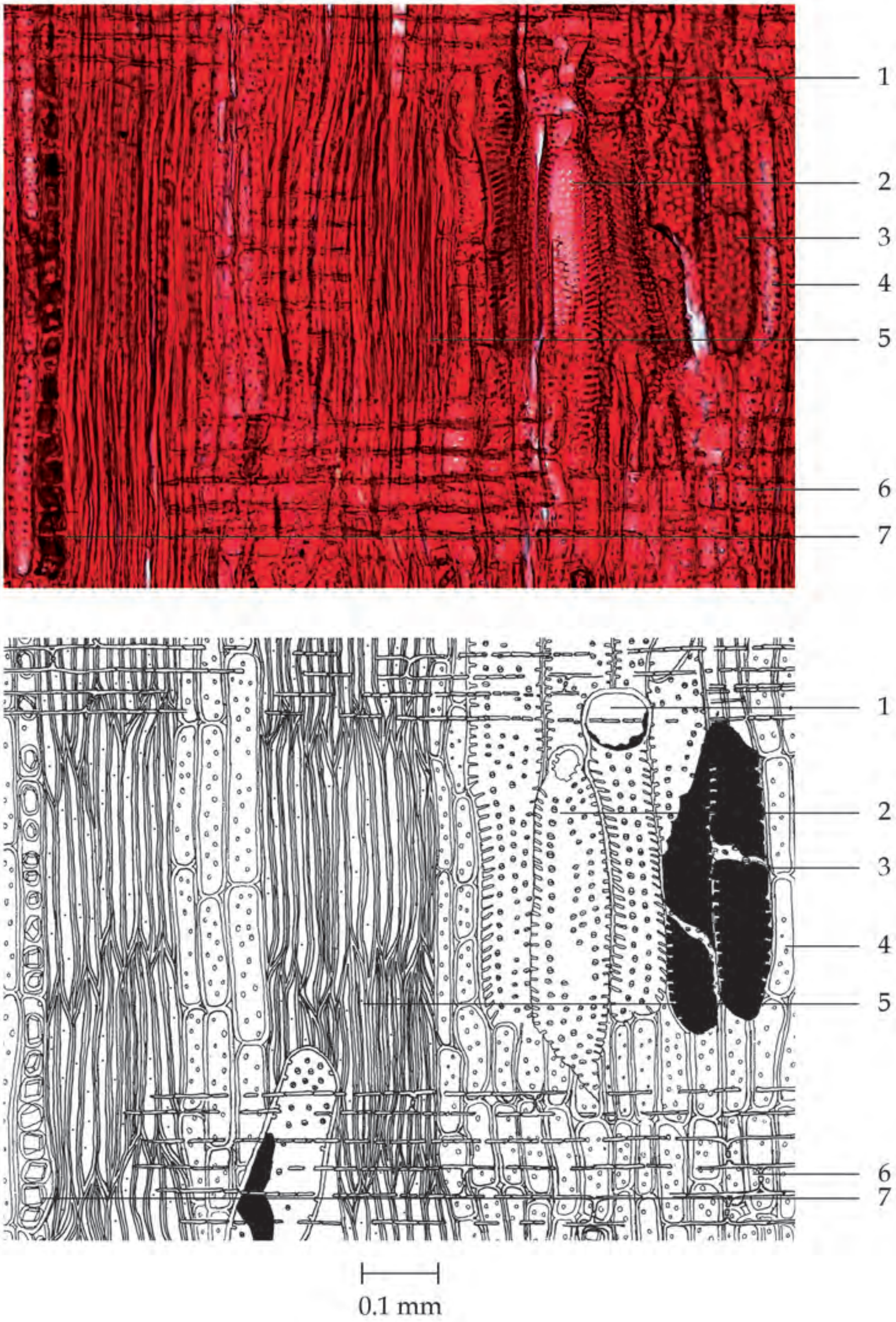


Fig. 2c Photomicrograph and Line Drawing of Radial Longitudinal Section of the Heartwood of *Pterocarpus santalinus* L. f.

1. simple perforation
2. bordered-pitted vessel
3. red resin in vessel
4. xylem parenchyma
5. fibre
6. xylem ray, perpendicular to xylem parenchyma
7. prismatic crystal of calcium oxalate in xylem parenchyma

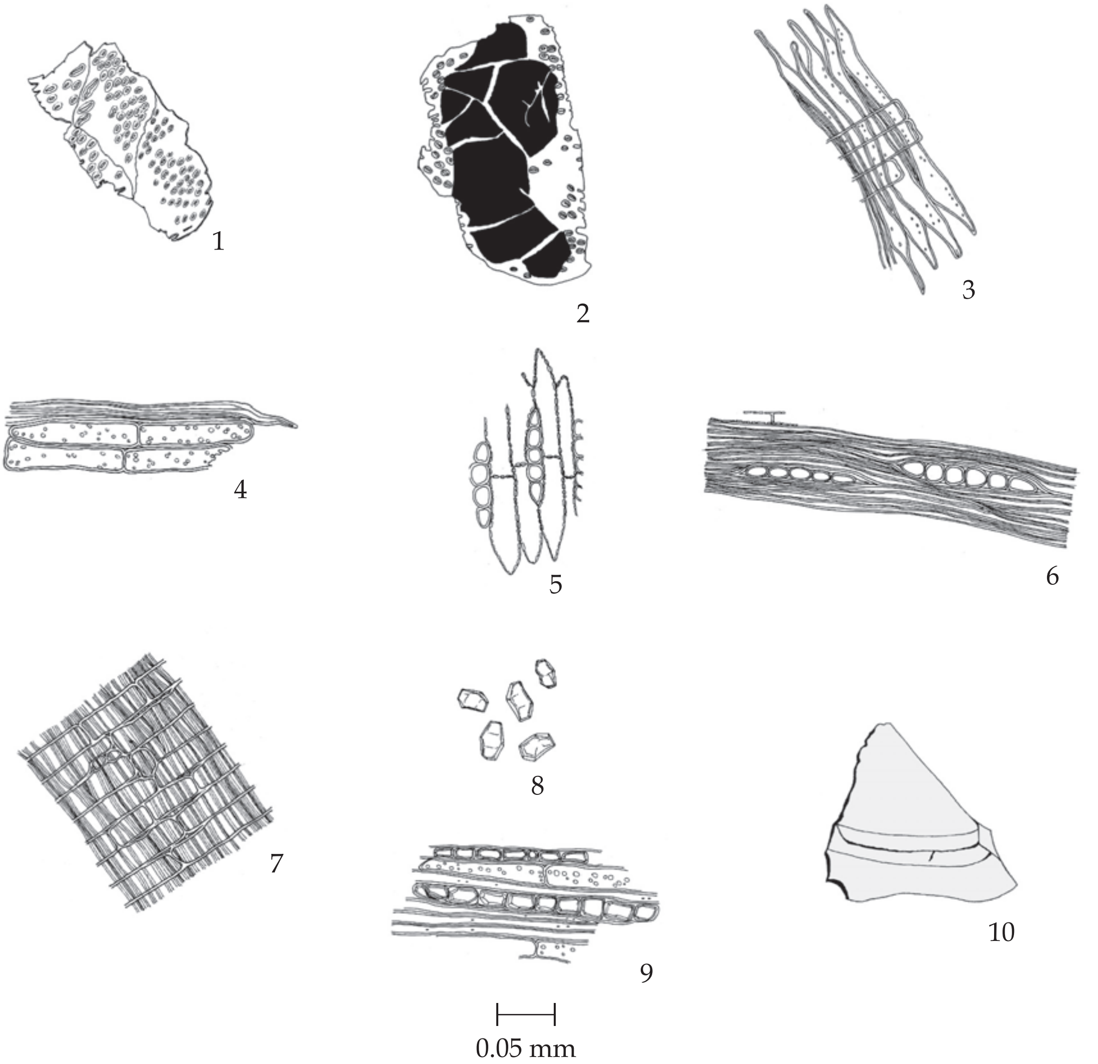


Fig. 2d Line Drawings of Powdered Drug of the Heartwood of *Pterocarpus santalinus* L. f.

1. fragment of bordered-pitted vessel
2. fragment of vessel with red resin
3. group of fibres perpendicular to xylem ray
4. xylem parenchyma and fibres
5. xylem parenchyma and xylem rays in tangential longitudinal view
6. xylem rays and fibres in tangential longitudinal view
7. xylem ray and fibres in radial longitudinal view
8. prismatic crystals of calcium oxalate
9. fibres and prismatic crystals of calcium oxalate in xylem parenchyma
10. fragment of red resinous substance

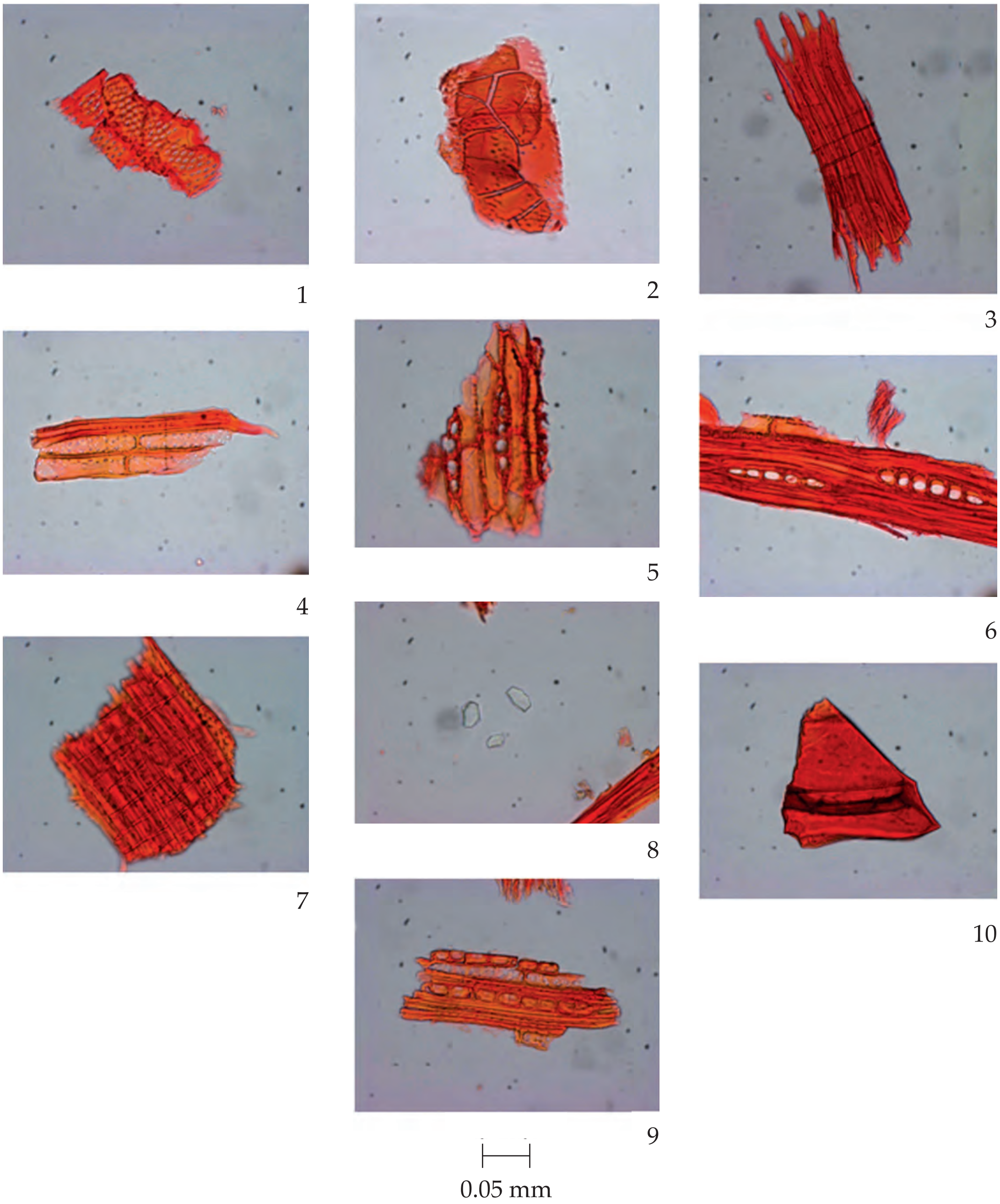


Fig. 2e Photomicrographs of Powdered Drug of the Heartwood of *Pterocarpus santalinus* L. f.

1. fragment of bordered-pitted vessel
2. fragment of vessel with red resin
3. group of fibres perpendicular to xylem ray
4. xylem parenchyma and fibres
5. xylem parenchyma and xylem rays in tangential longitudinal view
6. xylem rays and fibres in tangential longitudinal view
7. xylem ray and fibres in radial longitudinal view
8. prismatic crystals of calcium oxalate
9. fibres and prismatic crystals of calcium oxalate in xylem parenchyma
10. fragment of red resinous substance

Identification

A. Reflux 1.2 g of the sample, in powder, with 30 ml of *methanol* for 30 minutes and filter (solution 1). Evaporate 2 ml of solution 1 to dryness. Dissolve the residue in 1 ml of *acetic anhydride*, slowly add a few drops of *sulfuric acid* and mix: a deep brownish red colour develops.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 80 volumes of *hexane* and 20 volumes of *ethyl acetate* as the mobile phase. Apply to the plate, 5 µl of the test solution prepared by evaporating about 20 ml of solution 1 to dryness and dissolving the residue in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter; several blue fluorescent spots are observed. Spray the plate with *anisaldehyde TS* and heat at 110° for 10 minutes; several spots of different colours appear (Table 1); see also Fig. 3.

Table 1 hR$_f$ Values of Components in Methanolic Extract of the Heartwood of *Pterocarpus santalinus* L. f.

Spot	hR$_f$ Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	7-9	–	light blue	violet
2	11-14	quenching	–	–
3	14-18	–	light blue	violet
4	20-24	quenching	–	brownish purple
5	25-29	–	light blue	–
6	32-35	–	blue	–
7	48-53	quenching	light blue	brownish purple
8	62-68	–	intense blue	–
9	69-72	–	–	pale violet
10	75-79	–	light blue	–
11	79-82	–	–	violet
12	83-87	–	light blue	–
13	87-90	–	–	pale violet
14	95-98	–	–	pale violet
15	98-99	–	–	violet

Loss on drying Not more than 8.0 per cent w/w after drying at 105° for 5 hours (Appendix 4.15).

Foreign matter Not more than 0.5 per cent w/w (Appendix 7.2).

Total ash Not more than 1.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 12.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 1.0 per cent w/w (Appendix 7.12).

Dose 3 to 5 g a day.

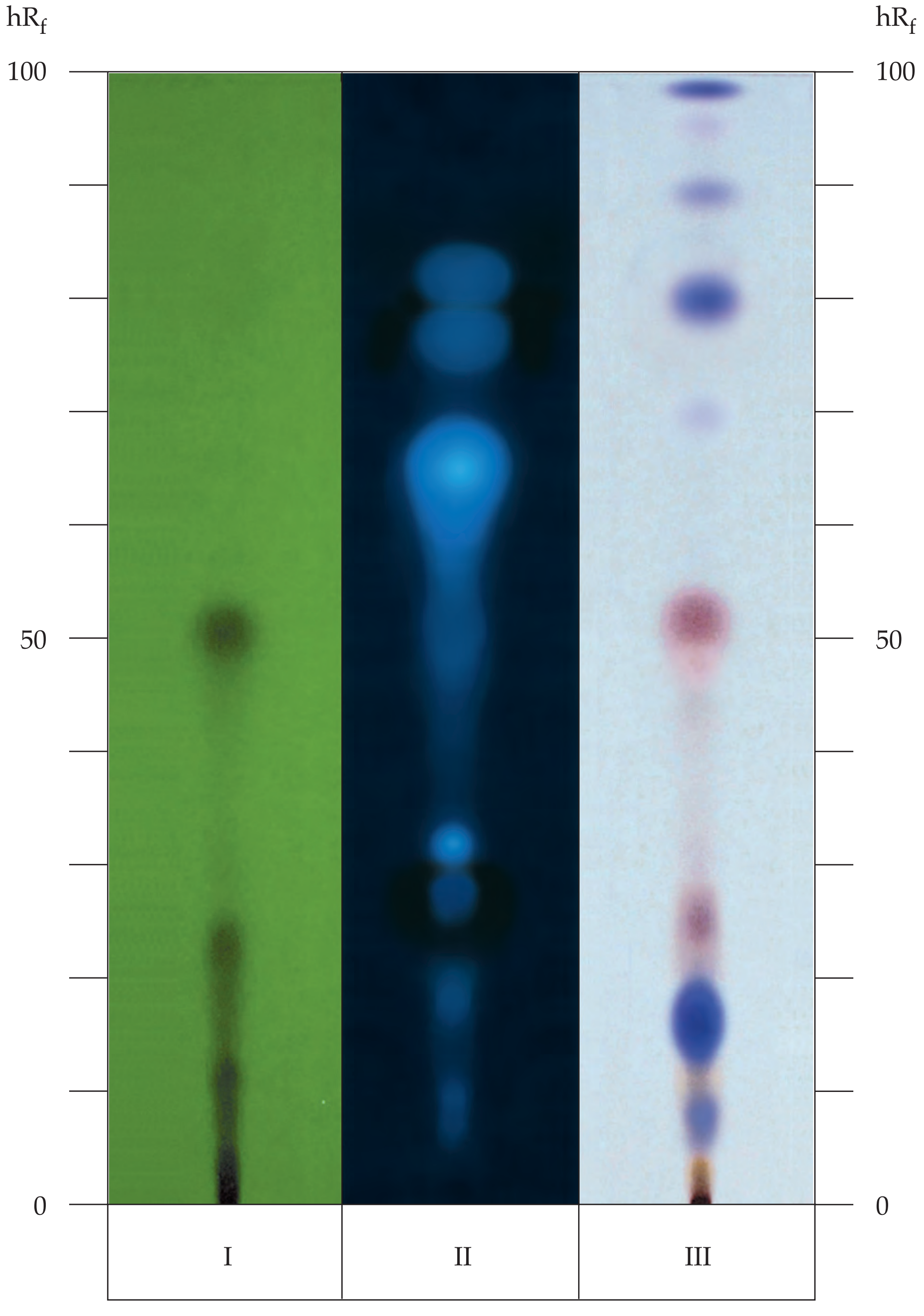


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Heartwood of *Pterocarpus santalinus* L. f.

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *anisaldehyde TS*

จันทน์ขาว (CHAN KHAO)

แก่นจันทน์ (KAEN CHAN), จันทน์หิมาลัย (CHAN HIMALAI), แก่นจันทน์เทศ (KAEN CHAN THET)
Santali Albi Lignum
Sandalwood

Synonyms White Sandalwood, Yellow Sandalwood

Category Cardiotonic, stomachic, nervotonic.

Sandalwood is the dried heartwood of *Santalum album* L. (*S. album* Rumph., *S. ovata* R. Br., *S. ovatum* Miq., *S. myrtifolium* L., *Sirium myrtifolium* L.) (Family Santalaceae), Herbarium Specimen Number: DMSC 5178, BKF 168153, Crude Drug Number: DMSc 0935.

Constituents Sandalwood contains volatile oil, of which α- and β-santalol are major component. The volatile oil also contains santenone, santalone, α-terpineol, etc.

Description of the plant (Figs. 1a, 1b) Hemiparasitic tree, up to 20 m high, glabrous; bark rough, cracked, dark grey or brownish black. Leaves simple, opposite or sub-opposite, elliptic-lanceolate or ovate, coriaceous, 4 to 11 cm long, 1.5 to 3.5 cm wide, apex acute or shortly acuminate, base obtuse, attenuate, margin undulate; petiole slender, about 1 cm long. Inflorescence paniculate, terminal or axillary, pedunculate; peduncle slender, tortuous. Flowers 9 to 15, receptacular, pedicellate; pedicel slender, angular, 1 to 3 mm long; perianth tube campanulate, 4- to 5-lobed, deltoid, 1.5 to 3.5 mm long, 1 to 1.5 mm wide, reflexed, whitish, turning reddish then crimson; stamens 4 or 5, opposite to perianth lobes, filament narrow, slightly dilated at the base, covering with white hair-tuft; ovary semi-inferior, style angular, stigma 3-lobed; nectary concave, deeply 5-lobed, protruding between the perianth segments, brownish, turning reddish then crimson. Fruit drupe, globose or subglobose, about 1 cm in diameter, green, turning red then purplish black, juicy when ripe; exocarp smooth; endocarp ribbed. Seed 1, globose or obovoid.

Description Odour, aromatic, strongly aromatic when burning; taste, slightly bitter.

Macroscopical (Fig. 1a) Cylindrical woody segment, varying in size and diameter, some somewhat curved. Externally pale yellow or yellowish brown, fine and smooth, some with nodal scars or longitudinal cracks. Texture compact, uneasily broken; transversely cut surface brownish yellow, showing annual rings; longitudinally cut surface shows straight grains.

Microscopical (Figs. 2a, 2b, 2c, 2d) Transverse section of the heartwood shows vessel, xylem parenchyma, xylem ray, and fibre. Vessel, single, large, thick-walled, and scattered. Xylem parenchyma, round, thick-walled, apotracheal, and some of which contain prismatic crystals of calcium oxalate. Xylem ray, 1 to 3 rows, some of which contain red or brownish oleoresin. Fibre, thick-walled.

Tangential and radial longitudinal sections of the heartwood show vessel, xylem parenchyma, fibre, fibre-tracheid and xylem ray. Vessel, large, with bordered-pitted and simple perforation plate. Xylem parenchyma, thick-walled, some of which contain prismatic crystals of calcium oxalate. Fibre, thick-walled, simple pitted. Fibre-tracheid, thick-walled, bordered-pitted with tapering ends. Xylem ray; in tangential longitudinal view, mostly multiseriate, oval, some of which contain red or brownish oleoresin; in radial longitudinal view, rectangular, perpendicular to other adjacent cells.

Fig. 1a *Santalum album* L.
1. habit 2. stem 3. flowering twig 4. fruiting twig 5. crude drug

Fig. 1b *Santalum album* L.
1. flowering twig 2. flower 3. fruit

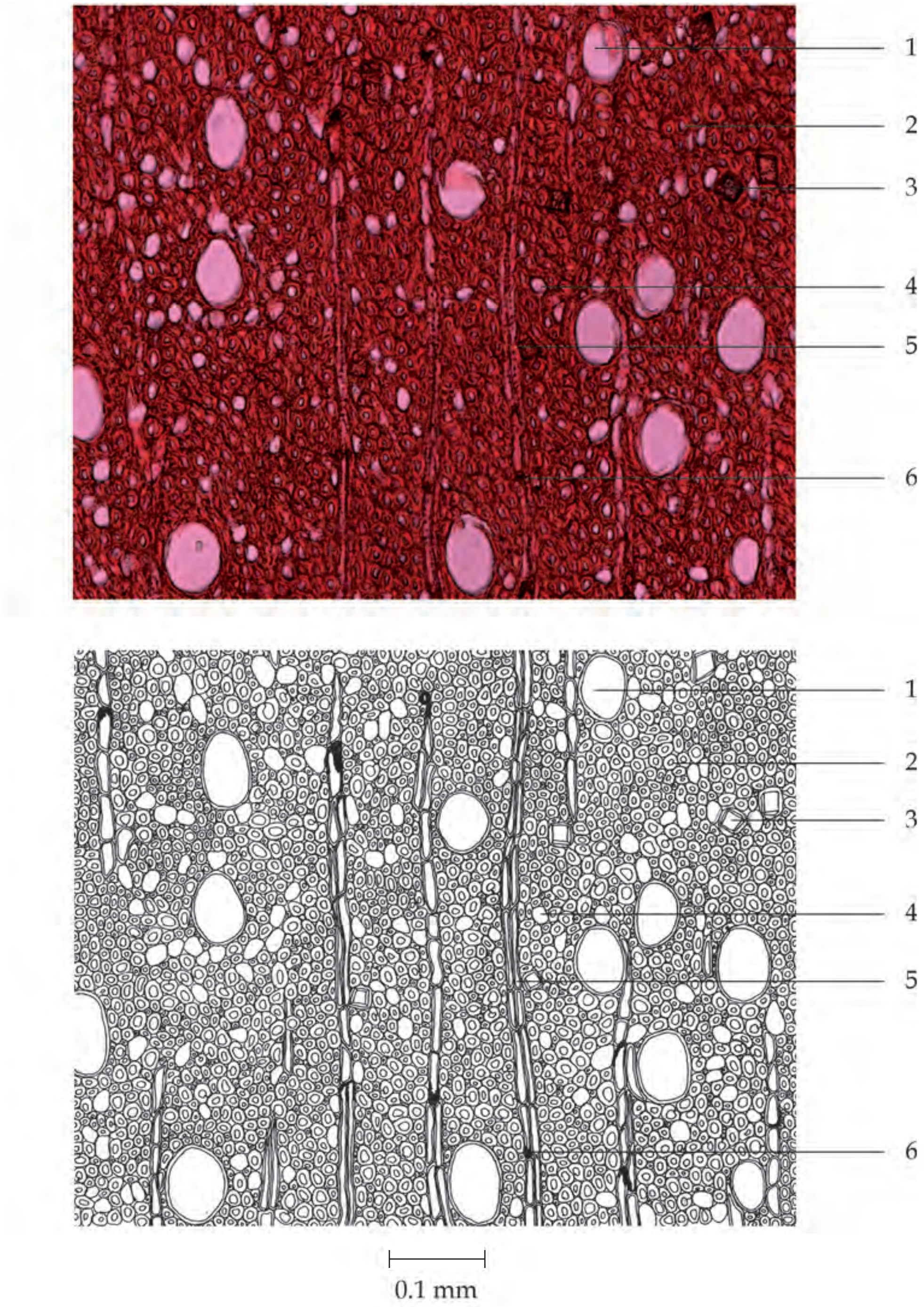


Fig. 2a Photomicrograph and Line Drawing of Transverse Section of the Heartwood of *Santalum album* L.

1. vessel
2. fibre
3. prismatic crystal of calcium oxalate
4. xylem parenchyma
5. xylem ray
6. oleoresin in xylem ray

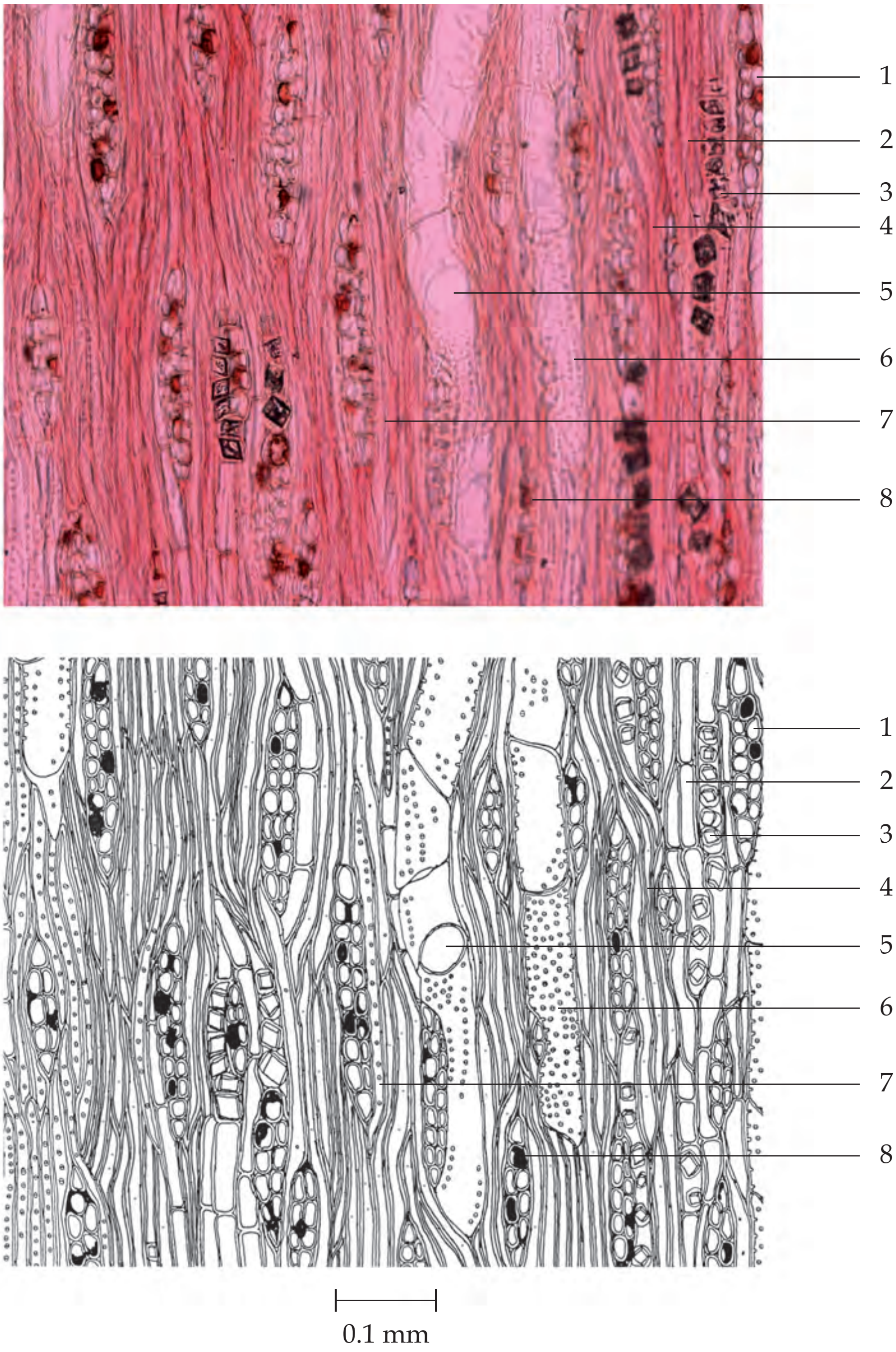


Fig. 2b Photomicrograph and Line Drawing of Tangential Longitudinal Section of the Heartwood of *Santalum album* L.

1. xylem ray
2. xylem parenchyma
3. prismatic crystal of calcium oxalate
4. fibre
5. simple perforation plate
6. bordered-pitted vessel
7. fibre-tracheid
8. oleoresin in xylem ray

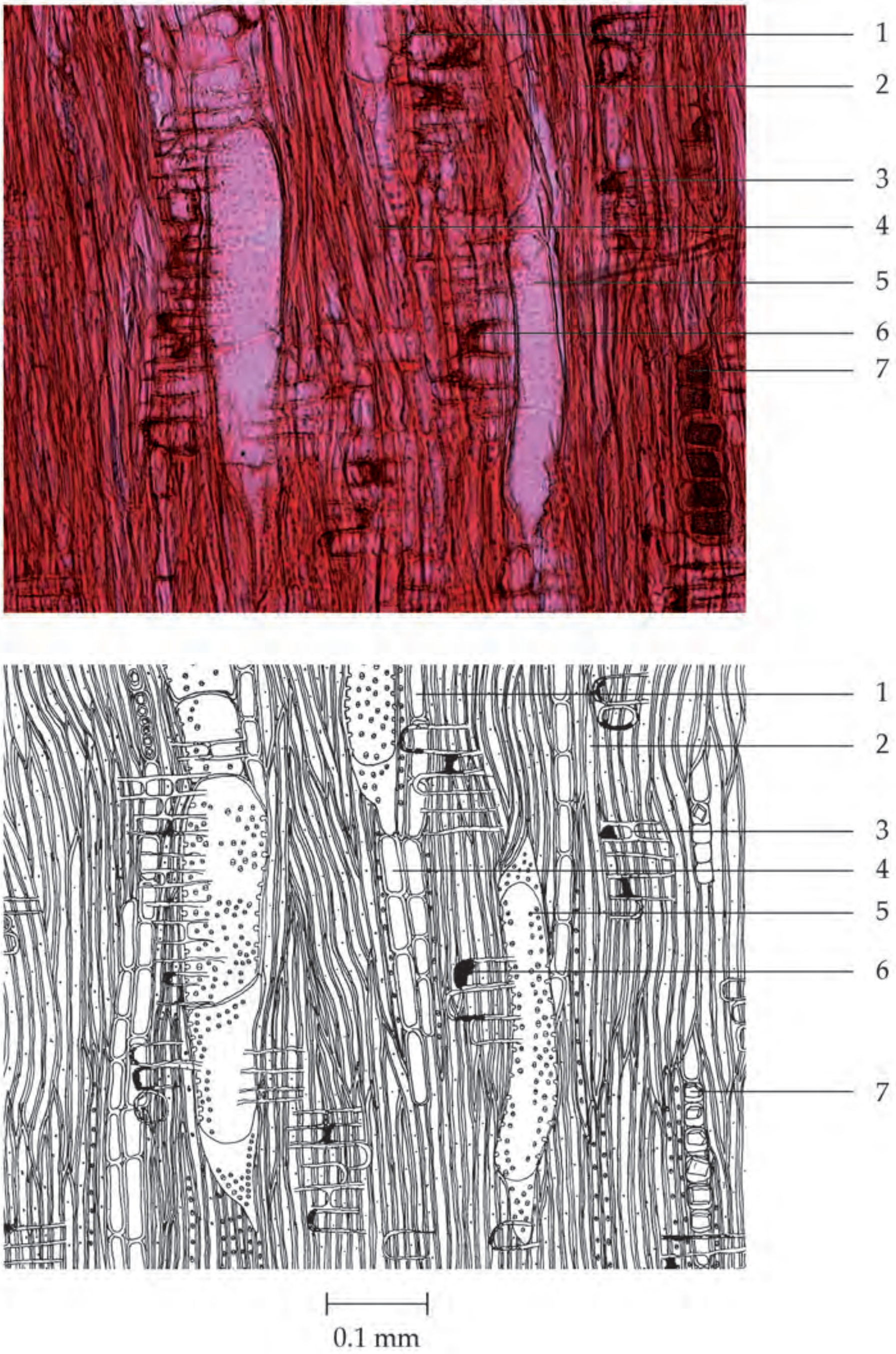


Fig. 2c Photomicrograph and Line Drawing of Radial Longitudinal Section of the Heartwood of *Santalum album* L.

1. fibre-tracheid
2. fibre
3. xylem ray
4. xylem parenchyma
5. bordered-pitted vessel
6. oleoresin in xylem
7. prismatic crystal of calcium oxalate

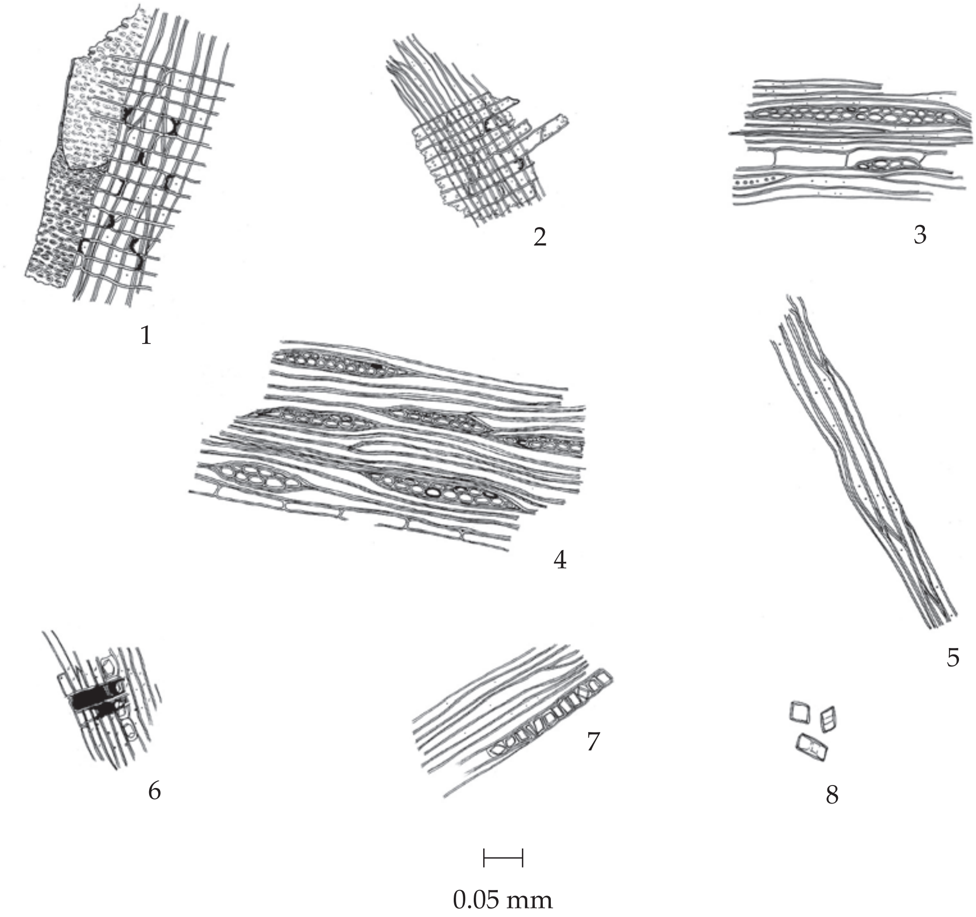


Fig. 2d Powdered Drug of the Heartwood of *Santalum album* L.

1. xylem in radial longitudinal view showing elongated vessel, fibres and xylem ray, some of which containing oleoresin
2. xylem ray, some of which containing oleoresin and fibres in radial longitudinal view
3. xylem in tangential longitudinal view showing fibres, parenchyma and xylem rays
4. xylem in tangential longitudinal view showing xylem rays and fibres
5. fibres
6. fibres associated with parenchyma containing oleoresin and prismatic crystals of calcium oxalate
7. fibres associated with xylem parenchyma containing prismatic crystals of calcium oxalate
8. prismatic crystals of calcium oxalate

Sandalwood in powder possesses the diagnostic microscopical characters of the unground drug.

Storage Sandalwood shall be stored in a cool and dry place, protected from light.

Identification

A. Reflux 1 g of the sample, in powder, with 20 ml of *methanol* for 15 minutes and filter (solution 1). Evaporate 2 ml of solution 1 to dryness. Dissolve the residue in 2 ml of *acetic anhydride*, and then slowly add 1 ml of *sulfuric acid* to form two layers: a brown colour forms at the zone of contact and the upper layer is green.

B. To 2 ml of solution 1, add a few drops of *iron(III) chloride TS*: a green colour is produced.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 90 volumes of *toluene* and 10 volumes of *ethyl acetate* as the mobile phase. Apply to the plate, 5 µl of the test solution prepared by refluxing 1 g of sample, in powder, with 30 ml of *dichloromethane* for 30 minutes and filtering. Evaporate the filtrate to dryness and dissolve the residue in 1 ml of *toluene*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter; one intense blue fluorescent spot is observed. Spray the plate with *anisaldehyde TS* and heat at 110° for 10 minutes; several spots of different colours appear (Table 1); see also Fig. 3.

Table 1 hR$_f$ Values of Components in Dichloromethane Extract of the Heartwood of *Santalum album* L.

Spot	hR$_f$ Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	15-22	–	–	violet
2	25-30	–	–	orange
3	32-38	–	–	violet
4	40-45	–	–	brown
5	45-48	–	–	pale violet
6	50-52	–	intense blue	–
7	58-64	–	–	brown
8	65-68	quenching	–	–
9	69-73	–	–	brown
10	73-76	–	–	pink
11	85-88	–	–	pale brown
12	94-97	weak quenching	–	violet

Water Not more than 9.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 0.5 per cent w/w (Appendix 7.2).

Total ash Not more than 1.5 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 3.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 3.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 1.0 per cent v/w (Appendix 7.3H). Use 30 g, in *coarse powder*, freshly prepared and accurately weighed. Use 300 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

Dose 2 to 6 g a day.

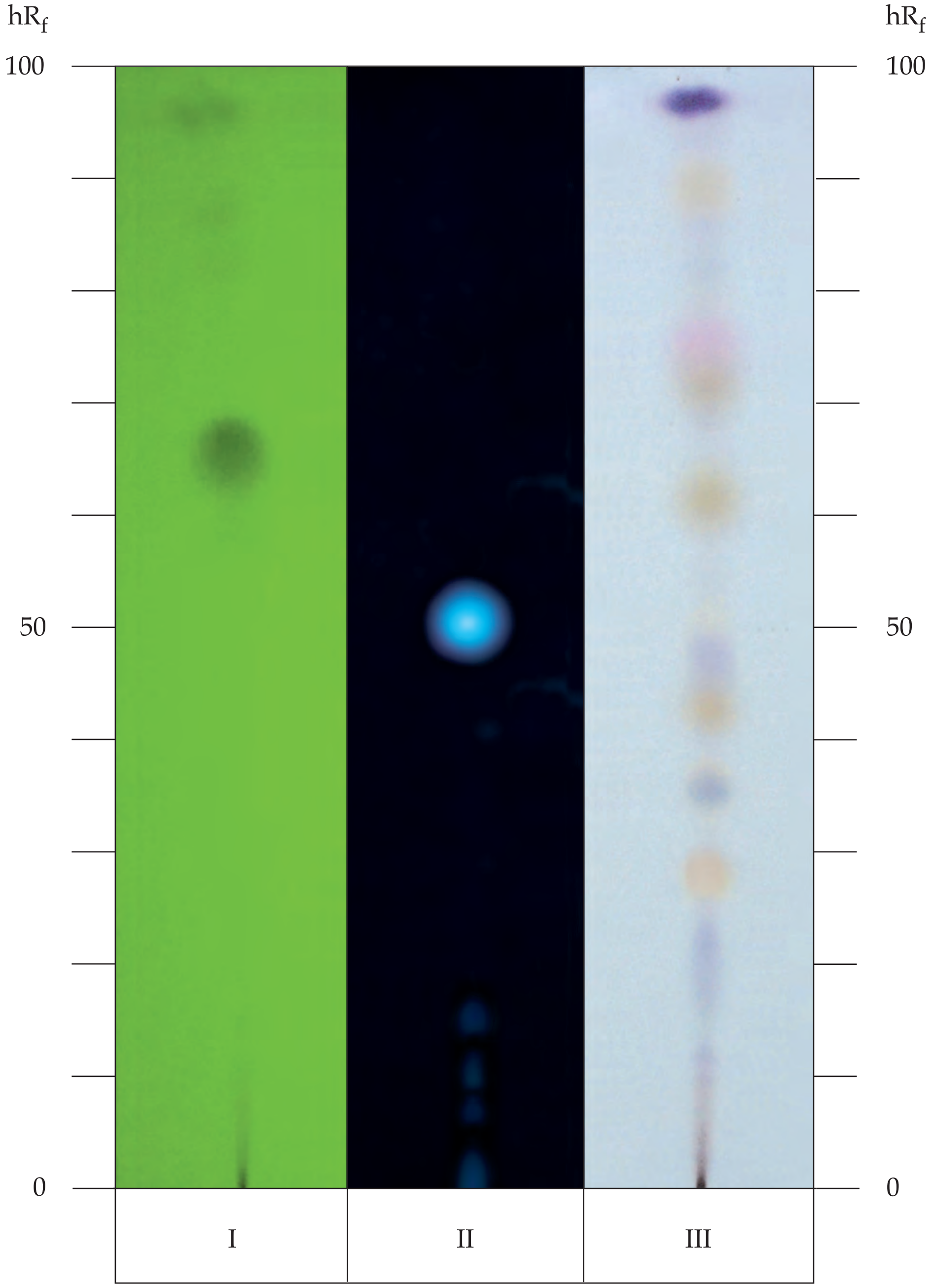


Fig. 3 Thin-layer Chromatogram of Dichloromethane Extract of the Heartwood of *Santalum album* L.

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *anisaldehyde TS*

ช้าพลู, ใบ (CHAPHLU, BAI)

ชะพลู, ใบ (CHAPHLU, BAI), ปูลิง, ใบ (PULING, BAI), พลูลิง, ใบ (PHLULING, BAI), ผักอีเลิด, ใบ (PHAK I LOET, BAI)
Piperis Sarmentosi Folium
Piper Sarmentosum Leaf

Category Carminative.

Piper Sarmentosum Leaf is the dried leaf of *Piper sarmentosum* Roxb. (Family Piperaceae), Herbarium Specimen Number: DMSC 2714, BKF 160077, Crude Drug Number: DMSc 0686.

Constituents Piper Sarmentosum Leaf contains phenylpropanoids.

Description of the plant (Figs. 1a, 1b) Herb, erect or creeping, often stoloniferous, swollen node. Leaves simple, alternate, stipulate; suborbicular ovate, or ovate-oblong, 7 to 15 cm long, 5 to 10 cm wide, surface glabrous or short hairs, apex acute to shortly acuminate, base cordate to obliquely obtuse or rounded, margin entire, slightly undulate, veins palmately 5- to 7-nerved, prominent on lower surface; petioles 2 to 5 cm long. Inflorescence leaf-opposed dense spike, generally unisexual. Male spikes white, 1.5 to 3 cm long; peduncle 0.5 to 1.5 cm long; stamens 2 to 3, filament very short, anthers subglobose. Female spikes whitish, 2 to 5 cm long; peduncle 0.5 to 1.5 cm long; stigmas 3 to 4. Fruits drupe, obovoid, dark green when riped. Seeds small.

Description Odour, characteristic; taste, slightly pungent.

Macroscopical (Fig. 1a) A mixture of entire and broken leaves. Entire leaf, ovate or obovate-oblong, 3.5 to 13 cm long, 2.5 to 7 cm wide; apex, acuminate; base, cordate or obtuse or obliquely obtuse; upper surface green to greenish brown, lower surface green to greyish green, lighter colour; petiole brown, 1 to 6 cm long.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the leaf shows upper epidermis, a single layer of cuticularized rectangular cells, polygonal in surface view, with few unicellular trichomes and pearl glands. Mesophyll, a single layer of palisade parenchyma and several layers of round spongy parenchyma with scattered small vascular bundles. Lower epidermis, a single layer of rectangular cells, polygonal and irregular shape in surface view; stomata, mostly anomocytic. Hypodermis, 1 to 2 layers of cells under upper and lower epidermises of midrib and nearby area, and leaf margin.

Transverse section through the midrib of lamina shows several layers of collenchyma underneath the epidermis, parenchyma and collateral vascular bundles. Trichomes, 1 to 3 cells uniseriate nonglandular, abundant at lower epidermis.

Piper Sarmentosum Leaf in powder possesses the diagnostic microscopical characters of the unground drug.

Packaging and storage Piper Sarmentosum Leaf shall be kept in well-closed containers, protected from light, and stored in a dry place.

Fig. 1a *Piper sarmentosum* Roxb.
1. habit 2. inflorescence 3. infructescence 4. crude drug

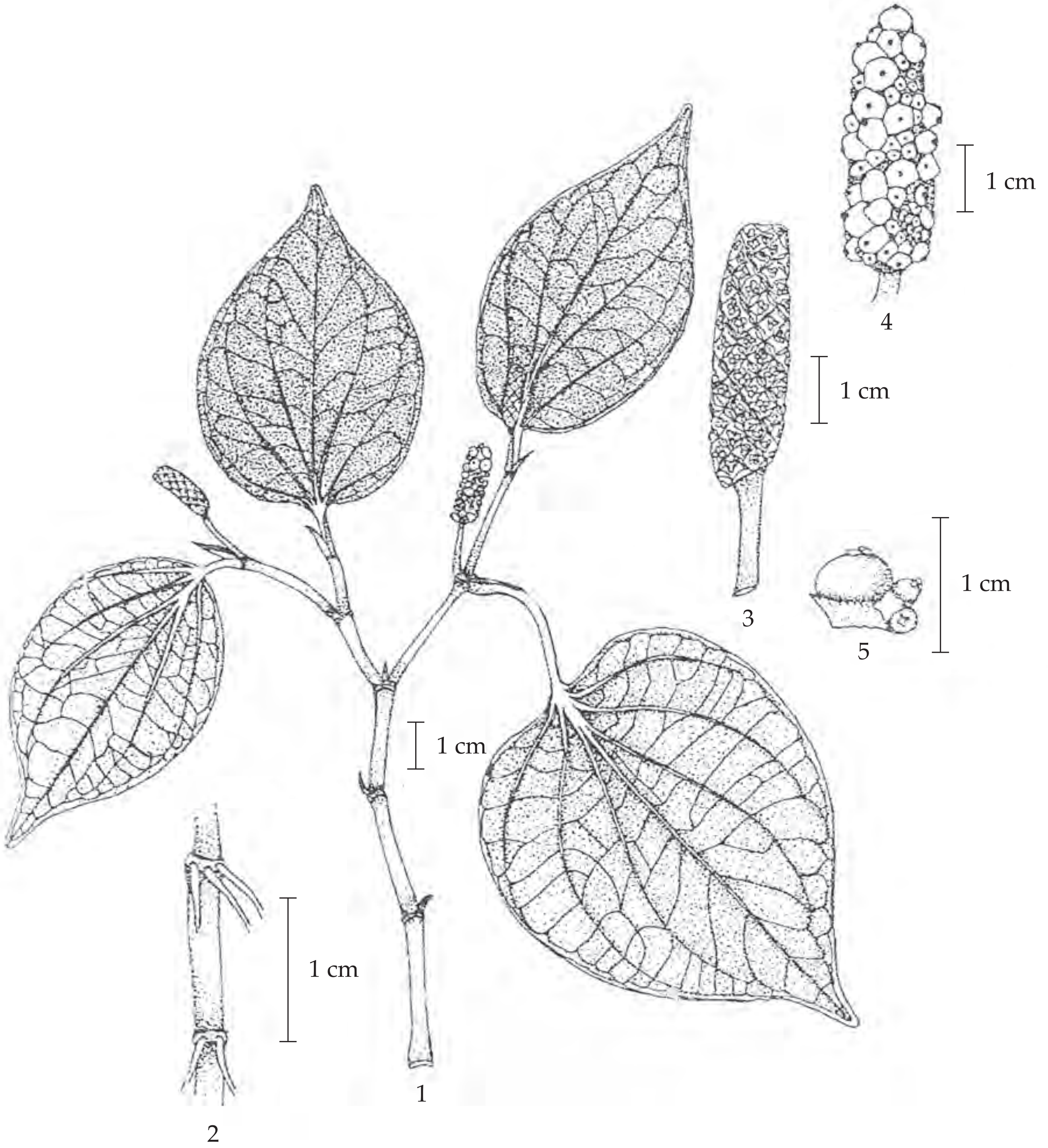
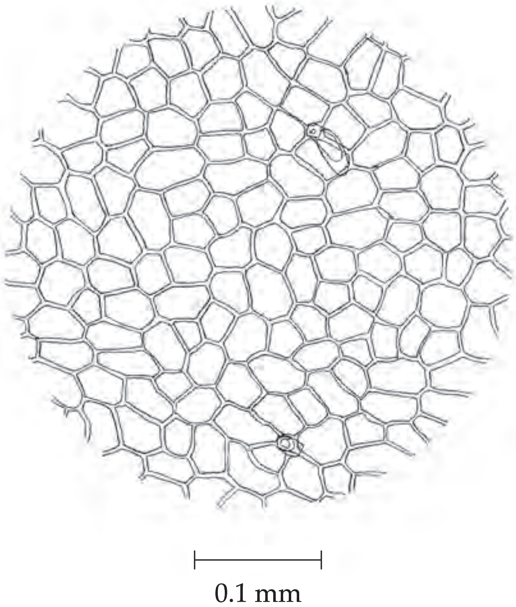
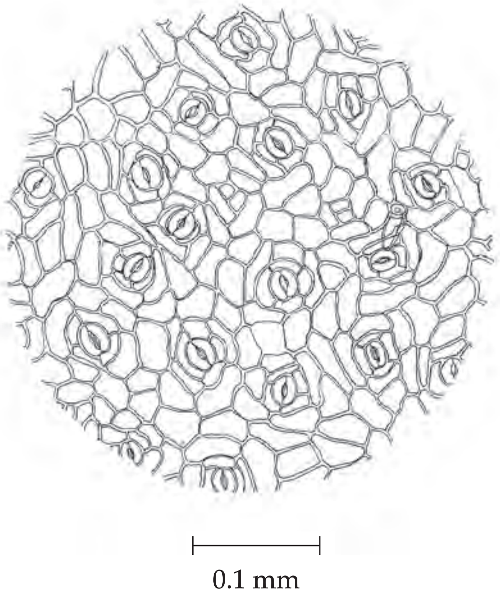


Fig. 1b *Piper sarmentosum* Roxb.
1. twig 2. stem with adventitious roots 3. inflorescence
4. infructescence 5. drupes

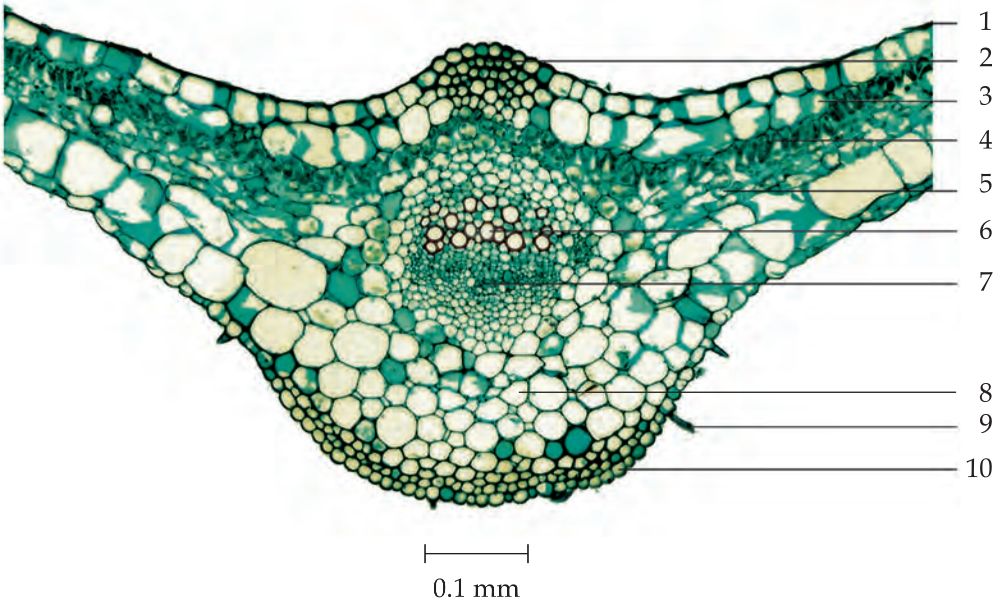


Upper Epidermis of the Lamina

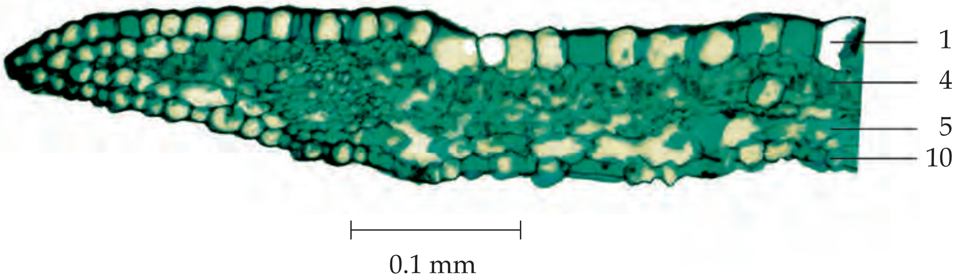


Lower Epidermis of the Lamina

Fig. 2a Epidermises of the Leaf of *Piper sarmentosum* Roxb.



Transverse Section of the Midrib



Transverse Section of the Margin

Fig. 2b Photomicrogrphs of Transverse Section of the Leaf of *Piper sarmentosum* Roxb. Stained with Safranin-Fast Green

1. upper epidermis
2. collenchymatous tissue
3. hypodermis
4. palisade cell
5. spongy cell
6. xylem tissue
7. phloem tissue
8. parenchyma in midrib
9. trichome
10. lower epidermis

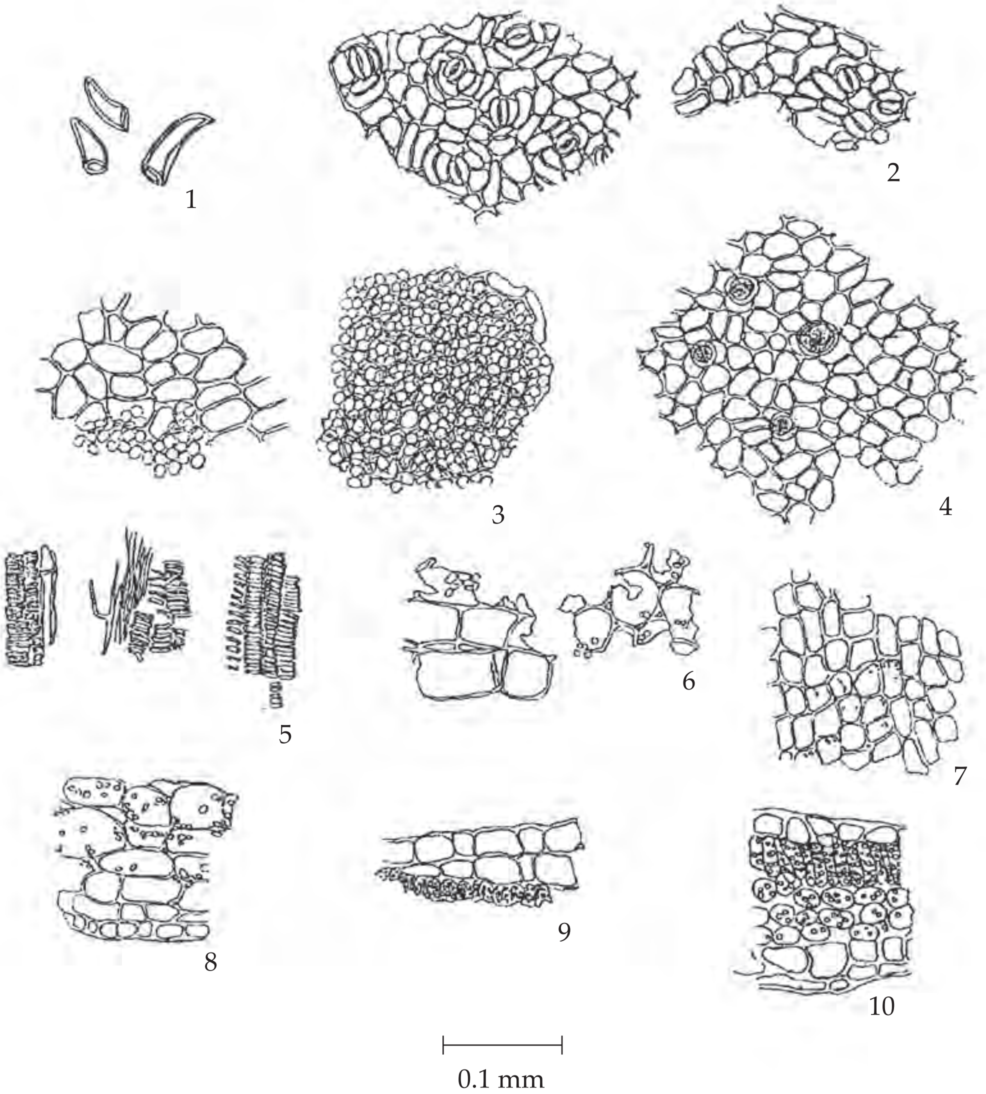


Fig. 2c Powdered Drug of the Leaves of *Piper sarmentosum* Roxb.

1. unicellular trichomes
2. lower epidermis consisting of mostly anomocytic stomata in surface view
3. upper epidermis and underlying palisade cells in surface view
4. upper epidermis with pearl glands in surface view
5. fibres and reticulate, spiral and scalariform vessels
6. parenchyma cells with starch grains
7. epidermal cells in surface view
8. lower epidermis with parenchyma cells
9. upper epidermis underlying hypodermis and palisade cells with chloroplast
10. leaf blade in transverse view

Identification

A. Macerate 500 mg of the sample, in *coarse powder*, with 25 ml of *ethanol* for 24 hours, filter, and evaporate the filtrate to dryness. Dissolve the residue in 10 ml of *dilute sulfuric acid* and filter (solution 1). To 2 ml of solution 1, add a few drops of *mercuric-potassium iodide TS*: a white precipitate is produced.

B. To 2 ml of solution 1, add a few drops of *modified Dragendorff TS1*: an orange precipitate is produced.

C. Boil 500 mg of the sample, in *coarse powder*, with 10 ml of *water* in a water-bath for 10 minutes and filter. To 2 ml of the filtrate, add 1 or 2 drops of a 1 per cent w/v solution of *iron(III) chloride*: a greenish grey precipitate develops.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 3 volumes of *ethyl acetate* and 1 volume of *toluene* as the mobile phase. Apply to the plate, 10 µl of the test solution prepared by macerating 500 mg of the sample, in *coarse powder*, with 25 ml of *ethanol* for 24 hours, filtering, and evaporating the filtrate to dryness. Dissolve the residue in 1 ml of *ethanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter; several fluorescent spots of different colours are observed. Spray the plate with *modified Dragendorff TS1*; several spots of different colours appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Leaves of *Piper sarmentosum* Roxb.

Spot	hR_f	Detection		
		UV 254	UV 366	*Modified Dragendorff TS1*
1	7-9	quenching	red	–
2	12-14	quenching	intense red	greyish blue
3	14-16	quenching	red	greyish blue
4	21-23	quenching	purple	orange
5	25-27	–	red	–
6	30-32	quenching	–	orange
7	33-35	weak quenching	–	–
8	37-39	–	red	–
9	41-43	–	light purple	–
10	46-48	weak quenching	green	–
11	57-59	weak quenching	–	–
12	60-62	–	intense blue	–
13	68-70	weak quenching	red	–
14	71-73	weak quenching	–	–
15	78-80	weak quenching	red	–
16	88-90	quenching	red	–
17	92-94	quenching	red	greyish blue
18	95-97	quenching	red	–

Loss on drying Not more than 10.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Acid-insoluble ash Not more than 7.0 per cent w/w (Appendix 7.6).

Total ash Not more than 20.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 7.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 20.0 per cent w/w (Appendix 7.12).

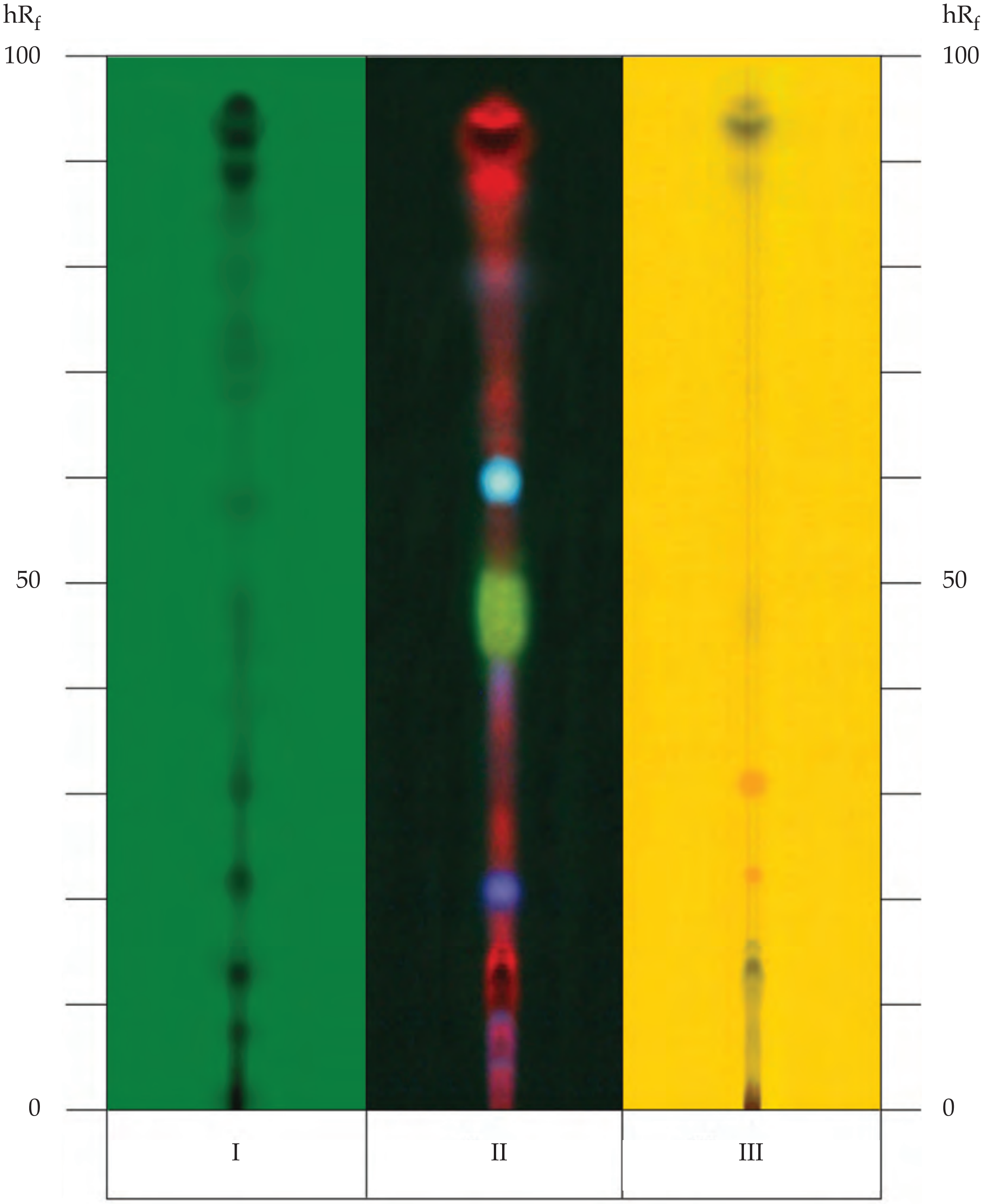


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Leaves of *Piper sarmentosum* Roxb.

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *modified Dragendorff TS1*

ชุมเห็ดไทย (CHUMHET THAI)

ชุมเห็ดเขาควาย (CHUMHET KHAO KHWAI), ชุมเห็ดเล็ก (CHUMHET LEK), ชุมเห็ดนา (CHUMHET NA)
Sennae Torae Semen
Cassia Seed

Category Laxative, diuretic.

Cassia Seed is the dried mature seed of *Senna tora* (L.) Roxb. (*Cassia tora* L.) (Family Leguminosae), Herbarium Specimen Number: DMSC 1514, Crude Drug Number: DMSc 427.

Constituents Cassia Seed contains anthraquinone aglycones (e.g., emodin, aloe-emodin, chrysophanol, physcione, rhein) and their glycosides. It also contains toralactone, sterols, mucilage, etc.

Description of the plant (Figs. 1a, 1b) Herb or undershrub up to 1 m high, nearly glabrous. Leaves with 2 to 4 pairs of leaflets; petioles 1 to 4 cm long; rhachis 2 to 3 cm long with a subulate, about 2 mm long gland between the two lower pairs of leaflets; stipules setaceous, 1 to 1.5 cm long, more or less caducous, leaflets increasing in size distally with a short petiolule, membranous, obovate, apex broadly rounded, base cuneate-rounded, 2 to 5 cm long, 1.5 to 2 cm wide. Inflorescence axillary, racemes short, 1- or few-flowered; bracts linear-acute, 2 to 3 mm long; pedicels 4 to 10 mm (enlarging in fruit); sepals 5, subequal, ovate, about 5 mm long, 2 to 4 mm wide; petals 5, yellow, unequal, obovate, short-clawed with rounded apex, up to 1 cm long, about 6 mm wide; stamens 7 to 10, 3 large, 4 medium, 3 staminodial or absent, rarely perfect, filaments 1.5 to 2 mm long, anthers 1.5 to 2.5 mm long, opening by apical pores, reduced stamens absent; ovary densely pubescent, style glabrous with truncate apex (stigma). Fruit terete, more or less falcate, 10 to 15 cm long, about 5 mm wide. Seeds 20 to 30, glossy, brown rhomboidal, 3 to 6 mm long, 2 to 3 mm wide.

Description Odour, foetid; taste, slightly bitter.

Macroscopical (Figs. 1a, 1b) Hard rhomboid-prismatic, 3 to 6 mm long, 2 to 3 mm wide, with a beak at one end. Externally greenish brown or yellowish brown, smooth and glossy, with pale brown bands occurring on both sides. The hilum and micropyle are located on the beak side.

Microscopical (Figs. 2a, 2b) Transverse and longitudinal sections of the seed reveal seed coat, cotyledon and embryo. Seed coat, covering with a thick cuticle layer, following by a layer of sclerified palisade cells, a layer of lagenosclereids and several layers of crushed macrosclereids. Endosperm, non-lignified, thick-walled cells, mostly with empty protoplasm. Cotyledons, two layers of dense, cylindrical, parenchymatous palisade cells and spheroidal spongy cells. Embryo, mostly with parenchyma cells. Crystals, rosette aggregate and prismatic, may be seen in macrosclereids, embryo and cotyledons.

Cassia Seed in powder possesses the diagnostic microscopical characters of the unground drug.

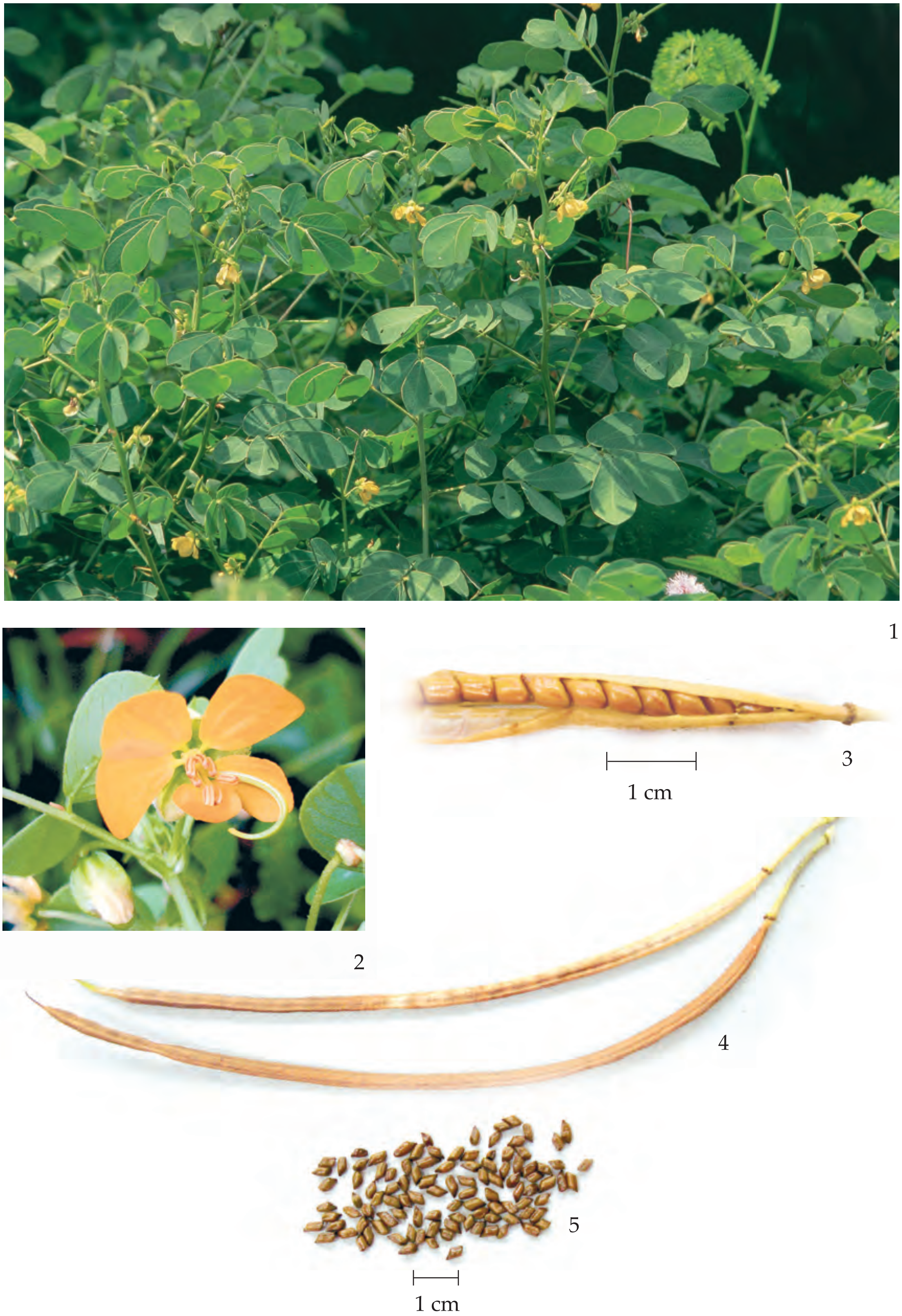


Fig. 1a *Senna tora* (L.) Roxb.
1. habit 2. flowering twig 3. young pod 4. mature pods 5. seeds

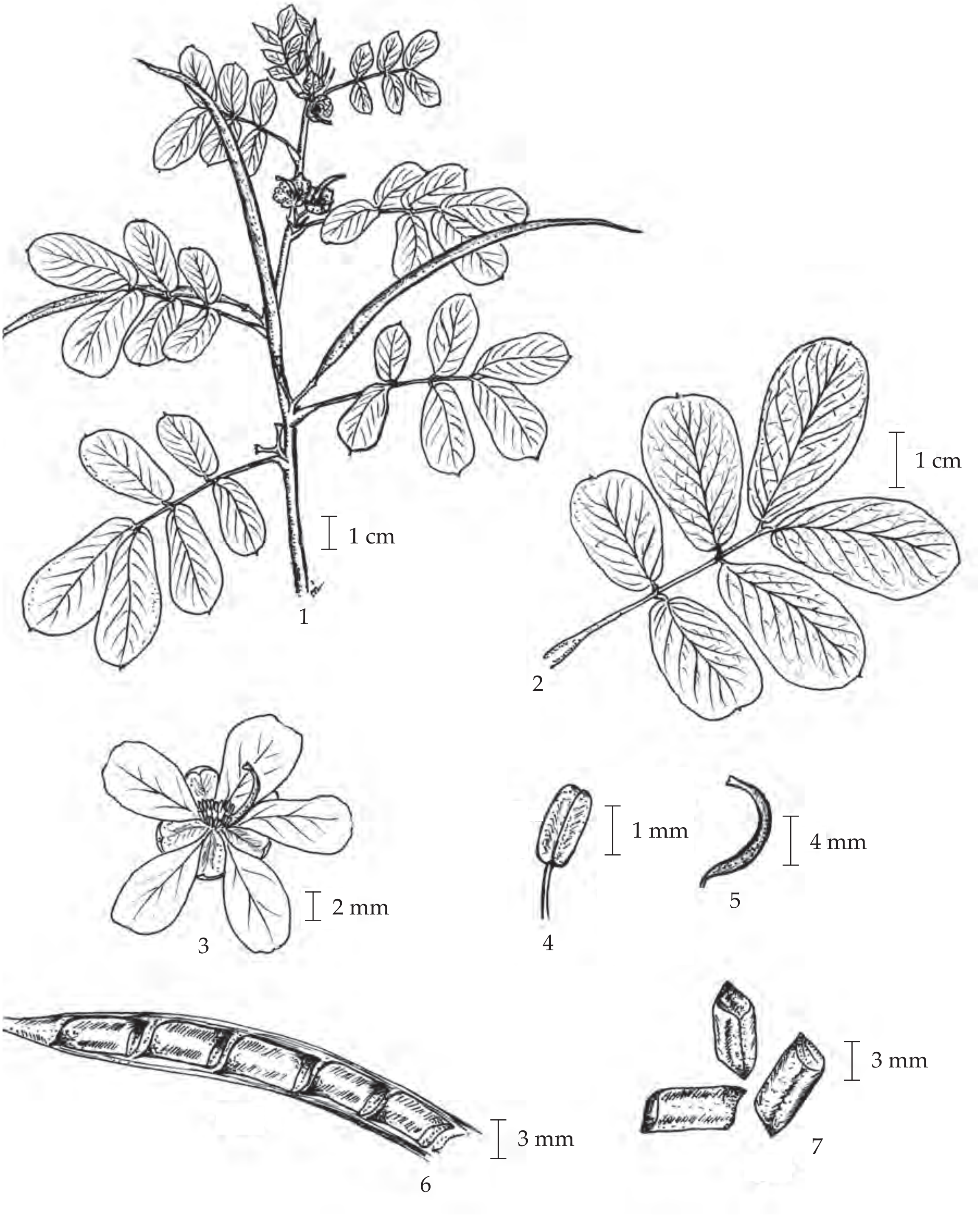


Fig. 1b *Senna tora* (L.) Roxb.
1. flowering top showing leaves and pods 2. compound leaf 3. flower
4. stamen 5. pistil 6. part of pod showing seeds 7. seeds

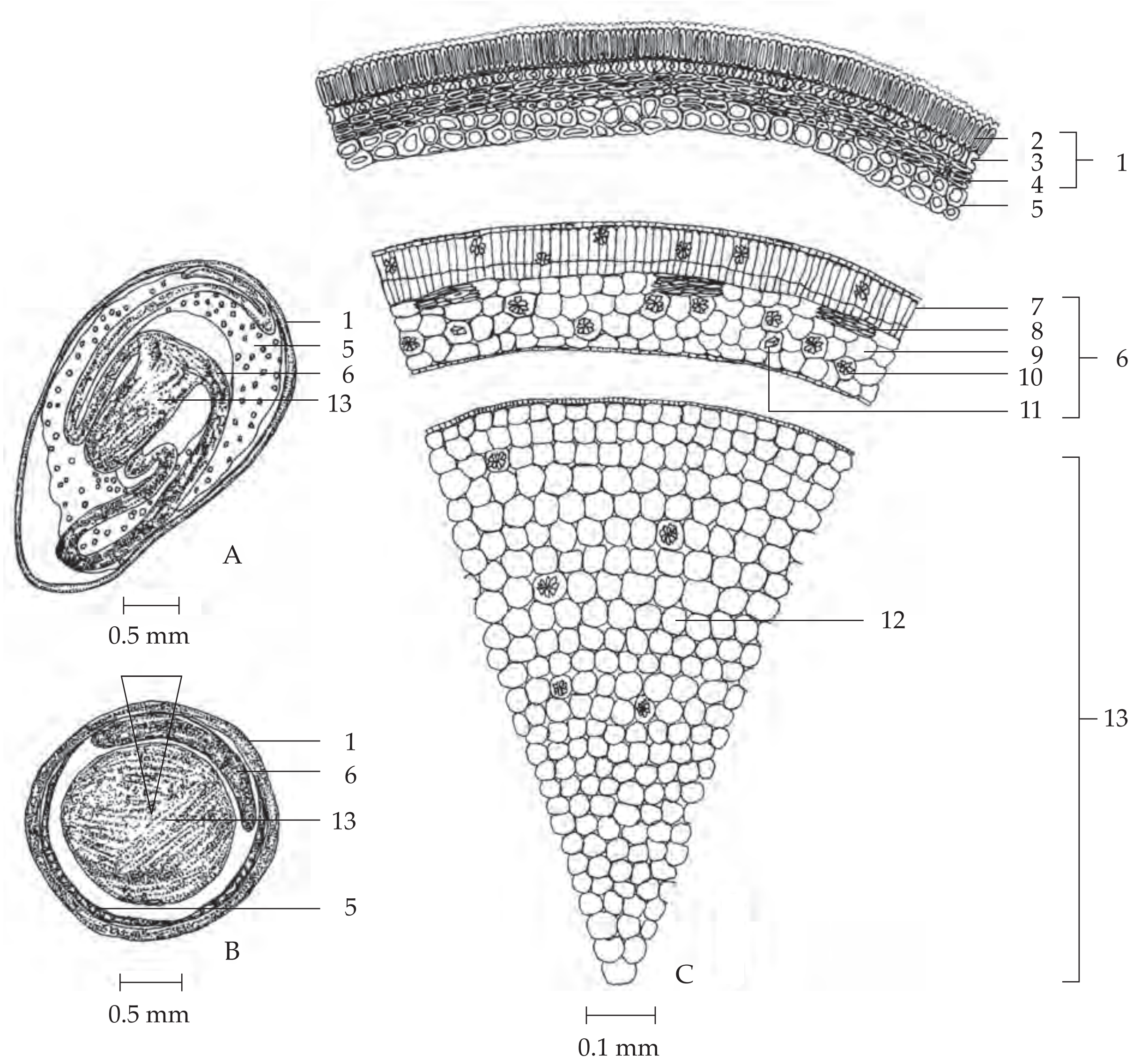


Fig. 2a Section of the Seed of *Senna tora* (L.) Roxb.

A. Diagram of Longitudinal Section
B. Diagram of Transverse Section
C. Sectional View of Transverse Section

1. seed coat covering with a cuticle layer
2. sclerified palisade cell
3. lagenosclereid
4. macrosclereid
5. thick-walled endosperm
6. cotyledon
7. palisade cell
8. vascular tissue
9. spongy cell
10. rosette aggregate crystal
11. prismatic crystal
12. parenchyma cell
13. embryo axis

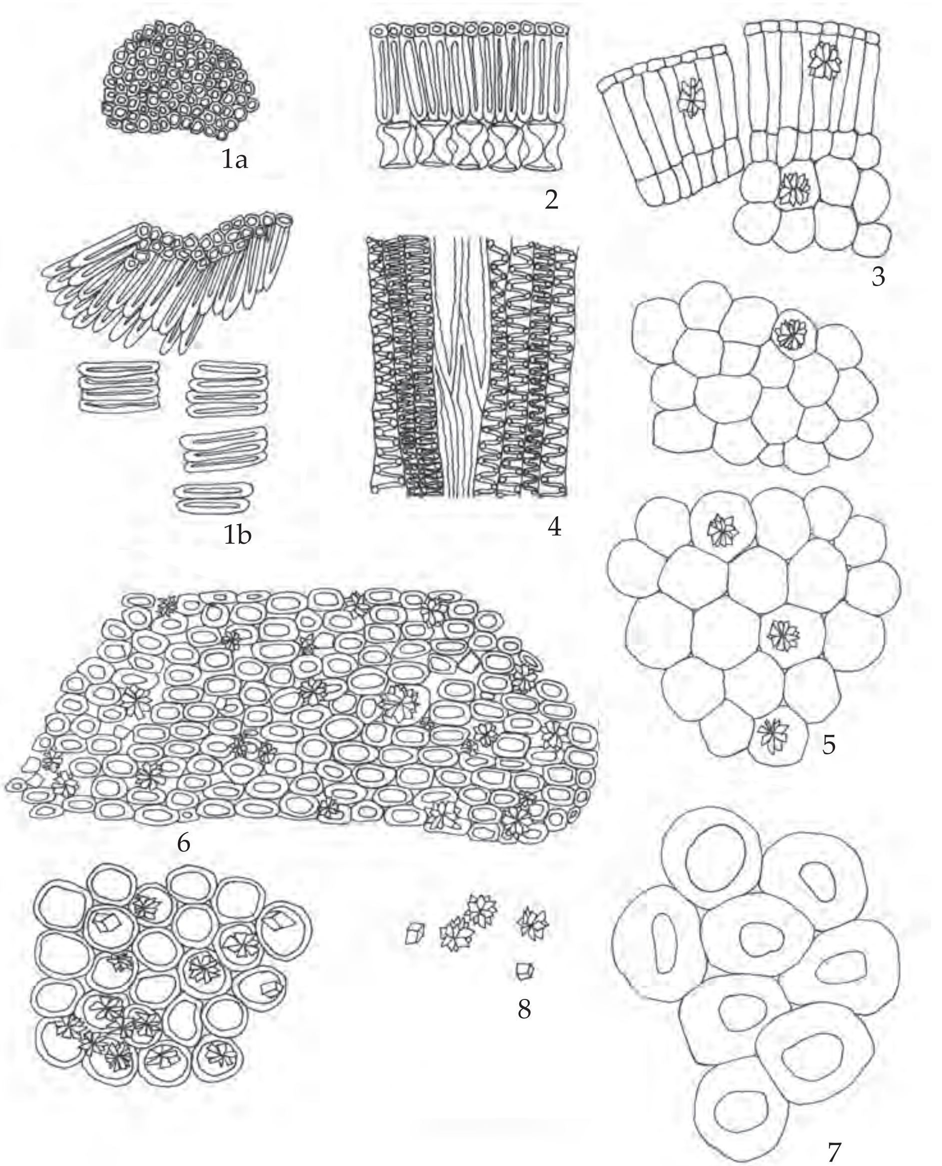


Fig. 2b Powdered Drug of the Seeds of *Senna tora* (L.) Roxb.

1. sclerified palisade cells in surface (1a) and tangential longitudinal (1b) views of seed coat
2. sclerified palisade cells with a covering cuticle layer and underlying langenosclerids
3. cotyledon in sectional view showing epidermal layer, palisade cells, spongy cells, and rosette aggregate crystals
4. vascular tissue in longitudinal view showing spiral vessels and fibres
5. parenchyma cells with rosette aggregate crystals
6. macrosclereids with scattering rosette aggregate and prismatic crystals
7. thick-walled endosperm cells
8. rosette aggregate and prismatic crystals

Warning

1. It should be used with caution in children, pregnant women or patients with inflammatory bowel disease.
2. It may cause drowsiness and/or mild abdominal discomfort such as colic or cramps. Excessive doses may produce nephritis.
3. Prolonged use should be avoided since it may result in diarrhea with excessive loss of water and electrolytes, particularly potassium.

Additional information Traditionally, it must be stir-fried[1] before use.

Packaging and storage Cassia Seed shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. To 100 mg of the sample, in *coarse powder*, add 25 ml of 2 M *hydrochloric acid*, heat on a water-bath for 15 minutes, and immediately filter through a plug of cotton wool. Allow the filtrate to cool and shake with 20 ml of *ether*. Separate the ether layer and shake with 10 ml of *ammonia TS*: the aqueous layer becomes pink-red.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 75 volumes of n-*hexane*, 25 volumes of *ethyl acetate* and 1 volume of *glacial acetic acid* as the mobile phase. Apply separately to the plate, 10 µl each of the following two solutions. Prepare solution (A) by refluxing 10 g of the defatted sample[2], in *coarse powder*, with 100 ml of *chloroform* for 1 hour. Filter the chloroform extract, evaporate the filtrate on a water-bath to dryness and dissolve the residue in 1 ml of *methanol*. For solution (B), dissolve 5 mg of *emodin* in 5 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 41 to 47), corresponding to the emodin spot from solution (B). Expose the plate to ammonia vapour; the spot due to emodin is reddish purple. Several other spots of different colours are also observed (Table 1); see also Fig. 3.

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[1] Place the clean crude drug in a pan or wok, and stir with gentle heat until slightly scented.

[2] Defat the sample by either extracting in a Soxhlet apparatus for 2 hours with *petroleum ether* (*boiling range, 40° to 60°*) or standing overnight with the petroleum ether without heating.

Table 1 hR_f Values of Components in Chloroform Extract of the Seeds of *Senna tora* (L.) Roxb.

Spot	hR_f Value	Detection	
		UV 254	Ammonia Vapour
1	19-25	quenching	violet
2	25-29	quenching	yellowish brown
3	32-38	quenching	reddish purple
4*	41-47	quenching	reddish purple
5	50-55	quenching	reddish purple
6	75-80	quenching	reddish purple
7	81-85	quenching	reddish purple

*emodin

Loss on drying Not more than 12.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 0.2 per cent w/w (Appendix 7.6).

Total ash Not more than 6.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 8.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 20.0 per cent w/w (Appendix 7.12).

Dose Stir-fried seeds, 10 to 13 g a day.

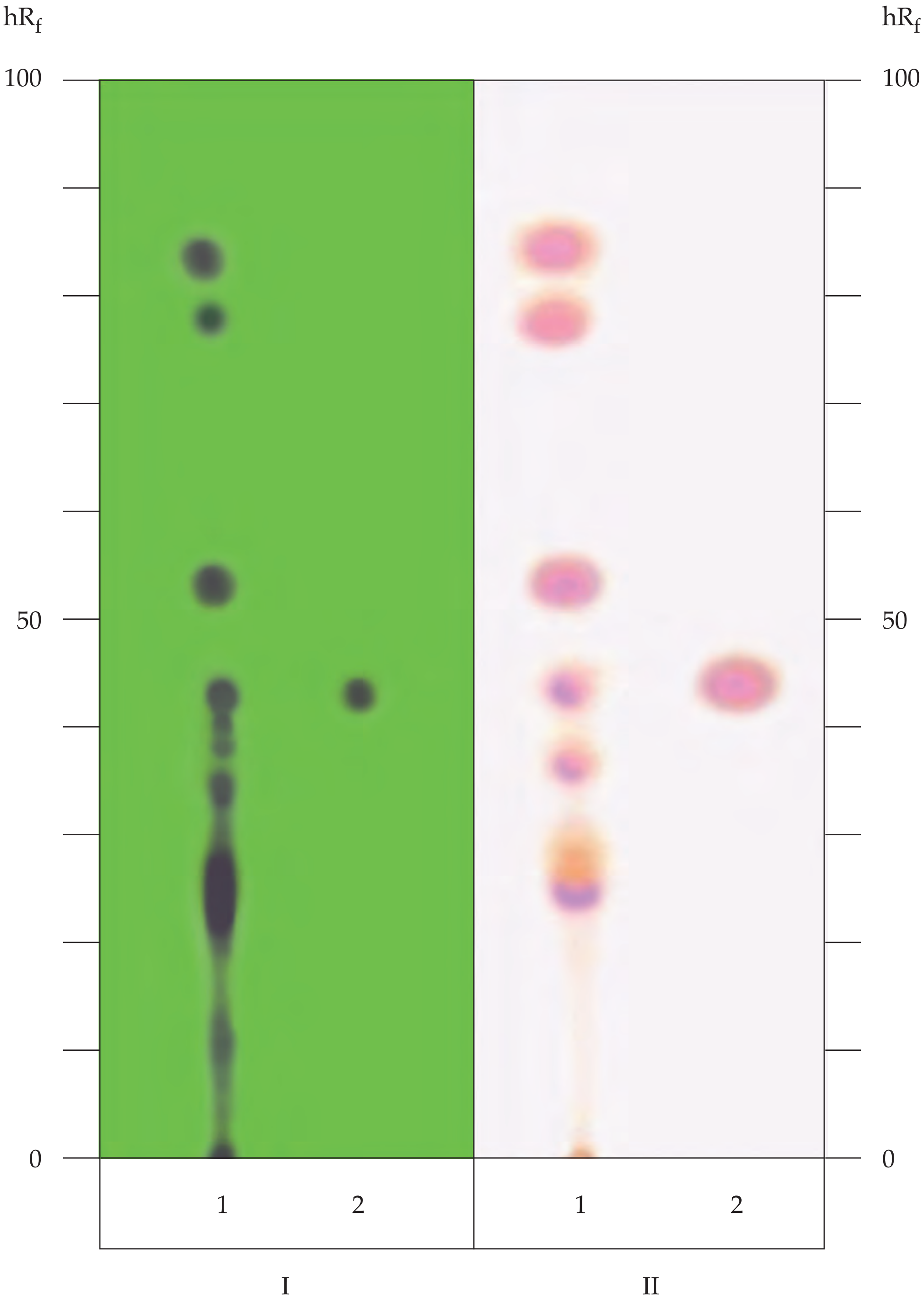


Fig. 3 Thin-layer Chromatogram of Chloroform Extract of the Seeds of *Senna tora* (L.) Roxb.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with ammonia vapour

ชุมเห็ดเทศ (CHUMHET THET)

Sennae Alatae Folium
Senna Alata Leaf

Synonym Ringworm Senna Leaf

Category Laxative, antifungal (ringworm).

Senna Alata Leaf is the dried leaflets of *Senna alata* (L.) Roxb. [*Cassia alata* L., *Herpetica alata* (L.) Raf., *Cassia bracteata* L. f.] (Family Leguminosae), Herbarium Specimen Number: DMSC 323.

Constituents Senna Alata Leaf contains anthraquinone aglycones (rhein, emodin, aloe-emodin, chrysophanol, and isochrysophanol), glycosides of rhein, of aloe-emodin and of physcione. It also contains kaempferol, β-sitosterol and sennosides A, B, C, and D.

Description of the plant (Fig. 1) Shrub, 1 to 5 m high with pubescent, horizontally spread branches. Leaves compound, leaflets 8 to 20 pairs; petiole robust 2 cm; rachis 30 to 60 cm; stipule auriculate, persistent, deltoid, 6 to 8 mm long; leaflet oblong-elliptic, rounded at both ends, 5 to 15 cm long, 3 to 7 cm wide, glabrous; petiolule robust, 2 to 3 mm. Inflorescence axillary, raceme, dense, robust, 20 to 50 cm long, 3 to 4 cm wide; bract caducous, 2 to 3 cm long, 1 to 2 cm wide; pedicel very short, 2 to 4 mm, sepal unequal, oblong, 10 to 20 mm long, 6 to 7 mm wide; petal bright yellow, ovate-orbicular to spathulate, short clawed, 2 cm long, 1 to 1.5 cm wide; stamens 9 to 10, 2 largest with thick filaments, 4 mm long and anthers 12 to 13 mm long, 4 stamens with filaments 2 mm long and anthers 4 to 5 mm long, opening by apical pores, 3 to 4 stamens reduced; ovary and style glabrous, stigma small. Fruit thick, flattened, winged, glabrous, septate, 10 to 15 cm long, 1.5 to 2 cm wide; wing 5 mm. Seed about 50, flattened, slightly quadrangular, 7 to 10 mm long, 5 to 8 mm.

Description Odour, slightly aromatic; taste, slightly bitter.

Macroscopical Senna Alata Leaf occurs as a mixture of entire and broken, greenish brown to brown leaflets and frequently rachillae of the compound leaf. Leaflets, 5 to 15 cm long, 3 to 7 cm wide, oblong to obovate, minutely mucronate at the apex, base oblique, margin entire, pinnate reticulate venation, rigidly subcoriaceous, obscurely downy beneath.

Microscopical (Figs. 2a, 2b) Transverse section of the leaflet shows epidermal cells with cuticle, slightly sinuous walls at both sides. Papillae, epidermal appendages, blurred and rarely seen at the upper, whereas clearly and densely at the lower. Covering trichomes with thickened and slightly warty walls, unicellular, occasionally found on the lamina, but more frequently found at the lower surface of the midrib. Stomata, paracytic at both epidermides. Palisade cells, columnar beneath upper epidermis. Spongy cells parenchymatous. Vascular bundles, xylem in the upper part and phloem in the lower part, surrounded by fibres with a crystal sheath containing calcium oxalate prisms. Vessels, lignified spiral and scalariform in the vein and more reticulate, pitted and bordered pitted in the midrib. Collenchyma, found beneath upper and lower epidermises in the midrib.

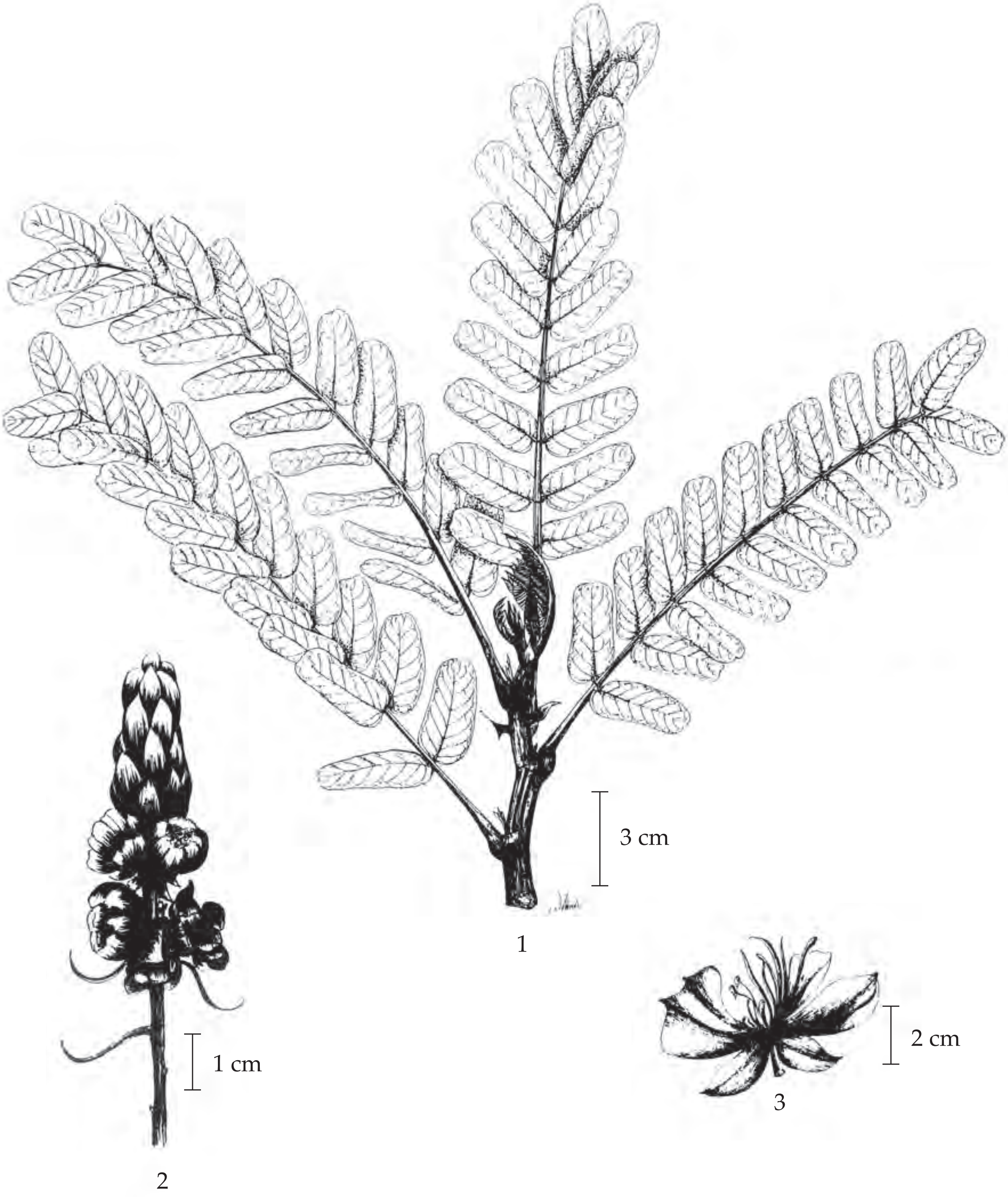


Fig. 1 *Senna alata* (L.) Roxb.
1. twig 2. inflorescence 3. flower

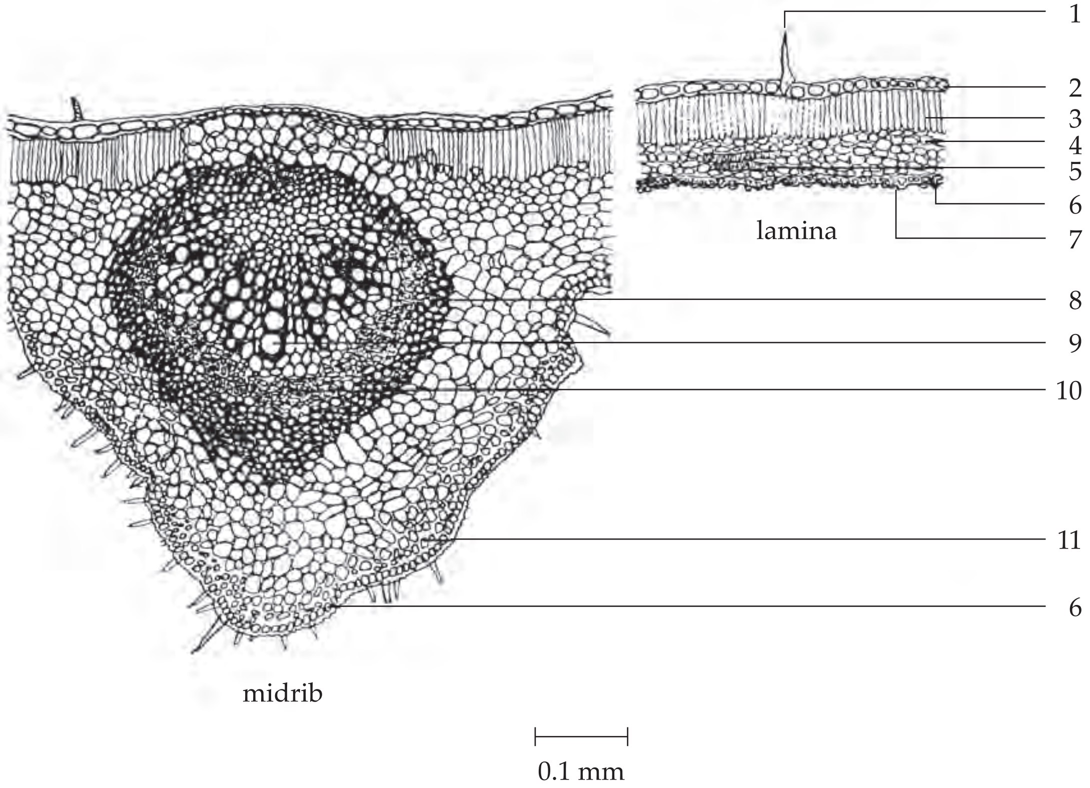


Fig. 2a Transverse Section of the Leaf of *Senna alata* (L.) Roxb.

1. unicellular trichome
2. upper epidermis
3. palisade cell
4. spongy cell
5. vascular bundle
6. lower epidermis
7. papilla
8. crystal sheath fibre
9. xylem
10. phloem
11. collenchyma

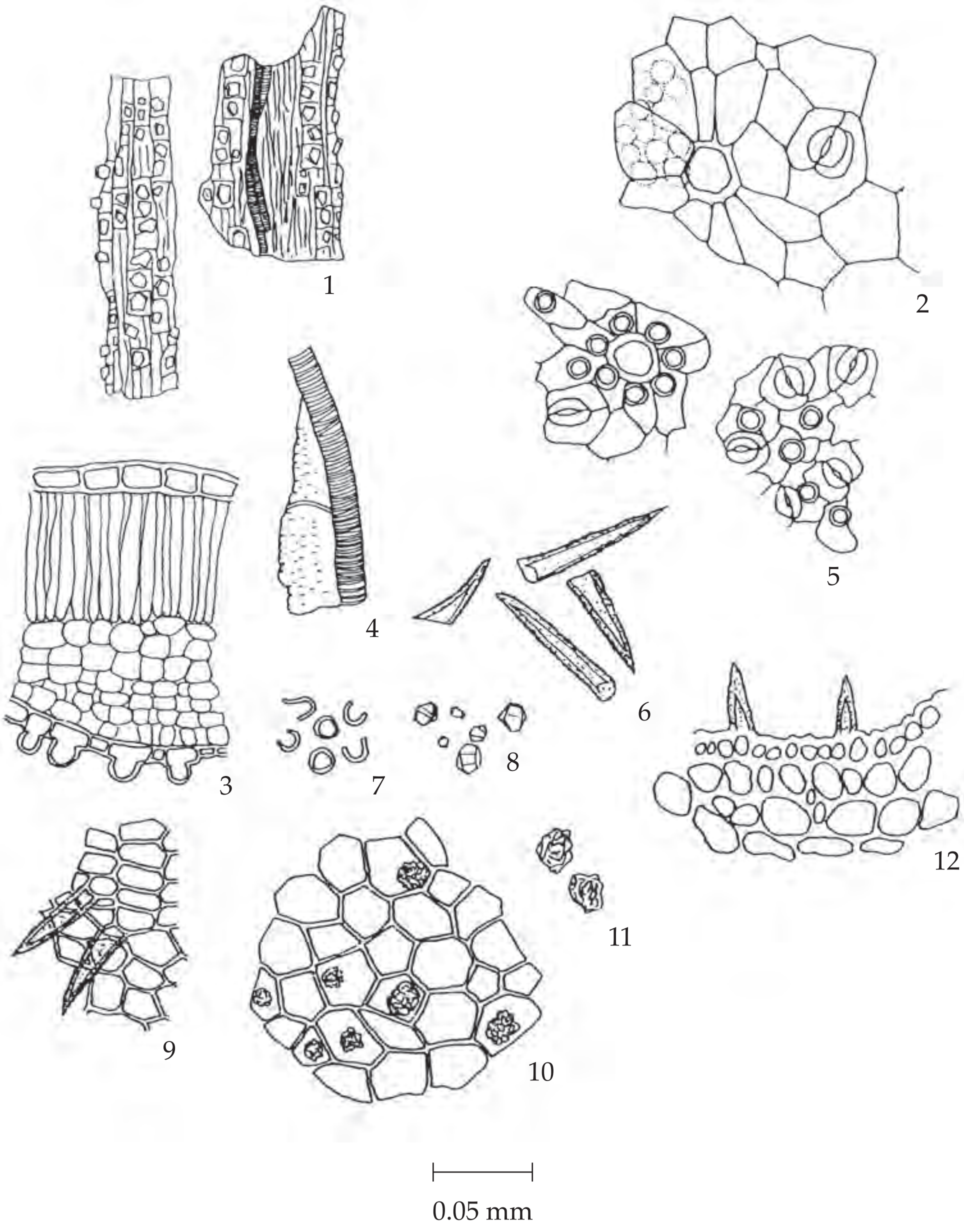


Fig. 2b Powdered Drug of the Leaves of *Senna alata* (L.) Roxb.

1. bundle of crystal sheath fibres
2. upper epidermis with stoma, cicatrix and underlying palisade cells
3. lamina in sectional view showing papillae
4. spiral and pitted vessels
5. lower epidermis with stoma, cicatrix and papillae
6. unicellular trichomes
7. fragments of papillae
8. prismatic crystals
9. epidermis with unicellular trichomes
10. parenchyma with cluster crystals
11. cluster crystals of calcium oxalate
12. collenchyma and epidermis with unicellular trichomes from midrib

Senna Alata Leaf in powder possesses the diagnostic microscopical characters of the unground drug.

Warning

1. It should be used with caution in children, or in patients with inflammatory bowel disease.
2. It may cause mild abdominal discomfort such as colic or cramps. Excessive doses may produce nephritis.
3. Prolonged use should be avoided since it may result in diarrhoea with excessive loss of water and electrolytes, particularly potassium.

Packaging and storage Senna Alata Leaf shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. To 100 mg of the sample, in powder, add 25 ml of 2 M *hydrochloric acid*, heat on a water-bath for 15 minutes, and immediately filter through a plug of cotton wool. Allow the filtrate to cool and shake with 20 ml of *ether*. Separate the ether layer and shake with 10 ml of *ammonia TS*: the aqueous layer becomes red.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 75 volumes of *petroleum ether (boiling range, 40° to 60°)*, 25 volumes of *ethyl acetate* and 1 volume of *anhydrous formic acid* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, 5 µl each of the following three solutions. Prepare solution (A) by refluxing 100 mg of the sample, in powder, with two 50-ml portions of *chloroform* on a water-bath for 15 minutes. Combine the chloroform extracts and evaporate to dryness. Dissolve the residue in 0.5 ml of *chloroform*. For solution (B), add 25 ml of 2 M *hydrochloric acid* to the marc obtained after the reflux and proceed as in the test for Identification A, beginning with "heat on a water-bath ..." to obtain the ether layer. Evaporate the ether layer to dryness. Dissolve the residue in 0.5 ml of *ether*. For solution (C) dissolve 1 mg of *rhein* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (366 nm), locating the spots. The chromatogram obtained with solutions (A) and (B) correspond in hR_f value and colour to that of rhein as shown in Table 1 or as of solution (C) (Table 1); see also Fig. 3. Expose the plate to ammonia vapour; the spots due to rhein are red. Other spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in the Extract of the Leaves of *Senna alata* (L.) Roxb.

Solution	Spot	hR_f Value	Detection	
			UV 366	Ammonia Vapour
A	1	4-8	–	pale green
	2	25-28	–	yellow
	3	29-32	–	yellow
	4*	34-38	orange	red
	5	53-58	–	pale green
	6	60-64	–	pale green
	7	74-77	–	pale green
	8	78-80	–	pale green
B	1	2-4	–	pale brown
	2	25-28	–	yellow
	3*	34-38	orange	red

*rhein

Loss on drying Not more than 11.0 per cent w/w after drying at 105° for 5 hours (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w of stems and other foreign matter (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 9.0 per cent w/w (Appendix 7.7).

Ethanol (50 per cent)-soluble extractive Not less than 21.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 18.0 per cent w/w (Appendix 7.12).

Hydroxyanthracene derivatives content Not less than 1.0 per cent w/w of hydroxyanthracene derivatives, calculated as rhein-8-glucoside on the dried basis, when determined by the following method.

Accurately weigh about 150 mg of Senna Alata Leaf, in *No. 150 powder*, and place in a 100-ml round-bottomed flask. Add 30.0 ml of *water*, mix, weigh, and place in a water-bath. Heat under a reflux condenser for 15 minutes. Allow to cool, weigh and adjust to the original weight with *water*. Centrifuge and transfer 20.0 ml of the supernatant liquid to a 150-ml separator. Add 0.1 ml of 2 M *hydrochloric acid* and shake with three 15-ml portions of *chloroform*. Allow to separate and discard the chloroform layer. Add 100 mg of *sodium hydrogencarbonate* and shake for 3 minutes. Centrifuge and transfer 10.0 ml of the supernatant liquid to a 100-ml round-bottomed flask with a ground glass neck. Add 20 ml of a 10.5 per cent w/v solution of *iron(III) chloride* and mix. Heat for 20 minutes under a reflux condenser in a water-bath with water level above that of the liquid in the flask, add 1 ml of *hydrochloric acid* and heat for a further 20 minutes with frequent shaking, to dissolve the precipitate. Cool, transfer the mixture to a separator and shake with three 25-ml portions of *ether* previously used to rinse the flask. Combine the ether layers and wash with two 15-ml portions of *water*. Transfer the ether layers to a 100-ml volumetric flask and dilute with *ether* to volume. Evaporate 25.0 ml carefully to dryness at low temperature and dissolve the residue in 10.0 ml of a 0.5 per cent w/v solution of *magnesium acetate* in *methanol*. Measure the absorbance of this solution at the maximum at about 515 nm (Appendix 2.2), using the magnesium acetate solution as the blank.

Calculate the percentage content of rhein-8-glucoside from the expression:

$$A \times 0.4283/w,$$

where A = the absorbance of the substance being examined at 515 nm, and

w = the weight in g of the substance being examined.

Dose 3 to 6 g once a day at bedtime.

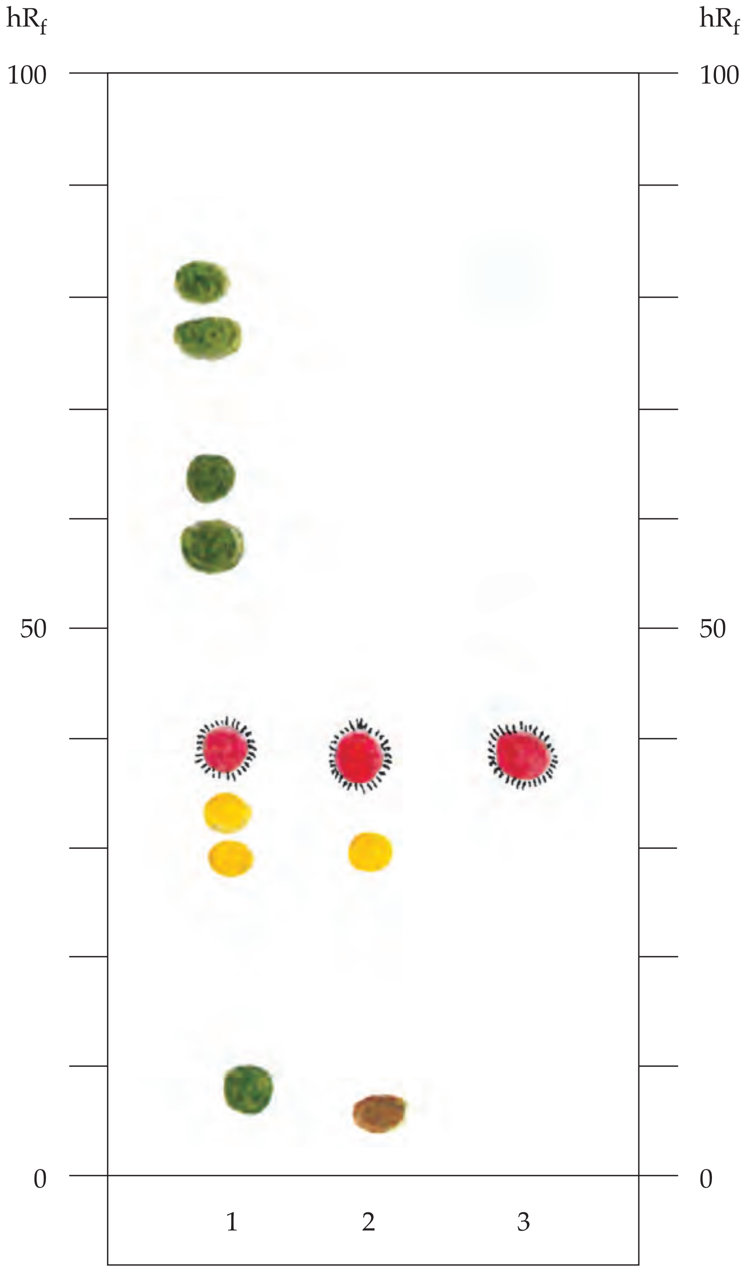


Fig. 3 Thin-layer Chromatogram of the Extracts of the Leaves of *Senna alata* (L.) Roxb.

1 = solution (A)
2 = solution (B)
3 = solution (C)
○ = detection with ammonia vapour
✺ = detection under UV light (366 nm)

ยาชงชุมเห็ดเทศ (YA CHONG CHUMHET THET)

Senna Alata Tea

Category Laxative.

Senna Alata Tea contains an amount of powdered Senna Alata Leaf equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the labelled content of hydroxyanthracene derivatives, calculated as rhein-8-glucoside.

Strength available 3 g (powder), supplied in a sachet.

Dose One or two sachets, prepared as an infusion by soaking each with 120 ml of boiling water for 10 minutes, once a day at bedtime.

Warning It should be used with caution in children under 12 years of age.

Packaging and storage Senna Alata Tea shall be kept in well-closed containers, protected from light.

Labelling The label on the container states (1) the equivalent amount of hydroxyanthracene derivatives, calculated as rhein-8-glucoside; (2) expiration date.

Identification

1. The tea contents exhibit diagnostic structures of the powdered drug described under *Senna Alata Leaf.*
2. The tea contents comply with the tests for Identification A and B described under *Senna Alata Leaf.*

Loss on drying Of the tea contents, not more than 11.0 per cent w/w after drying at 105° for 5 hours (Appendix 4.15).

Microbial limit Complies with the requirements for Category 5 in the “Limits for Microbial Contamination” (Appendix 10.5).

Assay Grind the contents of not less than 20 sachets of Senna Alata Tea to *fine powder*. Transfer about 150 mg, accurately weighed, to a 100-ml round-bottomed flask. Add 30.0 ml of *water*, mix, weigh, and place in a water-bath. Heat under a reflux condenser for 15 minutes. Allow to cool, weigh and adjust to the original weight with *water*. Centrifuge and transfer 20.0 ml of the supernatant liquid to a 150-ml separator. Add 0.1 ml of 2 M *hydrochloric acid* and shake with three 15-ml portions of *chloroform*. Allow to separate and discard each chloroform layer. Add 100 mg of *sodium hydrogencarbonate* and shake for 3 minutes. Centrifuge and transfer 10.0 ml of the supernatant liquid to a 100-ml round-bottomed flask. Add 20 ml of a 10.5 per cent w/v solution of *iron(III) chloride* and mix. Heat for 20 minutes under a reflux condenser in a water-bath with its water level above that of the liquid in the flask, add 1 ml of *hydrochloric acid* and heat for a further 20 minutes with frequent shaking, to dissolve the precipitate. Cool, transfer the mixture to a separator and shake with three 25-ml portions of *ether* previously used to rinse the flask. Combine the ether layers and wash with two 15-ml portions of *water*. Transfer the ether layers to a 100-ml volumetric flask and dilute with *ether* to volume. Evaporate 25.0 ml carefully to dryness at a low temperature and dissolve the residue in 10.0 ml of a 0.5 per cent w/v solution of *magnesium acetate* in *methanol*. Measure the absorbance of this solution at the maximum at about 515 nm

(Appendix 2.2), using the magnesium acetate solution as the blank. Calculate the quantity, in mg, of rhein-8-glucoside in the sample taken by the formula:

$$(446.37/284.22)\ (1200A/440),$$

in which 446.37 and 284.22 are the molecular weights of rhein-8-glucoside and rhein, respectively, A is the absorbance of the solution at 515 nm and 440 is the specific absorbance.

Other requirements Complies with the requirements described under "Herbal Teas" (Appendix 1.16H).

ดีปลี (DI PLI)

ดีปลีเชือก (DI PLI CHUEAK)
Piperis Retrofracti Fructus
Java Long Pepper

Category Carminative, expectorant, stomachic, anti-emetic, oxytocic.

Java Long Pepper is the dried ripe fruit-spike of *Piper retrofractum* Vahl (*P. chaba* Hunter) (Family Piperaceae), Herbarium Specimen Number: DMSC 48, 75, 456, 467.

Constituents Java Long Pepper contains piperine, piperlongumine, pyridine alkaloids, sesamin, and volatile oil.

Description of the plant (Figs. 1a, 1b) Climber, glabrous, rather fleshy, with the aid of adventitious roots, rarely 20 creeping; stems much branched, stout, cylindrical, thickened above nodes. Leaves simple, alternate, 3 to cm long, 2 to 13 cm wide, lower ones ovate or lanceolate with cordate base, upper ones oblong-oval to oblong with obtuse, cordate or cuneate base, unequal, all tapering or acuminate, entire, glabrous, reticulate venation sunk above and raised beneath; petioles of lower leaves 1.5 to 3 cm long, of the upper ones 0.5 to 1.5 cm long; stipules 1 to 1.5 cm long, membranous, lanceolate, obtuse, enclosing the bud but soon falling off. Inflorescence spikes, erect or patent; peduncles 0.7 to 2 cm long; bracts broadly oval-ovate, 1.5 to 2 mm long; flowers unisexual, bisexual; male spikes 2.5 to 8.5 cm long, stamens 2, rarely 3, very short; female spikes 1.7 to 3 cm long, stigmata 2 to 3, short, obtuse, persistent. Fruiting spikes cylindro-conic; berries connate and adnate to stalk of bract, broadly rounded, bright red. Seeds 2 to 2.5 mm in diameter.

Description Odour, aromatic; taste, pungent.

Macroscopical (Fig. 1a) Condensed fruiting spikes, reddish brown, subcylindrical, about 2.5 to 7.5 cm long and 5.0 to 8.0 mm in diameter, rather rough surface with persistent stigmata.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the fruit shows epicarp composed of a layer of epidermal cells of which the outer tangential wall thick, glandular trichomes and cuticle. Mesocarp composed of 3 to 4 layers of collenchyma of hypodermis in which stone cells scattered followed by zone of thin-walled parenchyma, some of which containing brownish substance, oil droplets and starch grains and a few layers of large oil cells. Endocarp composed of a single layer of sclerenchyma. Zone of ground parenchyma scattered with vascular bundles around the cavity at the centre of the fruit. Spermoderm composed of outer thick-walled epidermis, collapse parenchyma of middle epidermis and inner thin-walled epidermis. Perisperm, elongated reserve parenchyma cells containing numerous angular starch grains and rod-shaped crystals. Embryo embedded in the endosperm.

Java Long Pepper in powder possesses the diagnostic microscopical characters of the unground drug.

1

2

4

3

5

Fig. 1a *Piper retrofractum* Vahl
1. habit 2. flowering twig 3. ripening fruit-spikes 4. ripe fruit-spike 5. crude drug

Fig. 1b *Piper retrofractum* Vahl

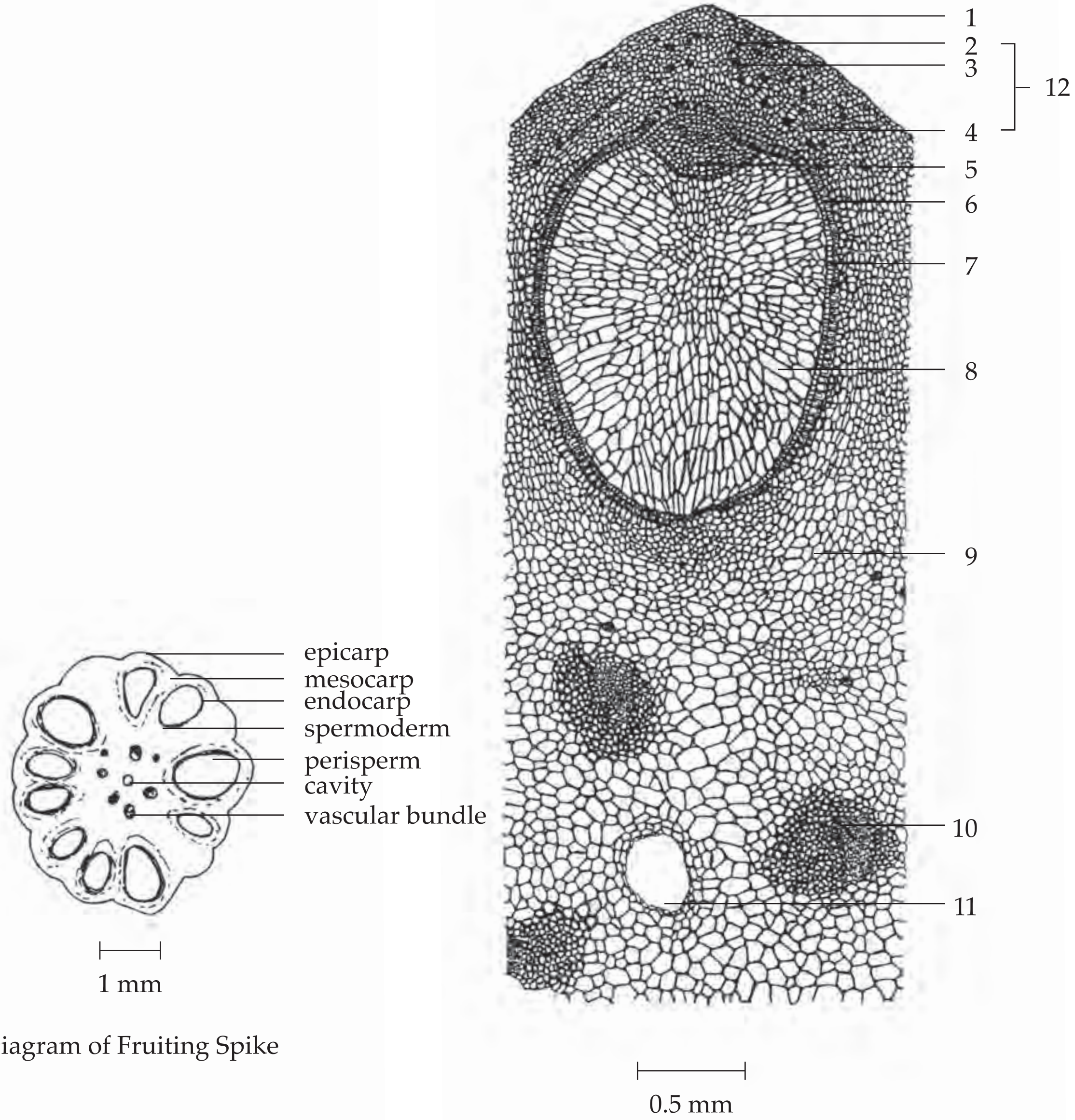


Diagram of Fruiting Spike

Fig. 2a Transverse Section of the Fruit of *Piper retrofractum* Vahl

1. epicarp
2. thick-walled parenchyma
3. sclereid
4. oil cell
5. embryo
6. sclerenchyma of endocarp
7. spermoderm
8. perisperm
9. ground parenchyma
10. vascular bundle
11. cavity
12. mesocarp

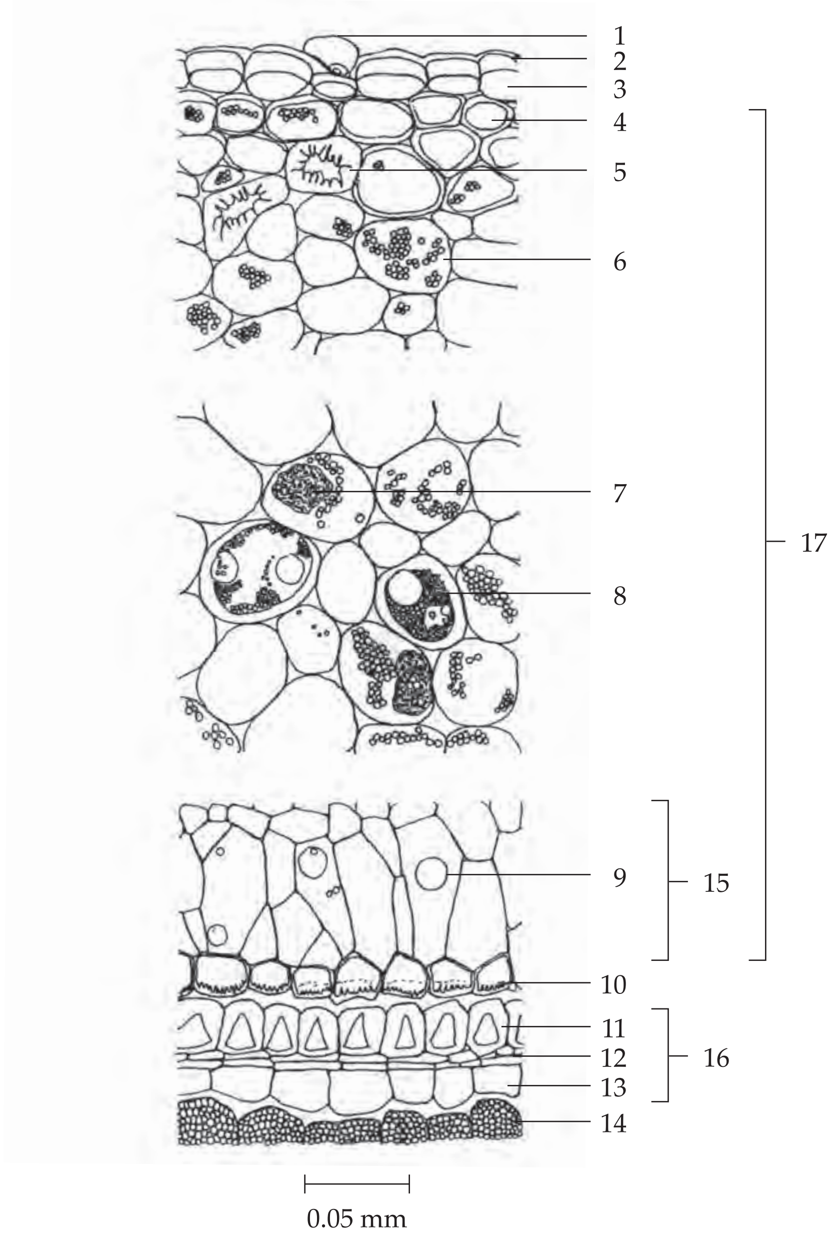


Fig. 2b Transverse Section of Epicarp, Mesocarp, Endocarp, and Spermoderm of the Fruit of *Piper retrofractum* Vahl

1. glandular trichome	9. oil droplet
2. cuticle	10. sclerenchyma of endocarp
3. epidermis	11. outer epidermis of spermoderm
4. thick-walled parenchyma	12. middle epidermis of spermoderm
5. sclereid	13. inner epidermis of spermoderm
6. parenchyma containing starch grains	14. perisperm
7. brownish substance	15. layer of large oil cells
8. oil cell	16. spermoderm
	17. mesocarp

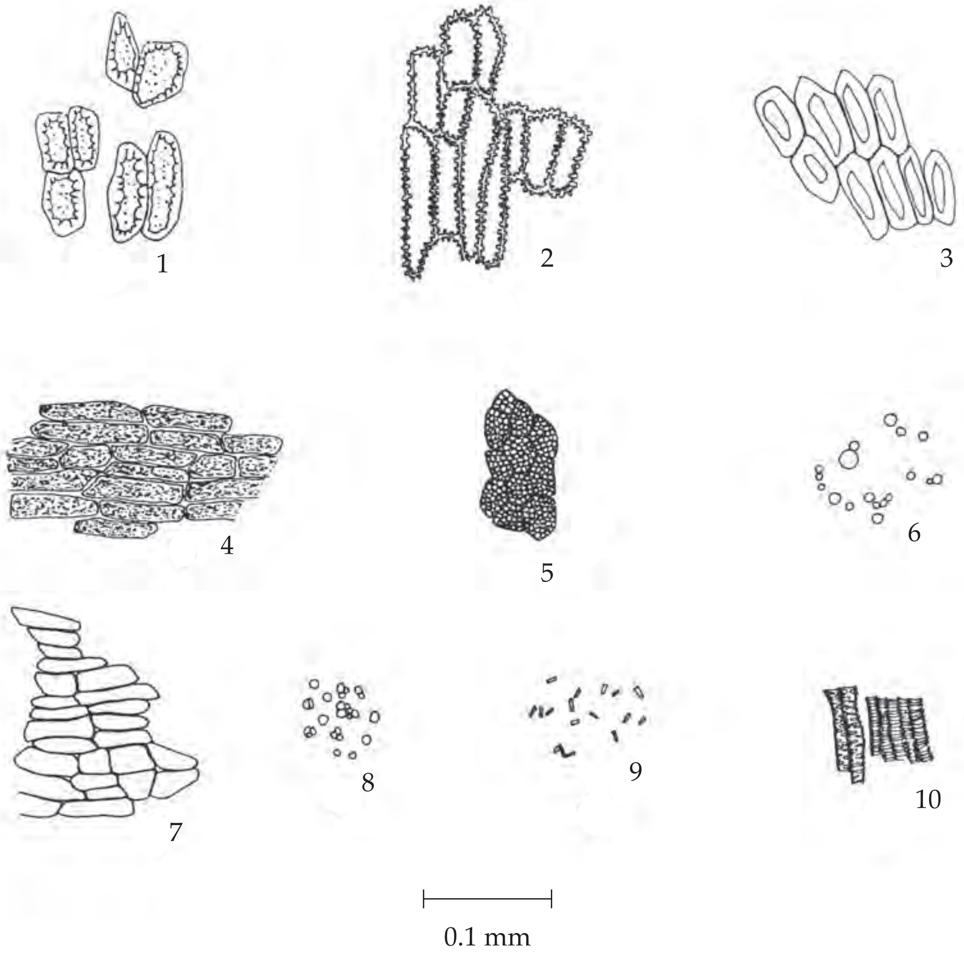


Fig. 2c Powdered Drug of the Fruits of *Piper retrofractum* Vahl

1. sclereids of mesocarp
2. sclerenchymatous endocarp
3. outer epidermis of spermoderm
4. inner epidermis of spermoderm
5. perisperm containing starch grains
6. oil globules
7. parenchyma
8. starch grains
9. rod-shaped crystals
10. vessels

Packaging and storage Java Long Pepper shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Reflux 1 g of the sample, in powder, with 25 ml of *ethanol* for 10 minutes and filter (solution 1). To 2 ml of solution 1, add a few drops of *ninhydrin TS* and warm in a water-bath for a few minutes: a purple colour is produced.

B. Evaporate 2 ml of solution 1 to dryness. Dissolve the residue in 2 ml of *acetic anhydride*, and then slowly add 1 ml of *sulfuric acid* to form two layers: a brown ring develops at the zone of contact.

C. Evaporate 2 ml of solution 1 to almost dryness and add a few drops of Marquis'reagent, prepared by adding a few drops of *formaldehyde solution* to 6 ml of *sulfuric acid*: a brownish red colour is produced.

D. To 100 mg of the sample, in powder, add 1 ml of *sulfuric acid*: a deep red colour is produced at first, turning to reddish brown and brown.

E. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 7 volumes of n-*hexane* and 3 volumes of *ethyl acetate* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate, 5 µl each of the following two solutions. Prepare solution (A) by macerating 500 mg of the sample, in powder, with 25 ml of *chloroform* for 15 minutes and filtering. Evaporate the filtrate to dryness and dissolve the residue in 2 ml of *chloroform*. For solution (B), dissolve 2 mg of *piperine* in 1 ml of *chloroform*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 20 to 22), corresponding to the piperine spot from solution (B), and several spots of higher hR_f values. Spray the plate with *modified Dragendorff TS2*; the spot due to piperine is orange and one orange spot is observed (Table 1); see also Fig. 3.

Repeat the same procedure on another plate but spray with *anisaldehyde TS*. The chromatogram obtained from solution (A) shows a green spot (hR_f value 20 to 22), corresponding to the piperine spot from solution (B), and several other spots of different colours are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Chloroform Extract of the Fruits of *Piper retrofractum* Vahl

Spot	hR_f Value	Detection		
		UV 254	*Modified Dragendorff TS2*	*Anisaldehyde TS*
1	5-6	–	–	pale violet
2*	20-22	quenching	orange	green
3	25-27	quenching	pale orange	green
4	31-33	quenching	–	violet
5	36-38	quenching	–	violet
6	43-44	quenching	–	purple
7	50-51	quenching	–	–
8	58-59	–	–	pale violet
9	60-61	quenching	–	–
10	64-66	–	–	violet
11	66-69	quenching	–	–
12	74-77	quenching	–	blue
13	91-95	–	–	violet

*piperine

Water Not more than 13.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Acid-insoluble ash Not more than 0.4 per cent w/w (Appendix 7.6).

Total ash Not more than 7.5 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 10.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 1.0 per cent v/w (Appendix 7.3 H). Use 25 g, in *fine powder,* freshly prepared and accurately weighed. Use 250 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

Alkaloids content Not less than 2.5 per cent w/w of alkaloids, calculated as piperine, when determined by the following method. (**Note** Use light-resistant glassware to reduce photodegradation of piperine in solution.)

Standard piperine solution Dissolve about 10 mg of *piperine*, accurately weighed, in sufficient *1,2-dichloroethane* to produce 100.0 ml.

Standard piperine curve Transfer into six 100-ml volumetric flasks, 1, 2, 3, 4, 5, and 6 ml, respectively, of *Standard piperine solution,* dilute to volume with *1,2-dichloroethane,* and mix. Measure the absorbances of the standard solutions relative to the blank at 342 nm (Appendix 2.2). Plot the readings and draw the curve of best fit.

Procedure Place about 500 mg of Java Long Pepper, in *fine powder* and accurately weighed, in a soxhlet apparatus. Add a sufficient quantity of *1,2-dichloroethane* and extract, until complete extraction of the alkaloids is effected (Appendix 7.4). Transfer the dichloroethane extract to a 100-ml volumetric flask and dilute with *1,2-dichloroethane* to volume. Transfer 2.0 ml of this solution to a 100-ml volumetric flask and dilute with *1,2-dichloroethane* to volume. Measure the absorbance of the resulting solution, at the maximun at about 342 nm (Appendix 2.2). By reference to the standard curve, calculate the content of alkaloids as piperine in the sample.

Dose 200 to 500 mg three times a day.

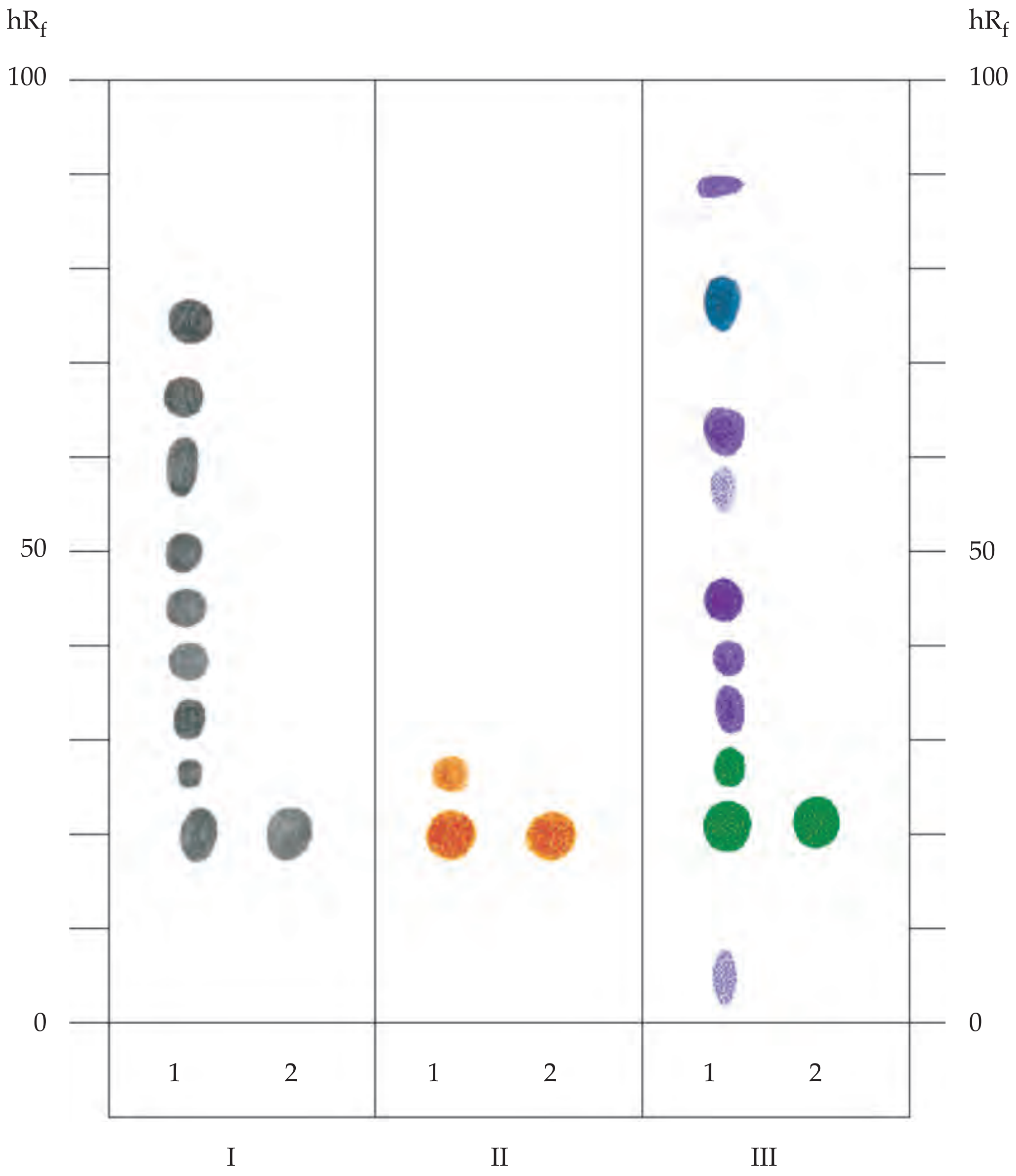


Fig. 3 Thin-layer Chromatogram of Chloroform Extract of the Fruits of *Piper retrofractum* Vahl

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with *modified Dragendorff TS2*
III = detection with *anisaldehyde TS*

ฟ้าทะลาย (FA THALAI)

น้ำลายพังพอน (NAMLAI PANGPON), สามสิบดี (SAM SIP DI), ฟ้าทะลายโจร (FA THALAI CHON)
Andrographitis Herba
Andrographis Herb

Category Antidiarrheal, antipyretic, anti-inflammatory in laryngitis.

Andrographis Herb is the dried aerial part of *Andrographis paniculata* (Burm. f.) Nees (*Justicia paniculata* Burm. f.) (Family Acanthaceae), Herbarium Specimen Number: DMSC 821, 854.

Constituents Andrographis Herb contains a large quantity of bitter diterpenoid lactones which mainly are andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide (dehydroandrographolide), dideoxyandrographolide (andrograpanin), etc. and their glycosides. It also contains β-sitosterol-D-glucoside and flavonoids.

Description of the plant (Fig. 1) Annual herb, up to 1 m high, erect; stem acutely quadrangular. Leaves simple, opposite, lanceolate, 2 to 12 cm long, 1 to 3 cm wide, acute, glabrous, entire-slightly undulate, upper ones often bracteiform; petiole short. Inflorescence patent, terminal and axillary, in panicle, 10 to 30 cm long; bract small, pedicel short. Flower: calyx 5-partite, small, linear; corolla tube narrow about 6 mm long, limb not shorter than the tube, bilabiate, upper lip oblong, white with a yellowish top, lower lip broadly cuneate, trifid white with violet marking; stamens 2, inserted in the throat and far exserted, anthers basally bearded, ovary superior, 2-celled, style far exserted. Fruit capsule erect, linear-oblong, 1 to 2 cm long, 2 to 5 mm wide, compressed, longitudinally furrowed on the broad faces, thinly glandular-hairy. Seed small, subquadrate.

Description Odour, slight and specific; taste, extremely bitter.

Macroscopical Dried herb occurs as a mixture of broken, crisp, mainly dark green lanceolate leaf and quadrangularly stem; capsule fruit and small flower occasionally found.

Microscopical (Figs. 2a, 2b, 2c, 2d) Surface and transverse sections of the leaf through lamina and midrib region show the following characters: Upper epidermis, a layer of cells; stomata absent; covering trichomes, glandular, unicellular and multicellular present; cicatrices rarely seen; lithocysts, fairly large 27 µm × 96 µm to 30 µm × 210 µm in size and 49 µm thick. Palisade cells, columnar. Collenchyma occurs in the midrib, beneath upper and lower epidermis. Spongy cells, parenchymatous. Vascular bundles, composed of lignified xylem in the upper part and phloem in the lower part. Vessels, spiral, scalariform and reticulate. Lower epidermis, a layer of wavy-walled cells; stomata, diacytic; covering trichomes and lithocysts present.

Transverse section of the stem shows the following characters: epidermis, a layer of cells; stomata, diacytic; covering trichomes and lithocysts present. Collenchyma, densely found at the corners of stem. Parenchyma, containing chlorophyll. Endodermis, a layer of thick-walled cells. Vessels, spiral, scalariform and pitted. Pith, large parenchyma cells.

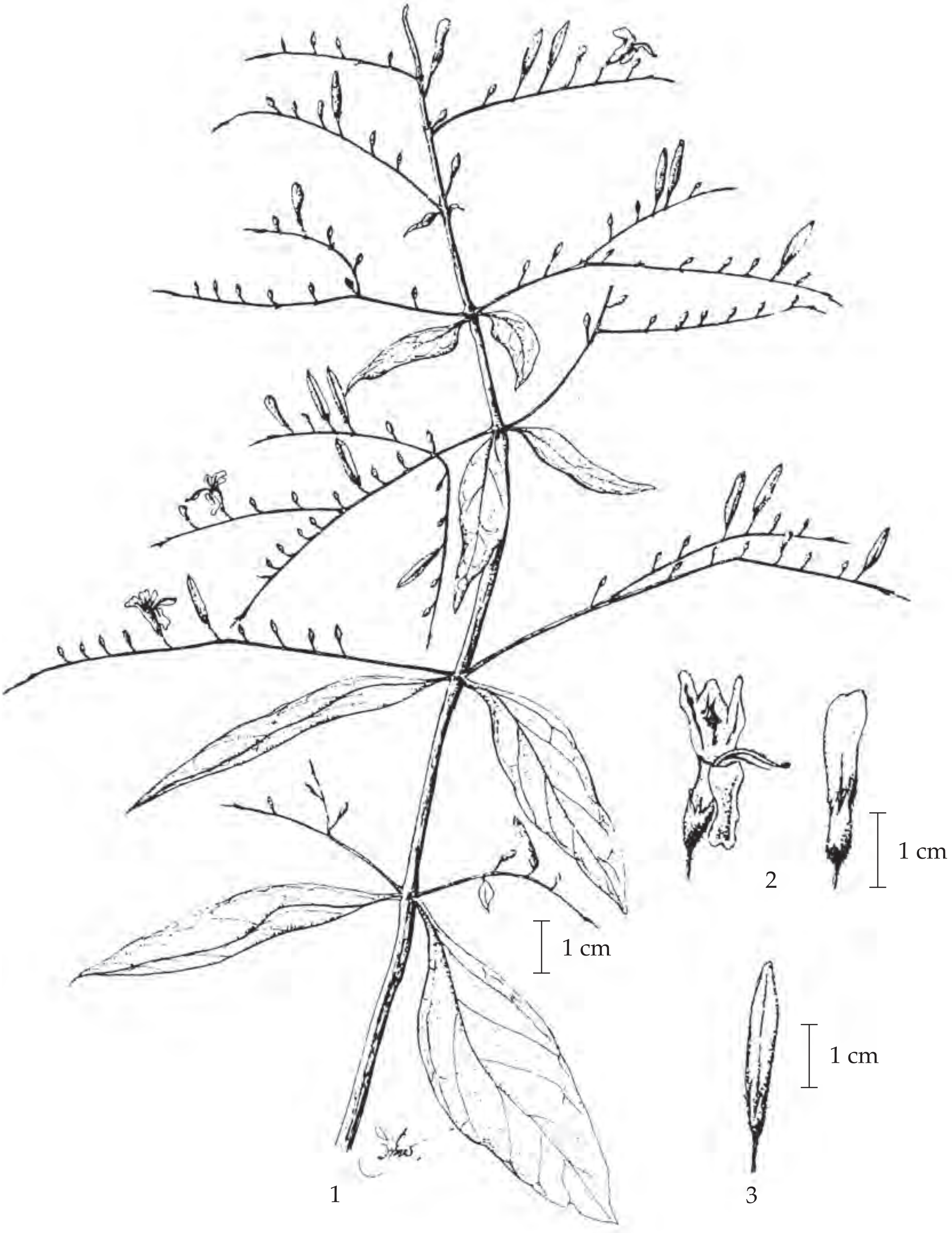
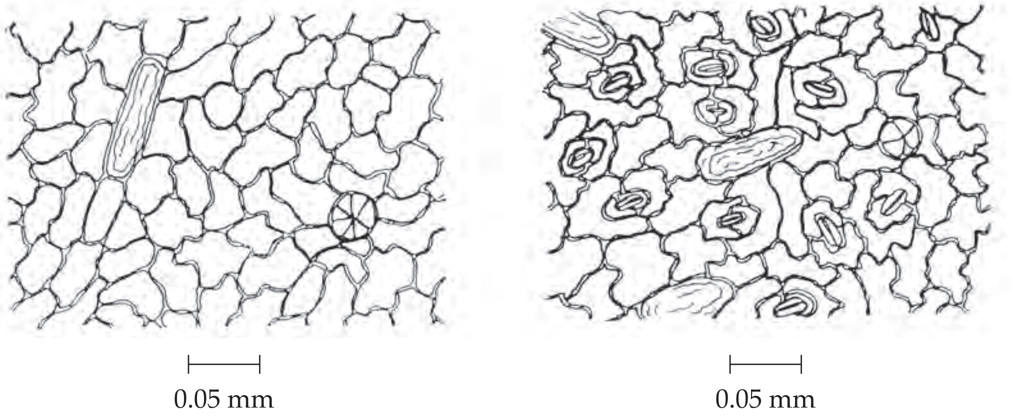


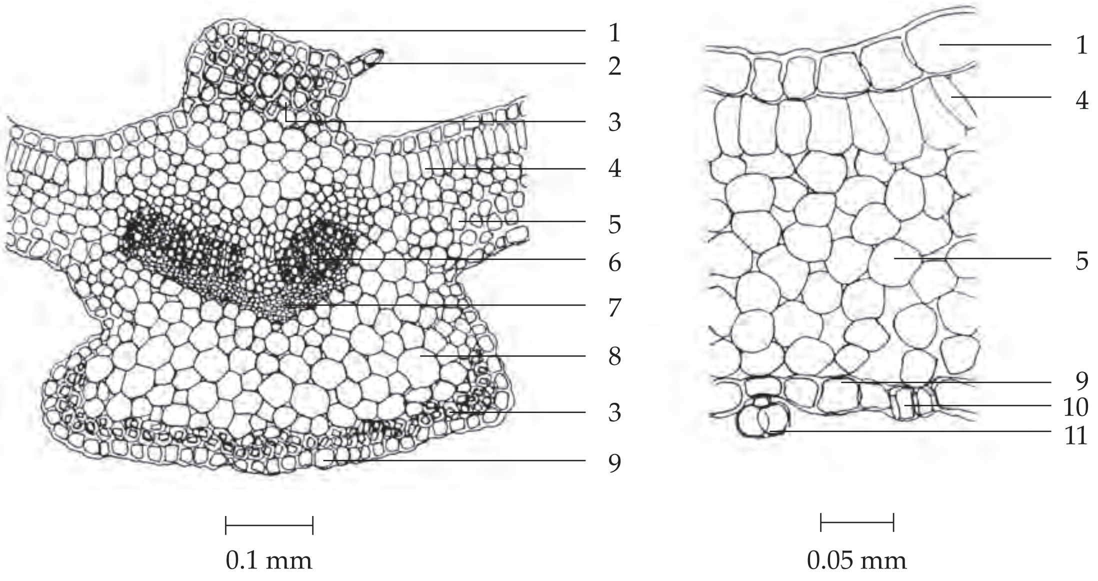
Fig. 1 *Andrographis paniculata* (Burm. f.) Nees
1. aerial part 2. flower 3. capsule



Upper Epidermis of the Lamina

Lower Epidermis of the Lamina

Fig. 2a Epidermises of the Leaf of *Andrographis paniculata* (Burm. f.) Nees



Transverse Section of the Midrib

Transverse Section of the Lamina

Fig. 2b Transverse Section of the Leaf of *Andrographis paniculata* (Burm. f.) Nees

1. upper epidermis
2. multicellular trichome
3. collenchyma
4. palisade cell
5. spongy cell
6. xylem
7. phloem
8. parenchyma
9. lower epidermis
10. stoma
11. glandular trichome

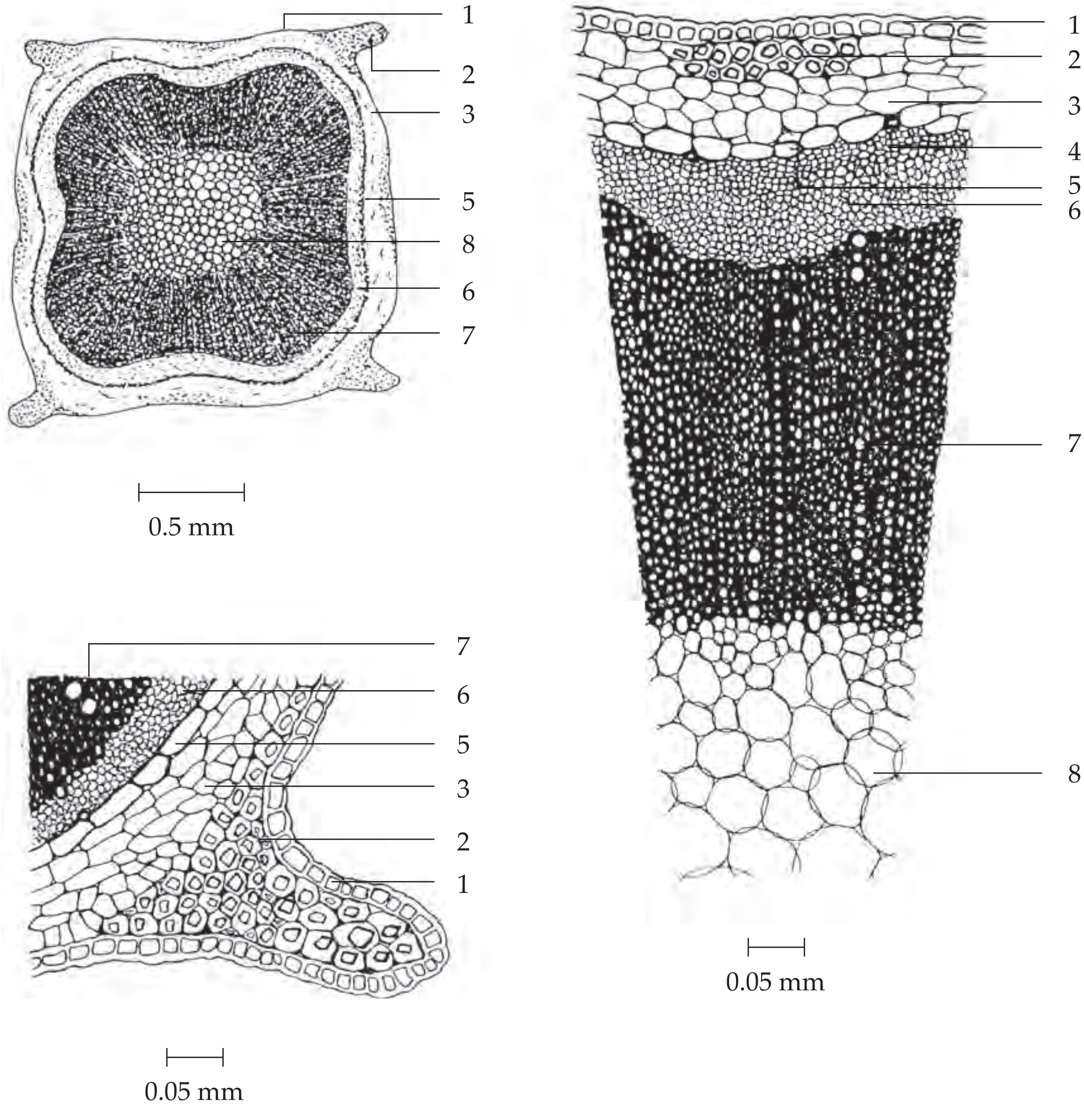


Fig. 2c Transverse Section of the Stem of *Andrographis paniculata* (Burm. f.) Nees

1. epidermis
2. collenchyma
3. cortical parenchyma
4. fibre
5. endodermis
6. phloem
7. xylem
8. pith parenchyma

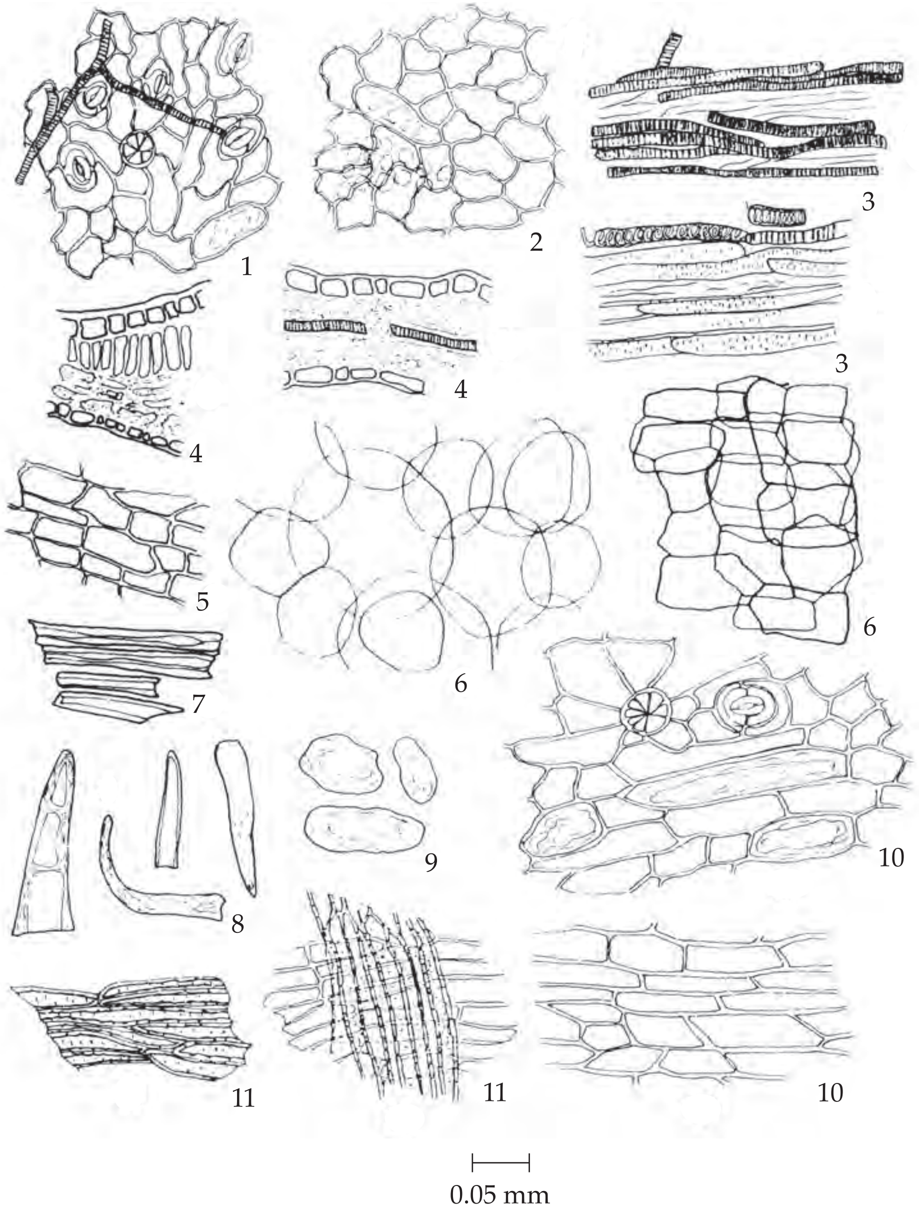


Fig. 2d Powdered Drug of the Leaves and Stems of *Andrographis paniculata* (Burm. f.) Nees

1. lower epidermis showing stomata lithocyst, glandular trichome
2. upper epidermis showing lithocyst, underlying palisade
3. spiral, scalariform and reticulate vessels
4. sectional views of lamina
5. midrib epidermis
6. transverse and longitudinal sections of parenchyma
7. fibre bundle
8. covering trichomes
9. cystolith
10. stem epidermis
11. layer of fibres in fruit wall

Andrographis Herb in powder possesses the diagnostic microscopical characters of the unground drug.

Packaging and storage Andrographis Herb shall be kept in well-closed containers, protected from light, and stored in a dry place. It should be used within 1 year and air-dried every 2 to 3 months.

Identification

A. To about 1 g of the sample, in powder, add 20 ml of *ethanol*, boil in a water-bath and filter. To the filtrate, add 300 mg of *decolorizing charcoal*, stir and filter (solution 1). To 1 ml of solution 1, add 2 drops of a 2 per cent w/v solution of *3,5-dinitrobenzoic acid* in *methanol* and 2 drops of a 5.7 per cent w/v solution of *potassium hydroxide* in *methanol*: a purplish red colour develops.

B. To 1 ml of solution 1, add several drops of a 6.5 per cent w/v solution of *potassium hydroxide* in *ethanol* until it shows a red colour. Set aside for 10 to 15 minutes: the colour is changed to yellow.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 85 volumes of *chloroform* and 15 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate, 5 µl each of the following solutions. Prepare solution (A) by boiling 1 g of the sample, in powder, with 20 ml of *ethanol* on a water-bath for 5 minutes, adding 300 mg of *decolorizing charcoal*, stirring, and filtering. Evaporate the filtrate under reduced pressure to dryness, and dissolve the residue in 1 ml of warm *ethanol (80 per cent)*. For solution (B), dissolve 2 mg of *andrographolide* in 1 ml of *ethanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained with solution (A) shows a quenching spot (hR_f values 52 to 56), corresponding to the andrographolide spots from solution (B) and other four spots of different hR_f values (Table 1); see also Fig. 3. Spray the plate with a 2 per cent w/v solution of *3,5-dinitrobenzoic acid* in *methanol* and then with an excess of a 5.7 per cent w/v solution of *potassium hydroxide* in *methanol*; the spot due to andrographolide is dark violet. Two dark violet spots due to the spot numbers 4 and 9 in Table 1 and other violet and dark violet spots are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Aerial Parts of *Andrographis paniculata* (Burm. f.) Nees

Spot	hR_f Value	Detection	
		UV 254	*3,5-Dinitrobenzoic Acid/ Potassium Hydroxide* in *Methanol*
1	1-5	–	dark violet
2	11-15	quenching	violet
3	18-22	quenching	violet
4*	28-32	–	dark violet
5	49-51	–	violet
6**	52-56	quenching	dark violet
7	57-59	quenching	violet
8	66-68	–	violet
9***	69-71	quenching	dark violet

*neoandrographolide
**andrographolide
*** dehydroandrographolide

Loss on drying Not more than 11.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Ethanol (85 per cent)-soluble extractive Not less than 13.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 18.0 per cent w/w (Appendix 7.12).

Total lactones content Not less than 6.0 per cent w/w of total lactones, calculated as andrographolide, when determined by the following method.

Procedure Place about 1 g of Andrographis Herb in *fine powder*, accurately weighed, in a 100-ml round-bottomed flask, add 50 ml of *ethanol (85 per cent)*, reflux in a water-bath for 2 hours, and filter. Wash the marc with sufficient amount of *ethanol (85 per cent)* until the last washing is almost colourless. Combine the washings and the filtrate and allow to cool. Add 1 ml of *basic lead acetate TS*, set aside for 15 minutes, filter, and wash the precipitate with *ethanol* until the last washing is no longer green. Combine the washings and the filtrate, add dropwise with swirling 1 ml of a 25 per cent w/v solution of *sodium sulfate* and mix well. Set aside for 1 hour, add 500 mg of *decolorizing charcoal*, and reflux in a water-bath for 10 minutes. Filter through the Büchner funnel containing 500 mg of *decolorizing charcoal* and wash with three 2-ml portions of hot *ethanol*. Combine the washings and the filtrate, add 20 ml of *distilled water,* allow to cool, and neutralize with 0.1 M *sodium hydroxide*, using *phenolphthalein TS* as indicator. Add 5.0 ml of 0.1 M *sodium hydroxide VS*, reflux in a water-bath for 30 minutes, allow to cool, and titrate with 0.05 M *hydrochloric acid VS*.

Perform a blank determination (Residual Titrations, Appendix 6.17). Each ml of 0.1 M *sodium hydroxide VS* is equivalent to 35.05 mg of total lactones calculated as andrographolide ($C_{20}H_{30}O_5$).

Andrographolide content Not less than 1.0 per cent w/w of andrographolide ($C_{20}H_{30}O_5$). Carry out the determination as described in the "High-pressure Liquid Chromatography" (Appendix 3.5).

Mobile phase Prepare a mixture of 52 volumes of *distilled water* and 48 volumes of *methanol*. Make adjustments if necessary.

Standard preparations Dissolve an accurately weighed quantity of *andrographolide* in sufficient *methanol* and dilute with *Mobile phase* to obtain a stock solution having a known concentration of about 200 µg per ml. Dilute this solution quantitatively, and stepwise with *Mobile phase* to obtain six solutions having known concentrations of 20, 40, 60, 80, 100, and 140 µg per ml.

Sample preparation Reflux about 400 mg of Andrographis Herb, in *fine powder*, accurately weighed, with 50 ml of a mixture of equal volumes of *dichloromethane* and *methanol* in a water-bath for 30 minutes. Filter and evaporate the filtrate at 50° under reduced pressure to dryness. Dissolve the residue in sufficient *methanol*, transfer quantitatively to a 100-ml volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter through a nylon membrane having a 0.45-µm porosity.

Chromatographic system The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm), (b) a guard column (20 mm × 3.9 mm) packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm), (c) *Mobile phase* at a flow rate of about 1 ml per minute, and (d) an ultraviolet photometer set at 224 nm.

To determine the suitability of the chromatopraphic system, chromatograph *Standard preparation* having a known concentration of 60 µg per ml and record the peak responses as directed under *Procedure* and *Calculation*: the symmetry factor for the andrographolide peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Procedure and **Calculation** Separately inject about 20 µl each of *Standard preparations* into the chromatograph, record the chromatograms and measure the responses for the major peaks. Plot the readings and draw the standard curve of best fit. Inject about 20 µl of *Sample preparation* into the chromatograph, record the chromatogram and measure the response for the major peak. By reference to the standard curve, calculate the content of andrographolide ($C_{20}H_{30}O_5$) in the Andrographis Herb taken.

Dose 0.5 to 2 g four times a day after meals and at bedtime.

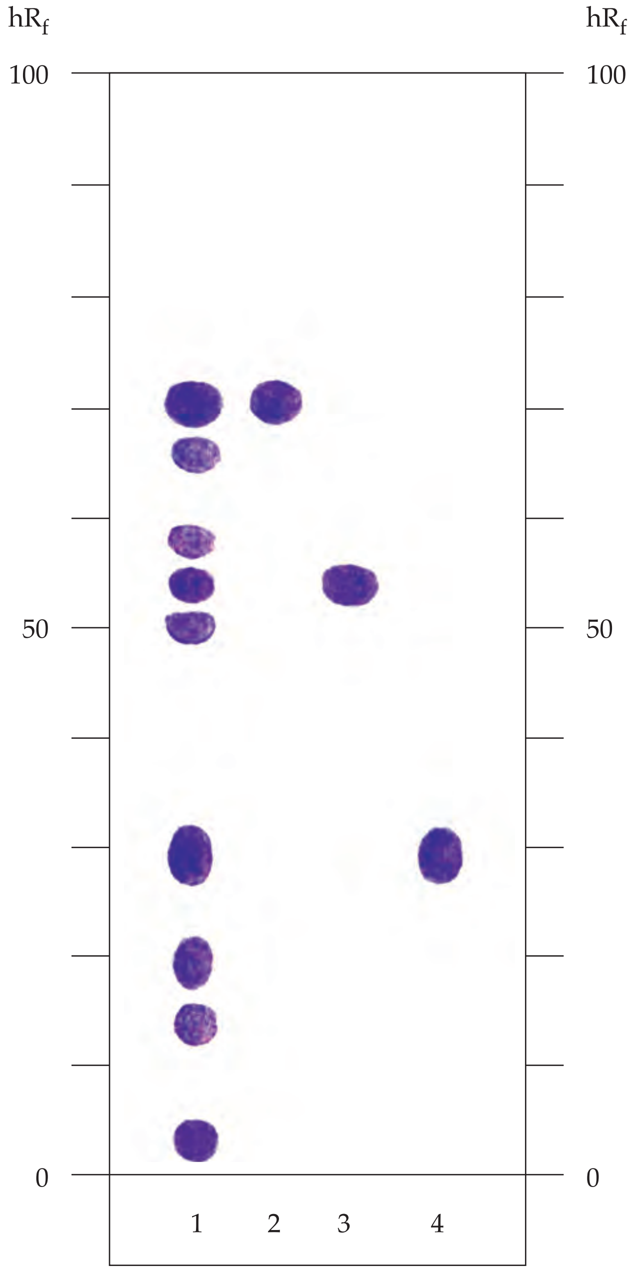


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Aerial Parts of *Andrographis paniculata* (Burm. f.) Nees, Detected with *3,5-Dinitrobenzoic Acid/Potassium Hydroxide* in *Methanol*.

1 = solution (A)
2 = a 0.4 per cent w/v solution of dehydroandrographolide in *ethanol*
3 = solution (B)
4 = a 0.2 per cent w/v solution of neoandrographolide in *ethanol*

ยาแคปซูลฟ้าทะลาย (FA THALAI CAPSULES)

Andrographis Capsules

Category Antidiarrheal, antipyretic, anti-inflammatory in laryngitis.

Andrographis Capsules contain an amount of powdered Andrographis Herb equivalent to not less than 80.0 per cent and not more than 120.0 per cent of the labelled content of total lactones, calculated as andrographolide ($C_{20}H_{30}O_5$).

Strengths available 250, 300, 350, and 500 mg (powder).

Dose Antidiarrheal: 0.5 to 2 g four times a day after meals and at bedtime.
Laryngitis: 0.75 to 1.5 g four times a day after meals and at bedtime.

Warning

1. It should not be used for more than 2 days as antidiarrheal and for more than 7 days as anti-inflammatory in laryngitis.
2. Concomitant use with anticoagulants, antiplatelets and anti-hypertensives should be avoided.

Packaging and storage Andrographis Capsules shall be kept in tightly closed containers, protected from light, and stored in a cool and dry place.

Labelling The label on the container states (1) the equivalent amount of total lactones, calculated as andrographolide; (2) expiration date.

Identification

1. The capsule contents exhibit diagnostic structures of the powdered drug described under *Andrographis Herb*.
2. The capsule contents comply with the tests for Identification A, B and C described under *Andrographis Herb*.

Loss on drying Of the capsule contents, not more than 11.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Microbial limit Comply with the requirements for Category 4 in the “Limits for Microbial Contamination” (Appendix 10.5).

Andrographolide content Not less than 1.0 per cent w/w of andrographolide ($C_{20}H_{30}O_5$). Carry out the determination as described in the “High-pressure Liquid Chromatography” (Appendix 3.5).

Mobile phase Prepare a mixture of 52 volumes of *distilled water* and 48 volumes of *methanol*. Make adjustments if necessary.

Standard preparations Dissolve an accurately weighed quantity of *andrographolide* in sufficient *methanol* and dilute with *Mobile phase* to obtain a stock solution having a known concentration of about 200 µg per ml. Dilute this solution quantitatively, and stepwise with *Mobile phase* to obtain six solutions having known concentrations of 20, 40, 60, 80, 100, and 140 µg per ml.

Assay preparation Remove, as completely as possible, the contents of not less than 20 Andrographis Capsules, and grind to *fine powder*. Transfer about 400 mg, accurately weighed, to a 100-ml round-bottomed flask. Add 50 ml of a mixture of equal volumes of *dichloromethane* and *methanol*, reflux in a water-bath for 30 minutes, and filter. Evaporate the filtrate at 50° under reduced pressure to dryness and dissolve the residue in sufficient *methanol*. Transfer quantitatively to a 100-ml volumetric flask, dilute with *Mobile phase* to volume and mix. Filter through a nylon membrane having a 0.45-μm porosity.

Chromatographic system The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 3.9 mm) packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 μm), (b) a guard column (20 mm × 3.9 mm) packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 μm), (c) *Mobile phase* at a flow rate of about 1 ml per minute, and (d) an ultraviolet photometer set at 224 nm.

To determine the suitability of the chromatopraphic system, chromatograph *Standard preparation* having a known concentration of 60 μg per ml and record the peak responses as directed under *Procedure* and *Calculation*: the symmetry factor for the andrographolide peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Procedure and **Calculation** Separately inject about 20 μl each of *Standard preparations* into the chromatograph, record the chromatograms and measure the responses for the major peaks. Plot the readings and draw the standard curve of best fit. Inject about 20 μl of *Assay preparation* into the chromatograph, record the chromatogram and measure the response for the major peak. By reference to the standard curve, calculate the content of andrographolide ($C_{20}H_{30}O_5$) in the portion of the Capsules taken.

Assay Grind the contents of not less than 20 Andrographis Capsules to *fine powder*. Transfer about 1 g, accurately weighed, to a 100-ml round-bottomed flask. Add 50 ml of *ethanol (85 per cent)*, reflux in a water-bath for 2 hours, and filter. Wash the marc with sufficient amount of *ethanol (85 per cent)* until the last washing is almost colourless. Combine the washings and the filtrate and allow to cool. Add 1 ml of *basic lead acetate TS*, set aside for 15 minutes, filter, and wash the precipitate with *ethanol* until the last washing is no longer green. Combine the washings and the filtrate, add dropwise with swirling 1 ml of a 25 per cent w/v solution of *sodium sulfate* and mix well. Set aside for 1 hour, add 500 mg of *decolorizing charcoal*, and reflux in a water-bath for 10 minutes. Filter through the Büchner funnel containing 500 mg of *decolorizing charcoal* and wash with three 2-ml portions of hot *ethanol*. Combine the washings and the filtrate, add 20 ml of *distilled water*, allow to cool, and neutralize with 0.1 M *sodium hydroxide*, using *phenolphthalein TS* as indicator. Add 5.0 ml of 0.1 M *sodium hydroxide VS*, reflux in a water-bath for 30 minutes, allow to cool, and titrate with 0.05 M *hydrochloric acid VS*. Perform a blank determination (Residual Titrations, Appendix 6.17). Each ml of 0.1 M *sodium hydroxide V*S is equivalent to 35.05 mg of total lactones calculated as andrographolide ($C_{20}H_{30}O_5$).

Other requirements Comply with the requirements described under "Capsules" (Appendix 1.16H).

หอม (HOM)

หอมแกง (HOM KAENG), หอมบัว (HOM BUA), หอมเล็ก (HOM LEK)
Allii Ascalonici Bulbus
Shallot

Category Carminative, expectorant.

Shallot is the air-dried bulb of *Allium ascalonicum* L. (*Allium cepa* var. *ascalonicum* Backer) (Family Amaryllidaceae), Herbarium Specimen Number: DMSC 5172, BKF 156785, Crude Drug Number: DMSc 1143.
(**Note** The samples used are air-dried in the well-ventilated shade until the outermost bulb scales become dried.)

Constituents Shallot contains volatile oil comprising sulfur-containing organic compounds (e.g., dipropyl disulfide, dipropyl trisulfide, methyl propyl trisulfide, diallyl disulfide), flavonoids and their glycosides, saponins, etc.

Description of the plant (Figs. 1a, 1b) Biennial herb, 20 to 50 cm high; subterranean bulbs ovoid, 1.5 to 5 cm long, 1 to 4 cm wide, often consisting of 1 to 3 bulblet(s), usually reddish purple. Leaves simple, alternate, basally clustered, blade terete or cylindrical, 10 to 40 cm long, 0.3 to 1 cm wide, apex acuminate, sheathing at base, shiny green. Inflorescence terminal, scapose, umbel-like, globose, subtended by a few membranous spathe-like bracts; peduncle 30 to 45 cm long; pedicel 1.5 to 2 cm long. Flower bisexual, small; tepals 6, white to greenish white, pink or purple, ovate-lanceolate or oblong-lanceolate, 4 to 9 mm long; stamens 6, biseriate, inner filaments dilated at base; ovary superior, consisting of 3 connated carpels, 3-loculed, ovules numerous in each locule, style 1, filiform. Fruit locucidal capsule, 3-lobed. Seed flat, black.

Description Odour, acrid and characteristic; taste, acrid.

Macroscopical (Fig. 1a) Ovoid bulbs, 1.5 to 5 cm long, 1 to 4 cm wide, often consisting of 1 to 3 bulblet(s), reddish purple.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the bulb shows upper and lower epidermis layers, mesophyll and vascular bundles. Upper and lower epidermises, small rectangular cells, some of which containing purple pigment. Mesophyll composed of parenchyma cells, some of which containing rod-shaped crystals and oil globules. Vascular bundles, in mesophyll layers, circularly arranged; vessels, annular, spiral and reticulate.

Shallot in powder possesses the diagnostic microscopical characters of the unground drug. Typical rod-shaped crystals in some parenchyma cells and small unbranched vessels are characteristic.

Additional information

1. It is usually harvested after one crop of cultivation (70 to 110 days) to obtain its bulbs although it is a biennial herb.
2. Traditionally, inhaling the vapour or soaking the head with warm water containing the freshly crushed bulbs could alleviate nasal congestion from common cold.

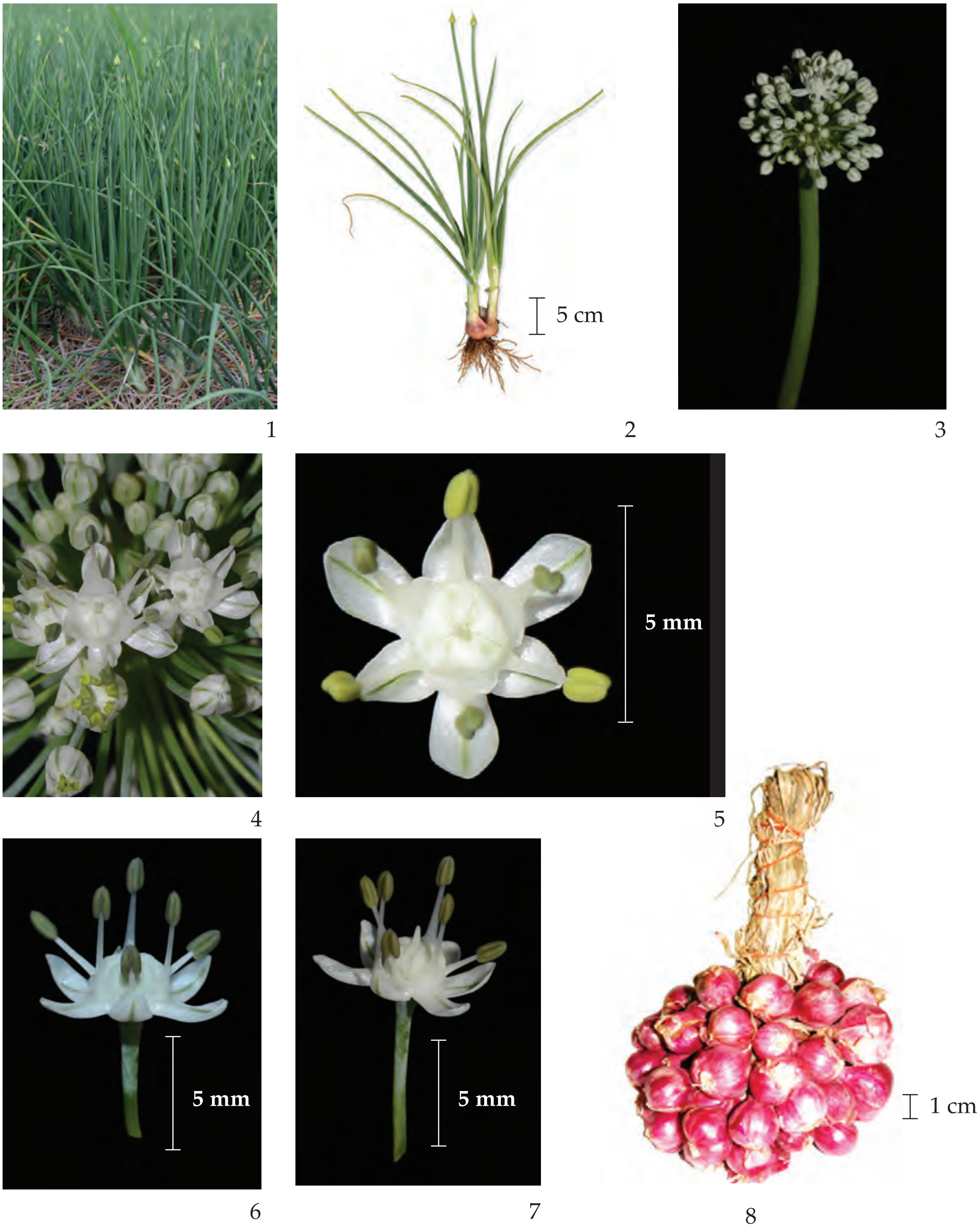


Fig. 1a *Allium ascalonicum* L.
1. cultivated plants 2. habit 3. inflorescence 4. flowers (in different stages)
5. flower (top view) 6. and 7. flower (side view) 8. crude drug

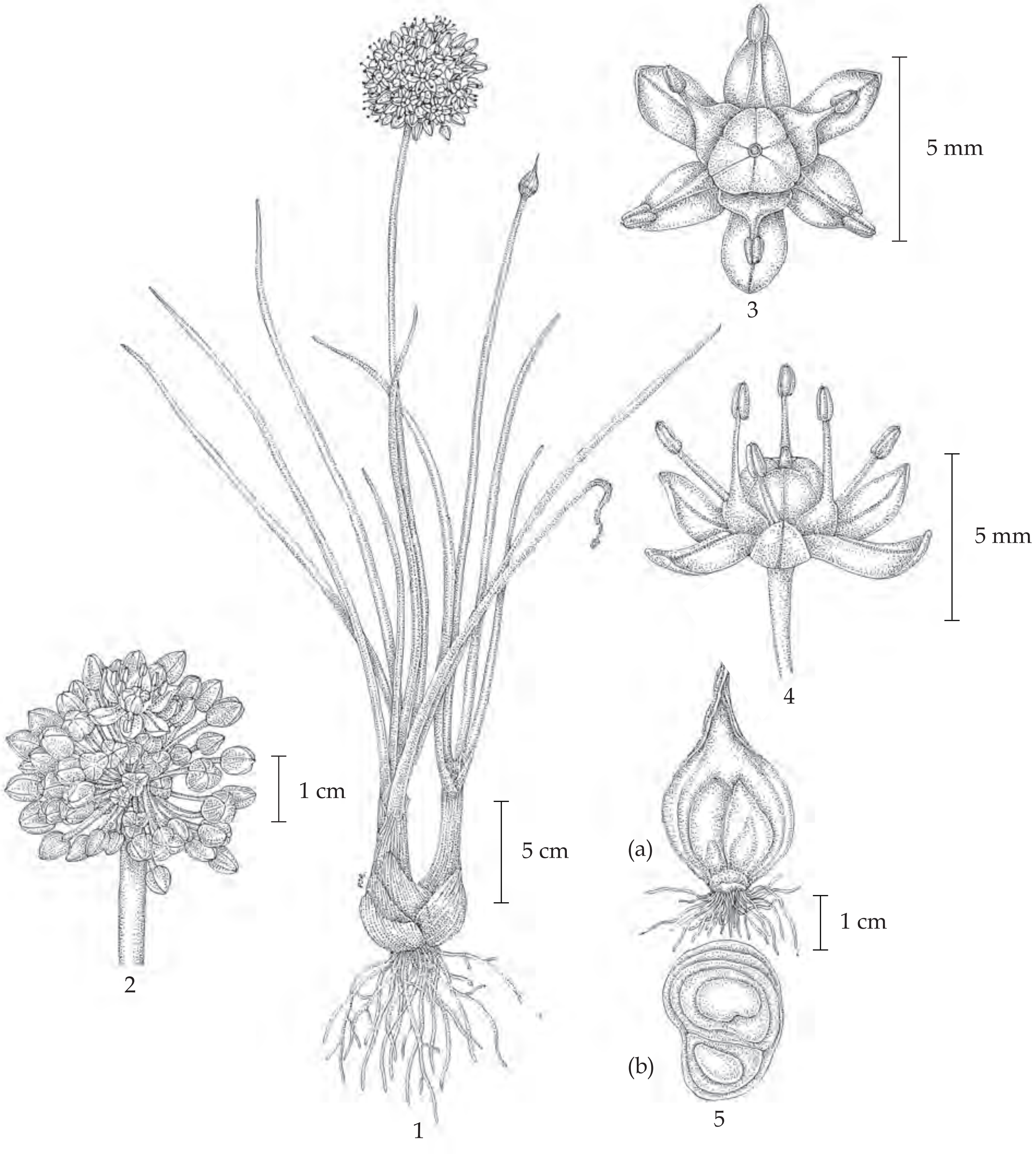


Fig. 1b *Allium ascalonicum* L.
1. habit, showing bulbs, leaves and inflorescences 2. inflorescence 3. flower (top view) 4. flowers (side view) 5. bulbs in longitudinal view (a) and sectional view (b)

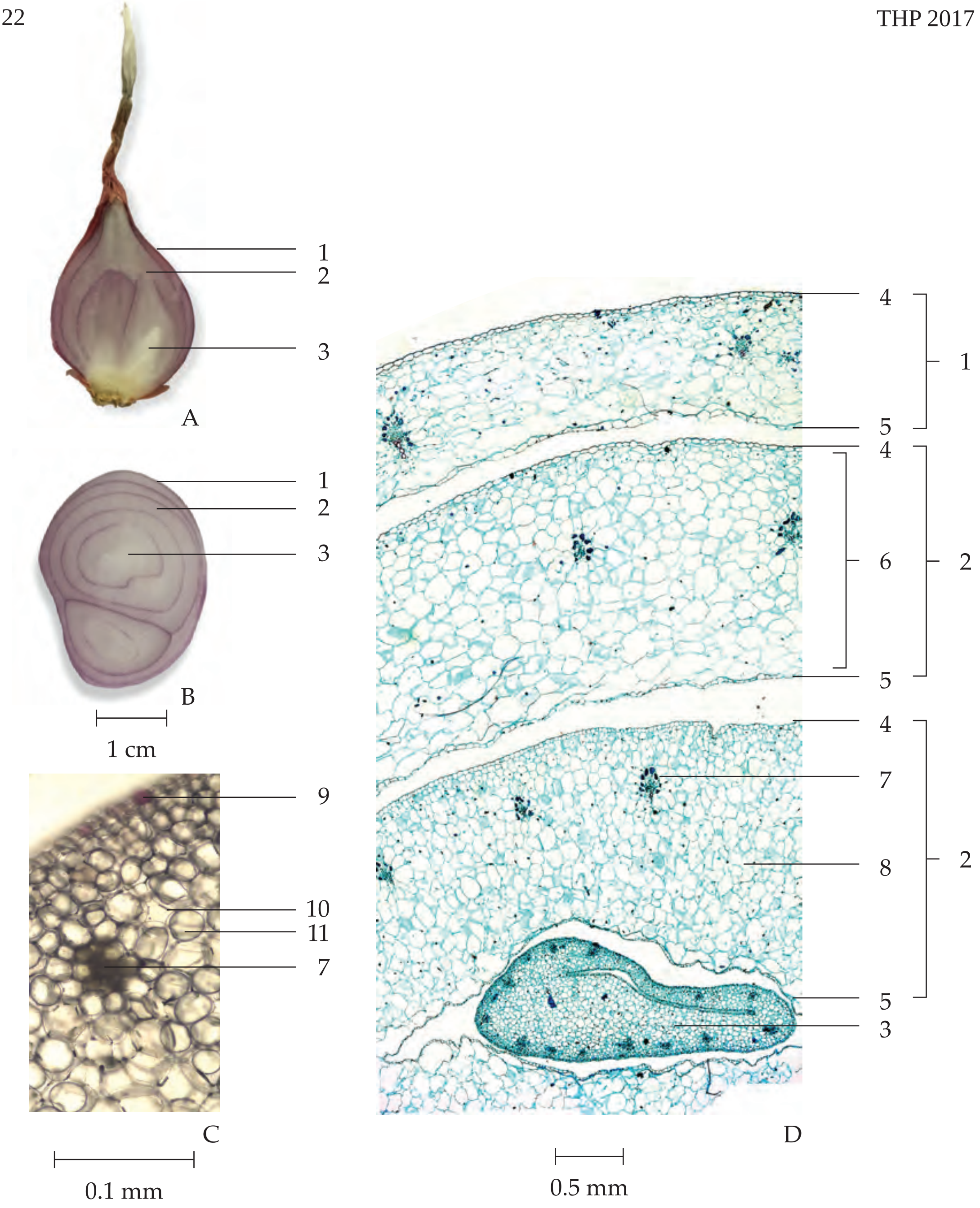


Fig. 2a Section of the Bulb of *Allium ascalonicum* L.

A. Longitudinal Section
B. Transverse Section
C. Photomicrograph of Transverse Section of the Outer Scale Leaf
D. Photomicrograph of Transverse Section of Part of the Bulb, Stained with Safranin-Fast Green

1. outer scale leaf
2. inner scale leaf
3. young shoot
4. lower epidermis
5. upper epidermis
6. mesophyll
7. vascular bundle
8. parenchyma
9. purple pigment
10. rod-shaped crystal
11. oil globule

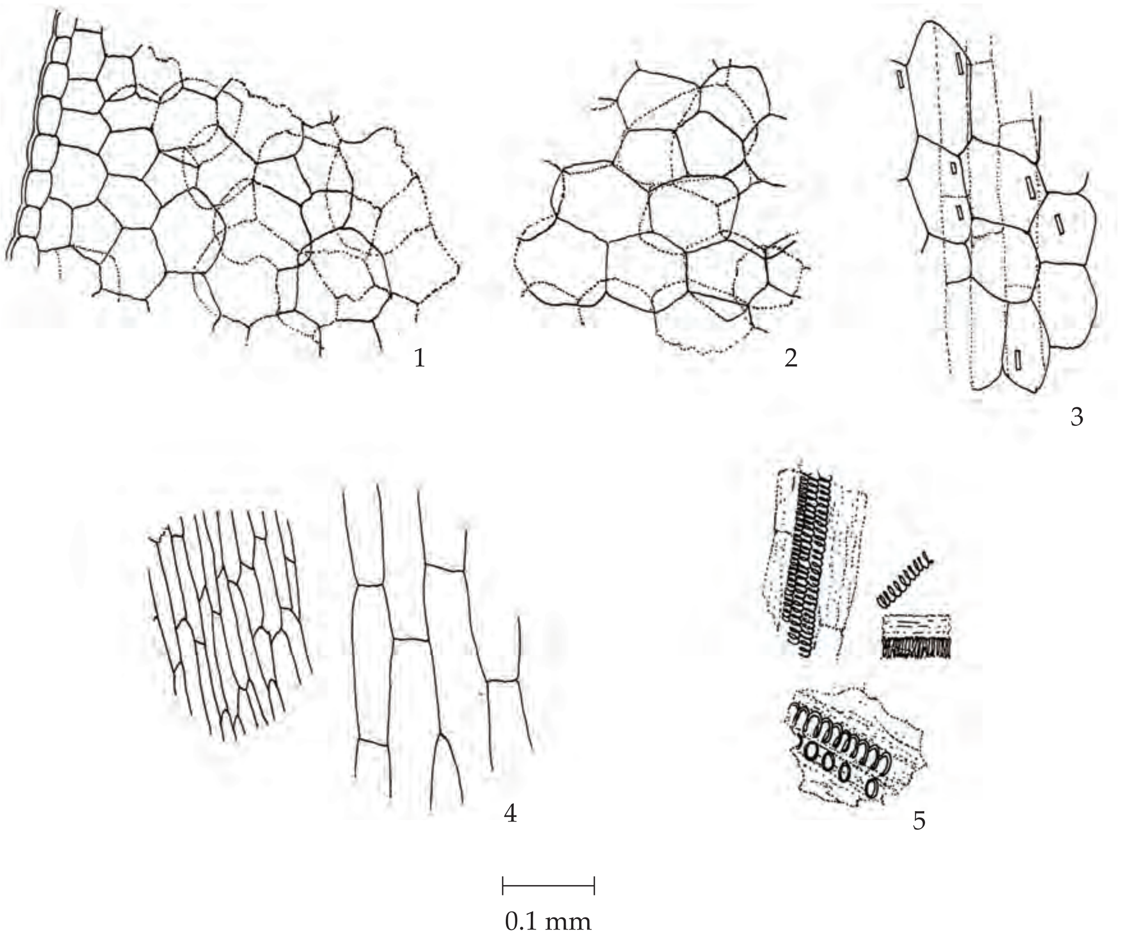


Fig. 2b Line Drawings of Powdered Drug of the Dried Bulbs of *Allium ascalonicum* L.

1. epidermis associated with parenchyma in sectional view
2. parenchyma
3. parenchyma, some containing rod-shaped crystals
4. epidermis in surface view
5. annular, spiral and reticulate vessels

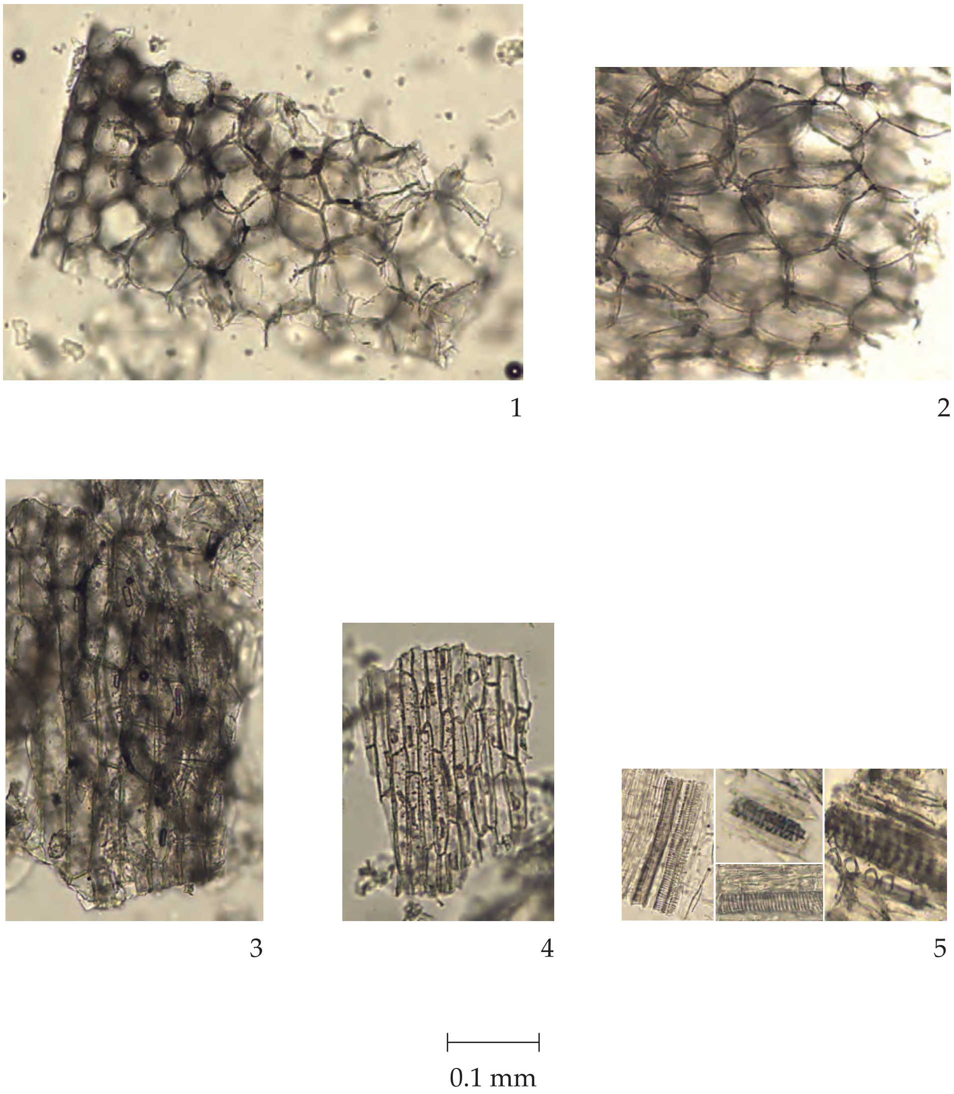


Fig. 2c Photomicrographs of Powdered Drug of the Dried Bulbs of *Allium ascalonicum* L.

1. epidermis associated with parenchyma in sectional view
2. parenchyma
3. parenchyma, some containing rod-shaped crystals
4. epidermis in surface view
5. annular, spiral and reticulate vessels

Packaging and storage Shallot shall be stored in a well-ventilated, dry place, protected from light; under these conditions it may be stored for 6 months after harvesting.

Identification

A. Shake 2 g of the crushed, peeled sample with 25 ml of *methanol* for 5 minutes and filter. Dilute 0.5 ml of the filtrate to 2 ml with *methanol*, add 2 drops of *ninhydrin TS* and immerse in a water-bath for a few minutes: a violet-blue colour appears.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 30 volumes of 1-*butanol*, 10 volumes of *water*, 10 volumes of 1-*propanol*, and 10 volumes of *glacial acetic acid* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply to the plate, 4 µl of the test solution prepared by refluxing 30 g of the crushed, peeled sample, with 50 ml of *ethanol* on a water-bath for 30 minutes. Cool, filter, evaporate the filtrate to dryness, and dissolve the residue in 1.0 ml of *ethanol*. After removal of the plate, allow it to dry in air. Spray the plate with *ethanolic ninhydrin TS* and heat at 110° for 10 minutes. Several spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Bulbs of *Allium ascalonicum* L.

Spot	hR_f Value	Detection
		Ethanolic Ninhydrin TS
1	5-8	orange
2	9-11	orange
3	18-20	pink
4	21-26	pink
5	26-29	pale yellow
6	32-36	reddish brown
7	36-40	reddish brown
8	44-49	reddish brown
9	50-53	reddish brown

Water Not more than 87.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Total ash Not more than 1.0 per cent w/w (Appendix 7.7).

Dose 15 to 30 g as a decoction.

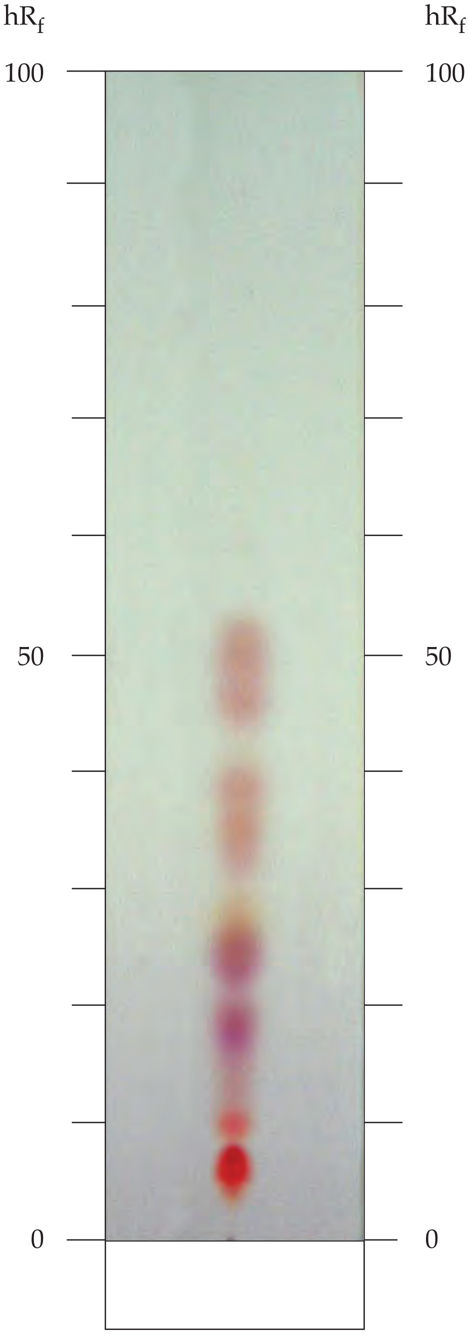


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Bulbs of *Allium ascalonicum* L., Detected with *Ethanolic Ninhydrin TS*

กะเพราแดง (KAPHRAO DAENG)

Ocimi Tenuiflori Folium
Holy Basil Leaf, Ocimum Tenuiflorum Leaf

Category Pharmaceutic aid (flavouring agent), carminative.

Holy Basil Leaf is the dried leaf of the red variety of *Ocimum tenuiflorum* L. [*O. sanctum* L., *O. monochorum* L., *O. album* Blanco, *O. flexuosum* Blanco, *O. nelsonii* Zippelius ex Spanoghe, *O. virgatum* Blanco, *O. brachiatum* Hasskarl, *Moschosma tenuiflorum* (L.) Heynh.] (Family Labiatae), Herbarium Specimen Number: DMSC 43, 418.

Constituents Holy Basil Leaf contains up to about 1.7 per cent of volatile oil, of which methyl eugenol, caryophyllene and methyl chavicol are its major components. It also contains β-carotene, sterols and fatty acids.

Description of the plant (Fig. 1) Herb 30 to 60 cm, erect, aromatic, much branched, twigs usually purplish, subquadrangular, clothed with soft spreading hairs, often woody at the base. Leaves simple, opposite, elliptic oblong or elliptic, 3 to 6 cm long, 1 to 2.5 cm wide, apex acute or obtuse, base acute or obtuse, margin entire or serrate, membranous, pubescent on both surfaces, low surface more hairy on midrib; petiole, 1 to 3 cm long, slender, hairy. Flower in terminal raceme of verticillasters 8 to 10 cm long; bracts 2 to 3 mm long, broadly ovate with acuminate apex, ciliated; pedicel 3 to 4.5 mm, slender, pubescent. Flower: calyx tubular-bilabiate, about 2.5 mm (in fruit 3 to 3.5 mm) long, glabrous inside (pubescent outside), the upper lip broadly obovate, flat, the lower lip 4-toothed, the 2 central with long slender awns which project beyond the upper lip and are much curved upwards; corolla purplish, tubular-bilabiate, the upper lip 4 of almost equal lobes, pubescent on the back, lower lip, the longest lobe and ascending; stamens 4, epipetalous, exserted, filaments slender, the upper pair with a small bearded basal appendage. Fruit small, 4-seeded nutlets; seed ovoid, brown, nearly smooth, about 1.2 mm long.

Description Odour, aromatic; taste, slightly pungent.

Macroscopical Leaves, wrinkled and broken; entire leaves, elliptic-oblong, 2 to 5 cm long and 1 to 3 cm wide; petiole 1 to 3 cm long, greenish brown.

Microscopical (Figs. 2a, 2b) Transverse section of the leaf shows upper epidermis, a single layer of rectangular cells containing reddish purple matters. Mesophyll consisting of a single layer of palisade parenchyma and several layers of more or less rounded spongy parenchyma, rich in chloroplastids. Through this region, scattered the small vascular bundles. Lower epidermis, a single layer of rectangular cells, no colouring matters, non-glandular and glandular trichomes occur as outgrowths from lower epidermis more abundant than upper epidermis. The non-glandular trichomes are uniseriate, 1 to 7 cells, some of the cells are more or less collapsed. The glandular trichomes have a 1-celled stalk and 2- to 4-celled glandular head. Transverse section through the midrib of the lamina shows the 2 or 3 layers of angular collenchyma underneath the epidermis, parenchyma and collateral vascular bundles. In surface view, the epidermides of upper and lower surfaces are wavy-walled polygonal cells with diacytic stomata.

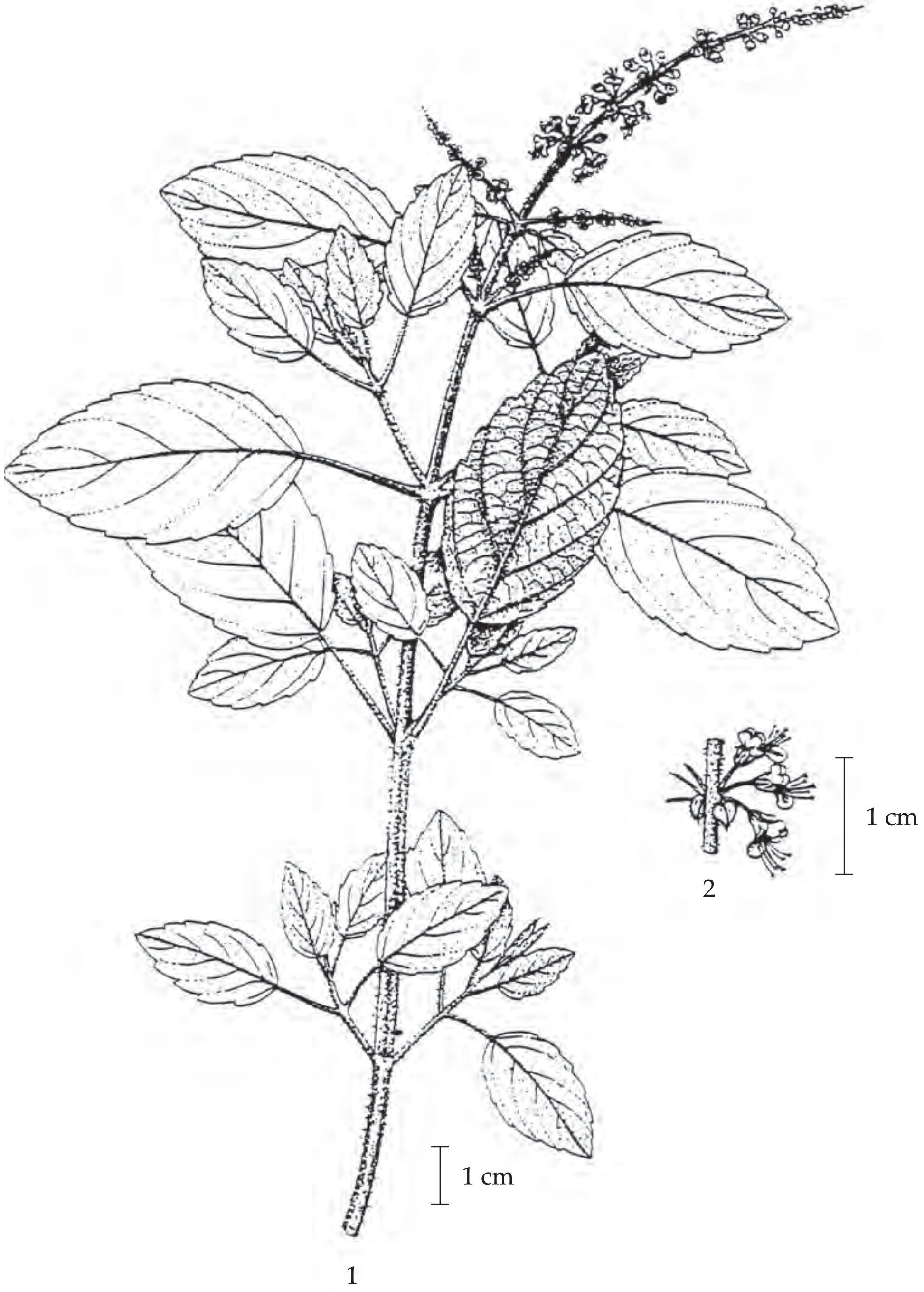


Fig. 1 *Ocimum tenuiflorum* L.
1. twig 2. inflorescence

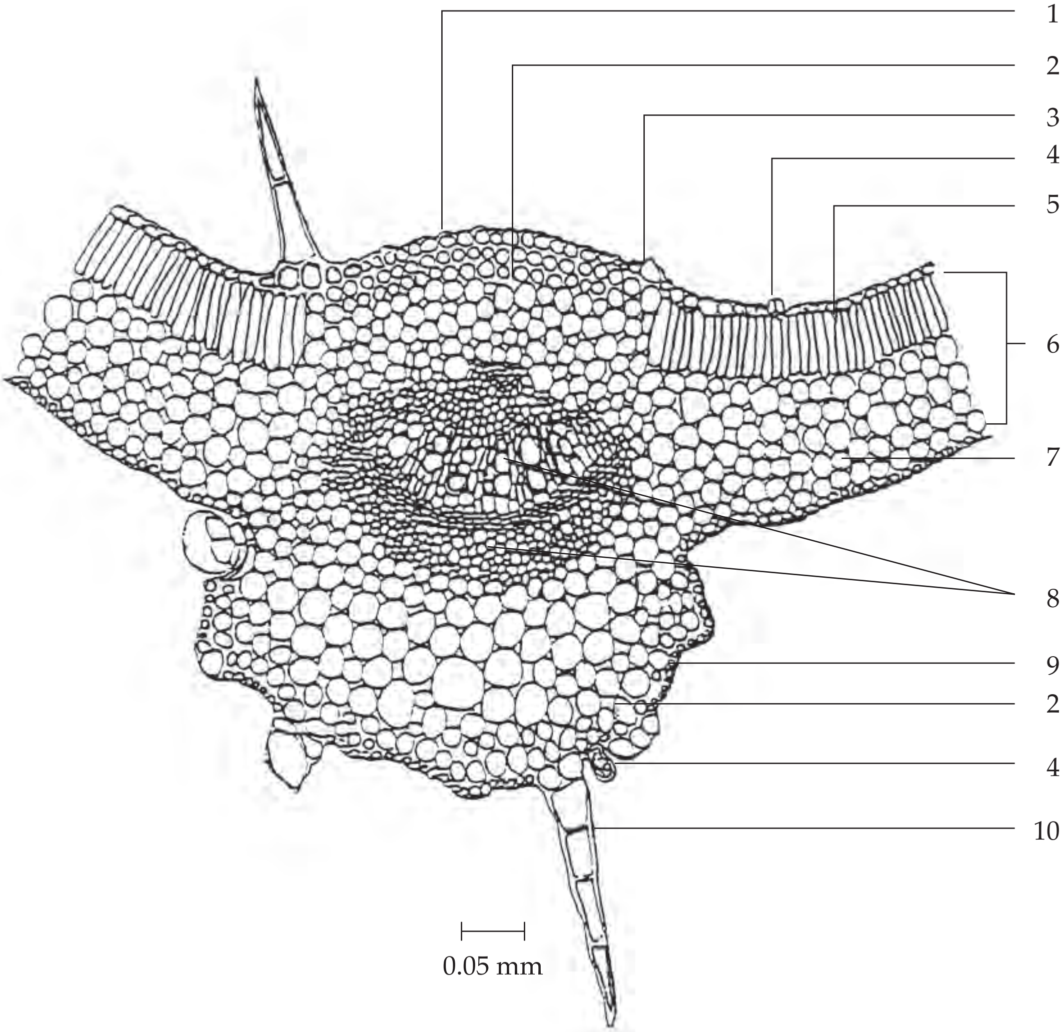


Fig. 2a Transverse Section of the Leaf of *Ocimum tenuiflorum* L.

1. upper epidermis	6. mesophyll
2. collenchyma	7. spongy parenchyma
3. unicellular trichome	8. vascular bundles
4. glandular trichomes	9. lower epidermis
5. palisade parenchyma	10. uniseriate multicellular trichome

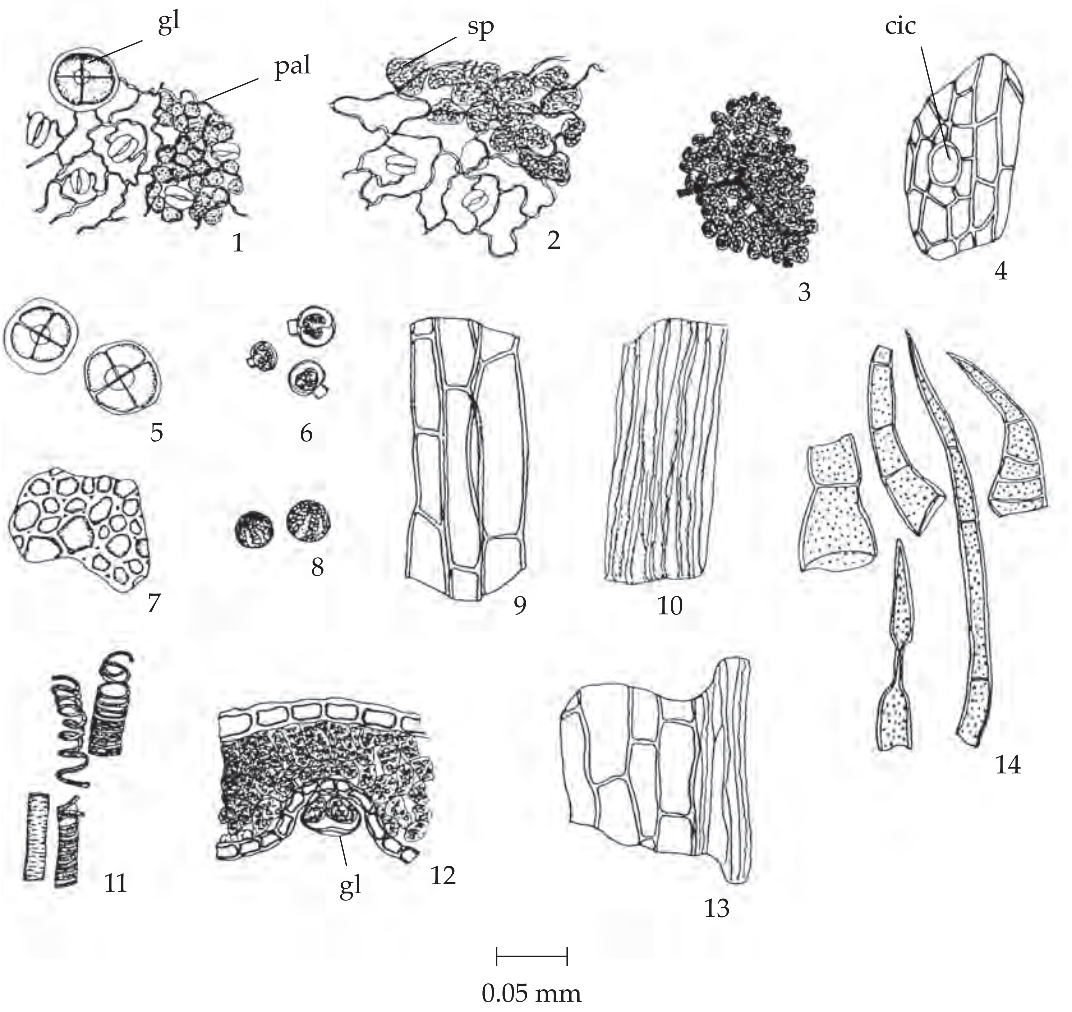


Fig. 2b Powdered Drug of the Leaves of *Ocimum tenuiflorum* L.

1. upper epidermis in surface view with diacytic stomata, glandular trichome and underlying palisade cells
2. lower epidermis with diacytic stomata and underlying spongy cells
3. spongy cells and veinlets
4. epidermis of the petiole in surface view, showing cicatrix
5. 4-celled head glandular trichomes
6. 2-celled head glandular trichomes
7. collenchyma from the petiole
8. pollen grains (rare)
9. parenchyma from stem in longitudinal view (rare)
10. group of lignified fibres
11. group of vessels
12. transverse section of lamina
13. parenchyma and fibres from the petiole
14. part of uniseriate multicellular trichomes showing collapsed cell

(gl = glandular trichome; pal = palisade cell; sp = spongy cells; cic = cicatrix)

Holy Basil Leaf in powder possesses the diagnostic microscopical characters of the unground drug.

Packaging and storage Holy Basil Leaf shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. To 500 mg of the sample, in powder, add 2 ml of *acetic anhydride*, warm on a water-bath for about 2 minutes, shake, and filter. Slowly add 1 ml of *sulfuric acid* to the filtrate to form a layer: a brownish red ring forms at the zone of contact.

B. Add 10 ml of *water* to 500 mg of the sample, in powder, heat for 2 minutes and filter. To 2 ml of the filtrate, add 1 drop of a 5 per cent w/v solution of *iron(III) chloride*: a green precipitate is produced.

C. Add 10 ml of *water* to 500 mg of the sample, in powder, boil for 2 minutes and filter. To 3 ml of the filtrate, add 1 drop of *potassium cupri-tartrate TS* and warm: a brick-red precipitate is produced.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 75 volumes of *chloroform* and 25 volumes of *benzene* as the mobile phase. Apply to the plate, 10 µl of the test solution, prepared by shaking 100 mg of the sample, in powder, with 2 ml of *dichloromethane* for about 15 minutes and filtering. Evaporate the filtrate on a water-bath to dryness and dissolve the residue in 0.5 ml of *toluene*. After removal of the plate, allow it to dry in air and spray with a 10 per cent w/v solution of *phosphomolybdic acid* in *ethanol*. Heat at 105° to 110° for 5 to 10 minutes. Several spots of different colours are observed (Table 1); see also Fig. 3.

Repeat the same procedure on another plate. After removal of the plate, allow it to dry in air. Spray the plate with *anisaldehyde TS*; several spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR$_f$ Values of Components in Dichloromethane Extract of the Leaves of *Ocimum tenuiflorum* L.

Spot	hR$_f$ Value	Detection	
		10 Per Cent W/V Solution of *Phosphomolybdic Acid* in *Ethanol*	*Anisaldehyde TS*
1	5-10	blue	violet
2	15-22	green	–
3	23-28	blue	pink
4	29-32	–	blue
5	33-35	green	blue
6	36-38	–	blue
7	39-45	–	green
8	50-55	blue	pink
9	56-65	blue	pale blue
10	66-70	–	green
11	75-80	blue	pink

Water Not more than 14.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Sulfated ash Not more than 20.0 per cent w/w (Appendix 5.3).

Ethanol-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 20.0 per cent w/w (Appendix 7.12).

Chloroform-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12H).

Volatile oil Not less than 1.0 per cent v/w (Appendix 7.3 H). Use 50 g of hand-crushed leaves, accurately weighed. Use 1000 ml of *water* as the distillation liquid and a 2.5-l round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

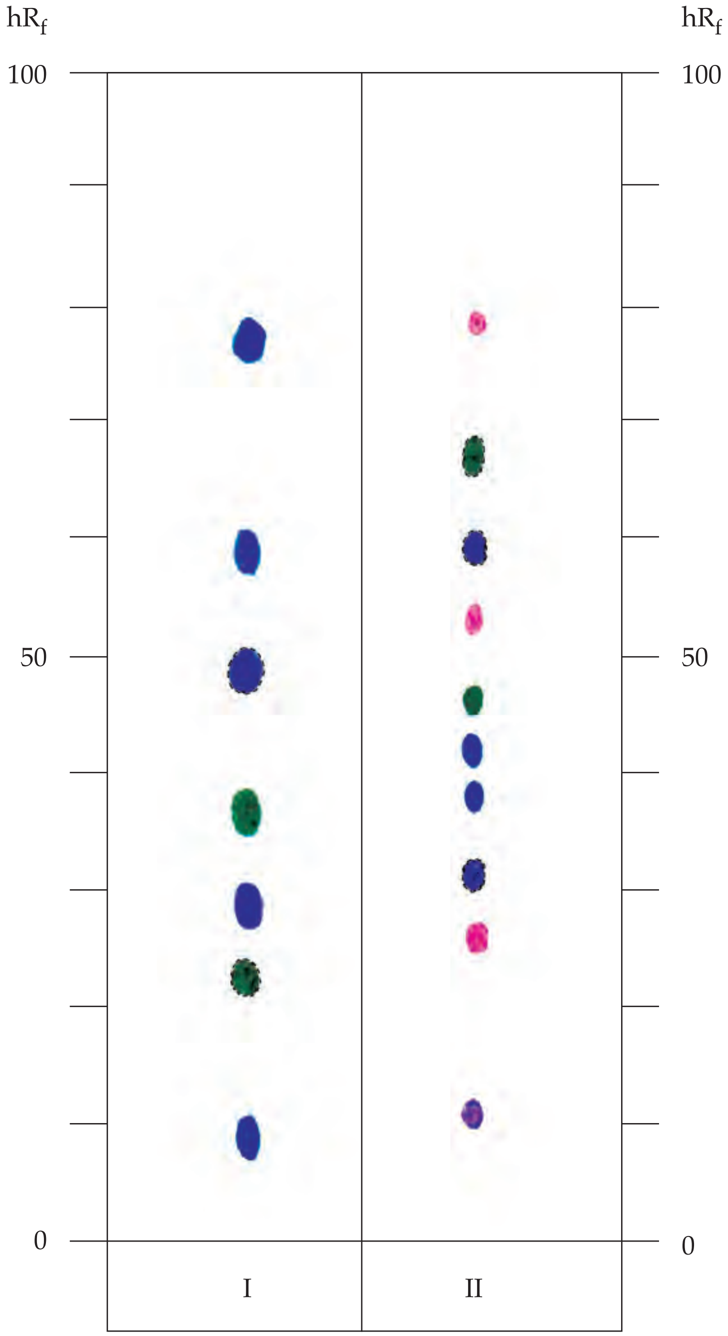


Fig. 3 Thin-layer Chromatogram of Dichloromethane Extract of the Leaves of *Ocimum tenuiflorum* L.

I = detection with a 10 per cent w/v solution of *phosphomolybdic acid* in *ethanol*
II = detection with *anisaldehyde TS*
◌ = spots developed in some samples

ขมิ้นชัน (KHAMIN CHAN)

Curcumae Longae Rhizoma, Curcumae Domesticae Rhizoma
Turmeric

Synonyms Curcuma, Yellow Root, Indian Saffron

Category Stomachic, carminative, pharmaceutic aid (colouring agent), astringent.

Turmeric is the dried rhizome of *Curcuma longa* L. (*C. domestica* Valeton) (Family Zingiberaceae), Herbarium Specimen Number: DMSC 31, 1410, 1458.

Constituents Turmeric contains yellow volatile oil, of which turmerone and zingiberene are its major components, and curcuminoids, of which curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin are its major components.

Description of the plant (Fig. 1) Perennial herb with a thick, ellipsoid-ovate rhizome, orange inside, giving rise to short blunt daughter rhizomes called fingers, leafy shoots to 1 m tall bearing 6 to 10 leaves. Leaves simple, glabrous, lamina, elliptic, oblong-elliptic or lanceolate, 30 to 45 cm long, 10 to 15 cm wide, apex acuminate, base narrow; petiole as long as lamina (rather abruptly broadened to leaf sheath, forming a pseudostem). Inflorescence scape from the apex of the rhizome; peduncle 15 cm long or more; spike 10 to 15 cm long and 5 to 7 cm in diameter; bract, white or white with green, 5 to 6 cm long, each subtending flowers; bracteoles thin, pale green and tinged with pink, elliptic to ovate, up to 3.5 cm long. Flowers as long as the bracts; calyx whitish tubular, unilateral split, unequally toothed; corolla white, tubular at base, upper half cup-shaped with 3 unequal lobes inserted on edge of cup lip; lateral staminode petaloid, oblong, folder under the dorsal petal, staminode and lip creamy-white with yellow median band, filament united to another about the middle of the pollen sac, spurred at base; ovary trilocular. Fruit capsule, globose to ellipsoid. Seed arillate.

Description

Macroscopical Dried rhizome occurs as an ovate, oblong or pear-shaped of round turmeric; cylindrical and often short-branched of long turmeric; the round about half as broad as long, the long 2 to 5 cm long and 1 to 2 cm thick; externally yellowish to yellowish brown, with root scars and annulations, the latter from the scars of leaf bases; fracture horny; internally orange-yellow to orange, waxy, showing a cortex separated from a central cylinder (about twice as broad as cortex) by a distinct endodermis; in both cortex and central cylinder, scattered bundles are seen.

Microscopical (Figs. 2a, 2b) Transverse section of the rhizome shows epidermis consisting of a layer of rectangular cells; covering trichomes, unicellular, up to 280 μm long. Hypodermis composed of 3 to 6 layers in the mature rhizome, but absent in the younger. Cork, 4 to 6 layers of rectangular cells. Cortex composed of thin-walled parenchymatous cells containing numerous starch grains, yellowish oil droplets and yellow colouring matter occasionally seen; starch grains, simple, flattened, rounded to oval or irregular in outline, very faint transverse striations could be seen in some granules. Endodermis, a layer of thin-walled cells. Stele, thin-walled parenchymatous cells containing numerous starch grains, yellowish oil droplets and yellow colouring matter. Fibrovascular bundles, non-lignified walled cells, scattered in cortex and stele; vessels, spiral, scalariform and reticulate.

Turmeric in powder possesses the diagnostic microscopical characters of the unground drug.

Fig. 1 *Curcuma longa* L.
1. habit 2. inflorescence 3. rhizome

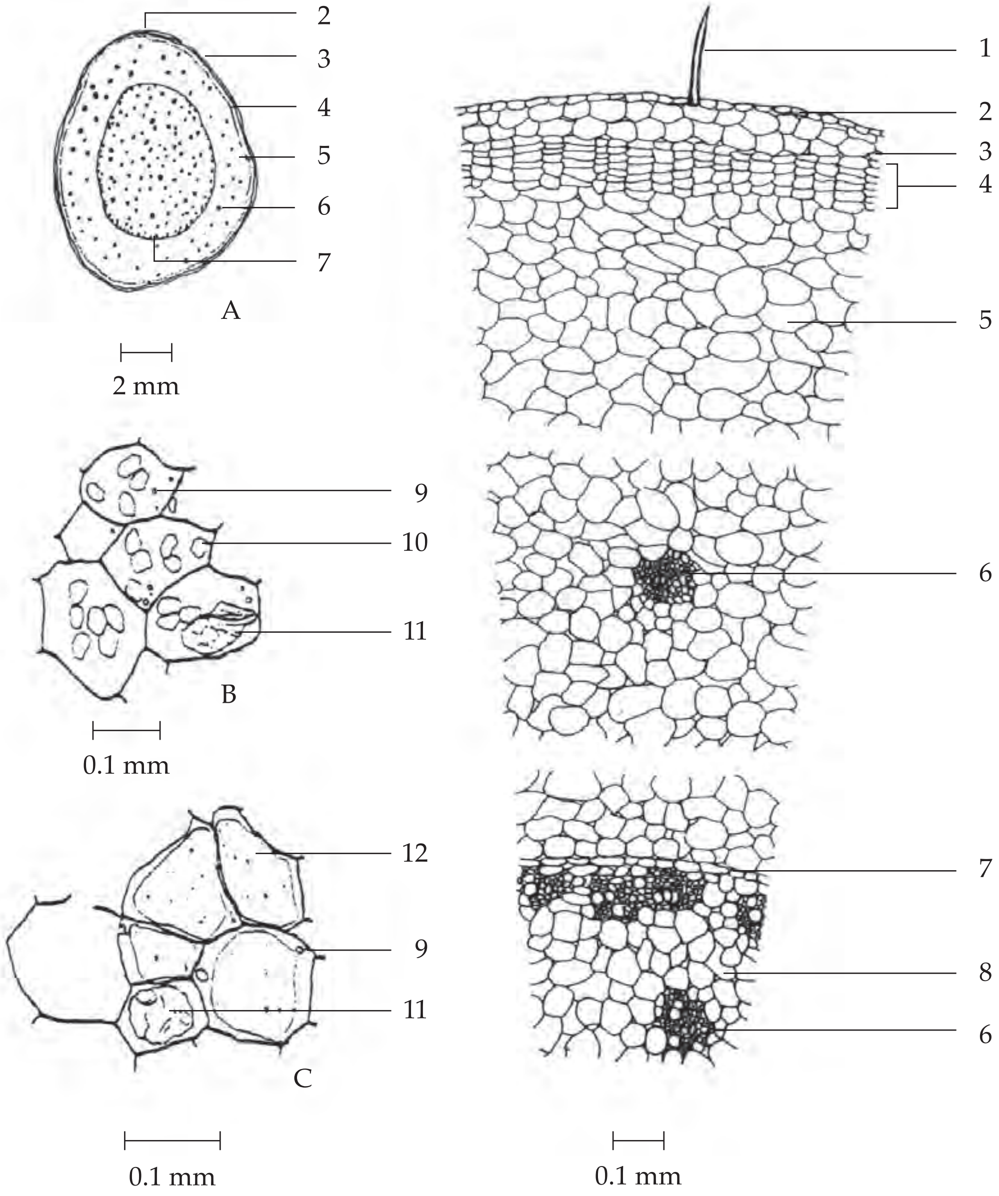
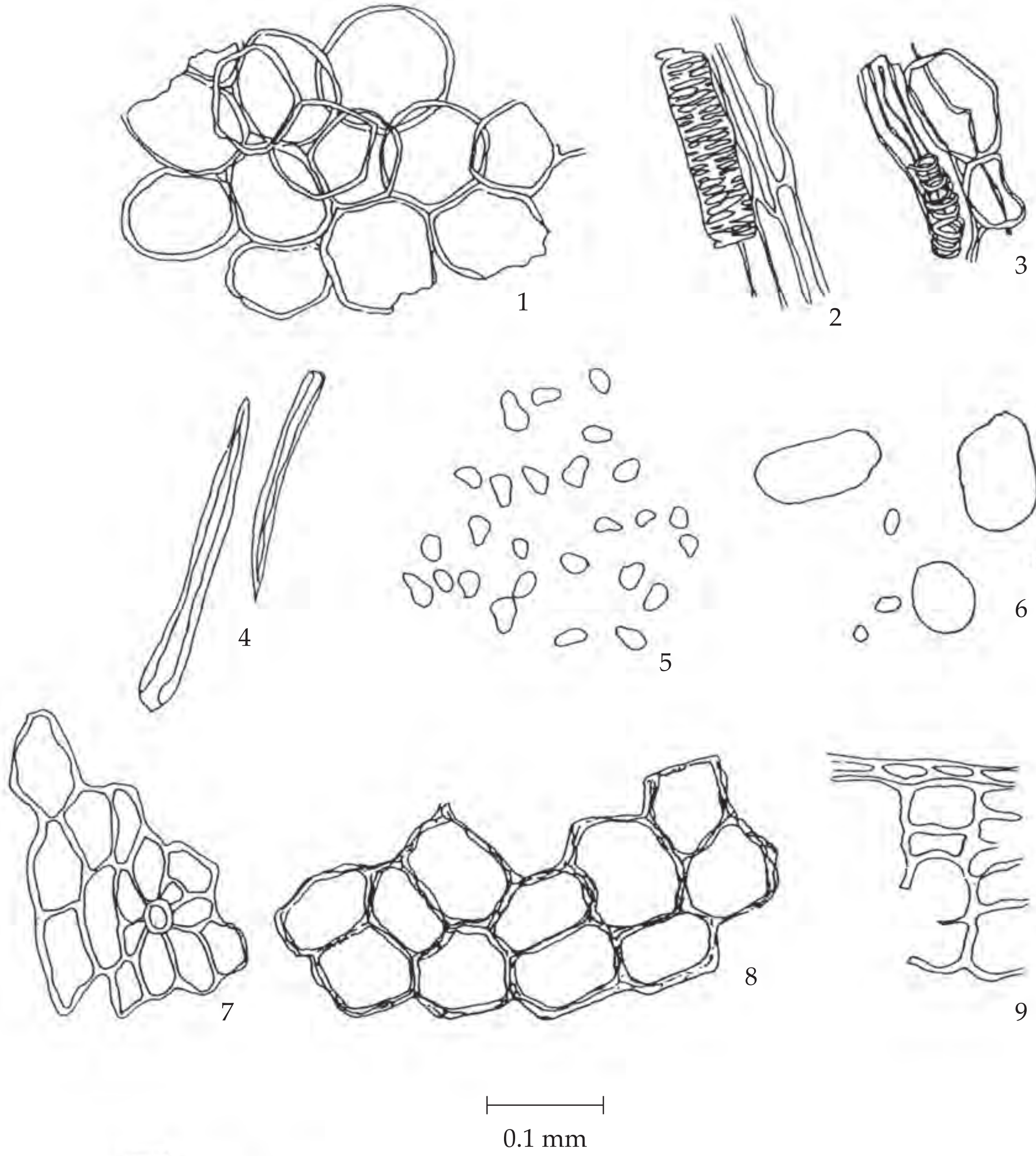


Fig. 2a Transverse Section of the Rhizome of *Curcuma longa* L.
A. Diagram
B. Parenchyma of Untreated Rhizome
C. Parenchyma of Steam-treated Rhizome
1. unicellular covering trichome
2. epidermis
3. hypodermis
4. cork layers
5. cortical parenchyma containing starch granules
6. vascular bundles
7. endodermis
8. stele parenchyma containing starch granules
9. oil droplet
10. starch granule
11. orange-yellow colouring matter
12. yellow gelatinized starch mass



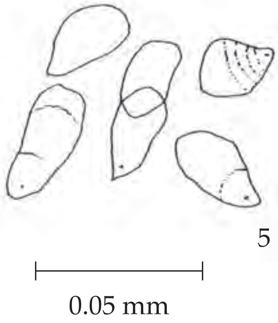


Fig. 2b Powdered Drug of the Rhizomes of *Curcuma longa* L.

1. parenchyma
2. reticulate vessel
3. spiral vessel
4. unicellular trichomes
5. starch granules
6. altered starch grains
7. epidermis in surface view
8. cork in surface view
9. epidermis and hypodermis in sectional view

Packaging and storage Turmeric shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Extract 10 mg of the sample, in powder, with 2 ml of *acetic anhydride*, add a few drops of *sulfuric acid* and observe under ultraviolet light (366 nm): the solution shows blood-red colour.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 49 volumes of *benzene*, 49 volumes of *chloroform* and 2 volumes of *ethanol* as the mobile phase and allowing the solvent front to ascend 17 cm above the line of application. Apply separately to the plate, 5 µl each of the following two solutions. Prepare solution (A) by placing 1 g of the sample, in powder, in a stoppered test-tube, adding 3 ml of *methanol* and shaking for a few minutes. Set aside for 1 hour and filter. For solution (B) dissolve 1 mg of *curcumin* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (366 nm), locating the spots. The chromatogram obtained with solution (A) shows a yellow-brown spot (hR_f value 28 to 34), corresponding to the curcumin spot from solution (B). Other two yellow-brown spots correspond in hR_f values to the spot numbers 2 and 3. Several spots of higher and lower hR_f values are observed (Table 1); see also Fig. 3. Spray the plate with a 10 per cent w/v solution of *phosphomolybdic acid* in *ethanol* and heat at 105° for 5 minutes; the spot due to curcumin is orange-brown. The spots due to those of numbers 2, 3, 10, 14 and 15 in Table 1 are orange, orange-brown, blue, blue, and blue, respectively. Other spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Rhizomes of *Curcuma longa* L.

Spot	hR_f Value	Detection	
		UV 366	*Phosphomolybdic Acid*
1	5-8	light brown	brown
2	11-15	yellow-brown	orange
3	17-20	yellow-brown	orange-brown
4	21-24	blue-green	blue
5	28-34	yellow-brown	orange-brown
6	35-38	blue-green	blue
7	39-42	yellow	pale yellow
8	44-46	–	blue
9	48-51	–	blue
10	52-53	–	blue
11	57-60	–	blue
12	62-66	–	blue
13	71-74	–	blue
14	80-85	–	blue
15	87-90	–	blue

2 = bisdesmethoxycurcumin
3 = desmethoxycurcumin
5 = *curcumin*
10 = curcumol
14 = *dl*-turmerone
15 = ar-curcumene (ar = aromatic)

Water Not more than 10.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 8.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 10.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 9.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 6.0 per cent v/w (Appendix 7.3H). Use 10 g, in *fine powder*, accurately weighed. Use 100 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube. Calculate the content of volatile oil with reference to the anhydrous substance.

Curcuminoids content Not less than 5.0 per cent w/w of curcuminoids, calculated as curcumin, when determined by the following method.

Standard curcumin solution Dissolve about 2 mg of *curcumin*, accurately weighed, in sufficient *methanol* to produce 5.0 ml.

Standard curcumin curve Transfer into five 10-ml volumetric flasks, 20, 40, 50, 60, and 80 μl, respectively, of *Standard curcumin solution*, dilute to volume with *methanol*, and mix. Measure the absorbances of the standard solutions relative to the blank at 420 nm (Appendix 2.2). Plot the readings and draw the curve of best fit.

Procedure Transfer about 300 mg of Turmeric, in *fine powder* and accurately weighed, into a 10-ml volumetric flask, add *tetrahydrofuran* to volume and mix. Set aside at room temperature for 24 hours with frequent shaking. Dilute 1.0 ml of the clear supernatant liquid with *methanol* to produce 25.0 ml. Transfer 1.0 ml of this solution into a 50-ml volumetric flask, dilute to volume with *methanol* and mix well. Measure the absorbance of the resulting solution at the maximum at adout 420 nm (Appendix 2.2). By reference to standard curve, calculale the content of as curcumin in the sample.

Dose 0.5 to 1 g four times a day after meals and at bedtime.

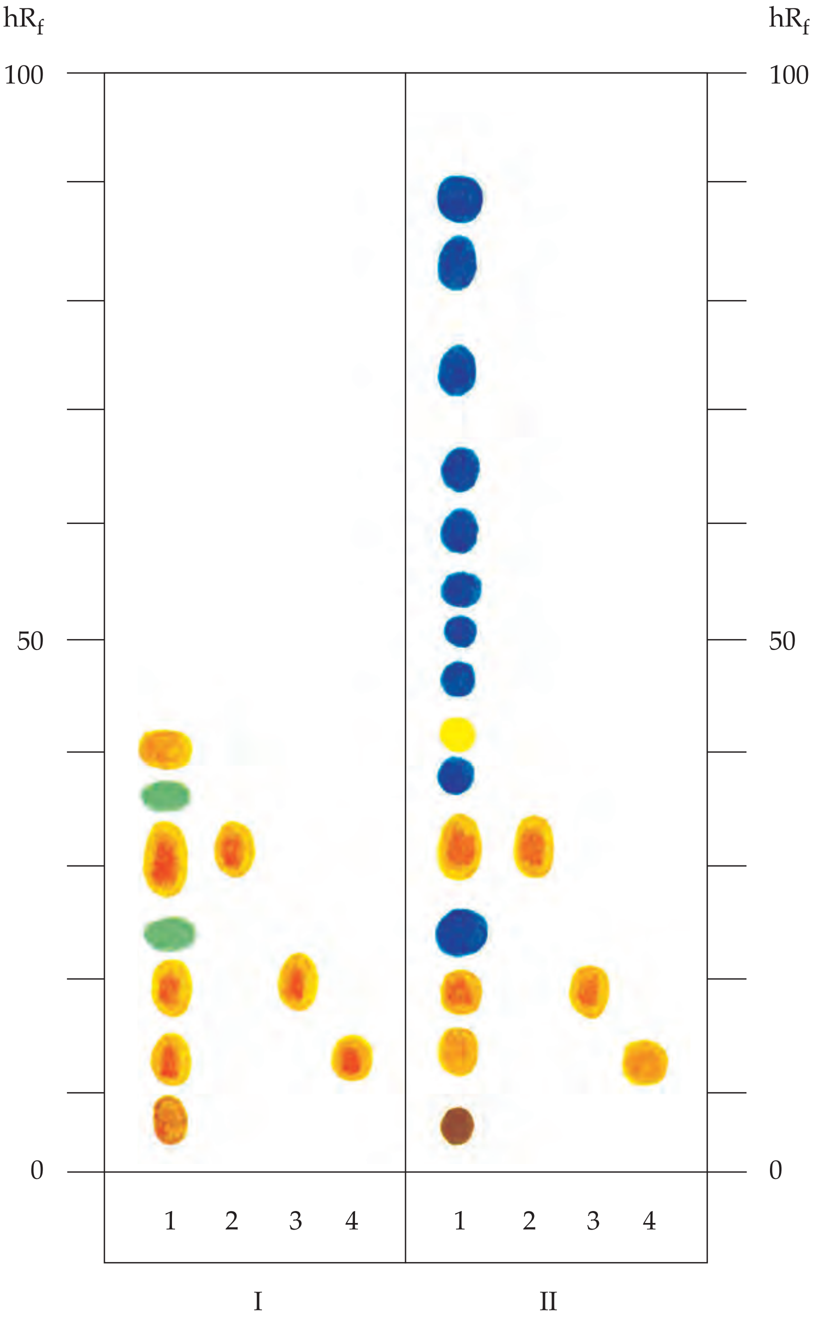


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Rhizomes of *Curcuma longa* L.

1 = solution (A)
2 = solution (B)
3 = a 0.1 per cent w/v solution of desmethoxycurcumin in *methanol*
4 = a 0.1 per cent w/v solution of bisdesmethoxycurcumin in *methanol*
I = detection under UV light (366 nm)
II = detection with a 10 per cent w/v solution of *phosphomolybdic acid* in *ethanol*

ยาแคปซูลขมิ้นชัน (KHAMIN CHAN CAPSULES)

Turmeric Capsules

Category Stomachic, carminative.

Turmeric Capsules contain an amount of powdered Turmeric equivalent to not less than 80.0 per cent of the labelled amount of volatile oil and not less than 90.0 per cent of the labelled amount of curcuminoids, calculated as curcumin ($C_{21}H_{20}O_6$).

Strength available 250 mg (powder).

Dose Two to four capsules four times a day after meals and at bedtime.

Warning

1. Long-term use is not recommended in pregnant women.
2. It should not be used in patients with bile duct obstruction except under medical supervision.
3. Concomitant use with anticoagulants and antiplatelets should be used with caution.

Packaging and storage Turmeric Capsules shall be kept in tightly closed containers, protected from light, in a dry place and at a temperature not exceeding 25°.

Labelling The label on the container states (1) the quantity equivalent to the amount of volatile oil and curcuminoids as curcumin; (2) expiration date.

Identification

1. The capsule contents exhibit diagnostic structures of the powdered drug described under *Turmeric*.
2. The capsule contents comply with the tests for Identification A and B described under *Turmeric*.

Water Of the capsule contents, not more than 10.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Microbial limit Comply with the requirements for Category 4 in the “Limits for Microbial Contamination” (Appendix 10.5).

Curcuminoids content Of the capsule contents, not less than 5.0 per cent w/w of curcuminoids, calculated as curcumin, when determined by the following method.

Standard curcumin solution Dissolve an accurately weighed quantity of *curcumin* in *methanol*. Dilute quantitatively with *methanol* to obtain a solution having a known concentration of about 400 μg of curcumin per ml.

Standard curcumin curve Transfer into five 10-ml volumetric flasks, 20, 40, 50, 60, and 80 μl, respectively, of *Standard curcumin solution*, dilute to volume with *methanol*, and mix. Measure the absorbances of the standard solutions at the maximum at about 420 nm, using *methanol* as the blank (Appendix 2.2). Plot the readings and draw the curve of best fit.

Sample preparation Weigh and mix the contents of not less than 20 Turmeric Capsules. Transfer an accurately weighed portion of the capsule contents, containing about 300 mg of turmeric, to a 10-ml volumetric flask. Add *tetrahydrofuran* to volume and mix. Set aside at room temperature for 24 hours with frequent shaking. Dilute 1.0 ml of the clear supernatant liquid with *methanol* to produce 25.0 ml. Transfer 1.0 ml of this solution into a 50-ml volumetric flask, dilute to volume with *methanol* and mix well.

Procedure and **Calculation** Measure the absorbance of *Sample preparation* at the maximum at about 420 nm, using *methanol* as the blank (Appendix 2.2). By reference to the Standard curcumin curve, calculate the content of curcuminoids as curcumin in the portion of Capsules taken.

Dissolution Carry out the test as described in the "Dissolution Test for Herbal Drug Preparations" (Appendix 4.24H).

Dissolution medium: 0.8 per cent w/v solution of *sodium lauryl sulfate* in 0.05 M *hydrochloric acid*; 900 ml. Prepare and use within 12 hours.

Apparatus 1: 100 rpm.

Time: 60 minutes.

Determine the amount of curcuminoids, calculated as curcumin ($C_{21}H_{20}O_6$) dissolved, using the following method.

Standard curcumin solution Prepare as directed in the test for *Curcuminoids content*.

Standard curcumin curve Transfer into six 10-ml volumetric flasks, 10, 20, 40, 50, 60, and 80 µl, respectively, of *Standard curcumin solution*, dilute to volume with *methanol*, and mix. Measure the absorbances of the standard solutions at the maximum at about 420 nm, using *methanol* as the blank (Appendix 2.2). Plot the readings and draw the curve of best fit.

Test solution Withdraw a 15-ml aliquot and filter. Combine 5.0 ml of the filtered solutions of the six individual capsules withdrawn as the pooled sample and dilute to 50.0 ml with *methanol*. Transfer 10.0 ml of this solution into a 50-ml volumetric flask, dilute to volume with *methanol* and mix well.

Procedure and **Calculation** Measure the absorbance of *Test solution* at the maximum at about 420 nm, using *methanol* as the blank (Appendix 2.2). By reference to the Standard curcumin curve, calculate the average content of curcuminoids as curcumin ($C_{21}H_{20}O_6$) dissolved on the anhydrous basis.

Tolerances Not less than 75 per cent of the labelled content of curcuminoids is dissolved in 60 minutes.

Assay

FOR VOLATILE OIL Grind the contents of not less than 20 Turmeric Capsules to *fine powder.* Transfer about 10 g, accurately weighed, to a 500-ml round-bottomed flask. Use 100 ml of *water* as the distillation liquid and distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube (Appendix 7.3H). Calculate the content of volatile oil, in ml, in the portion of the Capsules taken with reference to the anhydrous substance.

FOR CURCUMINOIDS Carry out the determination as described in the "Ultraviolet and Visible Spectrophotometry" (Appendix 2.2).

Standard curcumin solution Dissolve an accurately weighed quantity of *curcumin* in *methanol*. Dilute quantitatively with *methanol* to obtain a solution having a known concentration of about 400 µg of curcumin per ml.

Standard curcumin curve Transfer into five 10-ml volumetric flasks, 20, 40, 50, 60, and 80 µl of *Standard curcumin solution*, dilute to volume with *methanol*, and mix. Measure the absorbances of the standard solutions at the maximum at about 420 nm, using *methanol* as the blank. Plot the readings and draw the curve of best fit.

Assay preparation Weigh and mix the contents of not less than 20 Turmeric Capsules.

Transfer about 400 mg, accurately weighed, to a stoppered centrifuge tube. Add 10.0 ml of *tetrahydrofuran* and mix. Set aside at room temperature for 24 hours with frequent shaking. Dilute 1.0 ml of the clear supernatant liquid with *methanol* to produce 25.0 ml. Transfer 1.0 ml of this solution into a 50-ml volumetric flask, dilute to volume with *methanol* and mix well.

Procedure and **Calculation** Measure the absorbance of *Assay preparation* at the maximum at about 420 nm, using *methanol* as the blank. By reference to the Standard curcumin curve, calculate the content of curcuminoids as curcumin ($C_{21}H_{20}O_6$) in the portion of the Capsules taken.

Other requirements Comply with the requirements described under “Capsules” (Appendix l.16H).

ขมิ้นเครือ (KHAMIN KHRUEA)

Arcangelisiae Flavae Caulis
Arcangelisia Flava Stem

Category Stomachic, antidiarrheal, antibacterial.

Arcangelisia Flava Stem is the dried stem of *Arcangelisia flava* (L.) Merr. (Family Menispermaceae), Herbarium Specimen Number: DMSC 857, Crude Drug Number: DMSc 426.

Constituents Arcangelisia Flava Stem contains berberine as the major alkaloidal component. It also contains other isoquinoline alkaloids (e.g., columbamine, palmatine, jatrorrhizine), diterpenoids, etc.

Description of the plant (Figs. 1a, 1b) Large climber, woody, glabrous, dioecious; stem up to 5 cm in diameter, wood yellow, exuding yellow sap when cut, bearing prominent cup-like, petiole- scars. Leaves usually ovate, elliptic-ovate or broadly ovate, 10 to 25 cm long, 5.5 to 19 cm wide, base usually rounded, truncate or slightly cordate, apex abruptly acuminate, palmately 5-nerved at the base and with 1 to 3 pairs of lateral nerves, usually arising from above halfway along the midrib, both surfaces usually with a rather obscure reticulum, coriaceous; petioles 4 to 20 cm long, swollen at both ends, geniculate at base. Inflorescences axillary or cauliflorus paniculate slender, 10 to 50 cm long, lateral branches spicate to subspicate, 1 to 5 cm long. Male flowers sessile or subsessile, subtended by an ovate bracteole, about 2 mm long, strongly thickened at the base; outer sepals, 3 to 4, less than 1 mm long; inner sepals, 2 whorls of 3, larger, elliptic, ovate or narrowly obovate, 1.5 to 2.5 mm long; synandrium 0.5 to 1 mm long with a globose cluster of 9 to 12 anthers. Female flowers main sepals 6, narrowly oblong with the apex becoming reflexed, 2.5 to 4 mm long; staminodes minute, scale-like; carpels 3, 1.5 mm long; stigma broad, sessile, papillose. Infructescences cauliflorous, usually branched, 5 to 45 cm long, with thickened axis and branches, 3 to 6 mm in diameter, the fruits plus carpophores borne on the lateral branches, 1 to 3 borne together on a club-shaped, unbranched carpophore swollen at the apex, up to 4 cm. Fruit drupe, yellow, laterally slightly compressed, transversely subovoid, 2.2 to 3 cm long, 2.5 to 3.3 cm (long axis), 2 to 2.5 cm thick, drying finely rugulose, glabrous; endocarp woody. Seeds broadly ellipsoid, with ruminate endosperm, cotyledons much folded.

Description Odourless; taste, bitter.

Macroscopical (Fig. 1a) Cylindrical, segmented, or oblique pieces, externally brownish, rather smooth; sectional view golden yellow to yellowish when fresh and brownish yellow when dried, porous, with several successive concentric and distinctly radiate zones.

Microscopical (Figs. 2a, 2b) Transverse section of the stem shows several layers of rectangular, brownish cork cells and layers of lignified thick-walled, yellowish rectangular sclerenchymatous cells. Cortex, dark brown band of ovate parenchyma cells. Anomalous vascular tissues, several layers of tissue bands with distinct rays, each layer separated by layers of sclereids; phloem tissues, with groups of thick-walled sclereids and fibres in the outermost region; xylem tissues, simple pitted and bordered-pitted vessels, xylem fibres and rectangular or oblique or elongated xylem parenchyma containing prisms and starch grains. Pith, parenchyma cells containing prisms and starch grains.

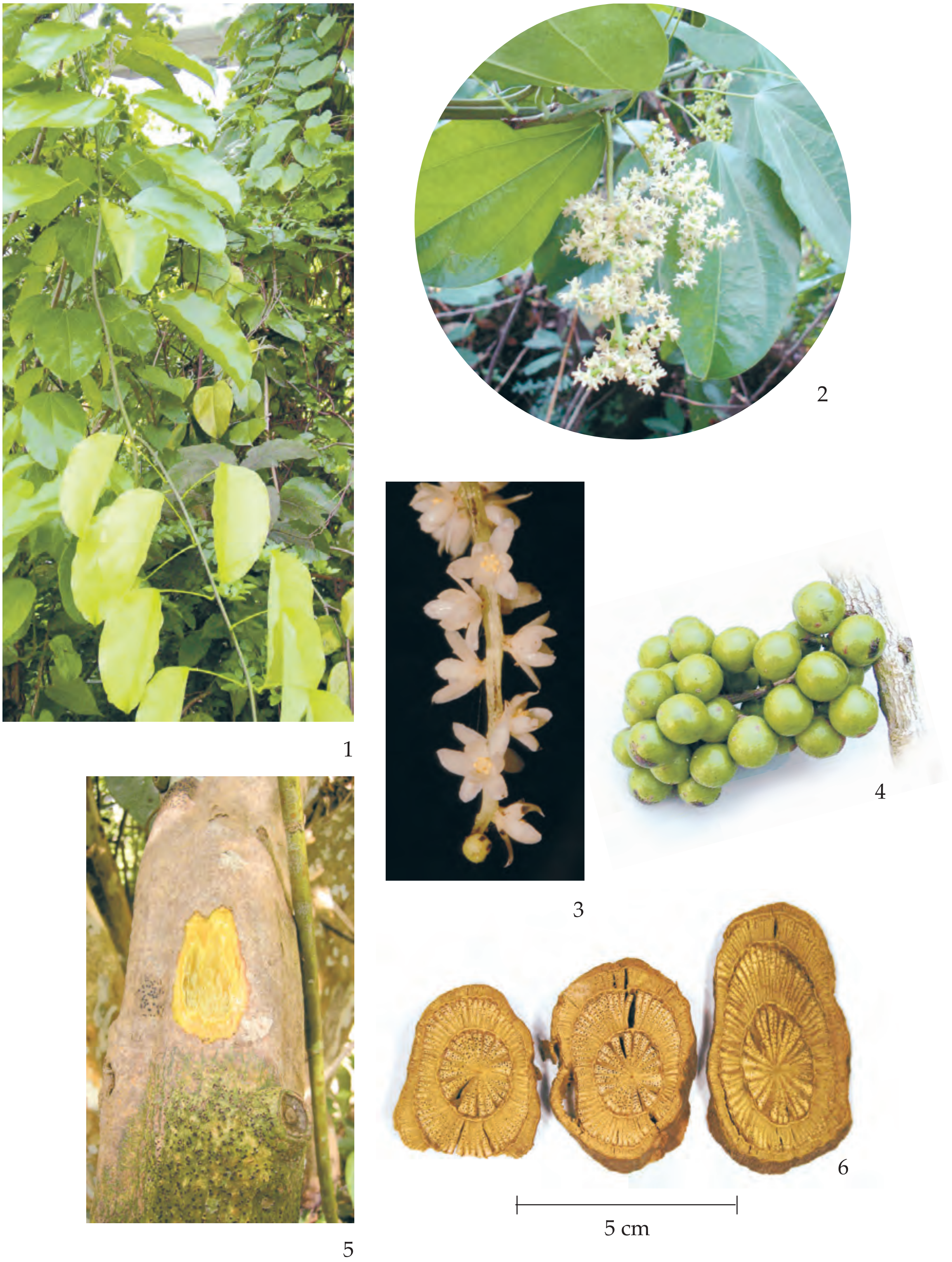


Fig. 1a *Arcangelisia flava* (L.) Merr.
1. habit 2. flowering twig 3. inflorescence 4. infructescence 5. stem 6. crude drug

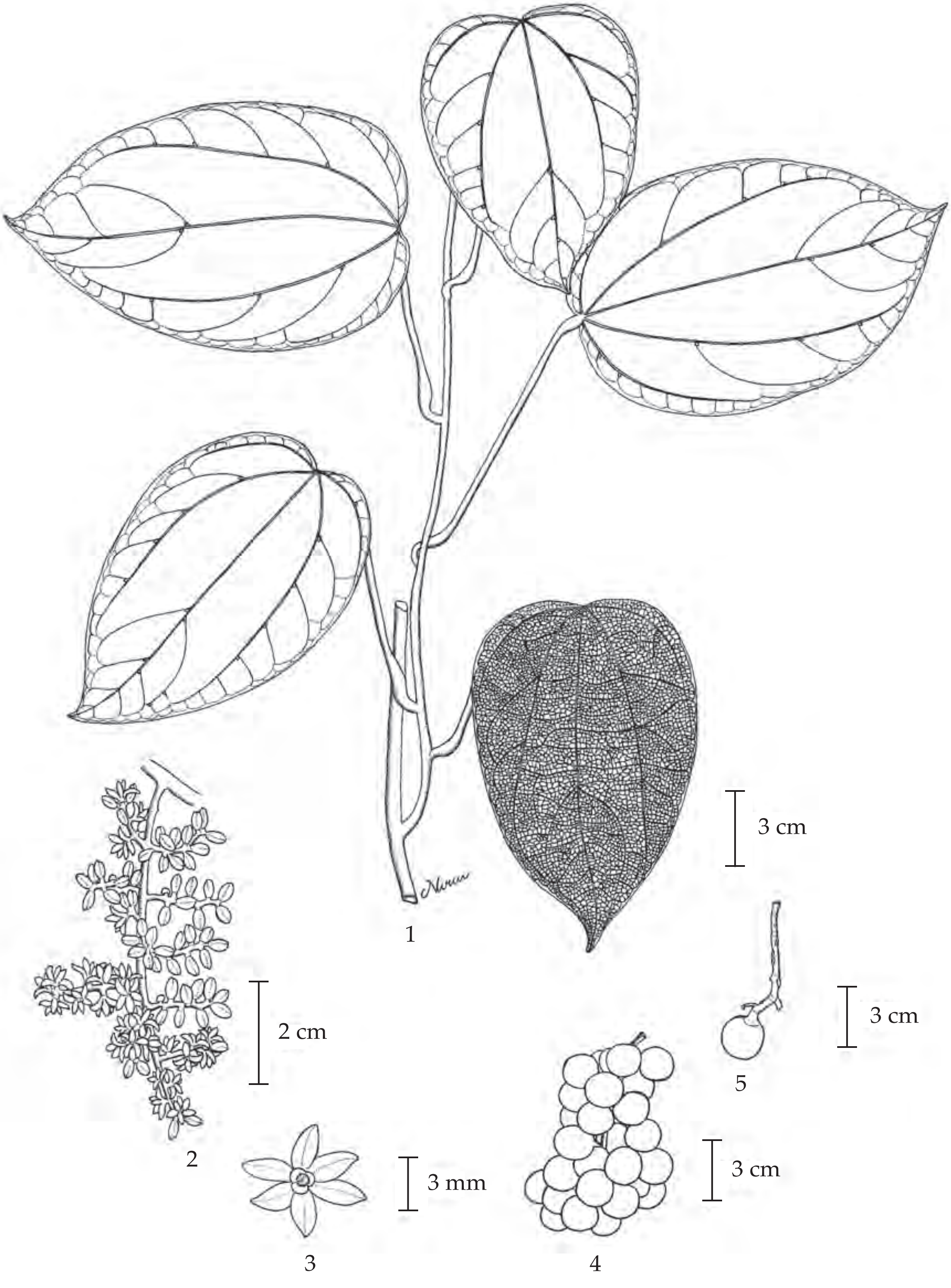


Fig. 1b *Arcangelisia flava* (L.) Merr.
1. part of vine showing leaves 2. female inflorescence 3. female flower 4. infructescence 5. fruit

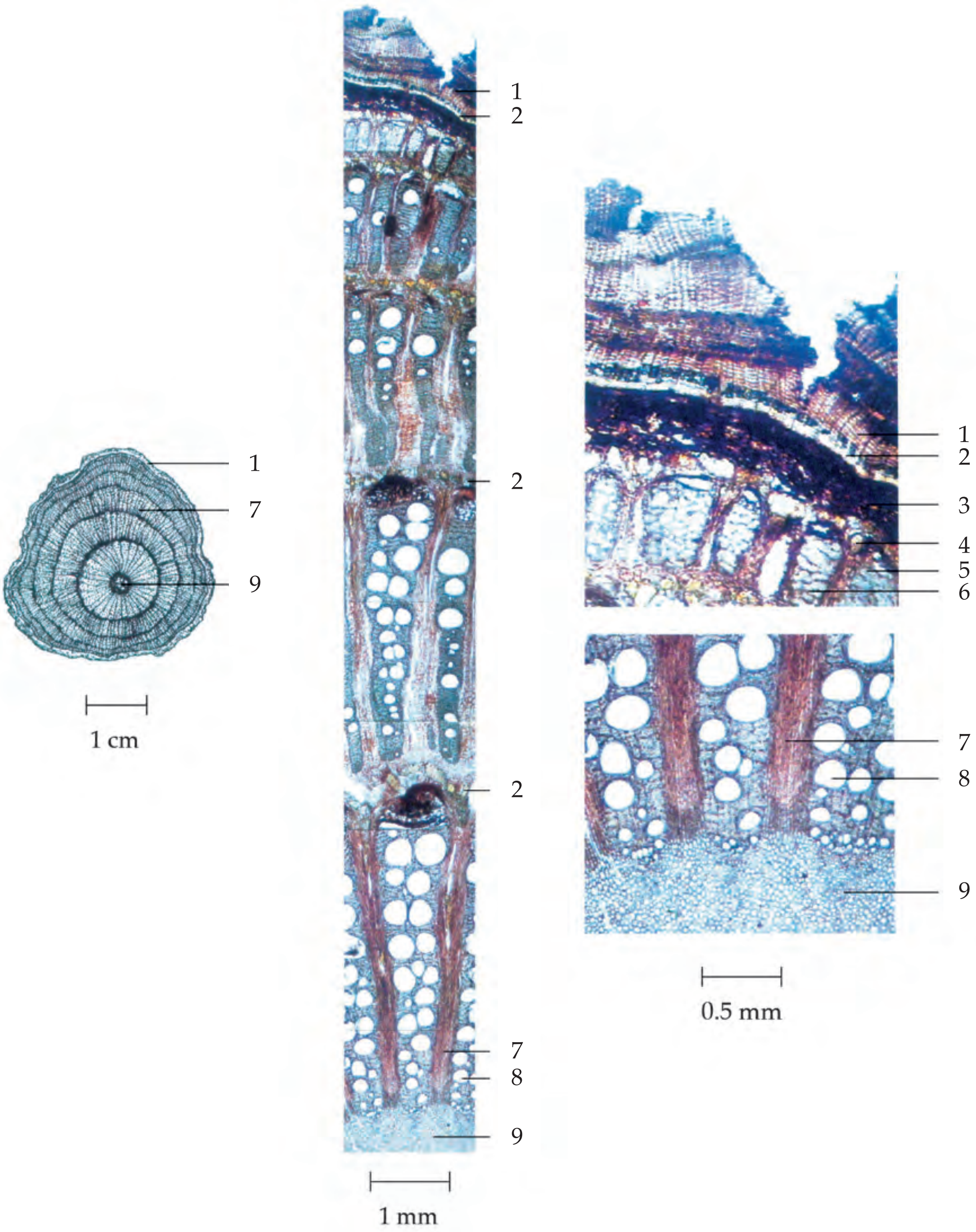


Fig. 2a Transverse Section of the Stem of *Arcangelisia flava* (L.) Merr.

1. cork
2. sclereid
3. cortical parenchyma
4. xylem sclereid
5. xylem fibre
6. vascular tissue
7. xylem ray
8. vessel
9. pith

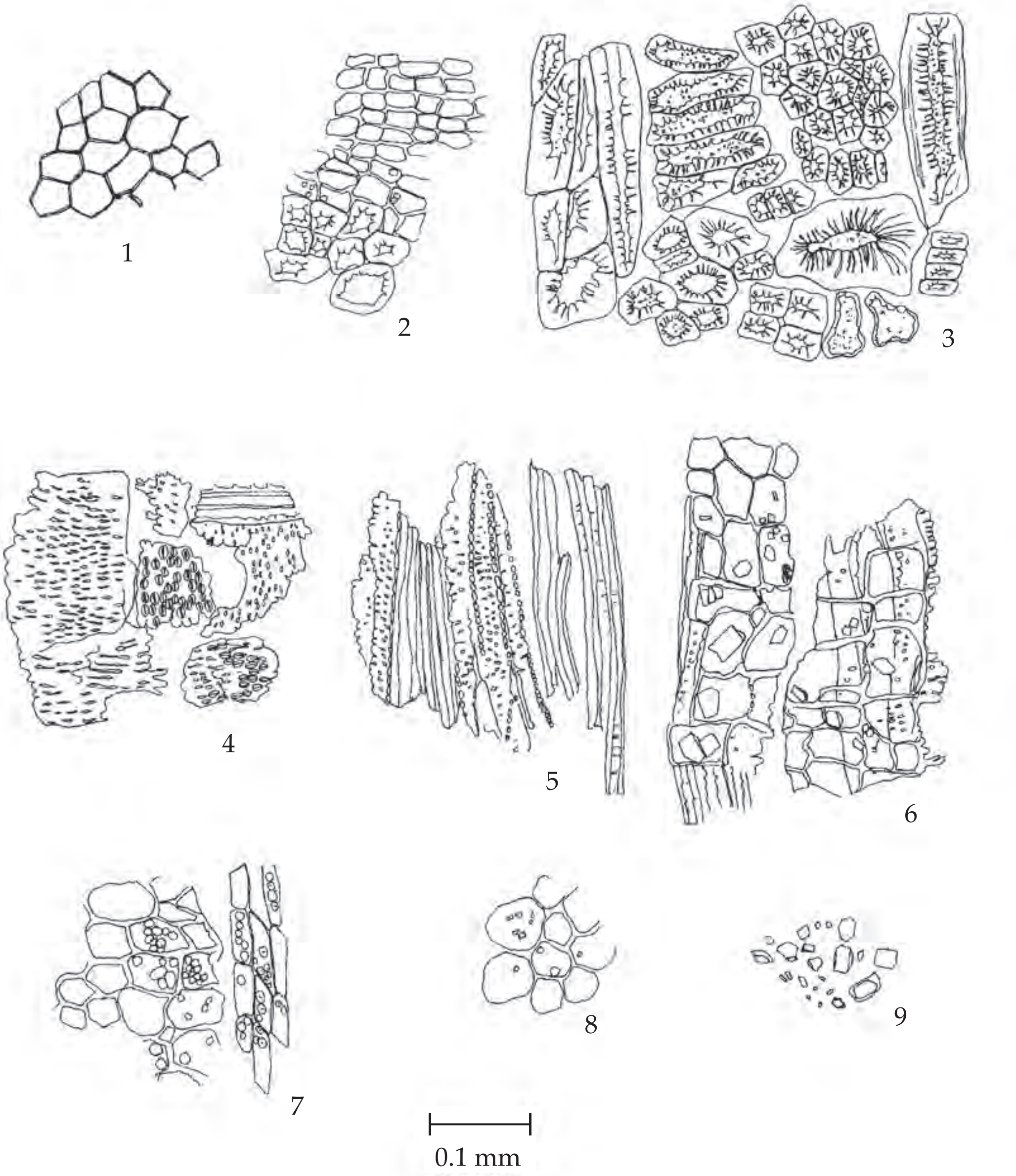


Fig. 2b Powdered Drug of the Stems of *Arcangelisia flava* (L.) Merr.

1. cork cells in surface view
2. cork cells in sectional view associated with sclereids
3. sclereids
4. simple pitted and bordered-pitted vessels
5. xylem fibres associated with pitted vessels
6. xylem ray in radial longitudinal section containing prismatic crystals
7. xylem parenchyma containing prismatic crystals and starch grains
8. pith parenchyma containing prismatic crystals and starch grains
9. prismatic crystals

Arcangelisia Flava Stem in powder possesses the diagnostic microscopical characters of the unground drug.

Packaging and storage Arcangelisia Flava Stem shall be kept in well-closed containers, protected form light, and stored in a dry place

Identification

A. Reflux 1 g of the sample, in *fine powder*, with 25 ml of *methanol* in a water-bath for 10 minutes, and filter (solution 1). Examine 2 ml of solution 1 under ultraviolet light (366 nm): a yellow fluorescence is observed.

B. To 2 ml of solution 1, add 0.5 ml of *nitric acid* and mix well: an orange colour develops.

C. Evaporate 2 ml of solution 1 to dryness, dissolve the residue in 2 ml of *ether*, add 0.5 ml of *hydrogen peroxide TS* (*10 volumes*), and mix. Carefully add 1 ml of *hydrochloric acid* to form a layer: a red ring forms at the zone of contact.

D. Evaporate 2 ml of solution 1 to dryness. Add a few drops of *modified Dragendorff TS2* to the residue: an orange precipitate is produced.

E. Evaporate 5 ml of solution 1 to dryness. Dissolve the residue in 2 ml of 1.5 M *sulfuric acid*, add a few drops of 0.02 M *potassium permanganate*, and warm on a water-bath: the colour of potassium permanganate is decolorized.

F. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 70 volumes of *1-butanol*, 20 volumes of *water* and 10 volumes of *glacial acetic acid* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate, 5 µl each of the following three solutions. Prepare solution (A) by refluxing 500 mg of the sample, in *fine powder*, with 20 ml of *methanol* for 5 minutes. Filter and evaporate the filtrate under reduced pressure to 5 ml. For solution (B), dissolve 1 mg of *berberine chloride* in 1 ml of *water*. For solution (C), dissolve 1 mg of *palmatine iodide* in 1 ml of *water*. After removal of the plate, allow it to dry in air and examine under daylight, locating the spots. The chromatogram obtained from solution (A) shows two yellow spots (hR_f values 52 to 54 and 44 to 47) corresponding to the berberine chloride spot from solution (B) and the palmatine iodide spot from solution (C), respectively. Subsequently examine the plate under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows two quenching spots, corresponding to the berberine chloride spot from solution (B) and the palmatine iodide spot from solution (C) and other three quenching spots of lower hR_fvalues. Examine the plate under ultraviolet light (366 nm); the spots due to berberine chloride and palmatine iodide show yellow fluorescences. Other severaal blue and one yellow spots are also observed. Spray the plate with a 10 per cent w/v solution of *phosphomolybdic acid* in *ethanol* and heat at 105° for 5 minutes; the spots corresponding to berberine chloride and palmatine iodide are brown. Other several blue spots are also observed (Table 1); see also Fig. 3.

Repeat the same procedure on another plate but spray with *modified Dragendorff TS*; the spots due to berberine chloride and palmatine iodide are orange (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Stems of *Arcangelisia flava* (L.) Merr.

Spot	hR_f Value	Detection				
		Daylight	UV 254	UV 366	*Phospho-molybdic Acid* in *Ethanol*	*Modified Dragendorff TS*
1	4-5	–	–	blue	–	–
2	7-8	–	quenching	blue	–	–
3	12-14	–	–	blue	–	–
4	14-15	–	quenching	blue	blue	–
5	19	–	–	–	blue	–
6	25-28	–	–	blue	–	–
7	32-34	–	quenching	blue	blue	–
8*	44-47	yellow	quenching	yellow	brown	orange
9**	52-54	yellow	quenching	yellow	brown	orange
10	57	–	–	blue	–	–
11	62-64	–	–	yellow	–	–
12	66-67	–	–	–	blue	–
13	82	–	–	–	blue	–
14	88	–	–	blue	–	–
15	96-97	–	–	–	dark blue	–
16	98	–	–	–	dark blue	–

*palmatine iodide

**berberine chloride

Loss on drying Not more than 10.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Acid-insoluble ash Not more than 0.5 per cent w/w (Appendix 7.6).

Total ash Not more than 3.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 4.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 4.0 per cent w/w (Appendix 7.12).

Chloroform-soluble extractive Not less than 1.0 per cent w/w (Appendix 7.12H).

Berberine content Not less than 2.0 per cent w/w, calculated on the dried basis, when determined by the following method.

Standard berberine chloride stock solution Transfer about 40 mg of *berberine chloride*, previously dried at 110° for 4 hours and accurately weighed, to a 50-ml volumetric flask, dissolve in 30 ml of *methanol*, dilute to volume with a mixture of 1 volume of *hydrochloric acid* and 100 volumes of *methanol*, and mix.

Standard berberine chloride solution Dilute *Standard berberine chloride stock solution* quantitatively and stepwise with a mixture of 1 volume of *hydrochloric acid* and 100 volumes of *methanol* to obtain solutions having known concentrations of 80, 160, 240, 320, and 400 µg per ml, respectively.

Standard berberine chloride curve Place 2.5 ml of each standard solution in separate aluminium oxide minicolumns (about 15 × 1 cm), pretreated by filling the minicolumns with 2.5 g of *neutral aluminium oxide* using wet method and washing with about 15 ml of *ethanol*. Elute each column with 15 ml of *ethanol* in portions, combine the eluates in a 25-ml volumetric flask and dilute to volume with *ethanol*. Pipette 10 ml of each solution into separate 25-ml volumetric flasks and dilute to volume with 0.5 M *sulfuric acid.* Measure the absorbances of the solutions obtained at the maximum at about 345 nm, using 0.5 M *sulfuric acid* as the blank (Appendix 2.2). Plot the readings and draw the curve of best fit.

Procedure Place 500 mg of Arcangelisia Flava Stem, in *fine powder* and accurately weighed, in a soxhlet extractor of appropriate size. Add 25 ml of a mixture of 1 volume of *hydrochloric acid* and 100 volumes of *methanol*, extract to colourless and allow to cool. Quantitatively transfer the extract to a 50-ml volumetric flask and dilute to volume with a mixture of 1 volume of *hydrochloric acid* and 100 volumes of *methanol*. Transfer 2.5 ml of this solution to an aluminium oxide minicolumn, and proceed as directed under *Standard berberine chloride curve*, beginning with “pretreated by...”. Read the absorbance of the resulting solution, and by reference to the Standard berberine chloride curve, calculate the content of berberine in the Arcangelisia Flava Stem taken using 407.85 and 336.37 as the molecular weights of berberine chloride and berberine, respectively.

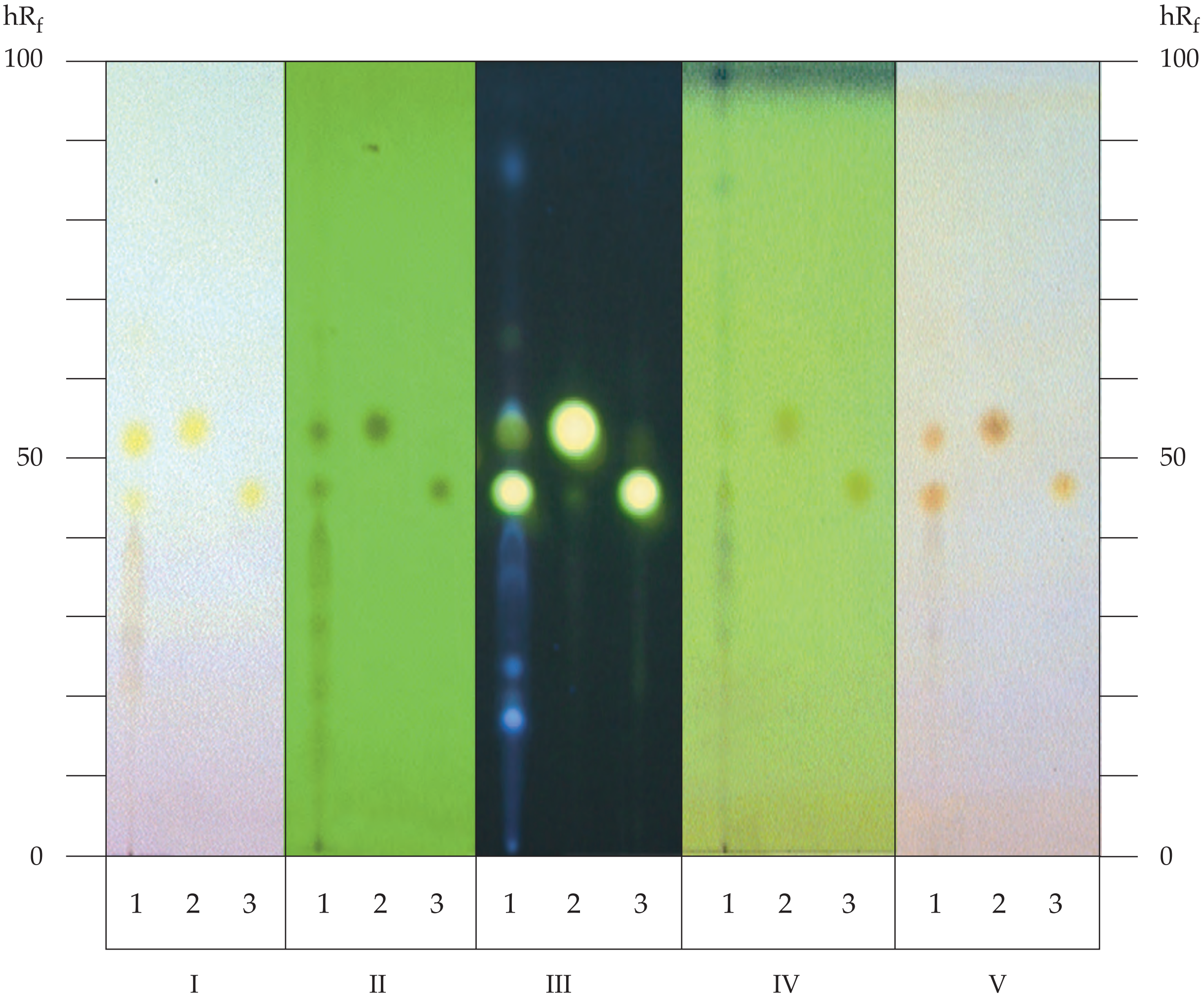


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Stems of *Arcangelisia flava* (L.) Merr.

1 = solution (A)
2 = solution (B)
3 = solution (C)
I = detection under daylight
II = detection under UV light (254 nm)
III = detection under UV light (366 nm)
IV = detection with *phosphomolybdic acid* in *ethanol*
V = detection with *modified Dragendorff TS*

ขมิ้นอ้อย (KHAMIN OI)

ขมิ้นขึ้น (KHAMIN KHUEN), ขมิ้นหัวขึ้น (KHAMIN HUA KHUEN)

Category Stomachic, antidiarrheal, emmenagogue.

Khamin-oi is the dried rhizome of *Curcuma* sp. "Khamin-oi" (Family Zingiberaceae), Crude Drug Number: DMSc 0271.

Constituents Khamin-oi contains curcuminoids, of which curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin are its major components. It also contains volatile oil.

Description of the plant (Figs. 1a, 1b) Perennial herb; rhizome well-developed mostly above ground, obconical to oblong-ovoid, branched, orange-yellow or pale yellow within, aromatic; leafy shoots up to 2 m high bearing 4 to 9 leaves. Leaves simple, oblong-lanceolate, up to 90 cm long, up to 25 cm wide, apex acuminate, glabrous or pubescent, base acute decurrent; petioles long, narrowly winged. Inflorescence scape from the rhizome, appearing before the leaves; peduncle 5 to 15 cm long; spikes cylindric, usually gradually widened to the apex, 10 to 18 cm long, 6 to 8 cm in diameter; primary bracts numerous, lower ones halfway connate, free part obliquely erect, 3 to 8 cm long, bracts usually increasing in length to the apex of spike, those of the lower bracts pale green or whitish, coma bract free and spreading, usually red-purple or violet; bracteoles hairy. Flowers pale yellow; calyx hairy, tubular, white or white with red teeth; corolla about 4 cm long, tube white or yellowish, lobes ovate or oblong, white with pink; labellum orbicular or obovate, bilobed, yellow or white with yellow median field; petaloid staminodes oblong-obovate, pale yellow; ovary inferior, trilocular. Fruits capsule, ovoid, trigonous. Seeds oblong, arillate.

Description Odour, characteristic and aromatic; taste, slightly bitter and pungent.

Macroscopical Dried slices of primary rhizomes and branched annulate tubers vary in size, shape and thickness; somewhat crooked, orange-yellowish brown to greyish brown; enclosed or nearly enclosed with outer cork layer, covered with coarse trichomes. Pseudoendodermal rings are seen in horizontal and oblique slices.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the fresh rhizome shows epidermis, composed of a layer of rectangular cells; covering trichomes, unicellular and uniseriate multicellular. Storied cork, several layers of rectangular cork cells. Cortex, composed of broad zone of thin-walled parenchyma cells, filled with numerous starch grains, some of which containing brownish yellow oleoresin or yellowish oil droplets. Pseudoendodermis, a circular layer of compressed rectangular cells, enclosing a broad zone of parenchyma cells similar to those in cortex. Vascular bundles, not yielding the *reaction* with *phloroglucinol TS*, scattering densely underneath endodermis; vessels, spiral, scalariform and reticulate.

Khamin-oi in powder possesses the diagnostic microscopical characters of the unground drug

Contra-indication It is contra-indicated in pregnant women.

Fig. 1a *Curcuma* sp. “Khamin-oi”
1. and 2. habit 3. inflorescence 4. and 5. crude drug

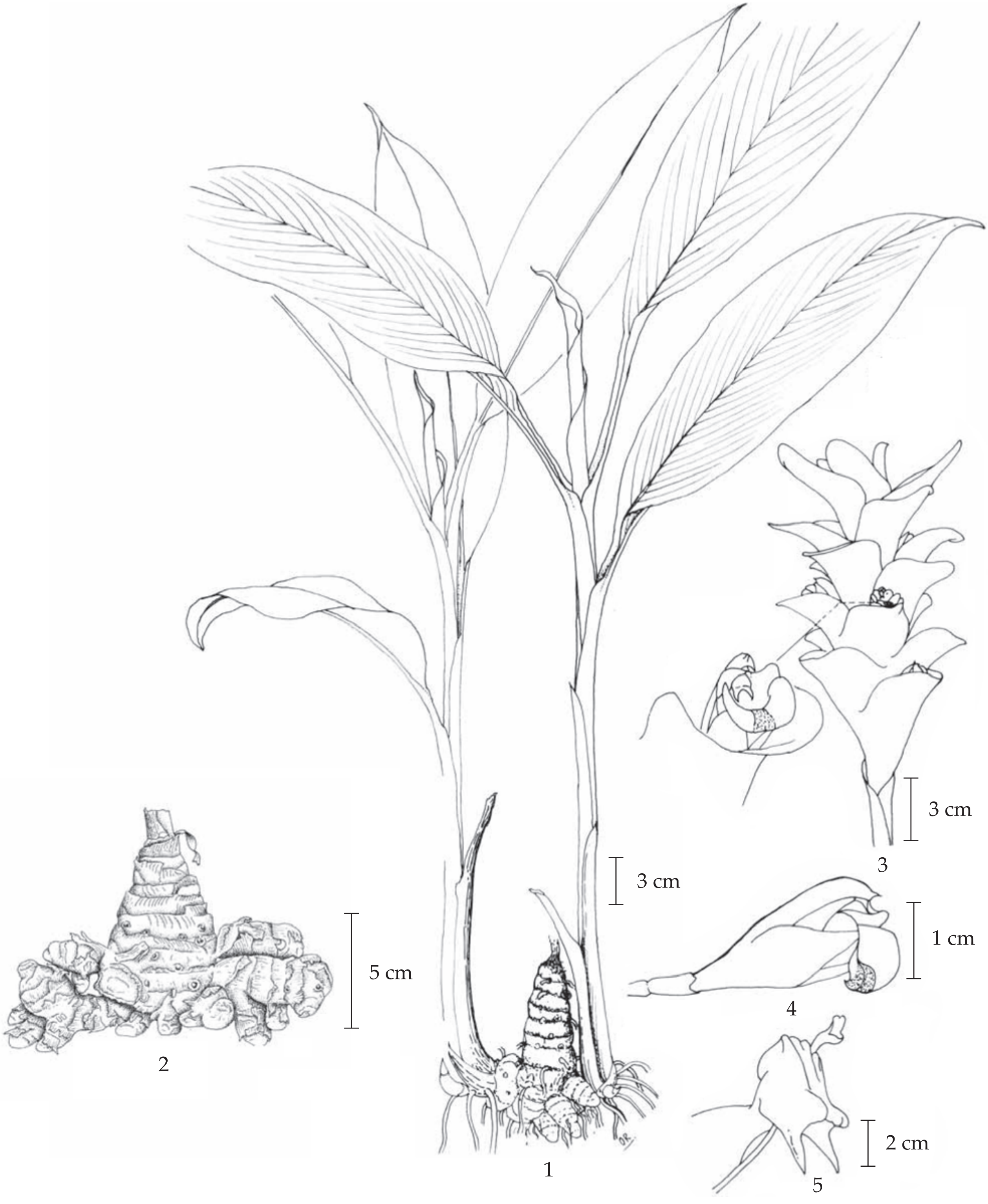


Fig. 1b *Curcuma* sp. "Khamin-oi"
1. habit 2. rhizome 3. scapose inflorescence (with the end of primary bract and single floret enlarged) 4. single floret 5. stamen with anther and spur, and stigma

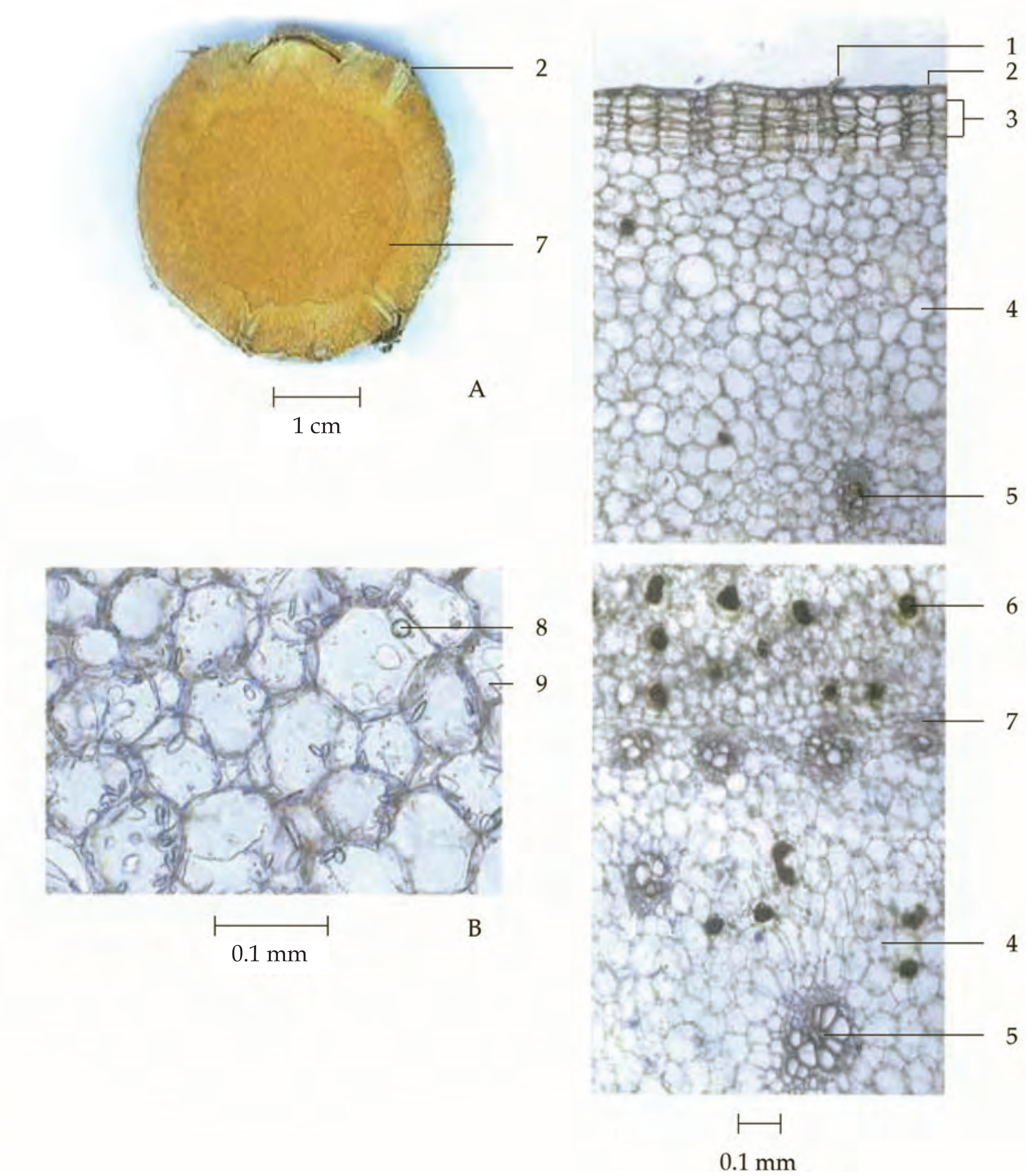


Fig. 2a Photomicrographs of Transverse Section of the Rhizome of *Curcuma* sp. “Khamin-oi”

A. Transverse Section of the Rhizome

B. Cortical Parenchyma

1. trichome
2. epidermis
3. cork layers
4. parenchyma
5. vascular bundle
6. orange-yellow colouring matter
7. pseudoendodermis
8. oil droplet
9. starch grain

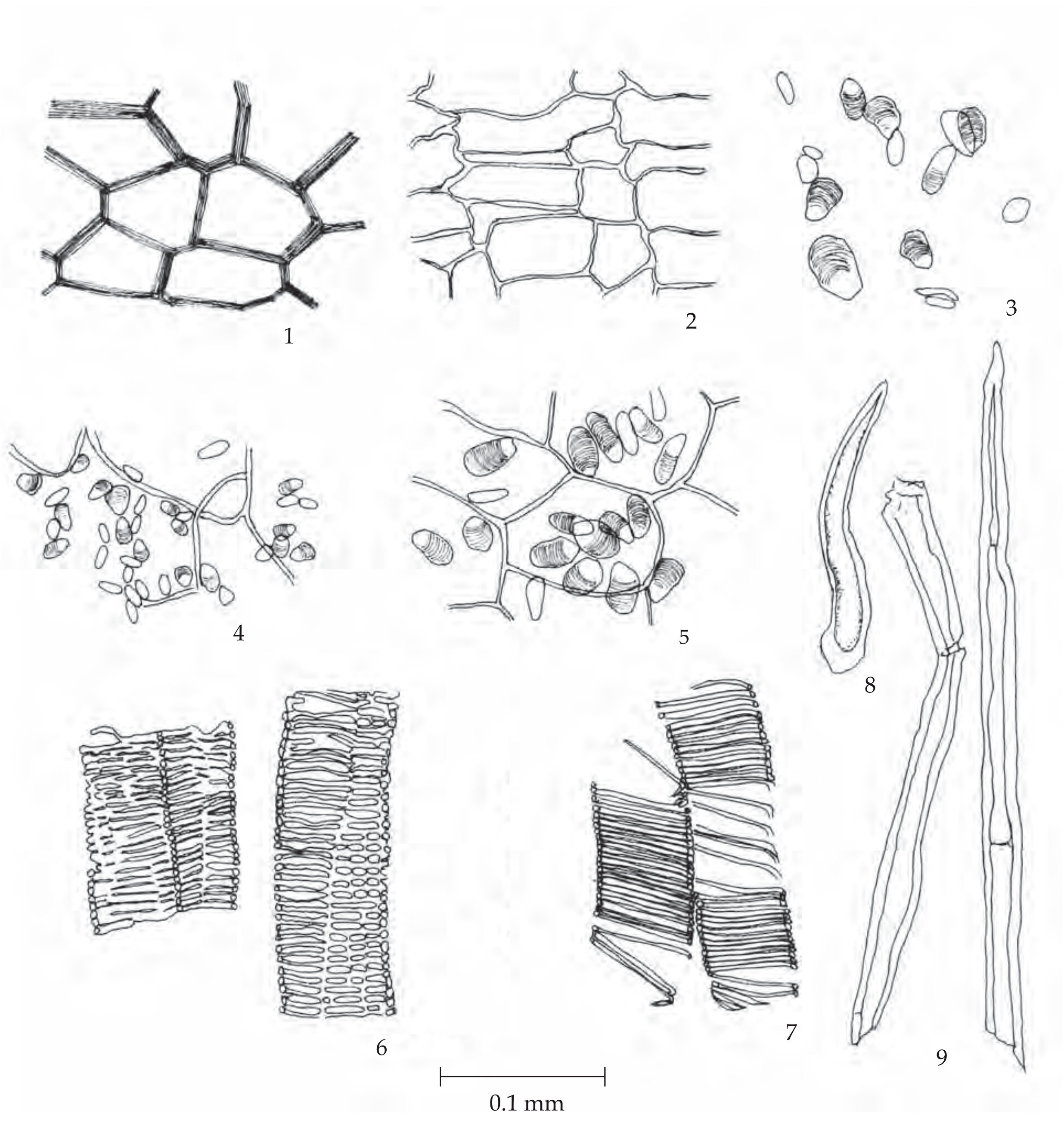


Fig. 2b Line Drawing of Powdered Drug of the Rhizomes of *Curcuma* sp. "Khamin-oi"

1. cork cells in surface view
2. cork cells in sectional view
3. starch grains
4. parenchyma cells containing small starch grains
5. parenchyma cells containing large starch grains
6. scalariform vessels
7. spiral vessels
8. unicellular trichome
9. fragments of uniseriate multicellular trichomes

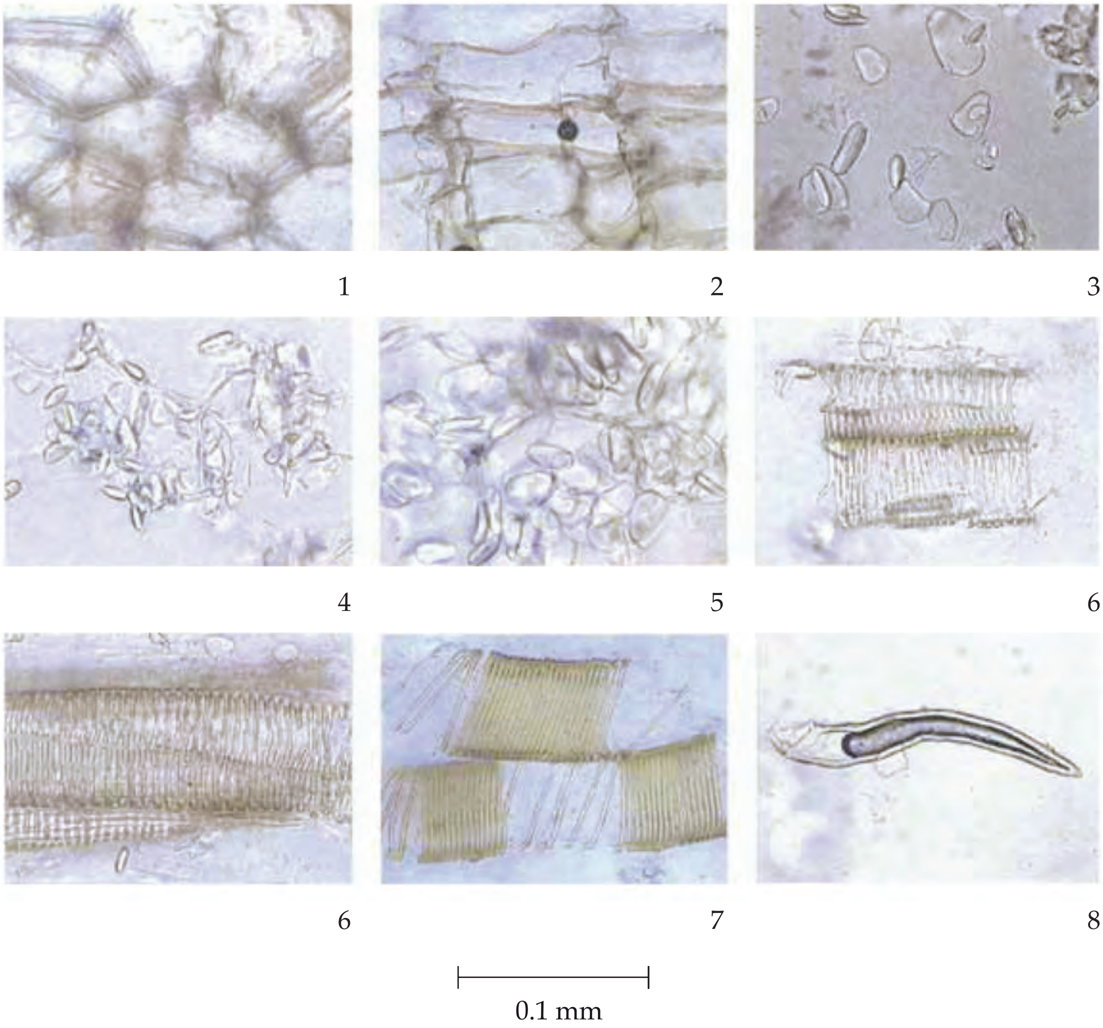


Fig. 2c Photomicrographs of Powdered Drug of the Rhizomes of *Curcuma* sp. “Khamin-oi”

1. cork cells in surface view
2. cork cells in sectional view
3. starch grains
4. parenchyma cells containing small starch grains
5. parenchyma cells containing large starch grains
6. scalariform vessels
7. spiral vessels
8. unicellular trichome

Packaging and storage Khamin-oi shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Extract 10 mg of the sample, in powder, with 2 ml of *acetic anhydride*, add a few drops of *sulfuric acid* and observe under ultraviolet light (366 nm): the solution shows blood-red colour.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 49 volumes of *toluene*, 49 volumes of *chloroform* and 2 volumes of *absolute ethanol* as the mobile phase and allowing the solvent front to ascend 17 cm above the line of application. Apply separately to the plate, 5 µl each of the following two solutions. Prepare solution (A) by placing 1 g of the sample, in powder, in a stoppered test-tube, adding 3 ml of *methanol* and shaking for a few minutes. Set aside for 1 hour and filter. For solution (B), dissolve 1 mg of *curcumin* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air, and examine under ultraviolet light (366 nm), locating the spots. The chromatogram obtained from solution (A) shows several spots of different colours. Two orange-brown spots (hR_f values 24 to 26 and 12 to 15) and one yellow spot (hR_f value 6 to 8) correspond to the spots of curcumin and its derivatives from solution (B). Spray the plate with a 10 per cent w/v solution of *phosphomolybdic acid* in *ethanol* and heat at 105° for about 10 minutes; the spots due to curcumin and its derivatives are brown. Other spots are blue (Table 1); see also Fig. 3.

Repeat the same procedure on another plate but spray with *vanillin-sulfuric acid TS* and heat at 105° for about 10 minutes. The chromatogram obtained from solution (A) shows three orange-brown spots, corresponding to the spots of curcumin and its derivatives from solution (B). Other spots are purple and violet (Table l); see also Fig. 3.

Table 1 hR_f Values of Component in Metanolic Extract of the Rhizomes of *Curcuma* sp. "Khamin-oi"

Spot	hR_f Value	Detection		
		UV 366	10 Per Cent W/V Solution of *Phosphomolybdic Acid* in *Ethanol*	*Vanillin-Sulfuric Acid TS*
1	4-6	orange-blue	blue	purple
2*	6-8	yellow	brown	orange-brown
3	8-10	–	blue	purple
4*	12-15	orange-brown	brown	orange-brown
5	15-18	blue-green	blue	violet
6	18-19	–	blue	violet
7*	24-26	orange-brown	brown	orange-brown
8	26-28	blue-green	blue	purple
9	32-35	–	blue	violet
10	35-38	–	blue	violet
11	41-44	–	blue	violet
12	44-46	–	blue	violet
13	44-51	blue-green	–	–
14	52-54	–	blue	violet
15	56-59	–	blue	violet
16	65-69	–	blue	purple
17	72-78	–	blue	violet

*curcumin and its derivatives

Water Not more than 11.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Acid-insoluble ash Not more than 6.0 per cent w/w (Appendix 7.6).

Sulfated ash Not more than 14.0 per cent w/w (Appendix 5.3).

Volatile oil Not less than 4.0 per cent v/w (Appendix 7.3H). Use 10 g, in *fine powder*, accurately weighed. Use 100 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube. Calculate the content of volatile oil with reference to the anhydrous substance.

Dose 4.5 to 9 g.

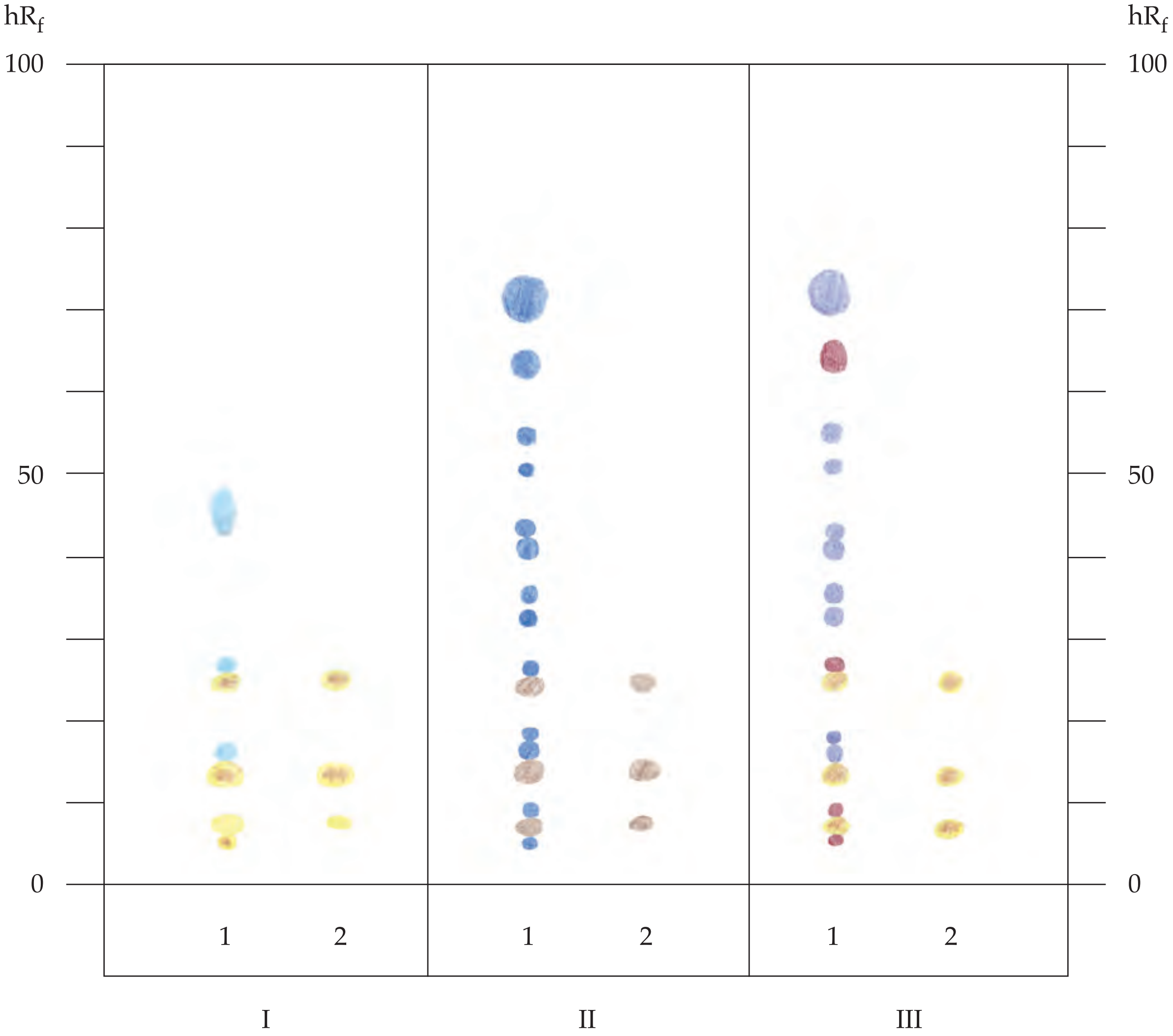


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Rhizomes of *Curcuma* sp. “Khamin-oi”

1 = solution (A)
2 = solution (B)
I = detection under UV light (366 nm)
II = detection with a 10 per cent w/v solution of *phosphomolybdic acid* in *ethanol*
III = detection with *vanillin-sulfuric acid TS*

ขิง (KHING)

Zingiberis Officinalis Rhizoma
Ginger

Category Carminative, antiflatulent.

Ginger is the dried rhizome of *Zingiber officinale* Roscoe (*Amomum zingiber* L.) (Family Zingiberaceae), Herbarium Specimen Number: BKF 193777, Crude Drug Number: DMSc 1145.

Constituents Ginger contains oleoresin predominantly consisting of gingerols, shogaols and volatile oil. Its volatile oil comprises zingiberene, borneol, geraneol, etc. It also contains fatty acids, starch, etc.

Description of the plant (Figs. 1a, 1b) Perennial herb; pseudostem up to 2 m high, leafy; subterraneous rhizome branched, thick, fleshy, yellowish inside, strongly aromatic. Leaves simple, alternate, sessile, lanceolate or linear-lanceolate, 10 to 30 cm long, 1.5 to 3 cm wide, apex acute or acuminate, base obtuse, sheathing; ligule membranous, 2 to 4 mm long, bilobed. Inflorescence arising from rhizome, spike elliptic to oblong, 4 to 9 cm long, 2 to 3 cm wide; peduncle erect, up to 25 cm long, with 5 to 9 green sheaths; bract imbricate, free green, incurved, globose, obrhombic or obovate, 2 to 3 cm long, 2 to 2.5 cm wide, apex obtuse, glabrous on both sides, cream to creamy yellow on margin and tip, sharp apex tip; bracteole elliptic-oblong, cirrated, 2.5 to 2.8 cm long, 1 to 1.5 cm wide, apex obtuse or teethed, glabrous; corolla tube 3-lobed, 2.2 to 2.5 cm long, glabrous, white, dorsal lobe 1.7 to 1.8 cm long, about 8 mm wide, lateral lobes 1.6 to 1.7 cm long, 4 to 5 mm wide; labellum conspicuously 3-lobed, midlobe rounded or obovate, about 1.6 cm long, 1.3 to 1.4 cm wide, apex truncate or emarginate, dark red with yellow blotches at base, side lobes about 7 mm long, 5 to 6 mm wide, apex acute, dark red with scattered creamy dots; anther about 1 cm long, about 3 mm wide, filament about 3 mm long, flattened, appendage 7 to 9 mm long, apex acute, dark red; stylode unequal, 7 to 8 mm long; ovary inferior, glabrous, 3-loculed, each locule with several ovules. Fruit capsule, oblong. Seeds numerous, blackish, arillate.

Description Odour, characteristic and aromatic; taste, pungent and aromatic.

Macroscopical (Fig. 1a) Unpeeled, horizontal, irregularly branched and vary in size. Externally light brown with longitudinal wrinkles and distinct annulated nodes; branched parts usually with remains of scale leaves, apex with stem scars or buds. Texture compact, fibrous; fracture yellowish to brownish.

Microscopical (Figs. 2a, 2b) Transverse section of the rhizome shows epidermis, storied cork, cortex, pseudoendodermis, and vascular bundles. Epidermis, a layer of thin-walled rectangular cells. Storied cork, layers of thin-walled, suberinized rectangular cells. Cortex, thin-walled parenchyma cells containing numerous starch grains and/or oil globules, some of which containing yellow oleoresin. Pseudoendodermis, a layer of small thin-walled rectangular cells. Vascular bundles, scattered in cortex, containing xylem and phloem tissues; vessels, mostly scalariform and reticulate.

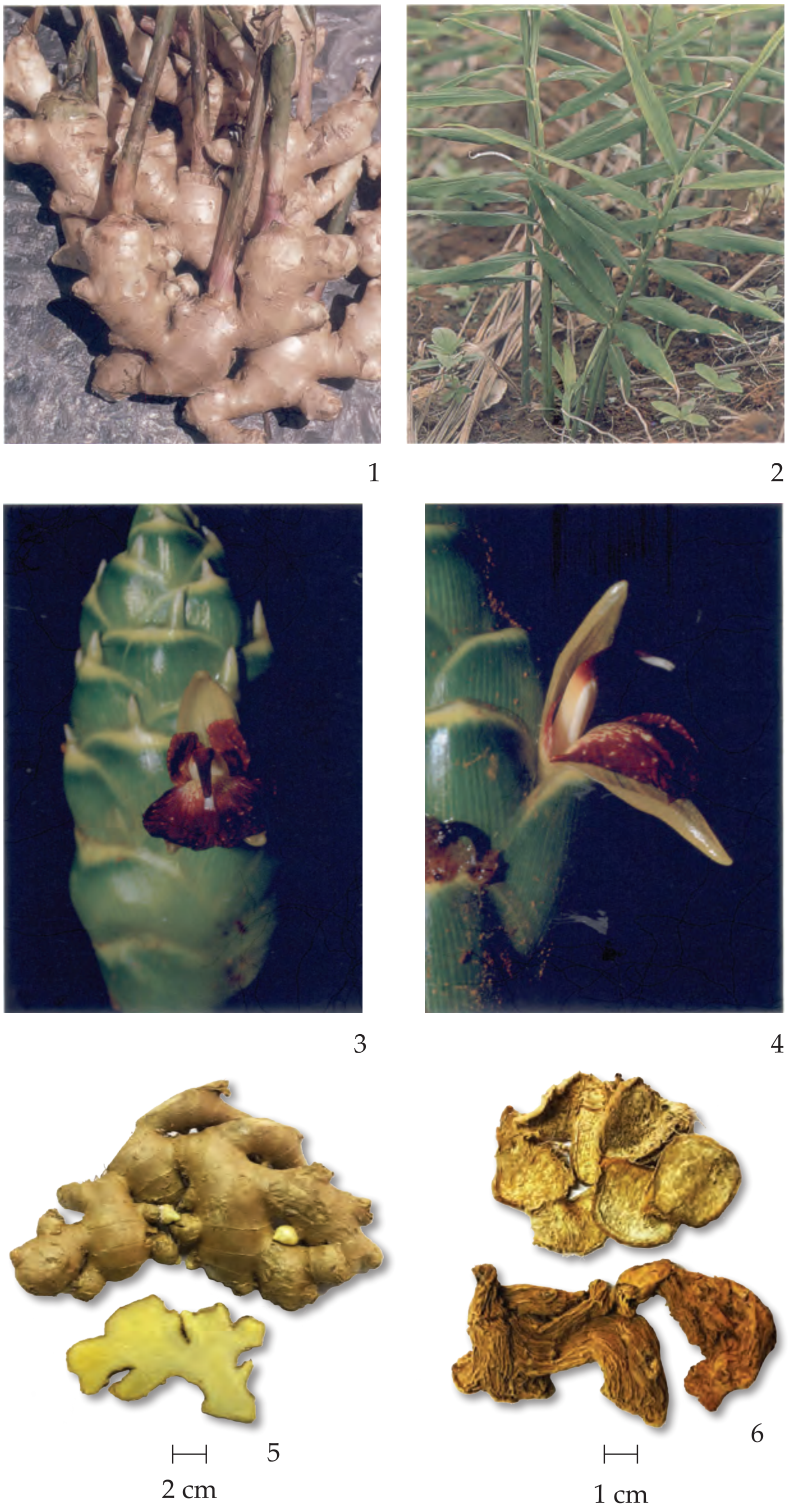


Fig. 1a *Zingiber officinale* Roscoe
1. rhizomes, showing lower part of pseudostems and young spike
2. pseudostems and leaf blades 3. spike with flowers, one in full bloom (front view)
4. flower (oblique view) 5. mature rhizome 6. crude drug

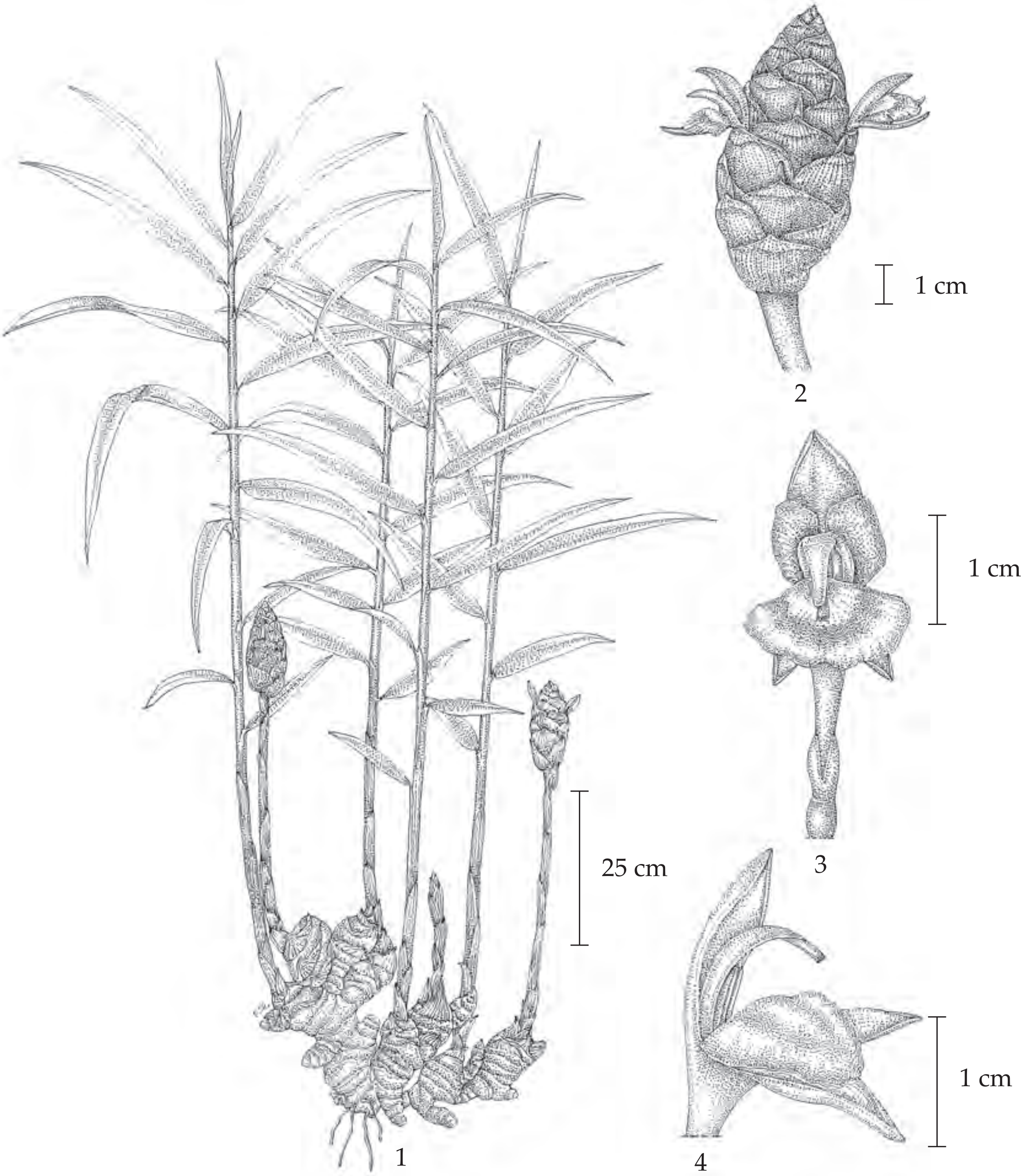


Fig. 1b *Zingiber officinale* Roscoe
1. habit, showing rhizomes, pseudostems, leaves, and spike 2. inflorescence (spike)
3. flower (front view) 4. flower (oblique view)

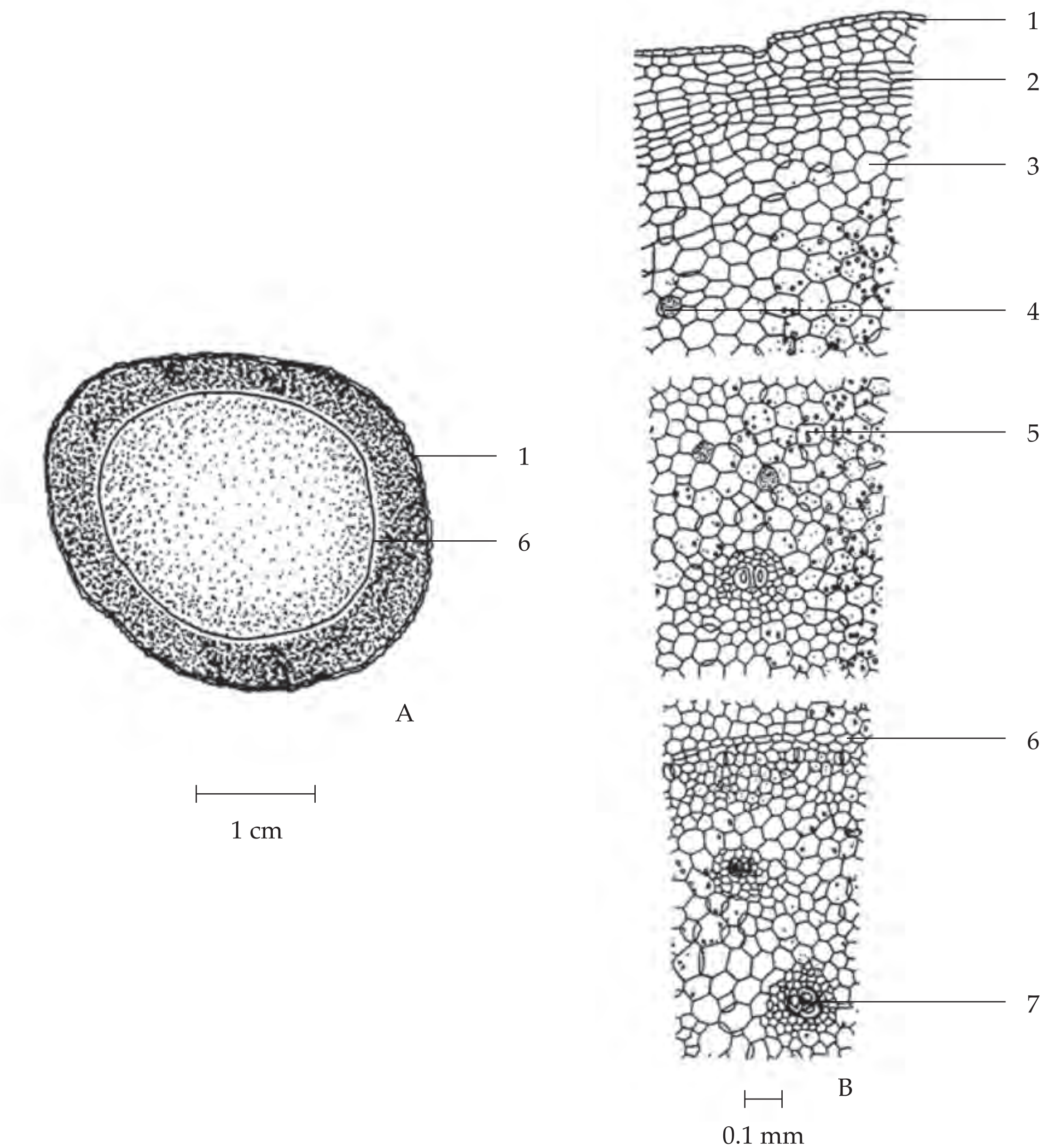


Fig. 2a Transverse Section of the Rhizome of *Zingiber officinale* Roscoe

A. Diagram

B. Part of Sectional View

1. epidermis
2. storied cork
3. parenchyma
4. parenchyma containing yellow oleoresin
5. parenchyma containing starch grains and oil globules
6. pseudoendodermis
7. vascular bundle

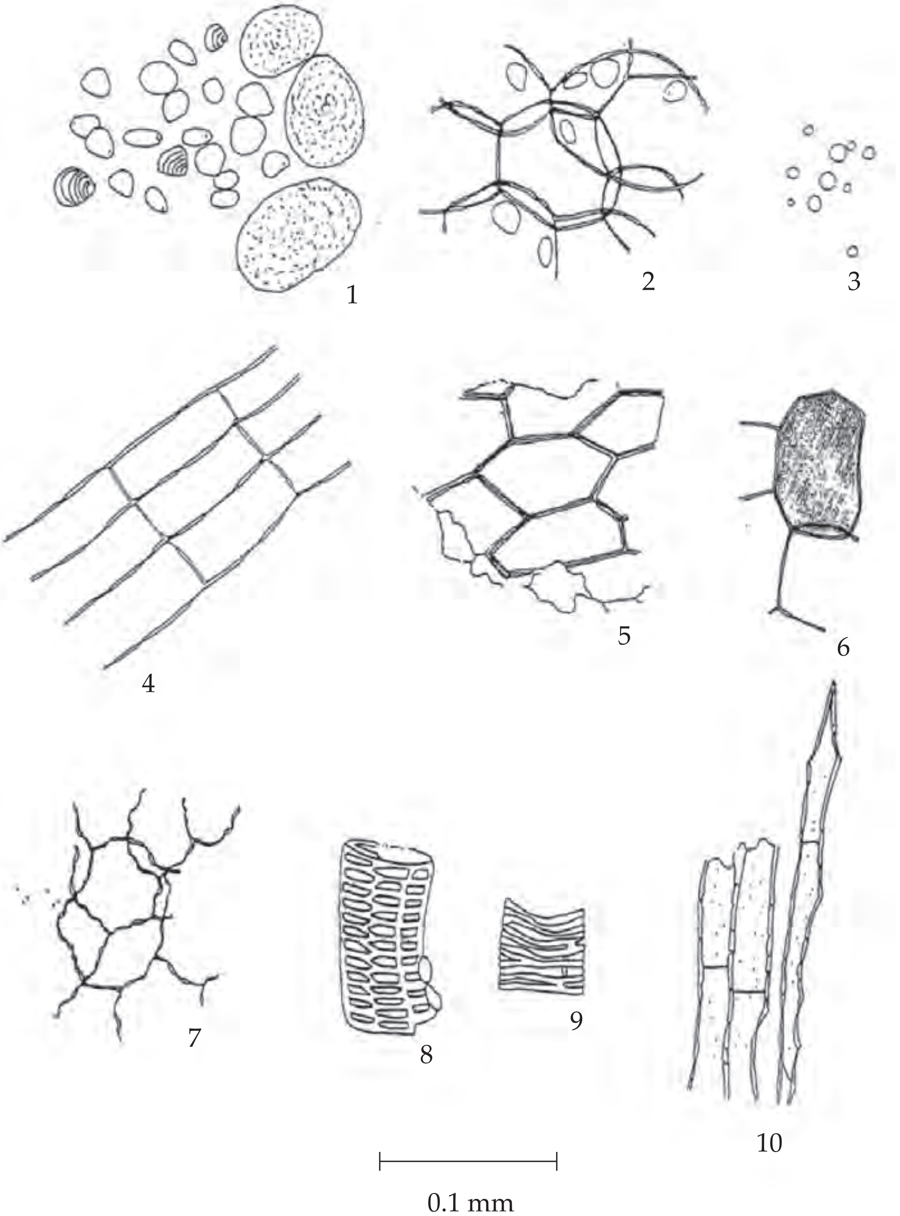


Fig. 2b Powdered Drug of the Rhizomes of *Zingiber officinale* Roscoe

1. starch grains and altered starch
2. parenchyma containing starch grains
3. oil globules
4. storied cork in sectional view
5. storied cork adjacent with parenchyma in surface view
6. parenchyma containing yellow oleoresin
7. parenchyma
8. scalariform vessel
9. reticulate vessel
10. septate fibres

Ginger in powder possesses the diagnostic microscopical characters of the unground drug. Layers of thin-walled cells of storied cork, parenchyma containing starch grains, and/or altered starch, and/or yellow oleoresin are characteristic.

Warning

1. It should not be used in children under 6 years of age and in pregnant women.
2. It should not be used in patients with bile duct obstruction except under medical supervision.
3. Concomitant use with anticoagulants and antiplatelets should be used with caution.

Additional information Ginger containing not less than 2 per cent v/w of volatile oil can be administered orally as an anti-emetic.

Packaging and storage Ginger shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Dissolve 50 mg of the residue obtained from *Ethanol-soluble extractive* in 25 ml of *water* and extract this solution with two 15-ml portions of *ether*. Combine the ether extracts and evaporate to dryness. To the residue, add 5 ml of a 75 per cent v/v solution of *sulfuric acid*, and 5 mg of *vanillin* and then mix well. Allow to stand for 15 minutes and add 5 ml of *water*: a bright blue colour develops.

B. To 1 g of the sample, in powder, add 5 ml of a 50 per cent v/v solution of *glacial acetic acid.* Shake vigorously for 15 minutes and filter. To the filtrate, add a few drops of *ammonium oxalate TS*: a slight turbidity is produced.

C. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 40 volumes of *hexane* and 60 volumes of *ether* as the mobile phase. Apply separately to the plate as bands of 15 mm, 20 µl each of the following two solutions. Prepare solution (A) by shaking 1 g of the sample, in powder, with 5 ml of *methanol* for 15 minutes and filtering. For solution (B), dissolve 10 µl of *citral* and 10 mg of *resorcinol*, as the reference compounds[1], in 10 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. The chromatogram obtained from solution (A) shows three quenching bands. Two quenching bands are between the bands due to resorcinol (hR_f value 30 to 33) and citral isomers (hR_f values 70 to 72 and 73 to 75) in the chromatogram obtained from solution (B) and one quenching band is below the resorcinol band. Examine the plate under ultraviolet light (366 nm) through the cut-off filter; one blue and several green-blue fluorescent bands are observed. Spray the plate with *vanillin-sulfuric acid TS2* and heat at 105° for 10 minutes. The chromatogram obtained from solution (A) shows one violet-grey, one brown-yellow and two violet bands below the red band due to resorcinol in the chromatogram obtained from solution (B). It also shows one dark violet band above the two violet bands due to citral isomers in the chromatogram obtained from solution (B) and two violet-grey and two violet bands between the bands due to resorcinol and citral isomers (Table 1); see also Fig. 3.

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[1] Citral and Resorcinol are not marker compounds for Ginger. They are used as the reference compounds for the purpose of TLC identification.

Table 1 hR_f Values of Components in Methanolic Extract of the Rhizomes of *Zingiber officinale* Roscoe

Band	hR_f Values	Detection		
		UV 254	UV 366	*Vanillin-Sulfuric Acid TS2*
1	4-5	–	–	violet-grey
2	13-15	–	blue	brown-yellow
3	20-22	weak quenching	green-blue	violet
4	23-25	–	green-blue	violet
5	35-37	–	green-blue	–
6	42-43	–	–	pale violet
7	44-46	–	green-blue	–
8	47-49	–	–	pale violet
9	53-55	quenching	green-blue	violet-grey
10	57-58	quenching	–	violet-grey
11	90-92	–	–	dark violet

Water Not more than 11.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 10.0 per cent w/w (Appendix 7.7).

Water-soluble ash Not less than 3.0 per cent w/w (Appendix 7.11).

Ethanol-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 13.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 0.8 per cent v/w, calculated on the anhydrous basis (Appendix 7.3H). Use 50 g, in *coarse powder,* freshly prepared and accurately weighed. Use 200 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

Dose 2 to 4 g a day.

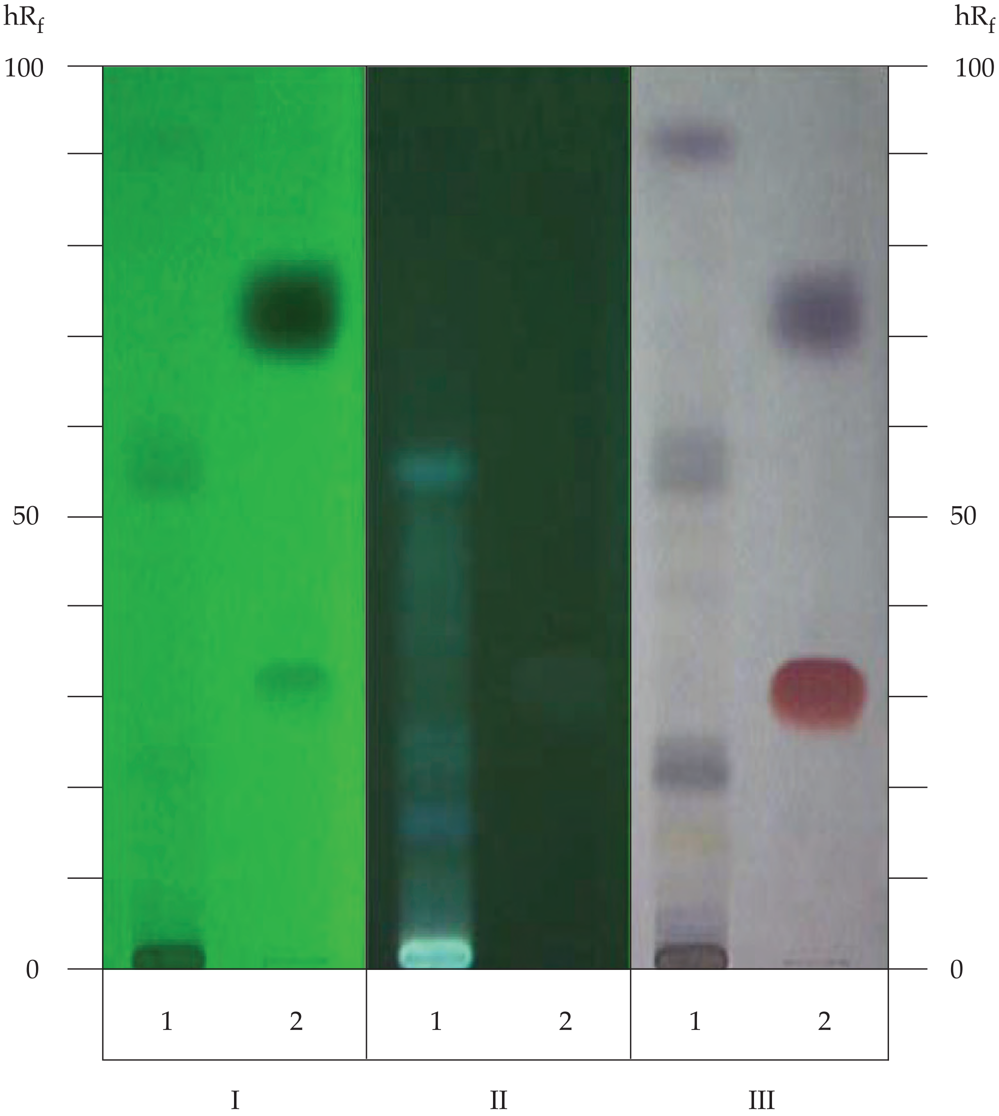

Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Rhizomes of *Zingiber officinale* Roscoe

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *vanillin-sulfuric acid TS2*

คูน, เนื้อในฝัก (KHUN, NUEA NAI FAK)

ลมแล้ง, เนื้อในฝัก (LOM LAENG, NUEA NAI FAK)
Cassiae Fistulae Pulpa
Purging Cassia Pulp

Synonyms Golden Shower Pulp, Indian Laburnum Pulp, Pudding Pine Pulp, Pudding Pipe Pulp, Purging Fistula Pulp, Riding Pipe Pulp

Category Laxative.

Purging Cassia Pulp is the aril of the ripe pod of *Cassia fistula* L. (Family Leguminosae), Herbarium Specimen Number: DMSC 5162, BKF 114900, Crude Drug Number: DMSc 1142.

Constituents Purging Cassia Pulp contains anthraquinones (e.g., rhein and its glycosides). It also contains flavonoids, tannins, etc.

Description of the plant (Figs. 1a, 1b) Deciduous tree, up to 20 m high; bark smooth, greenish grey when young, becoming rough and dark brown when mature. Leaves pinnately compound, 10 to 60 cm long; petiole 7 to 10 cm long; rachis 15 to 25 cm long; stipule small and caducous; leaflets opposite, 3- to 8-paired, ovate to ovate-oblong, 6 to 20 cm long, 3.5 to 9 cm wide, apex acute to acuminate, base cuneate, upper surface glabrous, lower surface silvery pubescent when young becoming glabrous when mature. Inflorescence raceme, axillary, drooping, lax, many-flowered, 20 to 40(–60) cm long; pedicel 1.5 to 5 cm long; bract about 1 cm long, caducous. Flower: sepals 5, ovate-elliptic, about 1 cm long, velutinous, reflexed at anthesis; petals 5, subequal, broadly ovate or obovate, 2 to 3.5 cm long, 1 to 2 cm wide, distinctly veined, short-clawed, yellow; stamens 10, 3 long, 4 short and 3 reduced forms, long stamens much curled and bearing large oblong anther, opening by apical and basal slits, short stamens with straight filaments, reduced stamens with minute anther; ovary stalked, velutinous, style velutinous, stigma small. Fruit pod, terete, 20 to 60 cm long, 1 to 2.5 cm wide, pendulous, indehiscent, blackish brown to black. Seeds numerous, elliptic, 7.5 to 9 mm long, 5 to 7 mm wide, flat, glossy brown, embedded in blackish pulp, separated by spongy septa.

Description Odour, characteristic; taste, unpleasantly sweet.

Macroscopical (Fig. 1a) Sticky lump of pulp, dark brown to black.

Microscopical [**Note** Even though the pulp (aril) is used as a crude drug, microscopical description of the young pod is provided here for better understanding of the entire structure enclosing the pulp and the seed within.] (Figs. 2a, 2b, 2c, 2d) Transverse section of the pod shows pericarp and seed. Pericarp, consisting of a rectangular epidermal layer (rarely with unicellular trichome), parenchyma cells some of which containing prismatic crystals, vascular bundles, and layers of elongated sclereids. Seed, containing aril and seed coat; aril, parenchyma cells, some of which containing rosette aggregate crystals; seed coat, covered with thick cuticle layer, layers of non-lignified macrosclereids and two layers of non-lignified lagenosclereids separated by layers of thick-walled parenchyma.

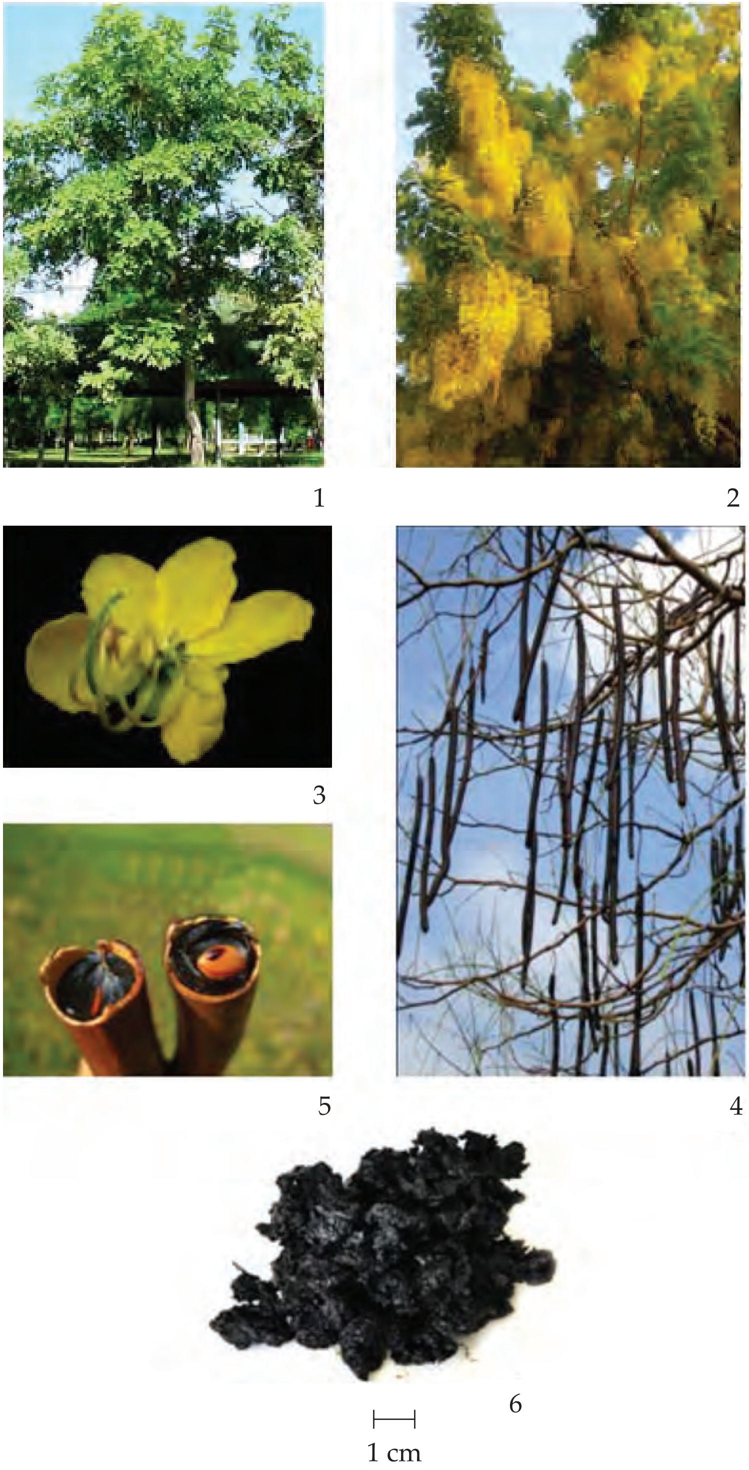

Fig. 1a *Cassia fistula* L.
1. habit 2. inflorescences 3. flower
4. ripe pods 5. pulp and seed inside ripe pod 6. crude drug

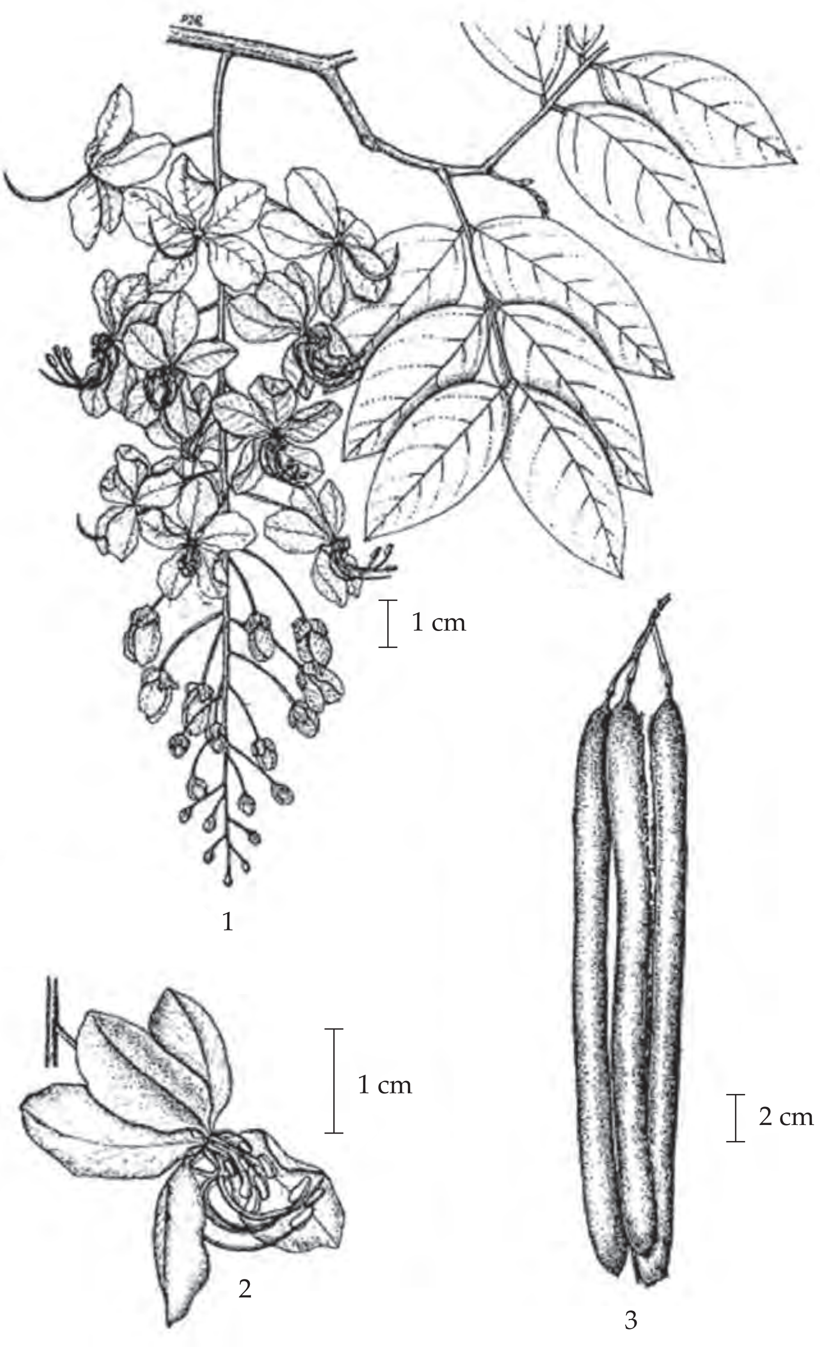

Fig. 1b *Cassia fistula* L.
1. flowering branch 2. flower 3. pods

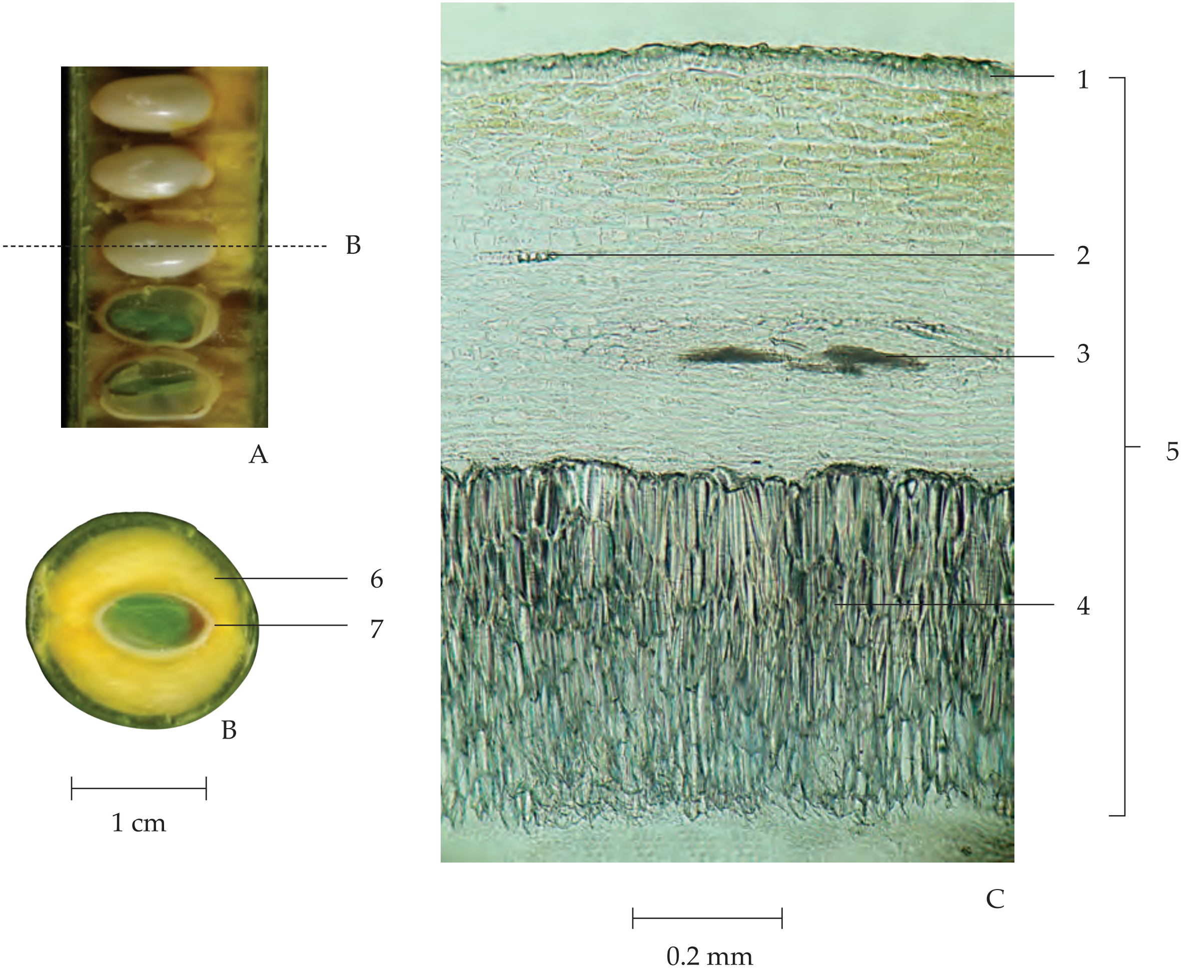

Fig. 2a Section of the Young Pod of *Cassia fistula* L.

A. Longitudinal Section of Part of the Pod
B. Transverse Section through the Seed
C. Photomicrograph of Transverse Section of the Pericarp

1. epidermis
2. prismatic crystal of calcium oxalate
3. group of spiral vessels
4. elongated sclereid
5. pericarp
6. pulp
7. seed coat

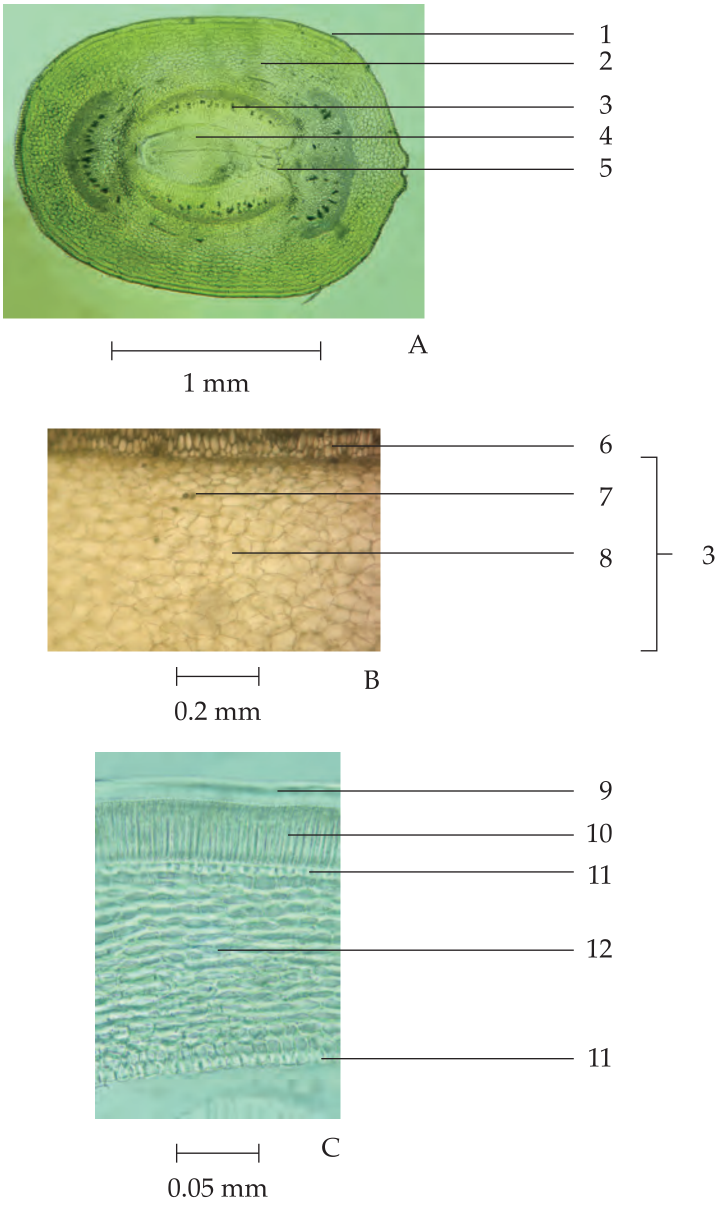

Fig. 2b Photomicrographs of Transverse Section of the Young Pod of *Cassia fistula* L.

A. Through the Seed and Funiculus
B. Part of the Pericarp and Pulp
C. Part of the Seed Coat

1. epidermis
2. pericarp
3. pulp
4. seed
5. funiculus
6. elongated sclereids of inner pericarp
7. rosette aggregate crystals
8. parenchyma of the pulp
9. cuticle
10. macrosclereids
11. lagenosclereids
12. thick-walled parenchyma

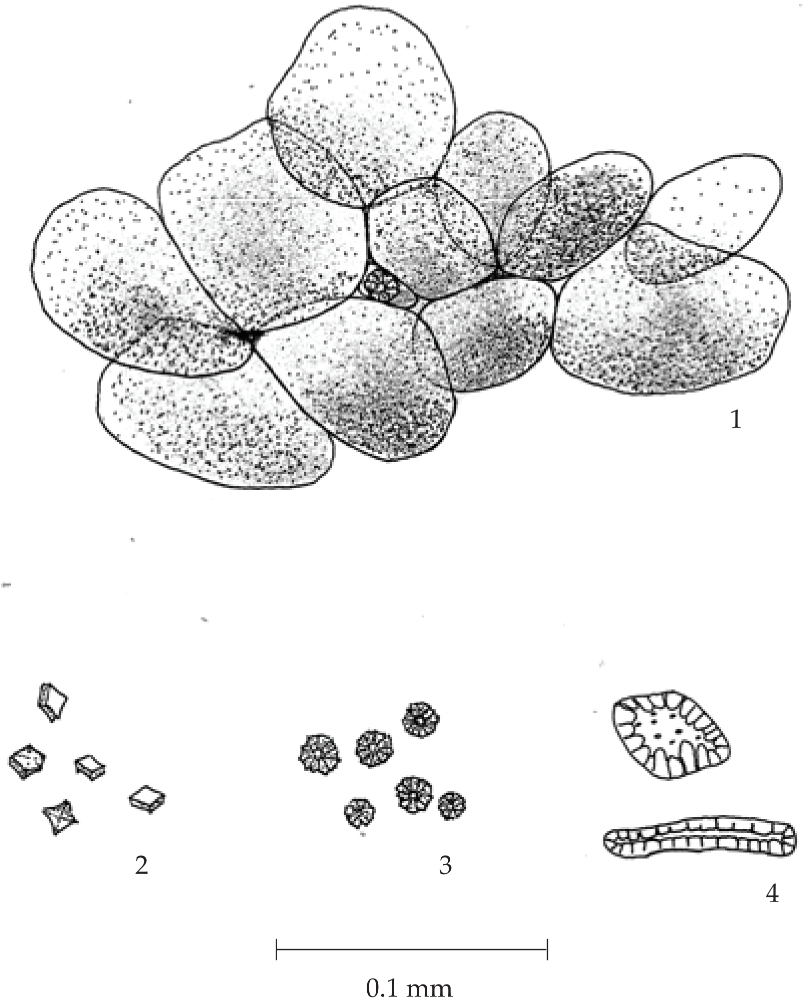

Fig. 2c Line Drawings of Powdered Drug of the Pulp of *Cassia fistula* L.
1. parenchyma containing microcrystals and brown pigment, some with rosette aggregate crystals
2. prismatic crystals
3. rosette aggregate crystals
4. sclereids

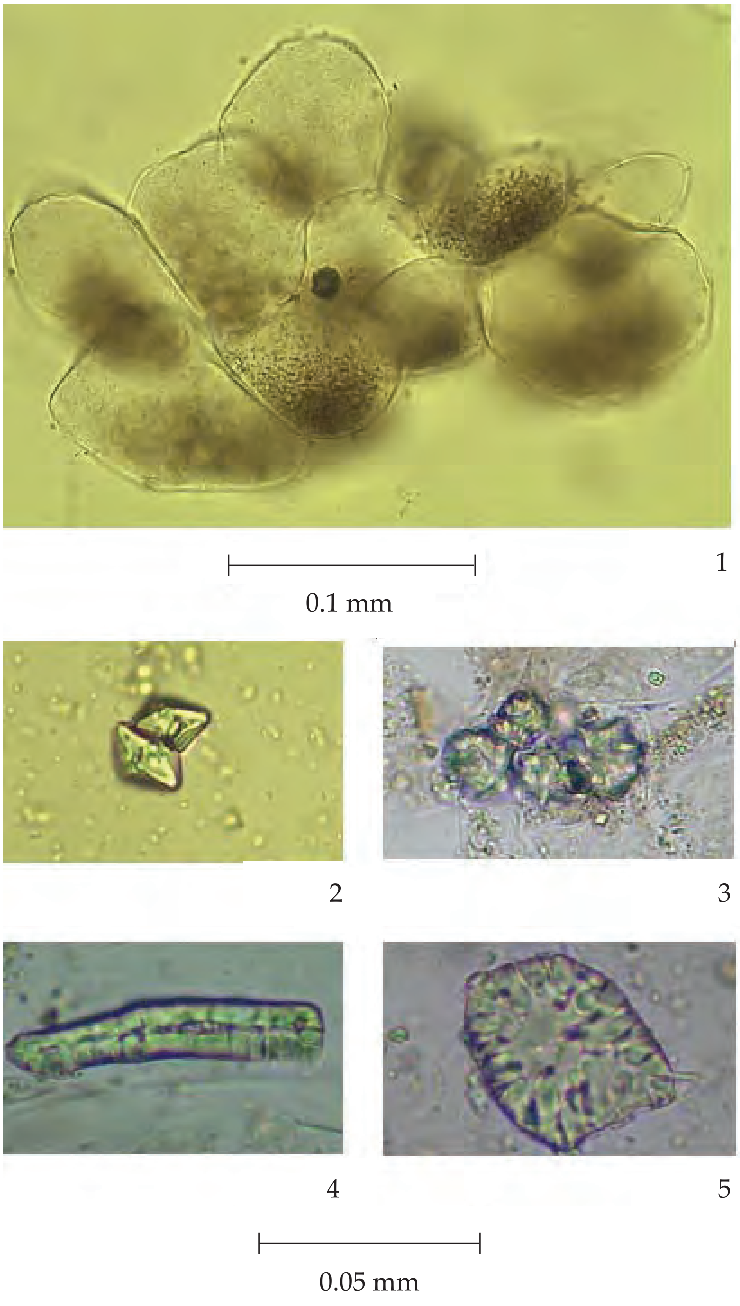

Fig. 2d Photomicrographs of Powdered Drug of the Pulp of *Cassia fistula* L.

1. parenchyma containing microcrystals and brown pigment, some with rosette aggregate crystals
2. prismatic crystals
3. rosette aggregate crystals in parenchyma
4. elongated sclereids
5. rhomboidal sclereid

Purging Cassia Pulp possesses the diagnostic microscopical characters of the aril of the drug. The parenchyma cells containing brownish to blackish granular mass is characteristic.

Contra-indication It is contra-indicated in patients with constipation from intestinal obstruction, diarrhoea, abdominal pain, nausea, and vomiting.

Warning

1. Prolonged use should be avoided since it may result in laxative dependence.
2. Pod shell and seeds should be removed from the pulp since they may cause vomiting.

Additional information

1. Although in Thai traditional remedies the crude drug is usually addressed as “ฝักคูน (FAK KHUN)” which means a purging cassia pod, only the pulp after removal of shell and seeds [เนื้อในฝักคูน (KHUN, NUEA NAI FAK)] is used in the preparations. Therefore, it is chosen to be the title of this monograph.
2. To obtain its maximum benefit, it is recommended that the pulp be freshly taken from the purging cassia pod.

Packaging and storage Purging Cassia Pulp shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. To 3 g of the sample, add 25 ml of 2 M *hydrochloric acid*, heat on a water-bath for 30 minutes, and immediately filter through a plug of cotton wool. Allow the filtrate to cool and shake with 20 ml of *dichloromethane*. Collect the dichloromethane layer and shake with 10 ml of *ammonia TS*: the aqueous layer becomes red.

B. Carry out the test as described in the “Thin-layer chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 100 volumes of *ethyl acetate*, 17 volumes of *methanol* and 3 volumes of *water* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, 4 µl each of the following solutions. Prepare solution (A) by refluxing 5 g of the sample with 50 ml of *chloroform* on a water-bath for 30 minutes, filtering and evaporating the filtrate to dryness. Dissolve the residue in 1.0 ml of *chloroform*. For solution (B) dissolve 3 mg of *rhein* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under daylight, locating the spots. The chromatogram obtained from solution (A) shows a yellow spot corresponding to the rhein spot (hR_f value 49 to 52) from solution (B). Subsequently examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot, corresponding to the rhein spot from solution (B), and other two quenching spots of the lower hR_f values. Examine the plate under ultraviolet light (366 nm); the spot due to rhein is a brownish purple; one blue fluorescent spot is also observed. Spray the plate with a 10 per cent w/v solution of *potassium hydroxide* in *ethanol*; the spot corresponding to rhein is reddish pink (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Chloroform Extract of the Pulp of the Ripe Pods of *Cassia fistula* L.

Spot	hR_f Value	Detection			
		Daylight	UV 254	UV 366	10 Per Cent W/V Solution of *Potassium Hydroxide* in *Ethanol*
1	25-28	–	quenching	–	–
2	30-32	–	–	blue	–
3	40-44	–	quenching	–	–
4*	49-52	yellow	quenching	brownish purple	reddish pink

*rhein

Loss on drying Not more than 14.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Total ash Not more than 4.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 66.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 67.0 per cent w/w (Appendix 7.12).

Dose 3 to 10 g at bed time.

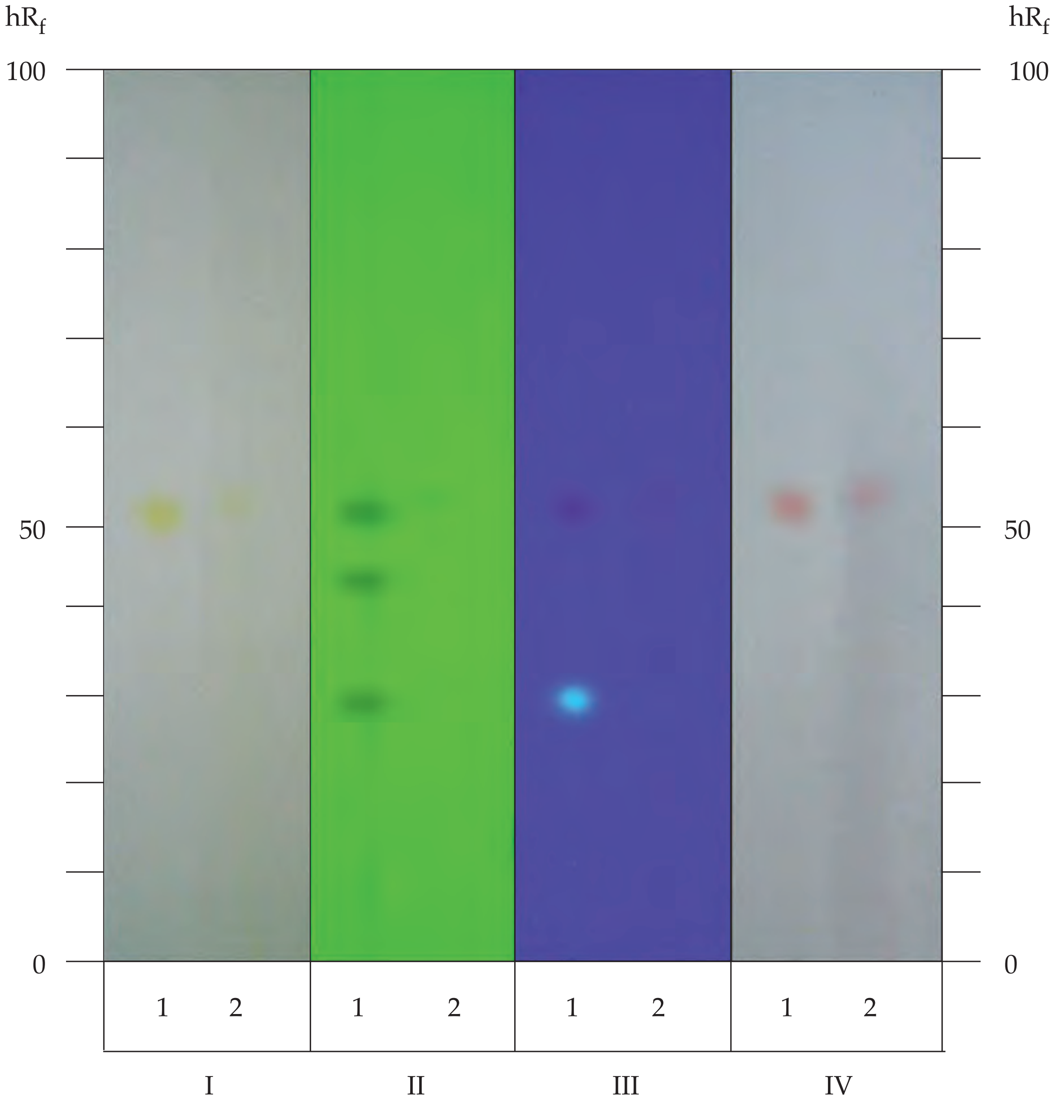

Fig. 3 Thin-layer Chromatography of Chloroform Extract of the Pulp of the Ripe Pods of *Cassia fistula* L.

1 = solution (A)
2 = solution (B)
I = detection under daylight
II = detection under UV light (254 nm)
III = detection under UV light (366 nm)
IV = detection with a 10 per cent w/v solution of *potassium hydroxide* in *ethanol*

โกฐเชียง (KOT CHIANG)

ตังกุยบ้วย (TANG KUI BOUA), กุยเหว่ย (KUIWEI)

Angelicae Sinensis Radix Lateralis
Chinese Angelica Lateral Root

Category Blood tonic, treatment of menstrual disorders.

Chinese Angelica Lateral Root is the dried lateral root of *Angelica sinensis* (Oliv.) Diels (*A. polymorpha* var. *sinensis* Oliv.) (Family Umbelliferae), Herbarium Specimen Number: see *Additional information 1*, Crude Drug Number: DMSc 1139.

Constituents Chinese Angelica Lateral Root contains alkyl phthalides (e.g., *Z*-ligustilide, *Z*-butylidenephthalide, angelicide) and phenylpropanoids (e.g., *E*-ferulic acid, coniferyl ferulate). It also contains terpenoids (e.g., carvacrol, *cis*-*β*-ocimene, *β*-cadinene), benzenoids (e.g., vanillic acid), polysaccharides, etc.

Description of the plant (Figs. 1a, 1b) Perennial herb, up to 1 m high; stem purplish green, linear striation, branched above, glabrous; root stout, conic or cylindrical, branched, lateral roots numerous. Leaves pinnately or ternately compound, alternate, blade ovate in outline, 10 to 30 cm long, 12 to 25 cm wide; pinnae 3 to 4 pairs; leaflets ovate or ovate-lanceolate, 2 to 3.5 cm long, 0.8 to 2.5 cm wide, 2- to 3-lobed, margin irregularly cuspidate-serrate, nerves and margins sparsely papillose to hairy; petioles 5 to 20 cm long, purplish green, base of petiole sheathing, inflated, ovate, margin membranous. Inflorescence compound umbel, terminal or axillary, umbels 10 to 30; peduncle 8 to 20 cm long, pubescent or subglabrous; bract(s) absent or 2, linear, unequal; bracteoles 2 to 4, linear, 3 to 5 mm long, glabrous. Flowers small, white, rarely purplish red, 13 to 36 per umbel; pedicel slender, 1 to 3 cm in fruit; calyx teeth obsolete; petals 5, obovate, apex incurved; ovary inferior, 2-loculed, 1-ovule per locule, stylopodium shortly conic. Fruit schizocarp, ellipsoid, ovoid or subglobose, 4 to 6 mm long, 3 to 4 mm wide, dorsal ribs prominent, filiform, lateral ribs broadly thin-winged, wings as wide as or wider than the body, vitta(e) 1 on each furrow, absent or 2 on commissure, split into 2 single-seeded mericarps. Seed plane or slightly concave.

Description Odour, strongly aromatic; taste sweet, slightly bitter and pungent.

Macroscopical (Fig. 1a) Lateral roots 0.3 to 1 cm in diameter, the upper portion thick and the lower portion thin, mostly twisted and exhibiting a few rootlet scars. Texture flexible, fracture yellowish white or yellowish brown, bark thick, showing some clefts and numerous brown-dotted secretory cavities, wood paler in colour, cambium ring yellowish brown.

Microscopical (Figs. 2a, 2b) Transverse section of the dried lateral root shows periderm, cortex, phloem, xylem, schizogenous oil cavities, longitudinal clefts, cambium, and pith. Periderm, 4 to 6 layers of thin-walled, rectangular cork cells. Cortex, narrow, with polygonal parenchyma cells, oil cavities and longitudinal clefts. Xylem, solitary or groups, 2 to 5, radially arranged, surrounded by small parenchyma; xylem rays 3 to 7 layers of cells. Phloem, broad, with polygonal parenchyma, oil cavities and longitudinal clefts. Cambium in a ring, several layers of small rectangular cells. Schizogenous oil cavities, abundant, scattered from phloem tissues to cortex, diameter increase from phloem tissues to outer part of cortex. Pith, distinct, several layers of parenchyma cells filled with starch grains.

Fig. 1a *Angelica sinensis* (Oliv.) Diels
1. flowering and fruiting shoot 2. and 3. inflorescences 4. crude drug

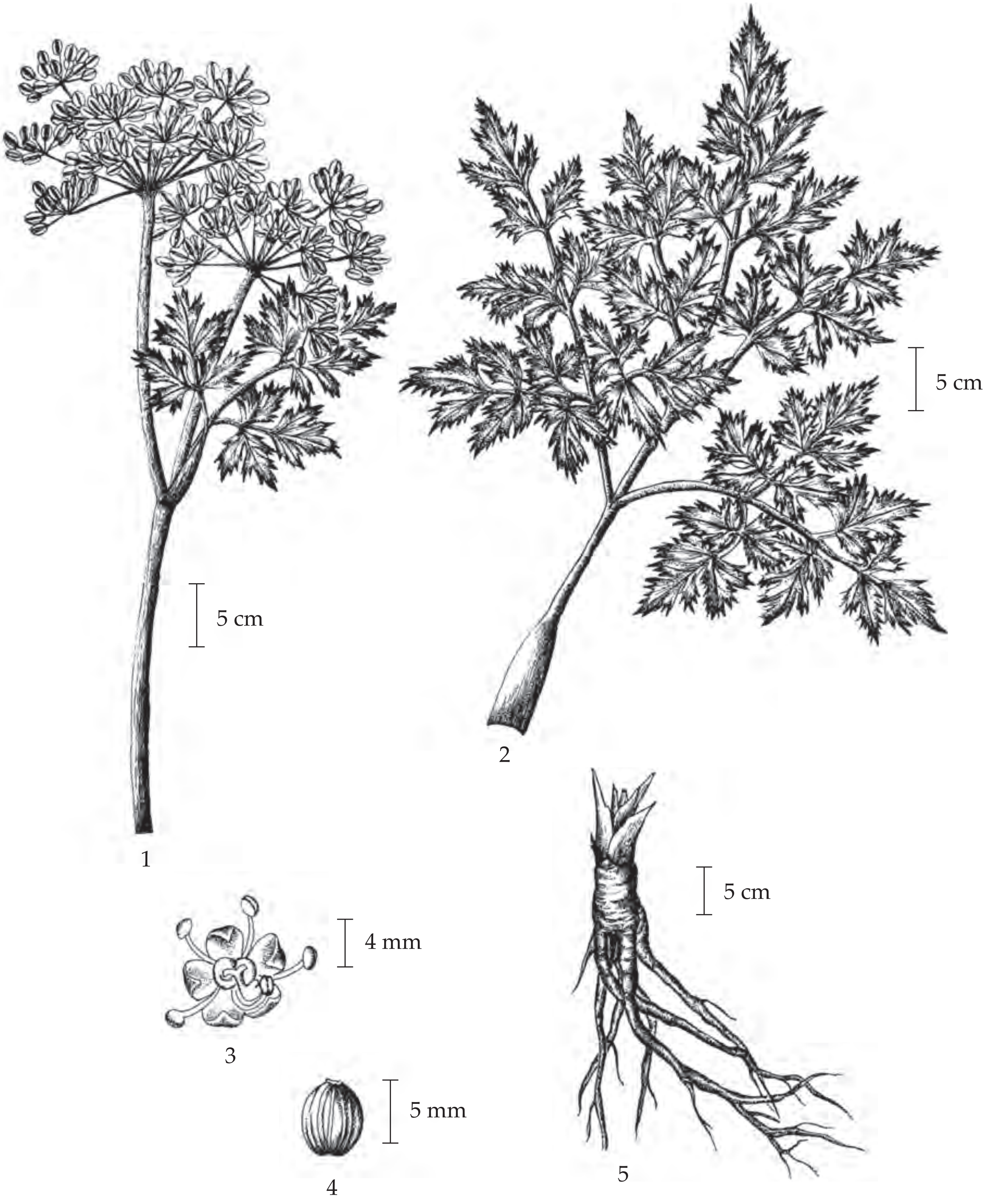

Fig. 1b *Angelica sinensis* (Oliv.) Diels
1. fruiting shoot 2. leaf 3. flower (top view) 4. schizocarp 5. root

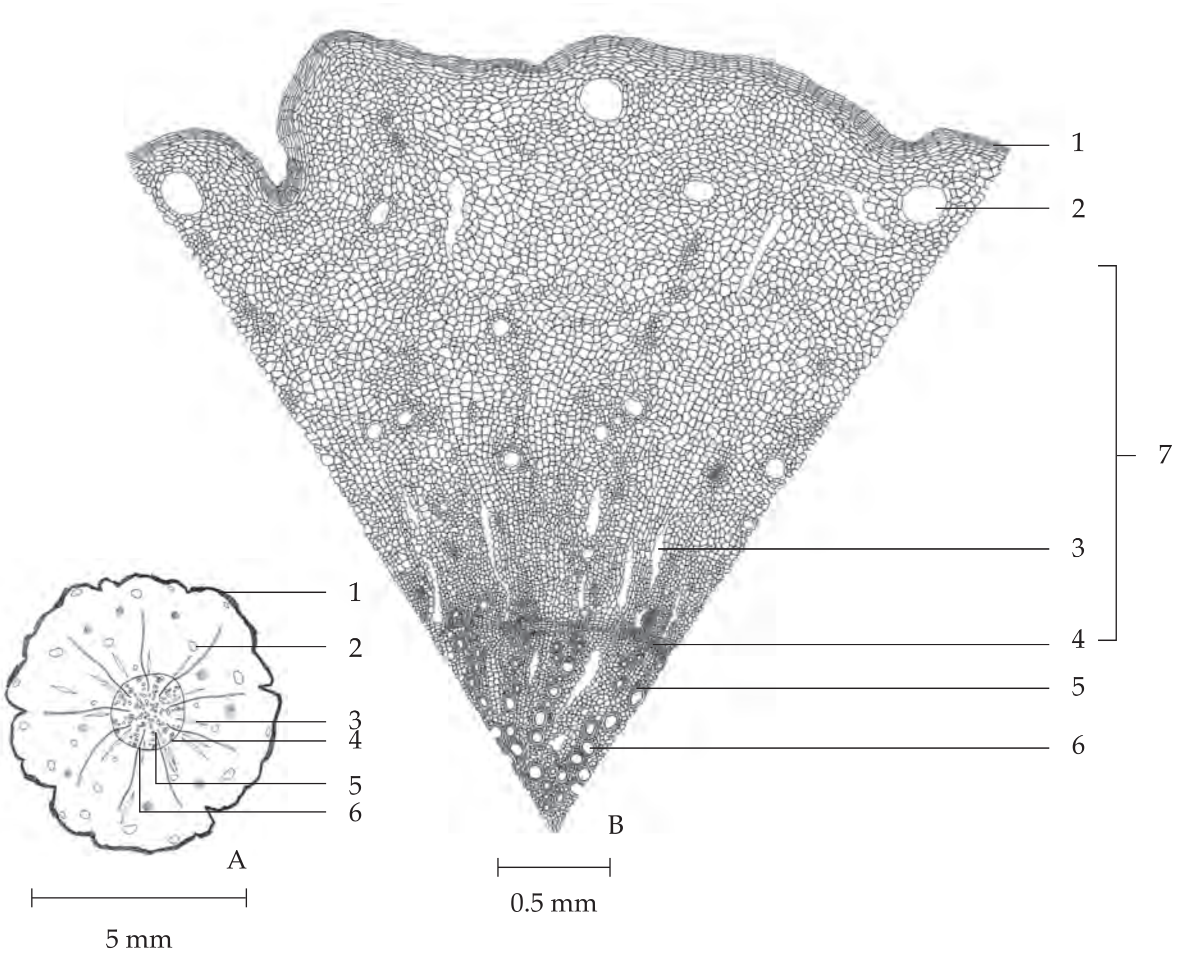

Fig. 2a Transverse Section of the Lateral Root of *Angelica sinensis* (Oliv.) Diels

A. Diagram

B. Part of Sectional View

1. cork
2. oil cavity
3. longitudinal cleft
4. cambium
5. xylem ray
6. vessel
7. phloem

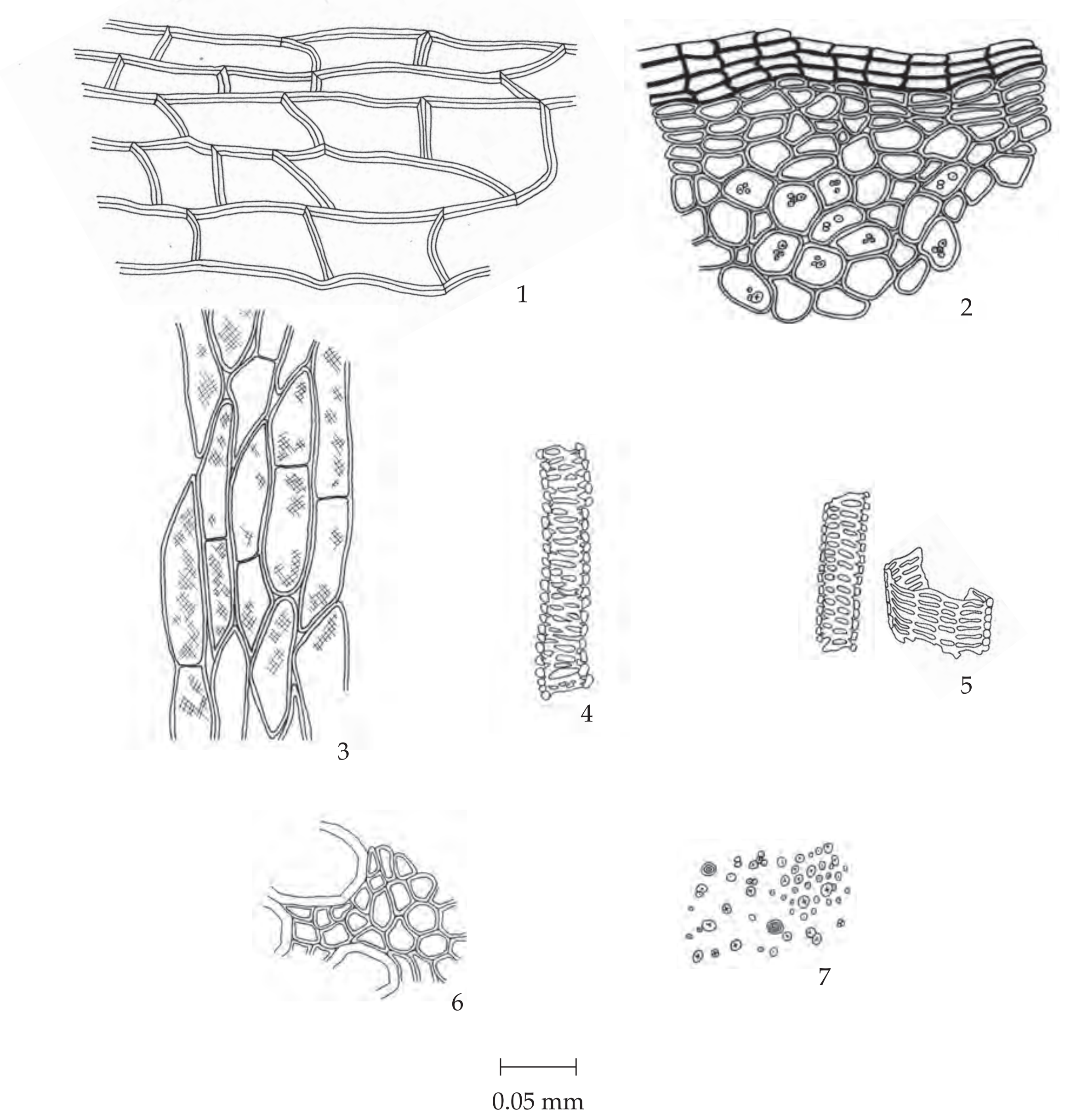

Fig. 2b Powdered Drug of the Lateral Roots of *Angelica sinensis* (Oliv.) Diels

1. cork in surface view
2. cork in sectional view associated with cortical parenchyma, some containing starch grains
3. fusiform-shaped parenchyma with very fine oblique criss-cross striations, some with septum
4. fragment of reticulate vessel
5. fragments of scalariform vessels
6. fragment of vessels in sectional view associated with parenchyma
7. starch grains

Chinese Angelica Lateral Root in powder possesses the diagnostic microscopical characters of the unground drug. Fusiform phloem parenchyma with slightly thick-walled, very fine oblique criss-cross striations and thin-walled septum is characteristic. Large and thin-walled cork, or cork associated with parenchyma, is frequently observed.

Contra-indication

1. It is contra-indicated in patients prior to surgery and in patients with diarrhoea, hemorrhagic diseases or hypermenorrhoea.
2. It is contra-indicated in children and in pregnant and nursing women.

Warning

1. It may cause phototoxicity, i.e. eczema, burns.
2. Caution should be exercised when it is to be used concomitantly with anticoagulant drugs such as warfarin and antiplatelet drugs.

Additional information

1. Chinese angelica plant is not native to nor commercially cultivated in Thailand. The plant yielding chinese angelica lateral root is here referred to the herbarium specimen, collector's number Aug. Henry 7143, deposited at New York Botanical Garden, New York, the United States of America. The photographic illustration of which can be seen at the Department of Medical Sciences Herbarium (DMSC), Nonthaburi, Thailand.
2. It is commonly used with other herbal drugs in Thai traditional herbal preparations.
3. It is recommended to keep in a refrigerator.

Packaging and storage Chinese Angelica Lateral Root shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 1 g of the sample, in powder, with 10 ml of *ethanol* for 30 minutes and filter (solution 1). To 2 ml of solution 1, add a few drops of *iron(III) chloride TS*: a green colour is produced.

B. Drop solution 1 on a filter paper and examine under ultraviolet light (366 nm): a blue fluorescence is produced.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 86 volumes of *toluene*, 14 volumes of *ethyl acetate* and 5 volumes of *formic acid* as the mobile phase. Apply separately to the plate as bands of 10 mm, 25 µl of solution (A) and 10 µl of solution (B). Prepare solution (A) by refluxing 1 g of the sample, in powder, with 10 ml of *dichloromethane* for 30 minutes and filtering. Evaporate the filtrate until dryness and dissolve the residue in 1 ml of *toluene*. For solution (B), dissolve 1 mg of *ferulic acid* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. The chromatogram obtained from solution (A) shows a quenching band (hR_f value 31 to 38) corresponding to the ferulic acid band from solution (B). Other several quenching bands are also observed. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter; the band corresponding to ferulic acid shows violet fluorescence. Other several fluorescent bands of different colours are also observed. Spray plate with *anisaldehyde TS* and heat at 105° for 10 minutes; the band corresponding to ferulic acid is violet. Other several bands of different colours also appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Dichloromethane Extract of the Lateral Roots of *Angelica sinensis* (Oliv.) Diels

Band	hR_f Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	2-5	–	–	violet
2	5-9	–	–	green
3	7-13	–	–	violet
4	15-20	–	–	violet
5	26-32	–	violet	–
6	29-35	–	yellow	–
7*	31-38	quenching	violet	violet
8	39-46	quenching	violet	grey
9	52-58	weak quenching	light blue	green
10	57-63	–	–	violet
11	57-65	weak quenching	blue	–
12	64-71	weak quenching	intense blue	green
13	69-75	–	–	green
14	71-79	quenching	intense blue	yellow
15	79-82	quenching	–	–
16	89-95	quenching	–	dark green

*ferulic acid

Water Not more than 14.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Total ash Not more than 7.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 12.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 52.0 per cent w/w (Appendix 7.12).

Dose 6 to 12 g, as a decoction, a day.

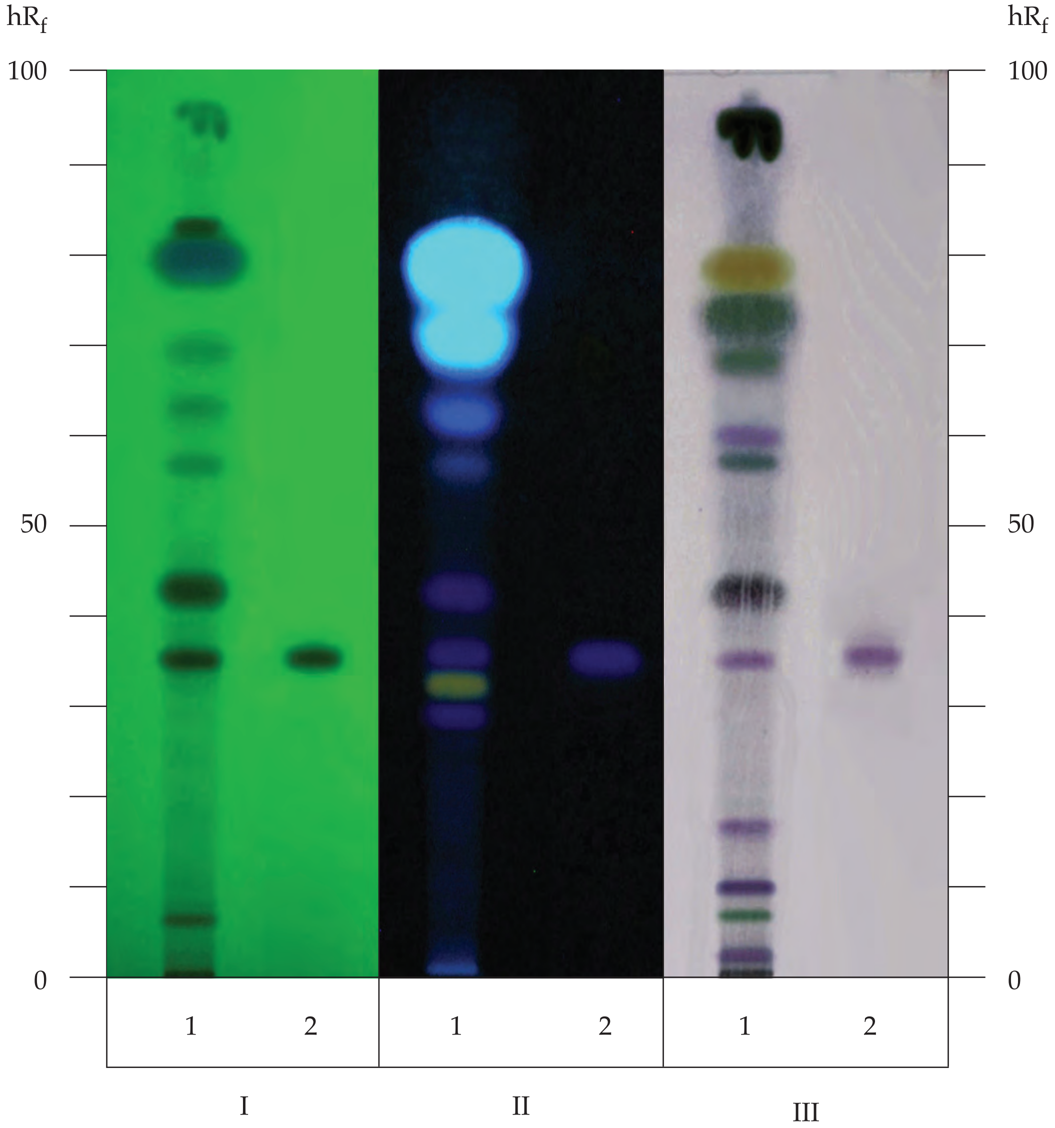

Fig. 3 Thin-layer Chromatogram of Dichloromethane Extract of the Lateral Roots of *Angelica sinensis* (Oliv.) Diels

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *anisaldehyde TS*

โกฐจุฬาลัมพา (KOT CHULA LAMPHA)

โกฐจุฬา (KOT CHULA), โกดจุฬาลัมพา (KOT CHULA LAMPHA)[1], โกฐจุฬาลัมพาจีน (KOT CHULA LAMPHA CHIN), ชิงเฮา (QINGHAO)
Artemisiae Annuae Herba
Sweet Wormwood Herb

Synonyms Annual Wormwood, Chinese Wormwood

Category Antipyretic.

Sweet Wormwood Herb is the dried aerial part of *Artemisia annua* L. (Family Compositae), Herbarium Specimen Number: see *Additional information 1*, Crude Drug Number: DMSc 1147.

(**Note** Sweet Wormwood Herb should be harvested during the blooming period.)

Constituents Sweet Wormwood Herb contains sesquiterpenoids (e.g., artemisinin, artemisinic acid, arteannuin B). It also contains volatile oil, flavonoids, coumarins, etc.

Description of the plant (Figs. 1a, 1b) Annual herb, 70 to 150 cm high, fragrant; stem furrowed, much branched, puberulous, soon glabrous. Leaves simple, spirally arranged, oblong or ovate in outline, 3 or 4 pinnatipartite, glandular-dotted; petioles of middle cauline leaves 1 to 2 cm long; radical leaves withering early; lowermost cauline leaves ovate or triangular-ovate, 3 to 7 cm long, 2 to 6 cm wide; segments 5 to 10 pairs; lobule deeply serrate; tooth triangular, 1 to 2 mm long, 0.5 mm wide; midvein prominent adaxially; rachis narrowly winged, sparsely serrate; middle cauline leaves 2 or 3-pinnati or pectinatisect; upper leaves and leafy bract 1 or 2 pinnatipartite. Inflorescences in broad pyramidal paniculate; capitula globose, numerous, 1 to 3 mm in diameter, nodding, shortly pedicellate; bracts linear, oval-acuminate or oval; invoucre globose, glabrous; phyllaries 2 to 3 seriate, the outer ones narrowly oblong, green, the inner elliptic or obovate, margin hyaline. Marginal florets pistillate, 10 to 18 minute; disc florets bisexual, 10 to 30, minute, dark yellow or yellow. Achenes ellipsoid-ovoid, faintly-nerved, glabrous.

Description Odour, characteristic and aromatic; taste, slightly bitter.

Macroscopical (Fig. 1a) Stems, vary in diameter and length, cylindrical, furrowed, greenish brown to brown; texture slightly hard, fracture medullated in the centre. Leaves, greenish brown to brown, rolled and crumpled, easily broken. Capitula abundant, 2 to 3 mm in diameter.

Microscopical (Figs. 2a, 2b, 2c, 2d, 2e) Transverse section of the fresh leaflet shows epidermis, mesophyll, and vascular bundle. Epidermis, a layer of rectangular cells with glandular trichomes and slightly-raised stomata, T-shape trichomes may be seen. Mesophyll, bifacial, 1 to 3 layers of palisade cells and thin-walled parenchyma cells in between. Vascular bundle, xylem in the upper part and phloem in the lower part.

Transverse section of the fresh stem illustrates epidermal layer, cortex, vascular tissue, and pith. Epidermal layer, rectangular cells, covering with cuticle layer. Cortex, few layers of angular collenchyma cells, groups of angular collenchyma cells and groups of cortical fibre cells in small ridges.

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[1] The two Thai names, โกฐจุฬาลัมพา and โกดจุฬาลัมพา, are spelled differently in Thai. However, both terms can be transcribed into the same English phonetic transcription “KOT CHULA LAMPHA”.

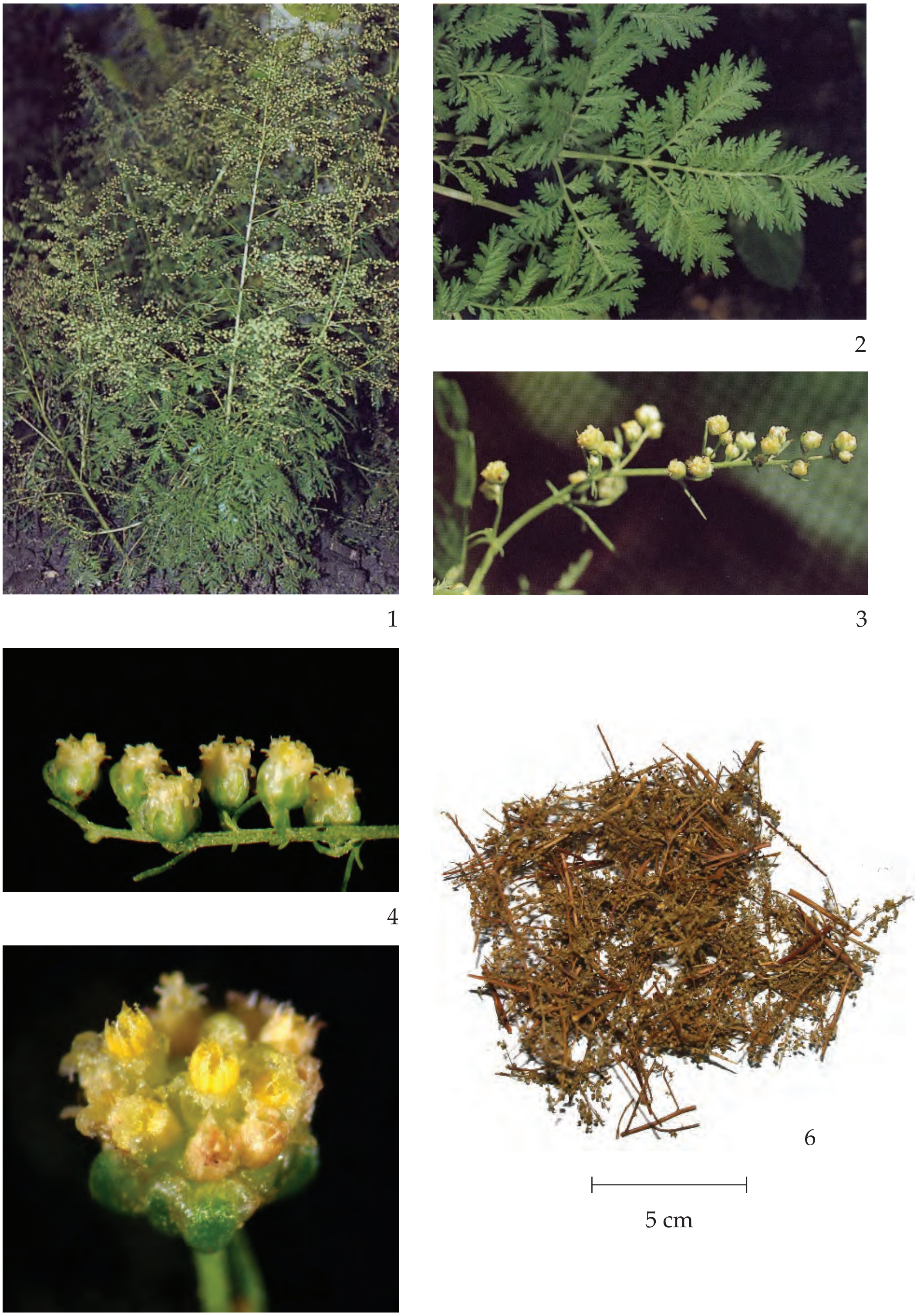


Fig. 1a *Artemisia annua* L.
1. habit 2. part of leaf 3. and 4. part of flowering twig 5. capitulum 6. crude drug

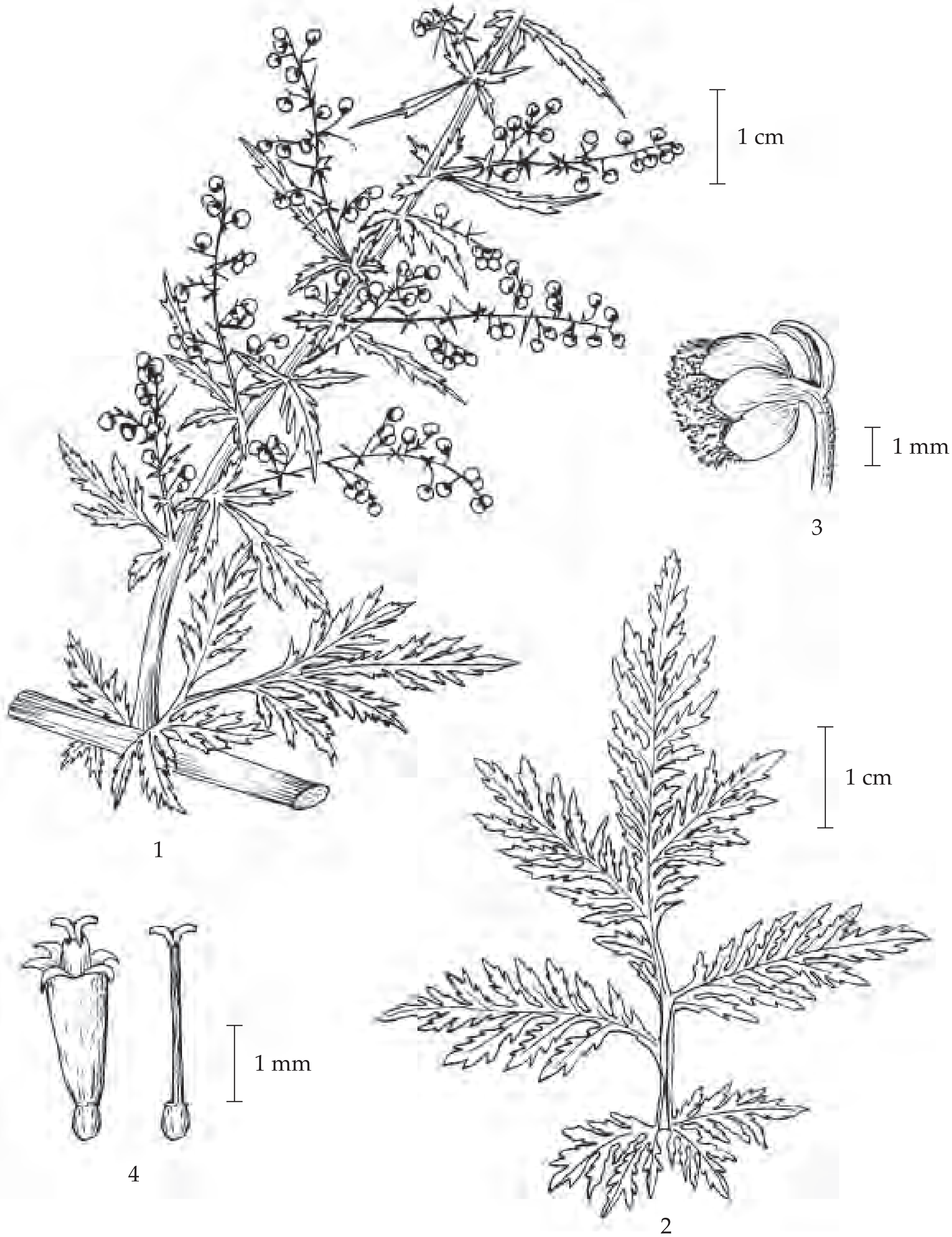
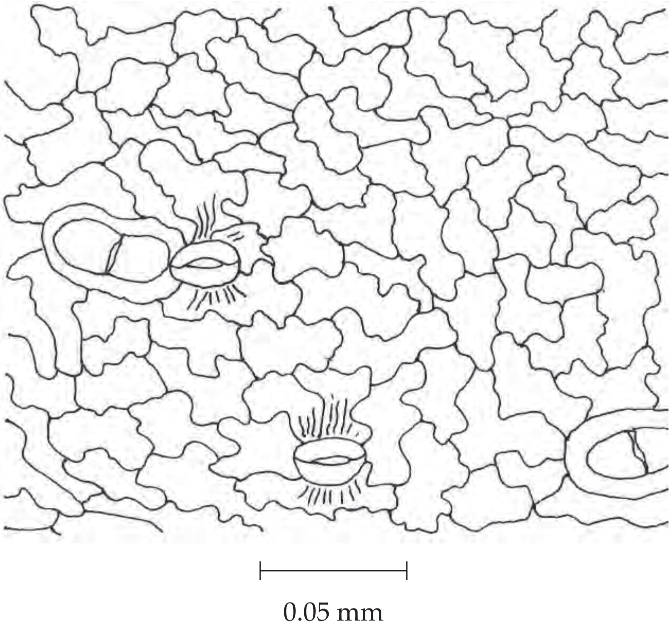
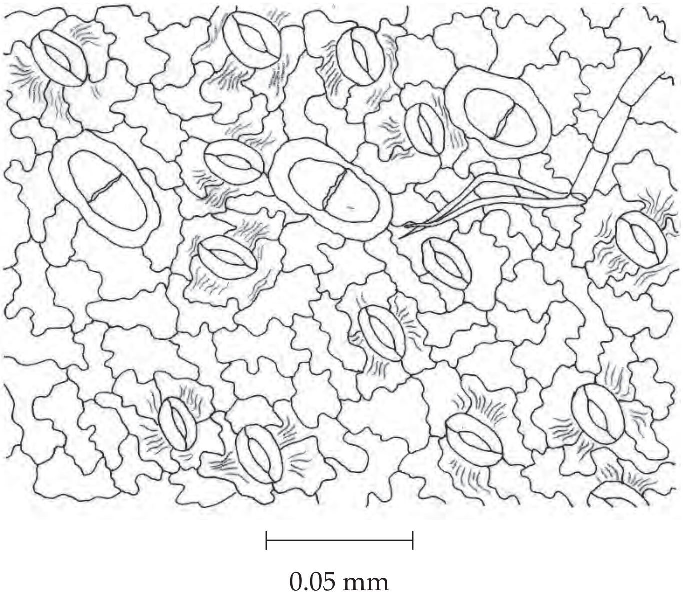


Fig. 1b *Artemisia annua* L.

1. flowering branch 2. part of leaf 3. capitulum 4. disc floret and pistil



Upper Epidermis of the Lamina



Lower Epidermis of the Lamina

Fig. 2a Epidermises of the Fresh Leaf of *Artemisia annua* L.

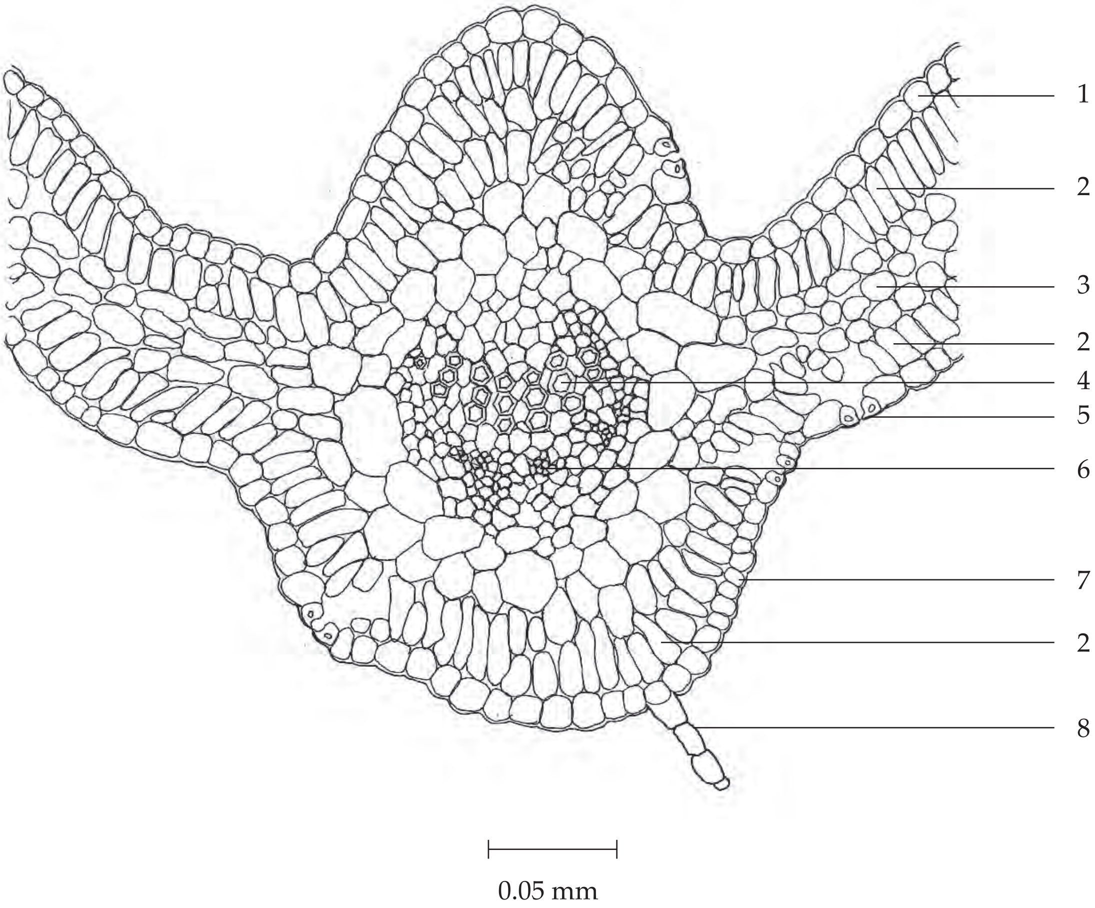


Fig. 2b Transverse Section of the Fresh Leaf of *Artemisia annua* L.

1. upper epidermis
2. cell
3. parenchyma
4. vessel
5. slightly-raised stoma
6. phloem cell
7. lower epidermis
8. stalk of T-shaped trichome

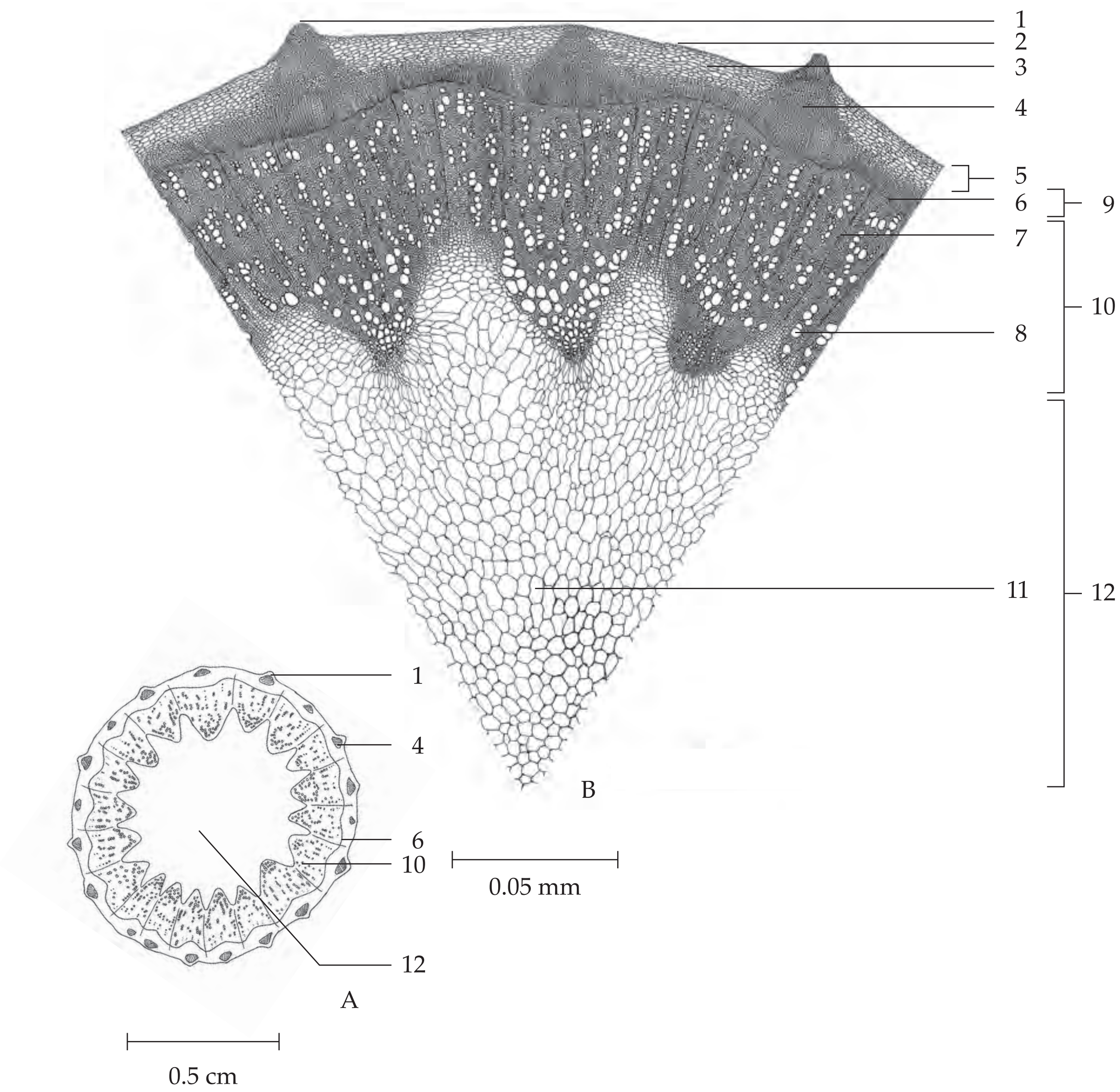


Fig. 2c Transverse Section of the Fresh Stem of *Artemisia annua* L.

A. Diagram
B. Part of Transverse Section

1. ridge
2. epidermis
3. angular collenchyma
4. cortical fibre
5. cortex
6. cambium
7. xylem ray
8. vessel
9. phloem tissue
10. xylem tissue
11. parenchyma
12. pith

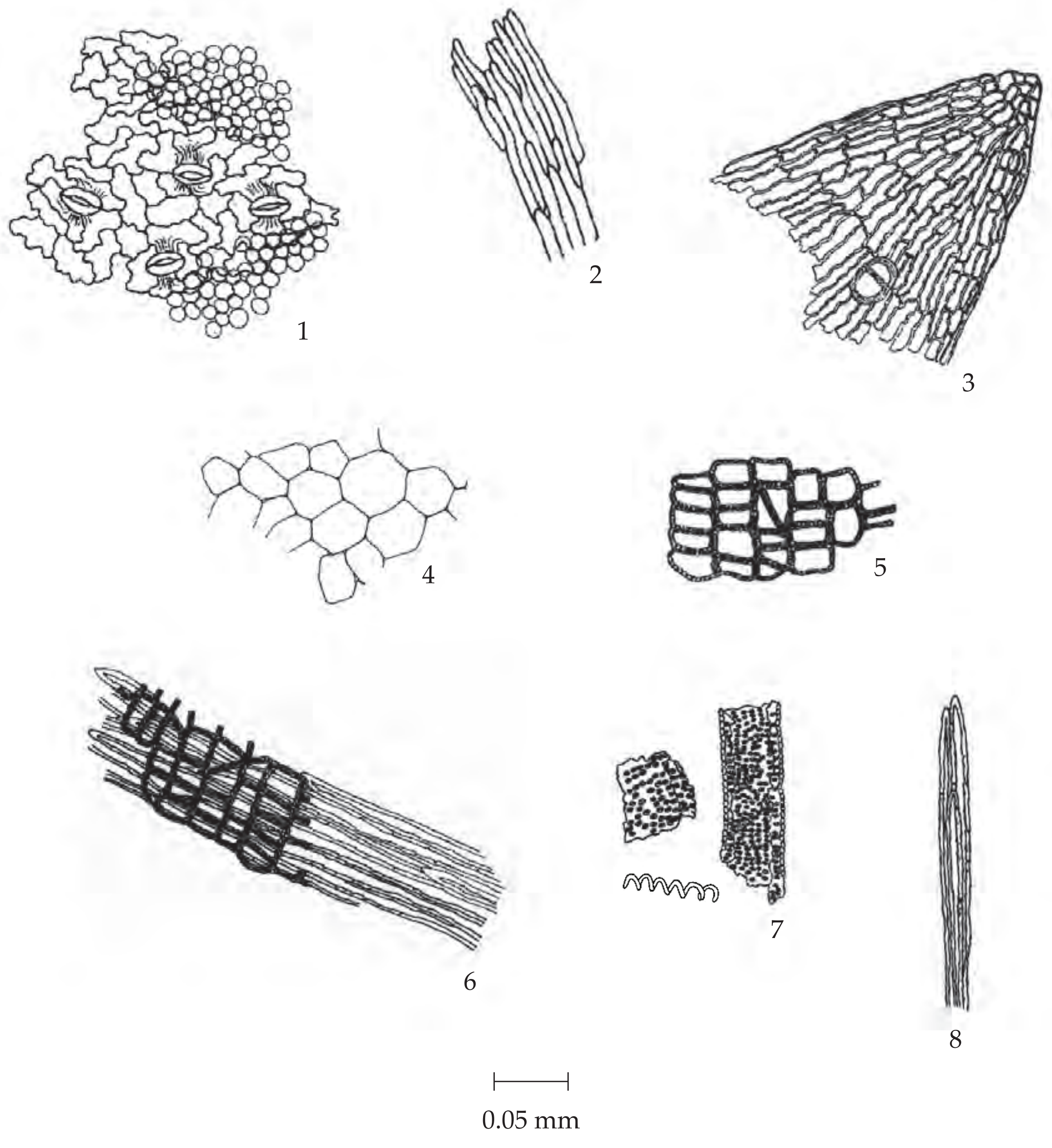


Fig. 2d Line Drawings of Powdered Drug of the Aerial Parts of *Artemisia annua* L.

1. epidermis with stomata and underlying palisade cells in surface view
2. epidermis of corolla in surface view
3. epidermis at apex of corolla showing glandular trichome in surface view
4. parenchyma
5. xylem parenchyma
6. fibres and xylem parenchyma in radial longitudinal view
7. fragment of bordered-pitted and spiral vessels
8. fibres

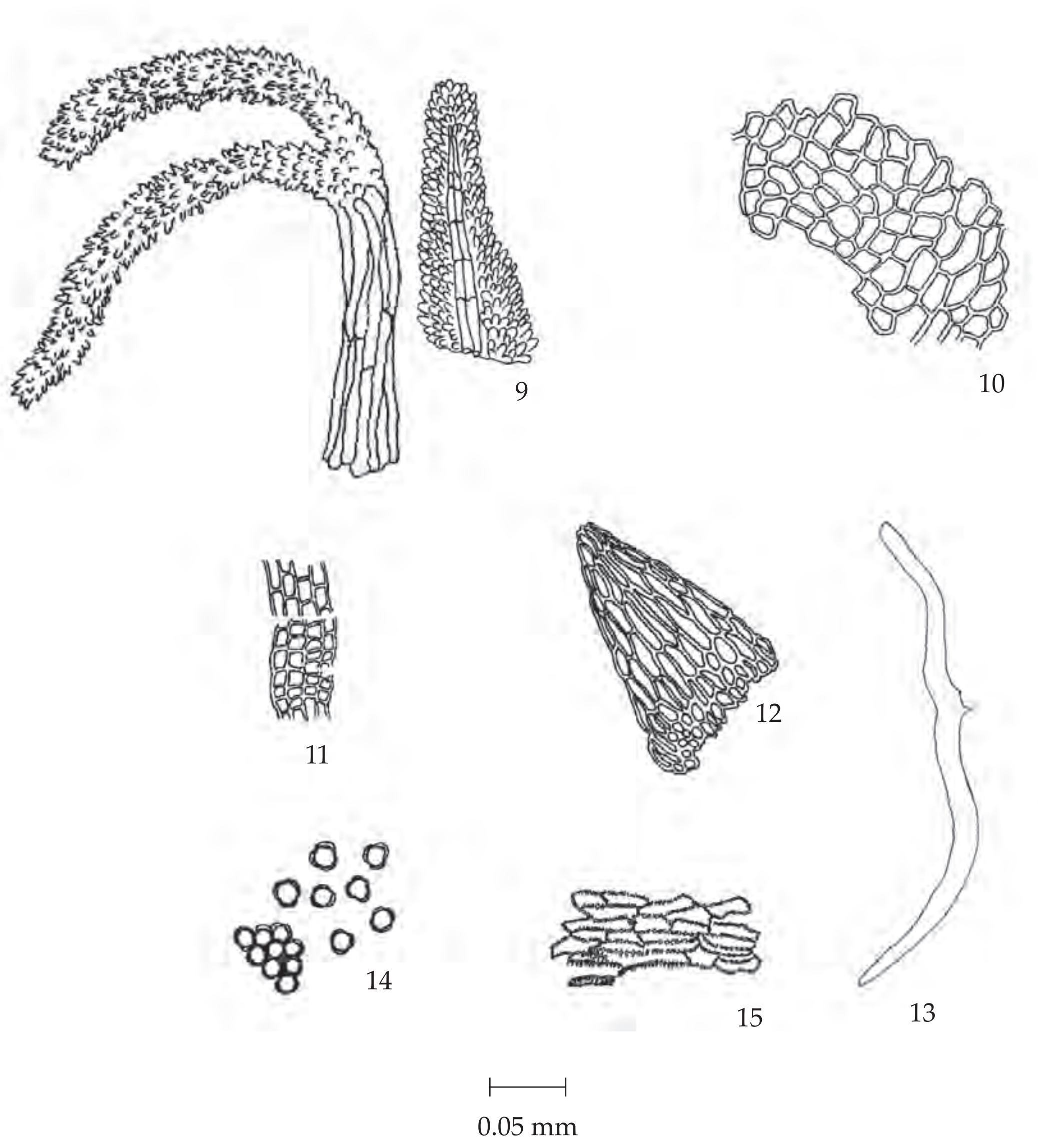


Fig. 2d Line Drawings of Powdered Drug of the Aerial Parts of *Artemisia annua* L. (continued)

9. papillosed stigma in surface view
10. ovary wall in surface view
11. filament of stamen in surface view
12. tip of anther lobe in surface view
13. part of T-shaped trichome
14. pollen grains
15. fibrous layer of anther sac in surface view

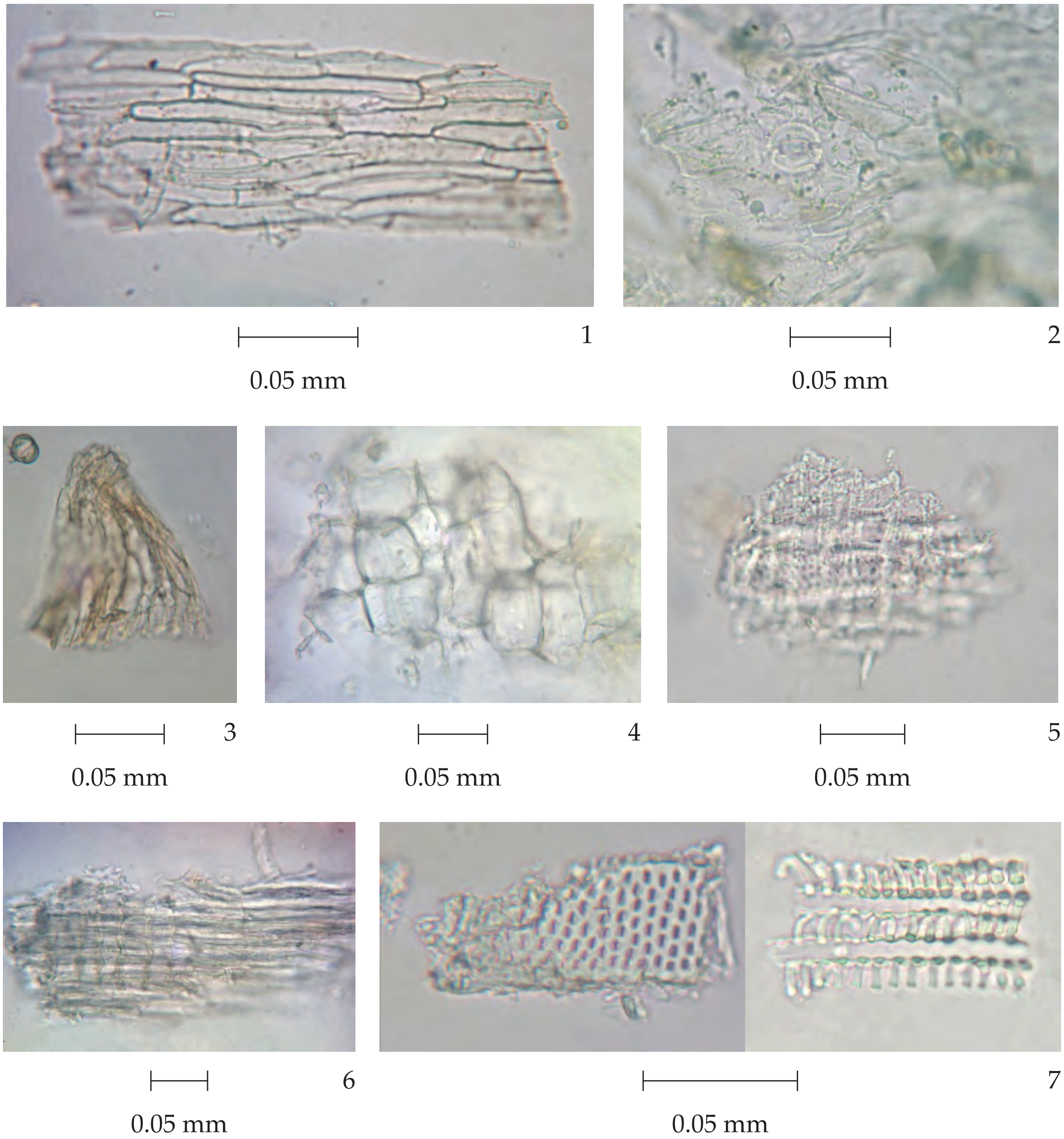


Fig. 2e Photomicrographs of Powdered Drug of the Aerial Parts of *Artemisia annua* L.

1. epidermis with stomata in surface view
2. epidermis of corolla in surface view
3. epidermis at apex of corolla in surface view
4. parenchyma
5. xylem parenchyma with underlying bordered-pitted vessels
6. fibres and xylem parenchyma in radial longitudinal view
7. bordered-pitted vessel and spiral vessels

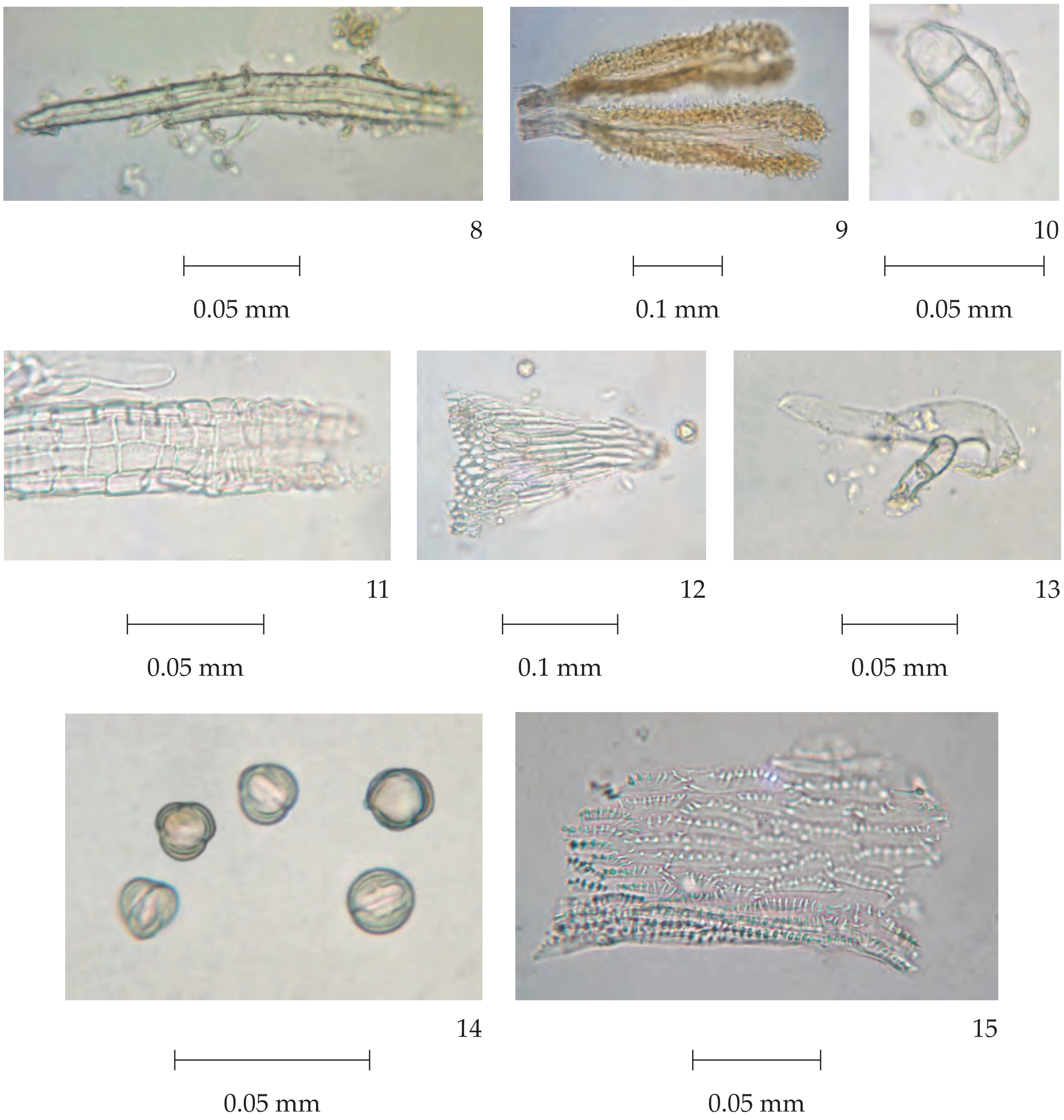


Fig. 2e Photomicrographs of Powdered Drug of the Aerial Parts of *Artemisia annua* L. (continued)

8. fibres
9. 2-branched papillosed stigmas in surface view
10. top view of 2-celled glandular trichome
11. filament in surface view
12. anther-lobed tip in surface view
13. T-shaped trichome
14. pollen grains
15. fibrous layer of anther sac in surface view

Vascular tissue, narrow zone of phloem tissue, vascular cambium, large zone of xylem tissue, and 1 to 3 layers of medullar rays. Pith, numerous, polygonal parenchyma cells.

Sweet Wormwood Herb in powder possesses the diagnostic microscopical characters of the unground drug. Two-celled glandular trichomes, 2-branched papillosed stigmas, tip of anther lobes, and triporate pollen grains are characteristic. Typical T-shaped trichomes of leaves may be seen.

Warning It should be used with caution in pregnant women and in patients with frequent diarrhoea.

Additional information

1. Sweet wormwood plant is not native to nor commercially cultivated in Thailand. The plant yielding sweet wormwood herb is here referred to the herbarium specimen number K000891904, collector's number A. Regel s.n., deposited at the Herbarium, Royal Botanic Gardens Kew (K), London, United Kingdom. The photographic illustration of which can be seen at the Department of Medical Sciences Herbarium (DMSC), Nonthaburi, Thailand.
2. It is commonly used with other herbal drugs in Thai traditional herbal preparations.
3. In Thai traditional drugstores, *A. pallens* Wall. ex Besser may be found as "Kot Chula Lampha".

Packaging and storage Sweet Wormwood Herb shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 1 g of the sample, in powder, with 10 ml of *ethanol* for 30 minutes and filter (solution 1). Drop solution 1 on a filter paper and examine under ultraviolet light (366 nm): a blue fluorescence is produced.

B. To 2 ml of solution 1, add 1 or 2 pieces of *magnesium ribbon*, 2 drops of *hydrochloric acid* and warm in a water-bath for a few minutes: a reddish colour is produced.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 30 volumes of *petroleum ether* (*boiling range, 60° to 80°*) and 20 volumes of *ether* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate as bands of 10 mm, 5 µl each of the following two solutions. Prepare solution (A) by refluxing 3 g of the sample, in powder, with 50 ml of *petroleum ether (boiling range, 60° to 80°)* for 1 hour, filtering and evaporating the filtrate to dryness. Dissolve the residue in 30 ml of n-*hexane.* Extract with three 10-ml portions of a 20 per cent v/v solution of *acetonitrile* and evaporate the combined extracts to dryness. Dissolve the residue in 0.5 ml of *ethanol*. For solution (B), dissolve 1 mg of *artemisinin* in 1 ml of *ethanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter; several fluorescent bands of different colours are observed. Spray the plate with a 10 per cent v/v solution of *sulfuric acid* in *ethanol,* heat at 105° for 10 minutes and examine under ultraviolet light (366 nm). The chromatogram obtained from solution (A) shows a yellow-green fluorescent band (hR_f value 34 to 38), corresponding to the artemisinin band from solution (B). Several other fluorescent bands of different colours are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in the Extract of the Aerial Parts of *Artemisia annua* L.

Band	hR_f Value	Detection		
		UV 254	UV 366	10 Per Cent V/V Solution of *Sulfuric Acid* in *Ethanol* and UV 366
1	5 - 7	blue	intense blue	intense blue
2	10 - 13	quenching	–	–
3	17 - 20	quenching	–	–
4	21 - 25	–	yellow	–
5	28 - 32	–	blue	blue
6*	34 - 38	–	–	yellow-green
7	44 - 48	–	yellow	yellow
8	75 - 79	–	yellow	–

*artemisinin

Water Not more than 11.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Total ash Not more than 7.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 2.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 6.0 per cent w/w (Appendix 7.12).

Dose 3 to 12 g, as a decoction, a day.

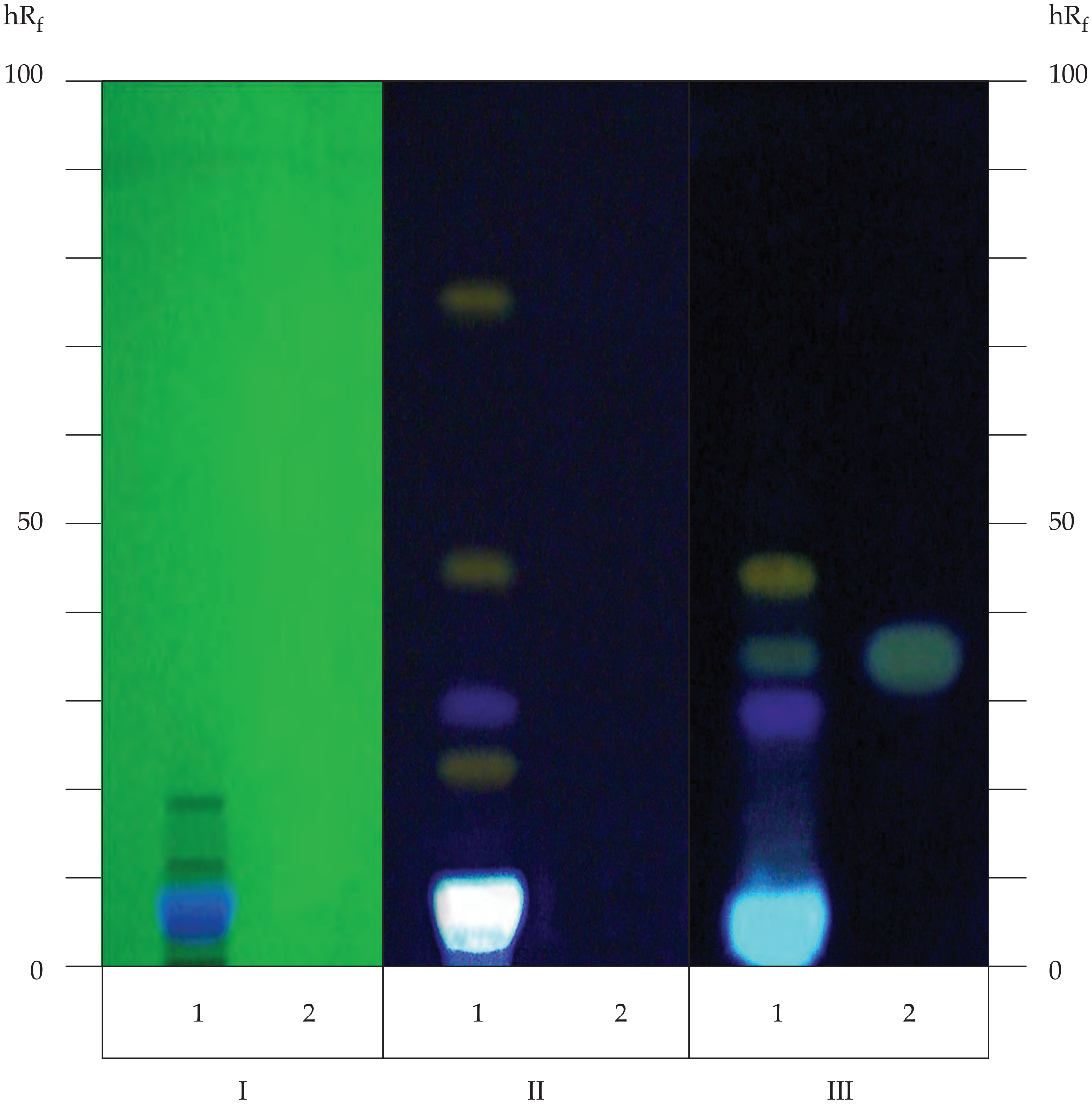


Fig. 3 Thin-layer Chromatogram of the Extract of the Aerial Parts of *Artemisia annua* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection under UV light (366 nm) after spraying with a 10 per cent v/v solution of *sulfuric acid* in *ethanol*.

โกฐหัวบัว (KOT HUA BUA)

โกฐหัวบัวน้อย (KOT HUA BUA NOI), โกฐหัวบัวใหญ่ (KOT HUA BUA YAI)
Chuanxiong Rhizoma
Sichuan Lovage Rhizome

Synonyms Szechuan Lovage Rhizome, Szechwan Lovage Rhizome

Category Carminative, blood tonic for menstrual disorder.

Sichuan Lovage Rhizome is the dried rhizome of *Ligusticum sinense* Oliv. cv. *Chuanxiong* (*Ligusticum chuanxiong* Hort.) (Family Umbelliferae), Herbarium Specimen Number: see *Additional information 1*, Crude Drug Number: DMSc 1148.

Constituents Sichuan Lovage Rhizome contains alkylphthalides (e.g., *Z*-ligustilide, senkyunolides). It also contains coniferyl ferulate, ferulic acid, levislolide A, volatile oil, etc.

Description of the plant (Figs. 1a, 1b) Perennial herb, 30 to 100 cm high; rhizome thick, apparently swollen at nodes, internodes short; stem erect, striate and branching. Leaves alternate, bipinnate or tripinnate; petioles sheathing at base, clasping the stem; basal one 10 to 20 cm long; blade triangular-ovate in outline, 15 to 20 cm long, 10 to 15 cm wide, ternate to 1- or 2-pinnated, primary pinnae 4 to 6 pairs, proximal pinnae remote, ultimate segments ovate or oblong-ovate, 2 to 3 cm long, 1 to 2 cm wide, margin irregularly serrate, glabrous on both surfaces except for the nerves; cauline leaves similar to basal, reduced, sessile, 1-pinnated. Inflorescences compound umbel, terminal and axillary, 6 to 8 cm across when anthesis; bracts 5 to 6(–10); rays 15 to 30, subequal, 3 to 5 cm; bracteoles 5 to 8, linear, shorter than pedicels, reflexed. Flowers small, white; calyx teeth obsolete; petals obovate, base cuneate; styles reflexed. Fruits schizocarp, oblong-ovoid, 2 to 3 mm long, 1.5 to 2 mm wide; dorsal and intermediate ribs prominent, filiform, lateral ribs narrowly winged; vittae 1 to 3(–4) in each furrow, 4 to 6 on commissure. Seeds smooth.

Description Odour, strongly aromatic; taste, bitter, pungent and slightly numb, and with a sweet aftertaste.

Macroscopical (Fig. 1a) Irregular knotty, subround and fist-like, 2 to 7 cm in diameter. Externally yellowish brown, greyish brown to blackish brown, rough and shrunken, dented scars or short remains of stems on the apex; numerous tuberculous rootlet scars on the lower part and at the nodes. Texture compact, uneasily broken, fracture yellowish white or greyish brown, scattered with yellowish brown oil dots.

Microscopical (Figs. 2a, 2b) Transverse section of the rhizome shows periderm, cortex, cambium, phloem, xylem, and pith. Periderm, over 10 layers of narrow rectangular cork. Cortex, numerous polygonal parenchyma cells containing simple and compound starch grains. Phloem, broad layers of polygonal parenchyma cells containing starch grains. Cambium, undulate rings of cambium cells. Pith, broad zone of slightly large polygonal parenchyma cells. Schizogenous oil canals, scattered in cortex, phloem, xylem, and pith.

Sichuan Lovage Rhizome in powder possesses the diagnostic microscopical characters of the unground drug. Dentate fibres, thick-walled parenchyma cells and numerous starch grains are characteristic.

1

4

5

2

3

6

2 cm

Fig. 1a *Ligusticum sinense* Oliv. cv. *Chuanxiong*
1. and 2. habit 3. plant showing swollen nodes 4. leaves 5. inflorescence 6. crude drug

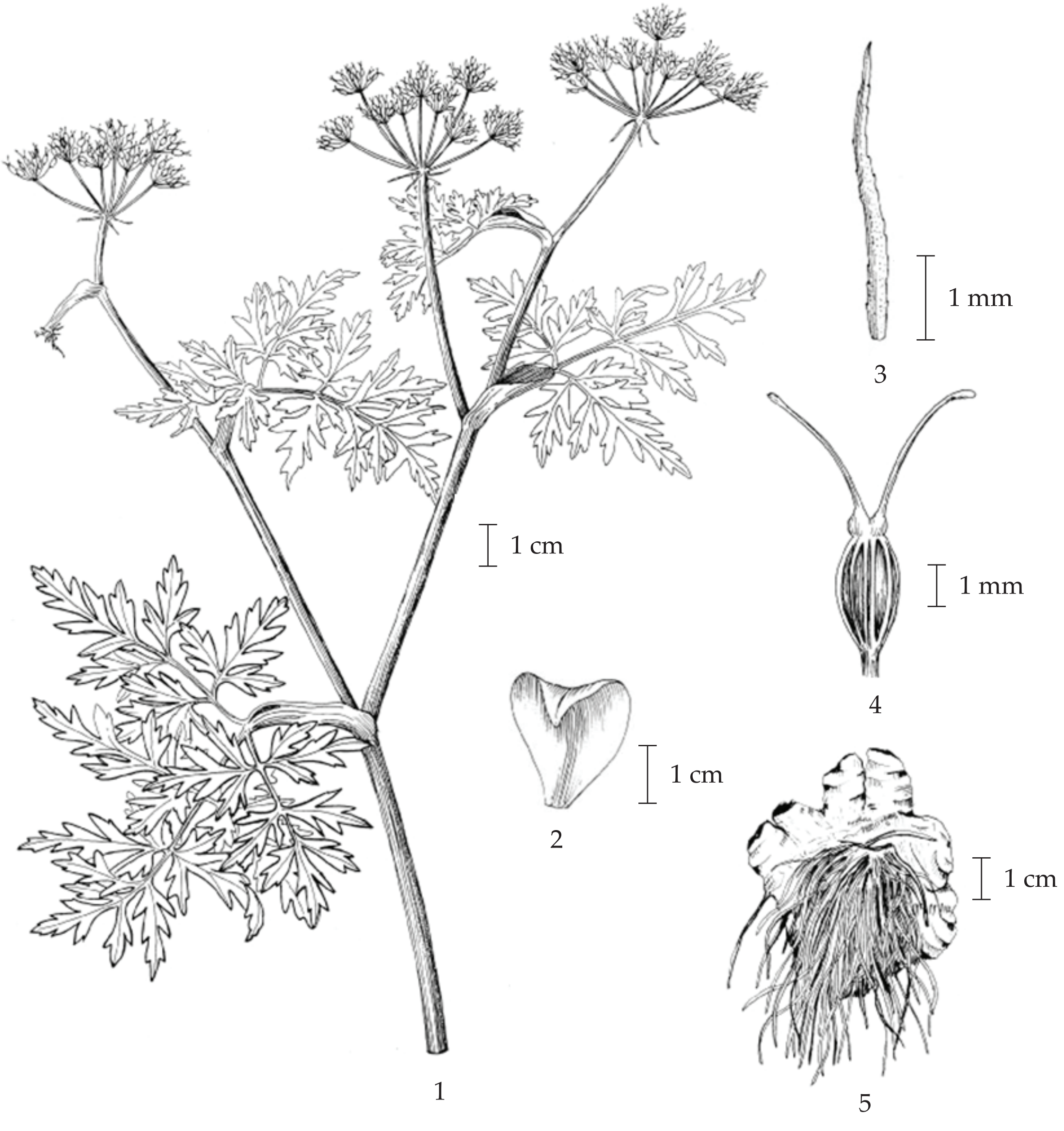


Fig. 1b *Ligusticum sinense* Oliv. cv. *Chuanxiong*
1. flowering branch 2. bract 3. bracteole 4. fruit 5. rhizome and roots

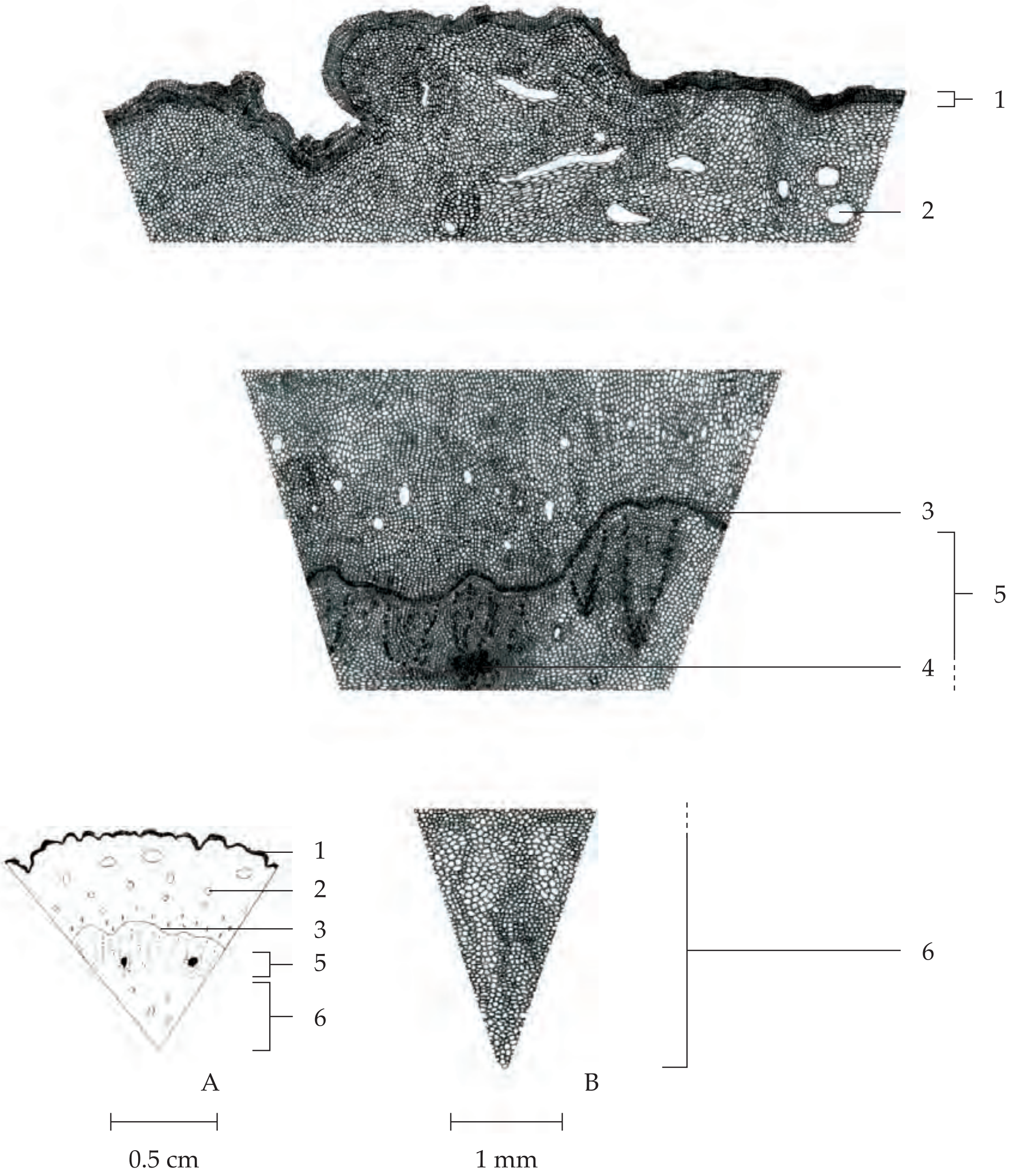


Fig. 2a Transverse Section of the Rhizome of *Ligusticum sinense* Oliv. cv. *Chuanxiong*

A. Diagram
B. Parts of Transverse Section

1. periderm	4. fibre
2. oil canal	5. xylem
3. cambium	6. pith

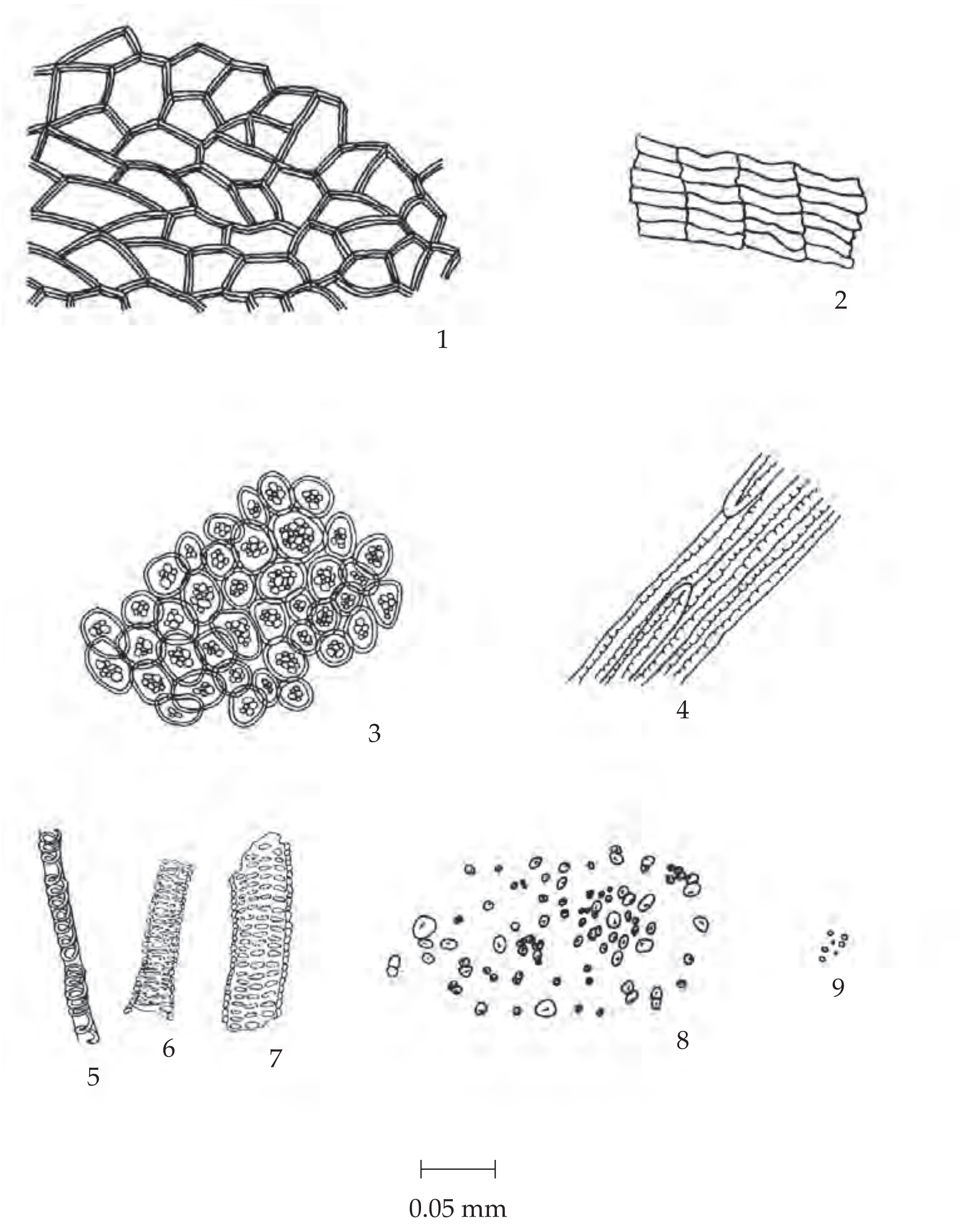


Fig. 2b Powdered Drug of the Rhizomes of *Ligusticum sinense* Oliv. cv. *Chuanxiong*

1. cork in surface view
2. cork in sectional view
3. thick-walled parenchyma cells containing starch grains
4. dentate fibres
5. spiral vessel
6. reticulate vessel
7. scalariform vessel
8. starch grains
9. oil droplets

Contra-indication It is contra-indicated in pregnant women with vaginal bleeding.

Warning Overdosage may cause vomiting and dizziness.

Additional information

1. Sichuan lovage plant is not native to nor commercially cultivated in Thailand. The plant yielding sichuan lovage rhizome is here referred to the herbarium specimen, collector's number Aug. Henry 66139A, deposited at the Institute of Botany (PE), Chinese Academy of Sciences, Beijing, People's Republic of China. The photographic illustration of which can be seen at the Department of Medical Sciences Herbarium (DMSC), Nonthaburi, Thailand.
2. It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Sichuan Lovage Rhizome shall be kept in well-closed containers, protected from light, and stored in a cool and dry place.

Identification

A. Reflux 1 g of the sample, in powder, with 10 ml of *ethanol* for 30 minutes and filter (solution 1). To 2 ml of solution 1, add a few drops of *iron(III) chloride TS*: a green colour is produced.

B. Drop solution 1 on a filter paper and examine the filter paper under ultraviolet light (366 nm): a blue fluorescence is produced.

C. Macerate 1 g of the sample, in powder, in 5 ml of *petroleum ether (boiling range, 60° to 80°)* and allow to stand overnight. Evaporate 1 ml of the supernatant to dryness and dissolve the residue in 1 ml of *methanol*. Add 2 or 3 drops of a 2 per cent w/v solution of *3,5-dinitrobenzsoic acid* in *methanol* and 2 drops of a saturated solution of *potassium hydroxide* in *methanol*: a purple colour is produced.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 86 volumes of *toluene*, 14 volumes of *ethyl acetate* and 5 volumes of *formic acid* as the mobile phase. Apply separately to the plate as bands of 10 mm, 20 µl of solution (A) and 10 µl of solution (B). Prepare solution (A) by refluxing 1 g of the sample, in powder, with 10 ml of *dichloromethane* for 30 minutes and filtering. Evaporate the filtrate until dryness and dissolve the residue in 1 ml of *toluene*. For solution (B), dissolve 1 mg of *ferulic acid* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air, and examine under ultraviolet light (254 nm), marking the quenching bands. The chromatogram obtained from solution (A) shows a quenching band (hR_f value 31 to 38), corresponding to the ferulic acid band from solution (B). Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter; the band due to ferulic acid shows a violet fluorescence. Other several fluorescent bands of different colours are also observed. Spray the plate with *anisaldehyde TS* and heat at 105° for 10 minutes; the band corresponding to ferulic acid is violet. Other several bands of different colours are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Dichloromethane Extract of the Rhizomes of *Ligusticum sinense* Oliv. cv. *Chuanxiong*

Band	hR_f Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	2-5	–	–	violet
2	5-9	quenching	–	green
3	7-13	–	–	violet
4	9-16	–	–	pink
5	11-18	quenching	–	green
6	15-20	–	–	violet
7	16-21	–	blue	–
8	26-32	–	violet	–
9	29-35	–	yellow	–
10*	31-38	quenching	violet	violet
11	39-46	quenching	violet	grey
12	52-58	–	blue	green
13	57-65	quenching	blue	–
14	64-71	quenching	blue	green
15	70 -76	–	–	green
16	71-79	blue	intense blue	yellow
17	79-86	quenching	–	–
18	89-95	quenching	–	green

*ferulic acid

Water Not more than 12.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Total ash Not more than 6.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 18.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 35.0 per cent w/w (Appendix 7.12).

Dose 3 to 9 g, as a decoction, a day.

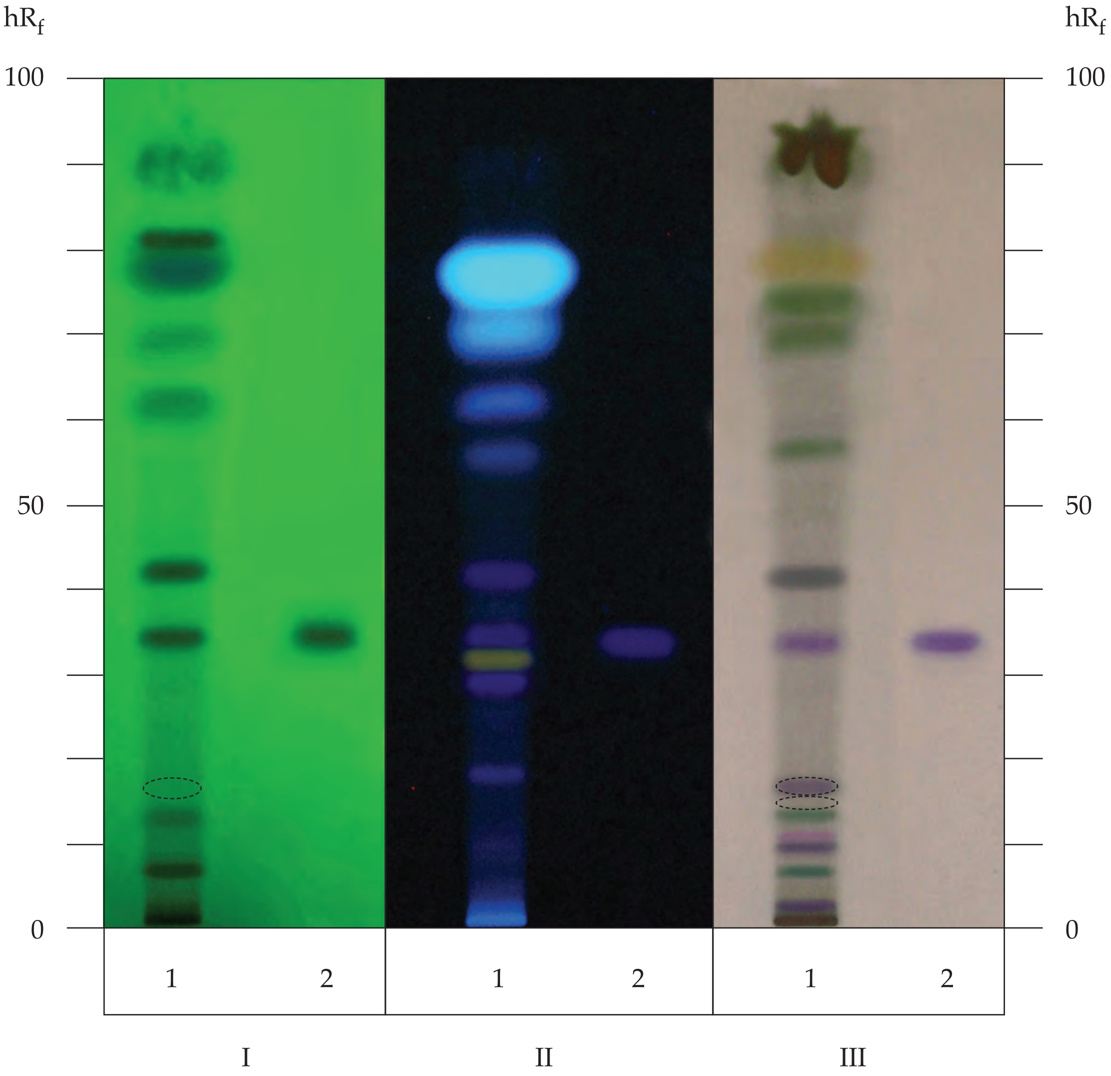


Fig. 3 Thin-layer Chromatogram of Dichloromethane Extract of the Rhizomes of *Ligusticum sinense* Oliv. cv. *Chuanxiong*

1	=	solution (A)
2	=	solution (B)
I	=	detection under UV light (254 nm)
II	=	detection under UV light (366 nm)
III	=	detection with *anisaldehyde TS*
⬭	=	bands developed in some samples

โกฐก้านพร้าว (KOT KAN PHRAO)

โกฐก้านมะพร้าว (KOT KAN MAPHRAO), โกดก้านพร้าว (KOT KAN PHRAO)
Neopicrorhizae Scrophulariiflorae Rhizoma
Figwortflower Picrorhiza Rhizome

Synonyms Katuka, Kutki

Category Antipyretic, stomachic.

Figwortflower Picrorhiza Rhizome is the dried rhizome of *Neopicrorhiza scrophulariiflora* (Pennell) Hong (*Picrorhiza scrophulariiflora* Pennell) (Family Plantaginaceae), Herbarium Specimen Number: see *Additional information 1*, Crude Drug Number: DMSc 0936.

Constituents Figwortflower Picrorhiza Rhizome contains iridoid glycosides (e.g., kutkoside, picrosides, picrorosides) and cucurbitacin triterpenoids. It also contains apocynin, cinnamic acid, vanillic acid, etc.

Description of the plant (Figs. 1a, 1b) Perennial herb, 4 to 30 cm high; rhizome elongated, 15 to 25 cm long, about 1 cm in diameter, giving rise to stems and roots; root stout. Leaves simple, spiral, basal leaves fasciculate, spathulate or narrowly elliptic, 5 to 15 cm long, upper leaves spathulate, 5 to 10 cm long, apex obtuse, base attenuate, margin serrate, leathery, black when dry. Inflorescence spike, terminal; scape semi-cylindrical, 5 to 15 cm long, naked or with few bracts below the inflorescence; bract elliptic or lanceolate, about half of the length of the sepal. Flowers numerous, pale blue to dark purple, hirsute; calyx nearly equally 5-partite; corolla 8 to 10 mm long, bilabiate, upper lip 1-lobed, hooked, emarginate, lower lip 3-lobed, about half the length of the upper lip; stamens 4, more or less equalling corolla, exerted from corolla tube, anther divergent; ovary superior, 2-loculed, ovules numerous per locule, style 1, stigma capitate. Fruit capsule, septicidally dehiscent, ellipsoid or ovoid, about 1 cm long, turgid, tapered at top. Seeds numerous, about 1 mm long.

Description Odour, characteristic; taste, extremely bitter.

Macroscopical (Fig. 1a) Dried rhizome, cylindrical, 2.5 to 12 cm long, about 1 cm wide, easily broken, externally greyish brown, rough, irregularly-wrinkled, sometimes bearing roots, or circular root scars and bud scales. Broken, surface black, surrounding with white vascular bundle.

Microscopical (Figs. 2a, 2b) Transverse section of the rhizome shows periderm, cortex, vascular tissue, and pith. Periderm, several layers of cork cells and some of which in the outer layer cracked. Cortex, broad zone of thin-walled polygonal parenchyma with scattered groups of fibres. Vascular tissue, secondary phloem, xylem and broad zone of medullary ray. Pith, numerous thin-walled polygonal parenchyma.

Figwortflower Picrorhiza Rhizome in powder possesses the diagnostic microscopical characters of the unground drug. Parenchyma with dark brown substance and beaded-wall parenchyma are characteristic.

1

2

1 cm

Fig. 1a *Neopicrorhiza scrophulariiflora* (Pennell) Hong
1. habit 2. crude drug

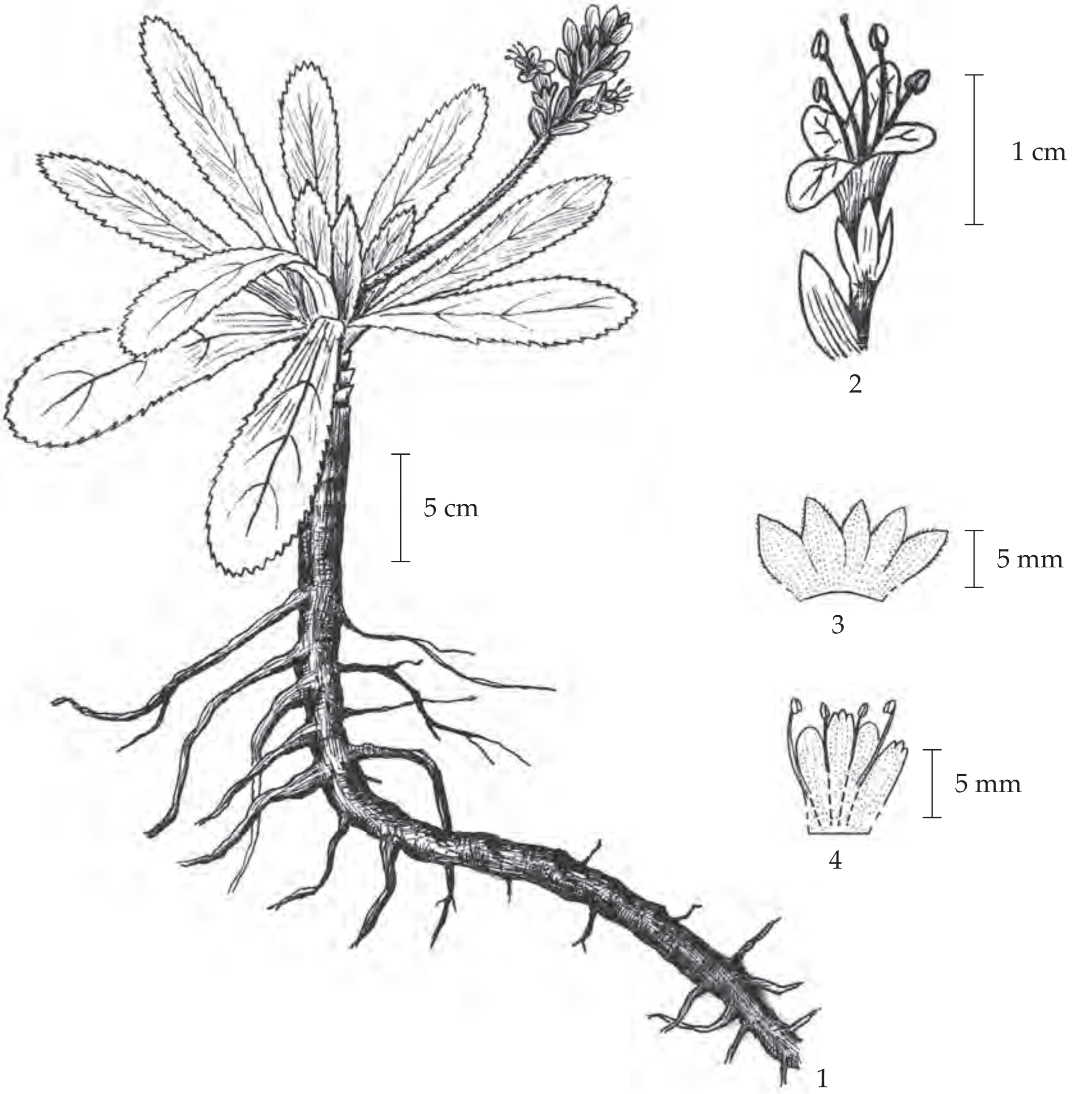


Fig. 1b *Neopicrorhiza scrophulariiflora* (Pennell) Hong
1. habit 2. blooming flower 3. opened calyx 4. opened corolla

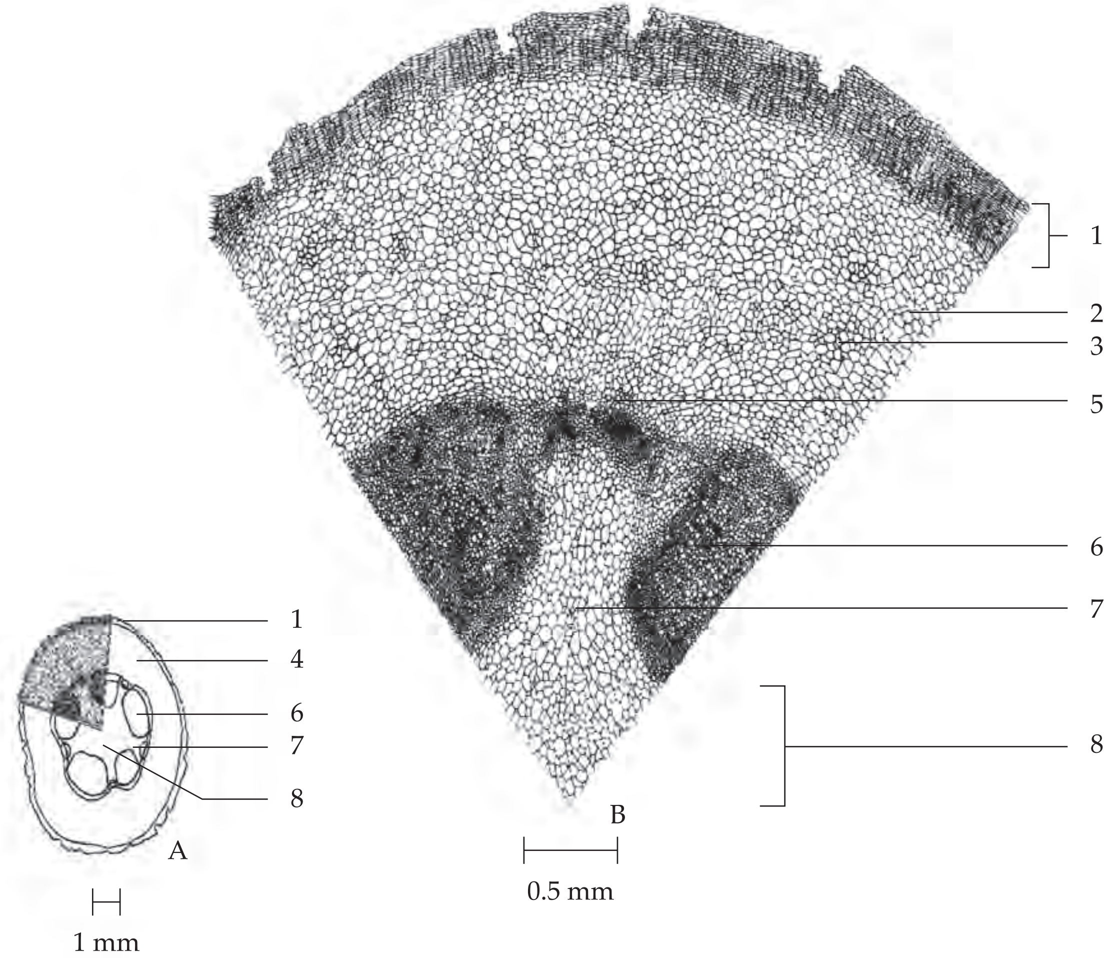


Fig. 2a Transverse Section of the Rhizome of *Neopicrorhiza scrophulariiflora* (Pennell) Hong

A. Diagram
B. Part of Transverse Section
1. periderm
2. parenchyma
3. group of fibres
4. cortex
5. phloem
6. xylem
7. medullary ray
8. pith

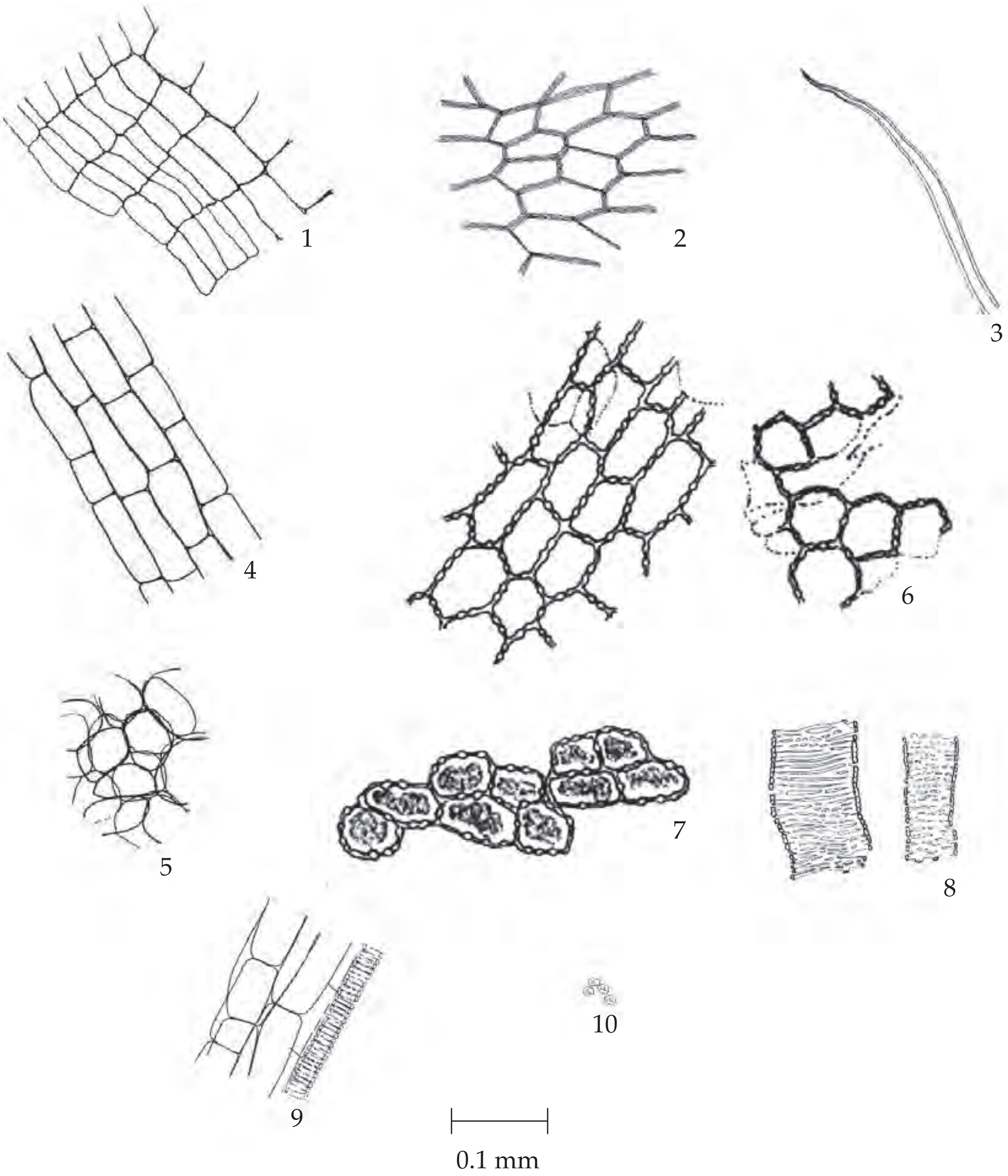


Fig. 2b Powdered Drug of the Rhizomes of *Neopicrorhiza scrophulariiflora* (Pennell) Hong

1. cork cells in sectional view
2. cork cells in surface view
3. fibre
4. parenchyma in longitudinal view
5. parenchyma in sectional view
6. beaded-wall parenchyma
7. beaded-wall parenchyma containing dark brown substance
8. reticulate and pitted vessels
9. parenchyma with attached vessel
10. starch grains

Additional information

1. Figwortflower picrorhiza plant is not native to nor commercially cultivated in Thailand. The plant yielding figwortflower picrorhiza rhizome is here referred to the herbarium specimen number 01434339 (PE), deposited at the Institute of Botany (PE), Chinese Academy of Sciences, Beijing, People's Republic of China. The photographic illustration of which can be seen at the Department of Medical Sciences Herbarium (DMSC), Nonthaburi, Thailand.
2. It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Figwortflower Picrorhiza Rhizome shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Heat 500 mg of the sample, in powder, with 10 ml of *water* in a water-bath for 10 minutes, allow to cool and filter. To 2 drops of the filtrate, add a few drops of a 1 per cent w/v solution of *iron(III) chloride*: a dark green colour is produced.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 50 volumes of *chloroform*, 30 volumes of *ethyl acetate*, 20 volumes of *methanol* and 1 volume of *strong ammonia solution* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply to the plate, 5 µl of the test solution prepared by refluxing 1 g of the sample, in powder, in 25 ml of *methanol* for 30 minutes, filtering and evaporating the filtrate to dryness. Dissolve the residue in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. Examine the plate under ultraviolet light (366 nm) through the cut-off filter. Several blue fluorescent spots are observed. Spray the plate with *anisaldehyde TS* and heat at 110° for 10 minutes. Several spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Rhizomes of *Neopicrorhiza scrophulariiflora* (Pennell) Hong

Spot	hR_f Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	4-10	quenching	blue	brown
2	20-27	quenching	intense blue	brown
3	29-34	quenching	–	brown
4	35-41	quenching	blue	violet
5	45-49	–	–	pale brown
6	47-51	quenching	intense blue	–
7	60-64	quenching	–	–
8	67-71	–	–	pale violet
9	76-79	–	blue	pale violet
10	83-87	–	–	pale violet

Loss on drying Not more than 11.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Total ash Not more than 5.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 30.0 per cent w/w (Appendix 7.12).

Dose 1 to 3 g of the powdered drug a day.

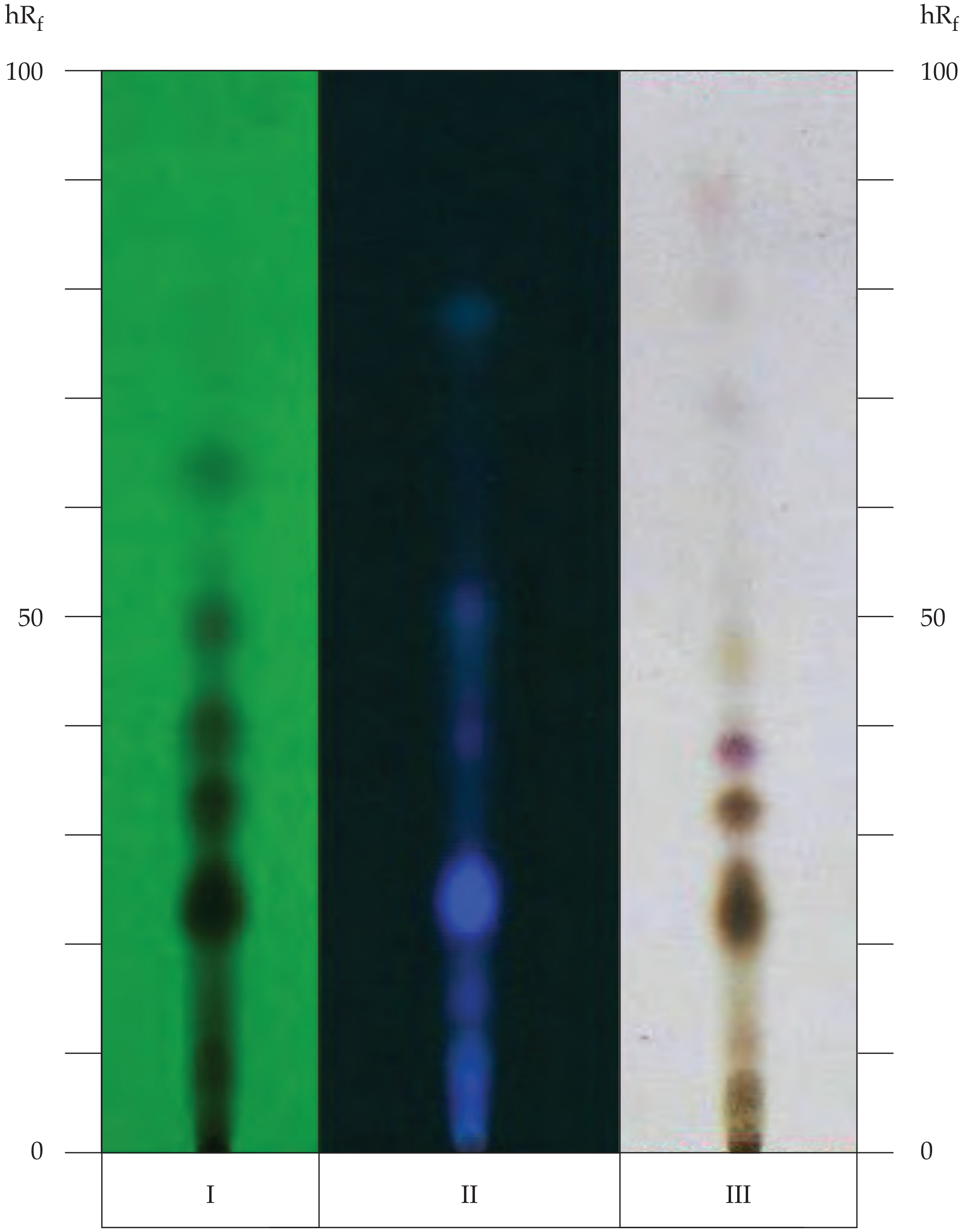


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of Rhizomes of *Neopicrorhiza scrophulariiflora* (Pennell) Hong

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *anisaldehyde TS*

โกฐเขมา (KOT KHAMAO)

โกฐหอม (KOT HOM)
Atractylodis Lanceae Rhizoma
Atractylodes Lancea Rhizome

Synonym Swordlike Atractylodes Rhizome

Category Stomachic.

Atractylodes Lancea Rhizome is the dried rhizome of *Atractylodes lancea* (Thunb.) DC. (*A. chinensis* (Bunge) Koidz.) (Family Compositae), Herbarium Specimen Number: see *Additional information 1*, Crude Drug Number: DMSc 1149.

Constituents Atractylodes Lancea Rhizome contains volatile oil, of which atractylodin and *β*-eudesmol are its major components. It also contains sesquiterpenoids (e.g., atractylenolides), triterpenoids, sterols, etc.

Description of the plant (Figs. 1a, 1b) Perennial herb, (15–)30 to 100 cm high; rhizome thick, prostrate or ascending, with numerous adventitious roots of even thickness; stem solitary or fascicled, unbranched or apically few-branched, sparsely arachnoid or glabrous. Leaves simple, alternate, green, concolorous, rigidly papery, glabrous, margin spiniform ciliate or spinosely toothed; basal leaves withered at anthesis; lower leaves subsessile to petiolate; petiole to 3.5 cm long; leaf blade 8 to 12 cm long, 5 to 8 cm wide, 3 to 5(–9)-pinnatifid or -binnatipartite, sometimes entire, lateral lobes elliptic, narrowly elliptic, or obovate-elliptic, terminal lobe orbicular, obovate, obliquely ovate, ovate, or elliptic; middle leaves with petiole 0.5 to 2.5 cm long, leaf blade obovate, narrowly obovate, obovate-elliptic, narrowly elliptic, or oblanceolate, base cuneate-attenuate, margin entire or basally sometimes with 1 or 2 triangular and spinose teeth; upper leaves sometimes triangular, with 1 or 2 spinose teeth; leaves subtending inflorescence pinnatipartite to pinnatisect. Capitula 1 to several, in terminal corymb; involucre campanulate, 1 to 1.5 cm in diameter; phyllaries 5 to 7 rows, margin sparsely arachnoid, apex rounded to obtuse, outer and outermost ones ovate to ovate-lanceolate, 3 to 6 mm long, 1.5 to 3 mm wide, middle ones ovate, ovate-elliptic, or elliptic, 6 to 10 mm long, 3 to 4 mm wide, inner ones elliptic to linear, 1.1 to 1.2 cm long, 0.2 to 0.3 mm wide, apically sometimes turning red; marginal florets pistillate; disc florets bisexual, corolla white, slightly tinged with purple, basal tube 9 mm long. Achenes obovoid; pappus brown to dirty-white, 7 to 8 mm long.

Description Odour, characteristic; taste, sweetish, pungent and bitter.

Macroscopical (Fig. 1a) Irregularly moniliform or nodular-cylindrical, somewhat curved, occasionally branched, 3 to 10 cm long, 1 to 2 cm in diameter. Externally greyish brown, wrinkled, transversely twisted-lined, with remains of rootlets, and stem scars or remains of stems attached at apex. Texture compact, fracture yellowish white or grayish white, scattered with many orange-yellow or brownish red oil cavities and crystallized out as white fine needle crystals after exposing for a long time.

Fig. 1a *Atractylodes lancea* (Thunb.) DC.
1. habit 2. and 3. flowering branches 4. crude drug

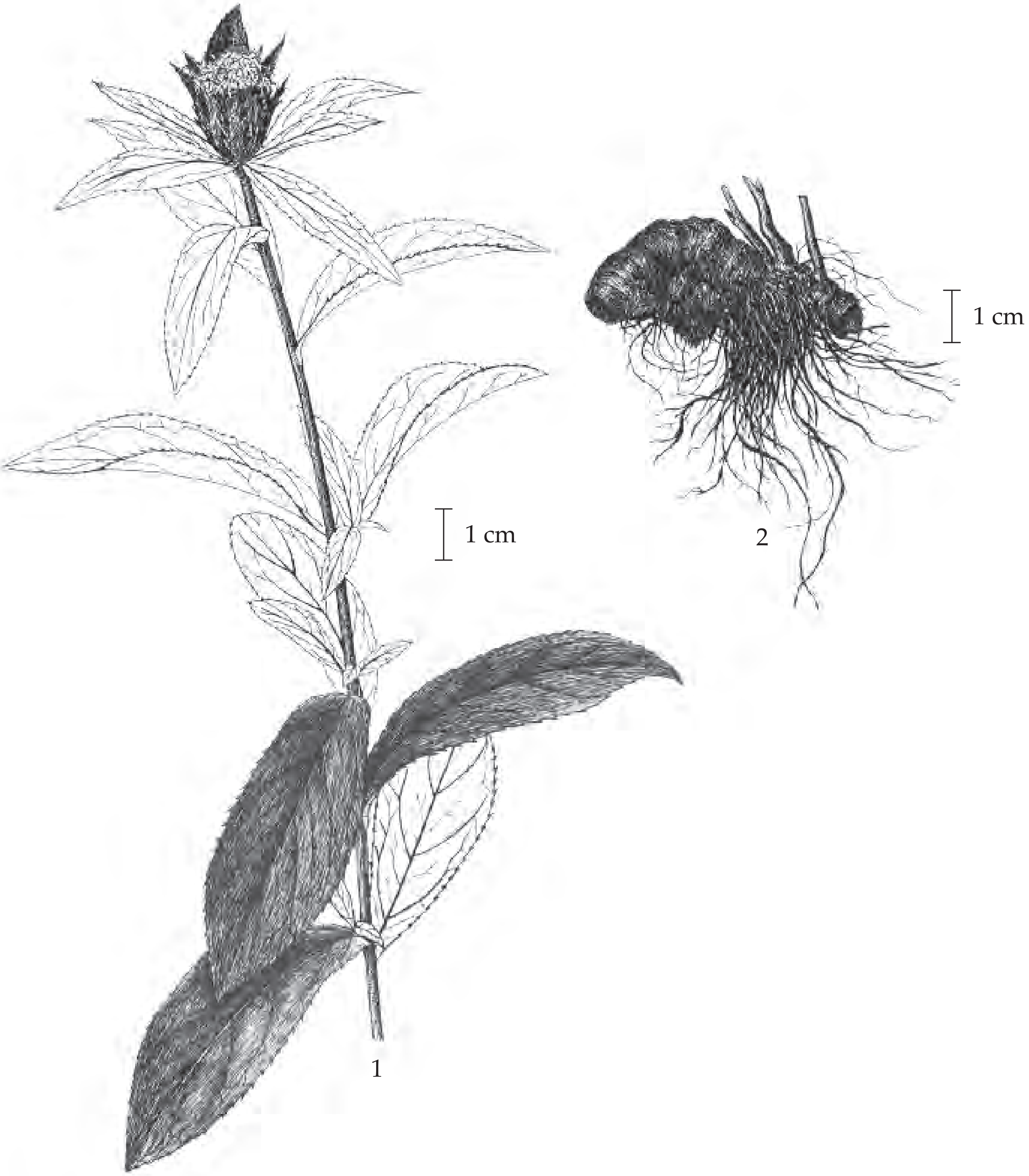


Fig. 1b *Atractylodes lancea* (Thunb.) DC.
1. flowering branch 2. rhizome and roots

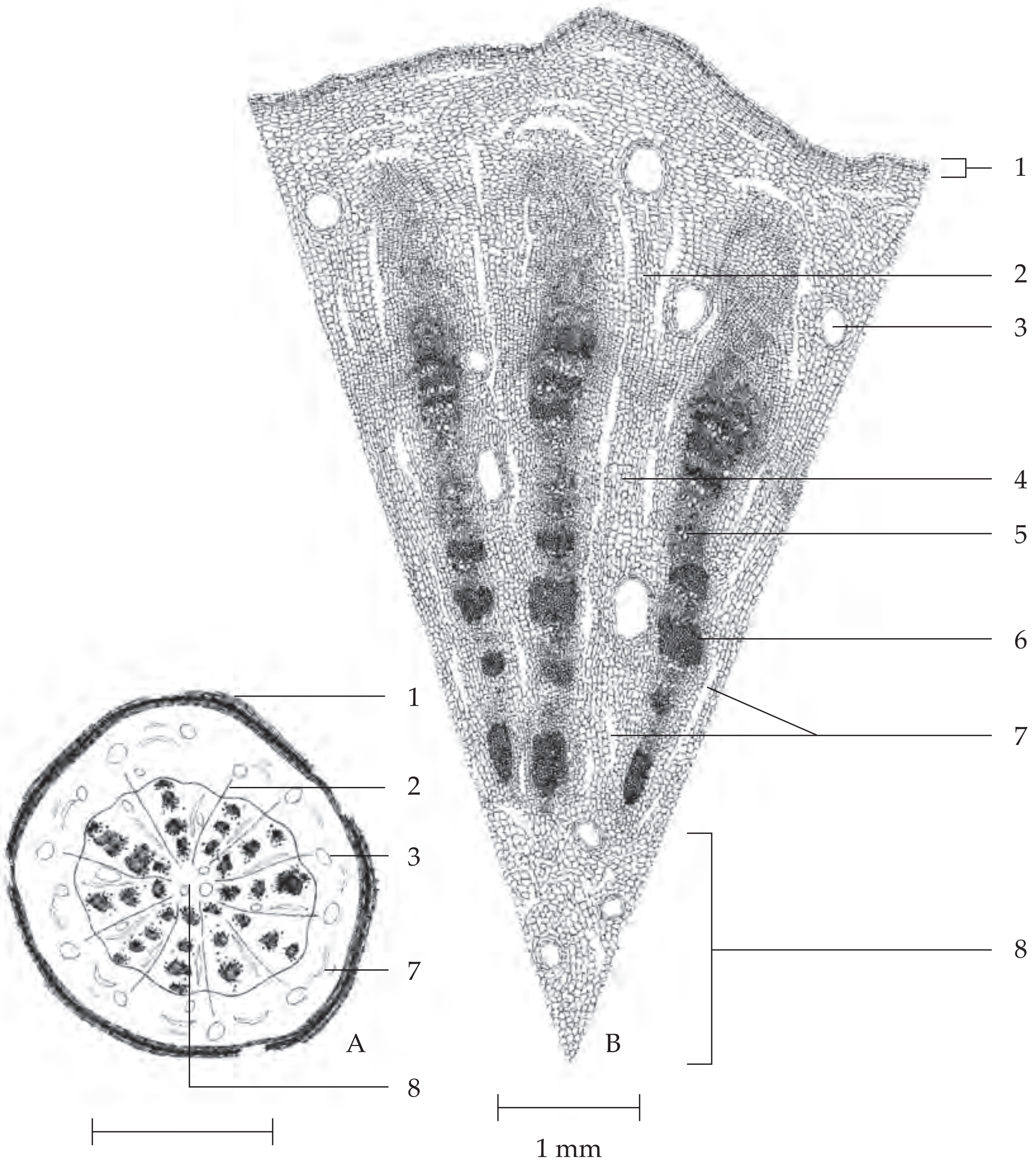


Fig. 2a Transverse Section of the Rhizome of *Atractylodes lancea* (Thunb.) DC.

A. Diagram
B. Part of Transverse Section

1. periderm
2. phloem ray
3. oil cavity
4. xylem ray
5. vessel
6. fibre
7. cracked cavity
8. pith

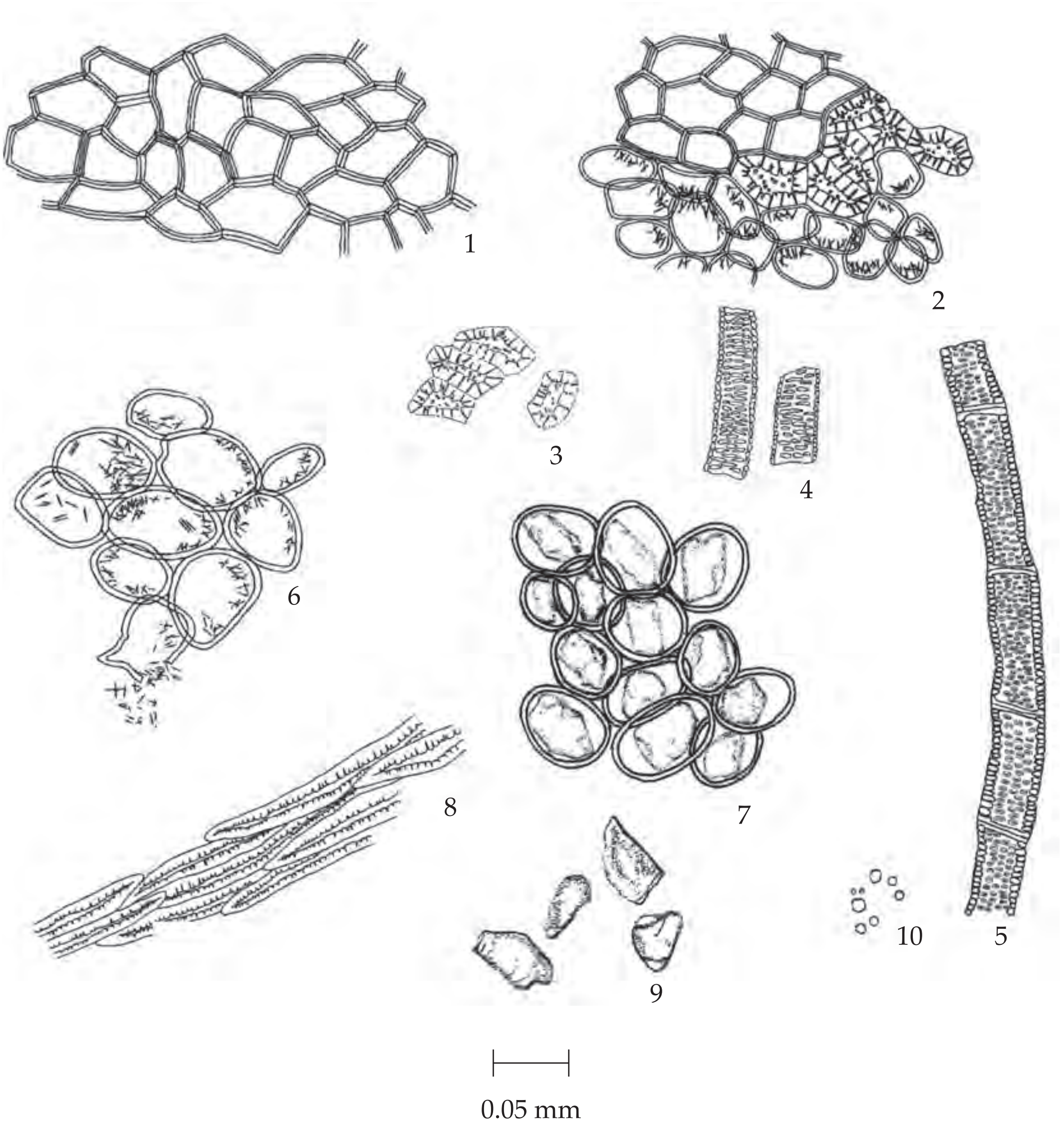


Fig. 2b Powdered Drug of the Rhizomes of *Atractylodes lancea* (Thunb.) DC.

1. cork in surface view
2. cork in surface view associated with sclereids and cortical parenchyma containing acicular crystals
3. sclereids
4. reticulate vessels
5. bordered-pitted vessel
6. parenchyma containing acicular crystals
7. parenchyma containing inulin
8. group of fusiform fibres
9. irregularly-shaped fragments of inulin
10. oil droplets

Microscopical (Figs. 2a, 2b) Transverse section of the rhizome shows periderm, cortex, phloem, xylem and pith. Periderm, several rows of rectangular corks cells and thick-walled sclereids. Cortex, narrow zone of parenchyma cells and cracked cavities. Phloem composed of phloem parenchyma cells, phloem rays and cracked cavities. Xylem comprised vessels, groups of fibres, xylem rays, and cracked cavities. Schizogenous brown oil cavities, scattered in cortex, phloem, xylem, and pith. Pith, numerous polygonal parenchyma cells.

Atractylodes Lancea Rhizome in powder possesses the diagnostic microscopical characters of the unground drug. Cork associated with sclereids, parenchyma cells containing oil droplets, irregularly-shaped fragment of inulin, acicular crystals, and reticulate and bordered-pitted vessels are commonly observed.

Warning It should be used with caution in patients with loose, watery stools.

Additional information

1. Atractylodes lancea plant is not native to nor commercially cultivated in Thailand. The plant yielding atractylodes lancea rhizome is here referred to the herbarium specimen, Herbarium Tsumara Laboratory 24217, deposited at the Herbarium Tsumara Laboratory, Japan. The photographic illustration of which can be seen at the Department of Medical Sciences Herbarium (DMSC), Nonthaburi, Thailand.
2. It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Atractylodes Lancea Rhizome shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Reflux 1 g of the sample, in powder, with 10 ml of *ethanol* for 30 minutes and filter. Evaporate 2 ml of the filtrate to dryness. Dissolve the residue in 2 ml of *acetic anhydride* and then slowly add 1 ml of *sulfuric acid* to make two layers: a purple colour appears in the upper layer.

B. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 70 volumes of *hexane*, 20 volumes of *acetone* and 10 volumes of *ethyl acetate* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply to the plate as a band of 10 mm, 5 μl of the test solution prepared by refluxing 1 g of the sample, in powder, with 10 ml of *dichloromethane* for 30 minutes, filtering and evaporating the filtrate to dryness. Dissolve the residue in 1 ml of *toluene.* After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter. Several fluorescent bands of different colours appear. Spray the plate with *anisaldehyde TS* and heat at 105° for 10 minutes. Several bands of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Dichloromethane Extract of the Rhizomes of *Atractylodes lancea* (Thunb.) DC.

Band	hR_f Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	12-16	quenching	–	grey
2	16-22	quenching	–	grey
3	24-28	quenching	blue	–
4	28-31	–	–	grey
5	32-35	quenching	–	–
6	36-38	–	–	grey
7	43-48	quenching	–	–
8	49-58	quenching	–	green
9	68-72	–	blue	blue
10	72-77	quenching	–	blue
11	78-84	quenching	light orange	grey
12	88-94	–	–	violet
13	92-96	–	–	green
14	94-98	–	–	violet

Water Not more than 11.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 7.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Total ash Not more than 7.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 12.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 35.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 1.4 per cent v/w (Appendix 7.3H). Use 25 g, in *coarse powder*, freshly prepared and accurately weighed. Use 250 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

Dose 3 to 9 g, as a decoction, a day.

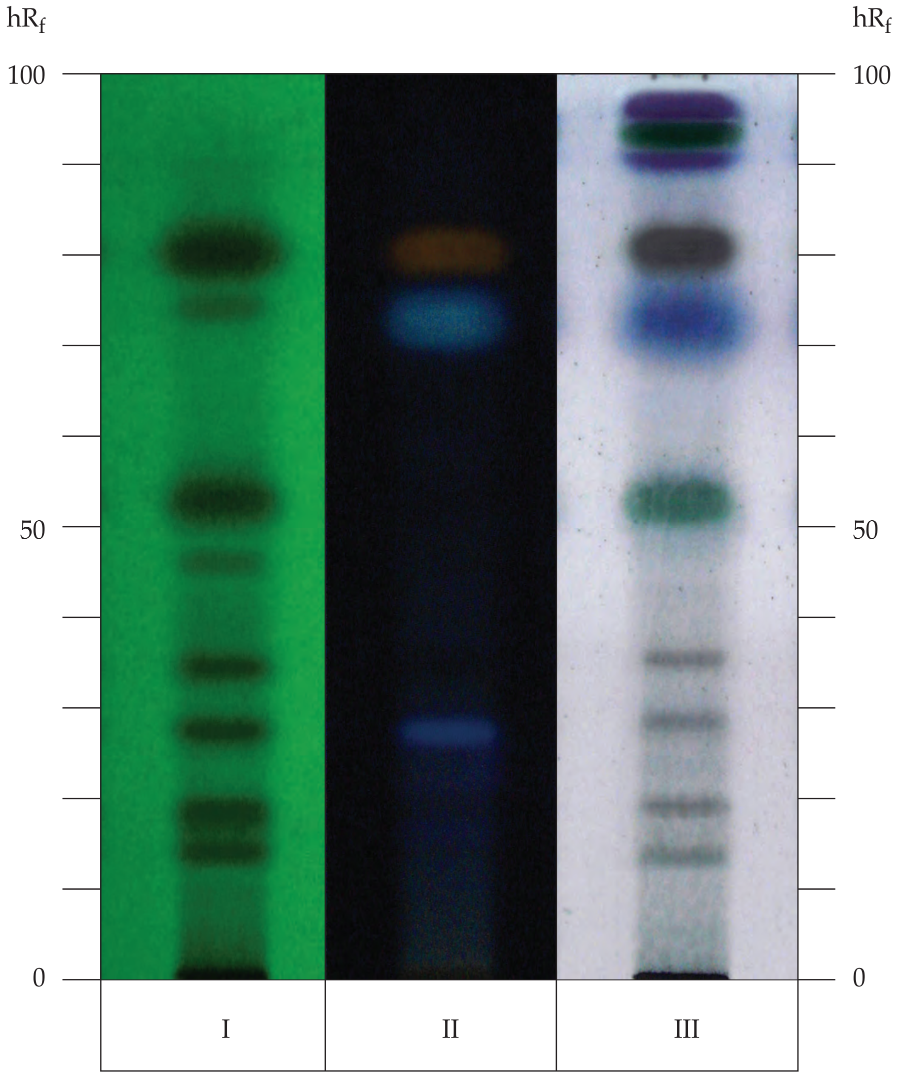


Fig. 3 Thin-layer Chromatogram of Dichloromethane Extract of the Rhizomes of *Atractylodes lancea* (Thunb.) DC.

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *anisaldehyde TS*

โกฐสอ (KOT SO)

โกฐสอจีน (KOT SO CHIN)
Angelicae Dahuricae Radix
Dahurian Angelica Root

Category Antipyretic, analgesic (for headache).

Dahurian Angelica Root is the dried root of *Angelica dahurica* (Hoffm.) Benth. & Hook. f. ex Franch. & Sav. var. *dahurica* (*A. macrocarpa* H. Wolff) (Family Umbelliferae), Herbarium Specimen Number: see *Additional Information 1*, Crude Drug Number: DMSc 1146.

Constituents Dahurian Angelica Root contains furanocoumarins such as imperatorin, isoimperatorin, phellopterin, etc.

Description of the plant (Figs. 1a, 1b) Perennial herb, 1 to 2.5 m high; stem purplish green, hollow, pubescent above; root stout, cylindrical. Leaves pinnately or ternately compound, alternate; blade triangular-ovate in outline, 10 to 50 cm long, 25 to 40 cm wide; leaflets sessile, terminal leaflet deeply 3-lobed, blade usually oblong or ovate-elliptic, 4 to 10 cm long, 1 to 5 cm wide, apex acute to acuminate, base slightly decurrent, margin white-cartilaginous, sharply serrate, pubescent along nerves adaxially, upper leaves reduced, basal and lower leaves long-petiolate, broadly dilated at base into saccate-inflated sheaths. Inflorescence compound umbel, 10 to 30 cm in diameter; peduncle 5 to 20 cm long, scabrous; bract(s) absent or 2, broadly lanceolate; bracteoles many, linear-lanceolate, scarious. Flowers white, 18 to 40(–70) per umbel; pedicellate, scabrous; calyx teeth obsolete; petals 5, obovate, apex incurved; ovary inferior, glabrous or pubescent, 2-loculed, 1 ovule per locule, stylopodium shortly conic. Fruit schizocarp, suborbicular, 4 to 7 mm long, 4 to 6 mm wide, dorsal ribs prominent, obtusely thick-rounded, much wider than furrow, lateral ribs broad-winged; vitta(e) 1 in each furrow, 2 on commissure; split into 2 single-seeded mericarps. Seed plane or slightly concave.

Description Odour, aromatic; taste, pungent and bitterish.

Macroscopical (Figs. 1a, 1b) Long-conical, 10 to 25 cm long, 1.5 to 2.5 cm in diameter. Externally greyish brown or yellowish brown, root stock obtusely quadrangular or subrounded, with longitudinal wrinkles, rootlet scars and lenticel-like transverse protrudings, some of them arranged in 4 longitudinal rows. Apex with dented stem scar. Texture compact, fracture white or greyish white and starchy, cambium ring brown, subsquare or subrounded, scattered with many brown oil dots in bark.

Microscopical (Figs. 2a, 2b) Transverse section of the root shows periderm, cortex, phloem, cambium, and xylem; with schizogenous oil cavities distributed throughout. Periderm, 5 to 10 layers of rectangular, thin- and slightly wavy-walled cork cells. Cortex, narrow, with polygonal or elliptical parenchyma cells. Phloem, a broad zone of polygonal parenchyma and phloem rays. Cambium layers, containing subsquare or subround cells. Xylem, containing vessels, radially arranged in row, with polygonal or subround xylem parenchyma cells and xylem rays. Medullary ray, 2 to 3 rows, rectangular and elliptical cells. Parenchyma cells, containing starch grains and some with prismatic crystals of calcium oxalate.

1

2

3

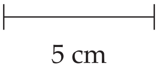


Fig. 1a *Angelica dahurica* (Hoffm.) Benth. & Hook. f. ex Franch. & Sav. var. *dahurica*
1. flowering and fruiting shoot 2. flowering twig 3. crude drug

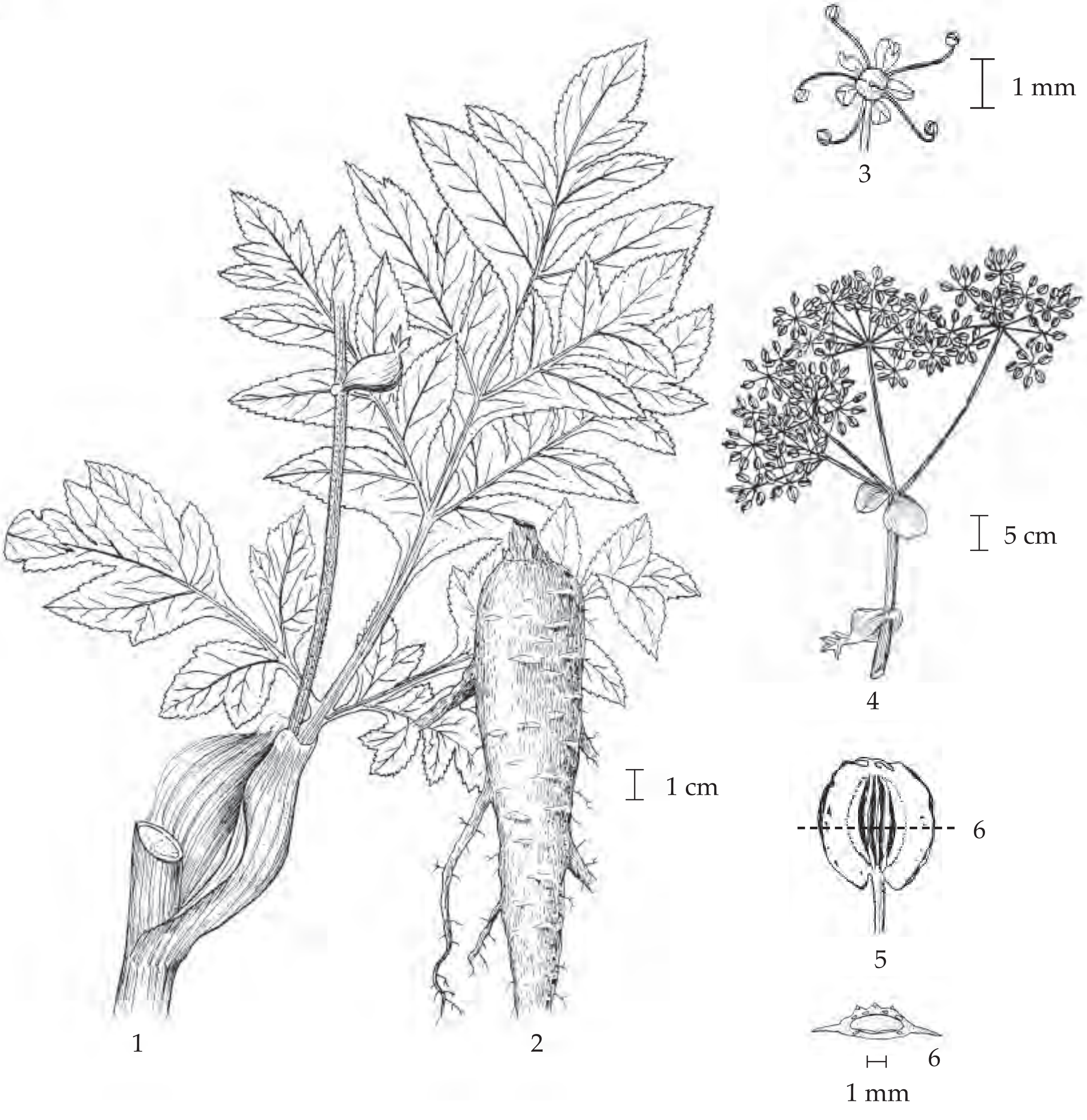


Fig. 1b *Angelica dahurica* (Hoffm.) Benth. & Hook. f. ex Franch. & Sav. var. *dahurica*
1. part of stem showing leaf with characteristic leaf sheath 2. root 3. flower
4. infructescence 5. fruit (mericarp) 6. fruit (cross section)

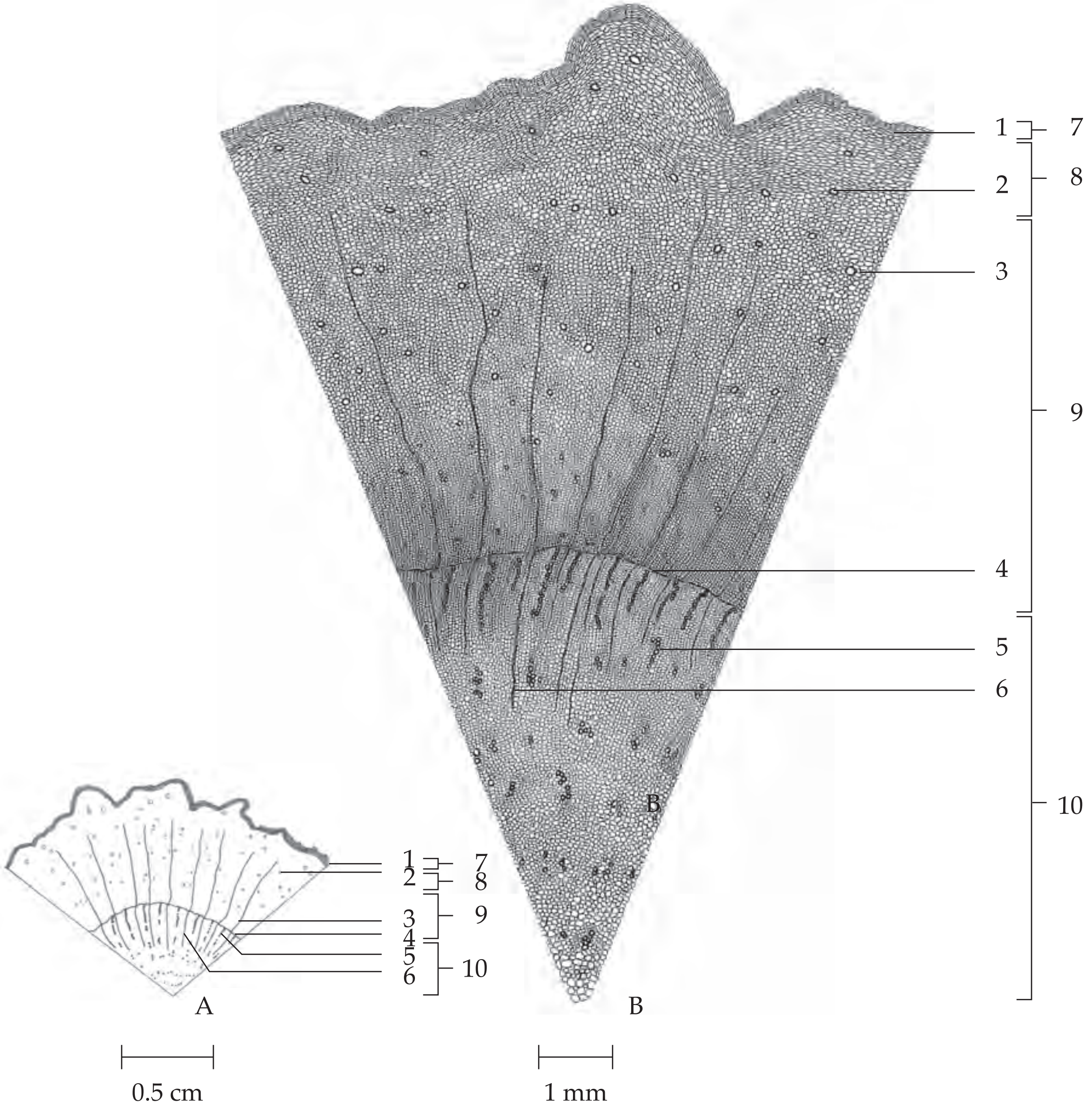


Fig. 2a Transverse Section of the Root of *Angelica dahurica* (Hoffm.) Benth. & Hook. f. ex Franch. & Sav. var. *dahurica*

A. Part of Diagram
B. Part of Sectional View

1. cork
2. oil cavity
3. phloem ray
4. cambium
5. vessel
6. xylem ray
7. periderm
8. cortex
9. phloem
10. xylem

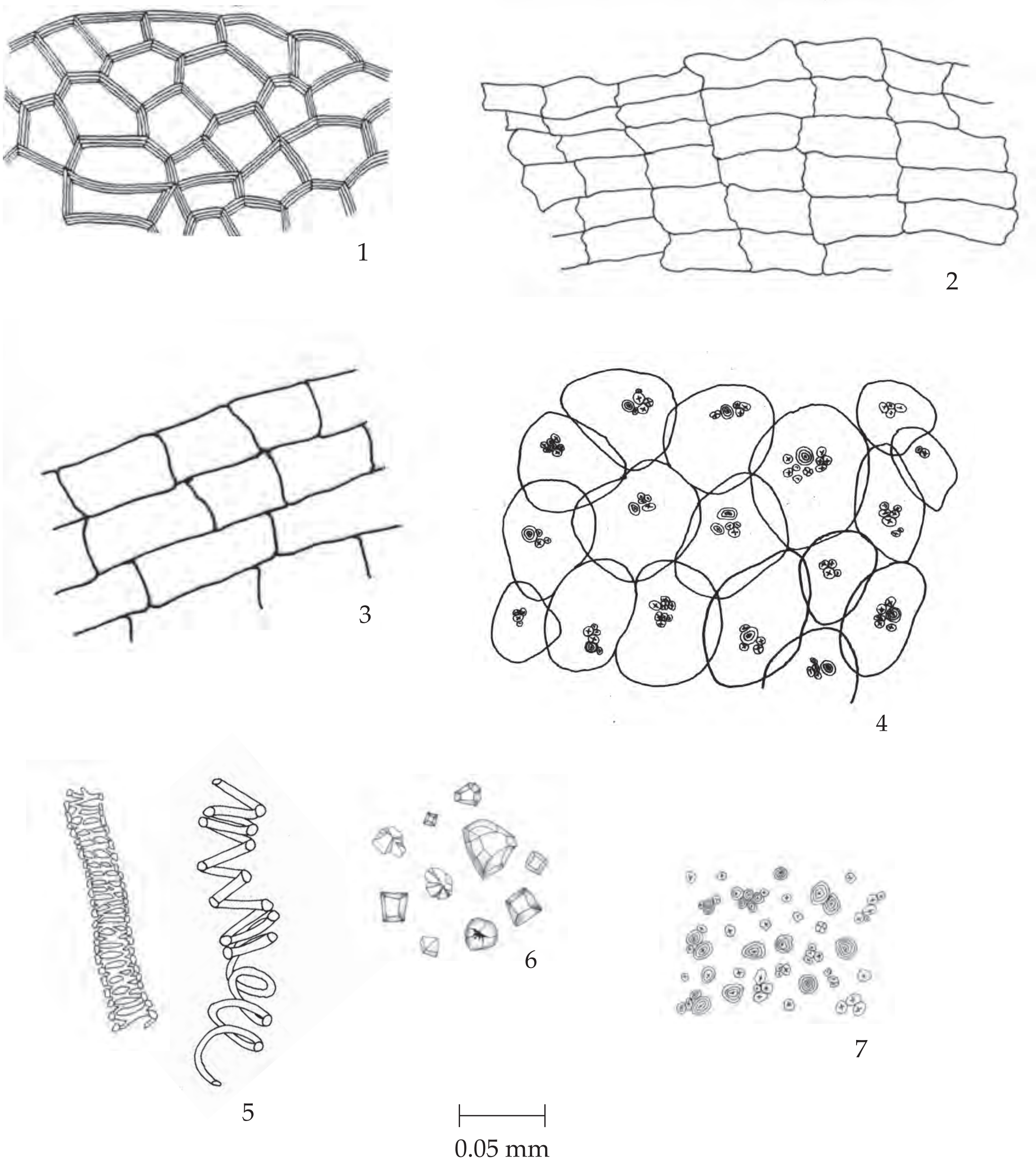


Fig. 2b Powdered Drug of the Roots of *Angelica dahurica* (Hoffm.) Benth. & Hook. f. ex Franch. & Sav. var. *dahurica*

1. cork in surface view
2. cork in sectional view
3. parenchyma in longitudinal view
4. parenchyma in sectional view containing starch grains
5. reticulate and spiral vessels
6. prismatic crystals of calcium oxalate
7. starch grains

Dahurian Angelica Root in powder possesses the diagnostic microscopical characters of the unground drug. Thin-walled cork cells, abundant parenchyma cells (mostly with starch grains and/or prismatic crystals of calcium oxalate), and abundant starch grains of different shapes and sizes (with prominent lamella and hilum), are commonly found.

Additional information

1. Dahurian angelica plant is not native to nor commercially cultivated in Thailand. The plant yielding dahurian angelica root is here referred to the herbarium specimen number K001097137, collector's number C.J. Maximowicz s.n., deposited at the Herbarium, Royal Botanic Gardens Kew (K), London, United Kingdom. The photographic illustration of which can be seen at the Department of Medical Sciences Herbarium (DMSC), Nonthaburi, Thailand.
2. Two varieties of *Angelica dahurica* (Hoffm.) Benth. & Hook. f. ex Franch. & Sav., var. *dahurica* and var. *formosana* (Boiss.) Shan et Yuan, are official in the Pharmacopoeia of the People's Republic of China 2010. However, only the prior variety is imported from China and sold in Thai herbal markets as KOT SO.
3. It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Dahurian Angelica Root shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. To 200 mg of the sample, in powder, add 5 ml of *ethanol*, shake, allow to stand for 5 minutes, and filter. Drop the filtrate on a filter paper moistened with 1 M *sodium hydroxide* and examine under ultraviolet light (366 nm): a green fluorescence is produced.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 75 volumes of *toluene*, 25 volumes of *ethyl acetate* and 5 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate, as bands of 10 mm, 20 µl of solution (A) and 10 µl of solution (B). Prepare solution (A) by refluxing 1 g of the sample, in powder, with 10 ml of *dichloromethane* for 30 minutes and filtering. Evaporate the filtrate to dryness and dissolve the residue in 1 ml of *toluene*. For solution (B), dissolve 1 mg of *imperatorin* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. The chromatogram obtained from solution (A) shows a quenching band (hR_f value 66 to 76), corresponding to the imperatorin band from solution (B), and several quenching bands are also observed (Table 1); see also Fig. 3. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter; the band due to imperatorin exhibits a yellow fluorescence. Other several fluorescent bands are also observed. Spray the plate with a 5 per cent w/v solution of *potassium hydroxide*, and examine the plate under ultraviolet light (366 nm) through the cut-off filter; the band corresponding to imperatorin is orange fluorescence. Several fluorescent bands of different colours are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Dichloromethane Extract of the Roots of *Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav. var. *dahurica*

Band	hR_f Value	Detection		
		UV 254	UV 366	5 Per Cent W/V Solution of *Potassium Hydroxide* and UV 366
1	13-17	quenching	yellow	yellow
2	17-20	–	violet	blue
3	24-27	–	violet	blue
4	30-34	–	violet	blue
5	33-42	–	–	blue
6	41-44	–	–	blue
7	43-48	quenching	blue	blue
8	49-53	quenching	–	–
9	51-57	–	intense blue	intense blue
10	59-62	quenching	yellow	yellow
11	65-69	–	–	blue
12*	66-76	quenching	yellow	orange
13	75-80	–	violet	violet
14	77-83	quenching	yellow	yellow
15	81-85	–	violet	violet

*imperatorin

Water Not more than 14.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Total ash Not more than 5.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 4.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 15.0 per cent w/w (Appendix 7.12).

Dose 3 to 9 g, as a decoction, a day.

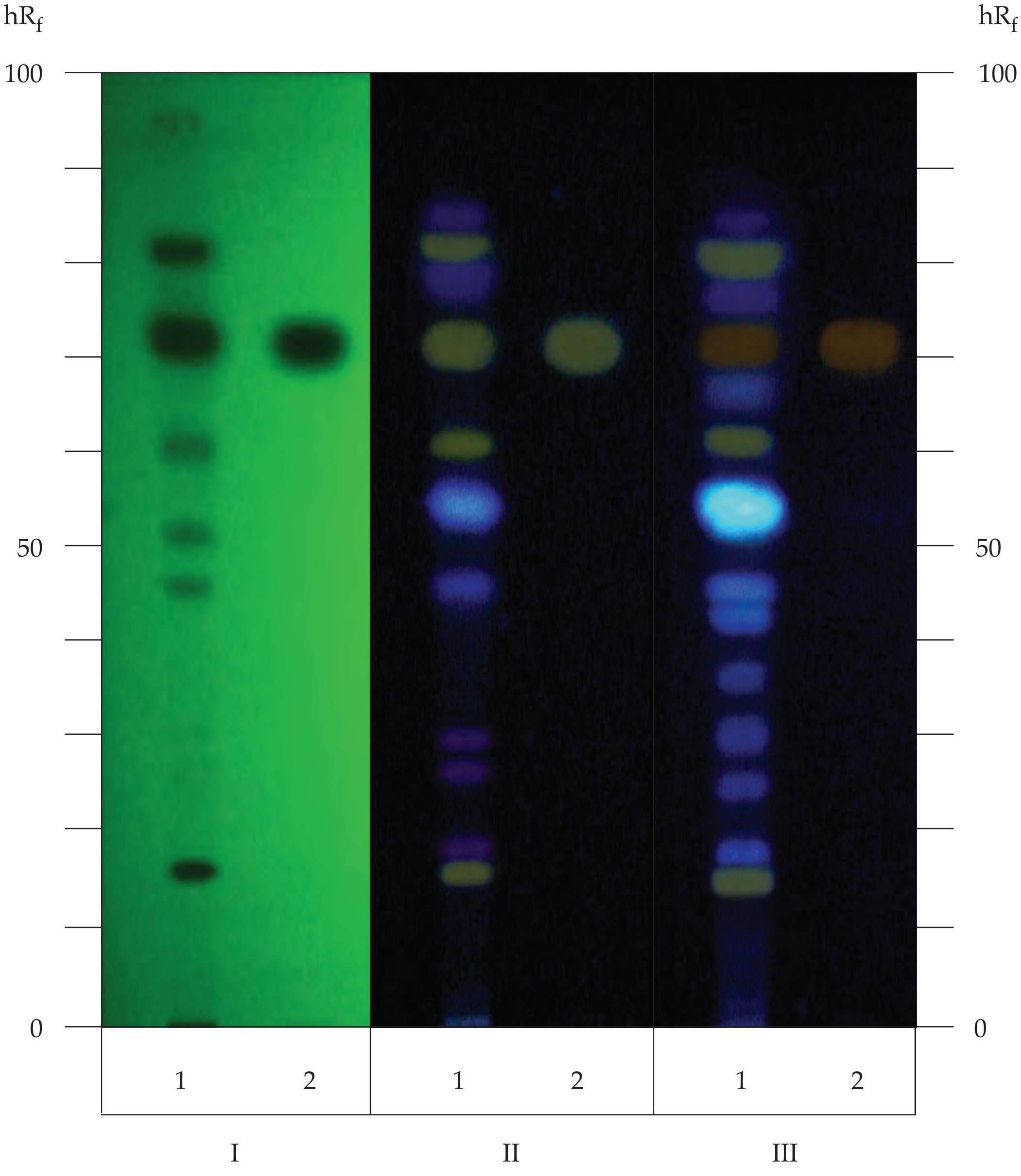


Fig. 3 Thin-layer Chromatogram of Dichloromethane Extract of the Roots of *Angelica dahurica* (Hoffm.) Benth. & Hook. f. ex Franch. & Sav. var. *dahurica*

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection under UV light (366 nm) after spraying with a 5 per cent w/v solution of *potassium hydroxide*

กระชายดำ (KRACHAI DAM)

Kaempferiae Parviflorae Rhizoma
Kaempferia Parviflora Rhizome

Category Tonic, carminative.

Kaempferia Parviflora Rhizome is the dried rhizome of *Kaempferia parviflora* Wall. ex Baker [*K. rubromarginata* (S. Q. Tong) R. J. Searle, *Stahlianthus rubromarginatus* S. Q. Tong] (Family Zingiberaceae), Herbarium Specimen Number: DMSC 1575, Crude Drug Number: DMSc 548.

Constituents Kaempferia Parviflora Rhizome contains volatile oil, of which borneol is major component. It also contains flavonoids, anthocyanins, etc.

Description of the plant (Figs. 1a, 1b) Herb, up to 25 cm tall; rhizome, subglobose to globose, light to dark purple within, with several succulent roots in a fascicle. Leaves one to several; blades ovate or elliptic, slightly unequal sided, 7 to 20 cm long, 4 to 9 cm wide, apex acute or mucronate, base subcordate, upper surface glabrous, under surface hairy; leaf-sheaths 6 to 12 cm long, margin membranous, green or with red-tinted; bladeless sheaths greenish, purple-tinted or purple; ligule broadly triangular, about 2 mm long, membranous, caducous. Inflorescence enclosed by two innermost leaf-sheaths or leaf-sheath and the bladeless sheath, usually elongate; peduncle 5 to 6 cm long; bracts oblong 1.7 to 2.3 cm long, about 6 mm wide, glabrous, apex rounded; bracteoles linear, 6 to 12 mm long, 1 to 2 mm wide, glabrous, apex rounded. Flowers many, up to 20; calyx 1.8 to 2.2 cm long, finely hairy, apex bifid; corolla-tube about 3 cm long, lobes linear; dorsal lobe about 1.2 cm long, about 3 mm wide, apex hooded, aristate, lateral lobes slightly smaller, apex rounded; staminodes white, oblong, 1 to 1.3 cm long, about 3 mm wide, apex acute or rounded. Labellum, white to light purple, darker at the base, obovate, 1.2 to 1.5 cm long, 8 to 9 mm wide, apex emarginate; stamen with very short filament, about 1 mm long; anther about 2 mm long, anther-crest suborbicular, entire or emarginate, 1 to 1.5 mm long, about 2 mm wide; ovary about 2 mm long, about 1 mm wide, hairy; stylodes filiform, about 5 mm long.

Description Odour, characteristic and aromatic; taste, slightly bitter.

Macroscopical (Figs. 1a, 1b) Subglobose to globose horizontally continuous rhizomes, sometimes with roots and rootlets; outer surface slightly wrinkled, brown to dark brown, with scars of pseudostems; fracture light to dark purple, mealy. Some occur as transverse slices, vary in shape and size.

Microscopical (Figs. 2a, 2b) Transverse section of the rhizome shows several layers of corky parenchyma cells. Cortex, broad zone of parenchyma cells, filled with numerous simple starch grains and purple anthocyanins, some of which containing yellowish oleoresins with small particles. Pseudoendodermis, layers of thin-walled rectangular cells. Vascular bundles, scattered; fibres, non-lignified; vessels, spiral, scalariform and reticulate, non-lignified.

Kaempferia Parviflora Rhizome in powder possesses the diagnostic microscopical characters of the unground drug.

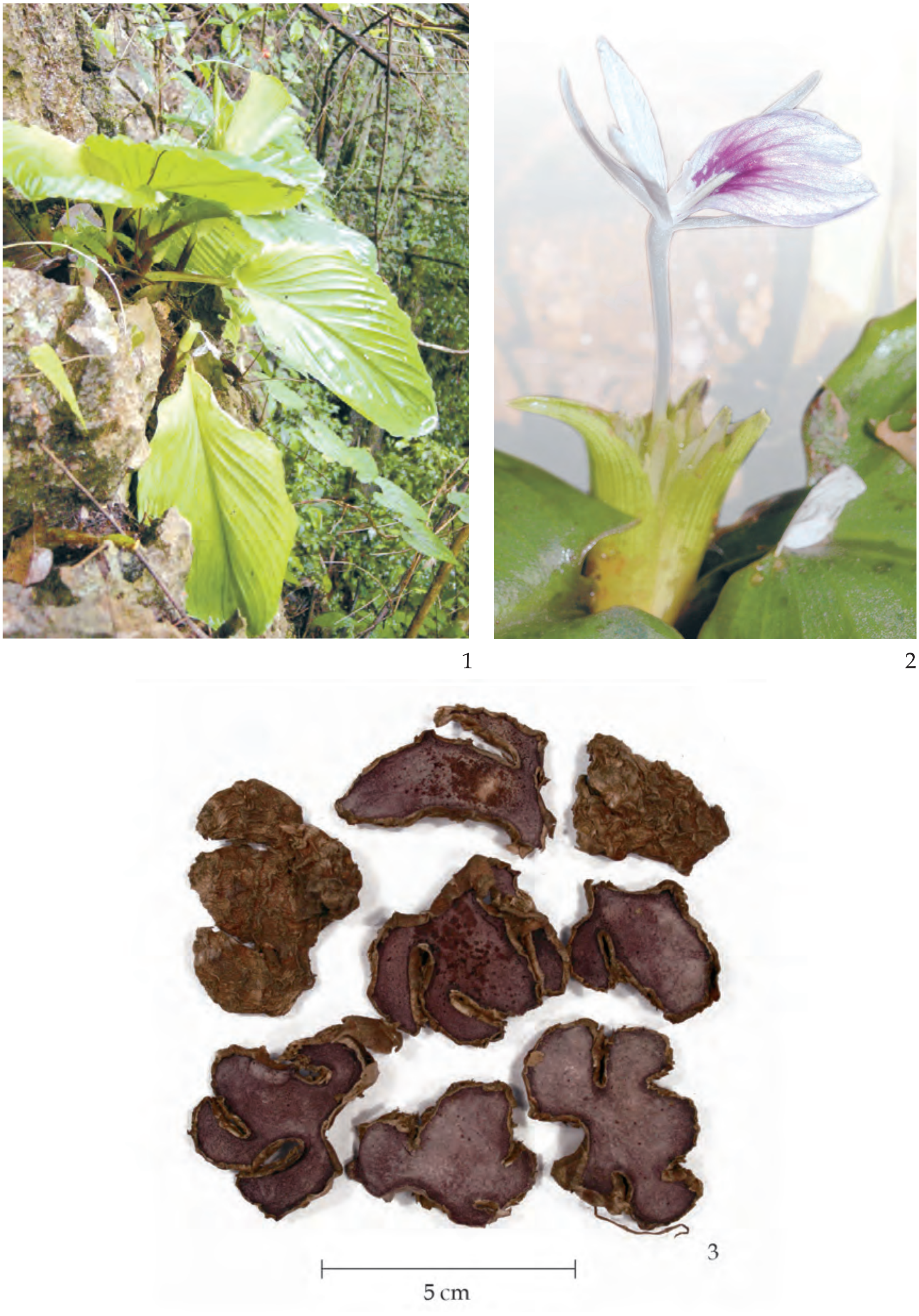


Fig. 1a *Kaempferia parviflora* Wall. ex Baker
1. habit 2. flower 3. crude drug

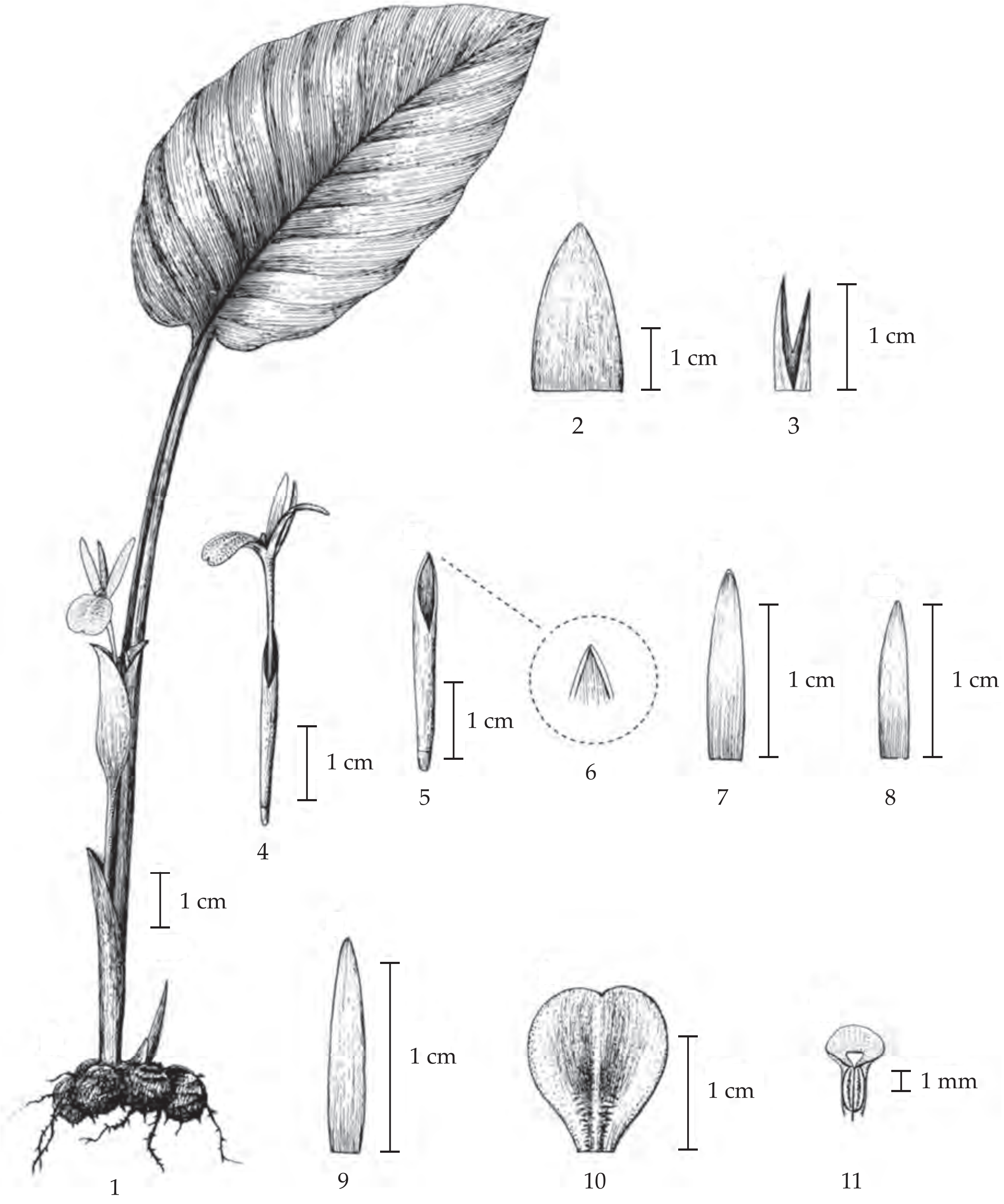


Fig. 1b *Kaempferia parviflora* Wall. ex Baker
1. habit 2. bract 3. bracteole 4. flower 5. ovary and calyx tube 6. apex of calyx tube
7. dorsal corolla lobe 8. lateral corolla lobe 9. lateral staminode
10. labellum 11. anther, stigma and anther crest

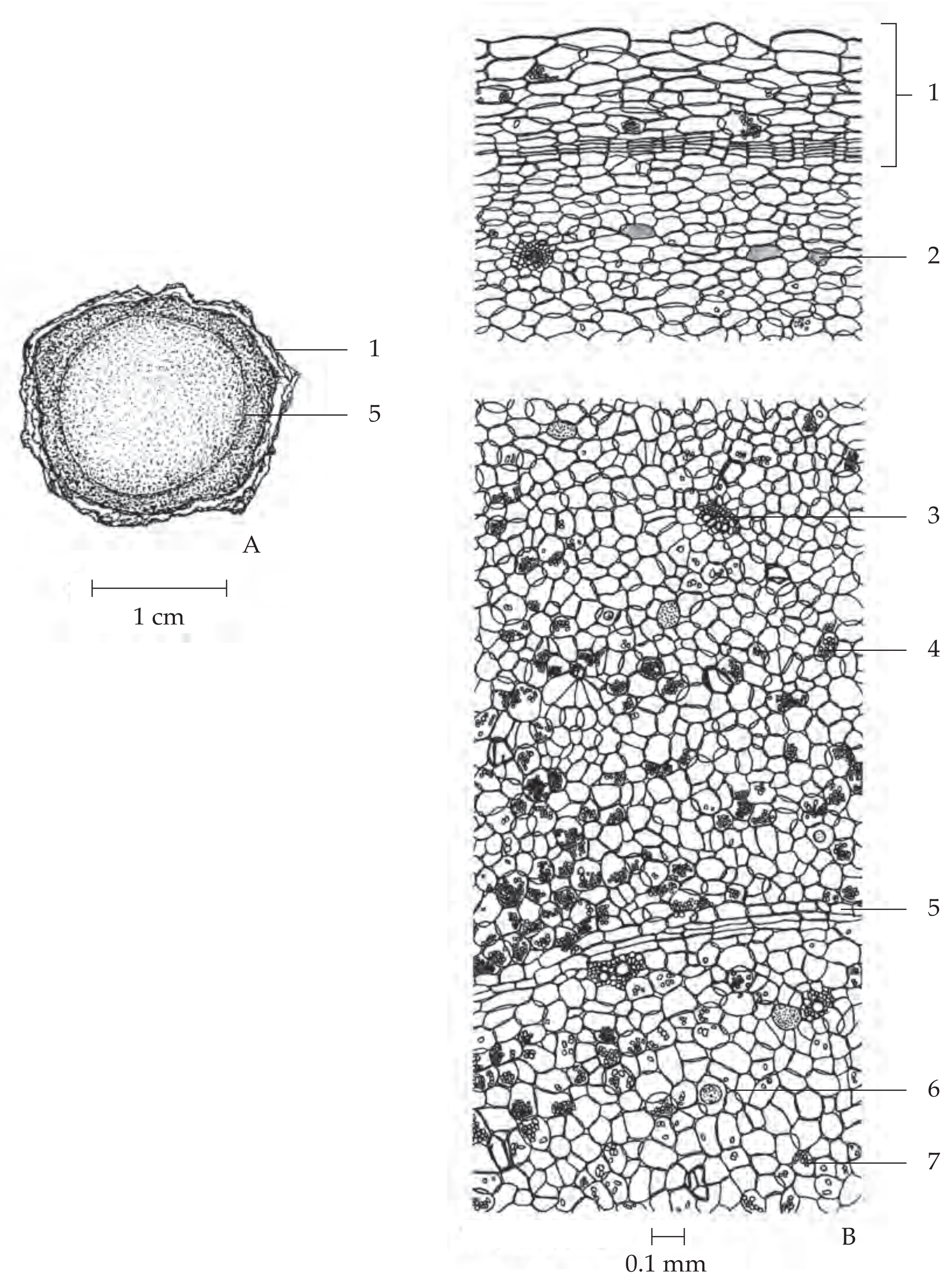


Fig. 2a Transverse Section of the Rhizome of *Kaempferia parviflora* Wall. ex Baker

A. Diagram

B. Part of Transverse Section

1. corky parenchymatous layer
2. parenchyma with purple matter of anthocyanins
3. vascular bundle
4. parenchyma containing starch grains
5. pseudoendodermis
6. parenchyma containing yellow oleoresin
7. starch grains

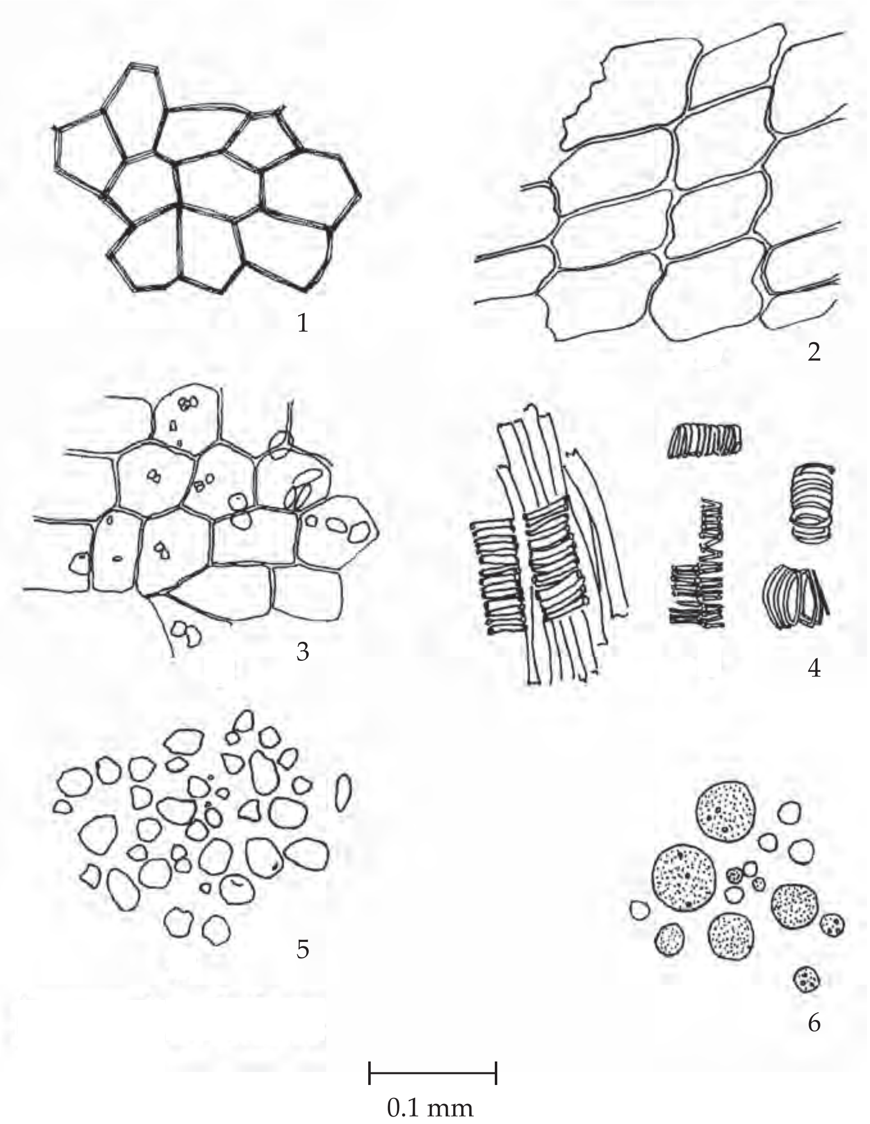


Fig. 2b Powdered Drug of the Rhizomes of *Kaempferia parviflora* Wall. ex Baker

1. corky parenchyma cells in surface view
2. corky parenchyma cells in sectional view
3. parenchyma cells containing starch grains
4. spiral vessels and reticulate vessels associated with fibres
5. starch grains
6. yellowish oleoresins containing small particles

Packaging and storage Kaempferia Parviflora Rhizome shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 1 g of the sample, in *fine powder*, with 20 ml of *ethanol* for 5 minutes and filter (solution 1). To 5 ml of solution 1, add 1 g of *decolorizing charcoal*, mix and filter. Add 2 drops of a freshly prepared 1 per cent w/v solution of *vanillin* in *ethanolic sulfuric acid*, mix well and heat in a water-bath for 2 minutes: a blue colour is produced.

B. To 2 ml of solution 1, add 1 drop of a 5 per cent w/v solution of *potassium hydroxide*: a green to blue colour is produced and changed to red after adding 1 drop of a 20 per cent v/v solution of *sulfuric acid*.

C. Apply 2 drops of solution 1 to a filter paper and air-dry. Add to the same spot, 1 drop of *ethanolic ninhydrin TS* and dry in a current of hot air: a violet colour develops.

D. To 2 ml of solution 1, add 1 piece of *magnesium ribbon*, shake well and mix with 2 drops of *hydrochloric acid*: a red colour develops.

E. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 60 volumes of n-*hexane*, 30 volumes of *ethyl acetate* and 5 volumes of *formic acid* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply to the plate, 5 µl of the test solution prepared by refluxing 500 mg of the sample, in *fine powder*, with 10 ml of *methanol* for 5 minutes and filtering. Dilute the filtrate with *methanol* to 10 ml. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. Several blue spots also appear. Examine the plate under ultraviolet light (366 nm) through the cut-off filter, five blue fluorescent spots of different hR_f values are observed. Heat the plate at 80° for 10 minutes and then spray with *natural products (NP) TS* while the plate is still warm. Subsequently spray the plate with *polyethyleneglycol (PEG) TS* and observe the colours of the spots under ultraviolet light (366 nm) through the cut-off filter within 5 to 15 minutes. Several fluorescent spots of different colours appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Rhizomes of *Kaempferia parviflora* Wall. ex Baker

Spot	hR_f Value	Detection		
		UV 254	UV 366	UV 366 after spraying with *NP/PEG TS*
1	17-26	blue	blue	blue
2	29-35	blue	blue	blue
3	36-40	blue	blue	blue
4	41-46	blue	blue	blue
5	46-52	blue	blue	blue
6	64-69	weak quenching	–	yellow
7	74-78	weak quenching	–	yellow-green
8	78-82	weak quenching	–	yellow
9	83-86	weak quenching	–	yellow
10	87-90	weak quenching	–	orange

Water Not more than 10.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Total ash Not more than 6.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 8.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 17.0 per cent w/w (Appendix 7.12).

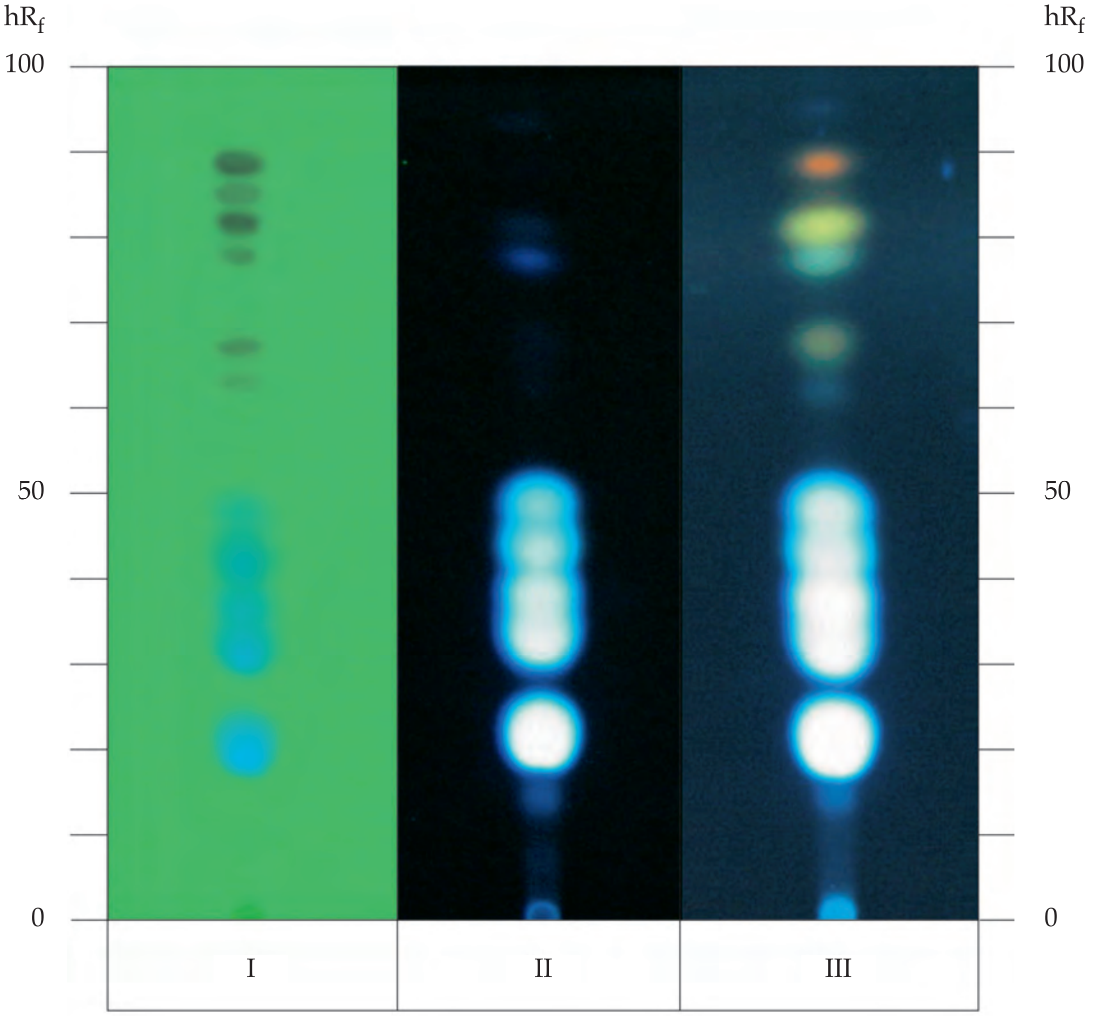


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Rhizomes of *Kaempferia parviflora* Wall. ex Baker

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection under UV light (366 nm) after spraying with *NP/PEG TS*

กระเจี๊ยบแดง (KRACHIAP DAENG)

กระเจี๊ยบเปรี้ยว (KRACHIAP PRIAO), ส้มปู (SOM PU)
Hibisci Sabdariffae Calycis et Epicalycis
Roselle

Synonyms Jamica Sorrel, Red Sorrel, Sorrel

Category Diuretic.

Roselle is the dried persistent calyx and epicalyx collected during fruiting of *Hibiscus sabdariffa* L. (Family Malvaceae), Crude Drug Number: DMSc 0437.
(**Note** Only dried reddish to dark red Roselle is recommended for medicinal uses.)

Constituents Roselle contains anthocyanins, of which delphinidin-3-sambubioside and cyanidin-3-sambubioside are major components. It also contains organic acids (e.g., hydroxycitric acid, hibiscus acid, citric acid, malic acid), phenolic acids (e.g., protocatechuic acid), flavonoids, mucilages, pectins, potassium salts, etc.

Description of the plant (Figs. 1a, 1b) Annual herb or subshrub, 1 to 2(–5) m high; stem erect, cylindrical, branched, woody at base, green when young becoming reddish to purplish with age, glabrous or covered with aculeate warts or caducous bristles. Leaves simple, alternate, dimorphic, proximal leaves ovate, margin serrate, distal leaves palmately 3- to 5-lobed, 6 to 15 cm long, lobes lanceolate, elliptic to oblong, 2 to 8 cm long, 0.5 to 1.5 cm wide, apex obtuse or acuminate, base round or broadly cuneate, margin serrate or dentate, blade glabrous or pubescent, basal veins 3 to 5, green or red, bearing a gland at base of midrib; petiole 4 to 15 cm long, somewhat spiny near the top; stipule filliform, about 1 cm long. Flower solitary, axillary; pedicels less than 3 cm long; epicalyx reddish, 8- to 12-lobed, 6 to 10 mm long, connate at base, adnate to the base of calyx, lobe lanceolate, 0.5 to 1.8 cm long, 2 to 3 mm wide, sparsely hirsute, with spiny appendix near apex; calyx fleshy, reddish or purplish, hirsute, lobes 5, connate below the middle into cup-shaped, each lobe triangular, with a prominent costa extending as far as its apex, base of each sinus between lobes terminated by a bifurcate nerve and bearing a distinct nectary gland, calyx after anthesis becoming thick-fleshy, 2.5 to 5.5 cm long, distinctly longer than fruit; corolla pink or yellow with dark red centre, petals 5, obovate, 4 to 6 cm long, 1 to 4 cm wide; staminal tube 1.5 to 2 cm long, anthers along the tube, pistils in the tube; ovary superior, 5-loculed, ovules 3 to many per locule, style branches 5, stigmas capitate. Fruit capsule, reddish, ovoid to globose, 1.5 to 3 cm long, 1 to 1.5 cm wide, loculicidally 5-valved, beaked, sparsely to densely appressed hairy, enclosed by red, fleshy, accrescent calyx. Seeds numerous, reniform, 2 to 6 mm long, brownish to blackish.

Description Odour, mild, berry-like; taste, sour.

Macroscopical (Fig. 1a) Dried calyx and epicalyx, irregularly shaped and sized, reddish to dark red, somewhat hard. A nectary found in the lower surface at the basal part of each calyx.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the epicalyx shows epidermis layer, cortex, vascular bundles and pith. Epidermis, a layer of small rectangular cells and some with unicellular trichomes. Cortex, several thin-walled of polygonal parenchyma cells, some containing anthocyanins or rosette aggregate crystals. Vascular bundles, collateral. Aerenchyma, some containing rosette aggregate crystals located at the innermost region.

Fig. 1a *Hibiscus sabdariffa* L.
1. habit 2. flower 3. persistent calyx and epicalyx 4. crude drug

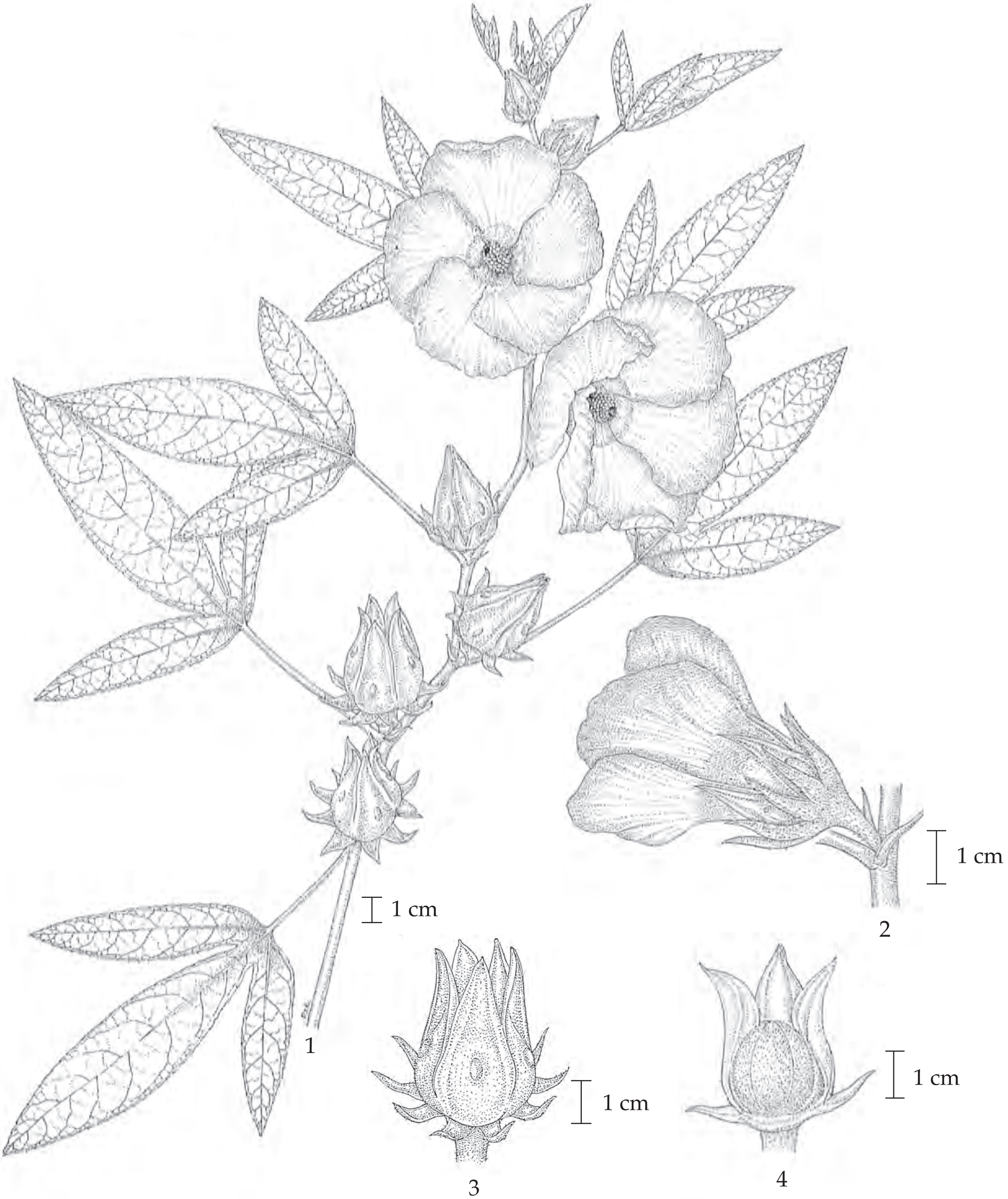


Fig. 1b *Hibiscus sabdariffa* L.
1. flowering and fruiting twig 2. flower 3. persistent calyx and epicalyx
4. persistent calyx and epicalyx, part of calyx removed showing fruit

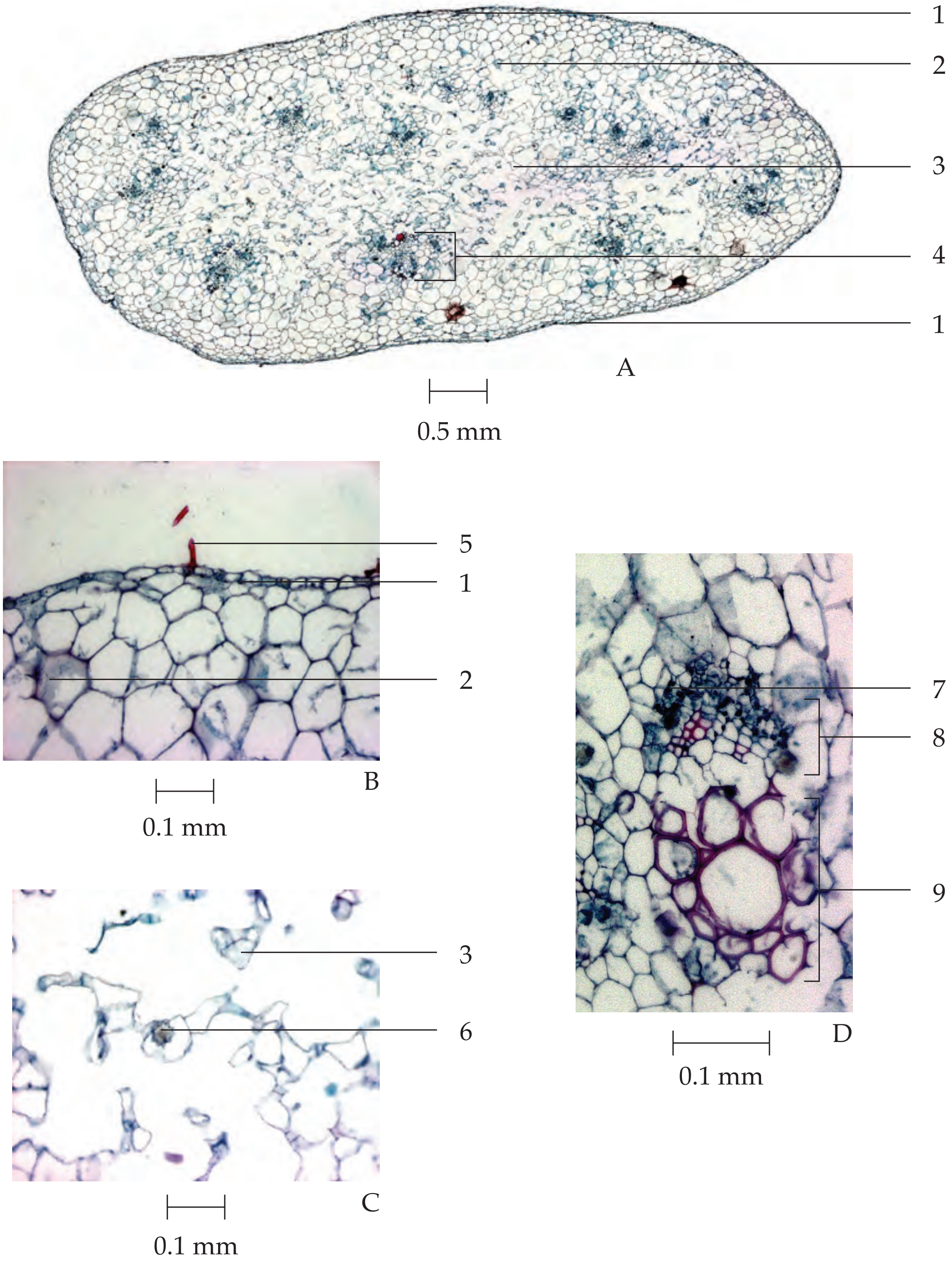


Fig. 2a Photomicrographs of Transverse Section of the Epicalyx of *Hibiscus sabdariffa* L., Stained with Safranin-Fast Green

A. Whole Epicalyx
B. Outermost Part
C. Innermost Part
D. Vascular Bundle

1. epidermis
2. parenchyma with anthocyanins
3. aerenchyma
4. vascular bundle
5. unicellular trichome
6. rosette aggregate crystal
7. fibre
8. phloem
9. xylem

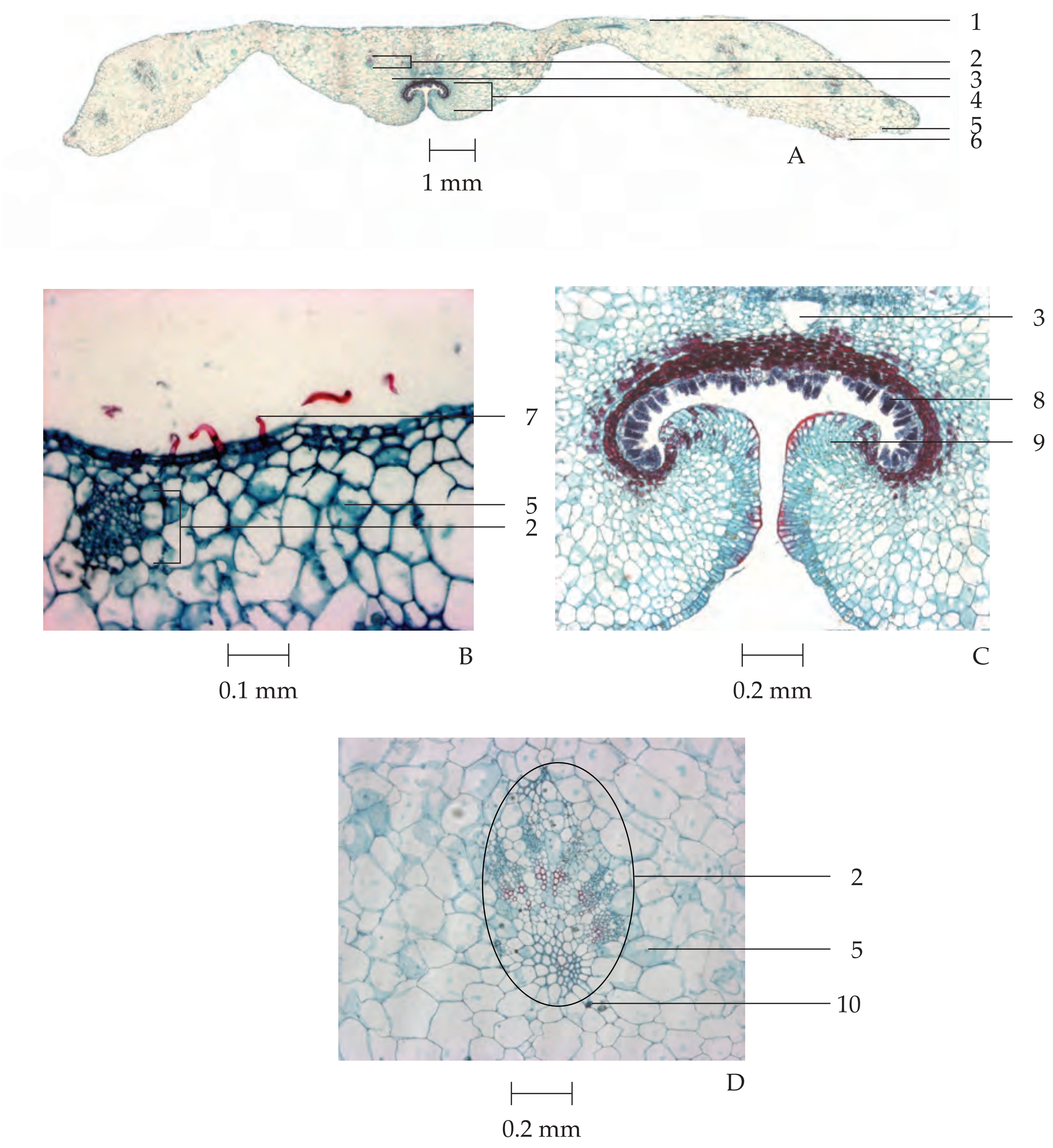


Fig. 2b Photomicrographs of Transverse Section of the Calyx of *Hibiscus sabdariffa* L., Stained with Safranin-Fast Green

A. Whole Calyx through the Nectary
B. Outermost Part
C. Nectary
D. Innermost Part

1. upper epidermis
2. vascular bundle
3. schizolysigenous duct
4. nectary
5. parenchyma with anthocyanins
6. lower epidermis
7. unicellular trichome
8. glandular trichome
9. columnar parenchyma
10. rosette aggregate crystal

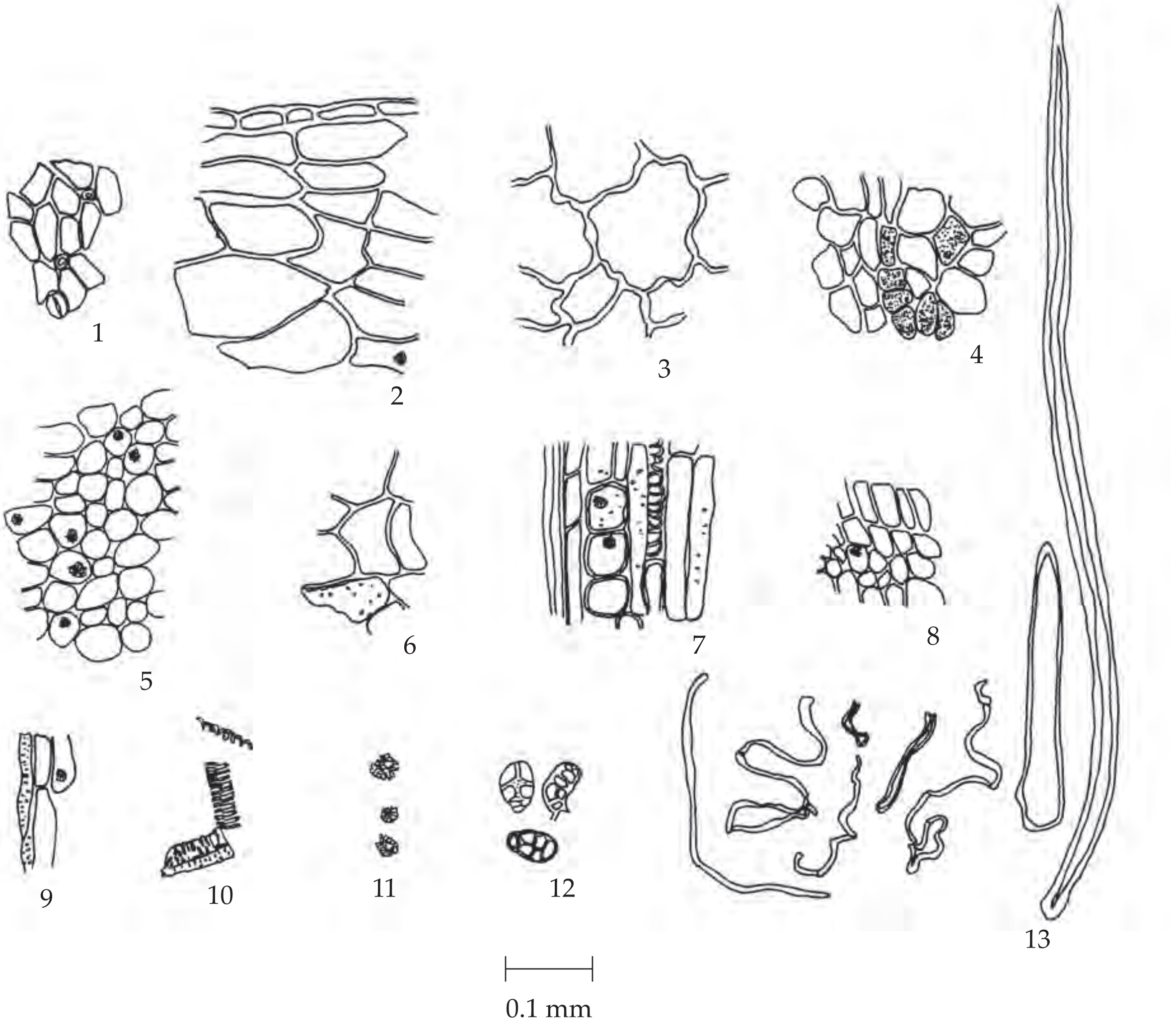


Fig. 2c Powdered Drug of the Calyces and Epicalyces of *Hibiscus sabdariffa* L.

1. lower epidermis, stomata and cicatrices
2. epidermis associated with parenchyma and rosette aggregate crystal in sectional view
3. parenchyma
4. parenchyma, some containing anthocyanins
5. parenchyma, some containing rosette aggregate crystals
6. parenchyma and pitted parenchyma
7. parenchyma containing rosette aggregate crystals, associated with fibres and vessel
8. columnar parenchyma of nectary
9. parenchyma and pitted vessels
10. spiral and pitted vessels
11. rosette aggregate crystals
12. glandular trichomes
13. unicellular trichomes

Transverse section of the calyx through the nectary shows a layer of upper and lower epidermises, cortex, vascular bundles, and nectary. Upper and lower epidermises, small rectangular cells with some modified unicellular trichomes. Cortex, round parenchyma cells, some containing anthocyanins, rosette aggregate crystals, and schizolysigenous ducts. Vascular bundles, collateral, containing phloem and xylem tissues. Nectary, composed of glandular trichome, small rectangular epidermis and several layers of slightly thick-walled, columnar parenchyma cells, connected with large vascular bundles and schizolysigenous ducts.

Roselle in powder possesses the diagnostic microscopical characters of the unground drug. Columnar parenchyma of nectary, unicellular and glandular trichomes, rosette aggregate crystals, and parenchyma containing anthocyanins are characteristic.

Contra-indication It is contra-indicated in patients with impaired renal function.

Packaging and storage Roselle shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. To 300 mg of the sample, in powder, add 8 ml of *water*, shake for 15 minutes and filter (solution 1). To 0.2 ml of solution 1, add 2 M *sodium hydroxide* dropwise: a bluish green colour develops and changes to red after immediately adding a few drops of 2 M *hydrochloric acid*.

B. To 0.2 ml of solution 1, add 4 drops of *iron(III) chloride TS*: a brownish green colour develops.

C. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 60 volumes of *ethyl acetate*, 20 volumes of *2-propanol*, 20 volumes of *water*, and 10 volumes of *formic acid* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply to the plate as a band of 10 mm, 10 µl of the test solution freshly prepared by macerating 1 g of the sample, in powder, in 10 ml of a 0.1 per cent v/v solution of *hydrochloric acid* in *methanol*, shaking frequently for 15 minutes and filtering. After removal of the plate, allow it to dry in air and examine under daylight. Two violet and several purple bands are observed. Spray the plate with a 5 per cent v/v solution of *sulfuric acid* in *methanol* and heat at 100° for 10 minutes; two pink and other several bands of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Calyces and Epicalyces of *Hibiscus sabdariffa* L.

Band	hR_f Value	Detection	
		Daylight	5 Per Cent V/V Solution of *Sulfuric Acid* in *Methanol*
1	3-5	pale violet	–
2	5-7	–	pale brown
3	5-8	pale purple	–
4	11-13	–	pale brown
5	12-14	pale purple	–
6	15-22	pale purple	–
7	17-22	–	grey
8	31-35	violet	pink
9	34-39	–	pale grey
10	40-44	purple	pink
11	43-47	–	pale grey
12	56-59	–	pale grey
13	70-73	–	pale violet
14	73-75	–	pale brown
15	89-90	–	pale violet
16	93-95	–	grey

Loss on drying Not more than 12.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Total ash Not more than 10.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 7.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 45.0 per cent w/w (Appendix 7.12).

Acid content Not less than 12.0 per cent w/w of acids, calculated as citric acid on the dried basis, when determined by the following method.

Shake 1 g of Roselle, in *No. 250 powder*, accurately weighed, with 100.0 ml of *carbon dioxide-free water* for 15 minutes and filter. To 50.0 ml of the filtrate, add 100 ml of *carbon dioxide free-water*. Titrate with 0.1 M *sodium hydroxide VS* until pH 7.0, determining the end-point potentiometrically (Appendix 6.4). Perform a blank determination, and make any necessary correction. Each ml of 0.1 M *sodium hydroxide VS* is equivalent to 6.4 mg of citric acid.

Dose 1.5 to 3 g of powdered roselle, taken as an infusion, three times a day.

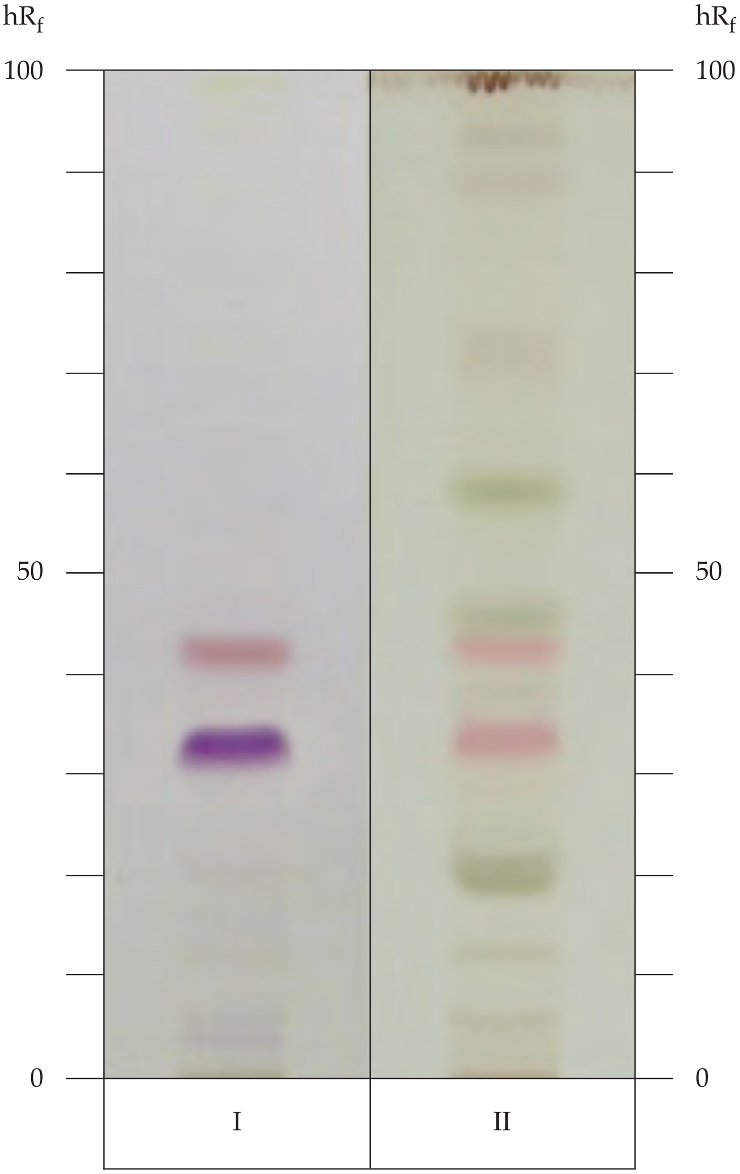


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Calyces and Epicalyces of *Hibiscus sabdariffa* L.

I = detection under daylight

II = detection with a 5 per cent v/v solution of *sulfuric acid* in *methanol*

กระเทียม (KRATHIAM)

Allii Sativi Bulbus
Garlic

Category Carminative, expectorant, antimicrobial (topical), antihyperlipidemic.

Garlic is the fresh compound bulb of *Allium sativum* L. (Family Amaryllidaceae), Herbarium Specimen Number: DMSC 901.

Constituents Garlic contains 0.1 to 0.3 per cent of volatile oil containing allyl disulfide and diallyl disulfide, other organic sulfur compounds including alliin and allicin. It also contains alliinase, mucilage, albumin, vitamins, and thioglycosides.

Description of the plant (Figs. 1a, 1b) Herb, 30 to 60 cm high; subterraneous bulb broadly ovoid, 1 to 4 cm in diameter, often consisting of several crowded bulbils, enclosed by whitish or purplish sheath. Leaves simple, alternate, linear, up to 60 cm long, 0.4 to 2.5 cm wide, longitudinally folded with keel on lower surface, sheathing at base. Inflorescences umbel, globose, with large spathe, usually with bulb-like nodules; peduncle up to 60 cm high. Flower small; tepals 6, oblong, acuminate, white to greenish white, or purple; stamens 6. Fruits capsule small, 3-valved.

Description Odour, aromatic and characteristic; taste, pungent and persistent.

Macroscopical (Fig. 1a) Subglobular compound bulb 3 to 5 cm broad, consisting of 8 to 20 cloves, the whole surrounded by 2 to 5 layers of white scale leaves attached to a flattened circular base. Cloves ovoid and 3- to 4-sided, summit acute, narrowed into a thread-like portion of fibre, base truncate. Each clove is covered with a white scale leaf and a pinkish white epidermis easily separated from the solid portion, consisting of two flaky scale leaves and two yellowish green conduplicate foliage leaves.

Microscopical (Fig. 2) Outer and inner scale leaves have outer epidermis of elongated, subrectangular cells with beaded walls; outer epidermis of inner scale are markedly sclerenchymatous. The hypodermal cells are elongated and thick-walled with triangular intercellular spaces at the corners. Each cell contains a prism of calcium oxalate 20 to 50 µm long. Broad zone of large thin-walled parenchyma in which vascular bundles scattered. Inner epidermis consists of elongated beaded walled cells. Scale leaf cells lignified. Fleshy leaf bases have a thin-walled epidermis, a mesophyll of oval parenchymatous cells and faintly lignified annular and spiral vessels.

Fig. 1a *Allium sativum* L.
1. habit 2. inflorescence 3. fruits 4. fresh bulbs and roots 5. crude drug

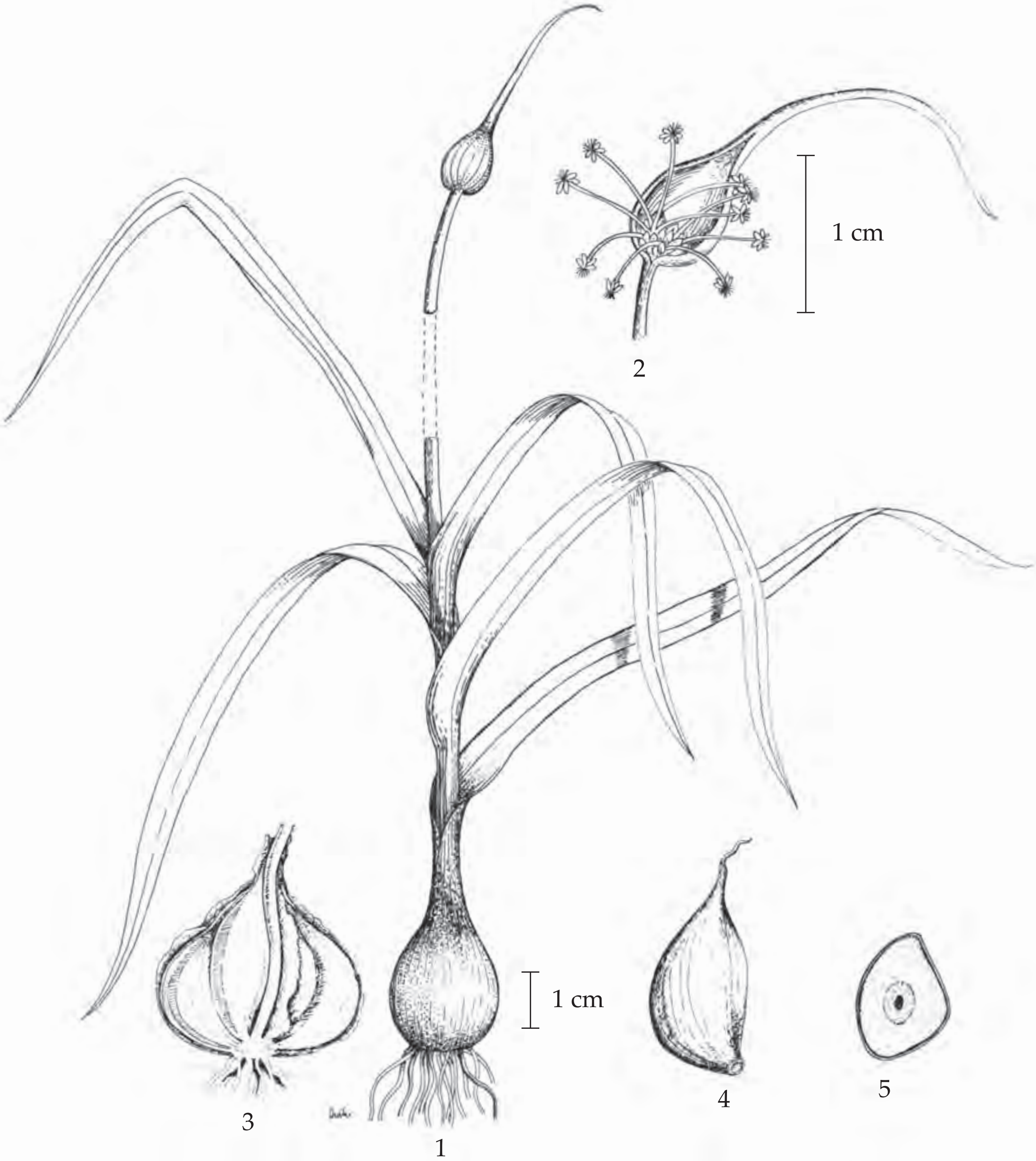


Fig. 1b *Allium sativum* L.
1. habit 2. inflorescence 3. garlic bulb 4. garlic clove 5. cross section of garlic clove

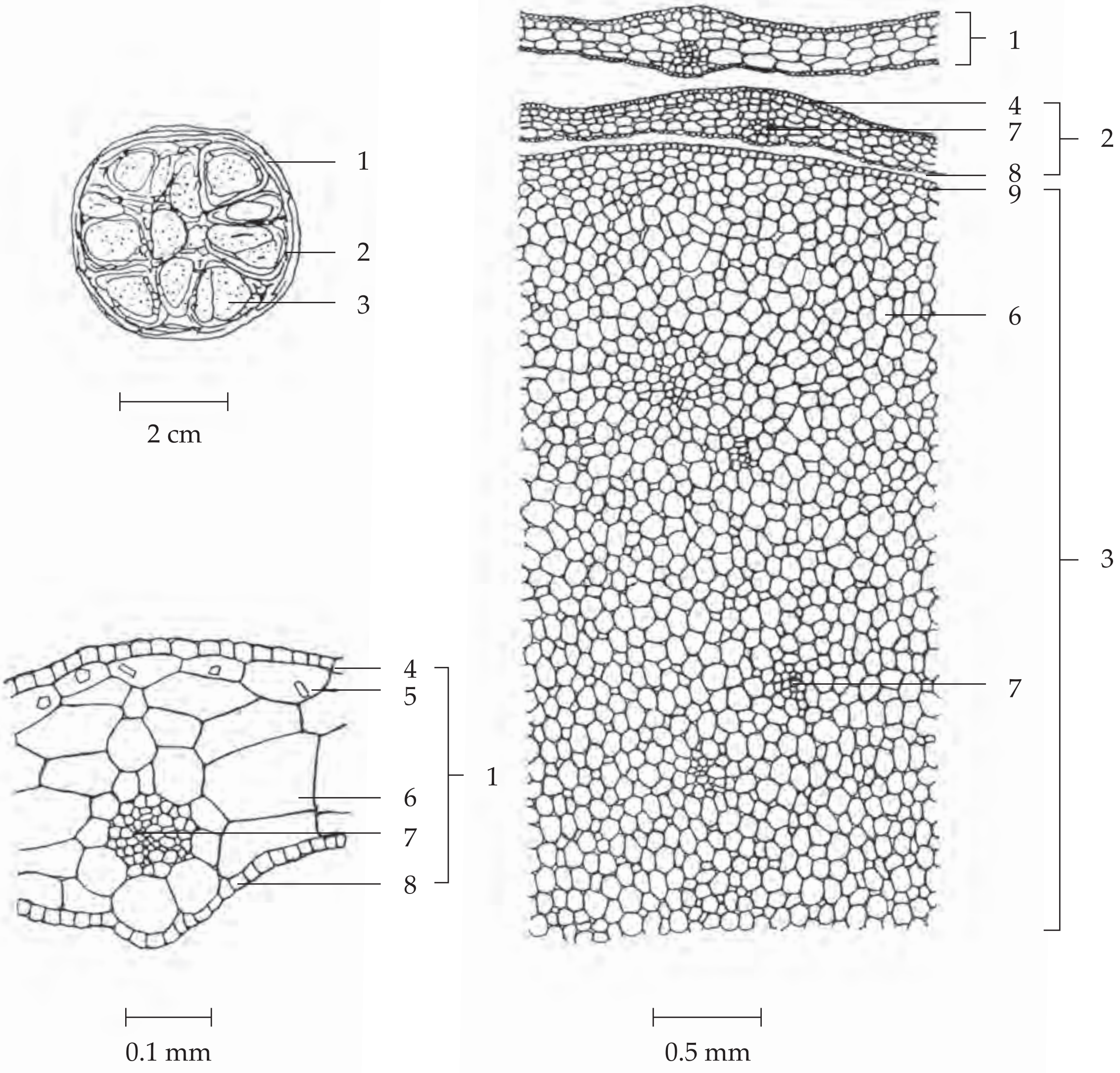


Fig. 2 Transverse Section of the Bulblet of *Allium sativum* L.

1. outer scale leaf
2. inner scale leaf
3. fleshy leaf base
4. outer epidermis
5. hypodermis containing prism
6. parenchyma cell
7. vascular bundle
8. inner epidermis
9. epidermis

Storage Garlic shall be stored in a well-ventilated, dry place, protected from light; under these conditions it may be stored for about 6 months after harvesting.

Identification

A. Shake 500 mg of crushed, peeled sample with 25 ml of *methanol* for 5 minutes and filter. Dilute 0.5 ml of the filtrate to 2 ml with *methanol*, add 2 drops of *ninhydrin TS* and immerse in a water-bath for a few minutes: a violet-blue colour is produced.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 30 volumes of *1-butanol*, 10 volumes of *water*, 10 volumes of *1-propanol*, and 10 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate, 20 µl each of the following two solutions. Prepare solution (A) by refluxing 1 g of the crushed, peeled sample, with 10 ml of an 80 per cent v/v solution of *methanol* on a water-bath for 15 minutes, cooling and filtering. For solution (B), dissolve 25 mg of L-*methionine* in 100 ml of *methanol*. After removal of the plate and allow it to dry in air. Spray with *ethanolic ninhydrin TS*, heat at 105° for about 10 minutes and examine in daylight. Major spots relative to L-*methionine* (RR_f 1) are approximately as follows: purplish brown (RR_f 0.9), purplish brown (RR_f 0.8), orange-brown (RR_f 0.6), pale orange (RR_f 0.38).

In case L-*methionine* cannot be obtained, follow the same procedure but use only solution (A). Several spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Mathanolic Extract of the Cloves of *Allium sativum* L.

Spot	hR_f Value	Detection
		Ethanolic Ninhydrin TS
1	10-13	reddish orange
2	17-26	pale orange
3	22-30	violet
4	28-38	orange-brown
5	34-39	purple
6	41-47	purplish brown
7	50-54	purplish brown

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Total ash Not more than 5.0 per cent w/w (Appendix 7.7).

GARLIC CLOVES

Allii Sativi Bulbletus.

Description Odour, aromatic; taste, pungent and persistent.

Macroscopical Cloves ovoid and 3- to 4-sided, summit acute, narrowed into a thread like portion of fibre, base truncate. Each clove is covered with a white scale leaf and a pinkish white or purplish white epidermis easily separated from the solid portion, consisting of two flaky scale leaves and two yellowish green conduplicate foliage leaves.

Microscopical (Fig. 2) Inner epidermis consists of bead-walled elongated cells, hypodermis of thick-walled elongated cells with triangular intercellular spaces at the corners. Scale leaf cells lignified. Fleshy scale leaves have a thin-walled epidermis, a mesophyll of oval parenchymatous cells and faintly lignified annular and spiral vessels.

Identification Complies with the test for Identification described under Garlic.

Water Not more than 68.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter The proportion of scale leaves, degenerated cloves and other foreign matter does not exceed 3.0 per cent w/w, of which the degenerated cloves do not exceed 1.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 2.5 per cent w/w (Appendix 7.7).

Water-soluble extractive Not less than 15.0 per cent w/w (Appendix 7.12).

Dose 2 to 4 g three times a day.

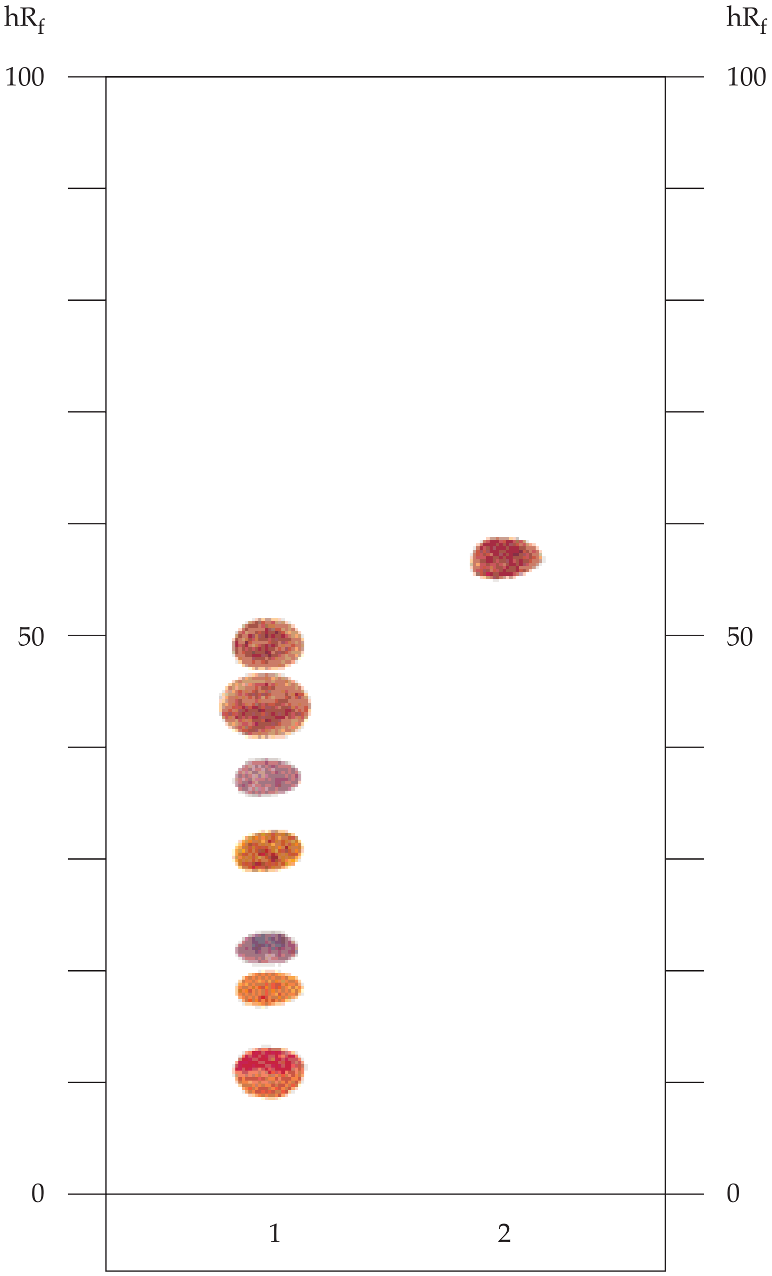


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Cloves of *Allium sativum* L., Detected with *Ethanolic Ninhydrin TS*.

1 = solution (A)
2 = solution (B)

ลักจั่น (LAKKACHAN)

ลักกะจั่น, ลักจันทน์, ลักกะจันทน์ (LAKKACHAN)[1]
Dracaenae Cochinchinensis Lignum
Dracaena Cochinchinensis Wood

Category Antipyretic, anti-inflammatory.

Dracaena Cochinchinensis Wood is the dried reddish fungal-infected wood of *Dracaena cochinchinensis* (Lour.) S. C. Chen (*Aletris cochinchinensis* Lour., *Dracaena loureiroi* Gagnep., *Pleomele cochinchinensis* Merr. ex Gagnep.) (Family Dracaenaceae), Herbarium Specimen Number: DMSC 5179, BKF 168152, Crude Drug Number: DMSc 0934.

Constituents Dracaenae Cochinchinensis Lignum contains flavonoids such as loureirin A and loureirin B, stilbenoids, steroids, etc.

Description of the plant (Figs. 1a, 1b) Tree, 5 to 15 m high; stem more or less branched, internode short, bark smooth, greyish white, becoming greyish brown with age. Leaves simple, spirally arranged, crowded at the top, sword-shaped, 30 to 100 cm long, 2 to 5 cm wide, apex acute, base completely covering internode, margin entire, blade leathery. Inflorescence terminal, panicle, more than 40 cm long, drooping; rachis densely papillose-pubescent. Flower whitish, in clusters of 2 to 5; pedicel 3 to 6 mm long, articulate distally; perianth campanulate, 6 to 8 mm long, tube 1.5 to 2 mm long, lobes 6, 5 to 6 mm long; stamens 6, inserted in tube of perianth, filament reddish, flat, 0.5 to 0.7 mm wide, anther versatile; ovary superior, 3-loculed, ovule(s) 1 or 2 per locule, style slender, stigma capitate. Fruit berry, subglobose, 8 to 12 mm wide, orange when ripe. Seed(s) 1 to 3.

Description Odour, slightly aromatic; taste, slightly astringent.

Macroscopical (Fig. 1a) Irregular pieces of woody segment, smooth or rough, easily broken, brownish red; longitudinal surface spirally-grained.

Microscopical (Figs. 2a, 2b, 2c, 2d) Transverse section of the wood shows parenchyma cells and scattered vascular bundles. Vascular bundle, concentric and amphivasal, surrounded by thick-walled fibre-tracheids; vessels, thick-walled, with orange to reddish resinous mass; phloem tissue, small and thin-walled, located in the centre. Parenchyma cell, thin-walled, round or ovate, some containing raphides or infected by fungal mycelia.

Longitudinal section of the wood shows parenchyma cells and vascular bundles. Vessels, elongated, thick-walled with scalariform, reticulate or bordered pits, some containing orange to reddish resinous mass. Fibre-tracheid, elongated, thick-walled with bordered pits, some containing orange to reddish resinous mass. Phloem tissue, elongated, thin-walled cells. Parenchyma cell, ovate or rectangular, slightly thick-walled, some containing raphides or fungal mycelia.

Dracaena Cochinchinensis Wood in powder possesses the diagnostic microscopical characters of the unground drug.

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[1] The four Thai names, i.e. ลักกะจั่น, ลักจันทน์, and ลักกะจันทน์, spelled and pronounced differently can be transcribed into the same English phonetic transcription “LAKKACHAN”.

Fig. 1a *Dracaena cochinchinensis* (Lour.) S. C. Chen
1. flowering branches 2. inflorescence 3. infructescences
4. ripe fruits and seeds 5. crude drug

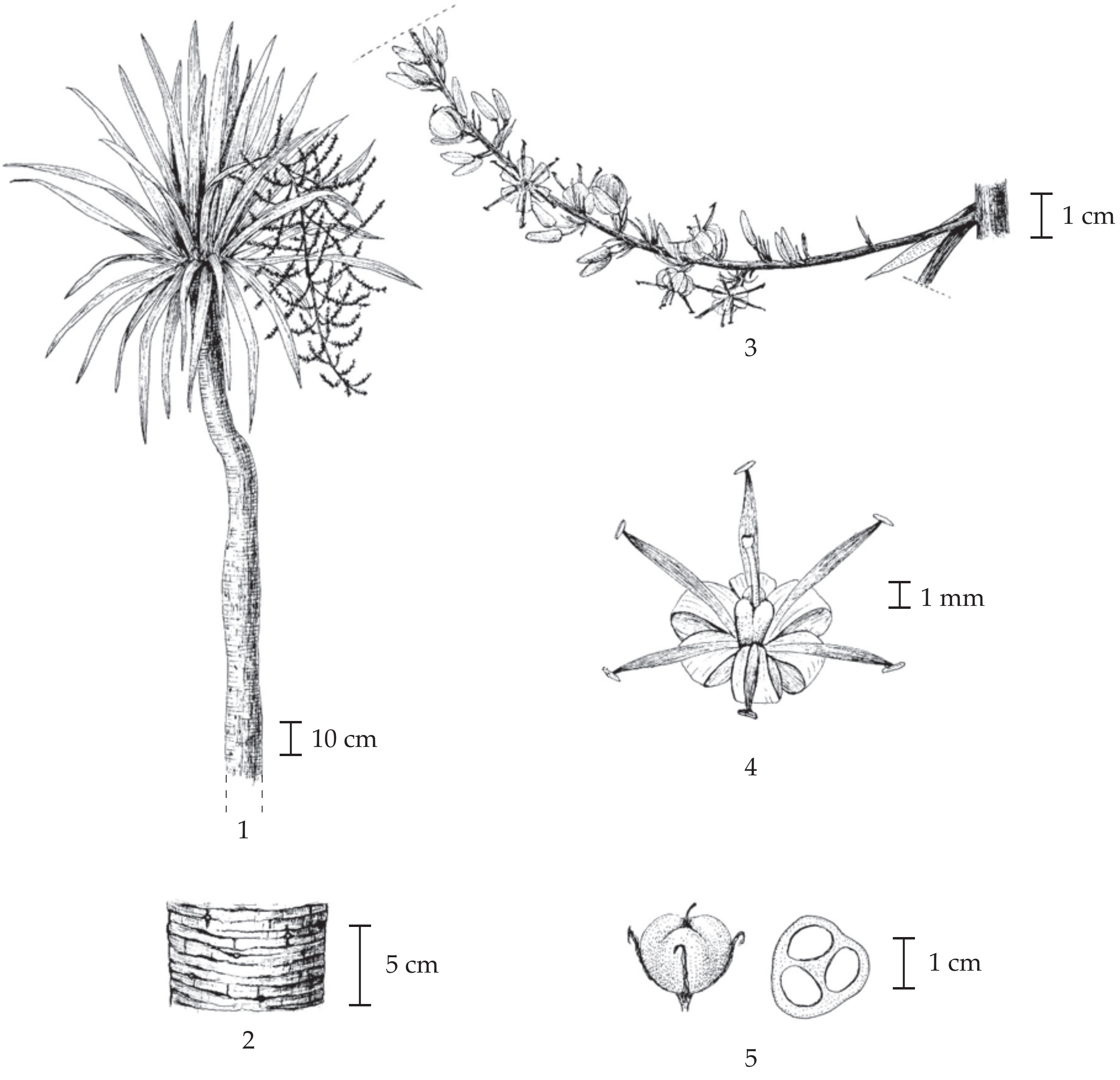


Fig. 1b *Dracaena cochinchinensis* (Lour.) S. C. Chen
1. flowering branch 2. surface of stem 3. part of inflorescence
4. flower 5. fruit and cross section of fruit

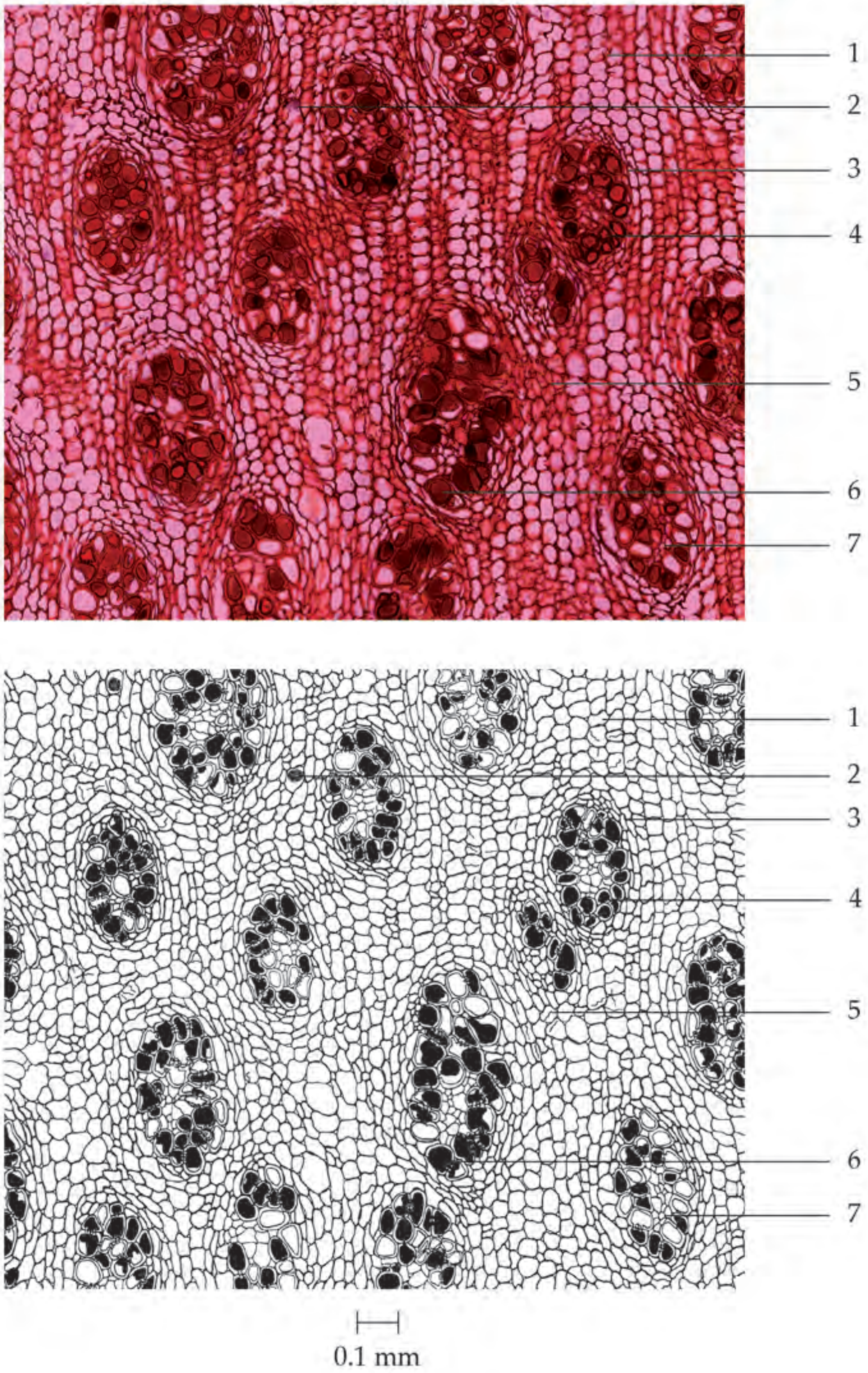


Fig. 2a Photomicrograph and Line Drawing of Transverse Section of the Wood of *Dracaena cochinchinensis* (Lour.) S. C. Chen

1. parenchyma cell
2. raphide
3. fibre-tracheid
4. vessel
5. fungal mycelia in parenchyma cell
6. orange to reddish resin in vessel
7. phloem tissue

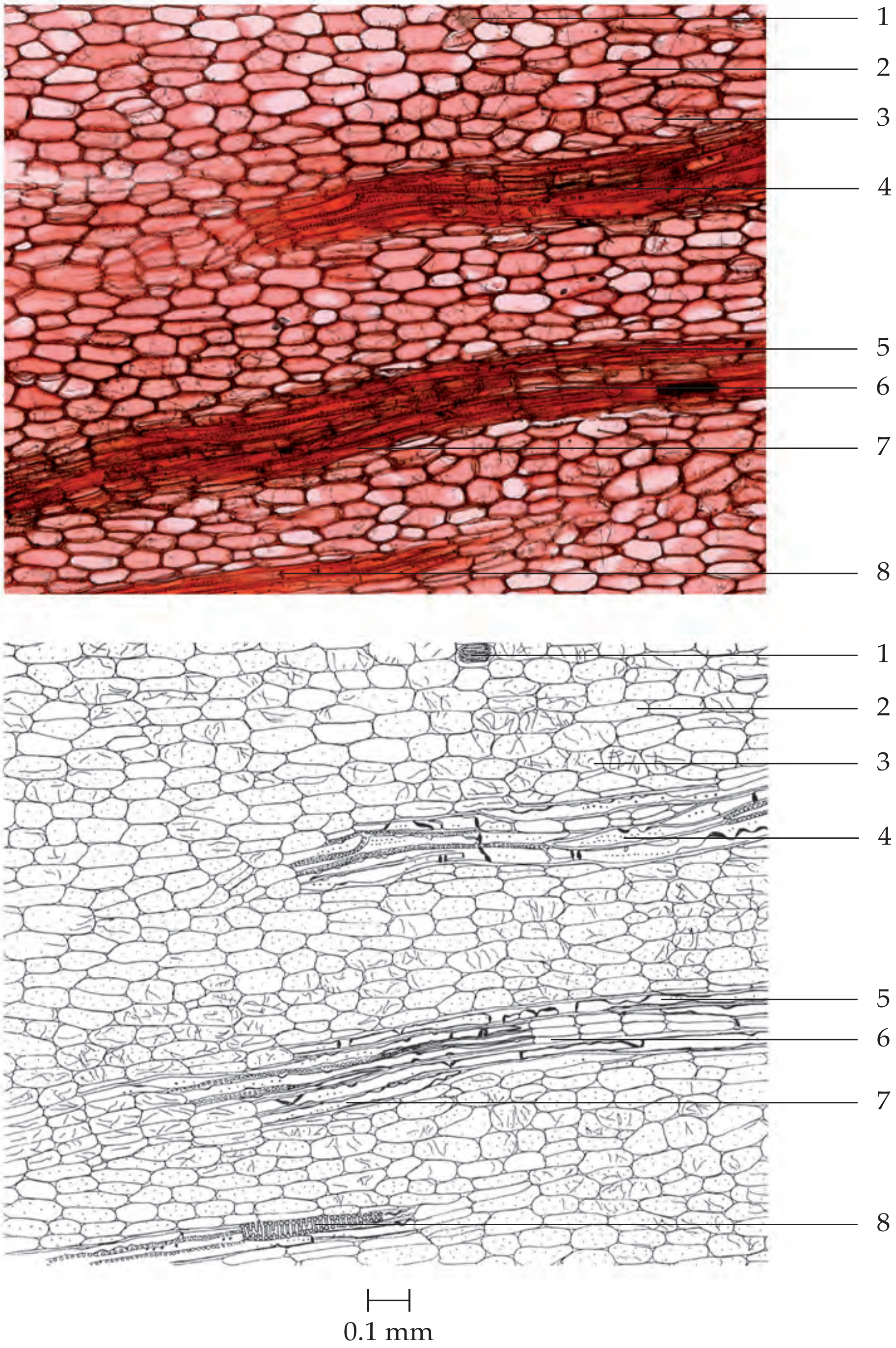


Fig. 2b Photomicrograph and Line Drawing of Longitudinal Section of the Wood of *Dracaena cochinchinensis* (Lour.) S. C. Chen

1. raphide
2. parenchyma cell
3. fungal mycelia in parenchyma cell
4. pitted vessel
5. orange to reddish resin in vessel
6. phloem tissue
7. fibre-tracheid
8. reticulate vessel

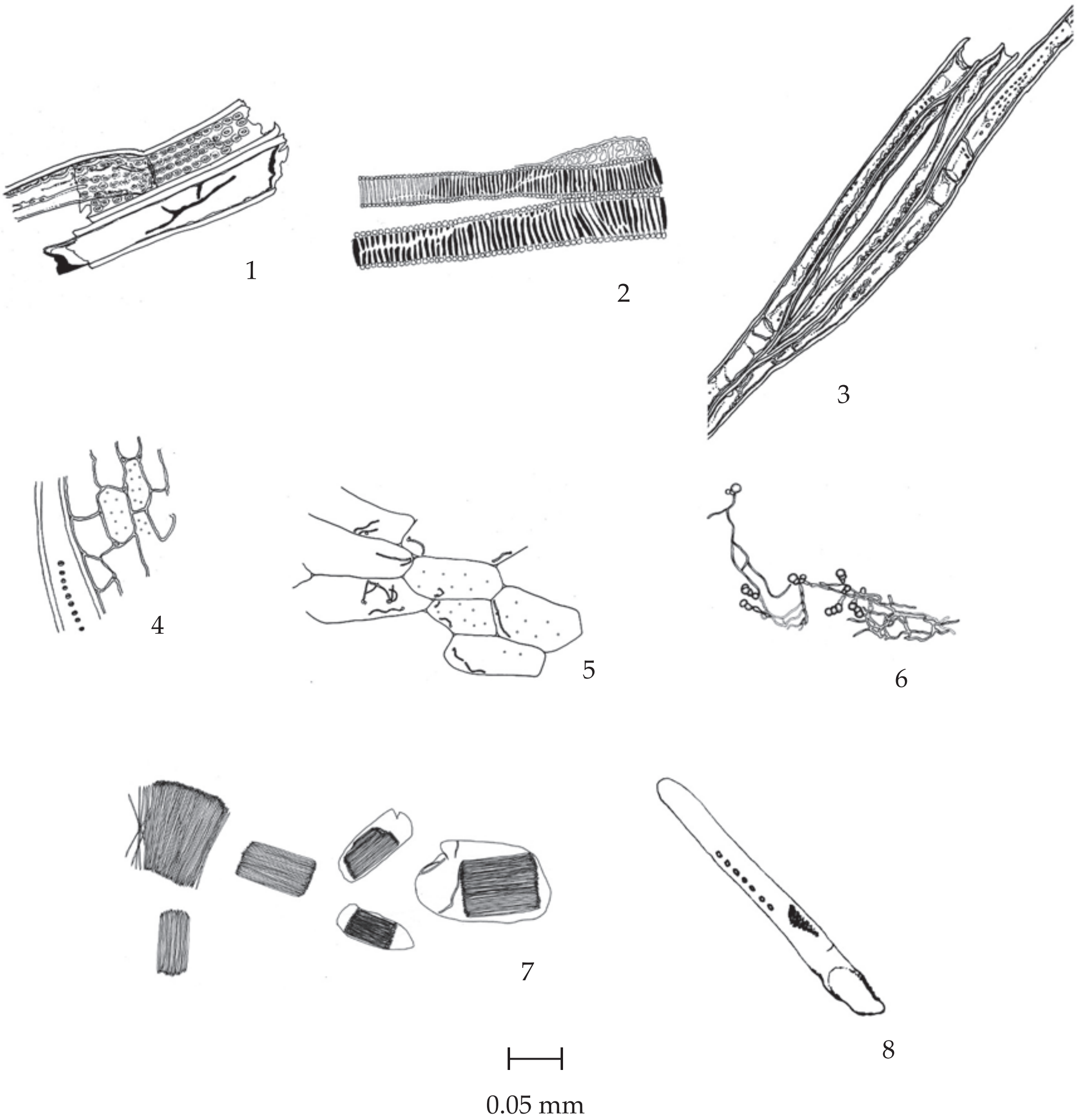


Fig. 2c Line Drawings of Powdered Drug of the Wood of *Dracaena cochinchinensis* (Lour.) S. C. Chen

1. bordered-pitted vessel
2. scalariform, reticulate and bordered-pitted vessels
3. fibre-tracheids
4. parenchyma cells and fibre-tracheid
5. fungal mycelia in parenchyma cells
6. fungal mycelia
7. raphides
8. orange to reddish resin in vessel

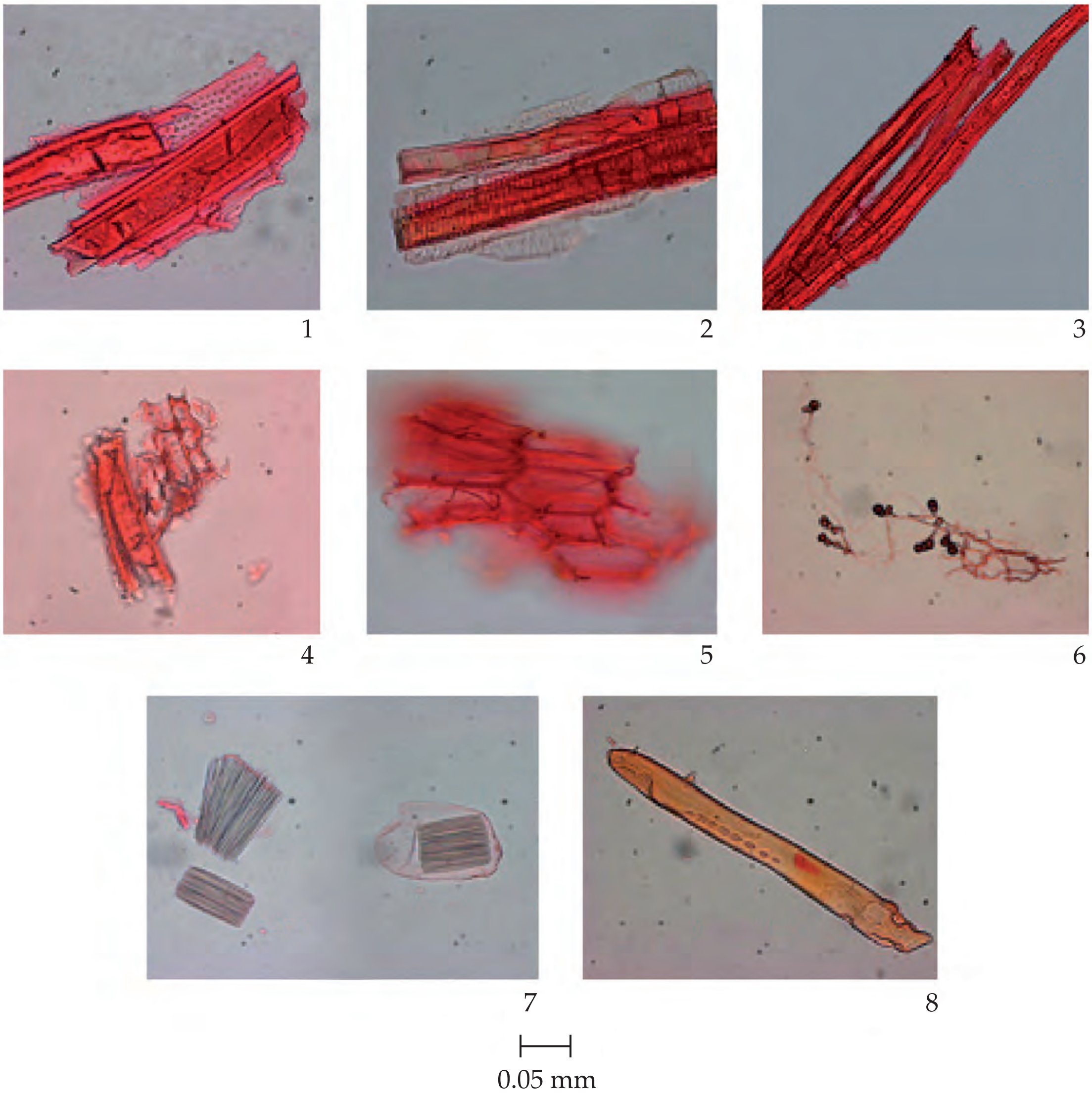


Fig. 2d Photomicrographs of Powdered Drug of the Wood of *Dracaena cochinchinensis* (Lour.) S. C. Chen

1. bordered-pitted vessel
2. scalariform, reticulate and bordered-pitted vessels
3. fibre-tracheids
4. parenchyma cells and fibre-tracheid
5. fungal mycelia in parenchyma cells
6. fungal mycelia
7. raphides
8. orange to reddish resin in vessel

Additional information It commercially available as a substitute for Chan Daeng (red sandalwood) in Thai traditional medicine.

Packaging and storage Dracaena Cochinchinensis Wood shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 1 g of the sample, in powder, with 30 ml of *methanol* for 30 minutes and filter (solution 1). Evaporate 2 ml of solution 1 to dryness. Dissolve the residue in 1 ml of *acetic anhydride*, slowly add a few drops of *sulfuric acid* and mix: a green colour develops.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 50 volumes of *hexane*, 50 volumes of *ethyl acetate* and 2.5 volumes of *methanol* as the mobile phase. Apply to the plate, 5 µl of the test solution prepared by evaporating about 20 ml of solution 1 to dryness and dissolving the residue in 2 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter; several blue fluorescent spots are observed. Spray the plate with *anisaldehyde TS* and heat at 110° for 10 minutes; several spots of different colours appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Wood of *Dracaena cochinchinensis* (Lour.) S. C. Chen

Spot	hR_f Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	16-19	–	bright blue	–
2	19-22	quenching	–	purplish brown
3	33-44	quenching	–	orange
4	45-52	quenching	blue	purplish brown
5	56-62	weak quenching	–	purplish brown
6	65-69	weak quenching	–	orange
7	69-72	–	light blue	–
8	72-74	quenching	–	purplish brown
9	74-79	weak quenching	–	pink
10	79-82	weak quenching	light blue	purplish brown
11	87-90	–	blue	pale violet
12	95-96	–	–	pale violet
13	97-98	–	–	pale violet

Loss on drying Not more than 9.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 0.5 per cent w/w (Appendix 7.2).

Total ash Not more than 1.2 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 17.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 2.0 per cent w/w (Appendix 7.12).

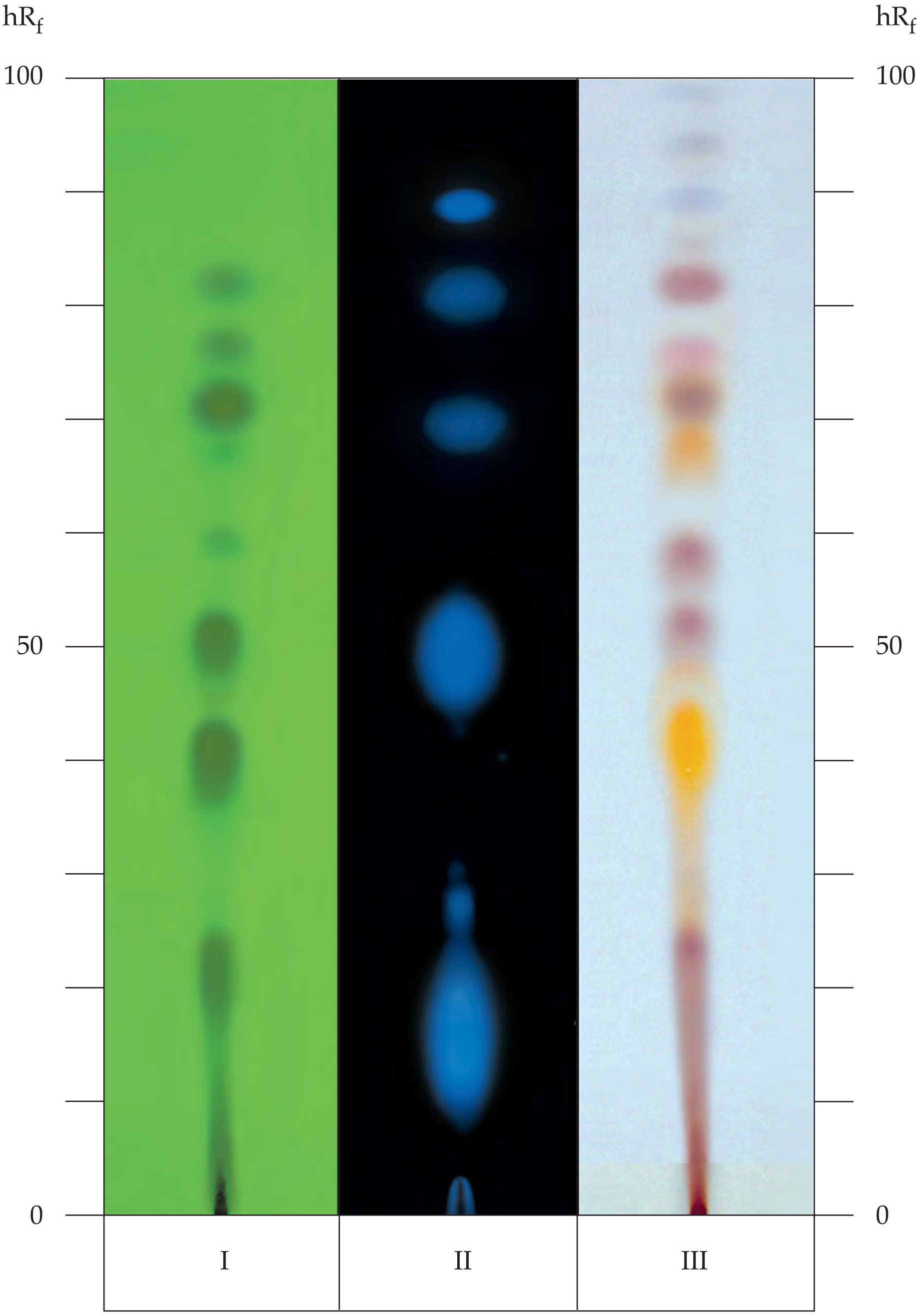


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Wood of *Dracaena cochinchinensis* (Lour.) S. C. Chen

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *anisaldehyde TS*

มะขามป้อม (MAKHAM POM)

Phyllanthi Emblicae Fructus
Emblic Myrobalan

Category Expectorant, laxative with secondary astringent action, antiscorbutic.

Emblic Myrobalan is the dried mature fruit of *Phyllanthus emblica* L. (*Emblica officinalis* Gaertn.) (Family Euphorbiaceae), Herbarium Specimen Number: DMSC 904.

Constituents Emblic Myrobalan contains ascorbic acid, rutin, mucic acid, gallic acid, phyllemblic acid, etc.

Description of the plant (Figs. 1a, 1b) Small or medium-sized tree, up to 20 m high, deciduous, with crooked trunk and spreading branches; bark greenish gray, peeling off in conchoidal flakes; branchlets glabrous or finely pubescent 10 to 20 cm long. Leaves imbricate when young, subsessile, 0.5 to 2.5 cm by 1.5 to 5.5 mm, closely set along the branchlets, distichous, light green, glabrous, narrowly-linear, obtuse, having appearance of pinnate leaves; stipules minute, ovate, finely acute. Flowers small, monoecious, apetalous, greenish yellow, in axillary fascicles on the leaf-bearing branchlets, often on the naked portion below the leaves, with fimbricate bracts at the base. Male flowers numerous, on short slender pedicel; calyx-lobes 6, oblong, obtuse, 1.2 mm long; anthers 3, filaments united in a short central column; disk-glands 6 alternating with the calyx-segments. Female flowers few, subsessile or sessile; calyx as in the male; ovary 3-celled, half immersed in the lacerate, cup-shaped disc, style connate at the base, stigma 3, bilobed, lobes dilated, recurved. Fruits sessile, 1.3 to 2.7 cm in diameter, fleshy, globose or depress globose, with 6 longitudinal faint lines, glabrous, lucid, pale yellow; endocarp of triangular cocci, bony, dehiscent, with 3 short bundles of vascular tissue at the base. Seeds 6, trigonous.

Description Odour, slightly aromatic; taste, slightly sour and astringent.

Macroscopical (Fig. 1a) Entire fruit, subspherical, wrinkle, about 1.3 to 2 cm in diameter, often dehiscent; fruit pulp dark brown to black, coriaceous; endocarp, brown, hard, globular, acutely hexangular; testa brown; seed brown, bony, trigonous.

Microscopical (Figs. 2a, 2b) Transverse section of the fruit pulp shows a layer of epicarp. Parenchyma, thin-walled ground tissue, containing prismatic crystals in some cells. Sclereid, lignified, occurring in 3 forms: rectangular sclereid, occurring in groups of a large number of cells near epicarp, containing water-soluble grey masses; spherical sclereid, occurring as single cell or in small groups of 2 to 10 cells; fibrous sclereid, occurring in bundles of 2 to 5 cells. Vascular bundle, lignified, composed of fibres and spiral vessels. Tannin granules, found in parenchyma, more often in the inner layer than in the outer layer.

Emblic Myrobalan in powder possesses the diagnostic microscopical characters of the fruit pulp with additional characters: sclerenchyma; thick-walled parenchyma, containing reddish masses.

1

2

3

4

1 cm

Fig. 1a *Phyllanthus emblica* L.
1. habit 2. flowering twig 3. fruiting twig 4. crude drug

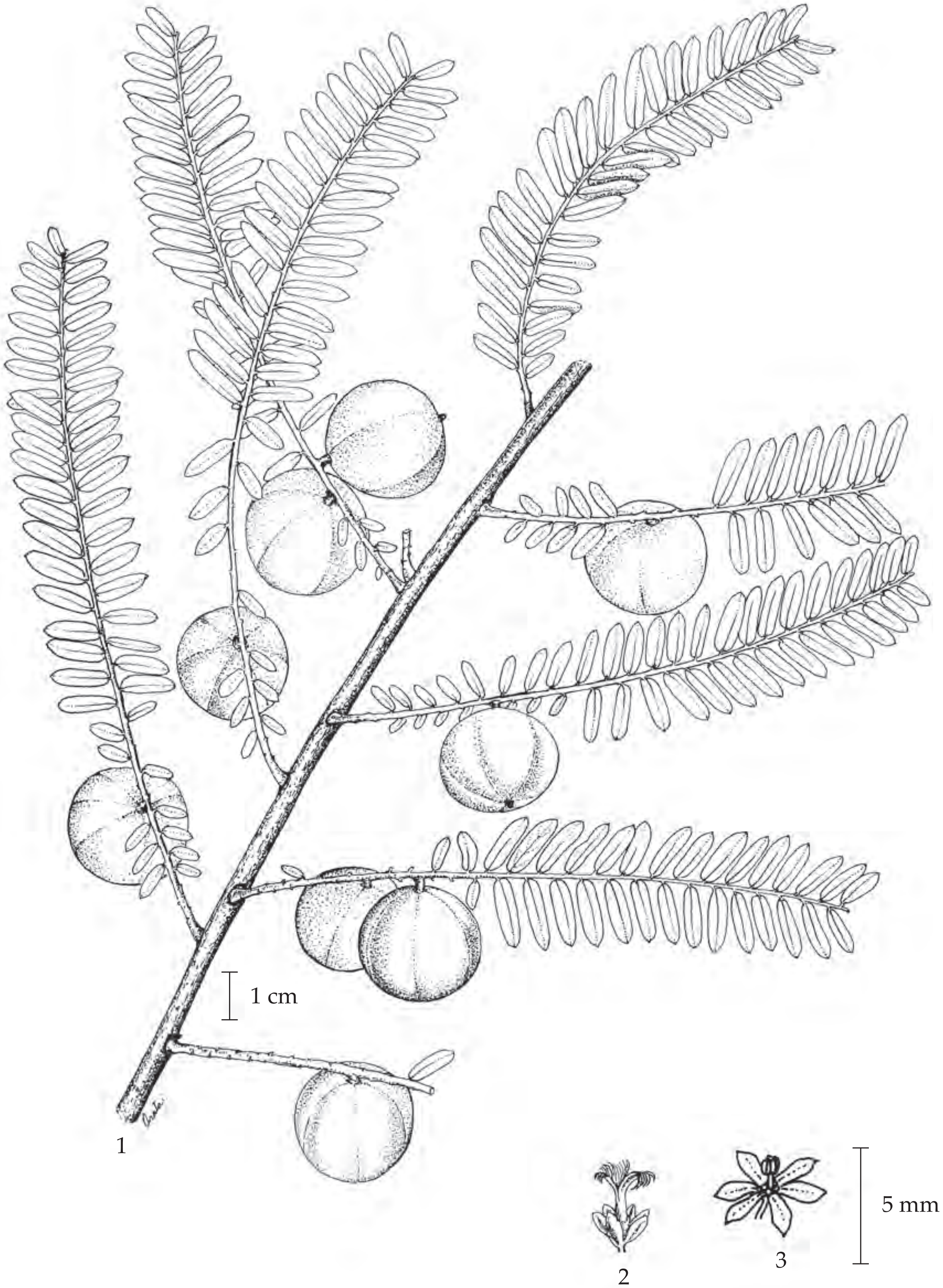


Fig. 1b *Phyllanthus emblica* L.
1. fruiting twig 2. female flower 3. male flower

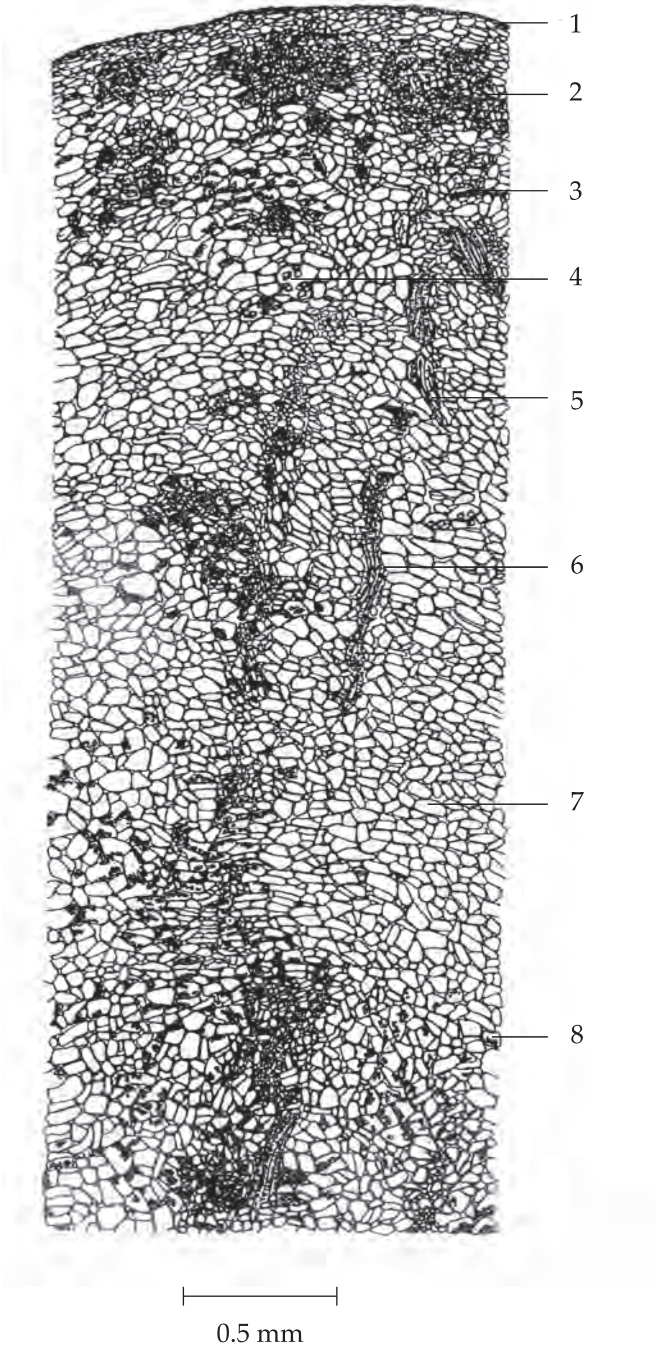


Fig. 2a Transverse Section of the Fruit Pulp of *Phyllanthus emblica* L.

1. epicarp
2. group of sclereids containing grey masses
3. sclereid
4. parenchyma containing prismatic crystals
5. fibrous sclereid
6. vascular bundle
7. parenchyma
8. parenchyma containing tannin granules

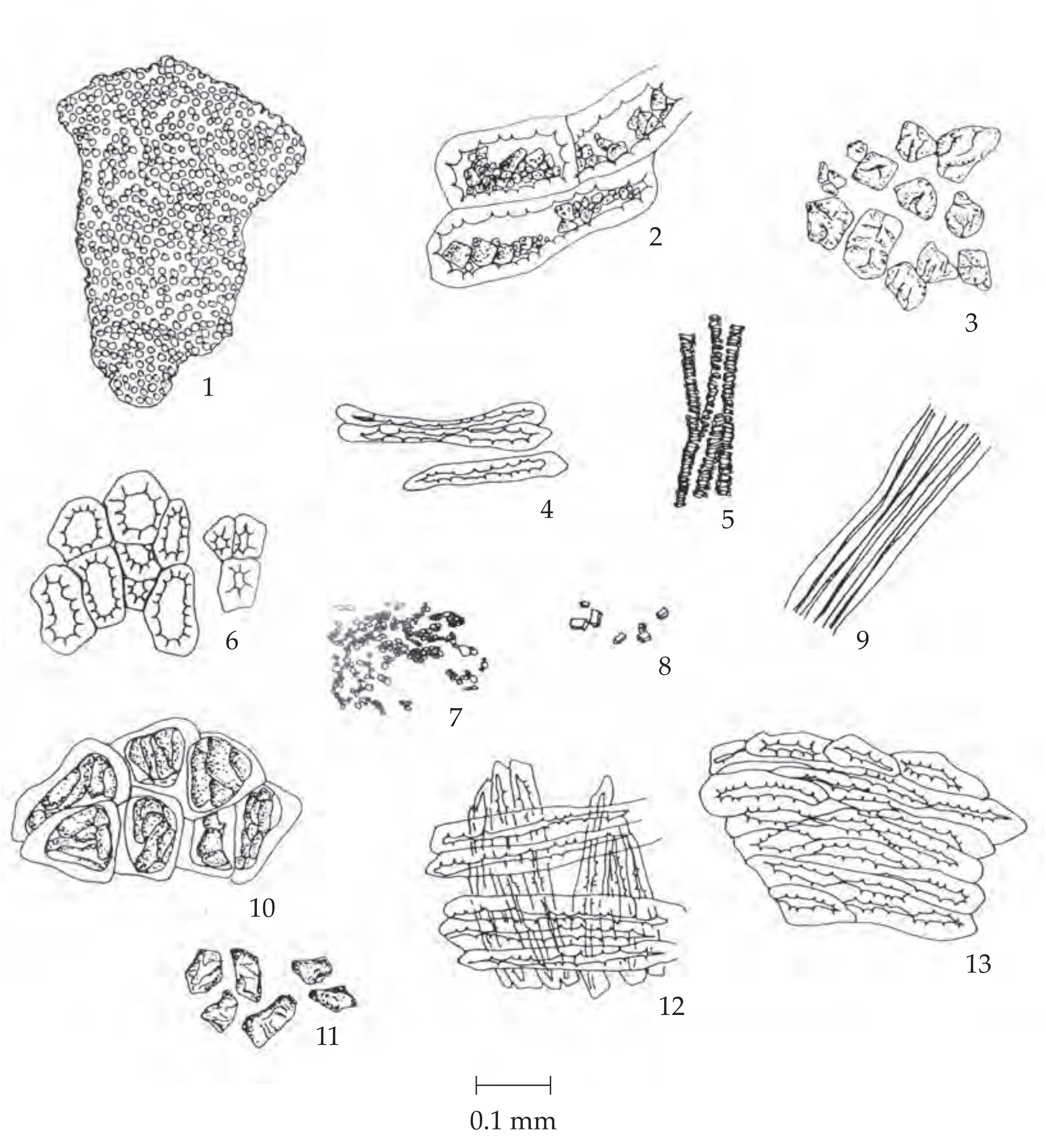


Fig. 2b Powdered Drug of the Fruit Pulp of *Phyllanthus emblica* L.

1. epicarp in surface view
2. sclereids containing grey masses
3. grey masses
4. fibrous sclereids
5. spiral thickenings
6. sclereids
7. tannin granules
8. prismatic crystals
9. fragment of fibres
10. thick-walled parenchyma containing reddish masses
11. reddish masses
12. fibrous sclereids from seed coat
13. sclerenchyma from seed coat

Additional information As an expectorant, it is suggested to frequently sip a juice freshly prepared by squeezing 10 to 30 fresh fruits of emblic myrobalan.

Packaging and storage Emblic Myrobalan shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 1 g of the sample, in powder, with 20 ml of *water* for 10 minutes and filter (solution 1). To 2 ml of solution 1, add a few drops of *sodium hydrogencarbonate TS* and *iron(II) sulfate TS*: a deep violet colour develops. Then add 0.5 ml of a 6 per cent v/v solution of *sulfuric acid*: a deep violet colour disappears.

B. To 2 ml of solution 1, add 1 ml of *ethanol* and shake well: a white gelatinous mass is produced.

C. Reflux 1 g of the sample, in powder, with 25 ml of *ethanol* for 15 minutes, and filter (solution 2). To 2 ml of solution 2, add a few drops of *iron(III) chloride TS*: a blue-black colour develops.

D. To 1 ml of solution 2, add a few drops of *fuming nitric acid*: a red colour develops.

E. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 5 volumes of *toluene*, 4 volumes of *ethyl formate* and 1 volume of *formic acid* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, 20 µl of solution (A) and 1 µl of solution (B). Prepare solution (A) by refluxing 500 mg of the sample, in powder, with 10 µl of *ethanol* for 10 minutes and filtering. To the filtrate, add 500 mg of *decolorizing charcoal*, reflux for a few minutes, filter, wash the filter paper and the funnel with small amount of *ethanol*, and adjust to 10 ml with *ethanol*. For solution (B), dissolve 3 mg of *gallic acid* in 1 ml of *ethanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots.

The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 43 to 44), corresponding to the gallic acid spot from solution (B), and one spot of lower hR_f value. Spray the plate with *iron(III) chloride TS*; the spot due to gallic acid is blue-black and the other blue-black spot is observed (Table 1); see also Fig. 3.

Table 1 hR$_f$ Values of Components in Ethanolic Extract of the Fruits of *Phyllanthus emblica* L.

Spot	hR$_f$ Value	Detection	
		UV 254	*Iron(III) Chloride TS*
1	4-6	quenching	blue-black
2*	43-44	quenching	blue-black

*gallic acid

Loss on drying Not more than 9.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 4.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 16.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 26.0 per cent w/w (Appendix 7.12).

Tannins content Not less than 20.0 per cent w/w (Appendix 7.12H). Use 4 g of Emblic Myrobalan, in *fine powder*, accurately weighed.

Dose 6 to 12 g a day, as a decoction, sipped as needed.

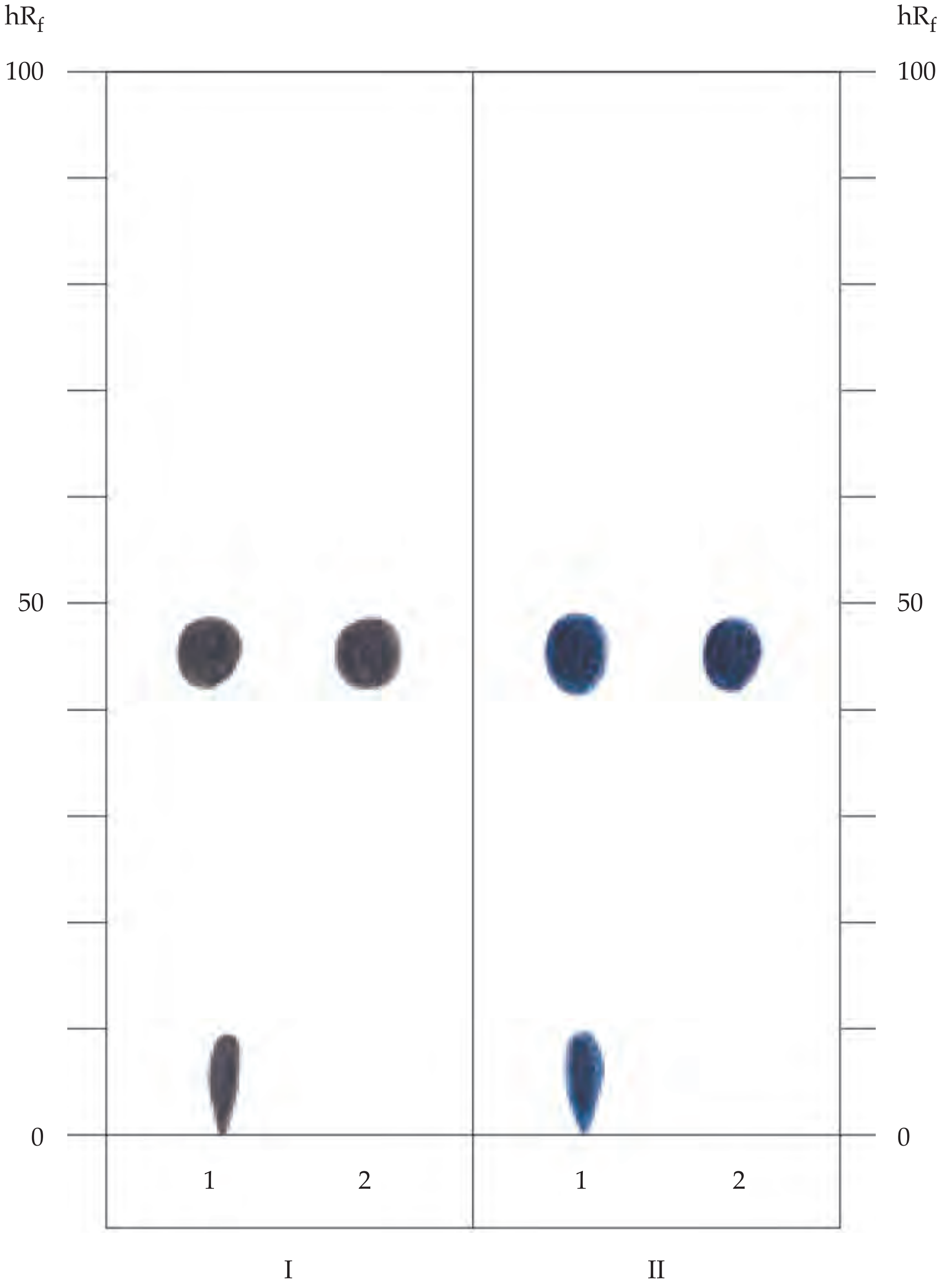


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Fruits of *Phyllanthus emblica* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with *iron(III) chloride TS*

มะกรูด, ใบ (MAKRUT, BAI)

Citri Hystricis Folium
Citrus Hystrix Leaf

Synonyms Kaffir Lime Leaf, Leech Lime Leaf, Porcupine Orange Leaf, Mauritius Papeda Leaf

Category Pharmaceutic aid (flavouring agent), carminative.

Citrus Hystrix Leaf is the dried leaf of *Citrus hystrix* DC. (*C. papeda* Miq., *C. tuberoides* J. W. Benn.) (Family Rutaceae), Herbarium Specimen Number: DMSC 342.

Constituents Citrus Hystrix Leaf contains volatile oil, of which citronellal, citronellol and citronellol acetate are its major components; sabinene, α- and β-pinene, α-phellandrene, limonene, α- and γ-terpinene, cymene, linalool, indole alkaloids are also found. It also contains rutin, hesperidin and diosmin.

Description of the plant (Figs. 1a, 1b) Shrub or small tree, 2 to 12 m high; branchlets compressed-angular when young; spine solitary, 0.5 to 1.5 cm long. Leaves alternate or spirally arranged, very young leaves light reddish purple; winged petioles obovate to obcordate-oblong, 1 to 8 cm long, 1 to 4.5 cm wide; lamina orbicular-ovate or ovate-oblong, 3 to 15 cm long, 2.5 to 6 cm wide, apex obtuse to obtusely acuminate or slightly emarginate, base cuneate or rounded, margin more or less crenate. Inflorescences axillary or terminal, 1- to 15-flowered, fragrant; pedicels 2 to 5 mm long; calyx cupular, 1 to 1.5 mm long, 4-lobed; petals 4 to 5, oval-oblong, 6 to 10 mm long, 3 to 5 mm wide, obtusely acuminate, yellowish white or suffused with violet, with scattered pellucid dots; stamens 24 to 30, free, filaments 2 to 6 mm long; ovary superior, subglobose. Fruits ellipsoid, ovoid or globose, 5 to 7 cm in diameter, irregular bumpy; fruit-pulp yellowish green, very sour, slightly bitter. Seeds ovoid-oblong, 1 to 1.5 cm long, 3 to 5 mm wide.

Description Odour, characteristic, aromatic; taste, slightly bitter.

Macroscopical (Fig. 1a) Citrus Hystrix Leaf occurs as a mixture of entire and broken, greenish brown to brown dried leaves. Petioles obovate, 1 to 8 cm long and 1 to 4.5 cm wide. Lamina ovate, 3 to 15 cm long and 2.5 to 6 cm wide; apex obtuse or retuse or slighty emarginate, undulate at margin.

Microscopical (Figs. 2a, 2b) Transverse section of the leaf shows upper epidermis, a layer of rectangular cells in sectional view or slightly wavy-walled cells in surface view, stomata absent. Mesophyll composed of 2 to 3 layers of palisade and several layers of spongy cells with idioblasts containing calcium oxalate prismatic crystals; fibrovascular bundle and schizolysigenous oil cavity containing oil droplets located in spongy tissues. Fibrovascular bundle composed of fibre surrounding amphicribal vascular bundle; xylem composed of spiral, reticulate, and bordered-pitted vessels and xylem parenchyma some of which containing prismatic crystals of calcium oxalate. Collenchyma, 2 to 3 layers of cells, located in the central part of midrib beneath lower epidermis. Lower epidermis, a layer of rectangular cells in sectional view or wavy-walled cells in surface view with anomocytic type of stomata.

Citrus Hystrix Leaf in powder possesses the diagnostic microscopical characters of the unground drug.

Fig. 1a *Citrus hystrix* DC.
1. habit 2. flowering twig 3. fruits 4. fresh leaves 5. crude drug

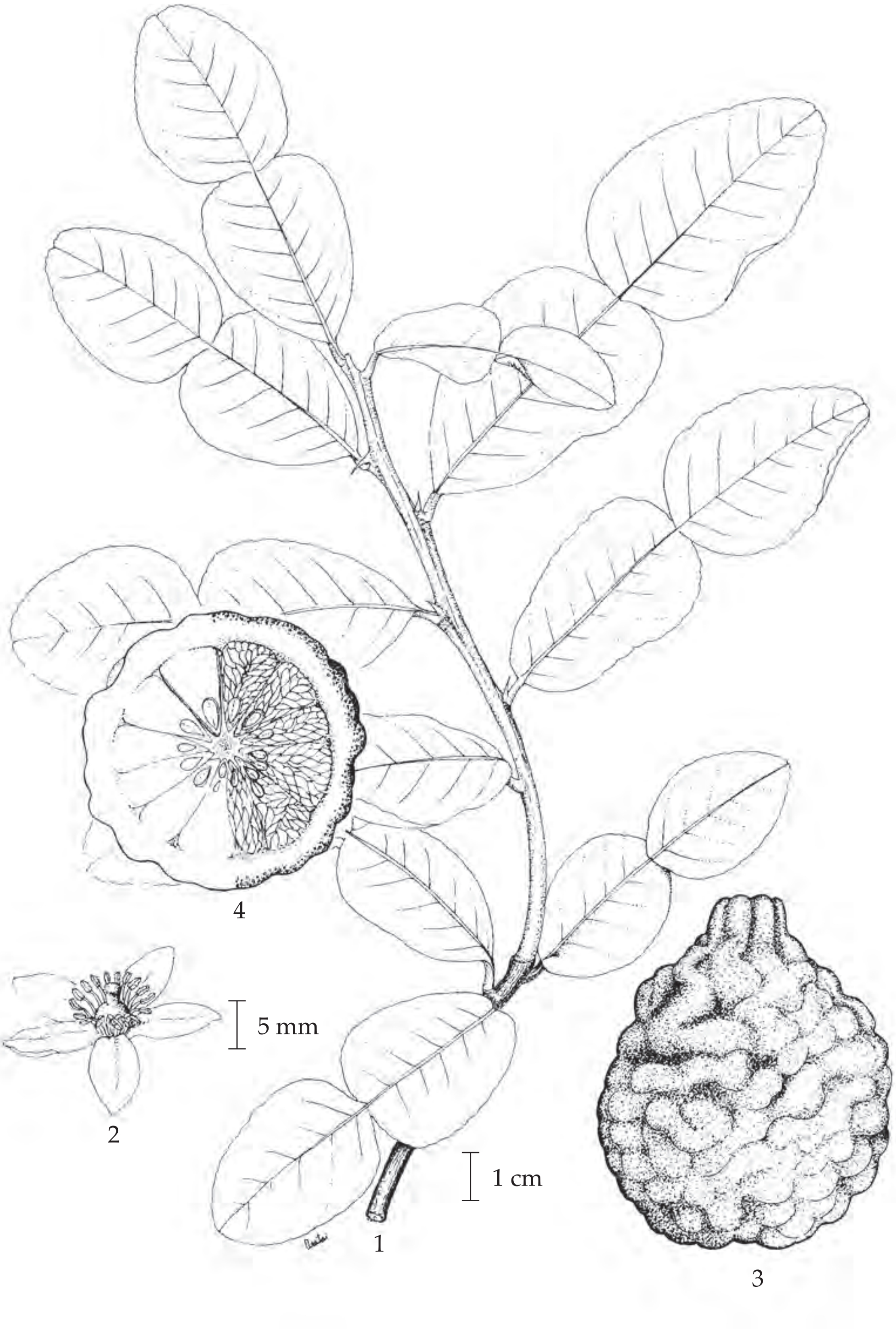
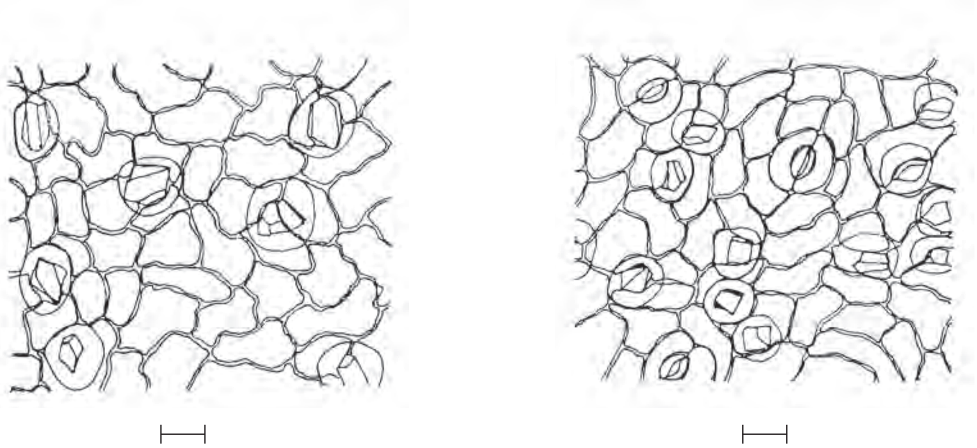


Fig. 1b *Citrus hystrix* DC.

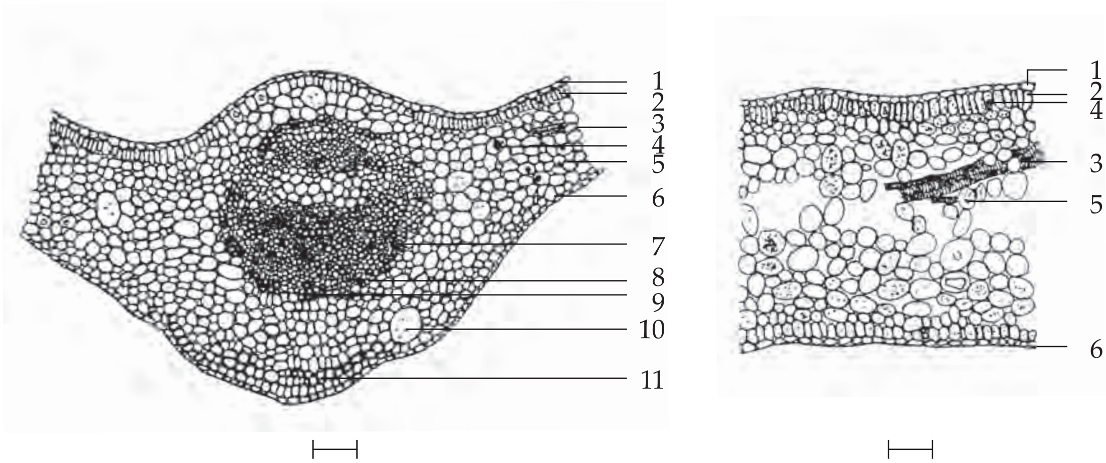
1. twig 2. flower 3. mature fruit 4. cross section of the fruit



Upper Epidermis of the Lamina

Lower Epidermis of the Lamina

Fig. 2a Epidermises of the Leaf of *Citrus hystrix* DC.



Transverse Section of the Midrib

Transverse Section of the Lamina

Fig. 2b Transverse Section of the Leaf of *Citrus hystrix* DC.

1. upper epidermis
2. palisade cell
3. vascular bundle
4. idioblast
5. spongy cell
6. lower epidermis
7. xylem
8. phloem
9. fibre
10. schizolysigenous oil cavity
11. collenchyma

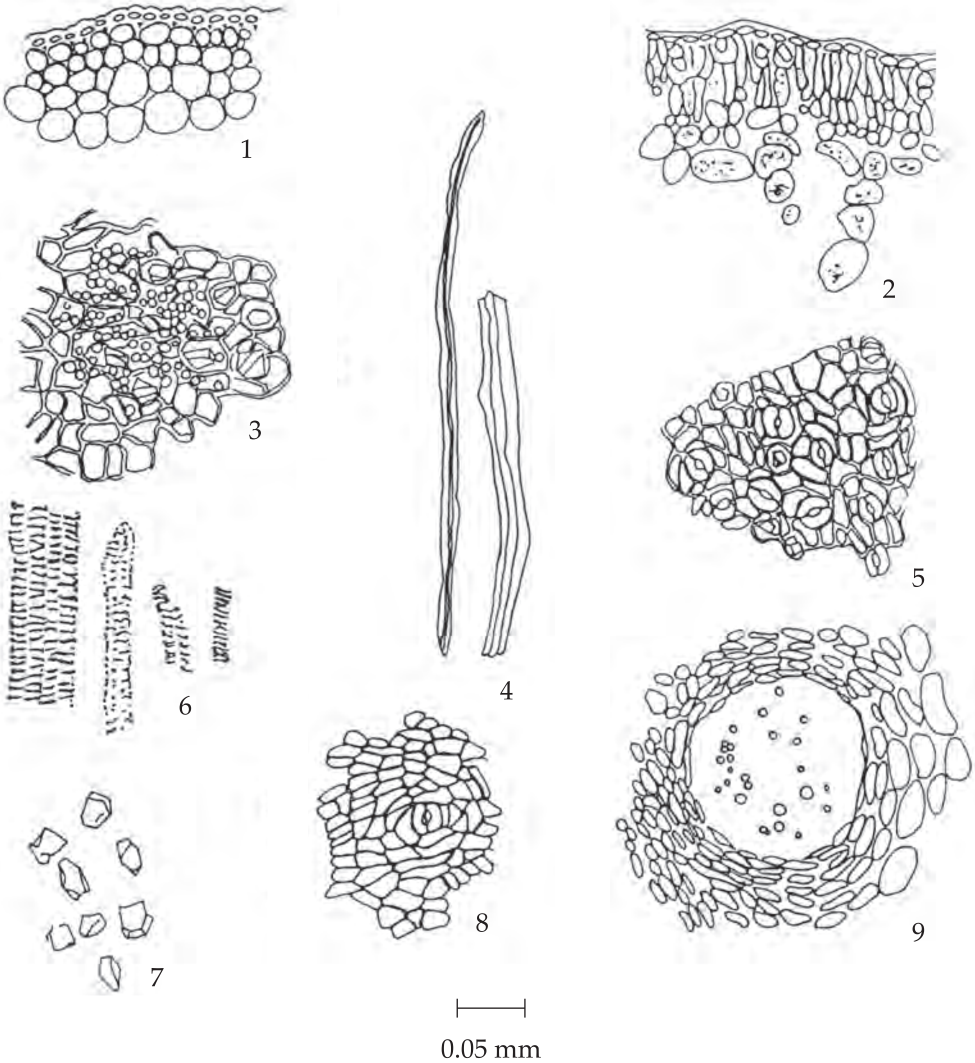


Fig. 2c Powdered Drug of the Leaves of *Citrus hystrix* DC.

1. epidermis an collenchyma in sectional view
2. lamina in sectional view
3. upper epidermis in surface view showing underlying palisade cells and idioblasts containing prismatic crystals of calcium oxalate
4. fibres
5. lower epidermis in surface view showing stomata and the underlying idioblasts containing prismatic crystals of calcium oxalate
6. vessels
7. prismatic crystals of calcium oxalate
8. epidermis of midrib in surface view
9. schizolysigenous oil cavity

Packaging and storage Citrus Hystrix Leaf shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place. It should be used within 1 year and air-dried every 2 to 3 months.

Identification

A. Reflux 1 g of the sample, in powder, with 30 ml of *ethanol* for 15 minutes, and filter (solution 1). To 2 ml of solution 1, add a few drops of *ammonium molybdate TS*: a yellowish green precipitate is produced.

B. To 2 ml of solution 1, add a few drops of a freshly prepared 1 per cent w/v solution of *iron(III) chloride*: a deep greenish brown colour is produced.

C. Extract 1 g of the sample, in powder, with 10 ml of *chloroform*, shake occasionally for 20 minutes, and filter. Evaporate 2 ml of the filtrate to dryness, dissolve the residue in 2 ml of *acetic anhydride*, and add slowly 1 ml of *sulfuric acid* to make two layers: a brown colour forms at the zone of contact and the upper layer is green.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and *chloroform* as the mobile phase. Apply to the plate, 10 µl of the test solution prepared by refluxing 1 g of the sample, in powder, with 30 ml of *ethanol* for 15 minutes, filtering, and concentrating the filtrate to 5 ml. After removal of the plate, allow it to dry in air, and examine under ultraviolet light (254 nm), marking the quenching spots. Examine the plate under ultraviolet light (366 nm) (Table 1); see also Fig. 3. Spray the plate with *anisaldehyde TS* and heat at 105° for 5 minutes. Several spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Leaves of *Citrus hystrix* DC.

Spot	hR_f Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	1-3	–	yellow	grey
2	3-6	–	red	–
3	3-7	quenching	–	bluish violet
4	6-8	–	red	–
5	9-12	quenching	pink	–
6	12-14	quenching	light violet	bluish violet
7	15-18	quenching	blue	–
8	18-20	–	red	–
9	20-22	–	blue	–
10	23-24	–	red	violet
11	25-27	–	green	–
12	28-32	–	red	–
13	31-35	–	–	pale violet
14	36-40	–	–	bluish violet
15	41-44	–	blue	–
16	44-46	quenching	–	–
17	57-61	–	–	violet
18	64-67	quenching	–	–
19	68-72	–	–	pale violet
20	72-76	–	–	bluish violet
21	76-79	quenching	–	violet
22	84-85	–	–	bluish violet
23	86-91	quenching	–	bluish violet

Water Not more than 11.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 3.0 per cent w/w (Appendix 7.6).

Total ash Not more than 17.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 6.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 18.0 per cent w/w (Appendix 7.12).

Chloroform-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12H).

Volatile oil Not less than 0.6 per cent v/w (Appendix 7.3H). Use 25 g of hand-crushed leaves, accurately weighed. Use 250 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

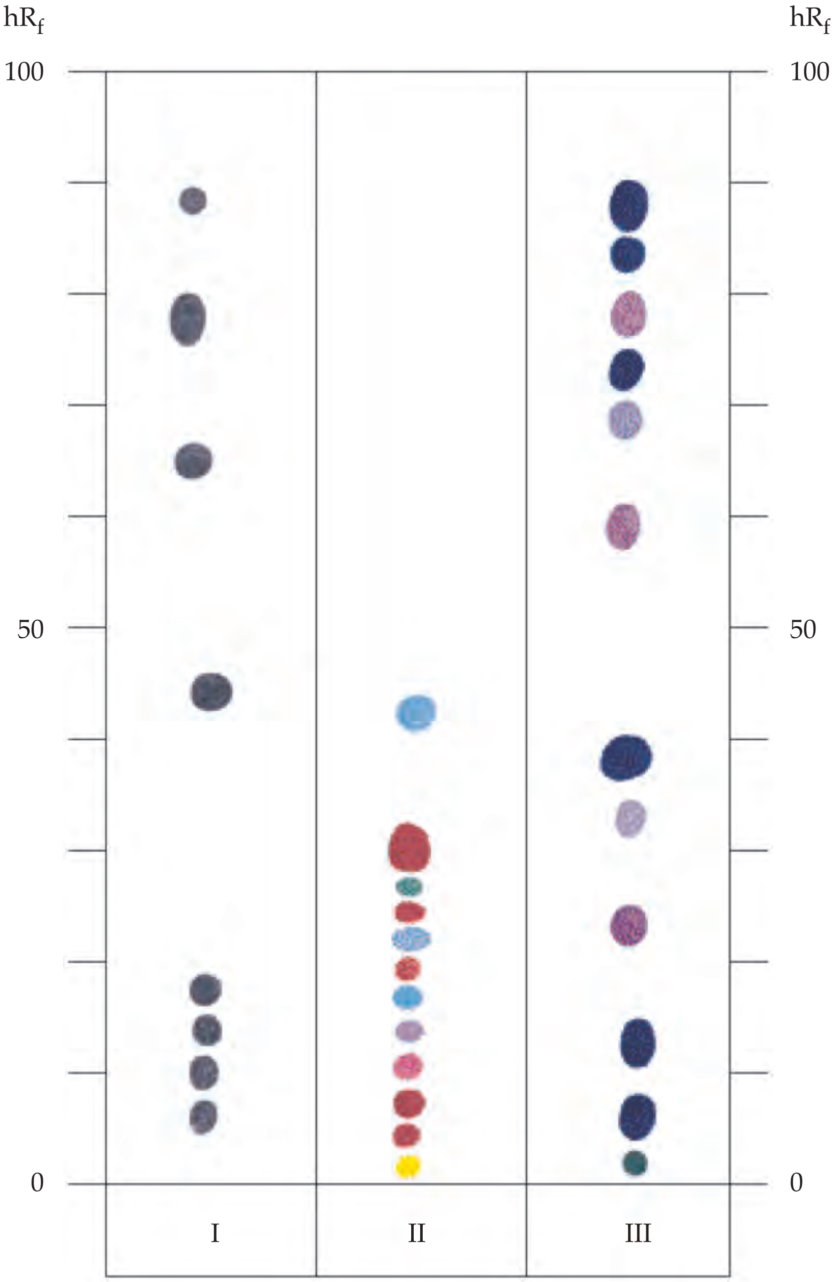


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Leaves of *Citrus hystrix* DC.

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *anisaldehyde TS*

มะกรูด, ผิว (MAKRUT, PHIO)

Citri Hystricis Pericarpium
Citrus Hystrix Peel

Synonyms Kaffir Lime Peel, Leech Lime Peel, Porcupine Orange Peel, Mauritius Papeda Peel

Category Pharmaceutic aid (flavouring agent), carminative.

Citrus Hystrix Peel is the dried peel of the unripe fruit of *Citrus hystrix* DC. (*C. papeda* Miq., *C. tuberoides* J. W. Benn.) (Family Rutaceae), Herbarium Specimen Number: DMSC 1459.

Constituents Citrus Hystrix Peel contains volatile oil, of which β-pinene, limonene, β-phellandrene, and citronellal are its major components. It also contains linalool, borneol, camphor, sabinene, germacrene D, aviprin, umbelliferone, and β-sitosterol.

Description of the plant See under *Makrut*, *Bai*, See also Fig. 1a.

Description Odour, characteristic, aromatic; taste, bitter.

Macroscopical (Fig. 1a) Citrus Hystrix Peel occurs as strips of dried fruit rind; outer surface dark green to brown, rough with pits of oil glands; inner surface exhibiting whitish spongy part.

Microscopical (Figs. 2a, 2b) Transverse section of the pericarp shows epicarp, a layer of rectangular cells covered by thick cuticle in sectional view or polygonal cells with anomocytic type of stomata in surface view. Mesocarp, the outer part composed of several layers of thick-walled parenchyma containing chromoplastids; the inner part composed of thick-walled spongy parenchyma; vascular bundles, scattered; vessels, lignified reticulate and spiral. Prismatic crystals of calcium oxalate and schizolysigenous oil cavities containing oil droplets are also found.

Citrus Hystrix Peel in powder possesses the diagnostic microscopical characters of the unground drug.

Packaging and storage Citrus Hystrix Peel shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place. It should be used within 1 year and air-dried every 2 to 3 months.

Fig. 1a *Citrus hystrix* DC.
1. habit 2. fruiting twig 3. crude drug

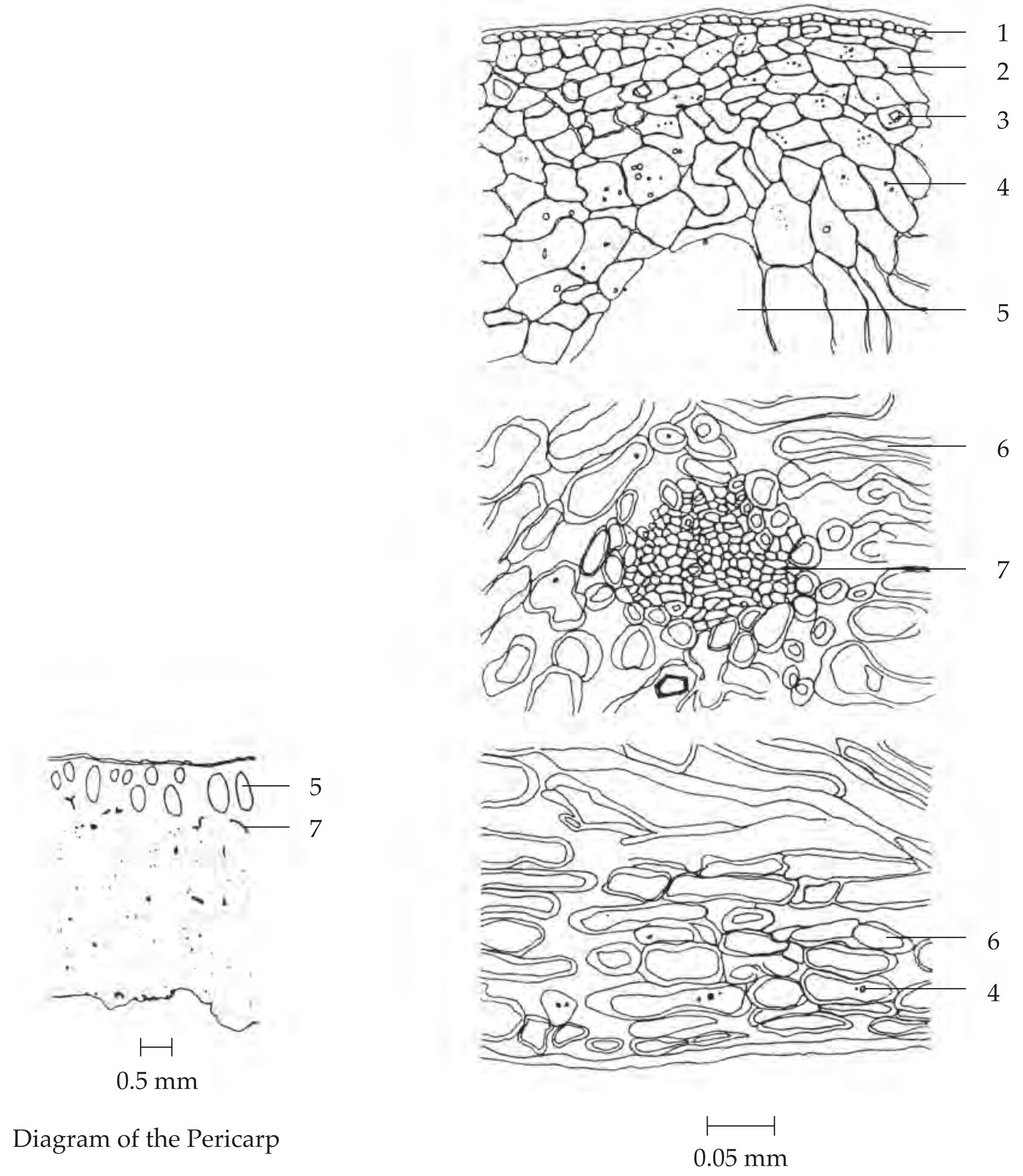


Diagram of the Pericarp

Fig. 2a Transverse Section of the Pericarp of *Citrus hystrix* DC.

1. epicarp with cuticle
2. thick-walled parenchyma containing chromoplastids
3. prismatic crystals of calcium oxalate
4. oil droplets
5. schizolysigenous oil cavity
6. thick-walled spongy parenchyma cells
7. vascular bundle

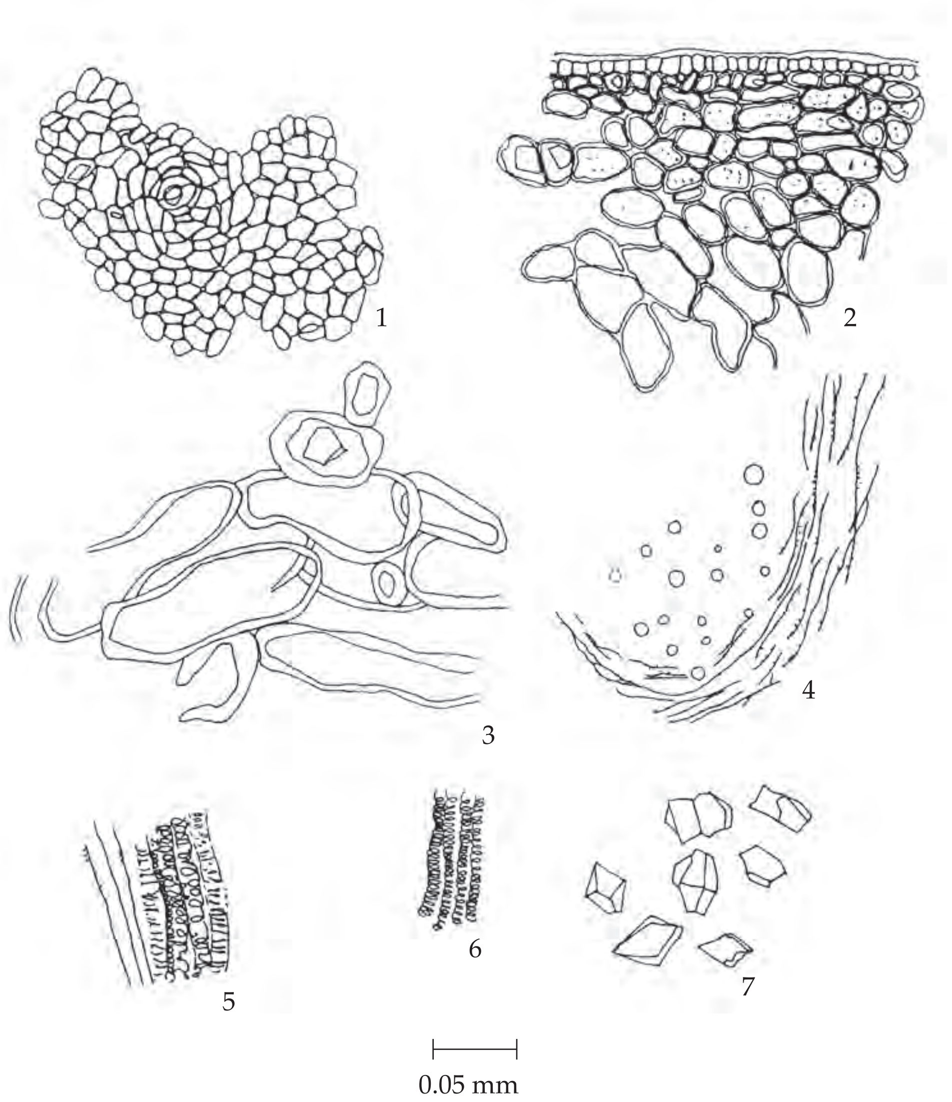


Fig. 2b Powdered Drug of the Pericarps of *Citrus hystrix* DC.

1. epicarp in surface view showing anomocytic stoma
2. pericarp in sectional view showing epicarp and parenchyma of mesocarp containing prismatic crystals and chromoplastids
3. thick-walled spongy cells with crystals
4. schizolysigenous oil cavity with oil droplets
5. spiral and reticulate vessels and fibres
6. spiral vessel thickenings
7. prismatic crystals of calcium oxalate

Identification

A. Reflux 1 g of the sample, in powder, with 30 ml of *ethanol* for 15 minutes, and filter (solution 1). To 2 ml of solution 1, add a few drops of *ammonium molybdate TS*: a bright yellow precipitate is produced.

B. To 2 ml of solution 1, add a few drops of a freshly prepared 1 per cent w/v solution of *iron(III) chloride*: a deep greenish brown colour is produced.

C. To 2 ml of solution 1, add a few drops of *ninhydrin TS* and warm on a water-bath for a few minutes: a reddish purple colour is produced.

D. Moisten 1 g of the sample, in powder, with 0.5 ml of *strong ammonia solution*, add 5 ml of *chloroform*, occasionally shaking for 10 minutes, and filter. Evaporate the filtrate until dryness and dissolve the residue in 1 ml of *ethanol*, and add a few drops of *modified Dragendorff TS2*: an orange precipitate is produced.

E. Extract 1 g of the sample, in powder, with 10 ml of *chloroform*, shake occasionally for 20 minutes, and filter. Evaporate 2 ml of the filtrate to dryness, dissolve the residue in 2 ml of *acetic anhydride*, and add slowly 1 ml of *sulfuric acid* to make two layers: a brown colour forms at the zone of contact and the upper layer is green.

F. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and *chloroform* as the mobile phase. Apply to the plate, 5 µl of the test solution prepared by moistening 1 g of the sample, in powder, with 1 ml of *strong ammonia solution* for a while, extracting with 10 ml of *chloroform* by occasionally shaking for 15 minutes, filtering, and concentrating the filtrate to 1 ml. After removal of the plate, allow it to dry in air, and examine under ultraviolet light (254 nm), marking the quenching spots. Examine the plate under ultraviolet light (366 nm) (Table 1); see also Fig. 3. Spray the plate with *anisaldehyde TS* and heat at 105° for 5 minutes. Several spots of different colours are observed (Table 1); see also Fig. 3.

Repeat the same procedure on other two plates but use a mixture of 20 volumes of *chloroform* and 1 volume of *methanol* as the mobile phase and allow the solvent front to ascend 12 cm above the line of application. After removal of the plates, allow them to dry in air, and spray one plate with *modified Dragendorff TS2*: two orange spots are observed. Spray another plate with *iodoplatinate TS*: one dark blue spot and one brown spot are observed (Table 1); see also Fig. 4.

Table 1 hR_f Values of Components in Chloroform Extract of the Peels of *Citrus hystrix* DC.

a. Mobile phase: *chloroform*

Spot	hR_f Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	0-2	–	light green	–
2	1-4	quenching	–	green
3	2-3	–	blue	–
4	4-5	–	blue	–
5	5-7	quenching	light green	bluish violet
6	7-9	quenching	light green	bluish violet
7	8-11	quenching	light green	brown
8	10-13	quenching	light green	bluish violet
9	12-15	quenching	light green	bluish violet
10	15-20	quenching	light green	green
11	20-26	quenching	light green	brown
12	25-28	–	red	–
13	29-31	–	–	bluish violet
14	33-34	–	–	blue
15	36-38	–	–	pink
16	36-39	–	blue	–
17	39-41	–	–	brown
18	41-48	quenching	green	violet
19	46-50	quenching	green	–
20	51-54	–	–	brown
21	58-61	–	–	pink
22	68-70	–	–	violet
23	73-76	–	–	bluish violet
24	77-80	–	–	violet
25	81-84	quenching	–	–
26	84-86	–	–	bluish violet
27	86-90	quenching	–	violet

b. Mobile phase: 20 volumes of *chloroform* and 1 volume of *methanol*

Spot	hR_f Value	Detection	
		Modified Dragendorff TS2	*Iodoplatinate TS*
1	33-40	orange	dark blue
2	87-92	orange	brown

Water Not more than 12.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 9.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 11.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 23.0 per cent w/w (Appendix 7.12).

Chloroform-soluble extractive Not less than 8.0 per cent w/w (Appendix 7.12H).

Volatile oil Not less than 2.0 per cent v/w (Appendix 7.3H). Use 25 g, in *coarse powder,* freshly prepared and accurately weighed. Use 250 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Dis til at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

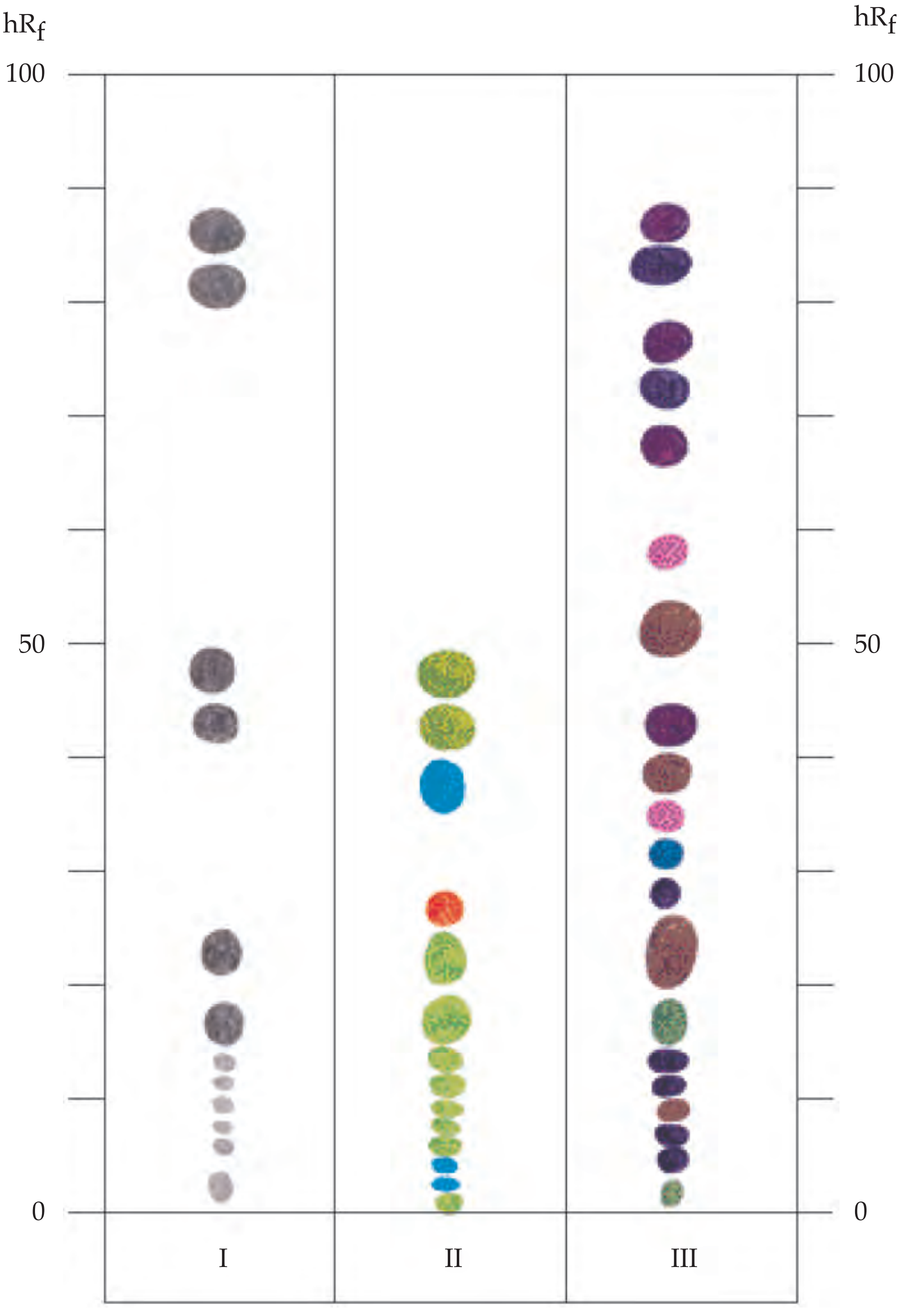


Fig. 3 Thin-layer Chromatogram of Chloroform Extract of the Peels of *Citrus hystrix* DC.

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *anisaldehyde TS*

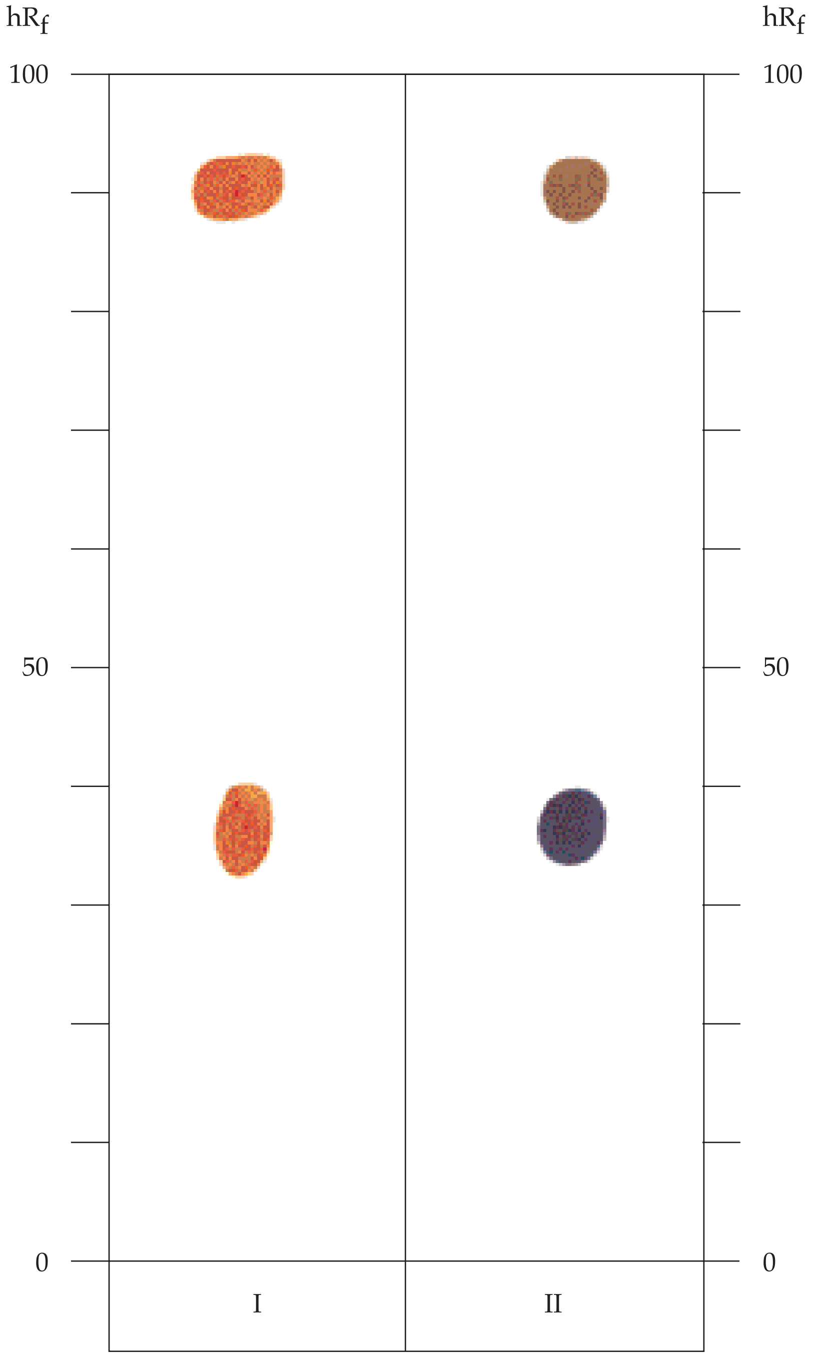


Fig. 4 Thin-layer Chromatogram of Chloroform Extract of the Peels of *Citrus hystrix* DC.

I = detection with *modified Dragendorff TS2*

II = detection with *iodoplatinate TS*

หมากสง (MAKSONG)

Arecae Catechi Semen
Areca Seed

Synonyms Areca Nut, Areca

Category Anthelmintic (taeniasis, ascariasis), antidiarrheal.

Areca Seed is the dried ripe seed of *Areca catechu* L. (Family Palmae), Herbarium Specimen Number: DMSC 1089.

Constituents Areca Seed contains the major alkaloid arecoline and other minor alkaloids which are arecolidine, arecaine (arecaidine), guvacine, guvacoline and isoguvacine. It also contains condensed tannins, (+)-catechin, (-)-epicatechin, fatty acids, and amino acids.

Description of the plant (Figs. 1a, 1b) Small to medium-sized tree, up to 30 m high; trunk solitary, straight, annulate, usually about 50 cm in circumference, uniformly thick. Leaves pinnate, 1 to 2 m long; petiole base expanding into a smooth, outside green, amplexicaul sheath, 75 to 100 cm long; leaflets numerous, 30 to 60 cm long, upper confluent glabrous, attached to the rachis in a vertical line. Inflorescences spadix, much branched, bearing male and female flowers; peduncle up to 60 cm high; rachis stout, compressed, branches with filiform tips. Male flowers numerous, sessile, without bracts, occupying the upper portion of the spikes; calyx 1-leaved, small, 3-cornered, 3-parted; petals 3, oblong, rigid, striated; stamens 6; anthers sagittate. Female flowers much larger, solitary, or 2 to 3, at or near the base of each ramification of the spadix, sessile, without bracts; sepals 3, cordate, rigid, fleshy, permanent; petals 3, sepaloid, permanent; staminodes 6, connate; style scarce any; stigma 3, short triangular. Fruits glabrous, ovoid or ellipsoid, 3 to 7 cm long, orange or scarlet when riped, supported by the persistent perianth, mesocarp fibrous. Seed 1, globose with truncate base; endosperm deeply ruminate; embryo basilar.

Description Odour, slight, characteristic; taste, astringent.

Macroscopical (Fig. 1a) Oblate or rounded-conical, 1.5 to 3.5 cm long, base 1.5 to 3 cm wide. Externally pale yellowish brown or pale reddish brown, with slightly concaved reticulate furrows, having a round, hollow micropyle in the centre of the base, beside which bearing an obvious scar-shaped hilum. Texture hard, uneasily broken; fractured surface showing marble-like striations alternated with brown seed coats and white endosperm.

Microscopical (Figs. 2a, 2b) Transverse section of the seed shows testa, several loosely packed layers of reddish brown cells with moderately thickened walls with a few scattered, small, rounded or slit-shaped pits. Testa rumination, several layers of large, pitted thin-walled cells, some of them filled with brown pigment and fixed oil globules, embedded with groups of vessels. Endosperm, large polygonal cells with beaded and porous cellulose walls, perforated by rounded to oval, conspicuous pits containing fixed oil globules and aleurone grains, some with rather smooth walls. Embryo, occupying the central region of the seed near the base.

Areca Seed in powder possesses the diagnostic microscopical characters of the unground drug.

Fig. 1a *Areca catechu* L.
1. habit 2. and 3. inflorescences 4. bunch of ripe fruits 5. crude drug

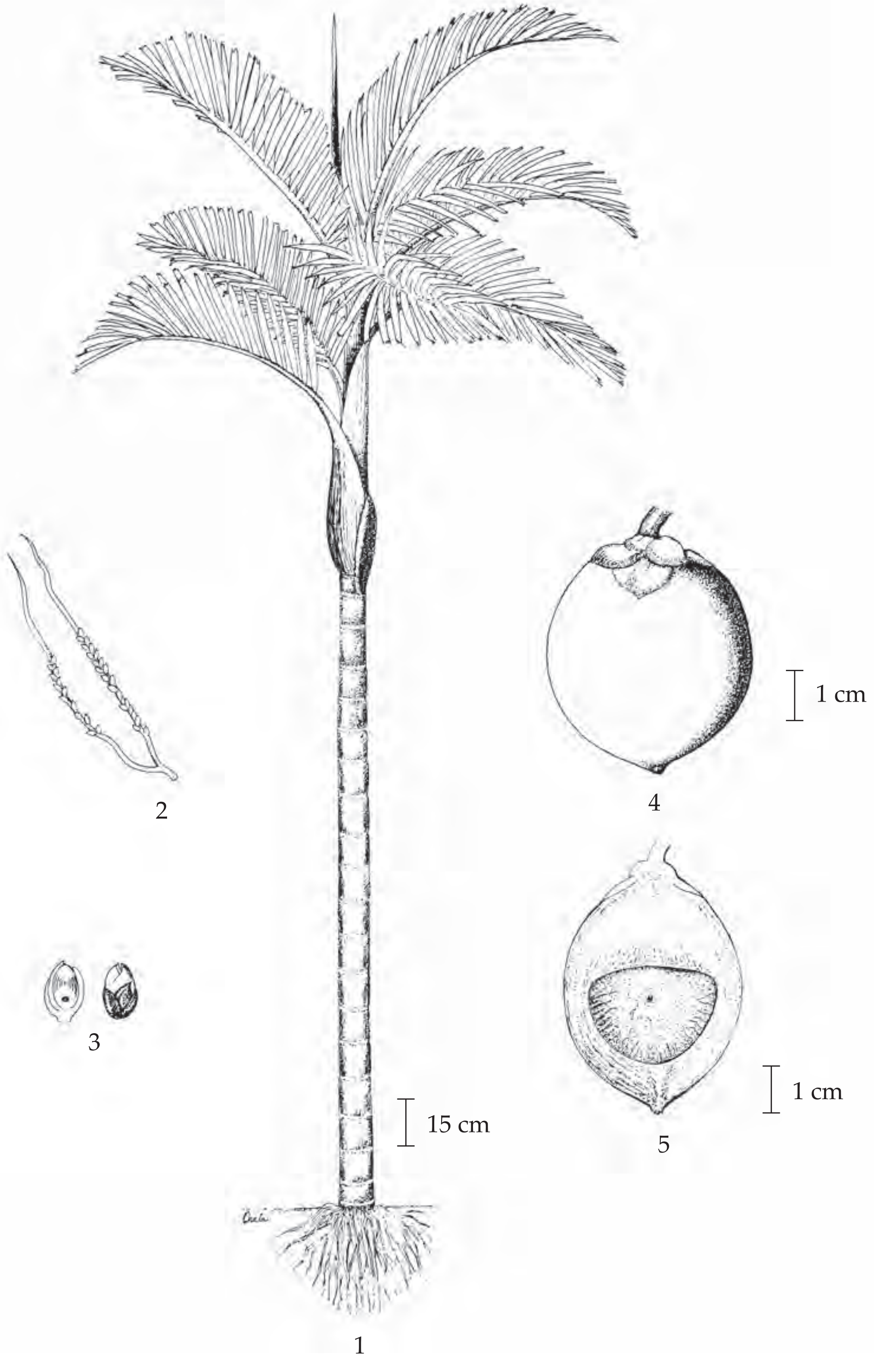


Fig. 1b *Areca catechu* L.
1. habit 2. inflorescences (scale omitted) 3. female flowers (scale omitted)
4. fruit 5. longitudinal section of the fruit

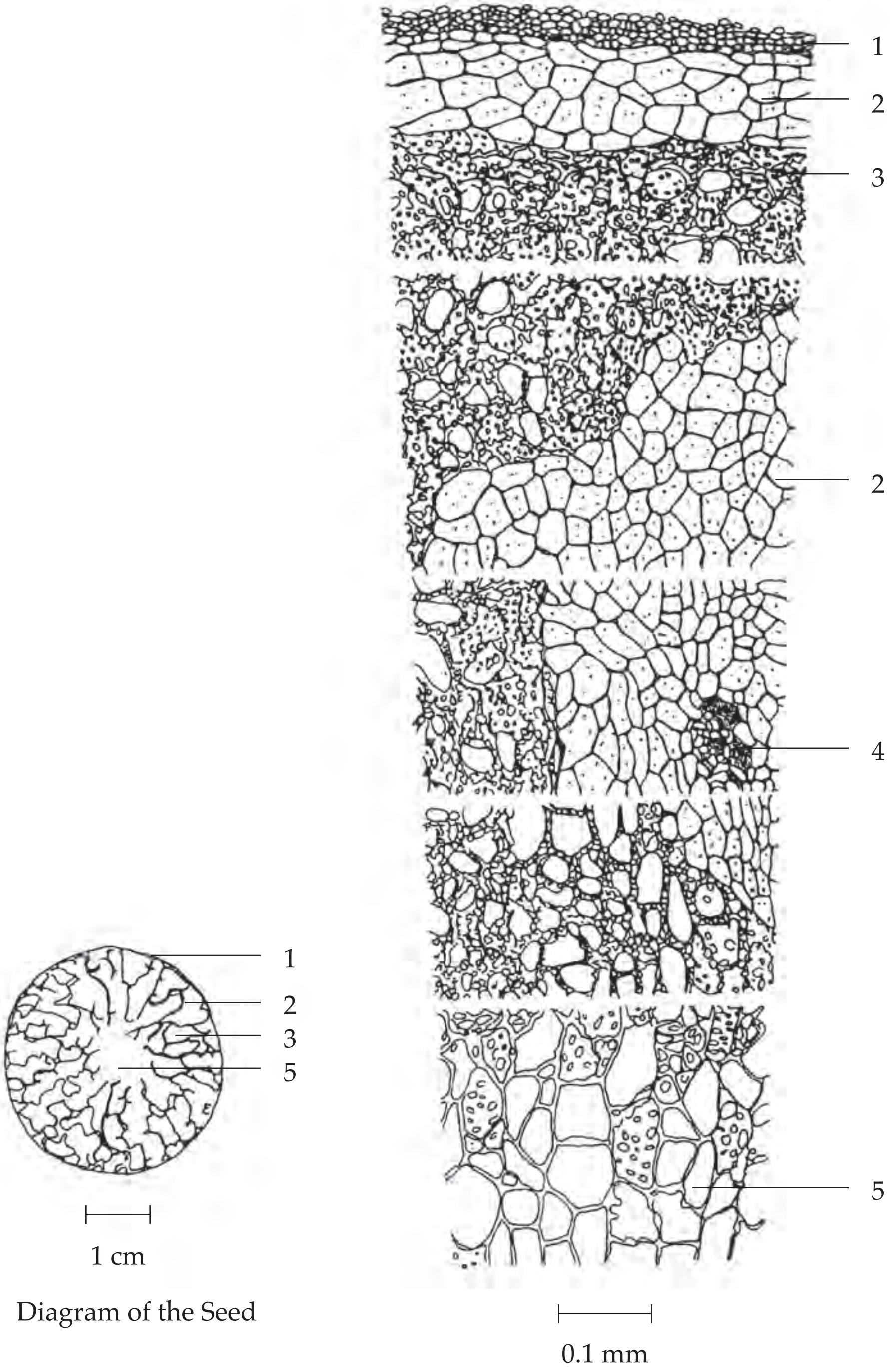


Diagram of the Seed

Fig. 2a Transverse Section of the Ripe Seed of *Areca catechu* L.

1. testa
2. testa rumination with yellowish brown pigment
3. endosperm with beaded and porous walls
4. vessel
5. endosperm

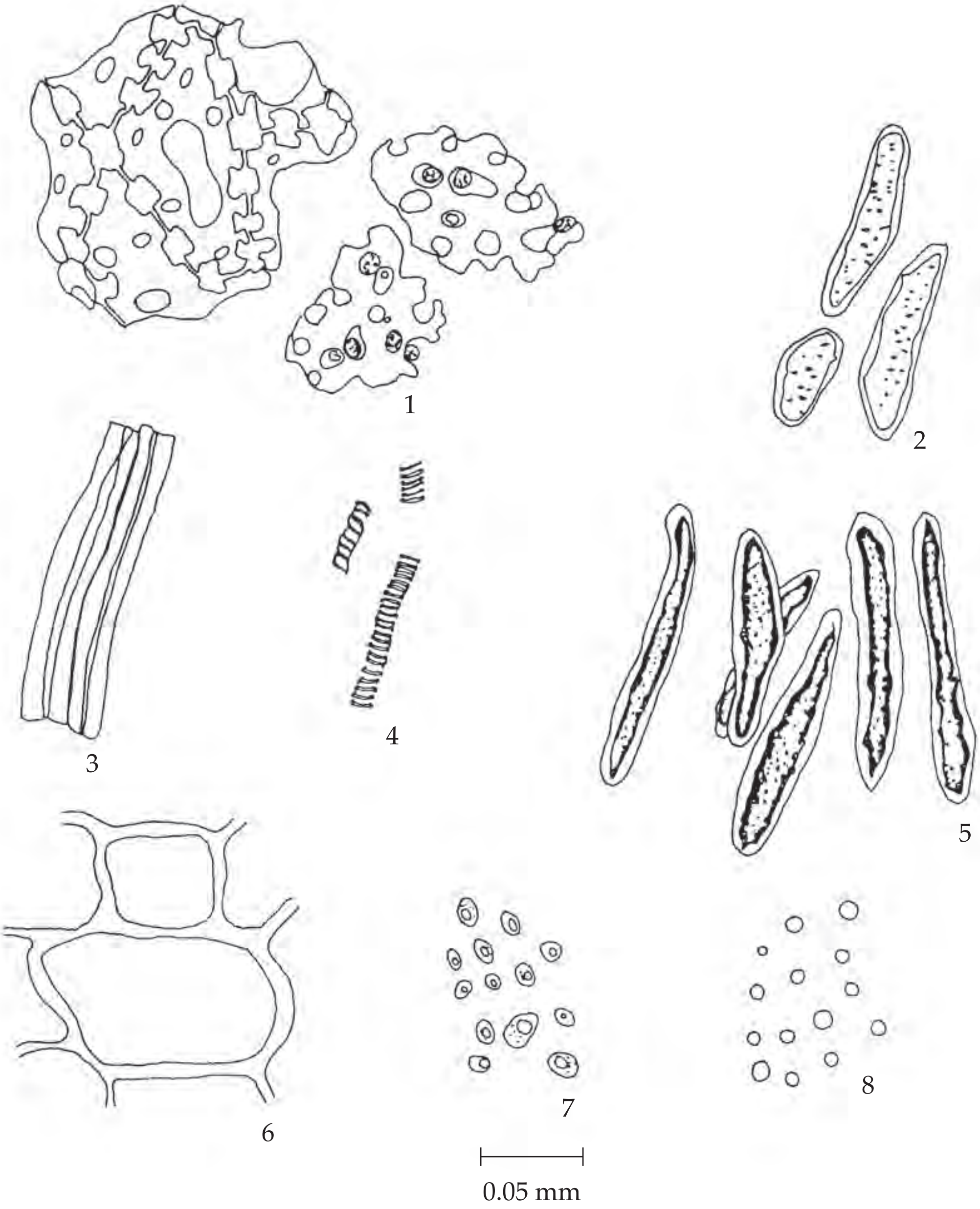


Fig. 2b Powdered Drug of the Seeds of *Areca catechu* L.

1. endosperm cells showing beaded and porous walls
2. testa rumination in longitudinal view
3. fibres
4. vessel thickenings
5. cells containing brown pigment
6. endosperm cells showing smooth walls
7. aleurone grains
8. oil droplets

Packaging and storage Areca Seed shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Macerate 1 g of the sample, in powder, with 20 ml of *water* for 15 minutes and filter. Dip a wooden stick in the filtrate for a few seconds and dry at room temperature. Dip again in *hydrochloric acid*, remove immediately and dry: the wooden stick stains red.

B. To 100 mg of the sample, in powder, add 25 ml of *water*, heat in a water-bath for 5 minutes and filter. To the filtrate add 2.5 ml of *hydrochloric acid* and 5 ml of *formaldehyde solution* and warm: a pale brown precipitate is produced. Cool and filter. To 5 ml of the filtrate, add 2.5 g of *sodium acetate* and 0.5 ml of *iron(III) chloride TS*, and shake well: a reddish brown colour is produced which does not change to blue.

C. Macerate 1 g of the sample, in powder, with 10 ml of *water* for 10 minutes and filter. To 2 ml of the filtrate, add a few drops of a 5 per cent w/v solution of *iron(III) chloride*, and then make slightly alkaline with a 4 per cent w/v solution of *sodium hydroxide*: the colour is changed from dark green to purple.

D. Reflux 1 g of the sample, in powder, with 20 ml of *chloroform* in a water-bath for 15 minutes and filter. Evaporate 2 ml of the filtrate to dryness and dissolve the residue in 2 ml of *acetic anhydride*. Carefully add 1 ml of *sulfuric acid* to form a layer: a brown colour develops at the zone of contact and the colour of the upper layer changes from violet to blue and finally to yellowish-green.

E. Moisten 2 g of the sample, in powder, with 2 ml of *strong ammonia solution*, add 20 ml of *ether*, shake well, set aside for 1 hour, and filter. Dry the filtrate over *anhydrous sodium sulfate*, filter, and evaporate to dryness. Dissolve the residue in 4 ml of *ethanol* (solution 1). To 1 ml of solution 1, add a few drops of *mercuric-potassium iodide TS*: a white precipitate is produced. To 1 ml of solution 1, add a few drops of *modified Dragendorff TS2*: a precipitate is produced.

F. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 75 volumes of *benzene*, 25 volumes of *methanol* and 1 volume of *formic acid* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate, 20 µl of each of the following solutions. Prepare solution (A) by boiling 500 mg of the sample, in powder, with 50 ml of *water* for a few minutes and filtering. Allow the filtrate to cool and shake with 30 ml of *ether*. Dry the ether layer over *anhydrous sodium sulfate*, filter, evaporate to dryness, and dissolve the residue in 1 ml of *ethanol*. For solution (B), dissolve 2 mg of *catechin* in 1 ml of *ethanol*. After removal of the plate, allow it to dry in air, and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 31 to 36), corresponding to the catechin spot from solution (B); other one or two spots are also observed (Table 1); see also Fig. 3. Spray the plate with *vanillin-hydrochloric acid TS*; the spot due to catechin is dark pink. Several other pink spots may appear (Table 1); see also Fig. 3.

Repeat the same procedure but use a mixture of 70 volumes of *toluene*, 20 volumes of *ethyl acetate* and 10 volumes of *diethylamine* as the mobile phase. Apply to the plate, 15 µl of solution 1 obtained from *test E*. After removal of the plate, allow it to dry in air, and examine under ultraviolet (254 nm), marking the quenching spots. The chromatogram shows three to four quenching spots (Table 2); see also Fig. 4. Spray the plate with *iodoplatinate TS*; several spots of different colours are observed (Table 2); see also Fig. 4.

Table 1 hR_f Values of Components in Water Extract of the Seeds of *Areca catechu* L.

Spot	hR_f Value	Detection	
		UV 254	*Vanillin-Hydrochloric Acid TS*
1	13-15	–	pale pink
2	19-22	–	pale pink
3	24-29	–	pale pink
4*	31-36	quenching	dark pink
5	49-52	quenching	pale pink
6	62-65	quenching	–

*catechin

Table 2 hR_f Values of Components in Ethereal Extract of the Seeds of *Areca catechu* L.

Spot	hR_f Value	Detection	
		UV 254	*Iodoplatinate TS*
1	20-23	quenching	dark orange
2	25-28	–	blue
3	33-37	quenching	wine-red
4	73-77	quenching	scarlet
5	87-91	–	blue
6	95-97	quenching	yellow

Loss on drying Not more than 12.0 per cent w/w (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 2.0 per cent w/ w (Appendix 7.7).

Ethanol-soluble extractive Not less than 25.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 20.0 per cent w/w (Appendix 7.12).

Hexane-soluble extractive Not less than 0.5 per cent w/w (Appendix 7.12H).

Alkaloids content Not less than 0.5 per cent w/w of alkaloids, calculated as arecoline, when determined by the following method.

Place 8 g of Areca Seed, in *coarse powder*, accurately weighed, in a conical flask with a stopper, add 80 ml of *ether* and shake. Add 4 ml of *ammonia TS,* shake for 10 minutes, add 10 g of *anhydrous sodium sulfate*, shake again for 5 minutes and allow to stand and precipitate. Transfer the ether solution to a separator, wash the residue with three successive 10-ml portions of *ether*. Combine the ether solutions, add 500 mg of *talc*, shake for 3 minutes, add 2.5 ml of *water*, and then shake for 3 minutes. Allow to stand until the supernatant ether solution is clear. Separate the ether solution, wash the aqueous layer with a small quantity of *ether*, combine the ether solutions and evaporate to about 20 ml at low temperature. Transfer to a separator, carefully add 20.0 ml of 0.01 M *sulfuric acid VS*, shake and allow to stand. Separate the ether layer, wash with three successive 5-ml portions of *water*. Combine the washings and the acid solution, filter, and wash the filter with *water*. Combine the washings and the acid solution, add several drops of *methyl red TS*, and titrate with 0.02 M *sodium hydroxide VS*. Perform a blank determination (Residual Titrations, Appendix 6.17). Each ml of 0.01 M *sulfuric acid VS* is equivalent to 3.104 mg of arecoline ($C_8H_{13}NO_2$).

Tannins content Not less than 24.0 per cent w/w (Appendix 7.21 H). Use 4 g of Areca Seed, in powder, accurately weighed.

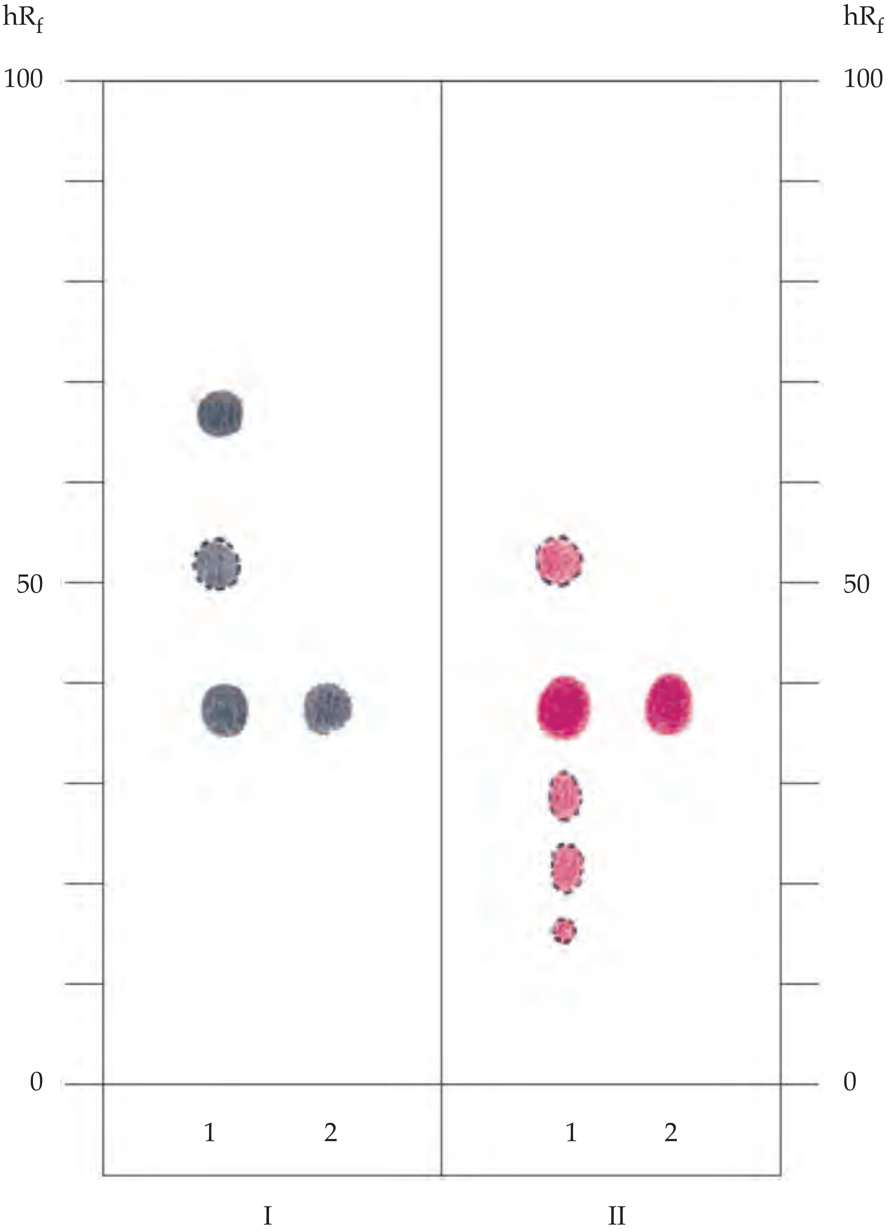


Fig. 3 Thin-layer Chromatogram of Water Extract of the Seeds of *Areca catechu* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with *vanillin-hydrochloric acid TS*
◌ = spots developed in some samples

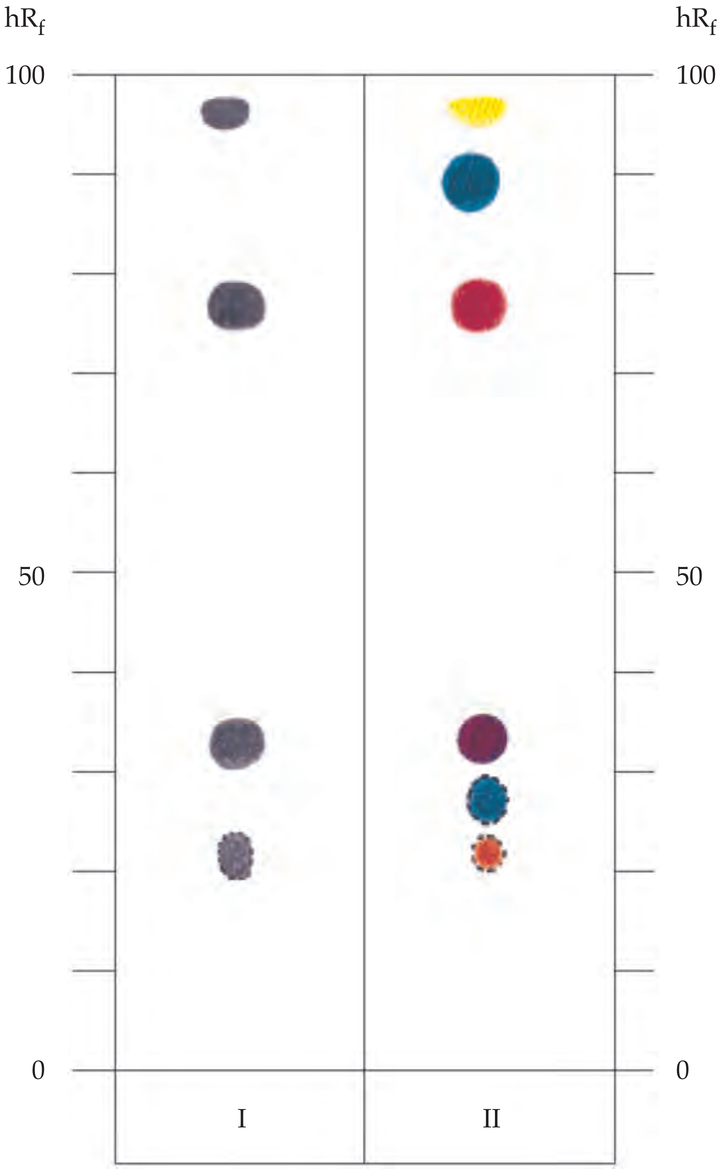


Fig. 4 Thin-layer Chromatogram of Ethereal Extract of the Seeds of *Areca catechu* L.
I = detection under UV light (254 nm)
II = detection with *iodoplatinate TS*
◌ = spots developed in some samples

มะระขี้นก (MARA KHI NOK)

มะระไทย (MARA THAI), มะไห่ (MA HAI)
Momordicae Charantiae Fructus
Thai Bitter Cucumber

Synonyms Thai Bitter Gourd, Thai Bitter Melon, Thai Bitter Squash

Category Bitter tonic, internal heat alleviating.

Thai Bitter Cucumber is the dried unripe fruit of *Momordica charantia* L. (*M. muricata* Willd.) (Family Cucurbitaceae), Herbarium Specimen Number: DMSC 5159, Crude Drug Number: DMSc 0889.

Constituents Thai Bitter Cucumber contains charantin, a mixture of sitosteryl and stigmasteryl glucosides, and bitter triterpenoids (e.g., momordicins). It also contains polypeptides (e.g., *p*-insulin), phenolic compounds, etc.

Description of the plant (Figs. 1a, 1b) Annual or perennial climber, monoecious, 3 to 4 m long, slender, angled, many-branched, pubescent; tendril unbranched, up to 20 cm long, slender. Leaves simple, spirally arranged, suborbicular to orbicular, 4 to 12 cm long, 2.5 to 12 cm wide, deeply palmately, 5- to 7(–9)-lobed, lobe ovate-oblong or ovate-elliptic, apex acute or obtuse, base cordate, margin crenate, mucronate or irregularly toothed, glabrous or pubescent, pellucidly dotted; petiole 1.5 to 7.5 cm long. Flower solitary, axillary, yellow; sepals 5; petals 5. Male flower peduncle, 2 to 6 cm long, slender; bract green; reniform to suborbicular, 0.5 to 1.5 cm wide, apex more or less mucronate, margin subentire; receptacle cup-shaped, 2 to 4 mm long and wide; sepal ovate-elliptic (-oblong), 4 to 6 mm long, 2 to 3 mm wide, acute, pale green; petal obovate to oblong, 1 to 2 cm long, 0.3 to 1.5 cm wide, apex more or less mucronate, basal scales 2; stamens 3, filament 1.5 to 2 mm long, free, inserted in the throat of cup-shaped receptacle, anthers coherent; disc cup-shaped, about 1.5 mm in diameter. Female flower peduncle 0.5 to 5 cm long; bract 0.1 to 1 cm in diameter; sepal oblong-lanceolate, 4 to 6 mm long, 2 to 3 mm wide; petal smaller than those of male flower, 0.7 to 1.2 cm long; ovary inferior, fusiform or narrowly rostrate, ribbed, warty or papillose, long-beaked, style about 2 mm long, stigma 3-lobed, lobe bifid or notched. Fruit pendulous, fusiform or ellipsoid, 2 to 8.5 cm long, up to 3.5 cm wide, tapering towards apex, beaked, longitudinally 8- to 10-ridged, tuberculate, orange when ripe, splitting incompletely with 3-valved exposing orange pulp; fruit stalk 3.5 to 15 cm long. Seeds few to numerous, oblong, 0.8 to 1.3 cm long, 5 to 9 mm wide, flattened, sculptured, whitish or brown, embedded in reddish pulp.

Description Odour, characteristic; taste, bitter.

Macroscopical (Fig. 1a) Transverse or oblique pieces, varying in size, up to 3 cm in diameter; externally warty, greenish to brownish; internally creamy white to pale brown.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the unripe fruit shows epicarp, mesocarp, endocarp, seed coat, and cotyledons. Epicarp, a layer of rectangular cells with stomata and glandular trichomes. Mesocarp, diffentiated in 3 zones: thin-walled parenchyma cells which increase in size from periphery towards the interior, the smaller

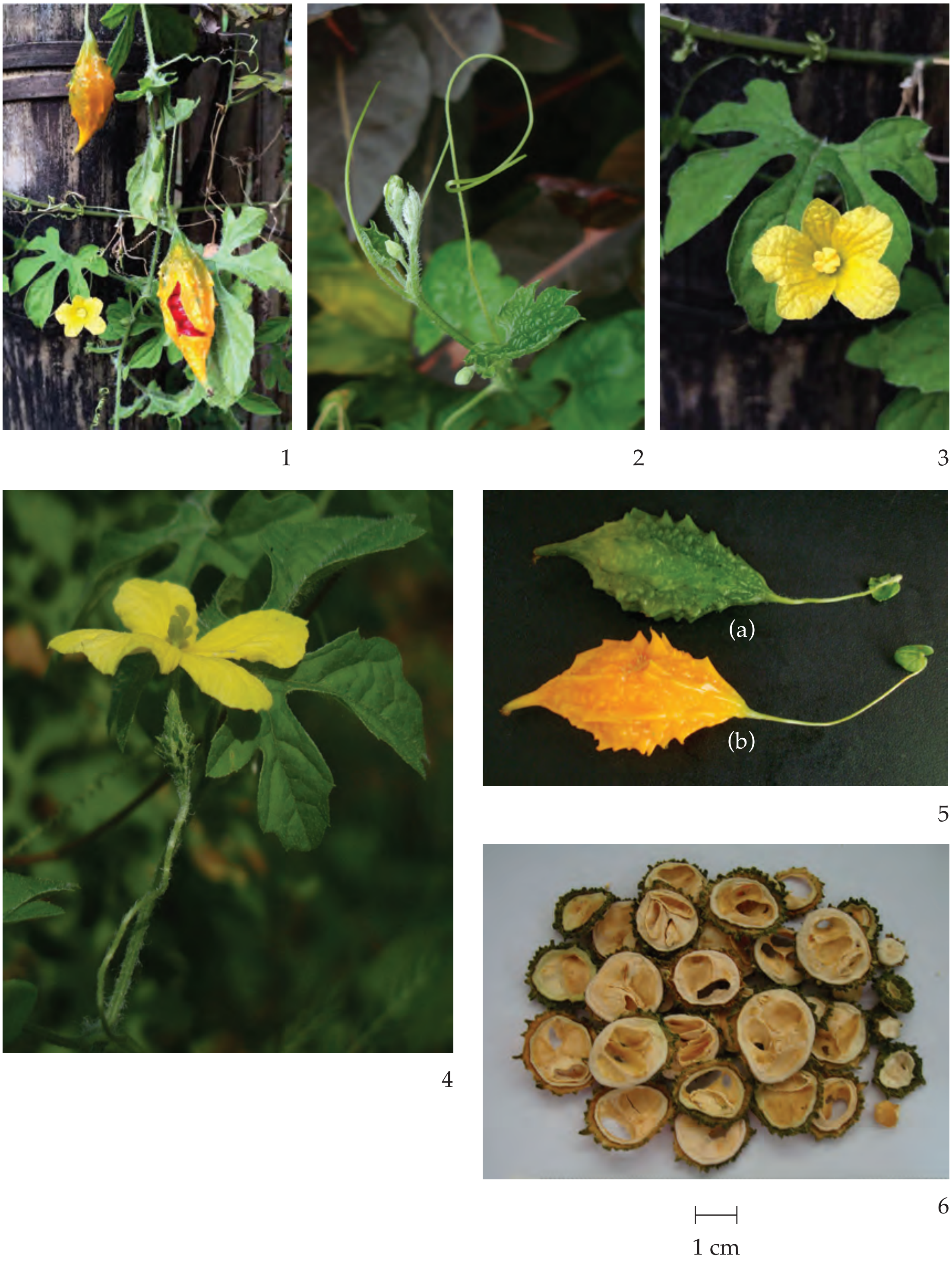


Fig. 1a *Momordica charantia* L.
1. flowering and fruiting vine 2. young shoot showing unbranched tendril
3. male flower 4. female flower 5. unripe fruit (a) and ripe fruit (b) 6. crude drug

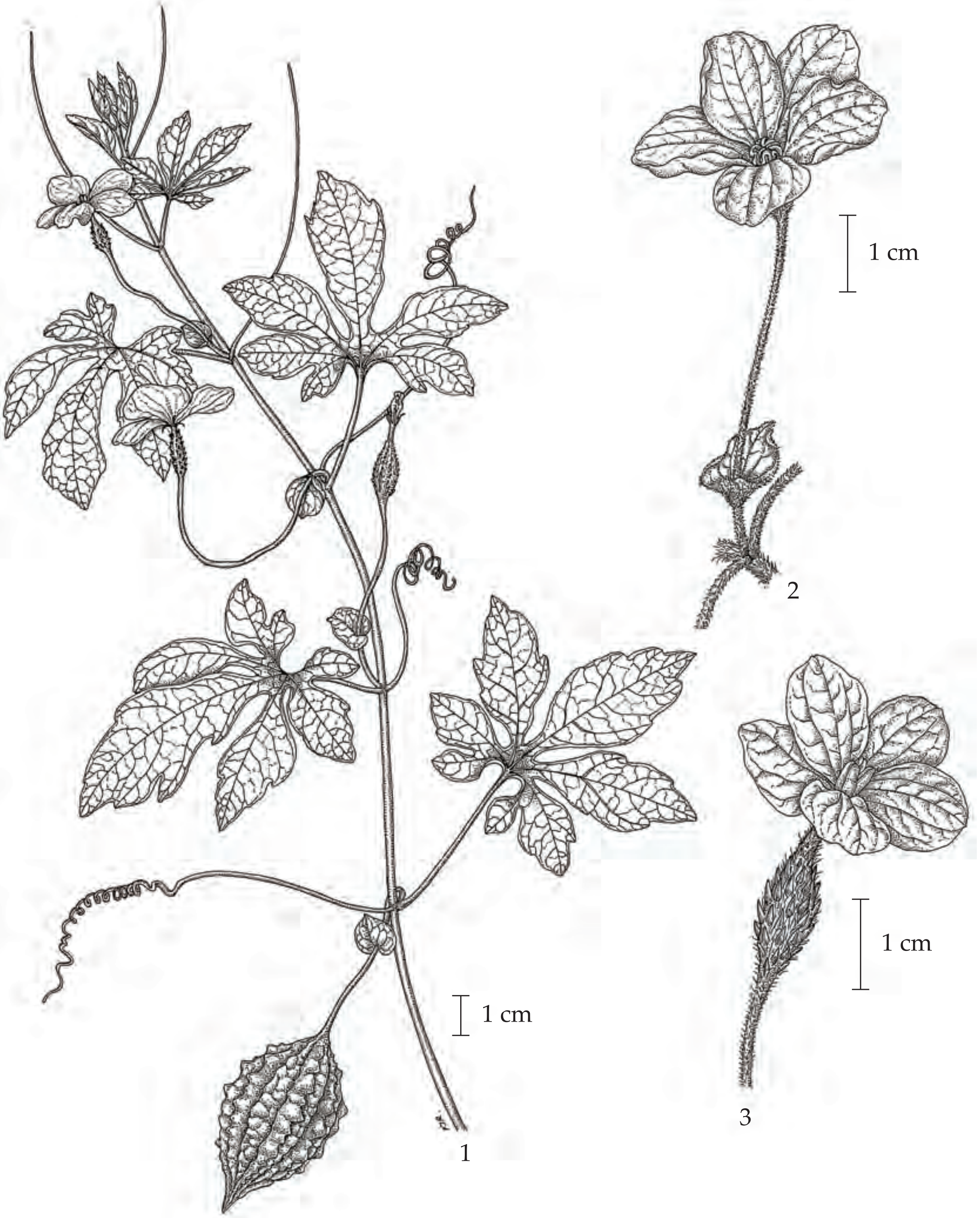


Fig. 1b *Momordica charantia* L.
1. vine, showing leaves, tendrils, female flowers and fruit
2. male flower 3. female flower

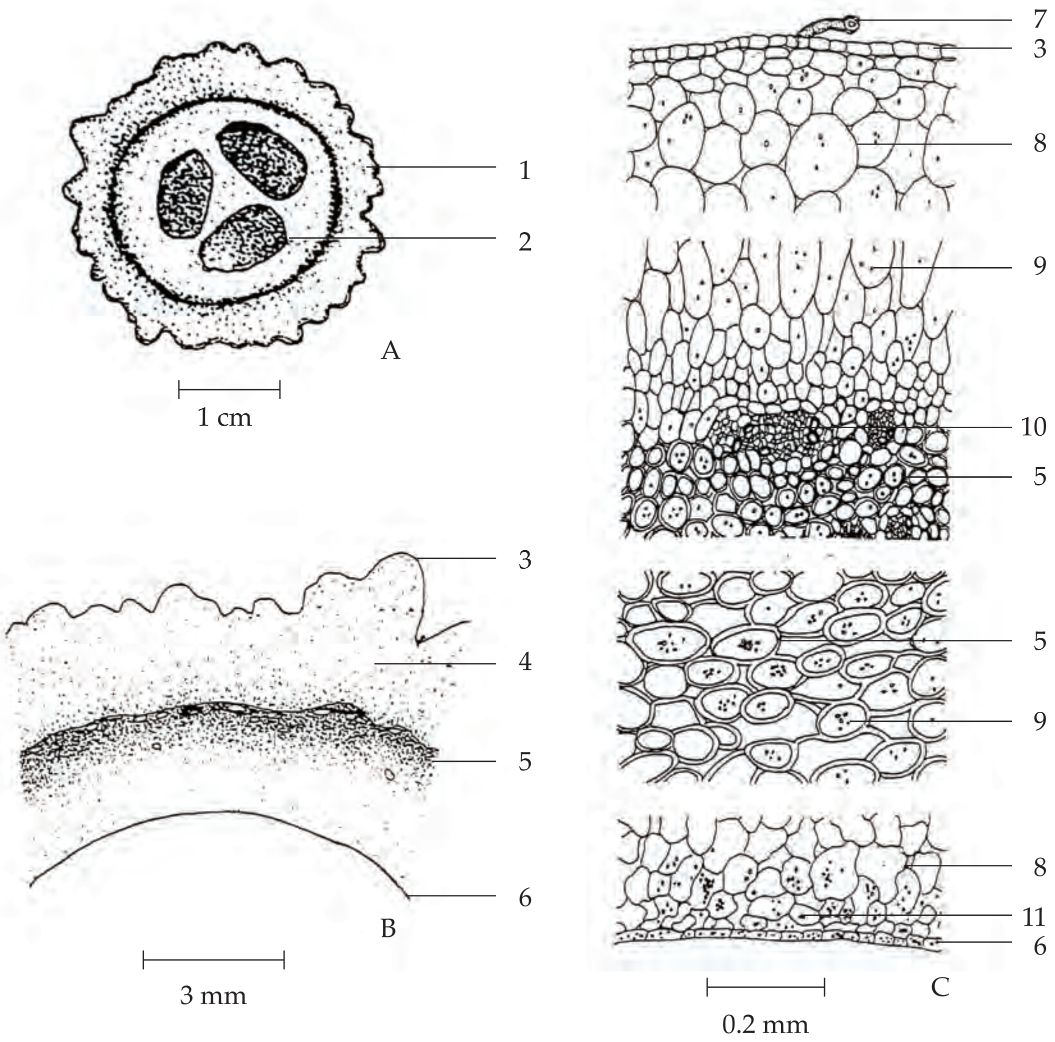


Fig. 2a Transverse Section of the Unripe Fruit of *Momordica charantia* L.

A. Diagram
B. Diagram of the Pericarp
C. Section of Part of Seed

1. pericarp
2. seed
3. epicarp
4. mesocarp
5. thick-walled parenchyma, some containing starch grains
6. endocarp
7. glandular trichome
8. thin-walled parenchyma, some containing starch grains
9. starch grains
10. vascular bundle
11. cuboidal protein crystal

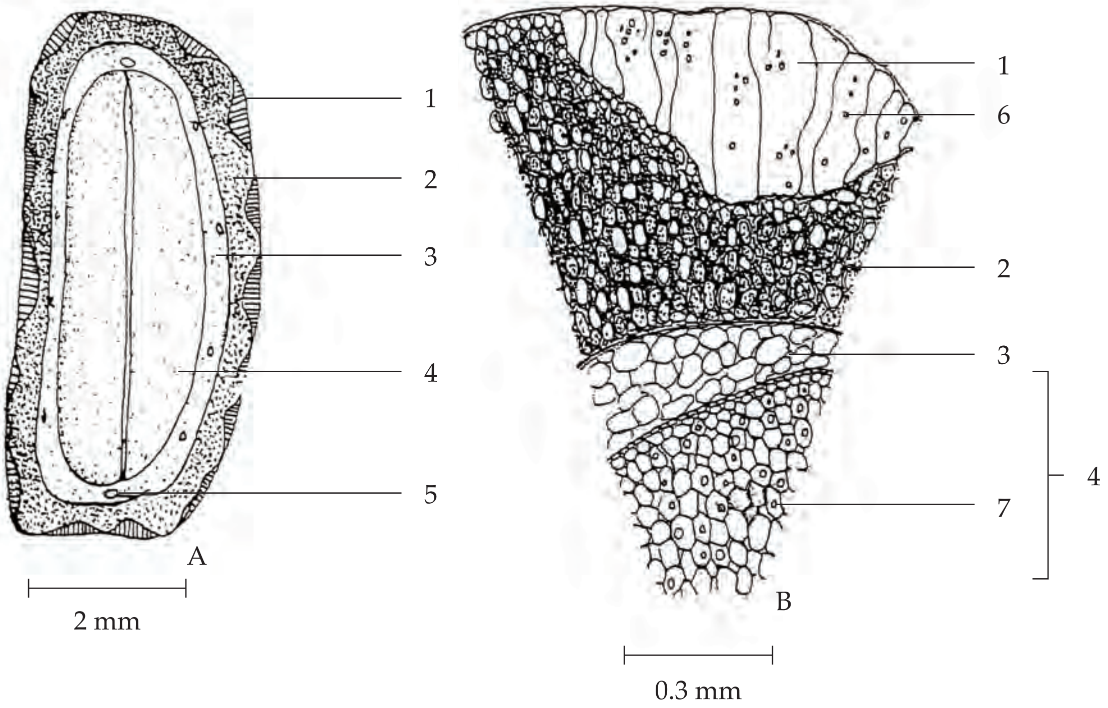


Fig. 2b Transverse Section of the Seed of *Momordica charantia* L.

A. Diagram
B. Section of Part of Seed

1. epidermis of seed coat
2. sclerenchyma of seed coat
3. parenchyma of seed coat
4. cotyledon
5. vascular bundle
6. starch grain
7. oil globule

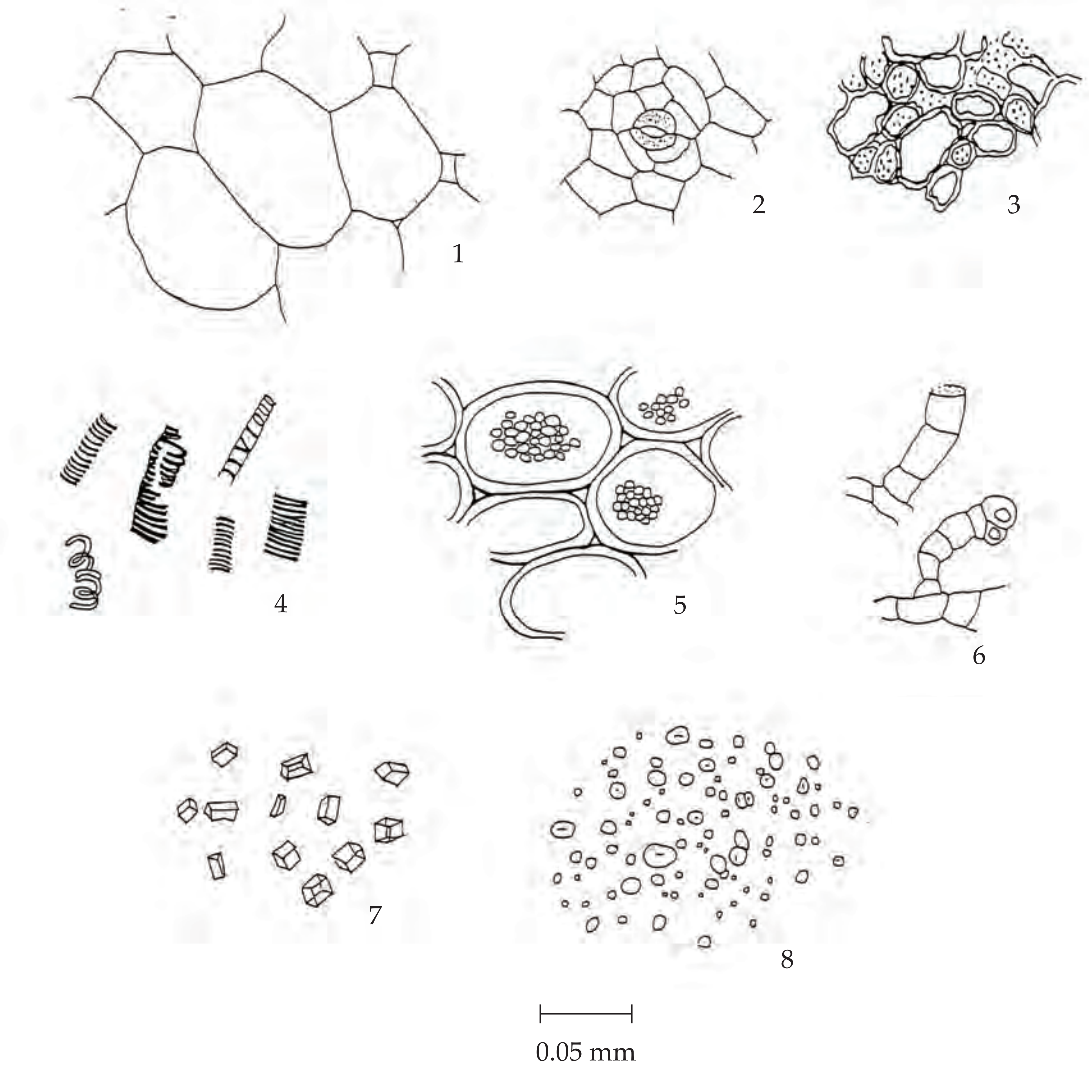


Fig. 2c Powdered Drug of the Unripe Fruits of *Momordica charantia* L.

1. thin-walled parenchyma
2. epicarp showing stoma
3. sclerenchyma of seed coat
4. annulo-spiral and spiral vessels
5. thick-walled parenchyma containing starch grains
6. uniseriate-stalked trichomes with and without gland
7. cuboidal protein crystals
8. starch grains

cells near the periphery contained most of chloroplasts, vascular bundles found at the innermost part of this zone; moderately thick-walled parenchyma cells, composed of several layers of loosely packed cells with intercellular spaces, containing numerous starch grains; and thin-walled parenchyma cells, several layers of cells containing cuboidal protein crystals and numerous starch grains. Endocarp, a layer of small thin-walled cells containing starch grains. Seed coat composed of epidermis, a layer of thin-walled cells filled with starch grains; sclerenchyma, several layers of lignified thick-walled or pitted cells containing starch grains; parenchyma, several layers of thin-walled cells. Cotyledons 2, composed of a layer of epidermis and reserved parenchyma cells which are fairly large and filled with oil globules.

Thai Bitter Cucumber in powder possesses the diagnostic microscopical characters of the unground drug. Thick-walled parenchyma cells containing starch grains; lignified thick-walled or pitted cells of sclerenchyma of seed coat; occasionally uniseriate-stalked glandular trichomes; and occasionally cuboidal protein crystals are characteristic.

Warning

1. It should not be used in nursing women and children unless recommended by a physician.
2. It should be used with caution in patients with existing liver diseases including alcoholic cirrhosis.
3. Concomitant use with oral hypoglycemic agents and insulin intake should be avoided due to hypoglycemic risks.

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Thai Bitter Cucumber shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 1 g of the sample, in powder, with 10 ml of *ethanol* for 15 minutes and filter. Evaporate the filtrate to dryness in a porcelain dish. Add 5 drops of *acetic anhydride* to the residue and then slowly add 1 drop of *sulfuric acid* along the inner side of the dish: a purple colour develops and gradually changes to brownish black.

B. Carry out the test as described in the “Thin-layer chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol* as the mobile phase and allowing the solvent front to ascend 9 cm above the line of application. Apply separately to the plate as bands of 7 mm, 20 µl each of the following two solutions. Prepare solution (A) by refluxing 1 g of the sample, in powder, with 5 ml of a mixture of equal volumes of *dichloromethane* and *methanol* in a water-bath and filtering. For solution (B), dissolve 1 mg of *charantin* in 1 ml of a mixture of equal volumes of *dichloromethane* and *methanol*. After removal of the plate, allow it to dry in air and examine the plate under ultraviolet light (366 nm) through the cut-off filter; two red fluorescent bands are observed. Spray the plate with a 10 per cent v/v solution of *sulfuric acid* in *ethanol* and heat at 110° for 1 to 2 minutes; the chromatogram obtained from solution (A) shows a violet band (hR_f value 28 to 30), corresponding to the charantin band from solution (B). Other several bands of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in the Extract of the Fruits of *Momordica charantia* L.

Band	hR_f Value	Detection	
		UV 366	10 Per Cent V/V Solution of *Sulfuric Acid* in *Ethanol*
1	9-10	–	pale violet
2	18-19	–	pale violet
3*	28-30	–	violet
4	50-53	–	pale purple
5	55-58	red	violet
6	58-61	light red	–
7	62-64	–	brownish yellow
8	72-75	–	pale brown

*charantin

Loss on drying Not more than 16.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 0.1 per cent w/w (Appendix 7.6).

Total ash Not more than 13.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 1.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 4.5 per cent w/w (Appendix 7.12).

Dose 500 mg to 1 g three times a day.

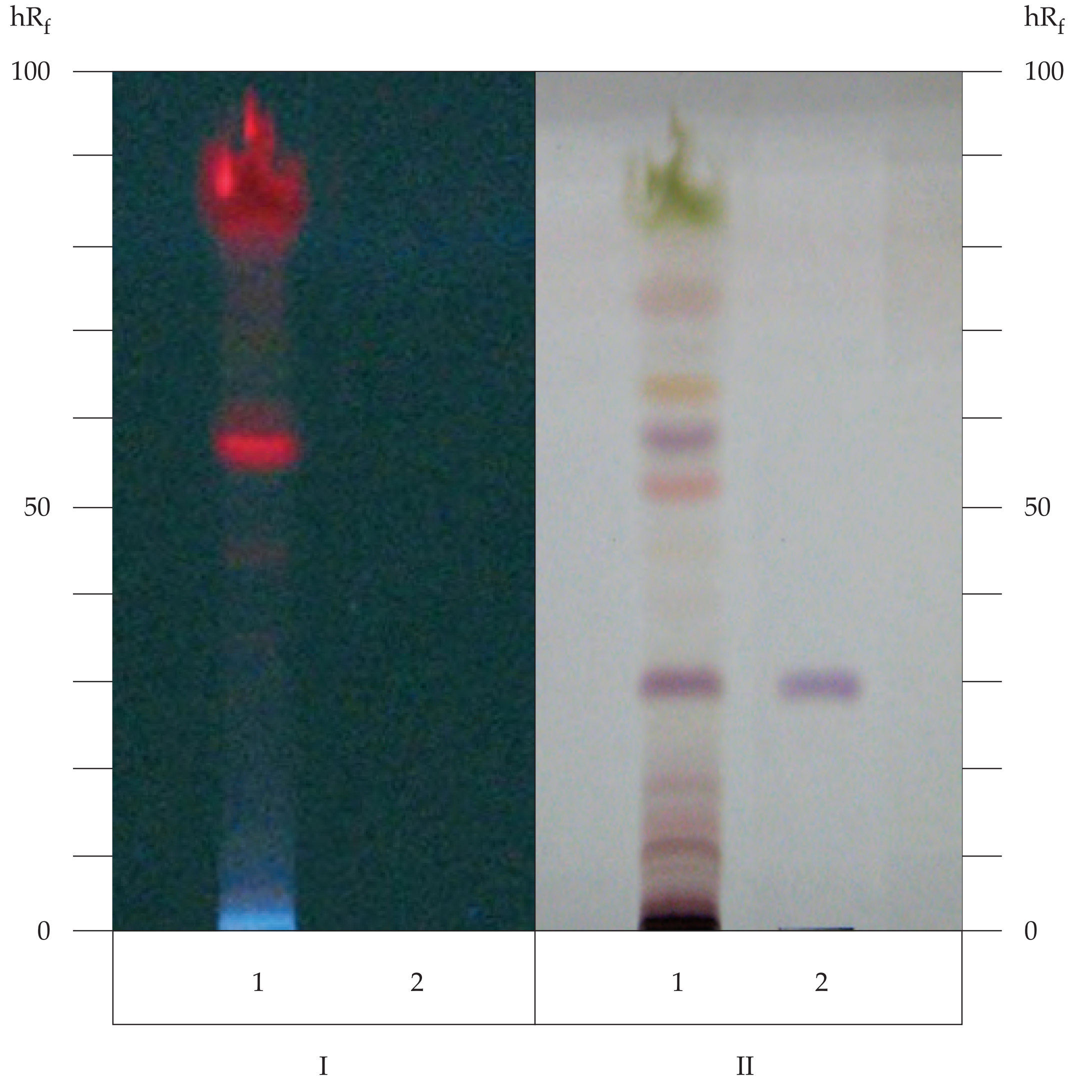


Fig. 3 Thin-layer Chromatogram of the Extract of the Fruits of *Momordica charantia* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (366 nm)
II = detection with a 10 per cent v/v solution of *sulfuric acid* in *ethanol*

มะแว้งเครือ (MAWAENG KRUEA)

Solani Trilobati Fructus
Solanum Trilobatum Fruit

Category Expectorant.

Solanum Trilobatum Fruit is the dried unripe fruit of *Solanum trilobatum* L. (Family Solanaceae), Herbarium Specimen Number: DMSC 147, 163.

Constituents Solanum Trilobatum Fruit contains solasodine.

Description of the plant (Fig. 1) Slender scrambling shrub, up to 3.6 m long; prickles hooked, broad-based, yellowish and numerous along the stems, otherwise almost glabrous; branches long divaricate, the young ones sometimes with a few stellate hairs, the older glabrous. Leaves simple, alternate, ovate or rotund-ovate, 2 to 7 cm long, 1 to 4 cm wide, obtuse, irregularly sinuate or 3- to 5-lobed, sparsely stellately hairy or glabrous; petiole and midrib with or without a few prickles, 1.3 to 3.8 cm long, base broadly cuneate-truncate, sometimes unequal. Inflorescences extra-axillary racemose cymes; peduncles short, 3- to 9-flowered. Flowers showy, violet-purple; pedicels 1 to 2 cm long, widely divergent, often prickly; calyx cyathiform, 3 to 4 mm long with narrow teeth, sparsely stellately hairy; corolla 1.2 to 1.6 cm long, tube 2 mm long, deeply lobed, stellate-pubescent outside, usually reflexed; stamens protruding, anthers 7 mm long, narrowly oblong, yellow, filaments 1.2 mm long; ovary 2-celled, ovoid or subglobose, style glabrous. Fruit berry, globose about 1 cm in diameter, mature berry green with white mottling, scarlet when ripe. Seeds numerous, compressed, 3 mm in diameter, slightly pitted.

Description

Macroscopical Nearly globular, from 6 to 10 mm in diameter, externally light brown to dark brown, more or less wrinkled. The base marked by a circular stalk scar, about 1 mm in diameter.

Microscopical (Figs. 2a, 2b) Transverse section of the fruit shows epidermis, a single layer of rectangular cells with cutinized outer walls. Hypodermis, 1 to 2 layers of rectangular cells. Two zones of parenchyma cells, the outer composed of 6 to 9 layers of thick-walled, polygonal cells containing chloroplastids, the inner composed of more layers of larger and thin-walled cells containing mucilage and numerous starch grains and small vascular bundles are scattered in this region. Epidermis of seed coat, a single layer of large lignified sclereids, more or less columnar shaped, inner walls are thicker with irregularly flamelike thickened appendages. Endosperm, small thin-walled, polygonal parenchyma cells containing oil droplets. Cotyledon, the several layers of small polygonal cells containing oil droplets.

Solanum Trilobatum Fruit in powder possesses the diagnostic microscopical characters of the unground drug.

Packaging and storage Solanum Trilobatum Fruit shall be kept in well-closed containers, protected from light, and stored in a dry place.

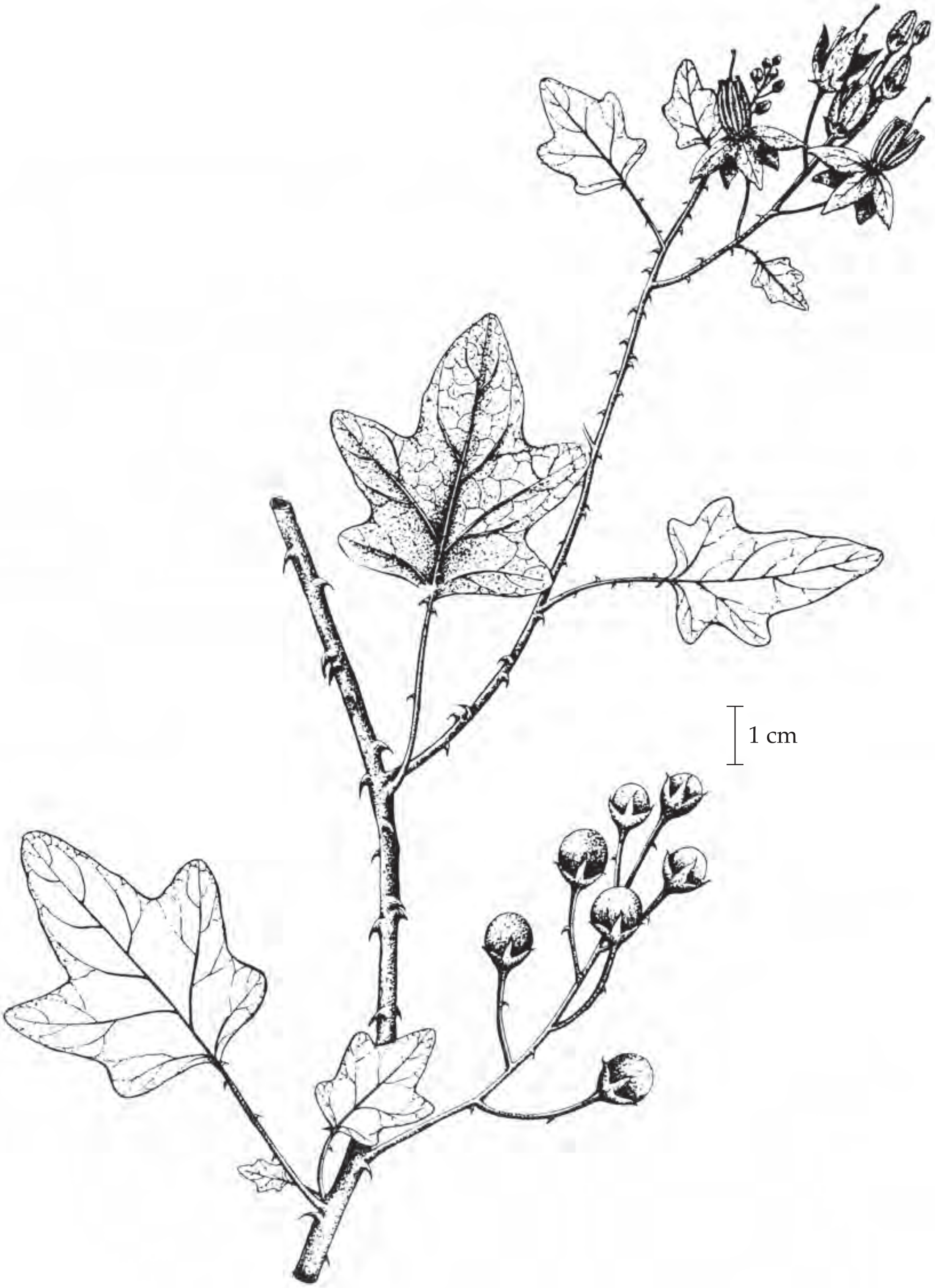


Fig. 1 *Solanum trilobatum* L.

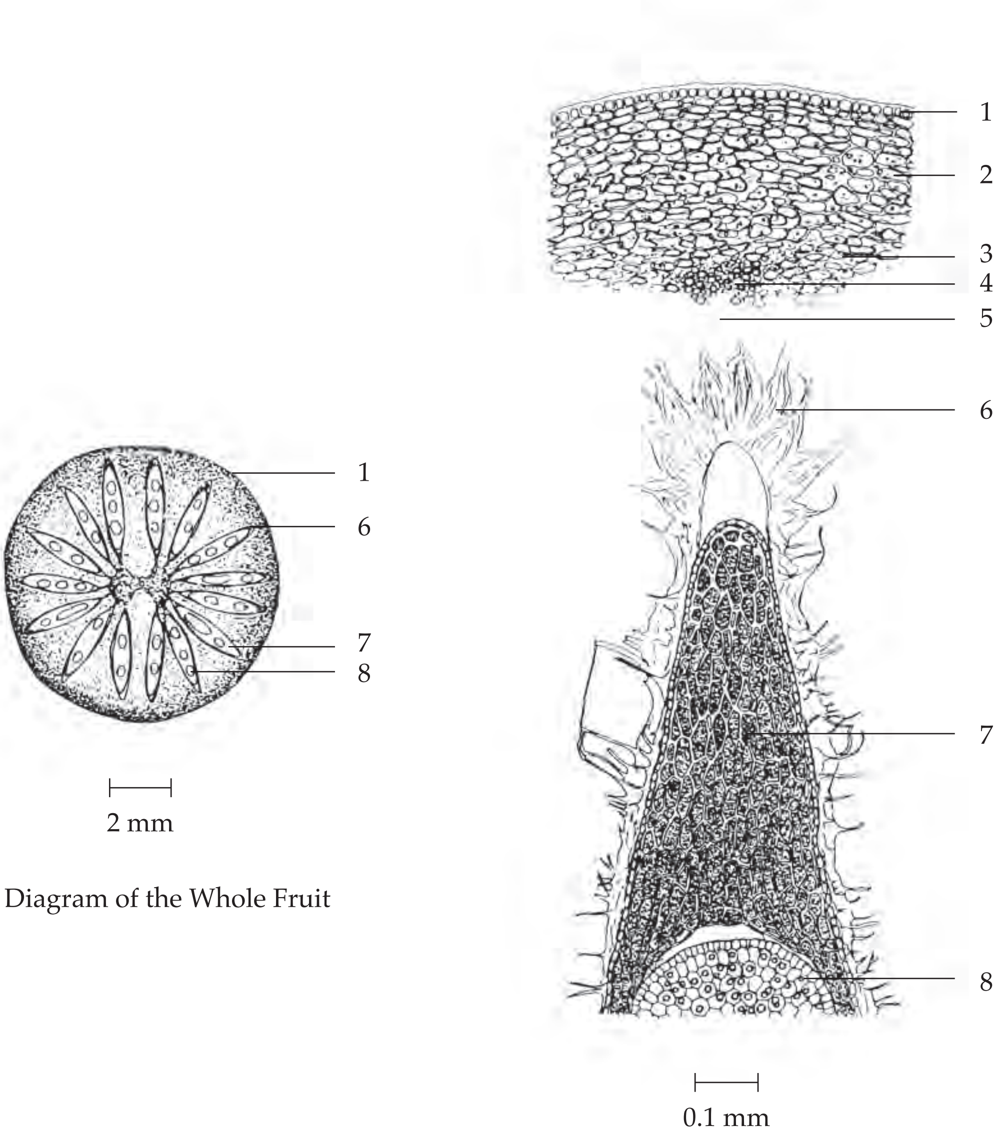


Diagram of the Whole Fruit

Fig. 2a Transverse Section of the Fruit of *Solanum trilobatum* L.

1. epidermis
2. thick-walled parenchyma containing starch grains and oil droplets
3. thin-walled parenchyma containing starch grains and oil droplets
4. vascular bundle
5. cavity
6. sclereids
7. endosperm containing oil droplets
8. embryo containing oil droplets

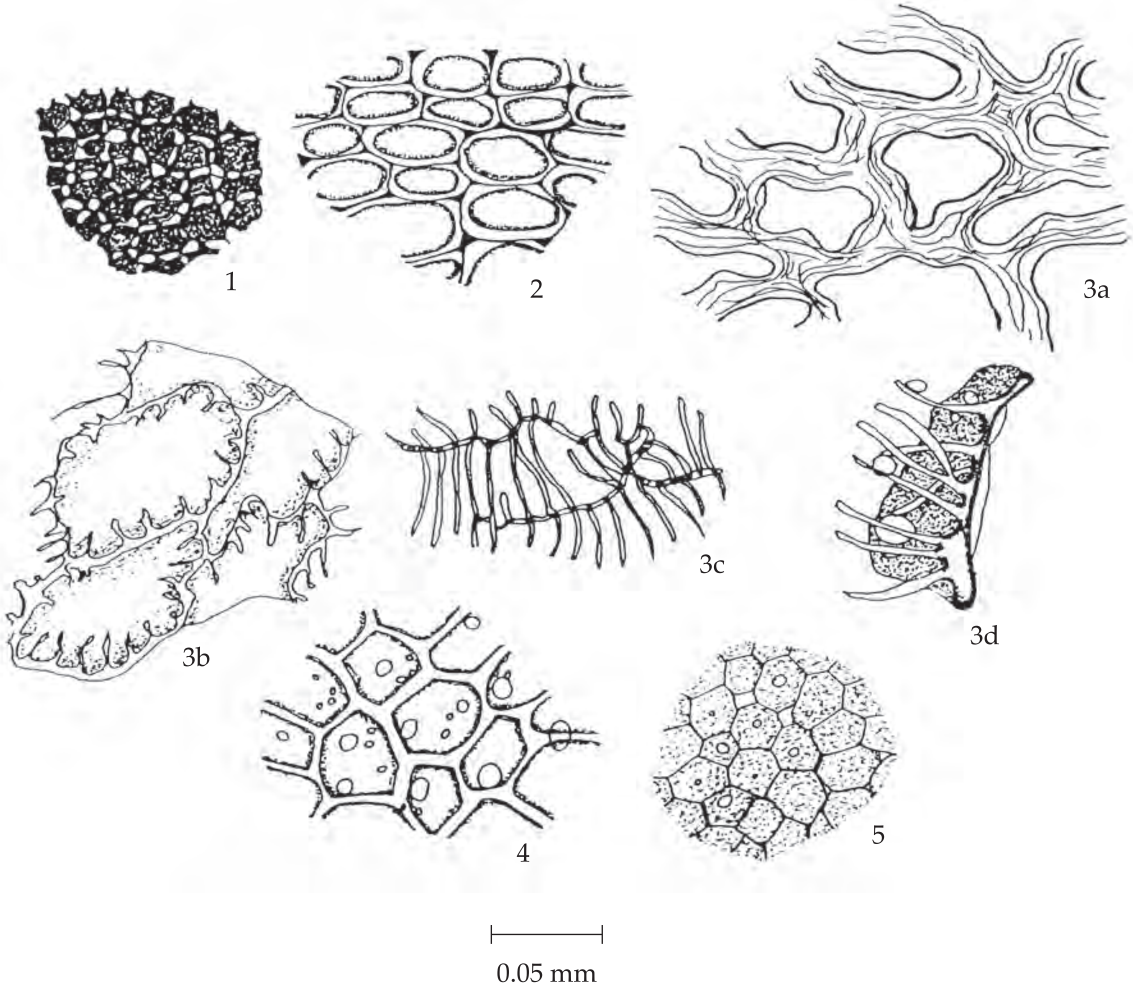


Fig. 2b Powdered Drug of the Fruits of *Solanum trilobatum* L.

1. epidermis of pericarp in surface view
2. thick-walled parenchyma of pericarp
3. sclerenchymatous layer of testa
   a. surface view seen from raphe
   b. surface view seen from below
   c. surface view seen from above
   d. sectional view
4. endosperm with oil droplets
5. embryo with oil droplets

Identification

A. Reflux 500 mg of the sample, in powder, with 10 ml of *ethanol* for 30 minutes, and filter. Evaporate 2 to 3 ml of the filtrate to dryness and add 2 to 3 drops of *acetic anhydride* and a few drops of *sulfuric acid*: a violet colour is produced.

B. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using s*ilica gel G* as the coating substance and a mixture of 55 volumes of *chloroform* and 45 volumes of *toluene* as the mobile phase. Apply to the plate, 10 µl of the test solution prepared by refluxing 5 g of the sample, in powder, with 10 ml of *methanol* for 20 minutes, and filtering. After removal of the plate, allow it to dry in air and examine under ultraviolet light (366 nm); two blue and two red fluorescent spots appear. Spray the plate with *vanillin-sulfuric acid TS* and heat at 120° for 5 to 10 minutes; six violet spots are observed (Table 1); see also Fig. 3.

Repeat the same procedure on another plate but using 90 volumes of *cyclohexane* and 10 volumes of *diethanolamine* as the mobile phase. After removal of the plate, allow it to dry in air and examine under ultraviolet light (366 nm); three light blue fluorescent spots appear. Spray the plate with *modified Dragendorff TS*; two orange-yellow spots are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Fruits of *Solanum trilobatum* L.

a. Mobile phase: 55 volumes of *chloroform* and 45 volumes of *toluene*

Spot	hR_f Value	Detection	
		UV 366	*Vanillin-Sulfuric Acid TS*
1	3-6	blue	–
2	5-7	red	–
3	5-7	–	violet
4	7-9	blue	–
5	8-12	–	violet
6	10-15	red	–
7	12-17	–	violet
8	15-21	–	violet
9	18-25	–	violet
10	43-53	–	violet

b. Mobile phase: 90 volumes of *cyclohexane* and 10 volumes of *diethanolamine*

Spot	hR_f Value	Detection	
		UV 366	*Modified Dragendorff TS*
1	2	light blue	–
2	23-24	–	orange-yellow
3	40-44	light blue	–
4	58-61	light blue	–
5	58-61	–	orange-yellow

Loss on drying Not more than 6.0 per cent w/w after drying at 100° to constant weight (Appendix 4.15).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Sulfated ash Not more than 11.0 per cent w/w (Appendix 5.3).

Ethanol-soluble extractive Not less than 3.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12).

Chloroform-soluble extractive Not less than 1.0 per cent w/w (Appendix 7.12H).

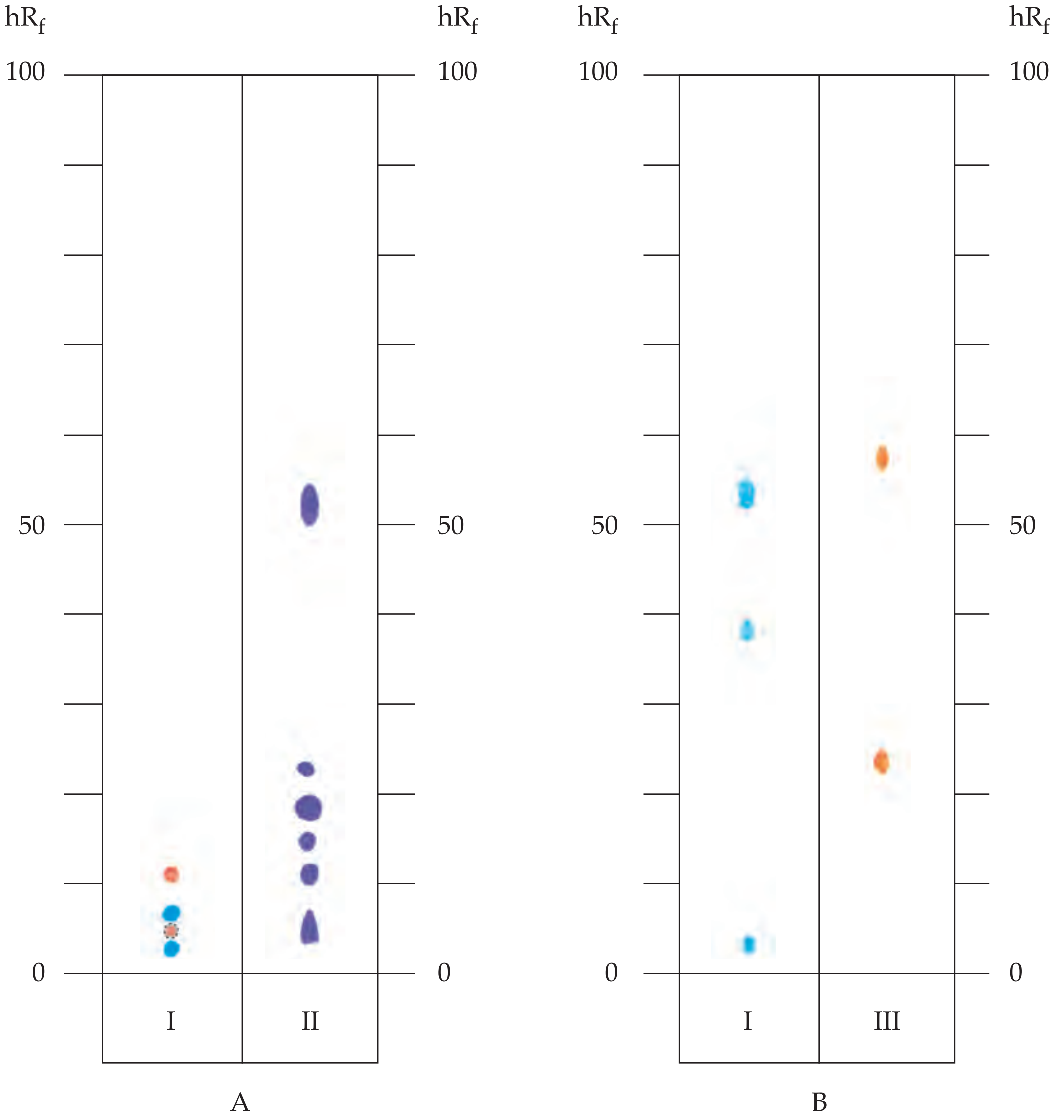


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Fruits of *Solanum trilobatum* L.

A = use 55 volumes of *chloroform* and 45 volumes of *toluene* as the mobile phase.
B = use 90 volumes of *cyclohexane* and 10 volumes of *diethanolamine* as the mobile phase.
I = detection under UV light (366 nm)
II = detection with *vanillin-sulfuric acid TS*
III = detection with *modified Dragendorff TS*
◌ = spots developed in some samples

หม่อน (MON)

Mori Albi Folium
Mulberry Leaf

Synonyms Moral Blanco Leaf, Russian Mulberry Leaf, Silkworm Mulberry Leaf, White Mulberry Leaf, White-fruited Mulberry Leaf

Category Mild antitussive.

Mulberry Leaf is the dried leaf of *Morus alba* L. (Family Moraceae), Herbarium Specimen Number: DMSC 5157, Crude Drug Number: DMSc 5431, 5432.

Constituents Mulberry Leaf contains flavonoids (e.g., rutin, quercetin 3-(6-malonyl) glucoside, isoquercitrin), alkaloids (e.g., 1-deoxynojirimycin), sterols, etc.

Description of the plant (Figs. 1a, 1b) Deciduous tree or shrub, 3 to 15(–20) m high; stem erect, branched; young branch greenish, pubescent; mature branch greyish green with lenticels; lenticel prominent, reddish or yellowish brown; stem bark thin, rough, shallowly furrowed, grey, reddish or yellowish brown; bud ovoid, 4 to 6 mm long. Leaves simple, alternate, variable in size and shape, usually ovate to broadly ovate, (2.5–)5 to 20(–30) cm long, 2.5 to 14 cm wide, apex acute, acuminate or obtuse, base truncate, round or cordate, margin serrate, serrate-crenate, or irregularly lobed, basal nerves 3, blade abaxially glabrous or sparingly pubescent along major veins or tufted in axils of principle lateral veins and midrib, adaxially glabrous or sparsely pubescent; petiole up to 5.5 cm long; stipule ovate to lanceolate, 5 to 9 mm, caducous. Inflorescence catkin, axillary, sordid white or greenish white, monoecious or dioecious; male catkin lax, 1 to 4 cm long; female catkin ovoid, 0.5 to 2 cm long; peduncle as long as the catkin. Male flower sessile; sepals 4, imbricate, ovoid, about 2.5 mm long, glabrous or hairy; stamens 4, filament inflexed in buds, anther globose to reniform, 2-loculed, papillose. Female flower sessile; sepals 4, imbricate, glabrous, marginally hairy; ovary 1-celled, glabrous, style, 0.5 to 1 mm long, 2-partite, stigma papillose. Fruit multiple, syncarpous, ovoid, ellipsoid or cylindric, 1 to 2.5 cm long, 0.5 to 1 cm wide, fleshy, becoming reddish, purplish, blackish or whitish when mature.

Description Odour, slightly aromatic; taste, slightly sweet with slightly bitter aftertaste.

Macroscopical (Fig. 1a) Lamina ovate to broadly ovate, irregularly lobed, 5 to 15 cm long, 4 to 10 cm wide, apex acute, acuminate or obtuse, base round to cordate, margin coarsely serrate to crenate, vein prominent; petiole, if present, slender, up to 5.5 cm long.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the leaf through the midrib and the lamina shows upper and lower epidermises, mesophyll, vascular tissue, and collenchyma. Upper epidermis, a layer of large rectangular cells; large lithocysts forming papillae, some of which boundary cells also contain cystoliths, covered with cuticle layer and unicellular trichomes. Mesophyll composed of a layer of palisade cells and layers of irregular spongy cells, xylem, phloem. Xylem composed of spiral and reticulate vessels. Collenchyma cells, located beneath upper and lower epidermises of the midrib. Lower epidermis, a layer of small oval cells; numerous anomocytic stomata, covered with cuticle layer and unicellular trichomes.

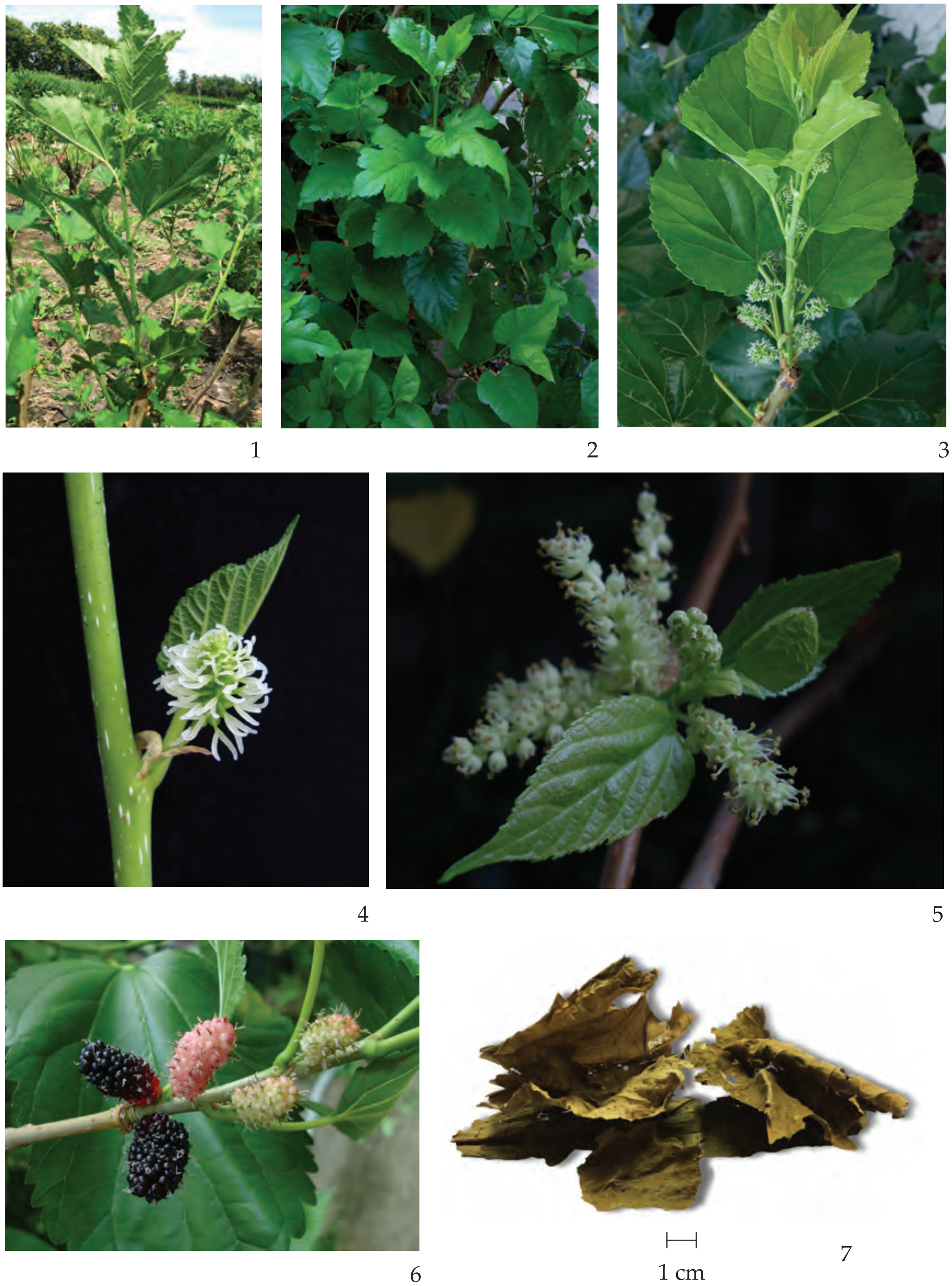


Fig. 1a *Morus alba* L.
1. habit 2. branch showing polymorphic leaves
3. female flowering branch 4. female inflorescence 5. male inflorescences
6. different stages of infructescences 7. crude drug

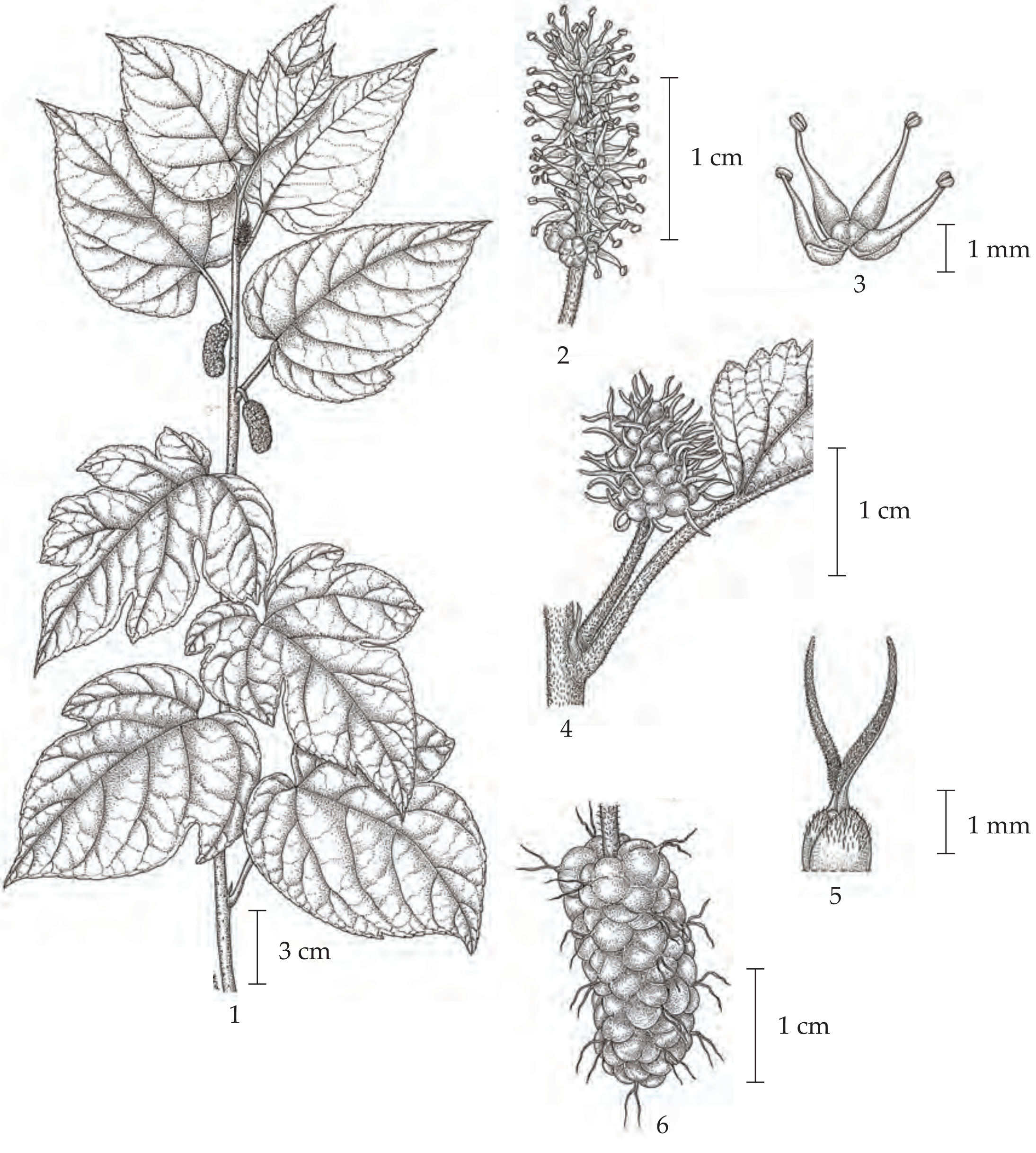
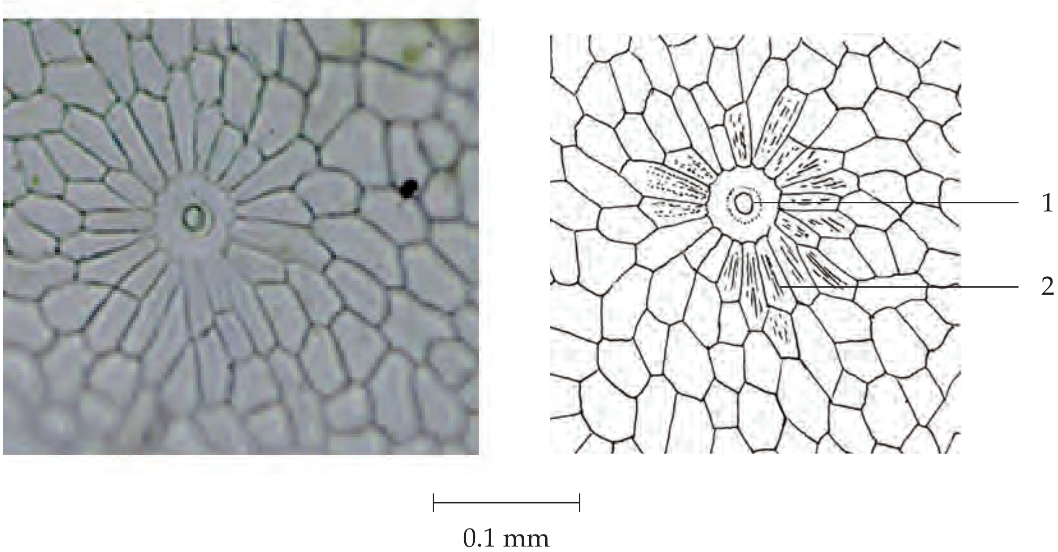
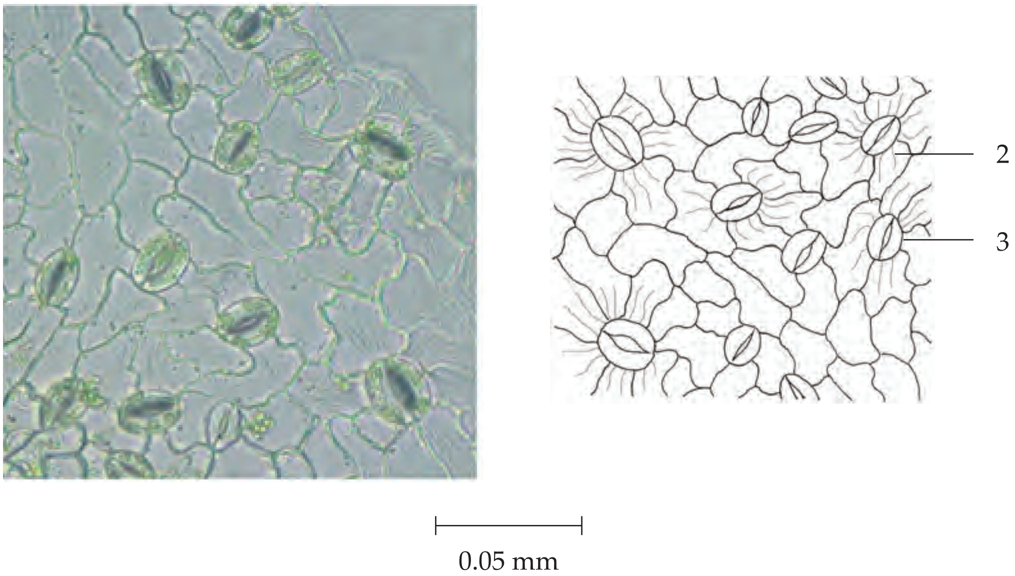


Fig. 1b *Morus alba* L.
1. flowering and fruiting branch showing polymorphic leaves
2. male inflorescence 3. male flower 4. female inflorescence
5. part of ovary, style and stigma 6. infructescence



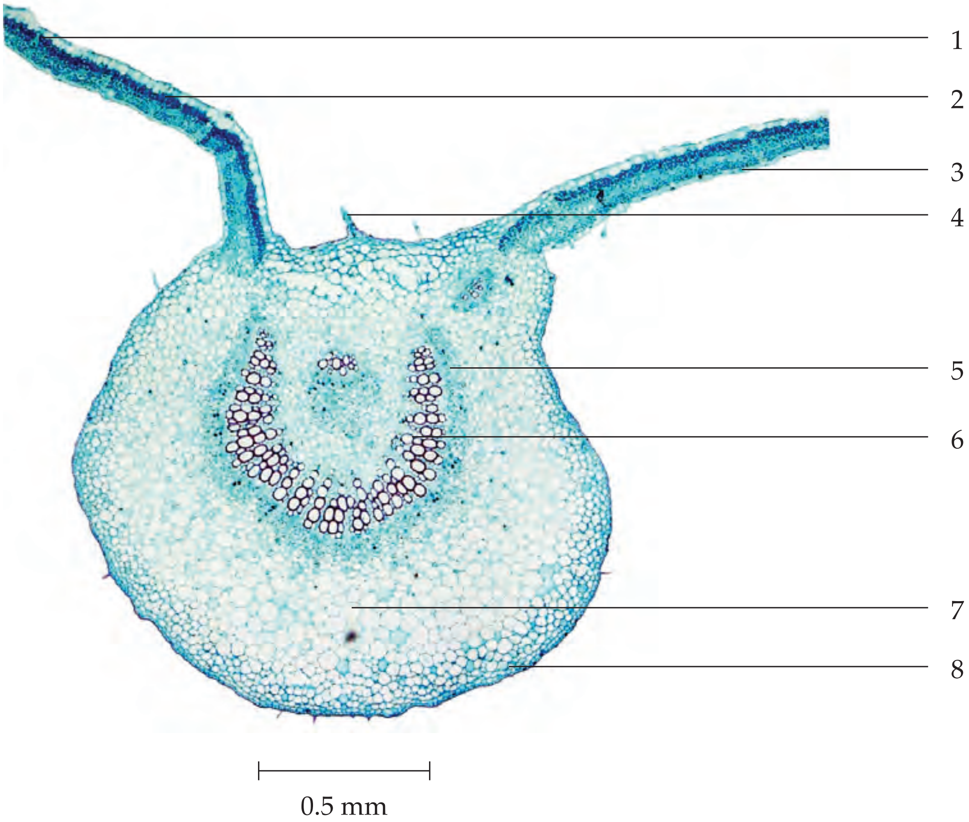
Upper Epidermis of the Lamina



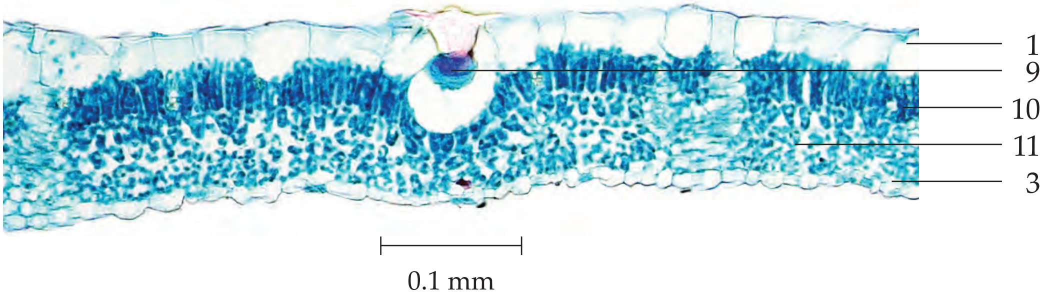
Lower Epidermis of the Lamina

Fig. 2a Photomicrographs and Line Drawings of Epidermises of the Leaf of *Morus alba* L.

1. cystolith trichome
2. striated cuticle
3. anomocytic stoma



Transverse Section of the Leaf through the Midrib



Transverse Section of the Lamina

Fig. 2b Photomicrographs of Transverse Section of the Leaf of *Morus alba* L., Stained with Safranin-Fast Green

1. upper epidermis
2. leaf blade
3. lower epidermis
4. trichome
5. phloem
6. xylem
7. parenchyma
8. collenchyma
9. cystolith
10. palisade cell
11. spongy cell

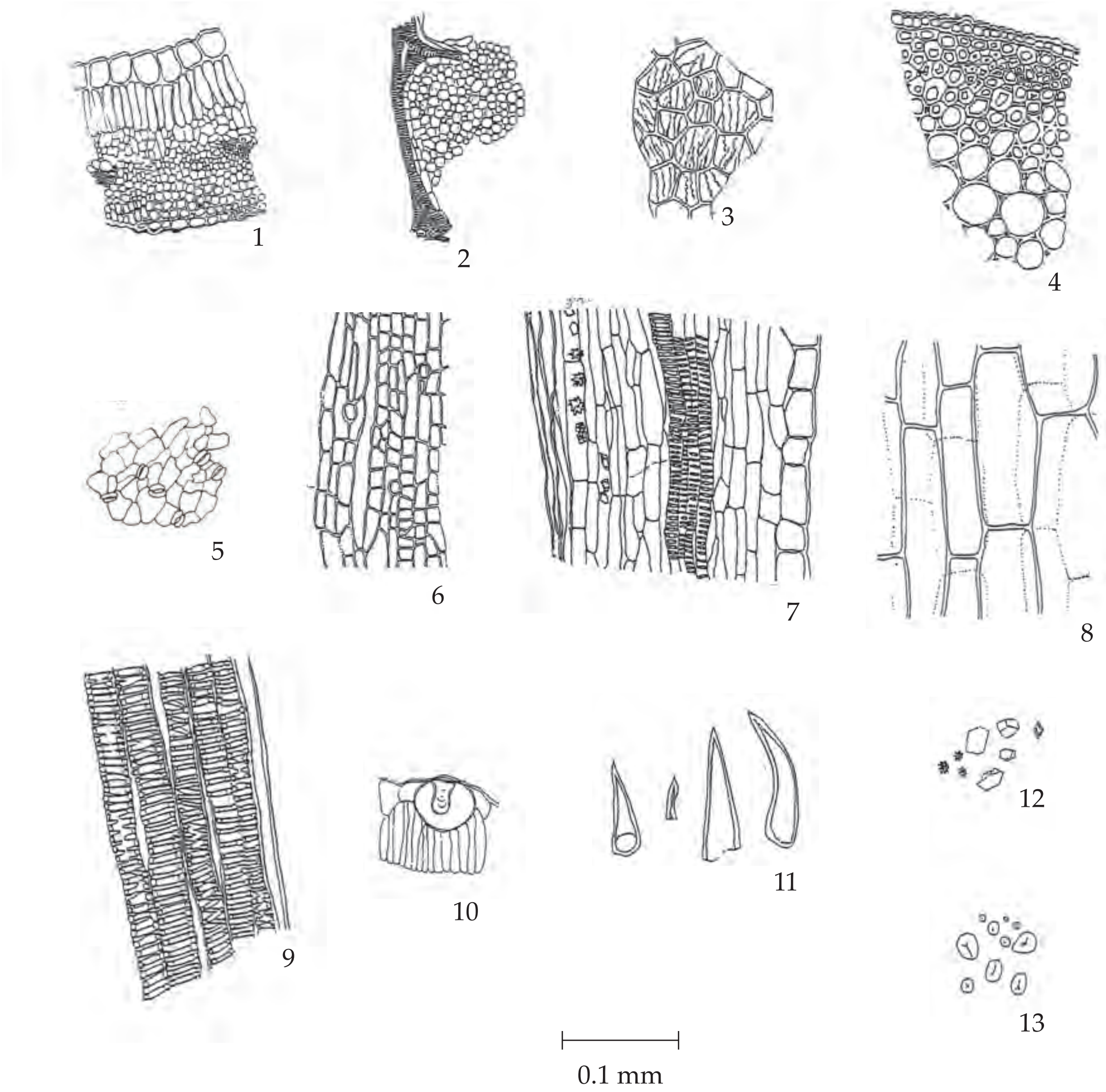


Fig. 2c Powdered Drug of the Leaves of *Morus alba* L.

1. lamina in sectional view showing epidermis, palisade cells, spongy cells, and vascular bundles
2. palisade cells and veinlet in surface view
3. upper epidermis with striated cuticle
4. epidermis associated with collenchyma of midrib
5. lower epidermis showing anomocytic stomata
6. epidermis and cicatrix, over vein in surface view
7. epidermis of petiole in longitudinal view associated with parenchyma, some containing rosette aggreagate or prismatic crystals and vascular tissue
8. rectangular epidermis and underlying parenchyma in surface view
9. fibre, spiral and reticulate vessels
10. epidermis associated with cystolith sac and layer of palisade cells
11. fragment of unicellular trichomes
12. rosette aggregate and prismatic crystals
13. starch grains

Mulberry Leaf in powder possesses the diagnostic microscopical characters of the unground drug. Upper epidermis, large rectangular cells, some with cystoliths; lower epidermis, small oval cells with anomocytic stomata; epidermis of the vein with cicatrix; epidermis with striated cuticle; and unicelluar trichomes are characteristic.

Packaging and storage Mulberry Leaf shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Warm 500 mg of the sample, in *No. 250 powder*, with 10 ml of *water* on a water-bath for 15 minutes and filter (solution 1). To 1 ml of solution 1, add 3 or 4 pieces of *magnesium ribbon*, shake well, and mix with 1 or 2 drops of *hydrochloric acid*: a brownish red colour develops.

B. To 2 ml of solution 1, add 1 or 2 drops of a 5 per cent w/v solution of *iron(III) chloride*: a greenish brown precipitate is produced.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 80 volumes of *ethyl acetate,* 20 volumes of *acetone*, 20 volumes of *formic acid* and 20 volumes of *water* as the mobile phase and allowing the solvent front to ascend 8.5 cm above the line of application. Apply separately to the plate as bands of 10 mm, 5 µl each of the following two solutions. Prepare solution (A) by shaking 1 g of the sample, in *No 250 powder*, in 10 ml of *ethanol (30 per cent)*, warming in a water-bath at 50° for 10 minutes, filtering, and washing the filtrate with three 10-ml portions of n-*hexane*. Evaporate the ethanolic layer on a water-bath to dryness and dissolve in 1 ml of *ethanol (30 per cent)*. For solution (B), dissolve 1 mg of *rutin* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under daylight, locating the bands. The chromatogram obtained from solution (A) shows a yellow band (hR$_f$ value 65 to 67), corresponding to the rutin band from solution (B), and other two yellow bands. Subsequently examine the plate under ultraviolet light (254 nm), marking the quenching bands. One quenching band, corresponding to the rutin band from solution (B) and other three quenching bands are observed. Examine the plate under ultraviolet light (366 nm) through the cut-off filter; two blue fluorescent bands are observed. Spray the plate with *anisaldehyde TS* and heat at 105° for 3 minutes; the band due to rutin is yellow. Other yellow and yellowish grey bands are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Leaves of *Morus alba* L.

Band	hR_f Value	Detection			
		Daylight	UV 254	UV 366	*Anisaldehyde TS*
1	14-20	–	–	–	yellowish grey
2	29-35	–	–	–	yellowish grey
3*	65-67	pale yellow	quenching	–	pale yellow
4	67-68	–	–	light blue	–
5	68-74	pale yellow	quenching	–	pale yellow
6	73-76	–	–	light blue	–
7	79-81	pale yellow	quenching	–	pale yellow
8	86-88	–	weak quenching	–	–

*rutin

Loss on drying Not more than 11.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 5.0 per cent w/w (Appendix 7.6).

Total ash Not more than 11.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 4.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 17.0 per cent w/w (Appendix 7.12).

Dose 5 to 10 g a day.

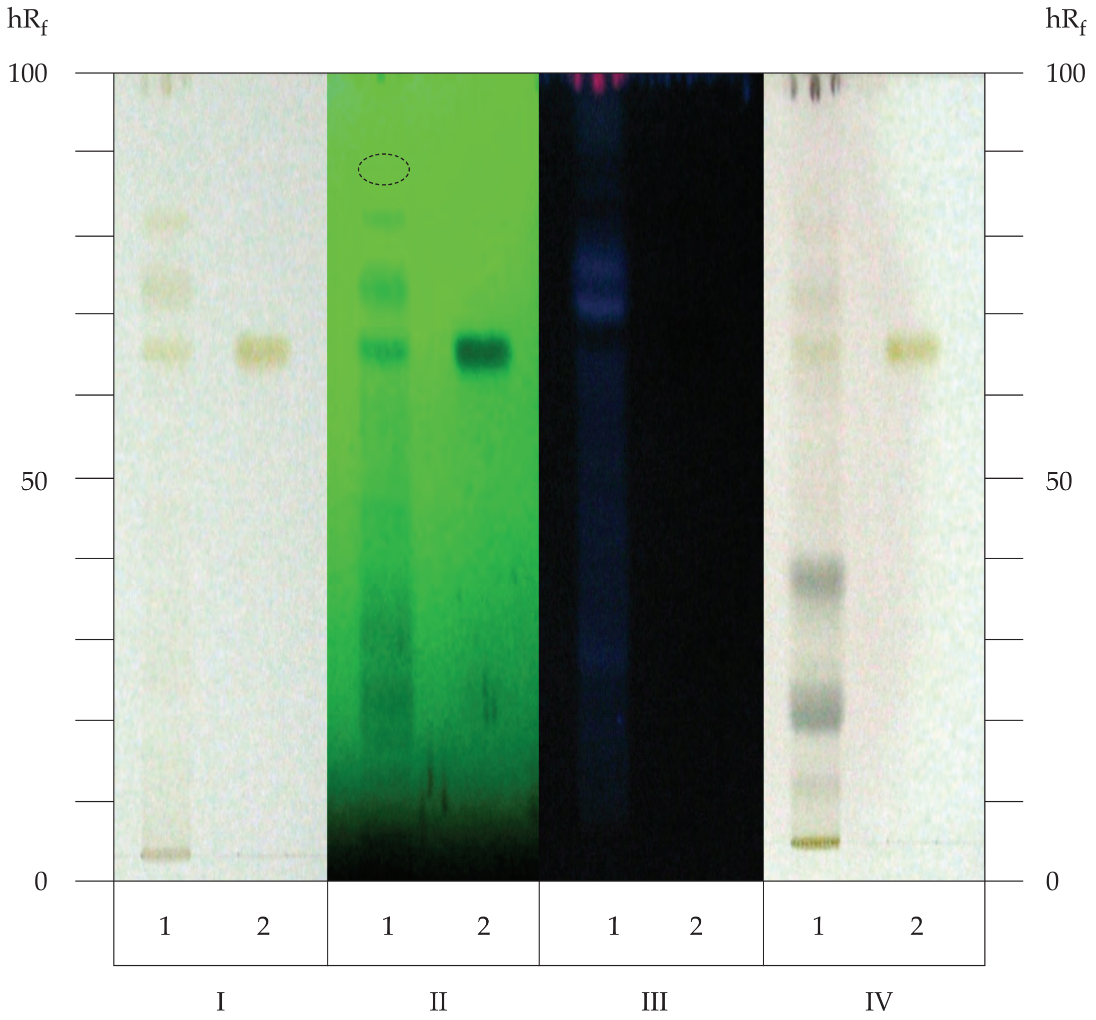


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Leaves of *Morus alba* L.

1 = solution (A)
2 = solution (B)
I = detection under daylight
II = detection under UV light (254 nm)
III = detection under UV light (366 nm)
IV = detection with *anisaldehyde TS*
◌ = band developed in some samples

พญายอ (PHAYA YO)

พญาปล้องทอง (PHAYA PLONG THONG), ผักมันไก่ (PHAK MAN KAI), เสลดพังพอนตัวเมีย (SALET PANGPHON TUAMIA), ผักลิ้นเขียด (PHAK LINKHIAT)

Clinacanthi Nutansis Folium
Clinacanthus Nutans Leaf

Category Anti-inflammatory, antiviral (Herpes simplex and Herpes zoster).

Clinacanthus Nutans Leaf is the dried leaf of *Clinacanthus nutans* (Burm. f.) Lindau (Family Acanthaceae), Herbarium Specimen Number: DMSC 5137, BKF 161293, Crude Drug Number: DMSc 0727.

Constituents Clinacanthus Nutans Leaf contains glycolipids, phaeophytins, triterpenoids, sterols, C-glycoside flavones, cerebrosides, sulfur-containing glucosides, etc.

Description of the plant (Figs. 1a, 1b) Scandent shrub, 1 to 3 m high, erect-drooping or clambering; branchlets finely pubescent. Leaves simple, decussate, narrowly elliptic, oblong, ovate or lanceolate, 2.5 to 13 cm long, 0.5 to 3.5 cm wide, apex long-acuminate, base obtuse, rounded, or truncate, often oblique, margin exsculptate-dentate or subentire, pubescent on the nerves; petioles 3 to 15 mm long. Inflorescence dense cymes, 5- to many-flowered, often terminating drooping-horizontal branches, resupinate; bracts narrow; calyx 5, deeply partite, segments narrow about 1 cm long; corollas 5, glandular-pubescent, about 3.5 cm long, dull red with green base, corolla-tube long curved, widened upwards, bilabiate, lower lip 3-lobed (turned upwards) with yellow streaks, apically sordidly yellow or greenish yellow; stamens 2, inserted in the throat, subequalling the upper lip, anthers 1-celled; ovary compressed, styles filiform, shortly bidentate. Fruit capsule, oblong, 4-seeded.

Description Odour, characteristic.

Macroscopical (Fig. 1a) A mixture of entire and broken, greenish brown to brown dried leaves.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the leaf shows upper epidermis, a layer of rectangular cells, irregular and slightly wavy-walled in surface view; lithocysts, oblong; stomata absent with glandular and non-glandular trichomes. Non-glandular trichomes, multicellular uniseriate, 1- to 12-celled with warted walls. Glandular trichomes, unicellular stalk, 6- to 8-celled head. Lower epidermis, almost similar to those of upper epidermis, slightly wavy walls in surface view; diacytic stomata, numerous, lithocysts and glandular trichomes. Mesophyll, a single layer of palisade cells and few layers of spongy cells, containing chloroplasts and globules of pale greenish yellow contents. Underneath both epidermises, 3 to 4 layers of angular collenchymatous cells in the midrib. Fibrovascular bundles composed of fibres, spiral vessels and xylem parenchyma.

Clinacanthus Nutans Leaf in powder possesses the diagnostic microscopical characters of the unground drug.

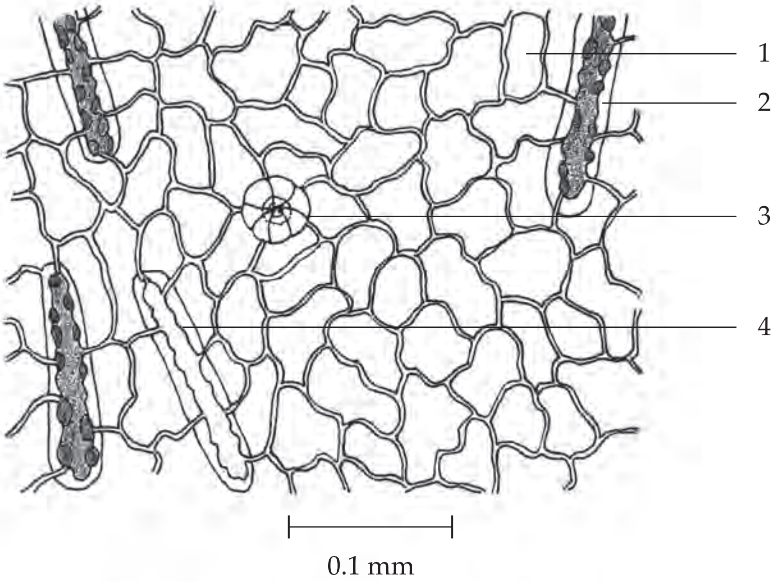
Warning It is not to be used on open wounds.

Additional information

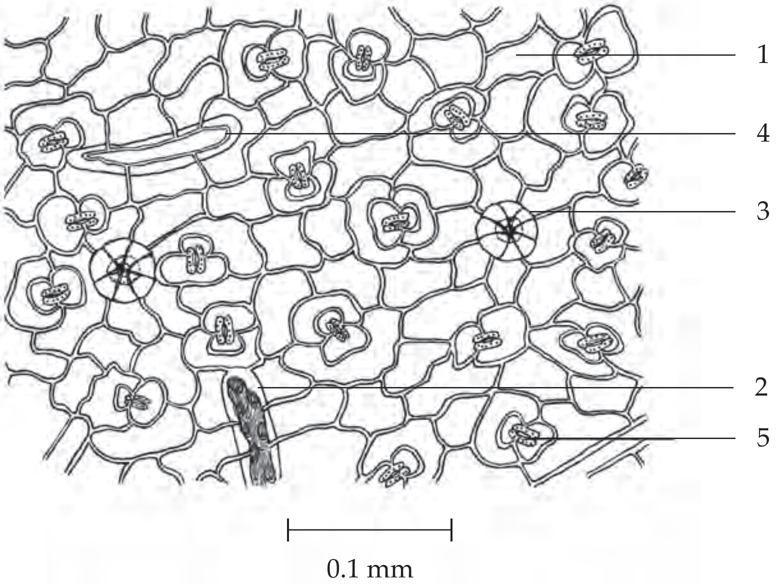
1. It should be used at the early signs or symptoms of inflammation or viral infection.
2. Ethnomedically, it has been used topically as an anti-inflammatory and antiviral agents. In these cases, 10 to 15 fresh or dried leaves, crushed and soaked with sufficient amount of *ethanol (40 per cent)* or Thai rice whiskey are applied directly to the affected areas as often as needed. Fresh leaves are also applied directly after being crushed.

Fig. 1a *Clinacanthus nutans* (Burm. f.) Lindau
1. habit 2. flowering twig 3. inflorescence 4. fresh leaves 5. crude drug

Fig. 1b *Clinacanthus nutans* (Burm. f.) Lindau
1. flowering twigs 2. flower



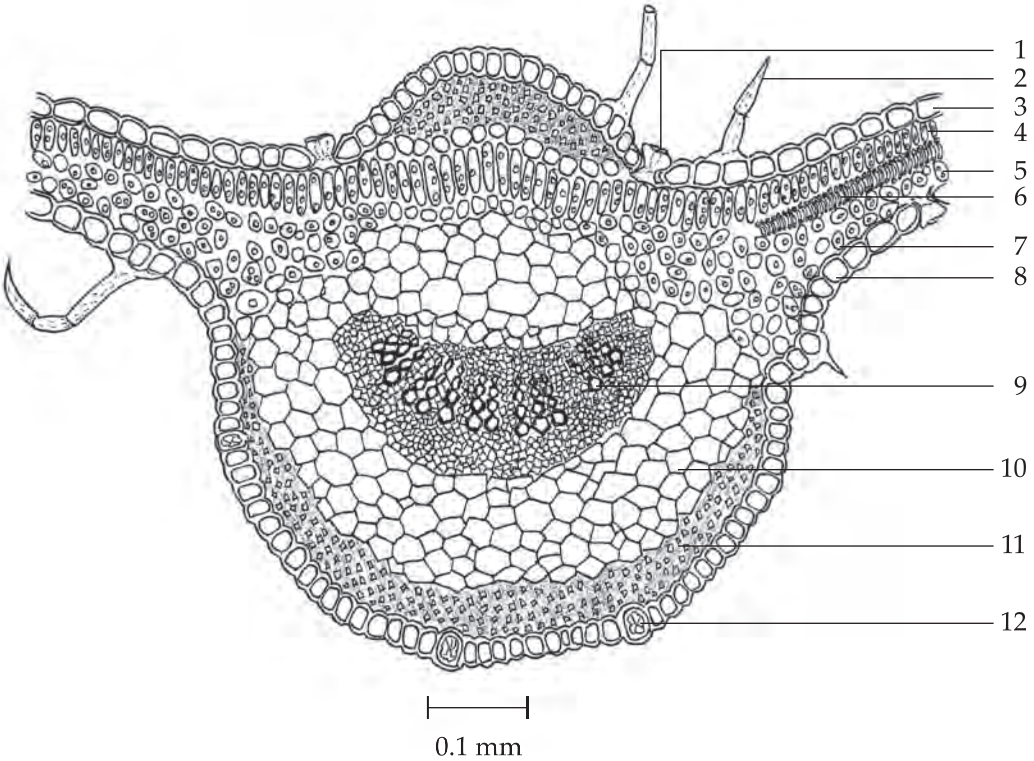
Upper Epidermis of the Lamina



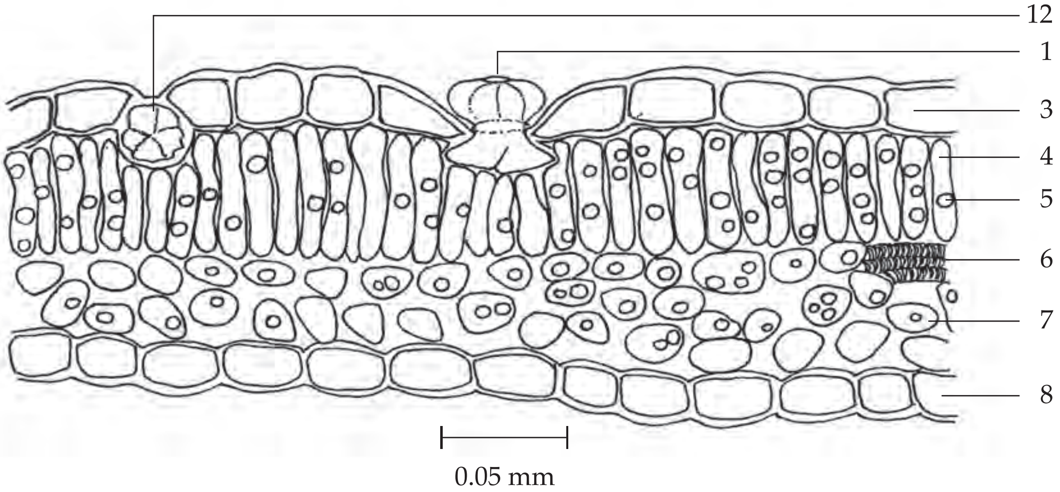
Lower Epidermis of the Lamina

Fig. 2a Epidermises of the Leaf of *Clinacanthus nutans* (Burm. f.) Lindau

1. epidermal cell
2. lithocyst
3. glandular trichome
4. lithocyst without cystolith
5. stoma



Transverse Section of the Midrib



Transverse Section of the Lamina

Fig. 2b Transverse Section of the Leaf of *Clinacanthus nutans* (Burm. f.) Lindau

1. glandular trichome
2. multicellular uniseriate trichome
3. upper epidermis
4. palisade cell
5. greenish yellow globule
6. spiral vessel
7. spongy cell
8. lower epidermis
9. xylem
10. parenchyma
11. collenchyma
12. lithocyst

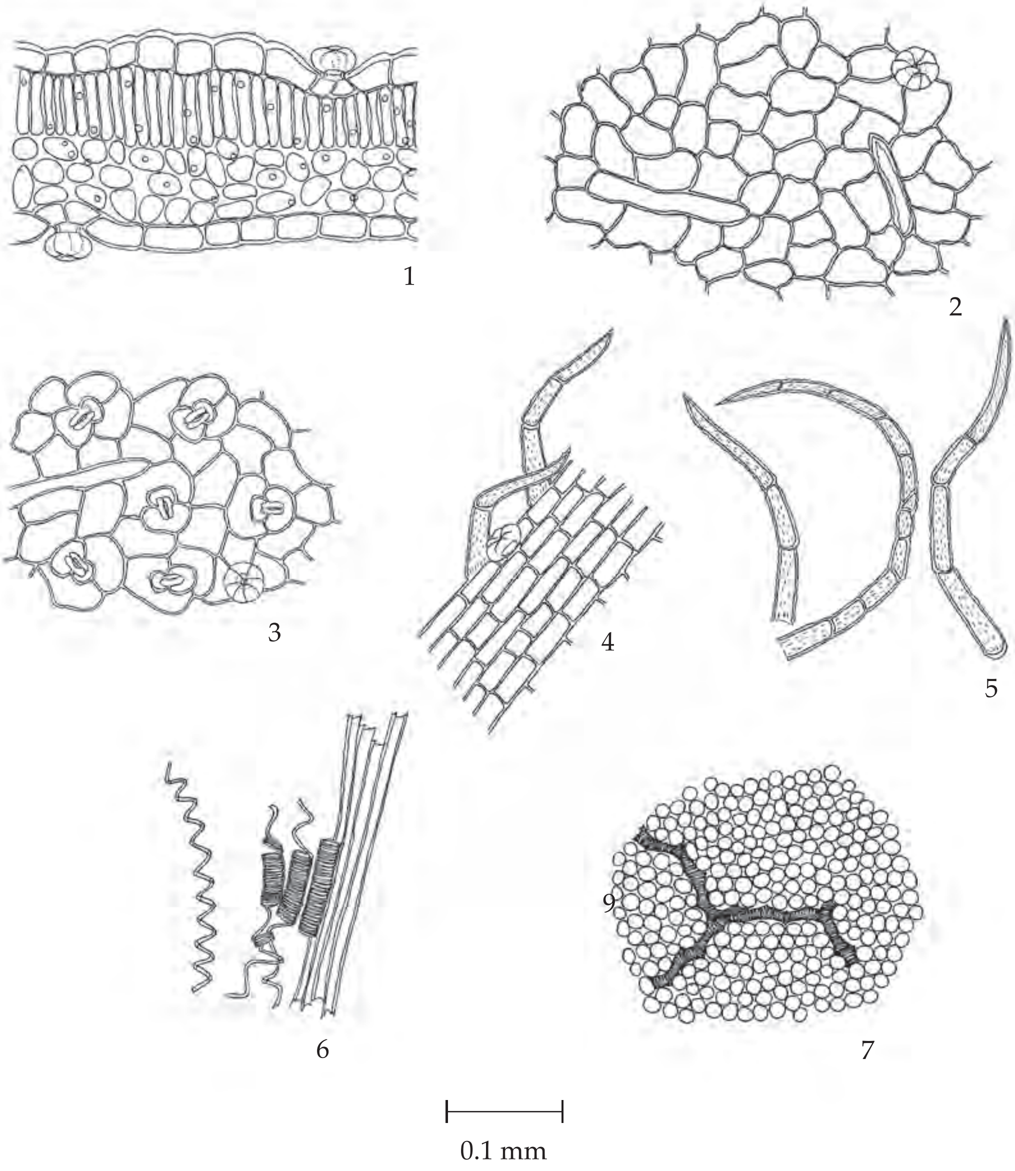


Fig. 2c Powdered Drug of the Leaves of *Clinacanthus nutans* (Burm. f.) Lindau

1. lamina in sectional view showing upper epidermis with underlying palisade cells and spongy cells containing greenish yellow globules, lower epidermis and glandular trichomes
2. upper epidermis in surface view with glandular trichome and lithocysts
3. lower epidermis in surface view showing diacytic stomata, glandular trichome and lithocyst without cystolith
4. epidermis of petiole with covering trichomes and glandular trichome
5. multicellular uniseriate trichomes
6. spiral vessels and group of fibres
7. palisade cells in surface view with veinlet

Packaging and storage Clinacanthus Nutans Leaf shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Warm 1 g of the sample, in *coarse powder*, with 20 ml of *water* in a water-bath for 10 minutes and filter (solution 1). To 2 ml of solution 1, add 1 drop of *iron(III) chloride TS*: a brown precipitate is produced.

B. To 2 ml of solution 1, add 1 ml of *potassium cupri-tartrate TS* and warm the mixture in a water-bath for a few minutes: a brick red precipitate is produced.

C. Warm 1 g of the sample, in *coarse powder*, with 20 ml of *ethanol* in a water-bath for 10 minutes and filter. Evaporate 5 ml of the filtrate to dryness. Dissolve the residue with 2 ml of *acetic anhydride* in a test-tube, and then slowly add 1 ml of *sulfuric acid* to form two layers: a greenish brown ring develops at the zone between the two layers.

D. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 80 volumes of *ethyl acetate* and 20 volumes of a 2 per cent v/v of *glacial acetic acid* in *methanol* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply to the plate as a band of 10 mm, 10 µl of the test solution. Prepare the test solution by macerating 2 g of the sample, in *coarse powder,* with 30 ml of a 70 per cent v/v solution of *methanol* overnight and filtering. Wash 20 ml of the filtrate by shaking with three 20-ml portions of *hexane,* discard the washings and concentrate to 5 ml. Add 15 ml of *water* and extract with three 20-ml portions of *ethyl acetate.* Combine the ethyl acetate extracts and evaporate to dryness. Dissolve the residue in 0.5 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. Several bands are observed. Examine the plate under ultraviolet light (366 nm) through the cut-off filter; several fluorescent bands of different colours are observed. Spray the plate with *phosphomolybdic acid TS* and heat at 105° for 5 minutes. Several bands of different colours are observed (Table 1); see also Fig. 3.

Repeat the same procedure on another plate. After removal of the plate and allow it to dry in air. Heat the plate at 80° for 10 minutes and then spray with *natural products (NP) TS* while the plate is still warm. Subsequently spray the plate with *polyethyleneglycol (PEG) TS* and observe the colours of the bands under ultraviolet light (366 nm) through the cut-off filter within 5 to 15 minutes. Several bands of different colours appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in the Extract of the Leaves of *Clinacanthus nutans* (Burm. f.) Lindau

Band	hR_f Value	Detection			
		UV 254	UV 366	*Phosphomolybdic Acid TS*	*NP/PEG TS* and UV 366
1	5-11	quenching	–	yellowish brown	yellow
2	14-17	–	light blue	–	light blue
3	20-24	weak quenching	–	–	–
4	24-30	–	–	pale greyish purple	yellowish green
5	30-37	weak quenching	–	yellowish brown	bluish green
6	40-44	–	–	–	yellowish green
7	44-47	weak quenching	–	pale yellowish brown	–
8	47-51	weak quenching	red	green	red
9	51-53	quenching	red	dark green	red
10	53-57	–	intense blue	pale green	intense blue
11	58-61	weak quenching	–	pale greyish purple	–
12	62-66	–	blue	–	light blue
13	66-70	quenching	–	dark purple	light blue
14	70-73	–	–	brown	–
15	75-80	quenching	red	purple	pinkish red
16	80-86	quenching	red	–	pinkish red

Loss on drying Not more than 12.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 18.0 per cent w/w (Appendix 7.7).

Ethanol (50 per cent)-soluble extractive Not less than 23.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 28.0 per cent w/w (Appendix 7.12).

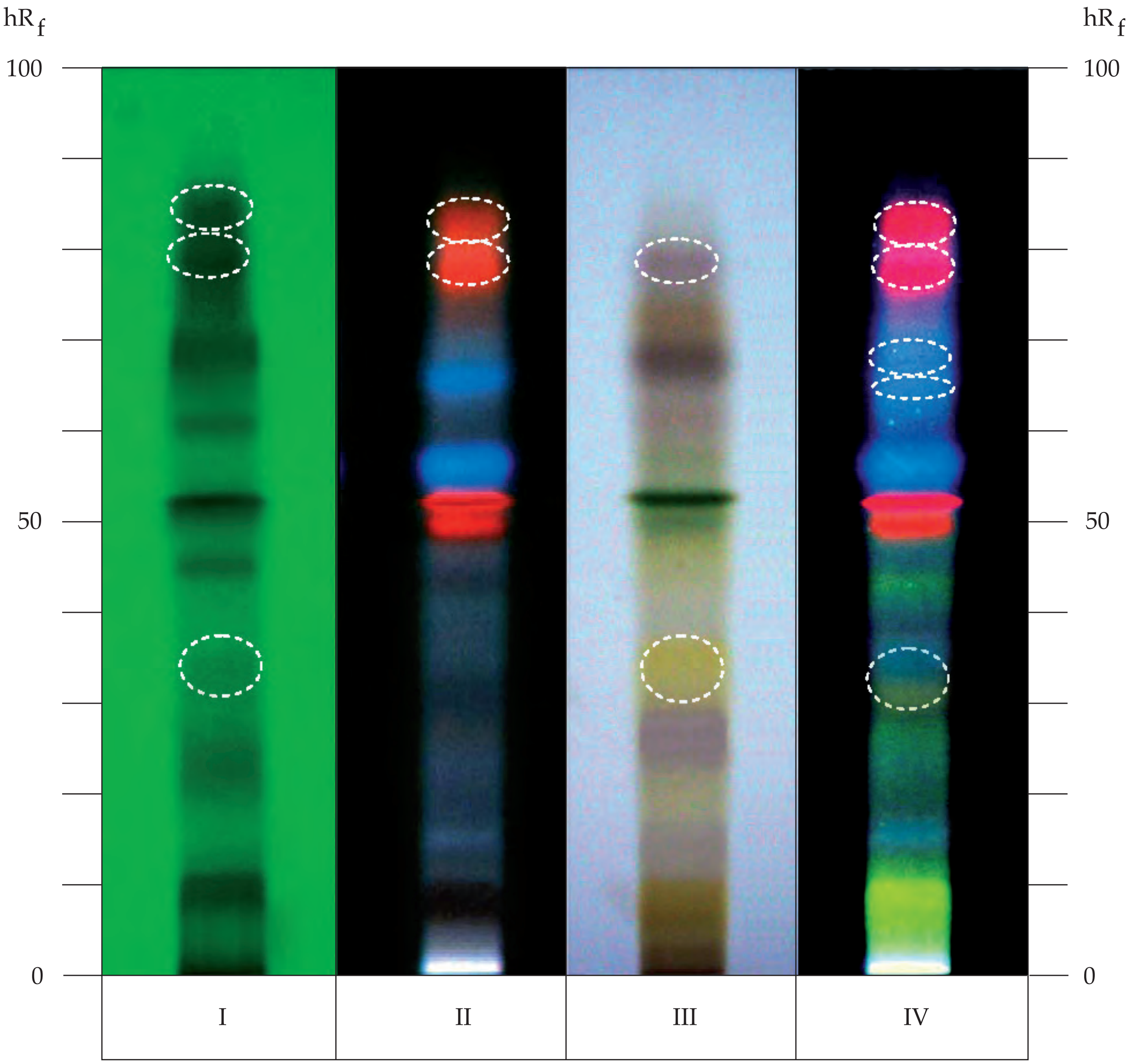


Fig. 3 Thin-layer Chromatogram of the Extract of the Leaves of *Clinacanthus nutans* (Burm. f.) Lindau

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *phosphomolybdic acid TS*
IV = detection under UV light (366 nm) after spraying with *NP/PEG TS*
◌ = bands developed in some samples

เพชรสังฆาต (PHET SANGKHAT)

เถาขั่นข้อ (THAO KHAN KHO), เถาสันชะควด (THAO SAN CHA KHUAT), เถาสามร้อยต่อ (THAO SAMROI TO)

Cissi Quadrangularis Caulis
Devil's Backbone

Synonyms Adamant Creeper Vine, Veldt Grape Vine

Category Alleviation of hemorrhoidal symptoms.

Devil's Backbone is the dried stem of *Cissus quadrangularis* L. (Family Vitaceae), Herbarium Specimen Number: DMSC 5158, Crude Drug Number: DMSc 0754.

Constituents Devil's Backbone contains triterpenoids (e.g., lupeol), flavonoids (e.g., quercetin, kaempferol), calcium oxalate, ascorbic acid, tannins, carotenoids, etc.

Description of the plant (Figs. 1a, 1b) Woody climber; stem green, succulent, quadrangular, angles winged, much constricted at nodes; tendril unequally bifurcate, 10 to 15 cm long, leaf-opposed; stipules 2, ovate, 3 to 4 mm long, 2 to 3 mm wide, caducous. Leaves simple, alternate, cordate to ovate, 4 to 7 cm long, 2.5 to 7 cm wide, apex obtuse to cuspidate, base auriculate to truncate, margin crenulate with minute teeth, coriaceous, with 3 main basal veins and 3 pairs of secondary veins, caducous; petiole up to 2 cm long, quadrangular. Inflorescence compound umbelliform cymes, leaf-opposed, up to 3 cm long, up to 5.5 cm wide; peduncle up to 2 cm long; pedicel up to 4 mm long. Flower small, reddish; calyx, cupuliform, inconspicuous lobes; petals 4, ovate, 2 to 3 mm long, 1 to 1.5 mm wide, apex hood-shaped; stamens 4, filament filiform, 0.7 to 1.3 mm long, anther elliptic-ovate; disc slightly 4-lobed; ovary superior, adnate to the disc, 2-loculed, ovules 2 per locule, style and stigma inconspicuous cylindrical. Fruit berry, mostly globose, 0.5 to 1 cm long, 0.5 to 1 cm wide, reddish purple to black. Seed(s) 1 or 2, orbicular or ellipsoid, about 5 mm long, about 4 mm wide, smooth.

Description Odour, mild; taste, bland.

Macroscopical (Fig. 1a) Part of quadrangular vine, varying in size and length, with or without node, pale brown colour; tendril occasionally found.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the fresh stem shows epidermal layer containing raised stomata, thick cuticle layer and underneath cork layer. Cortex layer composed of parenchyma some of which contain starch granules, rosette aggregate and raphide crystals, a group of thick-walled angular collenchyma in each ridge and air spaces. Fibrovascular bundle consisting of fibres, phloem, vascular cambium, and xylem.

Devil's Backbone in powder possesses the diagnostic microscopical characters of the unground drug.

Warning The fresh juice of the stem or the crushed-fresh stem irritates the skin and mucous membrane, and may cause itching.

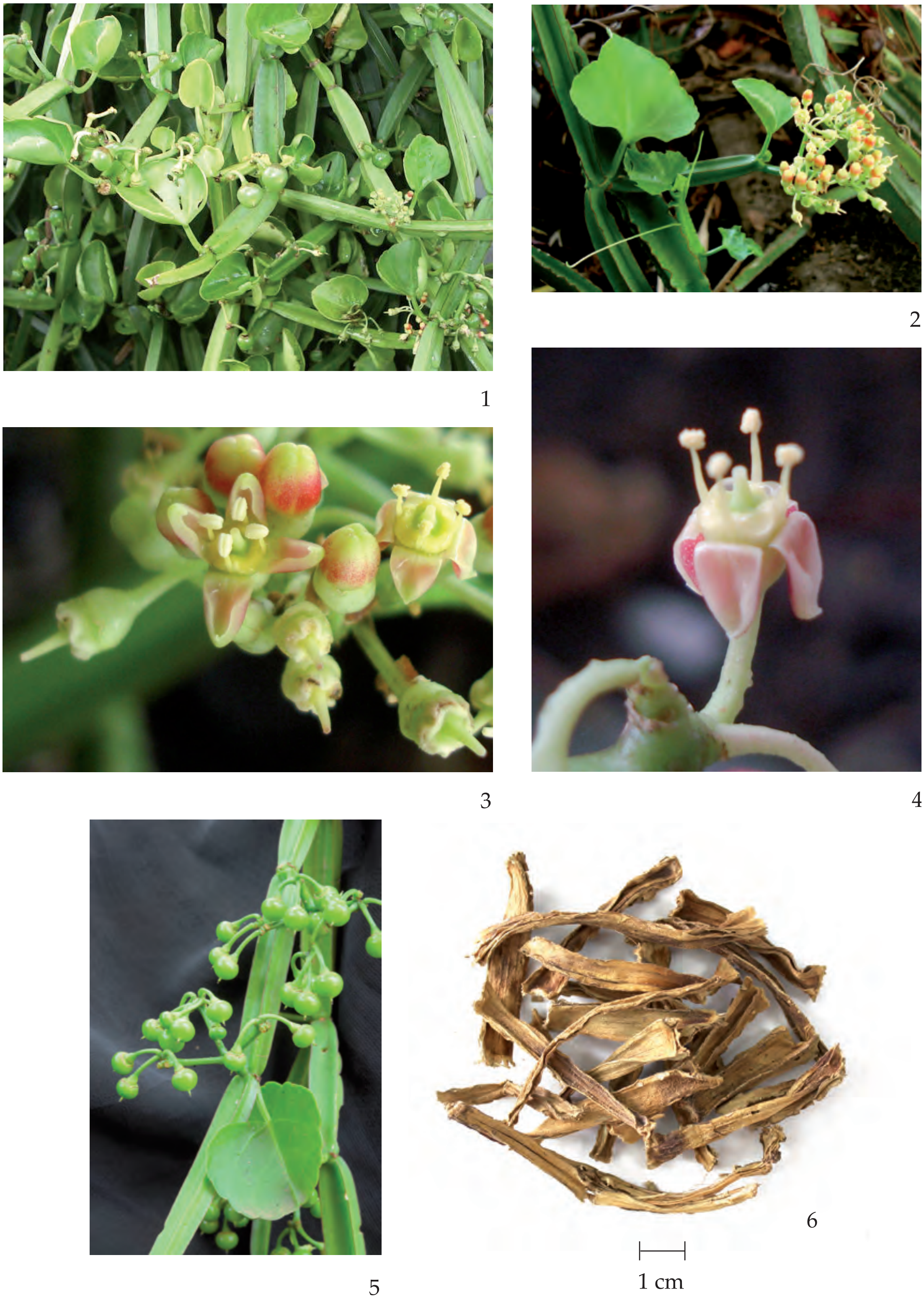


Fig. 1a *Cissus quadrangularis* L.
1. habit 2. flowering twig 3. top view of inflorescence 4. flower
5. infructescences 6. crude drug

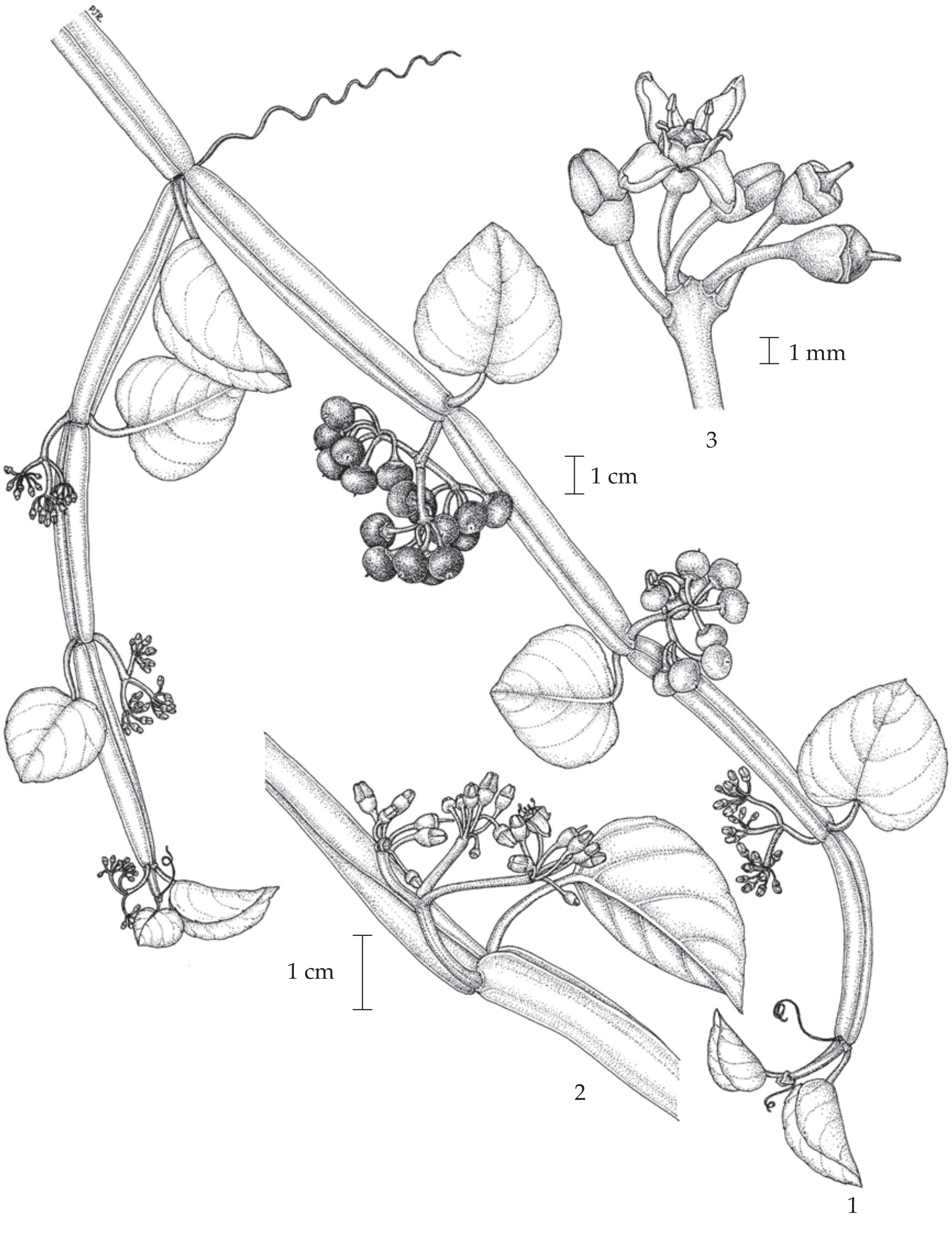


Fig. 1b *Cissus quadrangularis* L.
1. twig showing leaves, tendrils, inflorescences, and infructescences
2. inflorescence 3. flowers and young fruit

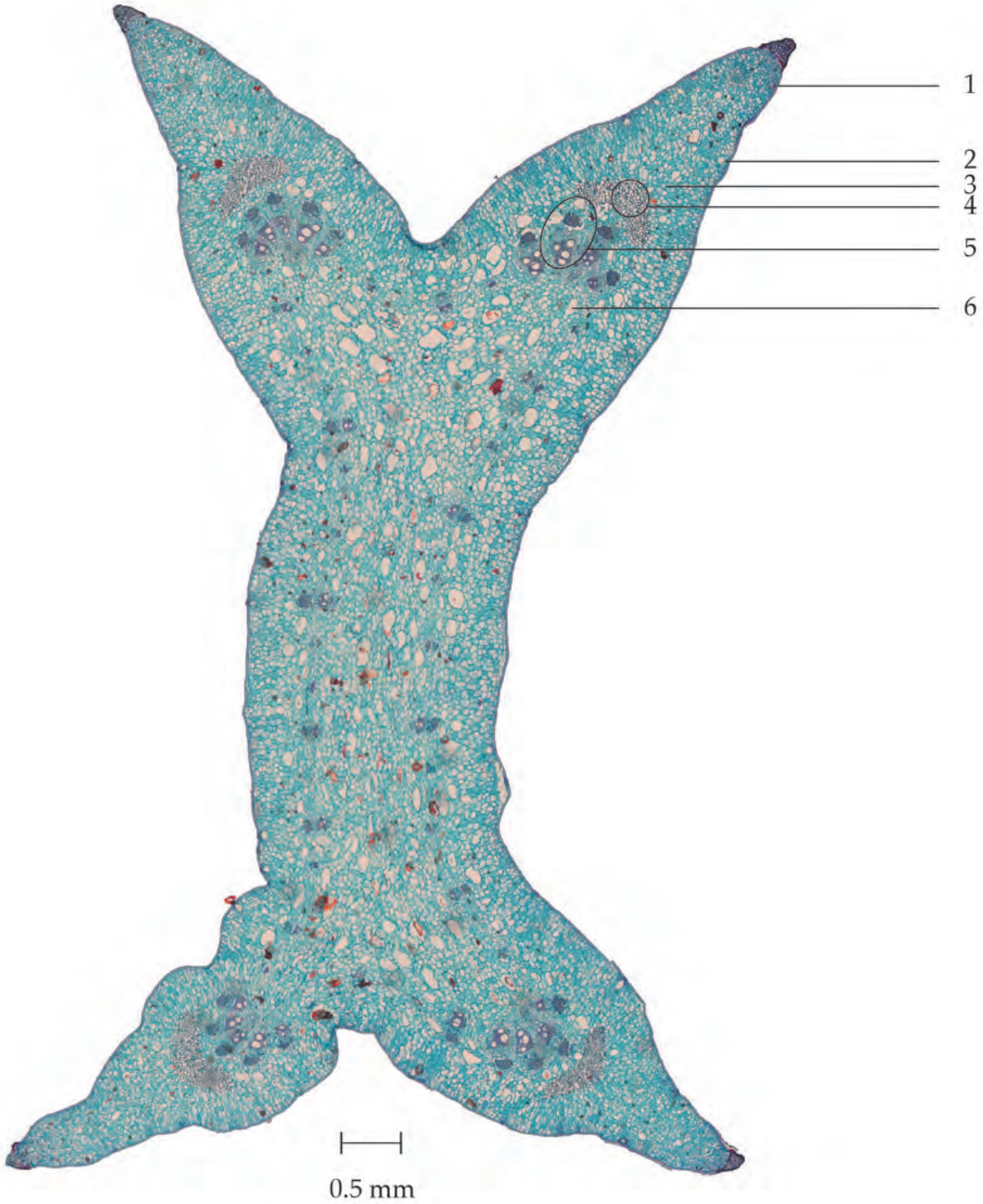


Fig. 2a Photomicrograph of Transverse Section of the Fresh Stem of *Cissus quadrangularis* L., Stained with Safranin-Fast Green

1. epidermis
2. raised stomata
3. parenchyma
4. group of angular collenchyma
5. vascular bundle
6. air space

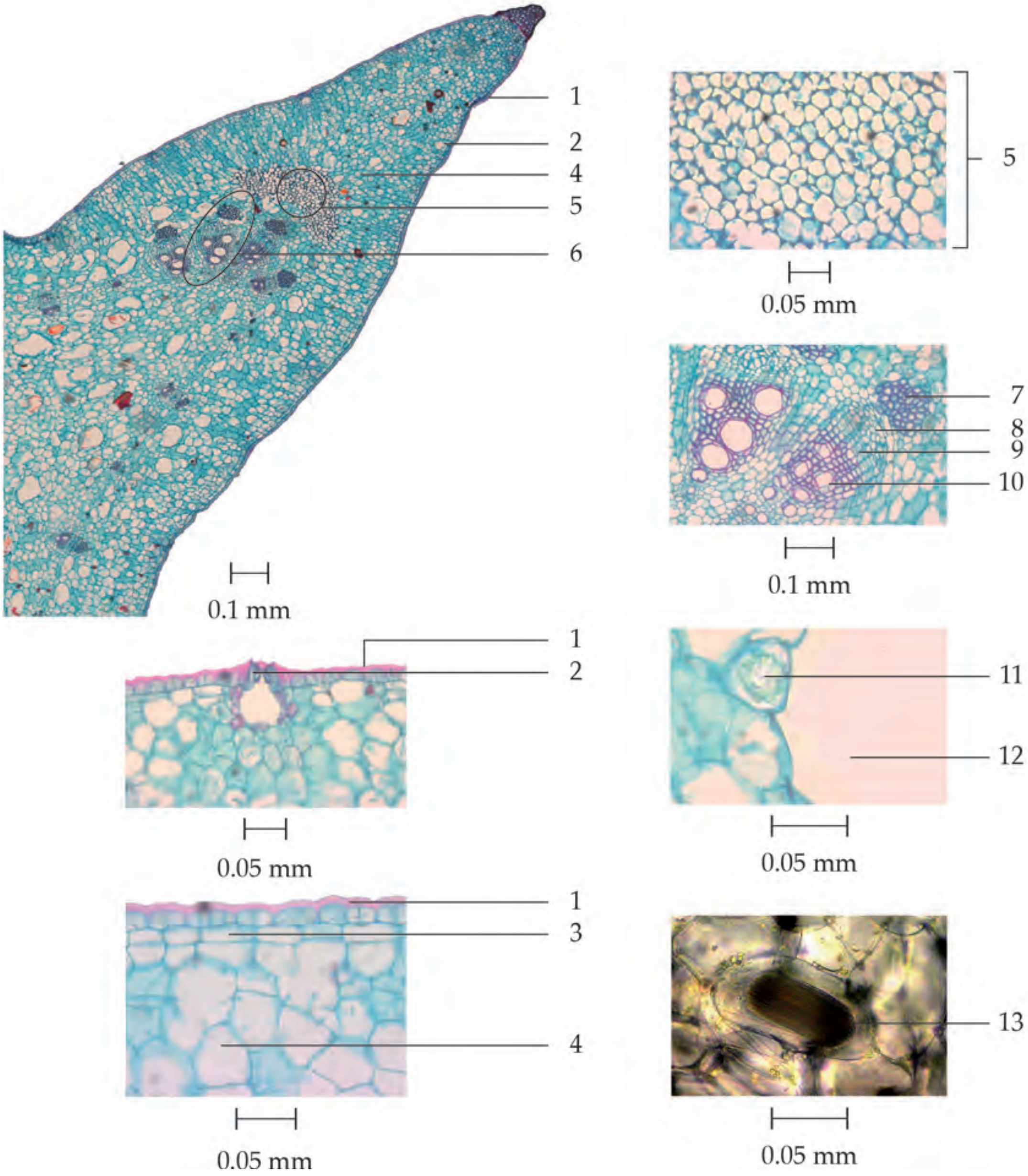


Fig. 2b Photomicrographs of Transverse Section of the Fresh Stem of *Cissus quadrangularis* L., Stained with Safranin-Fast Green

1. epidermis
2. raised stomata
3. cork cell
4. parenchyma cell
5. group of angular collenchyma
6. vascular bundle
7. fibre
8. phloem
9. vascular cambium
10. xylem
11. rosette aggregate crystal
12. air space
13. raphide

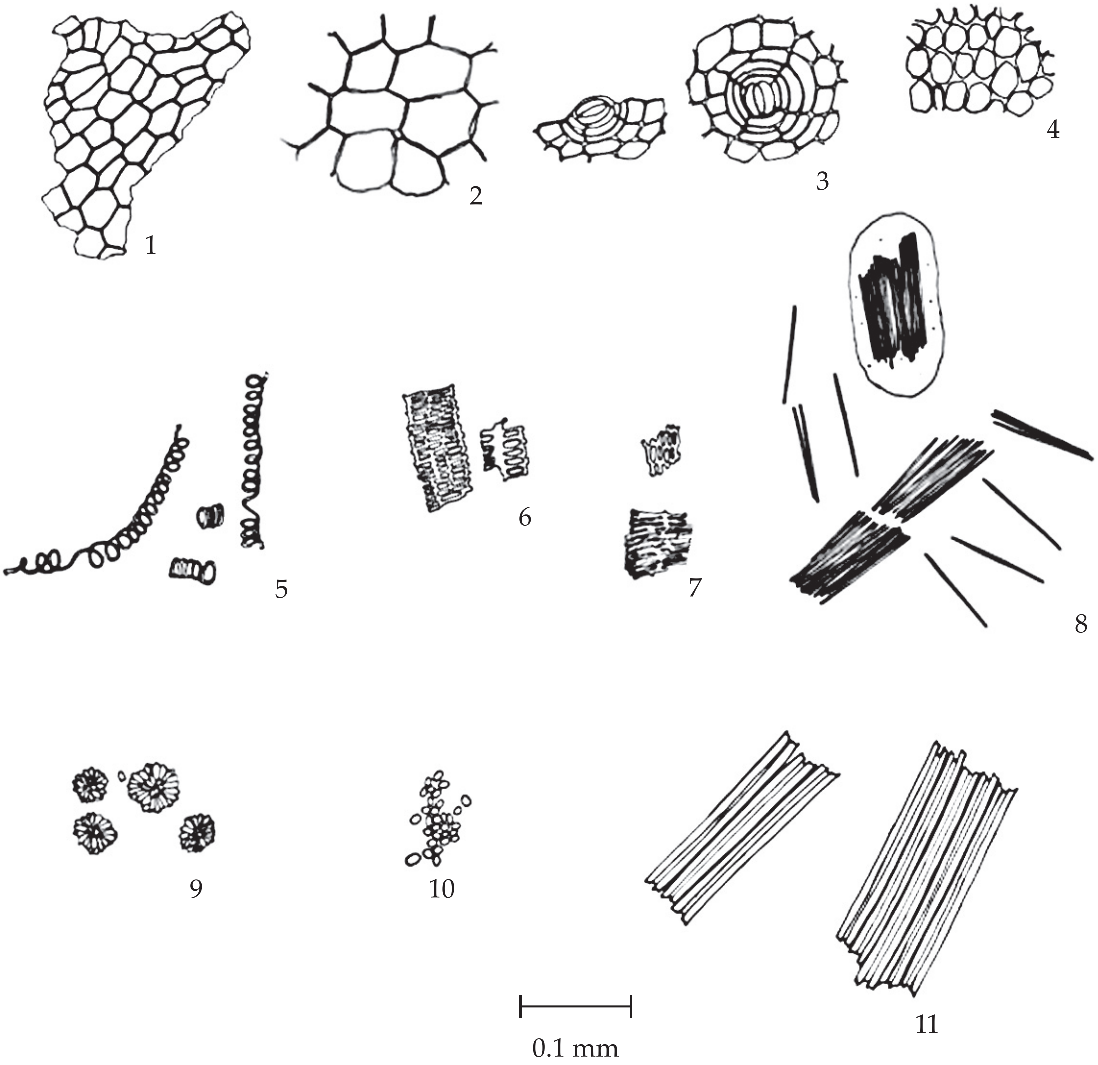


Fig. 2c Powdered Drug of the Stems of *Cissus quadrangularis* L.

1. epidermis in surface view
2. parenchyma cells
3. raised stomata in surface view and epidermis
4. fragment of collenchyma
5. spiral thickening wall of vessels
6. fragments of scalariform vessels
7. fragments of reticulate vessels
8. acicular crystals and raphides
9. rosette aggregate crystals
10. starch granules
11. bundles of fibres

Additional information

1. It is commonly used with other herbal drugs in the antihemorrhoidal remedies.
2. Traditionally, thin slices of one fresh internode wrapped with tamarind pulp or ripe banana are daily consumed for three consecutive days to treat hemorrhoid.

Packaging and storage Devil's Backbone shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. To 5 g of the sample, in powder, add 20 ml of *ethanol*, shake occasionally during 1 hour and filter (solution 1). To 2 ml of solution 1, add 4 or 5 pieces of *magnesium ribbon*, shake well and mix with a few drops of *hydrochloric acid*: a pink colour develops.

B. To 2 ml of solution 1, add a few drops of *iron(III) chloride TS*, and shake well: a blue colour develops.

C. Evaporate 2 ml of solution 1 to dryness, dissolve the residue in 2 ml of *acetic anhydride*, and then slowly add 1 ml of *sulfuric acid* to form two layers: a brown ring forms at the zone of contact and the upper layer is yellow which gradually changes to green.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 80 volumes of *hexane* and 10 volumes of *ethyl acetate* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate as bands of 5 mm, 5 µl each of the following two solutions. Prepare solution (A) by macerating 500 mg of the sample, in *No. 150 powder*, with 5 ml of *chloroform*, shaking frequently during 12 hours and filtering. Evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform*. For solution (B), dissolve 1 mg of *lupeol* in 1 ml of *chloroform*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. Subsequently examine the plate under ultraviolet light (366 nm); two reddish black bands are observed. Spray the plate with *anisaldehyde TS* and heat at 110° until colours are fully developed; the chromatogram obtained from solution (A) shows a pink band (hR_f value 34 to 36), corresponding to the lupeol band from solution (B). Several other bands of different colours are also observed (Table 1); see also Fig. 3.

Table 1 hR$_f$ Values of Components in Chloroform Extract of the Stems of *Cissus quadrangularis* L.

Band	hR$_f$ Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	4-6	quenching	reddish black	yellowish green
2	11-13	quenching	reddish black	blue-green
3	21-22	–	–	pink
4	25-26	weak quenching	–	–
5*	34-36	–	–	pink
6	39-40	–	–	pink
7	45-47	–	–	pale pink
8	85-86	–	–	pale pink

*lupeol

Loss on drying Not more than 9.0 per cent w/w after drying at 105° for 5 hours (Appendix 4.15).

Foreign matter Not more than 5.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Total ash Not more than 20.0 per cent w/w (Appendix 7.7).

Ethanol (80 per cent)-soluble extractive Not less than 2.5 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12).

Dose 3 to 6 g a day.

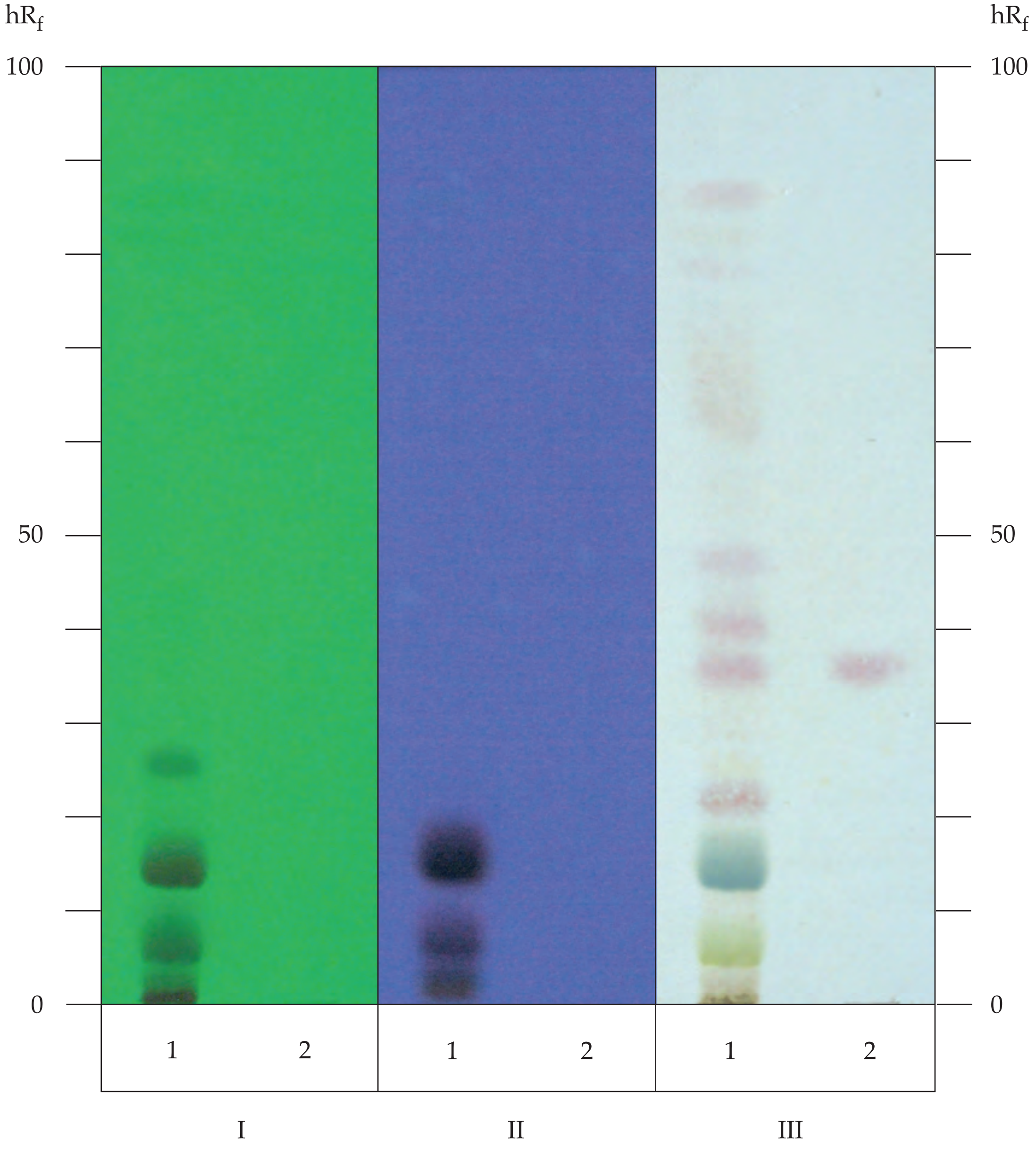


Fig. 3 Thin-layer Chromatogram of Chloroform Extract of the Stems of *Cissus quadrangularis* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *anisaldehyde TS*

พิกุล (PHIKUN)

ดอกกุน (DOK KUN), ดอกแก้ว (DOK KAEO), ดอกซางดง (DOK SANGDONG)
Mimusopsis Elengi Flos
Star Flower

Synonyms Bakul Flower, Bullet Wood Flower

Category Tonic, antipyretic.

Star Flower is the dried flower, predominantly corolla and stamen, of *Mimusops elengi* L. (Family Sapotaceae), Herbarium Specimen Number: DMSC 5161, Crude Drug Number: DMSc 0896.

Constituents Star Flower contains volatile oil, of which 2-phenylethanol is its major constituent. It also contains β-sitosterol, flavonoids, etc.

Description of the plant (Figs. 1a, 1b) Tree, up to 20 m tall, crown dense, round and spreading; bark dark grey to dark brown, cracked or longitudinally fissured; young twig pubescent, brownish. Leaves simple, spirally arranged, ovate or elliptic, 4 to 15 cm long, 2 to 7.5 cm wide, apex shortly acuminate, base cuneate or round, margin undulate, upper surface dark green and shining, lower surface paler; petiole 1.5 to 3.5 cm long, pubescent. Flower white or greenish white, fragrant, 1 to 6 in axil; peduncle 1 to 1.5 cm long, pubescent, brownish; sepals 8, arranged in 2 whorls of 4, outer one greenish brown, ovate-lanceolate, 7 to 8 mm long, about 4 mm wide, pubescent, inner one lighter, lanceolate, 7 to 8 mm long, 2.5 to 3 mm wide; corolla tube about 2 mm long, corolla lobes 8, lanceolate, 7 to 8 mm long, about 2 mm wide, each lobe with 2 dorsal petaloid appendages of the same shape and size as the corolla lobes; stamens 8, inserted at the top of corolla tube; staminodes 8, pubescent, alternating with the stamens; ovary superior, ovoid, 2 to 4.5 mm long, 2.5 to 3 mm wide, pubescent, style 7 to 8 mm long. Fruit berry, ovoid to ellipsoid, 1.5 to 3 cm long, 1 to 3 cm wide, glabrous, green when young and orange to red when ripe. Seed(s) mostly 1 or 2, ellipsoid to oblong, about 1.7 cm long, about 1 cm wide, dark brown.

Description Odour, strong and fragrant.

Macroscopical (Fig. 1a) Predominantly corolla and stamens, pale brown to brown; sepals arranged in 2 whorls, ovate-lanceolate, with brown hairs; corolla lobes and dorsal petaloid appendages lanceolate; stamens enclosed within corolla lobes.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the flower through the calyx, corolla and petaloid appendage shows upper epidermis, a layer of rectangular cells in the sepal and a layer of papillae with striation in the petal and the petaloid appendage; trichomes, T- and shaggy-shaped unicellular cells, containing oleoresin, numerous in calyx and a few in corolla; mesophyll composed of round parenchyma cells some of which contain oleoresin; vascular bundle, collateral, with phloem and xylem; vessel, spiral. Lower epidermis, similar to those of upper epidermis.

Longitudinal section of the central part of a young flower shows sepals, petals, stamens, ovary, style, and stigma.

Star Flower in powder possesses the diagnostic microscopical characters of the unground drug.

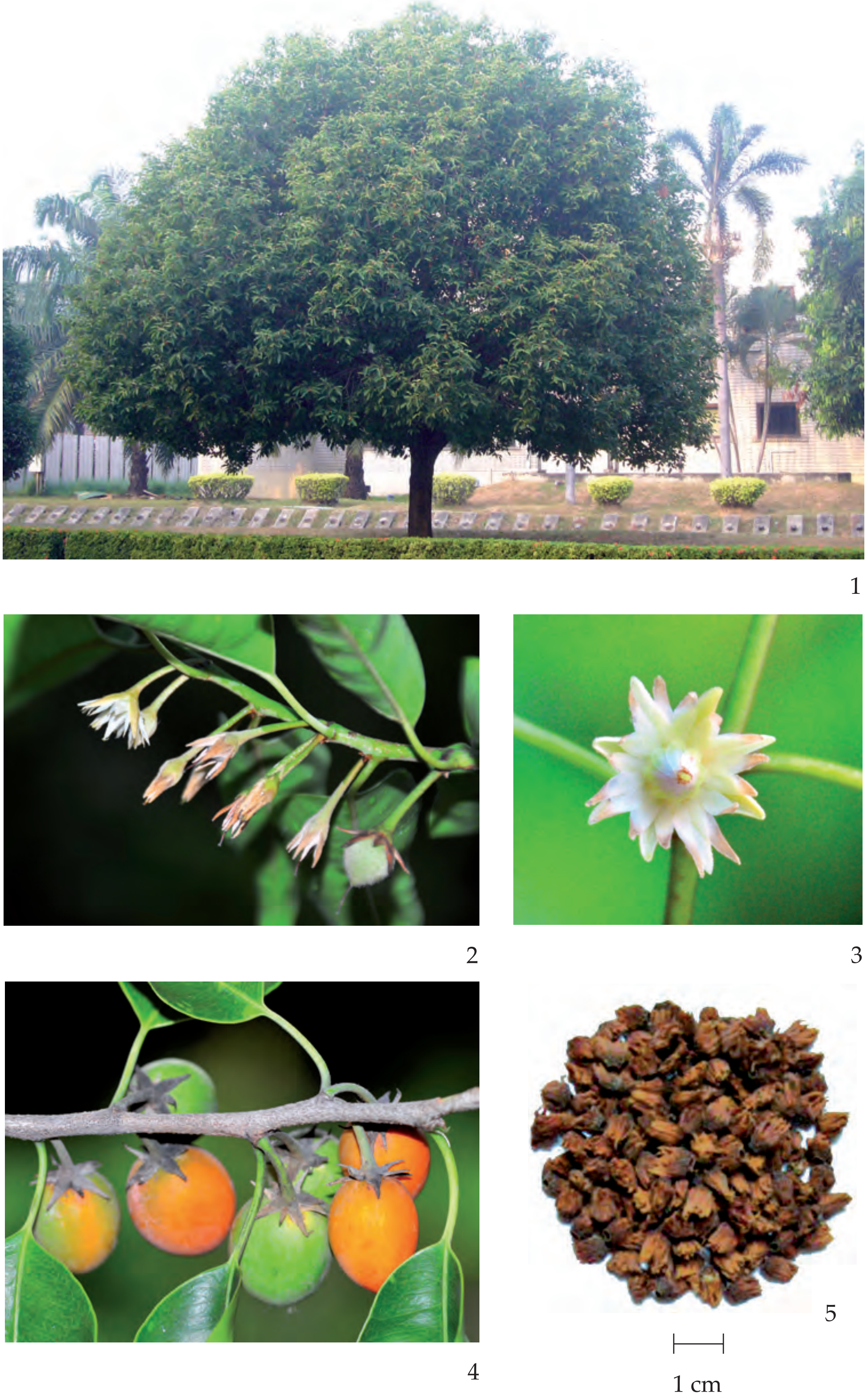


Fig. 1a *Mimusops elengi* L.
1. habit 2. flowering twig 3. flower 4. fruiting twig 5. crude drug

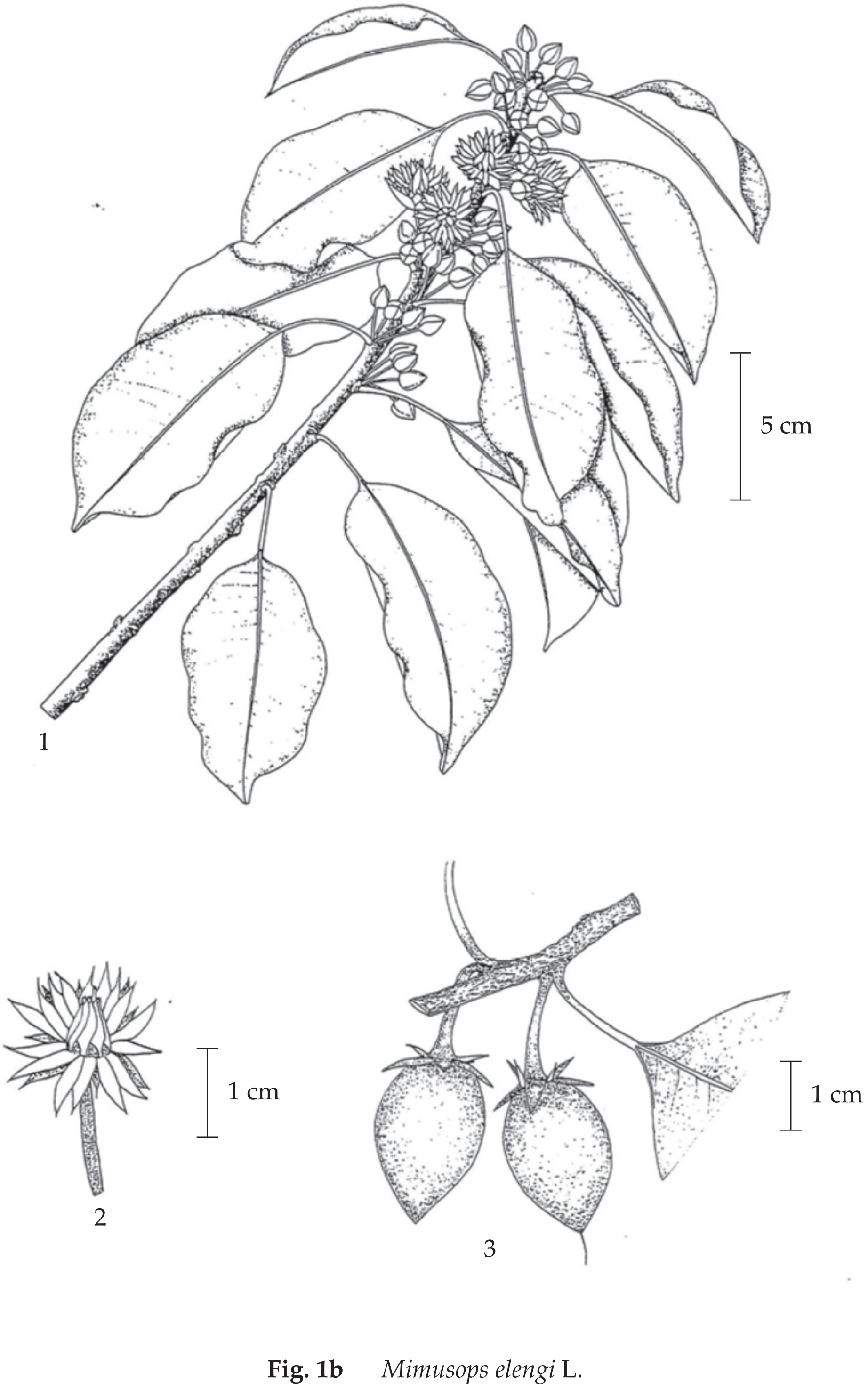


Fig. 1b *Mimusops elengi* L.
1. flowering twig 2. flower 3. fruits

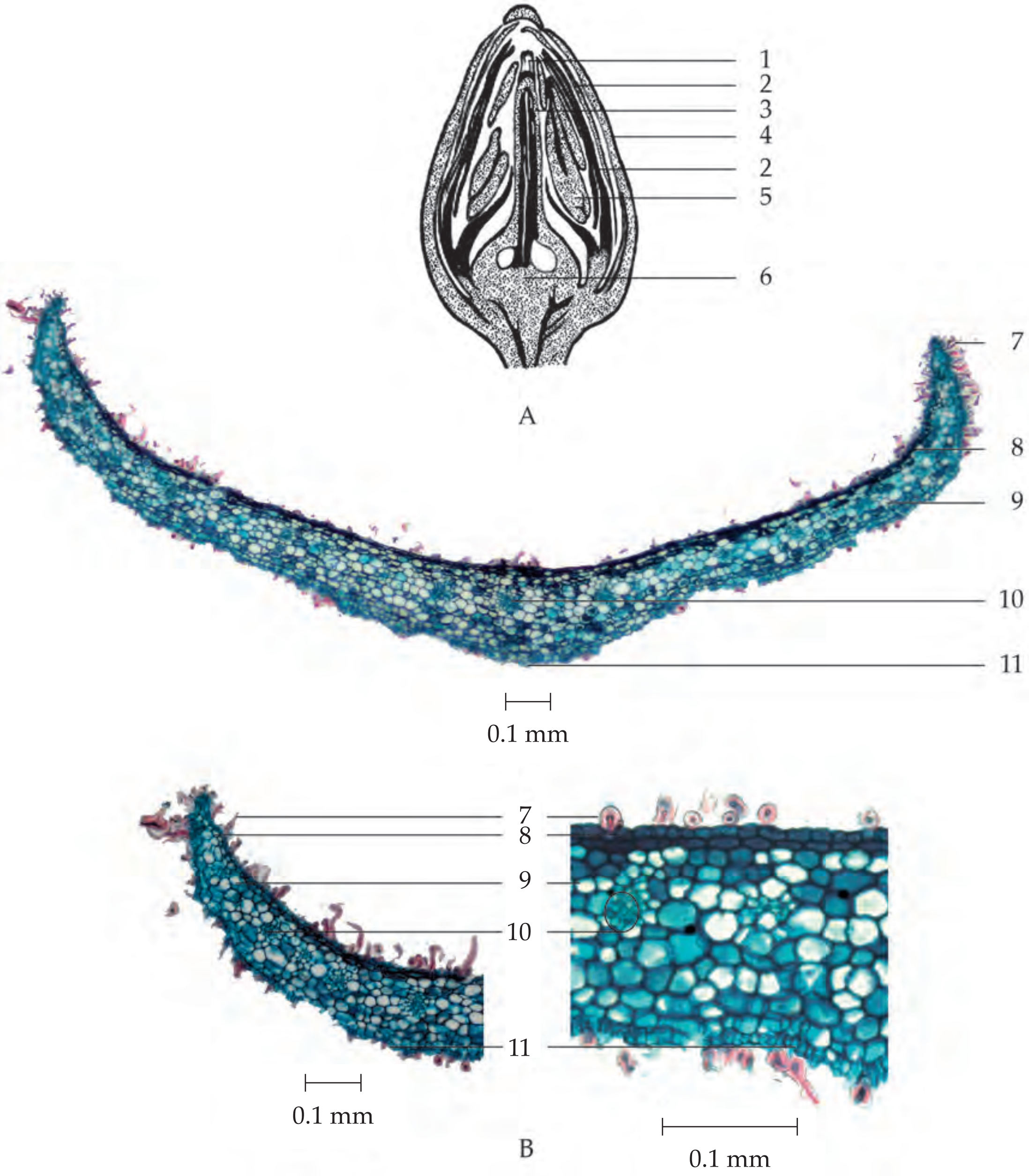


Fig. 2a Photomicrographs of Section of the Flower of *Mimusops elengi* L.
A. Longitudinal Section of a Young Flower
B. Transverse Section of a Sepal, Stained with Safranin-Fast Green
1. stigma
2. petal
3. style
4. sepal
5. stamen
6. ovary
7. trichome
8. upper epidermis
9. parenchyma cell
10. vascular bundle
11. lower epidermis

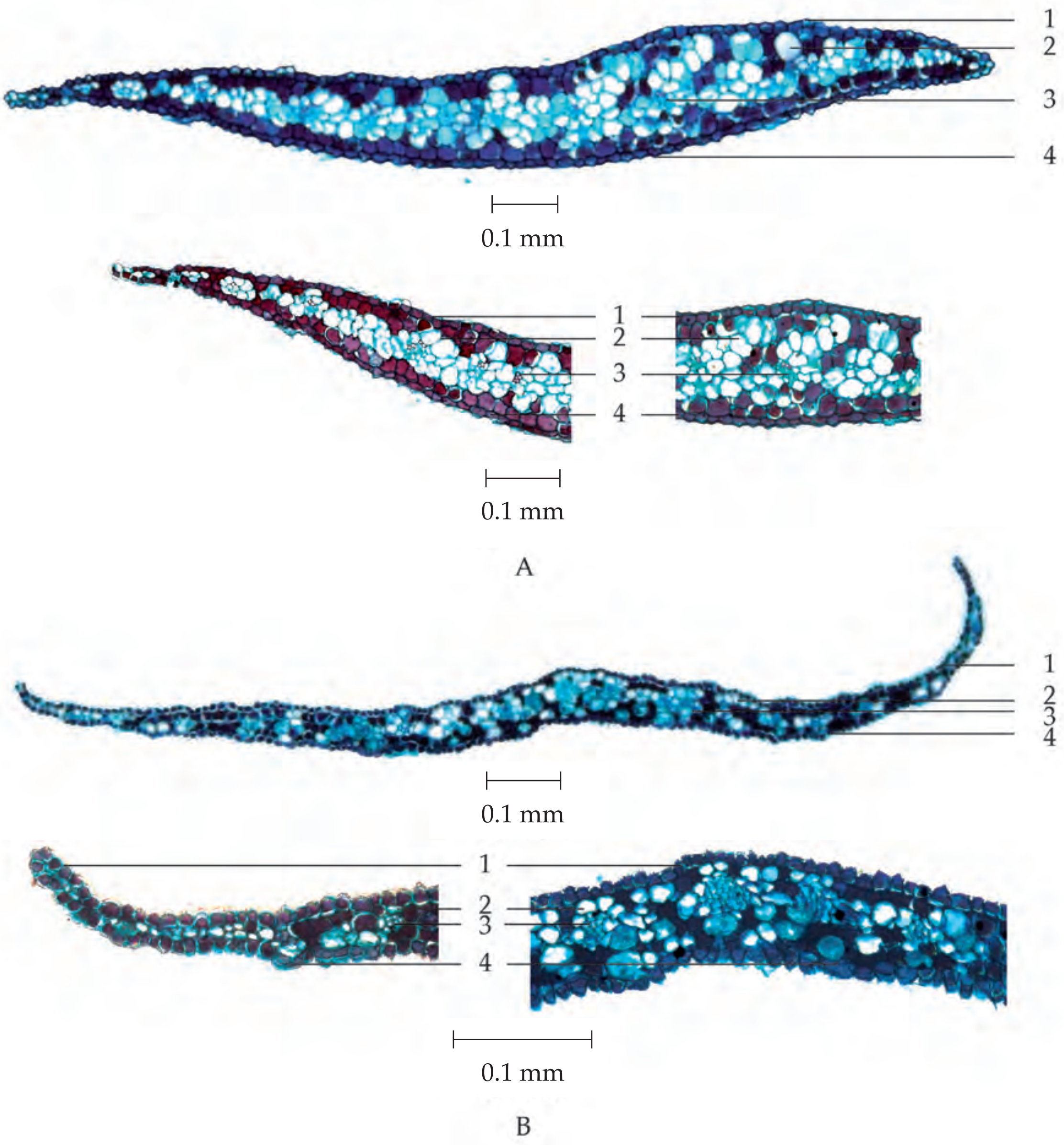


Fig. 2b Photomicrographs of Section of the Flower of *Mimusops elengi* L., Stained with Safranin-Fast Green

A. Transverse Section of a Petal

B. Transverse Section of a Petaloid Appendage

1. upper epidermis
2. parenchyma cell
3. vascular buddle
4. lower epidermis

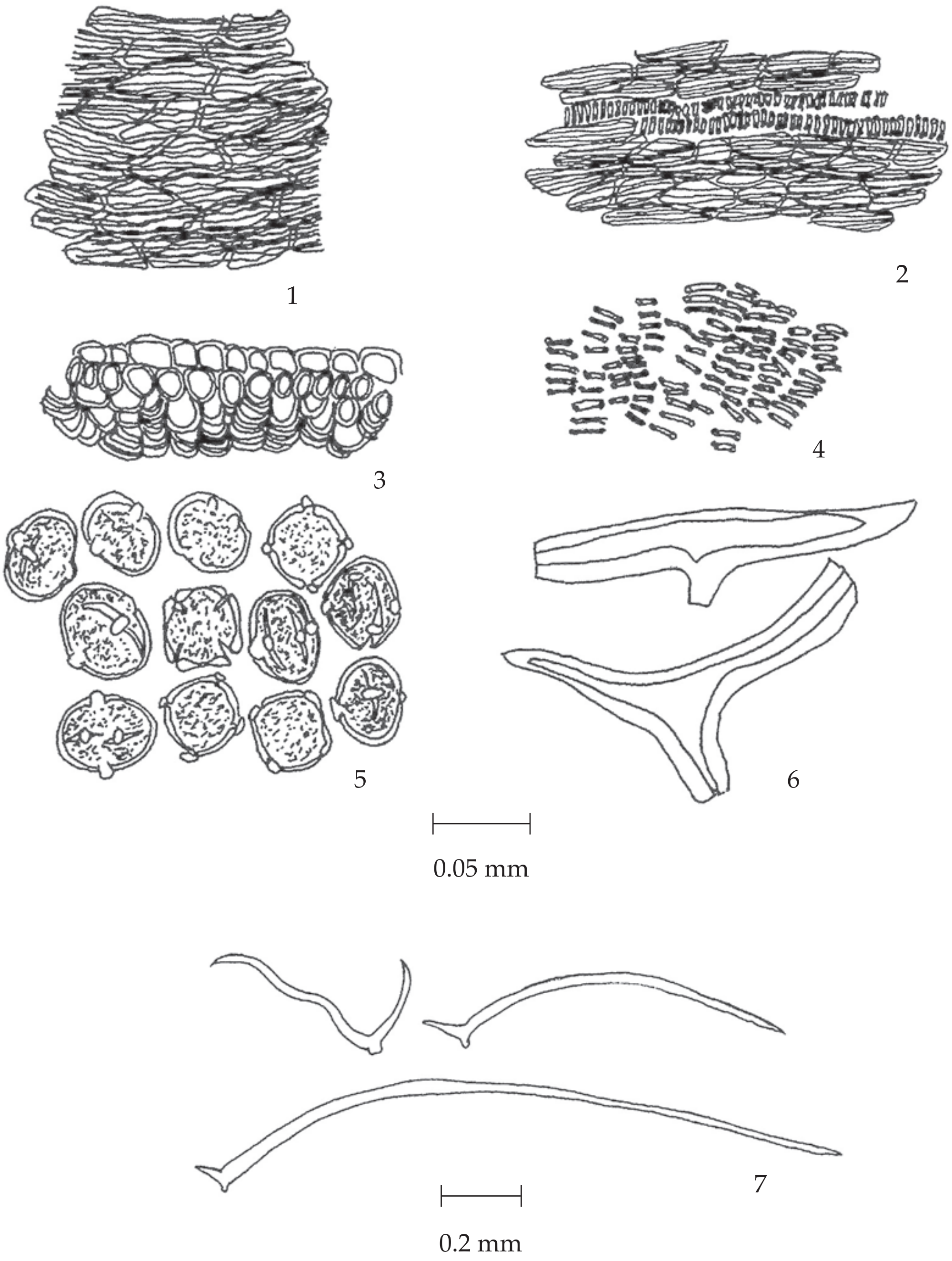


Fig. 2c Powdered Drug of the Flowers of *Mimusops elengi* L.

1. epidermal cells of corolla with striation in surface view
2. epidermal cells of corolla with striation, underlying with spiral vessels
3. exothecium associated with endothecium in sectional view
4. fragments of endothecium in surface view
5. pollen grains
6. fragments of unicellular trichomes
7. unicellular trichomes

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Star Flower shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Extract 2.5 g of the sample, in powder, with 10 ml of *methanol*, shake occasionally for about 20 minutes and filter (solution 1). To 1 ml of solution 1, add 1 or 2 pieces of *magnesium ribbon*, shake well and mix with 2 or 3 drops of *hydrochloric acid*: a pink colour is produced.

B. To 1 ml of solution 1, add 10 mg of *zinc powder* and 2 or 3 drops of *hydrochloric acid*: an orange colour develops.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 60 volumes of *ethyl acetate* and 40 volumes of *toluene* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply to the plate as a band of 5 mm, 30 µl of the test solution prepared by shaking vigorously 100 mg of the sample, in *No. 425 powder*, with 1 ml of *methanol* for 15 minutes, allowing to stand overnight, centrifuging for 5 minutes, and using the clear supernatant. After removal of the plate, allow it to dry in air and examine the plate under ultraviolet light (366 nm) through the cut-off filter; two blue fluorescent bands are observed. Spray the plate with a 2 per cent w/v solution of *vanillin* in *methanol*. Subsequently spray the plate with a 10 per cent v/v solution of *sulfuric acid* in *methanol* and heat at 120° for 10 minutes; several bands of different colours appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Flowers of *Mimusops elengi* L.

Band	hR_f Value	Detection	
		UV 366	*Vanillin* in *Methanol*/ *Sulfuric Acid* in *Methanol*
1	4-6	–	pale violet
2	14-16	–	pink
3	19-20	–	pale yellow
4	25-27	–	pale violet
5	53-55	–	pale brown
6	58-60	–	violet
7	68-70	blue	violet
8	75-77	blue	violet

Loss on drying Not more than 16.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 5.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 3.0 per cent w/w (Appendix 7.6).

Total ash Not more than 7.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 8.0 per cent w/w (Appendix 7.12).

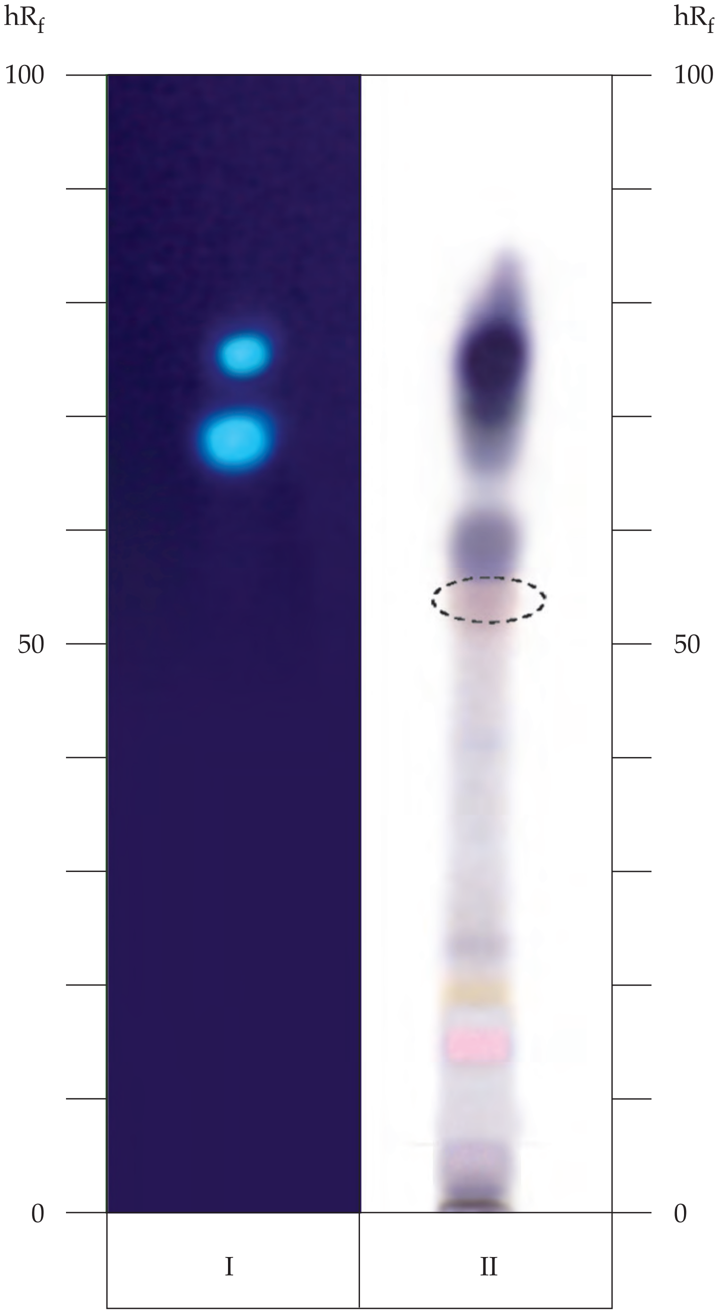


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Flowers of *Mimusops elengi* L.

I = detection under UV light (366 nm)
II = detection with *vanillin* in *methanol*/*sulfuric acid* in *methanol*
◌ = band developed in some samples

ไพล (PHLAI)

Zingiberis Montani Rhizoma
Zingiber Montanum Rhizome

Category Anti-inflammatory, counter-irritant, mosquito repellent.

Zingiber Montanum Rhizome is the dried rhizome of *Zingiber montanum* (J. König) Link ex A. Dietr. (*Z. cassumunar* Roxb.) (Family Zingiberaceae), Herbarium Specimen Number: DMSC 900.
(**Note** It should be harvested after three-year cultivation.)

Constituents Zingiber Montanum Rhizome contains cyclohexene derivatives, viz., *cis*-3-(3,4-dimethoxyphenyl)-4- [(*E*)-3,4-dimethoxystyryl]cyclohex -1-ene, *cis*-3-(2,4,5-trimethoxyphenyl)-4-[(*E*)-2,4,5-trimethoxystyryl]cyclohex-1-ene and *cis*-3-(3,4-dimethoxyphenyl) -4-[(*E*)-2,4,5-trimethoxystyryl]cyclohex-1-ene; naphthoquinone derivatives, viz., 8-(3,4-dimethoxyphenyl)-2-methoxynaphtho-1,4-quinone; phenylbutanoids, viz., (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-yl acetate and [(*E*)-4-(3,4-dimethoxyphenyl)]but-3-en-1-ol; vanillin; vanillic acid; veratric acid; curcumin; β-sitosterol; volatile oil consisting of α-pinene, β-pinene, sabinene, myrcene, α-terpinene, limonene, γ-terpinene, *p*-cymene, terpinolene, and terpinen-4-ol.

Description of the plant (Fig. 1) Herb, rhizome fleshy thick, greenish yellow to yellow inside, horizontal with many sessile tubers. Leafy stems 1 to 1.5 m tall. Leaves distichous, oblong-lanceolate, 20 to 30 cm long and 2 to 8 cm wide, pubescent below; ligule very short, bilobed, pubescent; sheath glabrous or hairy. Inflorescences scapose; peduncle 8 to 30 cm long, clothed with pubescent sheaths. Spike ovoid-ellipsoid; bracts greenish red, narrowly obovate or rhomboid, 2.5 to 3.5 cm long; bracteole shorter than bract, ovate, 3-dentate. Calyx truncate, glabrous. Corolla pale yellow, tube about 2.5 cm long, dorsal lobe cymbiform, lateral lobe linear-lanceolate. Labellum pale yellow, suborbicular, apex emarginate, lateral lobe ovate-oblong, appendage slightly longer than anther; stamen pale yellow. Ovary 3-celled, inferior. Fruit, small globose capsule.

Description Odour, pleasant aromatic; taste, pungent.

Macroscopical Transverse slices, 2 to 5 mm thick, greenish yellow with light brown skin; fracture fibrous, mealy; surface shows a narrow cortex separated by endodermis from a much wider stele.

Microscopical (Figs. 2a, 2b) Transverse section of rhizome: epidermis, a layer of rectangular cells. Outer cortex, parenchymatous cells. Cork, several layers of thin-walled, rectangular cells. Inner cortex, thin-walled parenchymatous cells containing numerous starch granules and oleoresin; starch granules, mostly simple, 30 to 75 µm long and 20 to 40 µm wide, accompanied with 2- to 6-compound grains, oblong to oval in outline with a small hilum at narrow end, very faint eccentric striations. Endodermis, a layer of thin-walled cells. Stele, thin-walled parenchymatous cells containing starch granules and oleoresin. Fibrovascular bundle, not yielding the *reaction* characteristic of lignin, scattered in inner cortex and stele; vessels, mostly scalariform and reticulate, spiral and annular.

Zingiber Montanum Rhizome in powder possesses the diagnostic microscopical characters of the unground drug.

Fig. 1 *Zingiber montanum* (J. König) Link ex A. Dietr.
1. habit 2. rhizome 3. crude drug

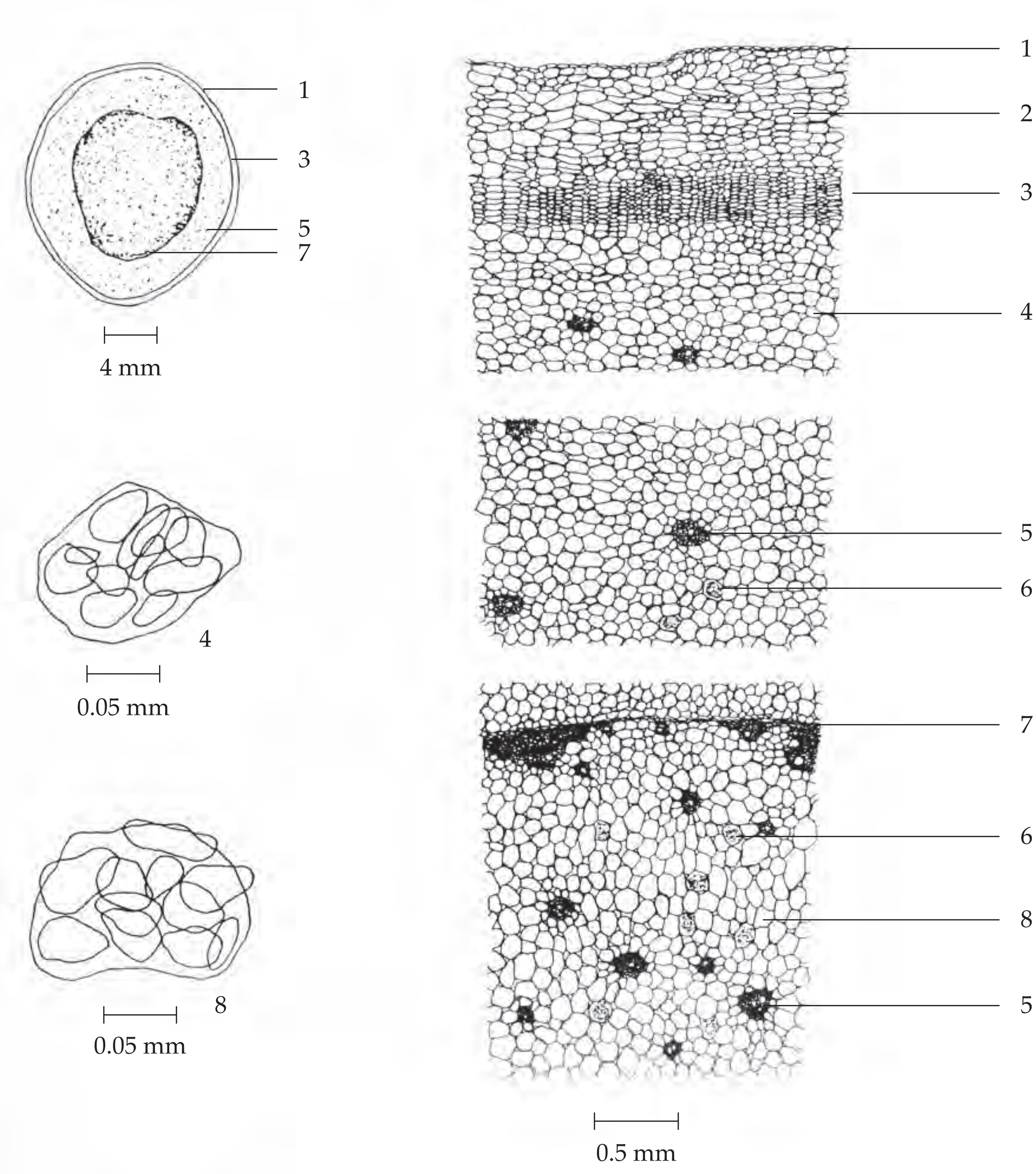


Fig. 2a Transverse Section of the Rhizome of *Zingiber montanum* (J. König) Link ex A. Dietr.

1. epidermis
2. outer cortical parenchyma
3. cork
4. inner cortical parenchyma containing starch granules
5. vascular bundles
6. oleoresins
7. endodermis
8. stele parenchyma containing starch granules

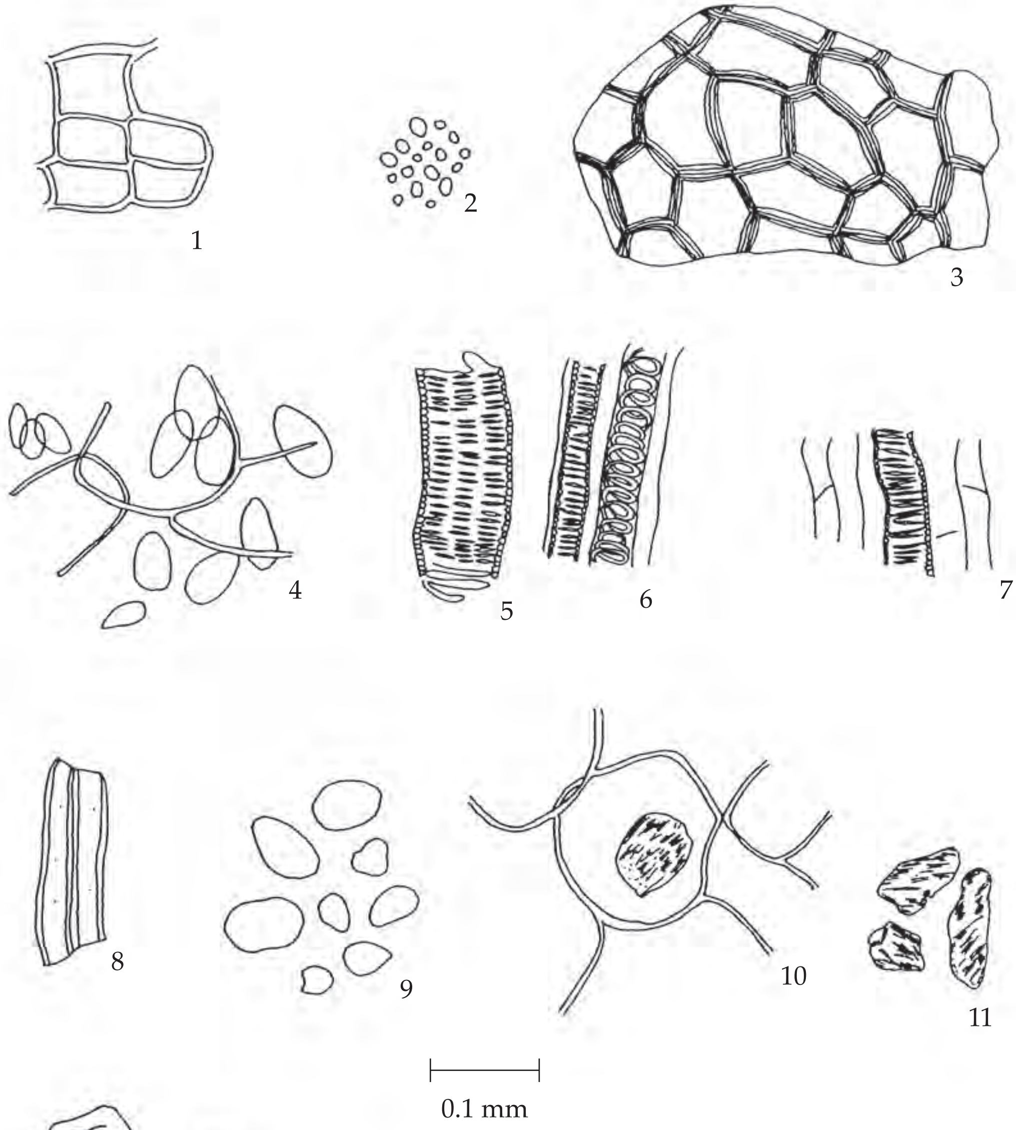


Fig. 2b Powdered Drug of the Rhizomes of *Zingiber montanum* (J. König) Link ex A. Dietr.

1. cork in sectional view
2. oil droplets
3. cork in surface view
4. parenchyma with starch granules
5. scalariform vessel
6. scalariform and spiral vessels
7. scalariform vessel with parenchyma
8. fibres
9. starch granules
10. parenchyma with oleoresin mass
11. oleoresin masses

Packaging and storage Zingiber Montanum Rhizome shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. To 500 mg of the sample, in powder, add 5 ml of *chloroform*, set aside for 5 minutes and filter (solution 1). Examine 1 ml of solution 1 under ultraviolet light (366 nm): a greenish yellow fluorescence is observed.

B. To 1 ml of solution 1, add 0.2 ml of *acetic anhydride-sulfuric acid TS*: a reddish brown colour is produced.

C. To 1 ml of solution 1, add 1 ml of *boric acid-methanol TS*: an orange colour is produced.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 70 volumes of *hexane* and 30 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, 2 µl of solution (A) and 3 µl of solution (B). Prepare solution (A) by extracting 2.5 g of the sample, in powder, with three 10-ml portions of *hexane*. Evaporate the hexane extract to dryness and dissolve the residue in 5 ml of *chloroform*. For solution (B) dissolve 1 mg of *curcumin* in 1 ml of *chloroform*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The spots obtained with solution (A) correspond in hR_f values to the spot numbers 2, 3, 5, 6, 7, and 8. Several spots of higher hR_f values are also observed (Table 1); see also Fig. 3. Spray the plate with *anisaldehyde TS* and heat at 110° for 10 to 15 minutes; the spots due to those of numbers 2, 3, 5, 6, 7, and 8 in Table 1 are brown, carmine-red, olive-green, wine-red, violet, and dark pink, respectively. Red spot due to curcumin (hR_f values 10 to 13) and other spots of different colours are observed (Table 1); see also Fig. 3.

Repeat the same procedure on another plate but spray with *vanillin-sulfuric acid TS* and heat at 110° for 10 to 15 minutes; the spots due to those of numbers 2, 3, 5, 6, 7, and 8 are olive-green, dark pink, purple, blue-violet, red, and light orange, respectively. Red-brown spot due to curcumin and other spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Hexane Extract of the Rhizomes of *Zingiber montanum* (J. König) Link ex A. Dietr.

Spot	hR_f Value	Detection		
		UV 254	*Anisaldehyde TS*	*Vanillin-Sulfuric Acid TS*
1*	10-13	–	red	red-brown
2	14-16	quenching	brown	olive-green
3	16-19	quenching	carmine-red	dark pink
4	20-22	–	violet	violet
5	24-28	quenching	olive-green	purple
6	31-35	quenching	wine-red	blue-violet
7	42-45	quenching	violet	red
8	49-52	quenching	dark pink	pale orange
9	58-61	quenching	blue-violet	violet
10	62-64	–	blue-violet	pink
11	68-72	quenching	dark blue-violet	blue-purple
12	73-76	quenching	pale orange	dark blue
13	84-88	–	brown-red	pale brown

*1 = curcumin
2 = 8-(3,4-dimethoxyphenyl)-2-methoxynaphtho-1,4-quinone
3 = [(*E*)-4-(3,4-dimethoxyphenyl)]but-3-en-1-ol
5 = *cis*-3-(2,4,5-trimethoxypheny1)-4-[(*E*)-2,4,5-trimethoxystyryl]cyclohex-1-ene
6 = *cis*-3-(3,4-dimethoxypheny1)-4-[(*E*)-2,4,5-trimethoxystyryl]cyclohex-1-ene
7 = *cis*-3-(3,4-dimethoxypheny1)-4-[(*E*)-3,4-dimethoxystyryl]cyclohex-1-ene
8 = (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-yl acetate

Water Not more than 13.0 per cent w/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 3.0 per cent w/w (Appendix 7.6).

Total ash Not more than 9.0 per cent w/w (Appendix 7.7).

Hexane-soluble extractive Not less than 3.0 per cent w/w (Appendix 7.12H).

Chloroform-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12H).

Volatile oil Not less than 2.0 per cent v/w (Appendix 7.3H). Use 10 g, in *coarse powder*, freshly prepared and accurately weighed. Use 100 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

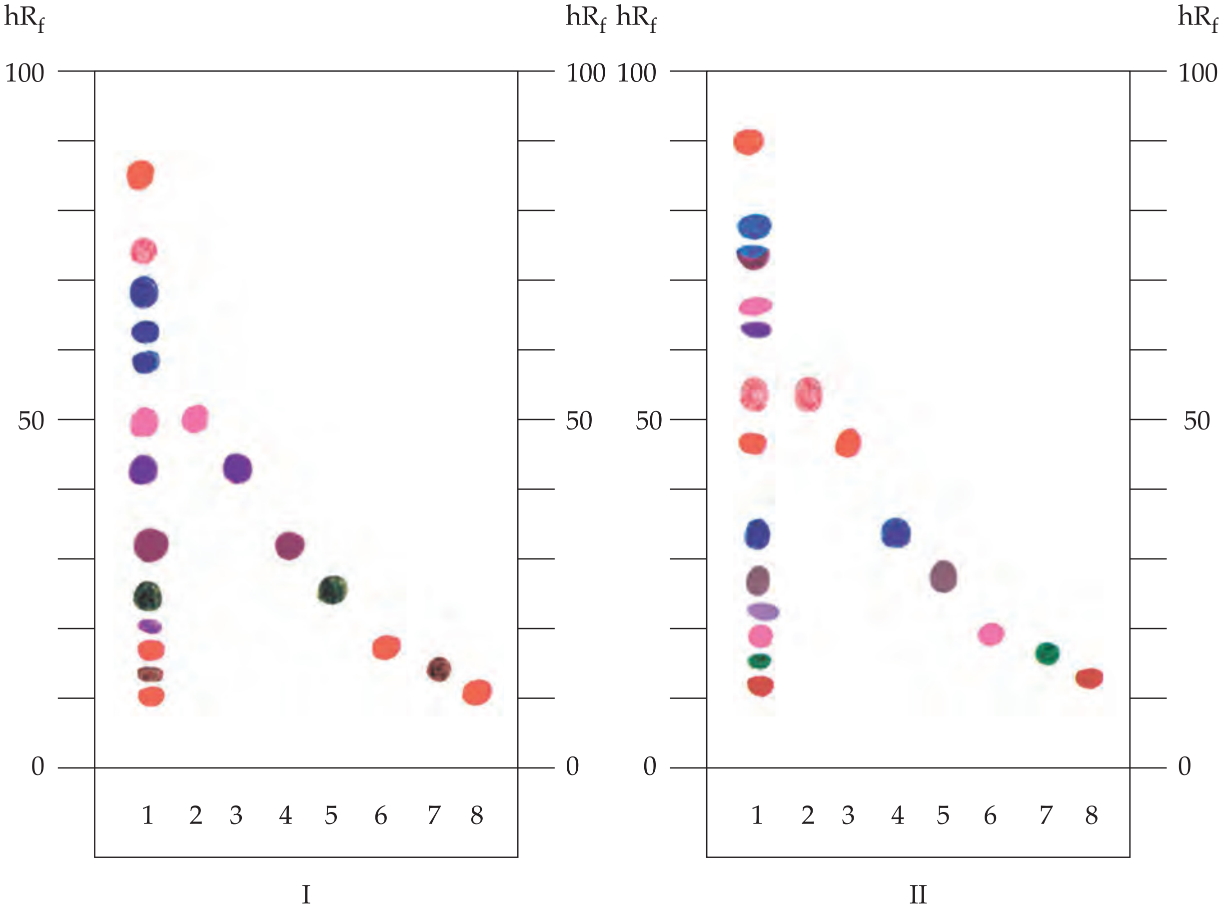


Fig. 3 Thin-layer Chromatogram of Hexane Extract of the Rhizomes of *Zingiber montanum* (J. König) Link ex A. Dietr.

1 = solution (A)
2 = a 0.1 per cent w/v solution of (*E*)-4-(3,4-dimethoxyphenyl) but-3-en-1-yl acetate in *chloroform*
3 = a 0.1 per cent w/v solution of *cis*-3-(3,4-dimethoxyphenyl)-4-[(*E*)-3,4-dimethoxys tyryl]cyclohex-1-ene in *chloroform*
4 = a 0.1 per cent w/v solution of *cis*-3-(3,4-dimethoxyphenyl)-4-[(*E*)-2,4,5-trimethoxys tyryl]cyclohex-1-ene in *chloroform*
5 = a 0.1 per cent w/v solution of *cis*-3-(2,4,5-trimethoxyphenyl)-4-[(*E*)-2,4,5-trimethoxys tyryl]cyclohex-1-ene in *chloroform*
6 = a 0.1 per cent w/v solution of [(*E*)-4-(3,4-dimethoxyphenyl)]but-3-en-1-ol in *chloroform*
7 = a 0.1 per cent w/v solution of 8-(3,4-dimethoxyphenyl)-2-methoxynaphtho-1,4-quinone in *chloroform*
8 = solution (B)
I = detection under *anisaldehyde TS*
II = detection with *vanillin-sulfuric acid TS*

พลู (PHLU)

Piperis Folium
Betel Leaf

Category Antifungal (topical), antiallergic (topical).

Betel Leaf is the dried leaf of *Piper betle* L. (Family Piperaceae), Herbarium Specimen Number: DMSC 902.

Constituents Betel Leaf contains volatile oil comprising cadinene, carvacrol, caryophyllene, chavibetol, chavicol, 1,8-cineole, estragole, eugenol, terpinyl acetate, etc. It also contains sitosterols, stigmasterol, vitamin C, oxalic acid, *d*(+)malic acid, *n*-hentriacontane, *n*-pentatriacontane, amino acids, pyridine alkaloids, tannins, minerals, etc.

Description of the plant (Figs. 1a, 1b) Woody climber with swollen nodes, up to 15 m high, dimorphic branching; orthotropic vegetative branches with adventitious roots; plagiotropic axillary fruiting branches without roots. Leaves simple, circular, ovate or ovate-oblong, 5 to 18 cm long, 3 to 12 cm wide; surface glabrous or with very short, thick white hairs, apex acuminate, base cordate or obliquely obtuse, margin entire, veins prominent on lower surface; petiole cylindrical, 1.2 to 2.5 cm long. Inflorescence in drooping, dense axillary spike, consisting of male and female flowers. Male spike 2.5 to 12 cm long; peduncle 1.5 to 3 cm long; stamens 2, very short. Female spike 2.5 to 12 cm long; peduncle 2.5 to 6 cm long; stigmas 3 to 5. Fruit berry, round with glabrous apex. Seed, suborbicular, 3.5 to 5 mm long.

Description Odour, aromatic; taste, pungent.

Macroscopical (Fig. 1a) Leaf, simple, circular, ovate or ovate-oblong, 5 to 18 cm long and 3 to 12 cm wide; acute acuminate; base cordate or obliquely obtuse; upper surface smooth, dull brown; lower surface dull, lighter colour.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the leaf shows upper epidermis, a layer of quadrangular cells, polygonal and straight-walled in surface view; cuticle thick, stoma absent with few glandular and non-glandular trichomes. Non-glandular trichome consists of a single short conical thick-walled cell tapering to the apex, and glandular trichome with a unicellular round head. Lower epidermis, almost similar to those of upper epidermis, polygonal and rather wavy walls in surface view; anomocytic stomata, numerous. Hypodermis, two to three layers of cells, some of which being oil glands. Mesophyll consists of a layer of palisade cells and a few layers of irregular spongy parenchyma containing abundant chloroplastids; oil glands, numerous. Vascular bundle, collateral, a few layer of collenchyma beneath upper and lower epidermis, schizogenous oil cavities, crystals and some starch granules in the midrib.

Betel Leaf in powder possesses the diagnostic microscopical characters of the unground drug.

Packaging and storage Betel Leaf shall be kept in well-closed containers, protected from light, and stored in a dry place.

Fig. 1a *Piper betle* L.
1. plant 2. flowering twig 3. leaves 4. crude drug

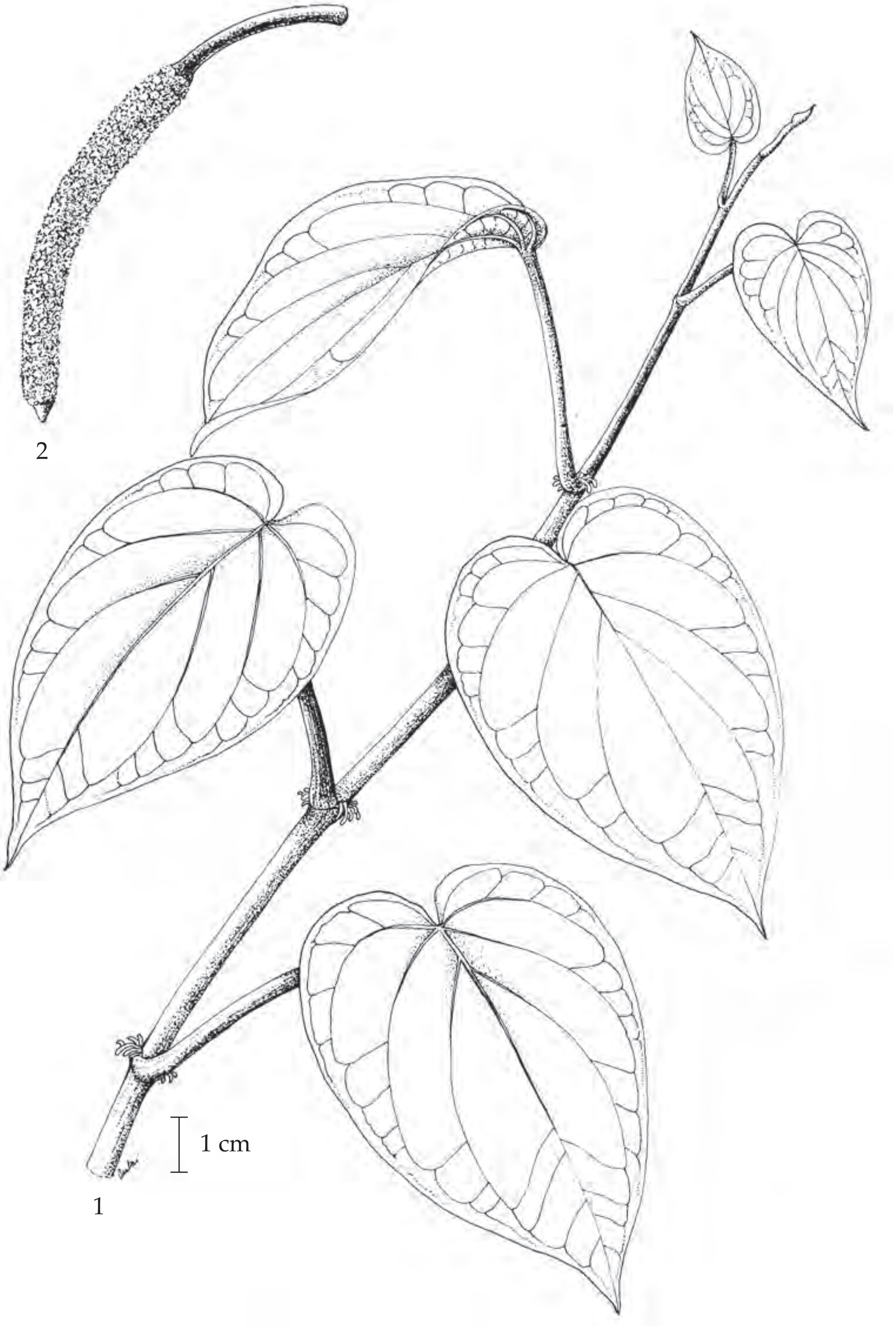
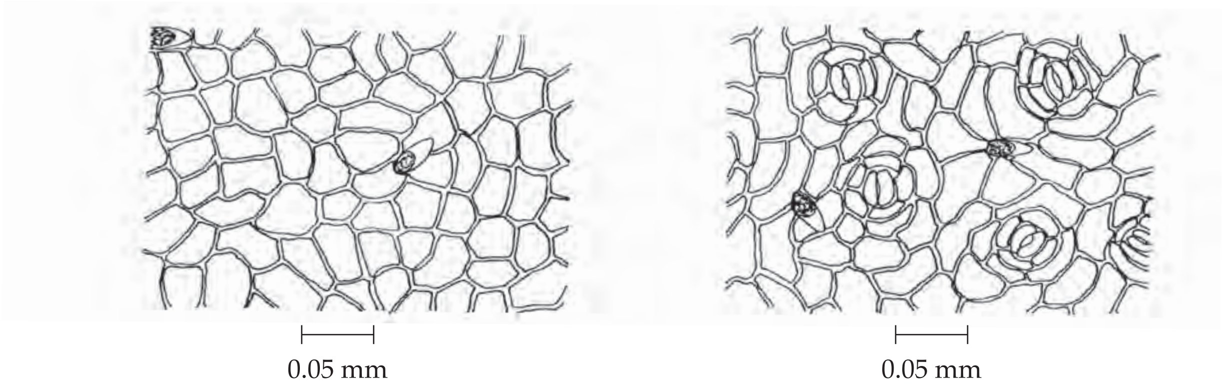


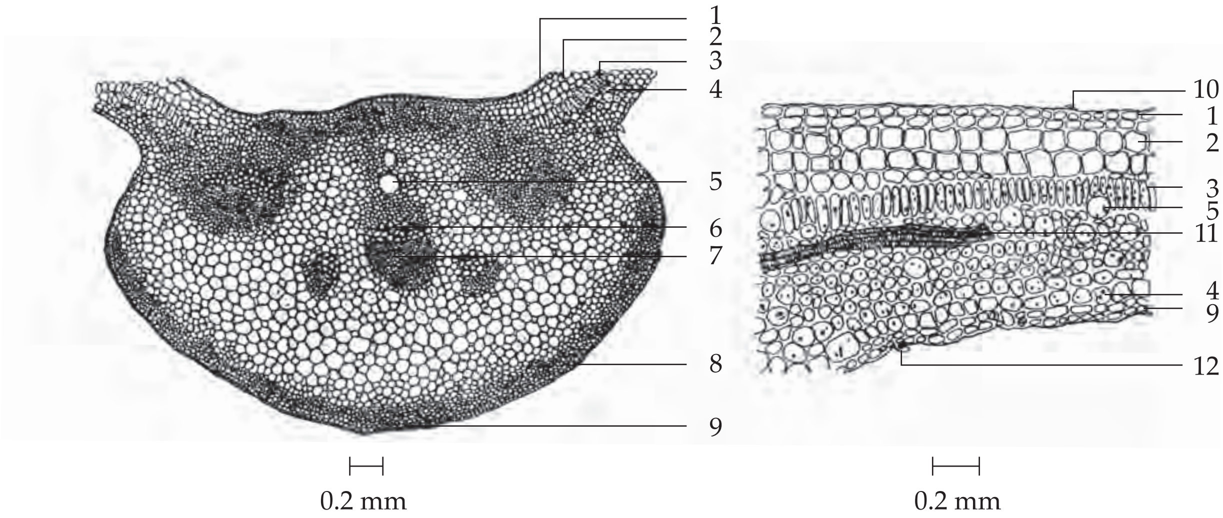
Fig. 1b *Piper betle* L.
1. young twig 2. inflorescence



Upper Epidermis of the Lamina

Lower Epidermis of the Lamina

Fig. 2a Epidermises of the Leaf of *Piper betle* L.



Transverse Section of the Midrib

Transverse Section of the Lamina

Fig. 2b Transverse Section of the Leaf of *Piper betle* L.

1. upper epidermis
2. hypodermis
3. palisade chlorenchyma
4. spongy chlorenchyma
5. oil gland
6. xylem
7. phloem
8. collenchyma
9. lower epidermis
10. non-glandular trichome
11. vascular bundle
12. glandular trichome

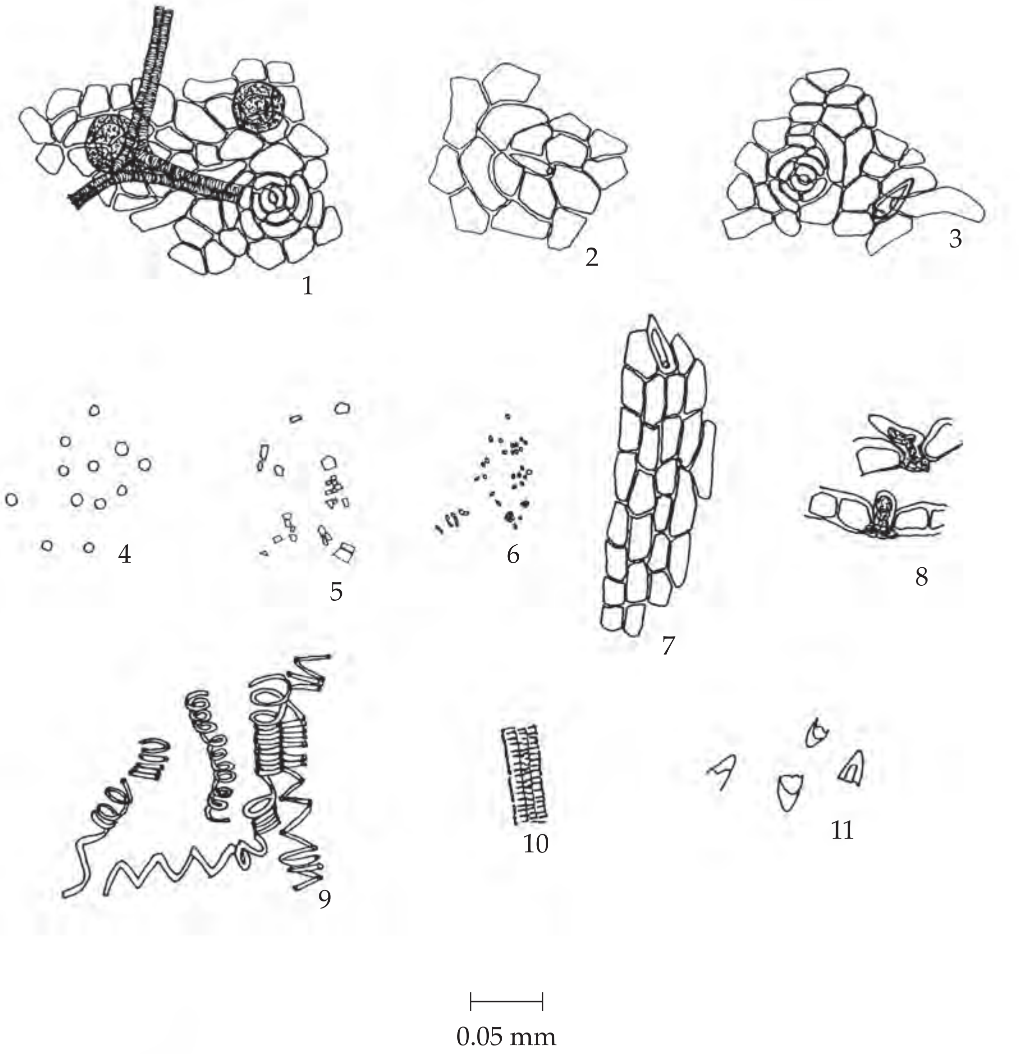


Fig. 2c Powdered Drug of the Leaves of *Piper betle* L.

1. lower epidermis in surface view showing anomocytic stoma, vascular bundle and oil glands
2. upper epidermis in surface view showing non-glandular trichome
3. lower epidermis in surface view showing stoma and non-glandular trichome
4. oil droplets
5. prismatic crystals of calcium oxalate
6. starch grains
7. epidermis of midrib with non-glandular trichome in surface view
8. glandular trichomes
9. spiral vessel thickenings
10. scalariform vessel
11. non-glandular trichomes

Identification

A. Macerate 5 g of the sample, in powder, with 50 ml of *water* for 15 minutes. Distil for about 3 to 4 hours. Extract the distillate with three successive 25-ml, 20-ml and 15-ml portions of *dichloromethane*. Filter over *anhydrous sodium sulfate*, evaporate to dryness. Add 1 ml of *chloroform* and 1 ml of *acetic anhydride* to the residue. Slowly add 1 ml of *sulfuric acid* to form a layer: a violet brown colour forms at the zone of contact.

B. Macerate 500 mg of the sample, in powder, with 10 ml of *ethanol* for 5 minutes and filter. Shake 5 ml of the filtrate with 200 mg of *decolorizing charcoal*, filter and add 1 drop of *iron(III) chloride TS* to 1 ml of the filtrate: a green colour is produced.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 75 volumes of *toluene* and 25 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, 1 µl each of the following solutions. Prepare solution (A) by macerating 2 g of the sample, in powder, with 20 ml of *chloroform* for 24 hours and filtering. Evaporate the filtrate under reduced pressure at 40° until dryness, and dissolve the residue in 2 ml of *chloroform*. For solution (B), dilute 5 µl of *eugenol* with *chloroform* to make 1 ml. After removal of the plate, allow it to dry in air, spray the plate with a 20 per cent w/v solution of *phosphomolybdic acid* in *ethanol* and heat at 105° for 5 minutes; a blue spot due to *eugenol* (hR_f value 60 to 62) and other four to six spots of blue and greenish blue colours are observed (Table 1); see also Fig. 3. When treated with ammonia vapour; the yellow background of the layer turns white.

Table 1 hR_f Values of Components in Chloroform Extract of the Leaves of *Piper betle* L.

Spot	hR_f Value	Detection
		20 Per Cent W/V Solution of *Phosphomolybdic Acid* in *Ethanol*.
1	33-38	blue
2	43-45	blue
3	55-57	greenish blue
4*	60-62	blue
5	63-69	greenish blue
6	66-67	greenish blue
7	78-88	blue

*eugenol

Water Not more than 8.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 7.0 per cent w/w (Appendix 7.6).

Total ash Not more than 14.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 4.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 14.0 per cent w/w (Appendix 7.12).

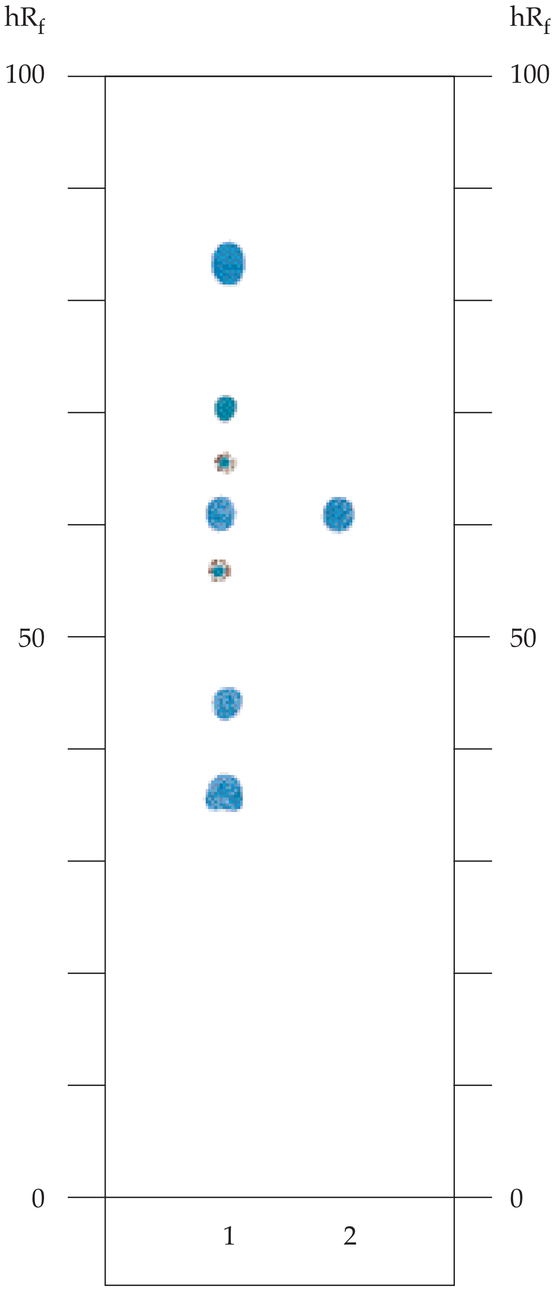


Fig. 3 Thin-layer Chromatogram of Chloroform Extract of the Leaves of *Piper betle* L. Detected with *Phosphomolybdic Acid* in *Ethanol*.

1 = solution (A)
2 = solution (B)
◌ = spots developed in some samples

พริกขี้หนู (PHRIK KHINU)

พริกกะเหรี่ยง (PHRIK KARIANG), พริกแกว (PHRIK KAEO), พริกขี้หนูสวน (PHRIK KHINU SUAN), พริกแด้ (PHRIK DAE)

Capsici Annui Fructus
Chilli Pepper

Synonyms Capsicum, Garden Pepper, Green Chilli Pepper, Red Chilli Pepper

Category Gastro-intestinal stimulant, counter-irritant.

Chilli Pepper is the dried ripe fruit of *Capsicum annuum* L. (*C. frutescens* L., *C. minimum* Roxb., *C. fastigiatum* Blume) (Family Solanaceae), Herbarium Specimen Number: BKF 193778, Crude Drug Number: DMSc 1144. It contains not less than 0.3 per cent of total capsaicinoids, calculated as capsaicin ($C_{18}H_{27}NO_3$), on the dried basis.

Constituents Chilli Pepper contains capsaicinoids (e.g., capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide), carotenoids, volatile oil, fixed oil, etc.

Description of the plant (Fig. 1a) Annual or perennial herb, up to 2 m high; stem erect, branched, terete, more or less angular, frequently woody at base. Leaves simple, alternate, ovate to ovate-lanceolate, 2 to 10 cm long, 1 to 5 cm wide, apex and base acuminate, margin entire; petiole 0.5 to 3 cm long. Flower solitary or in a cluster of 2 to 3, axillary or terminal, 5 to 9 mm wide, white, greenish or yellowish, nodding at anthesis; peduncle 1 to 2 cm long; calyx campanulate, entire or minutely 5-lobed, 2 to 3 mm long; corolla short tube, 5- to 7-lobed, 1 to 1.5 cm wide; stamens 5; filament 1 to 1.5 mm long, glabrous; anther 1 to 2 mm long, blue or purple; ovary superior, obtusely conical; style filiform, 1 to 3 mm long; stigma thickened. Fruit berry, narrowly conical, ovate-oblong, ellipsoid, or fusiform, 1 to 3 cm long, 0.3 to 1.5 cm wide, erect, green turning red when ripe. Seeds numerous, suborbicular, flat, 1.5 to 3 mm wide, creamy or pale yellow.

Description Odour, characteristic; taste, intensely pungent.

Macroscopical (Fig. 1a) Yellowish-orange to red, oblong-conical, ellipsoid, or fusiform, 1 to 3 cm long, 3 to 8 mm wide; outer surface glabrous, shrivelled.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the fruit shows epicarp, mesocarp, endocarp, septum separating two locules, and seeds. Epicarp, a layer of thick-walled rectangular epidermal cells covered with moderately thick cuticle layer. Mesocarp, several layers of polygonal thin-walled parenchyma cells and vascular bundles. Endocarp, very large thin-walled parenchyma cells and thin-walled sclereids. Septum, large thin-walled parenchyma cells and small slightly lignified sclereids. Seed composed of seed coat, a layer of thick-walled sclerenchyma cells; endosperm; and embryo with two cotyledons.

Chilli Pepper in powder possesses the diagnostic microscopical characters of the unground drug. Scattered numerous orange to red oil droplets; polygonal and moderately thickened epicarp; endocarp with slightly lignified polygonal to elongated, pitted-walled sclereids; yellowish green sclerenchymatous epidermis of testa, strongly lignified with large wavy, unevenly thickened and beaded walls are characteristic and frequently observed.

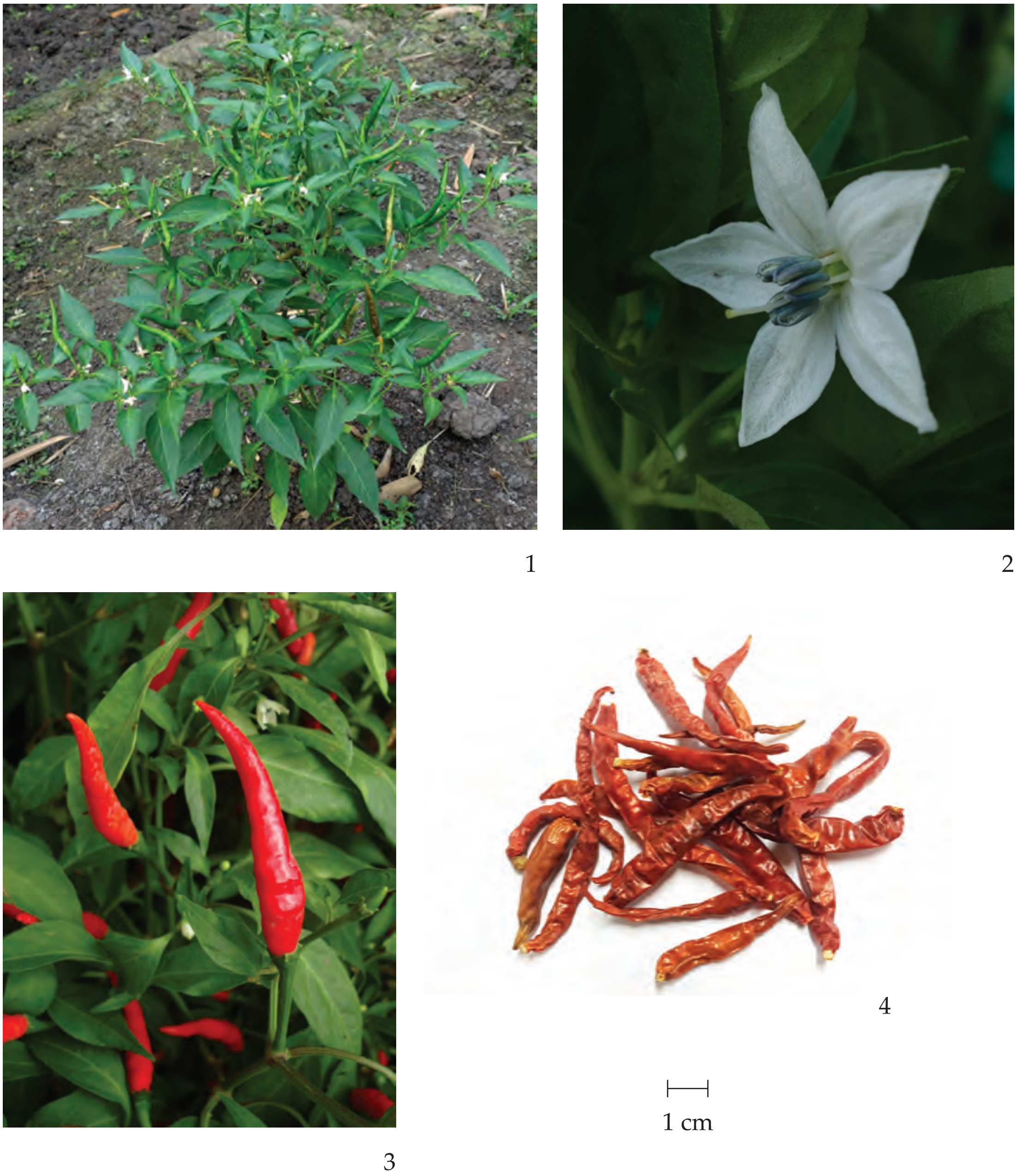


Fig. 1a *Capsicum annuum* L.
1. habit 2. flower 3. ripe fruits 4. crude drug

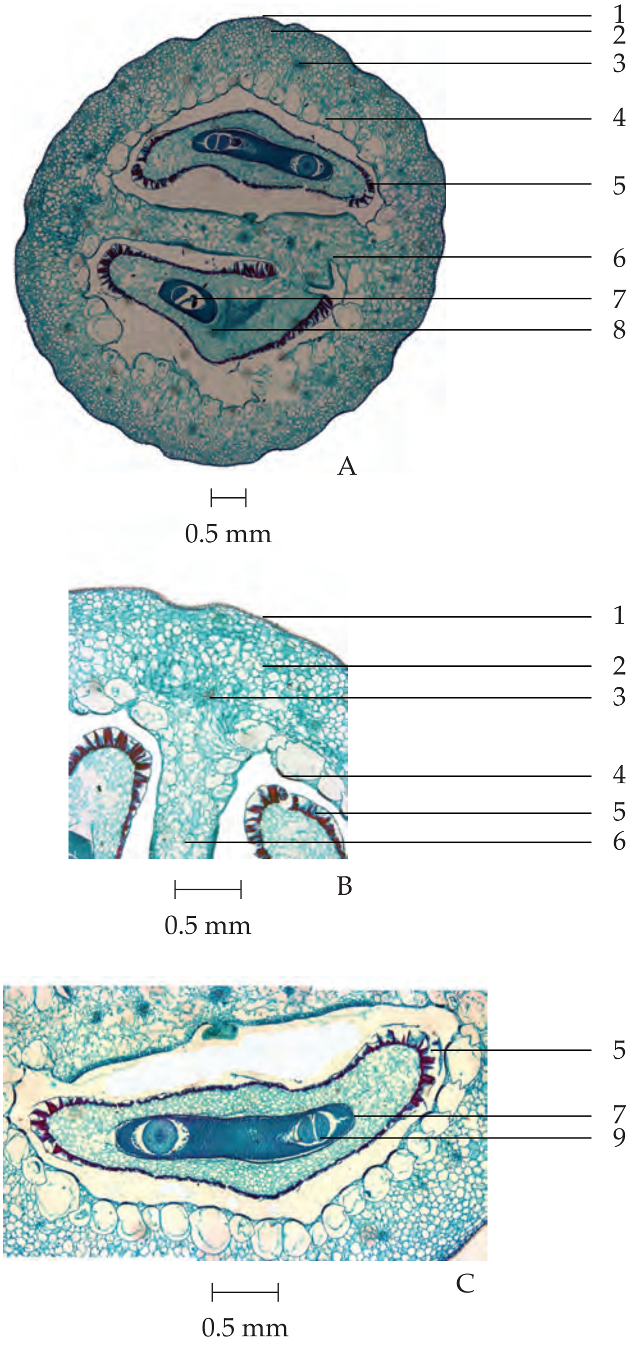


Fig. 2a Photomicrographs of Transverse Section of the Fruit of *Capsicum annuum* L., Stained with Safranin-Fast Green

A. Whole Fruit
B. Part of the Fruit
C. Seed

1. epicarp
2. mesocarp
3. vascular bundle
4. endocarp
5. seed coat
6. septum
7. endosperm
8. embryo
9. cotyledon

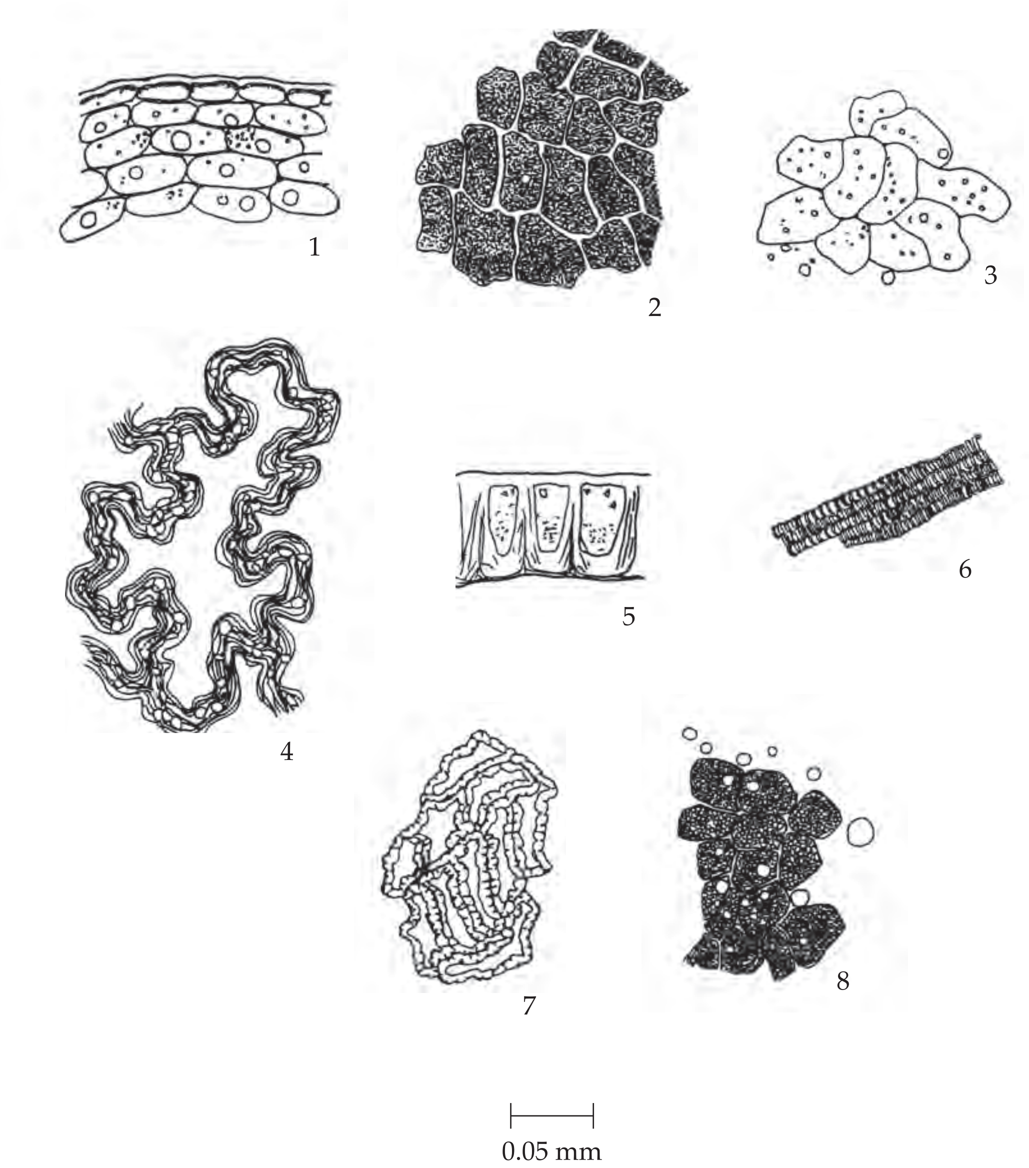


Fig. 2b Line Drawings of Powdered Drug of the Fruits of *Capsicum annuum* L.

1. epicarp and mesocarp with oil droplets in sectional view
2. epicarp in surface view
3. parenchyma of mesocarp with oil droplets and microcrystals
4. sclerenchymatous epidermis of testa in surface view
5. sclerenchymatous epidermis of testa in sectional view
6. spiral vessels
7. endocarp in surface view
8. endosperm containing aleurone grains and oil droplets

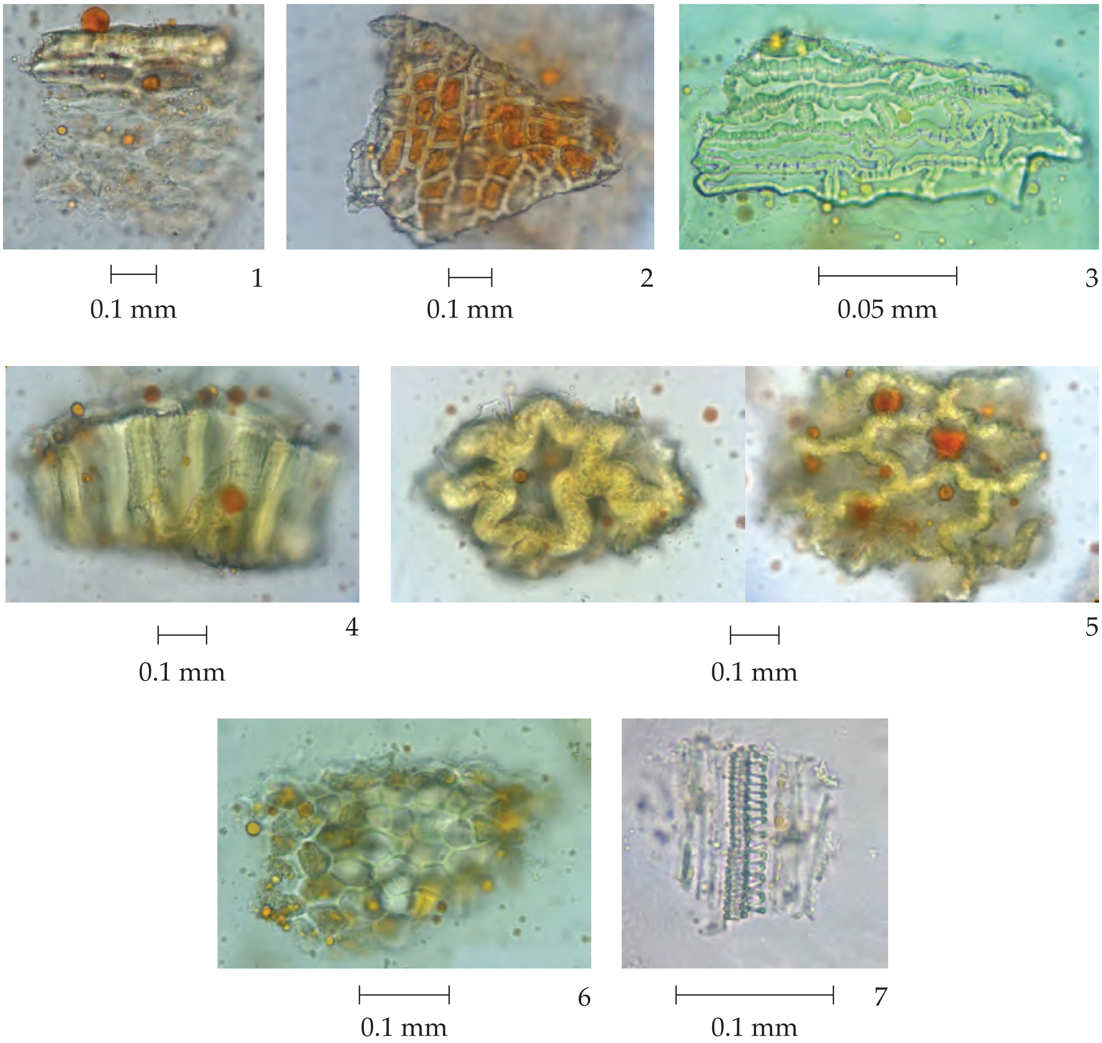


Fig. 2c Photomicrographs of Powdered Drug of the Fruits of *Capsicum annuum* L.

1. epicarp and mesocarp in sectional view with reddish orange to orange oil droplets
2. polygonal epicarp in surface view
3. endocarp in surface view
4. sclerenchymatous epidermis of testa in sectional view
5. sclerenchymatous epidermis of testa in surface view
6. endosperm containing aleurone grains and oil droplets
7. spiral vessels

Contra-indication Contact with eyes, nose, lip, wounded skin, and sensitive skin is contra-indicated when applied topically.

Warning

1. Transient erythema, burning and stinging without vesicular eruption at the application area may occur.
2. Excessive ingestion may cause hepatic or renal damage, gastro-enteritis.
3. Caution should be exercised when preparations of chilli pepper are to be used concomitantly with angiotensin-converting enzyme inhibitors, anticoagulants, low molecular weight heparins, thrombolytic agents, theophylline, barbiturates, and MAO inhibitors.
4. Risk-benefit should be considered if it is to be used in pregnant or nursing women.

Additional information Nonivamide content in Chilli Pepper should not exceed 5 per cent w/w of total capsaicinoids to prevent adulteration by the synthetic nonivamide.

Packaging and storage Chilli Pepper shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 5 g of the sample, in *coarse powder*, with 50 ml of *ethanol (80 per cent)* for 30 minutes and filter. Evaporate the filtrate to dryness and dissolve the residue in 5 ml of *ethanol (80 per cent)*. Add 25 ml of a 5 per cent v/v solution of *hydrochloric acid* and a few drops of *modified Dragendorff TS*: an orange precipitate is produced.

B. The retention time of the capsaicinoid peak in the chromatogram of the Assay preparation corresponds to the capsaicin peak of the Standard preparation, as obtained in the *Assay*.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 60 volumes of *toluene* and 40 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate as bands of 10 mm, 5 µl each of the following two solutions. Prepare solution (A) by refluxing 1 g of the sample, in *coarse powder*, with 25 ml of *methanol* for 30 minutes, filtering and evaporating the filtrate under reduced pressure to dryness. Dissolve the residue in 1 ml of *methanol*. For solution (B), dissolve 1 mg of *capsaicin* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. The chromatogram obtained from solution (A) shows a quenching band (hR_f value 29 to 31) corresponding to the capsaicin band from solution (B) and other two quenching bands. Subsequently spray the plate with *anisaldehyde TS* and heat at 105° for 3 minutes; the band due to capsaicin is purple. Other purple and violet bands are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Fruits of *Capsicum annuum* L.

Band	hR_f Value	Detection	
		UV 254	*Anisaldehyde TS*
1	9-11	weak quenching	pale purple
2	17-18	weak quenching	pale purple
3*	29-31	weak quenching	purple
4	52-55	–	violet
5	62-64	–	pale violet
6	75-77	–	pale violet
7	84-87	–	pale violet

*capsaicin

Loss on drying Not more than 12.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 1.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 10.0 per cent w/w (Appendix 7.7).

Assay Carry out the determination as described in the "High-pressure Liquid Chromatography" (Appendix 3.5).

Mobile phase Prepare a mixture of 2 volumes of *acetonitrile* and 3 volumes of a 1 per cent v/v solution of *formic acid*. Make adjustments if necessary.

Standard preparation Dissolve an accurately weighed quantity of Capsaicin RS in *ethanol* to obtain a solution having a known concentration of about 30 µg per ml.

Assay preparation Transfer about 500 mg of Chilli Pepper, in powder and accurately weighed, into a 50-ml centrifuge tube, add 30 ml of *methanol*, shake for 15 minutes, and centrifuge. Transfer a supernatant to a 50-ml volumetric flask. To the residue, add 10 ml of *methanol*, shake for 5 minutes and centrifuge. Transfer the supernatant to the volumetric flask. Repeat the extraction one more time with 10 ml of *methanol*. Dilute with *methanol* to volume, mix and filter through a nylon membrane having a 0.45-µm porosity.

Chromatographic system The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm) equipped with a similarly packed guard column, (b) *Mobile phase* at a flow rate of 1.4 ml per minute (The retention time is about 20 minutes for the main capsaicin peak.), and (c) an ultraviolet photometer set at 280 nm.

To determine the suitability of the chromatographic system, chromatograph *Standard preparation*, and record the peak response as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0 per cent, and the symmetry factor for the capsaicin peak is not more than 1.5. Chromatograph *Assay preparation* and record the peak response as directed under *Procedure*: the relative retention times are about 0.9 for nordihydrocapsaicin, 1.0 for capsaicin, and 1.6 for dihydrocapsaicin and the resolution factor, *R*, between the nordihydrocapsaicin peak and the capsaicin peak is not less than 1.2.

Procedure Separately inject equal volumes (about 20 µl) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms for a period of time that is twice that of the retention time of capsaicin, and measure the responses for the three peaks corresponding to nordihydrocapsaicin, capsaicin and dihydrocapsaicin.

Calculation Calculate the content of total capsaicinoids, expressed as capsaicin, in the portion of Chili Peper taken, using the sum of the peak responses for nordihydrocapsaicin, capsaicin and dihydrocapsaicin and the declared content of capsaicin ($C_{18}H_{27}NO_3$) in Capsaicin RS.

Dose 30 to 120 mg three times a day.

Topical, as a topical preparation containing about 0.025 per cent w/w of capsaicin to the affected areas, three or four times a day.

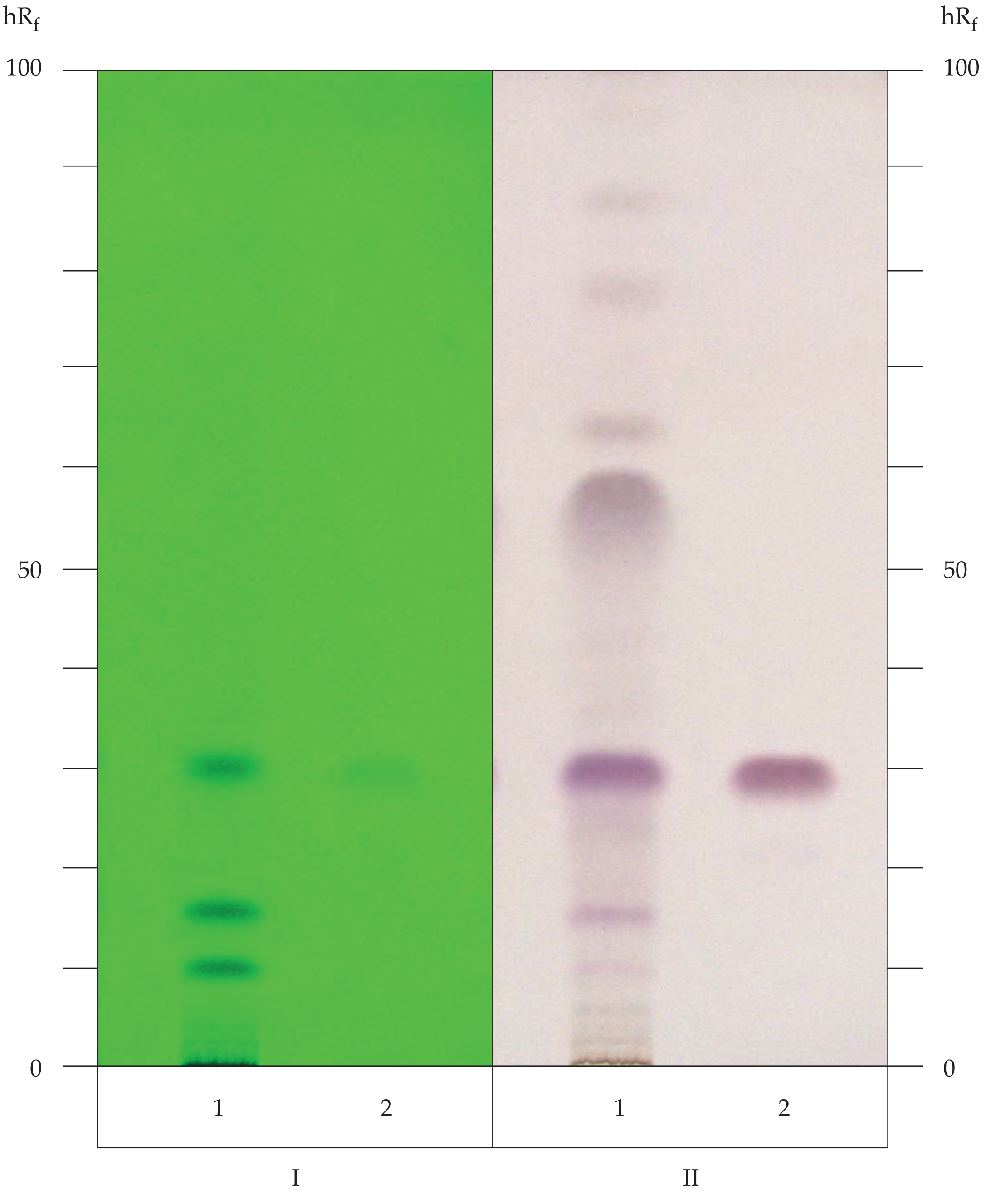


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Fruits of *Capsicum annuum* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with *anisaldehyde TS*

ชันน้ำมันพริกขี้หนู (CHAN NAMMAN PHRIK KHINU)

Capsicum Oleoresin

> ***Caution*** Capsicum Oleoresin is a powerful irritant, and even in minute quantities produces an intense burning sensation when it comes in contact with the eyes and tender parts of the skin. Care should be taken to protect the eyes and to prevent contact of the skin with Capsicum Oleoresin.

Category Counter-irritant.

Capsicum Oleoresin is an ethanolic extract of Chilli Pepper. It contains not less than 6.5 per cent of total capsaicinoids [capsaicin ($C_{18}H_{27}NO_3$), dihydrocapsaicin ($C_{18}H_{29}NO_3$) and nordihydrocapsaicin ($C_{18}H_{27}NO_3$)], calculated as capsaicin, ($C_{18}H_{27}NO_3$) on the anhydrous basis.

Description Dark red oily liquid.

Solubility Soluble in *acetone*, *chloroform*, *ethanol*, *ether*, and volatile oils. Soluble with opalescence in fixed oils.

Additional information Nonivamide content in Capsicum Oleoresin should not exceed 5 per cent w/w of total capsaicinoids to prevent adulteration by the synthetic nonivamide.

Packaging and storage Capsicum Oleoresin shall be kept in tightly closed containers, preferably in glass containers.

Labelling The label on the container stated that if separation occurs, it should be warmed and mixed before use.

Identification

A. The retention time of the capsaicinoid peak in the chromatogram of the Assay preparation corresponds to the capsaicin peak of the Standard preparation, as obtained in the *Assay*.

B. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 60 volumes of *toluene* and 40 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate as bands of 10 mm, 5 µl each of the following two solutions. Prepare solution (A) by diluting 50 mg of the sample with 5 ml of n-*hexane.* For solution (B), dissolve 1 mg of *capsaicin* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. The chromatogram obtained from solution (A) shows a quenching band (hR_f value 29 to 31) corresponding to the capsaicin band from solution (B) and other bands may be observed. Subsequently spray the plate with *anisaldehyde TS* and heat at 105° for 3 minutes; the band due to capsaicin is purple. Other two purple and four violet bands are also observed.

Water Not more than 8.0 per cent w/w (Karl Fischer Method, Appendix 4.12)

Assay Carry out the determination as described in the "High-pressure Liquid Chromatography" (Appendix 3.5)

Mobile phase Prepare a mixture of 40 volumes of *acetonitrile* and 60 volumes of a 0.1 per cent w/v solution of *phosphoric acid*. Make adjustments if necessary.

Standard preparation Prepare a solution of Capsaicin RS in *methanol* having a known concentration of about 200 µg per ml. Filter a portion of this solution through a 0.2-µm porosity filter.

Assay preparation Transfer about 1 g of Capsicum Oleoresin, accurately weighed, to a 100-ml volumetric flask, dissolve in and dilute with *methanol* to volume, and mix. Filter a portion of this solution through a 0.2-µm porosity filter.

Chromatographic system The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (1 to 10 µm) or monolithic rod, (b) *Mobile phase* at flow rate of 1 ml per minute, and (c) an ultraviolet photometer set at 280 nm.

To determine the suitability of the chromatographic system, chromatograph *Standard preparation*, and record the peak response as directed under *Procedure*: the symmetry factor for the capsaicin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent. Chromatograph *Assay preparation*, and record the peak response as directed under *Procedure*: the relative retention times are about 0.90 for nordihydrocapsaicin, 0.95 for nonivamide, 1.00 for capsaicin, and 1.30 for dihydrocapsaicin and the resolution factor, R, between the nonivamide peak and the capsaicin peak is not less than 1.5.

Procedure (**Note** Use peak areas where peak responses are indicated.) Separately inject equal volumes (about 10 µl) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the three major peaks.

Calculation Calculate the percentage of total capsaicinoids, as capsaicin ($C_{18}H_{27}NO_3$) in the Capsicum Oleoresin taken by the formula:

$$(C_S/C_U)(r_U/r_S) \times 100,$$

in which C_S is the concentration, in mg per ml, of Capsaicin RS in *Standard preparation*, C_U is the concentration, in mg per ml, of Capsicum Oleoresin in *Assay preparation*, r_U is the sum of the peak responses for nordihydrocapsaicin, capsaicin, and dihydrocapsaicin obtained from *Assay preparation* and r_S is the peak response of capsaicin obtained from *Standard preparation*.

Calculate the percentage of capsaicin in the Capsicum Oleoresin taken by the formula:

$$(C_S/C_U)(r_U/r_S) \times 100,$$

in which C_S is the concentration, in mg per ml, of Capsaicin RS in *Standard preparation*, C_U is the concentration, in mg per ml, of Capsicum Oleoresin in *Assay preparation*, r_U is the peak response for capsaicin obtained from *Assay preparation* and r_S is the peak response of capsaicin obtained from *Standard preparation*.

ยาเจลพริก (PHRIK GEL)

Capsicum Gel

Category Counter-irritant.

Capsicum Gel is Capsicum Oleoresin in a suitable hydrophilic gel base. It contains not less than 90.0 per cent and not more than 110.0 per cent of the labelled amount of capsaicin ($C_{18}H_{27}NO_3$)

Strength available 0.025 per cent w/w of capsaicin.

Dose Topical, to the affected areas, three or four times a day.

Warning

1. Contact with eyes, nose, lip, wounded skin, and sensitive skin should be avoided.
2. Transient erythema, burning and stinging without vesicular eruption at the application area may occur.

Packaging and storage Capsicum Gel shall be kept in collapsible tubes or in tightly closed containers, protected from light and stored at a temperature not exceeding 30°.

Labelling The label on the container states (1) the amount of capsaicin; (2) expiration date.

Identification The retention time of the major peak in the chromatogram of the Assay preparation corresponds to the capsaicin peak of the standard preparation, as obtained in the *Assay*.

Microbial limit Complies with the requirements for Category 3 in the “Limits for Microbial Contamination” (Appendix 10.5).

Assay Carry out the determination as described in the “High-pressure Liquid Chromatography” (Appendix 3.5).

Mobile phase Prepare a mixture of 2 volumes of *acetonitrile* and 3 volumes of a 1 per cent v/v solution of *formic acid*. Make adjustments if necessary.

Standard preparation Dissolve an accurately weighed quantity of Capsaicin RS in *ethanol* to obtain a solution having a known concentration of about 30 µg per ml.

Assay preparation Transfer an accurately weighed quantity of Capsicum Gel, containing about 1.5 mg of capsaicin, to a 125-ml Erlenmeyer flask. Add 25.0 ml of *ethanol*, shake well and sonicate for about 30 minutes. Quantitatively transfer the solution to a 50-ml volumetric flask, dilute with *ethanol* to volume and mix. Centrifuge the resulting solution at 855×*g* (3000 rpm) for 15 minutes. Pass a portion of the clear solution through a filter having a 0.5-µm or finer porosity.

Chromatographic system The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm) equipped with a similarly packed guard column, (b) *Mobile phase* at a flow rate of 1.4 ml per minute (The retention time is about 20 minutes for the main capsaicin peak.), and (c) an ultraviolet photometer set at 280 nm.

To determine the suitability of the chromatographic system, chromatograph *Standard preparation*, and record the peak response as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0 per cent, the column efficiency determined from the capsaicin peak is not less than 5000 theoretical plates, and the symmetry factor for the capsaicin peak is not more than 1.5. Chromatograph *Assay preparation* and record the peak response as directed under *Procedure*: the relative retention times are about 0.9 for nordihydrocapsaicin, 1.0 for capsaicin, and 1.6 for dihydrocapsaicin; and the resolution factor, *R*, between the nordihydrocapsaicin peak and the capsaicin peak is not less than 1.2

Procedure Separately inject equal volumes (about 20 μl) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms for a period of time that is twice that of the retention time of capsaicin, and measure the responses for the capsaicin peaks.

Calculation Calculate the content of capsaicin in the portion of the Gel taken, using declared content of $C_{18}H_{27}NO_3$ in Capsaicin RS.

Other requirements Complies with the requirements described under "Topical Preparations" (Appendix 1.16).

พริกไทยดำ (PHRIK THAI DAM)

Piperis Nigri Fructus, Piper Nigrum
Black Pepper

Synonyms Pepper, Pepper Corn, Piper

Category Aromatic, stomachic, carminative.

Black Pepper consists of the dried unripe fruit of *Piper nigrum* L. (Family Piperaceae), Herbarium Specimen Number: DMSC 667.

Constituents Black Pepper contains volatile oil, alkaloids, of which piperine and piperettine, are its major components.

Description of the plant (Fig. 1) Woody perennial climber; aromatic; branches stout, trailing and rooting at the nodes. Leaves simple, very variable in length, 12.5 to 17.5 cm long, 5.0 to 12.5 cm wide, sometimes glaucous beneath, apex acuminate, base acute rounded or cordate, equal or unequal, basal nerves 5 to 9 with one pair running to apex; petiole 1.2 to 3.7 cm, stout. Flowers usually dioecious, but often the female bears 2 anthers, and the male a pistillode, anther 2-celled. Fruiting spikes, 10 to 12 cm or more long. Fruits drupaceous, globose or ovoid, sessile, 1-seeded, 4 to 6 mm in diameter, orange red to reddish when ripe, seeds usually globose, testa thin.

Description Odour, aromatic; taste, pungent.

Macroscopical Subspherical, greyish brown to brownish black, superior, and about 3.5 to 6 mm in diameter; surface deeply and coarsely reticulately wrinkled; remains of a sessile stigma visible at the apex; vertical section of the fruit shows a thin, narrow, dark pericarp, within which is the whitish kernel of the single seed, to which the pericarp firmly adheres; kernel consisting almost entirely of perisperm, hollow at the centre, and, at its apex, surrounding a scanty endosperm in which the minute embryo is embedded.

Microscopical (Figs. 2a, 2b) Transverse section of the fruit shows epicarp composed of a layer of epidermal cells containing small rectangular crystals about 6 to 10 µm long and 2 to 3 layers of thickened stone cells with rather large lumina intermingled with thin-walled parenchymatous cells. Mesocarp composed of several layers of polygonal parenchymatous cells, some of which are oil cells which occur more in the inner part; vascular bundles located in the middle part of mesocarp. Endocarp, a layer of light brown, lignified beaker cells. Spermoderm composed of 2 to 3 layers of compressed elongated cells, the innermost layer composed of larger cells containing dark brown contents. Perisperm composed of cells containing numerous angular starch grains up to 5 µm in diameter, some of which containing oil globules, resin and monoclinic crystals.

Black Pepper in powder possesses the diagnostic microscopical characters of the unground drug.

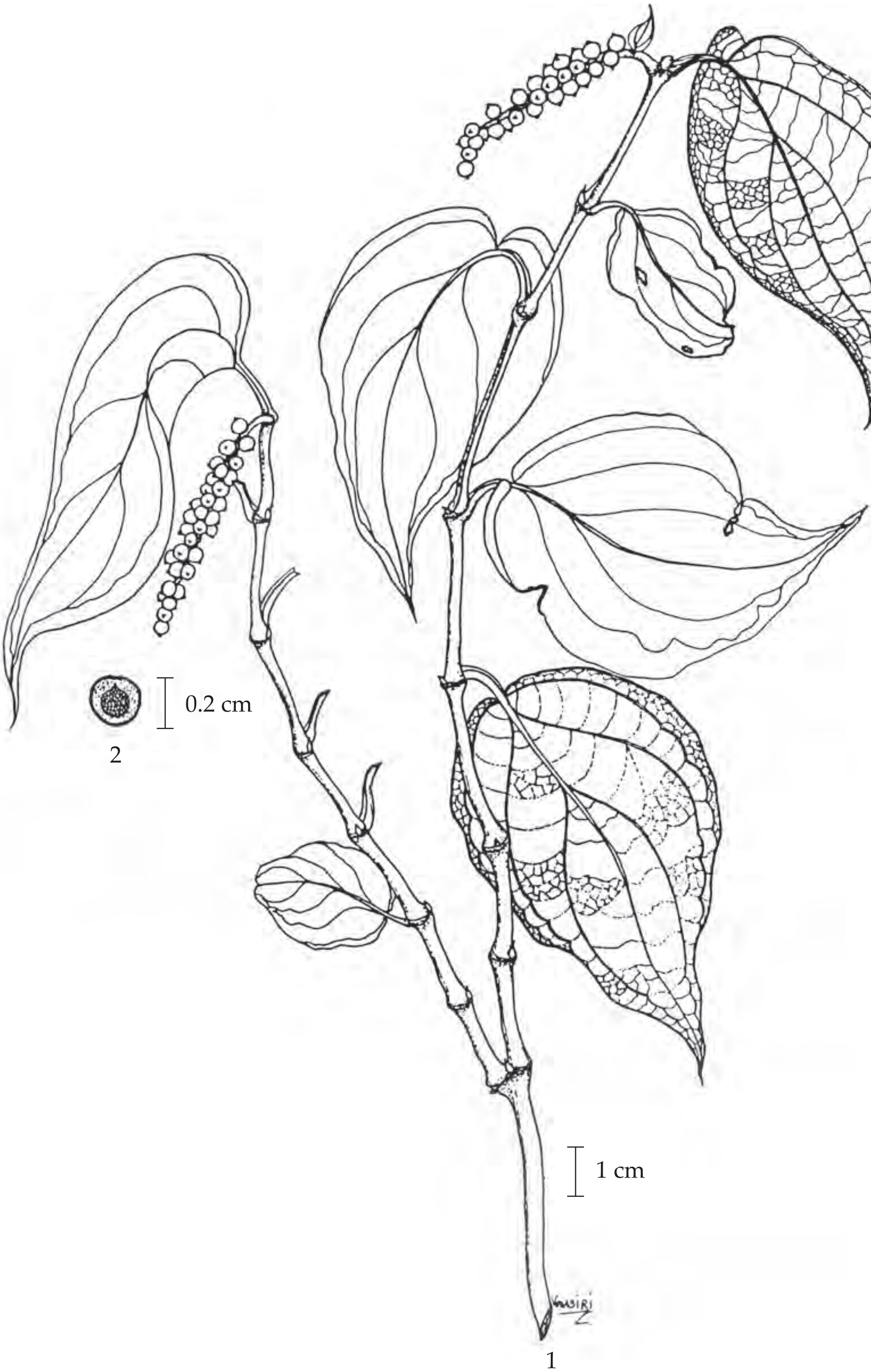


Fig. 1 *Piper nigrum* L.
1. twig 2. fruit

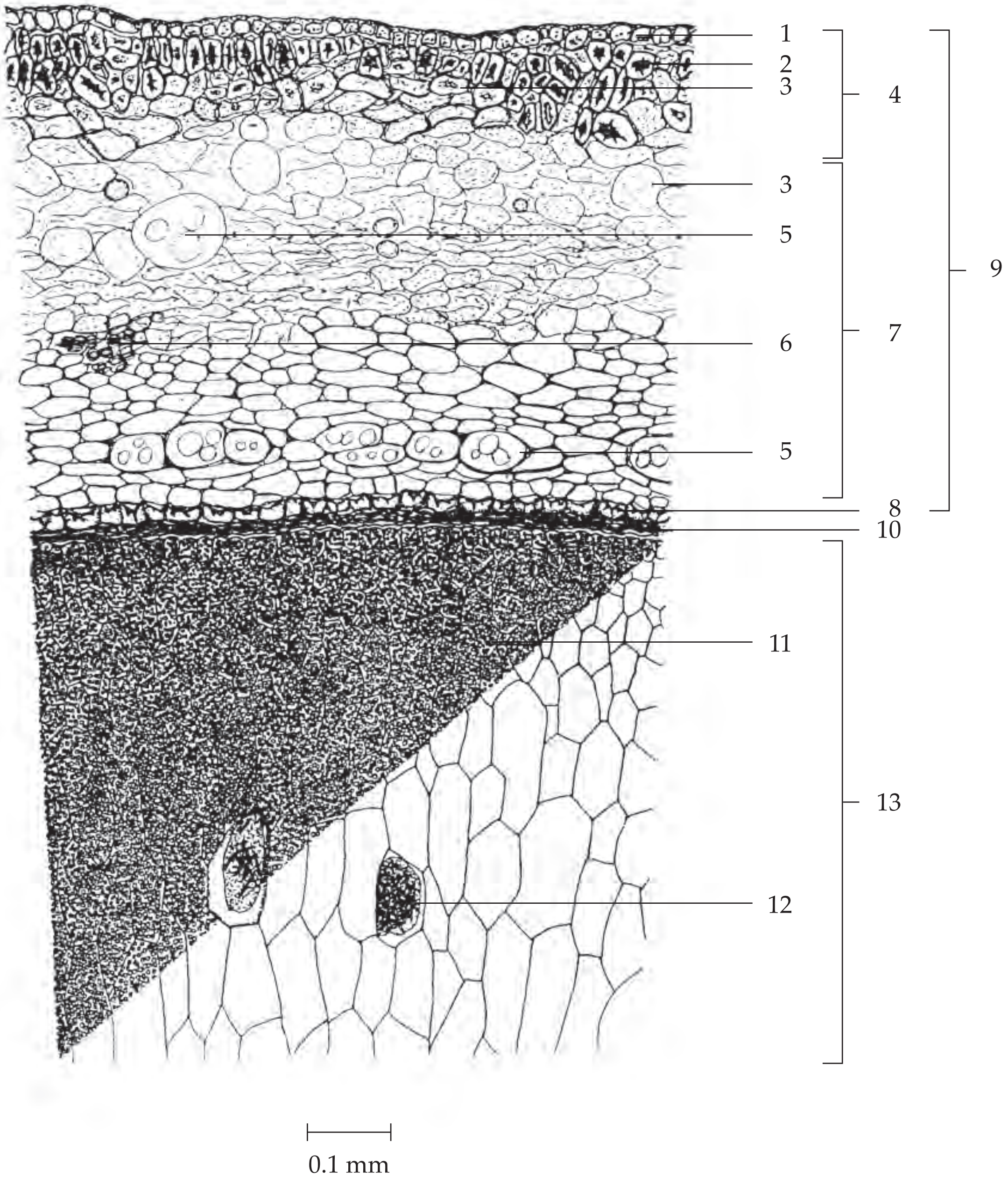


Fig. 2a Transverse Section of the Fruit of *Piper nigrum* L.

1. epidermis containing small crystals
2. stone cell
3. parenchyma
4. epicarp
5. oil cells
6. vascular bundle
7. mesocarp
8. endocarp
9. pericarp
10. spermoderm
11. cell containing starch grains
12. cell containing oil globules and monoclinic crystals
13. perisperm

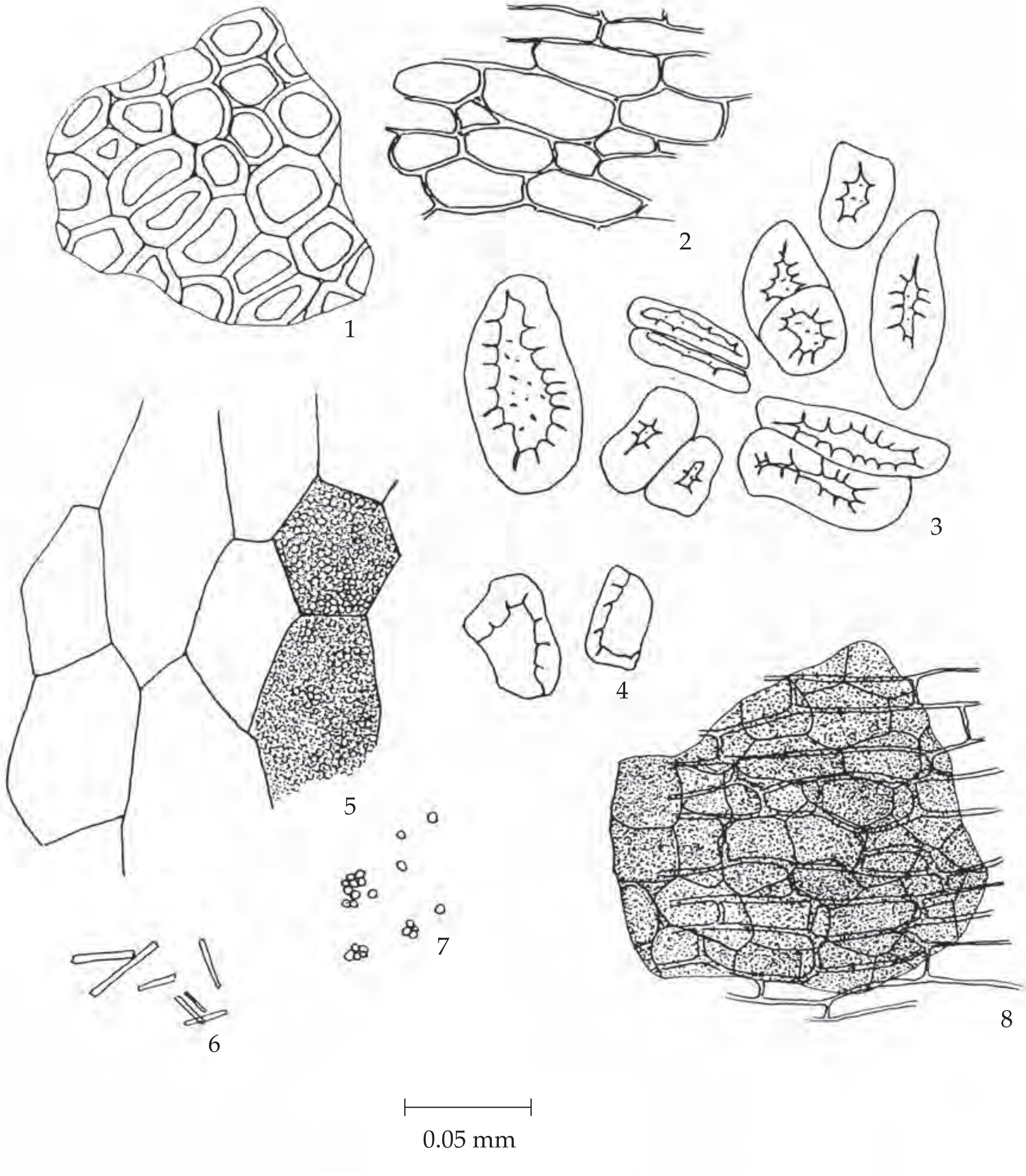


Fig. 2b Powdered Drug of the Fruits of *Piper nigrum* L.

1. epicarp in surface view
2. polygonal cells of mesocarp
3. stone cells
4. beaker cells of endocarp
5. perisperm containing starch grains
6. monoclinic crystals
7. starch grains
8. spermoderm showing underlying cells containing dark brown contents

Packaging and storage Black Pepper shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Reflux 1 g of the sample, in *No.150 powder*, with 20 ml of *chloroform* in a water-bath for 15 minutes, and filter (solution 1). Evaporate 2 ml of solution 1 to dryness, add 2 ml of *acetic anhydride*, shake well, and add carefully 1 ml of *sulfuric acid* to make two layers: a brownish red colour develops at the zone of contact.

B. Evaporate 2 ml of solution 1 to dryness. Dissolve the residue with 1 ml of *ethanol*, and add 0.5 ml of *sulfuric acid*: a brownish red colour is produced, and after set aside the colour changes to brown.

C. Evaporate 5 ml of solution 1 to dryness. Dissolve the residue with 0.5 ml of *ethanol*, and add 4 to 5 drops of *acetic potassium iodobismuthate TS*: an orange precipitate is produced.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 60 volumes of *hexane* and 40 volumes of *ethyl acetate* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate, 5 µl each of the following two solutions. Prepare solution (A) by macerating 500 mg of the sample, in *No.150 powder*, in a 5-ml volumetric flask with *chloroform* for 10 minutes, and shaking frequently. Filter and evaporate the filtrate to dryness. Dissolve the residue in 1 ml of *chloroform*. For solution (B) dissolve 1 mg of *piperine* in 1 ml of *chloroform*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained with solution (A) shows a quenching spot (hR_f value 28 to 38), corresponding to the piperine spot from solution (B), and several spots of higher and lower hR_f values. Spray the plate with *acetic potassium iodobismuthate TS*; the spot due to piperine is dark orange (Table 1); see also Fig. 3.

Repeat the same procedure on another plate but spray with a 50 per cent v/v solution of *sulfuric acid* in *methanol*; the spot due to piperine is yellow. After spraying, allow the plate to dry in air for 15 minutes and heat at 110° until colours are fully developed. The chromatogram obtained with solution (A) shows a brownish yellow spot (hR_f value 28 to 38), corresponding to the piperine spot from solution (B), and several other spots of different colours situated above and below that due to piperine (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Chloroform Extract of the Fruits of *Piper nigrum* L.

Spot	hR_f Value	Detection			
		UV 254	*Acetic Potassium Iodobismuthate TS*	*Sulfuric Acid* in *Methanol*	
				before heating	after heating
1	1-7	quenching	pale orange	yellow	–
2	8-12	quenching	orange	yellow	yellow, centre brown
3	10-15	quenching	orange	–	pale purple
4	15-19	quenching	orange	yellow	pale purple
5	21-23	–	–	–	blue
6	25-28	–	–	–	blue
7*	28-38	quenching	dark orange	yellow	yellow, centre brown
8	39-46	quenching	orange	–	yellow, centre brown
9	47-50	quenching	orange	yellow	yellow, centre brown
10	49-51	–	–	–	blue
11	53-57	quenching	–	–	bluish violet
12	59-63	quenching	–	–	bluish violet
13	64-68	quenching	pale orange	–	bluish violet
14	71-76	quenching	–	–	pale violet
15	78-83	quenching	–	–	pale violet
16	85-90	–	–	pale violet	–
17	96-99	–	–	–	pale purple

*piperine

Water Not more than 14.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Total ash Not more than 7.0 per cent w/w (Appendix 7.7).

Volatile oil Not less than 1.0 per cent v/w (Appendix 7.3H). Use 50 g, in *No.150 powder,* freshly prepared and accurately weighed. Use 250 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

Alkaloid content Not less than 5.0 per cent w/w of alkaloids, calculated as piperine, when determined by the following method.

Standard piperine solution Transfer about 20 mg of *piperine*, accurately weighed, into a 100-ml volumetric flask, add *chloroform* to dissolve and dilute to volume.

Standard piperine curve Transfer into five 25-ml volumetric flasks 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1.0 ml, respectively, of *Standard piperine solution* and add *chloroform* to make 1.0 ml. To each flask add 10.0 ml of *chromotropic acid TS*, shake vigorously and place in a water-bath for 30 minutes. Set aside for a few minutes, stopper, and allow to cool. Then add 10 ml of *dilute sulfuric acid*, mix well, allow to cool, and dilute with sufficient *dilute sulfuric acid* to volume. Measure the absorbances of the piperine-containing solutions relative to the blank at 570 nm (Appendix 2.2). Plot the readings and draw the curve of best fit.

Procedure Place 500 mg of Black Pepper, in *No.150 powder* and accurately weighed, in an extraction thimble and insert the thimble into a soxhlet extractor of appropriate size. Moisten with 0.5 ml of *chloroform*, mix, allow to stand for about 5 minutes, make alkaline with 0.5 ml of *ammonia TS*, and mix. Macerate for 6 to 12 hours or overnight, cover with a pledget of *absorbent cotton*, add a sufficient quantity of *chloroform*, and extract until complete extraction of the alkaloids is effected (Appendix 7.4). Transfer the total mass to a 250-ml volumetric flask, dilute with *chloroform* to volume and filter. Transfer 1.0 ml of the filtrate to a 25-ml volumetric flask, and proceed as directed under *Standard piperine curve*, beginning with “Add 10.0 ml of *chromotropic acid TS*, ...”. Read the absorbance of the resulting solution, and by reference to the Standard piperine curve, calculate the content of alkaloids as piperine in the sample.

Dose 250 to 500 mg three times a day after meals.

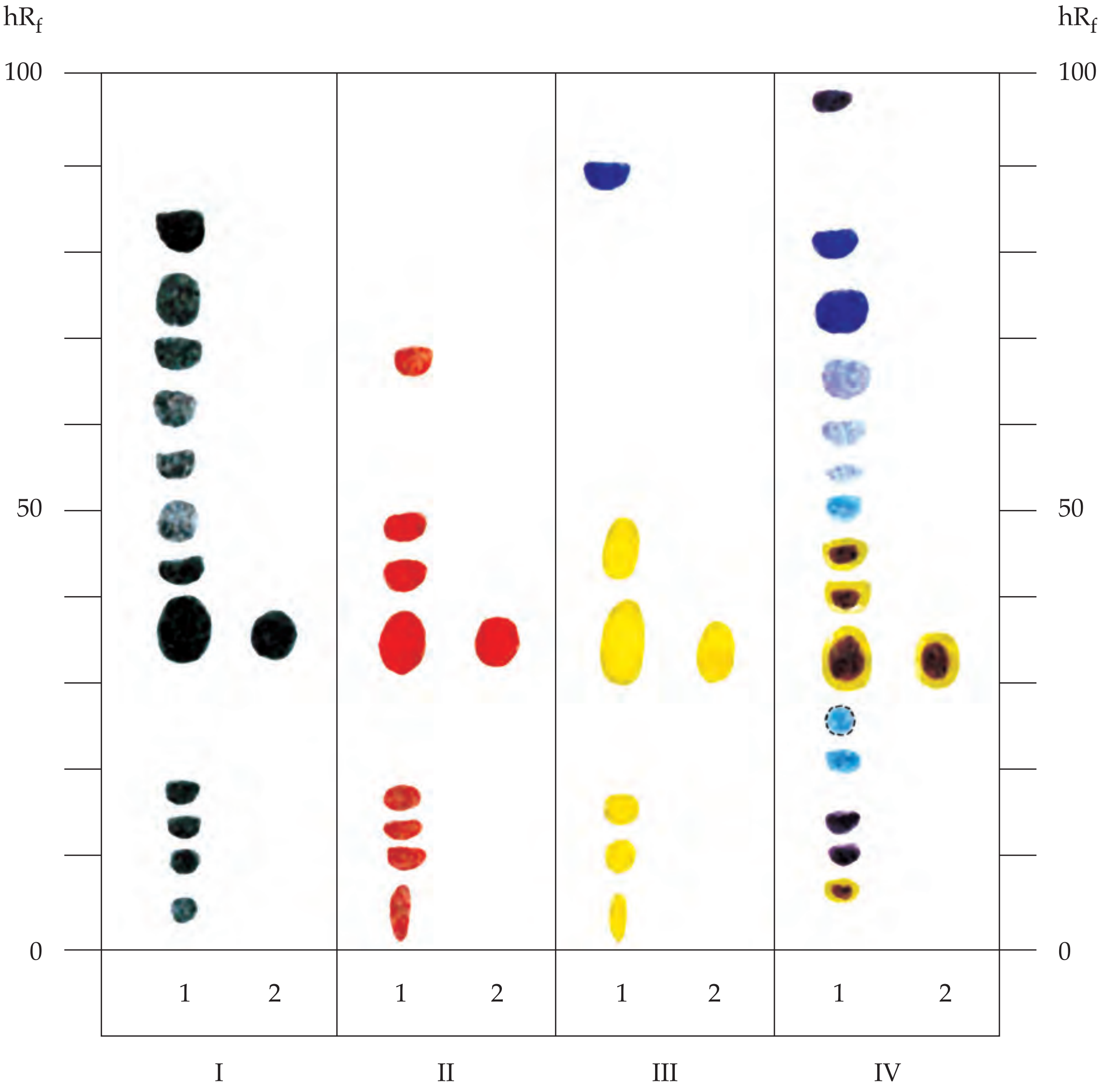


Fig. 3 Thin-layer Chromatogram of Chloroform Extract of the Fruits of *Piper nigrum* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with *acetic potassium iodobismuthate TS*
III = detection with a 50 per cent v/v solution of *sulfuric acid* in *methanol*
IV = detection with a 50 per cent v/v solution of *sulfuric acid* in *methanol* after heating
◌ = spots appear in some batches of the samples

พริกไทยล่อน (PHRIK THAI LON)

Piper Album
White Pepper

Category Aromatic, stomachic, carminative.

White Pepper consists of the dried ripe fruit, deprived of the outer part of the pericarp of *Piper nigrum* L. (Family Piperaceae), Herbarium Specimen Number: DMSC 667.

Constituents White Pepper contains the same constituents as black pepper but the amount of volatile oil is less.

Description of the plant; Packaging and storage See under *Black Pepper*.

Description Odour, aromatic; taste, pungent.

Macroscopical Spherical with a flattened and depressed apical region and slightly conical at the base; dull yellowish grey and about 4 to 5 mm in diameter; surface marked by 10 to 16 ridges running from the base to the depression at the apex, the ridges including the vascular bundles which occur in the middle layer of the pericarp; testa, exposed by scraping the residue of pericarp, appearing as a dark brown layer with a darker patch about 1.5 mm in diameter at the base; kernel consisting almost entirely of perisperm, showing, when broken, a darker greenish outer part and a lighter inner zone surrounding a central hollow; oil cells appearing as scattered bright points in the perisperm; minute embryo lying embedded in a small endosperm in a depression at the apex of the perisperm.

Microscopical The diagnostic characters are lignified beaker cells of endocarp; brown pigment layer of testa; abundant starch in compact polyhedral masses, individual grains being up to 7 μm in diameter; large oil cells, 50 to 60 μm in diameter; epidermal cells and hypodermal sclerenchyma of the outer part of the pericarp absent.

Identification

A. Complies with the tests for Identification A, B, C, and D described under *Black Pepper*.

B. Reflux 500 mg of the sample, in *fine powder*, with 5 ml of *ethanol* in a water-bath for 10 minutes, filter and evaporate the filtrate to 1 ml. Add a few drops of *ninhydrin TS*, and immerse in a water-bath for a few minute: a purplish red colour is produced.

Acid-insoluble ash Not more than 0.5 per cent w/w (Appendix 7.6).

Total ash Not more than 4.0 per cent w/w (Appendix 7.7).

Volatile oil Not less than 0.8 per cent v/w (Appendix 7.3H). Carry out the test as described under *Black Pepper*.

Water; Foreign matter; Alkaloid content Complies with the tests described under *Black Pepper.*

Dose 250 to 500 mg three times a day after meals.

รางจืด, ใบ (RANGCHUET, BAI)

ยาเขียว, ใบ (YAKHIAO, BAI), รางเย็น, ใบ (RANGYEN, BAI), เครือเขาเขียว, ใบ (KHRUEA KHAO KHIAO, BAI)
Thunbergiae Laurifoliae Folium
Blue Thunbergia Leaf

Synonym Laurel Clock Vine Leaf

Category Detoxificant, antipyretic.

Blue Thunbergia Leaf is the dried leaf of *Thunbergia laurifolia* Lindl. (Family Acanthaceae), Herbarium Specimen Number: BKF 160078, Crude Drug Number: DMSc 0688.

Constituents Blue Thunbergia Leaf contains caffeic acid, flavonoids, sterols, etc.

Description of the plant (Figs. 1a, 1b) Climber to woody climber; stem terete, glabrous to glabrescent. Leaves simple, opposite, oblong to ovate, 5 to 19 cm long, 2.5 to 9 cm wide, apex acute to acuminate, base cuneate, obtuse, truncate, or subcordate, margin entire or slightly crenate to dentate, basal veins 3 to 5, prominent on lower surface, both surfaces scabrous, glabrous or sparsely hispidulous, dark green above, lighter green beneath; petiole 1.5 to 6 cm long, sparsely hispidulous to nearly glabrous. Inflorescence pendulous, axillary or terminal raceme, 4 to 30 cm long; pedicel 1.5 to 3 cm long; bracteoles 2, ovate-lanceolate, 2 to 4.5 cm long, 1 to 2 cm wide, apex obtuse to acute, green with violet blotches. Flower blue to violet, showy, 6 to 8 cm in diameter; calyx green, 3 mm long, puberulous; corolla broadly funnelform, lobes 5, 3 to 4.5 cm long; stamens 4, didynamous, filaments puberulous; ovary superior, 2-loculed, each with 2 axile ovules, style 1, stigma 2-lobed. Fruit capsule, subglobose at base, about 1.5 cm long, about 1.0 cm wide, with a stout beak 2 cm long at the tip, splitting through the beak into 2 pieces. Seed(s) 1 or 2 per locule, plano-convex, about 1 cm in diameter.

Description Odour, mild; taste, bland.

Macroscopical (Fig. 1a) A mixture of entire and broken, brown to dark brown, oblong to ovate leaves.

Microscopical (Figs. 2a, 2b, 2c) Transverse sections of the leaf through the midrib and the lamina show upper epidermis, a layer of elongated rectangular cells, irregular and wavy-walled in surface view; diacytic stomata; glandular trichomes, unicellular stalk, 2-celled head; non-glandular trichomes, unicellular. Lower epidermis, wavy-walled in surface view; diacytic stomata, numerous; glandular trichomes, a few. Mesophyll, 2 or 3 layers of columnar palisade cells and several layers of irregular spongy cells. Underneath both epidermises, few layers of collenchyma cells associated with vascular tissues in the midrib. Vascular bundle, phloem in the upper part and xylem in the lower part, surrounded by bundle sheaths. Some bundle sheaths and parenchyma cells containing acicular crystals. Vessels, spiral, reticulate and pitted.

Blue Thunbergia Leaf in powder possesses the diagnostic microscopical characters of the unground drug.

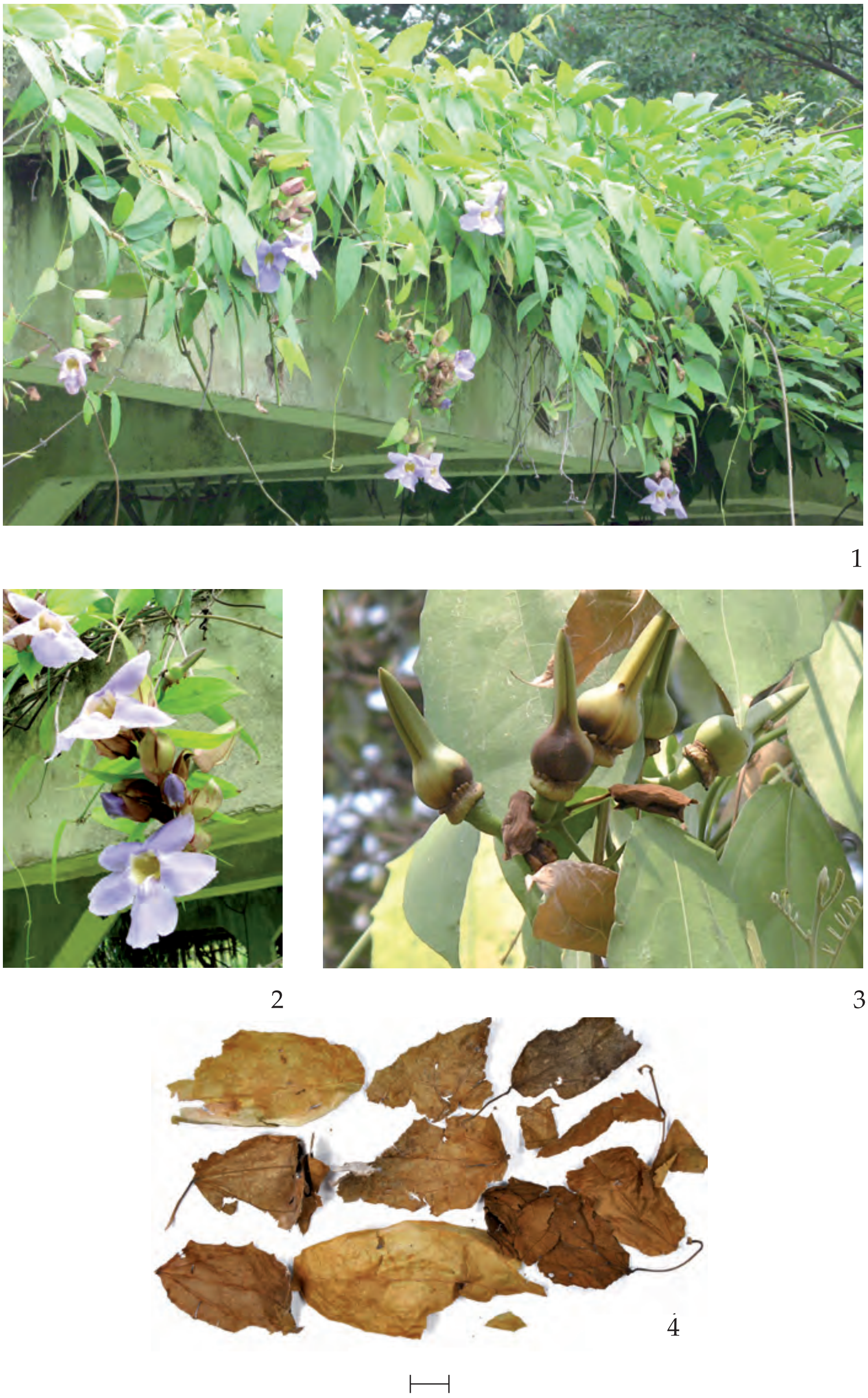
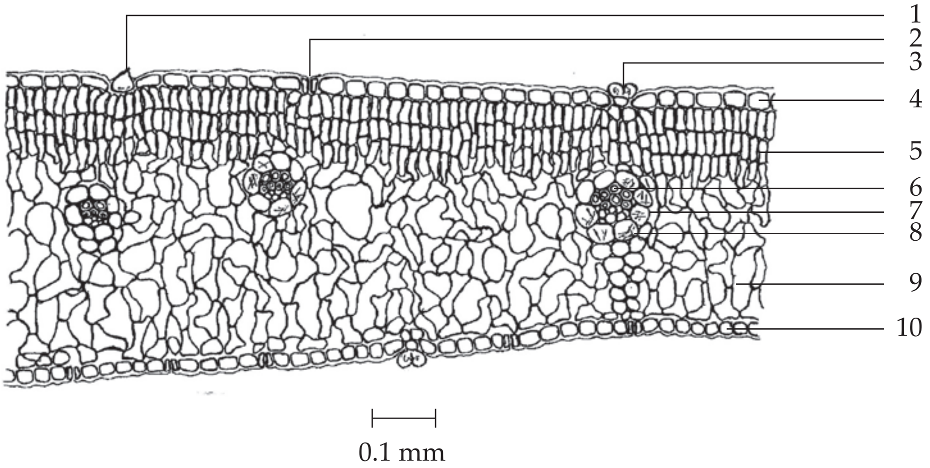
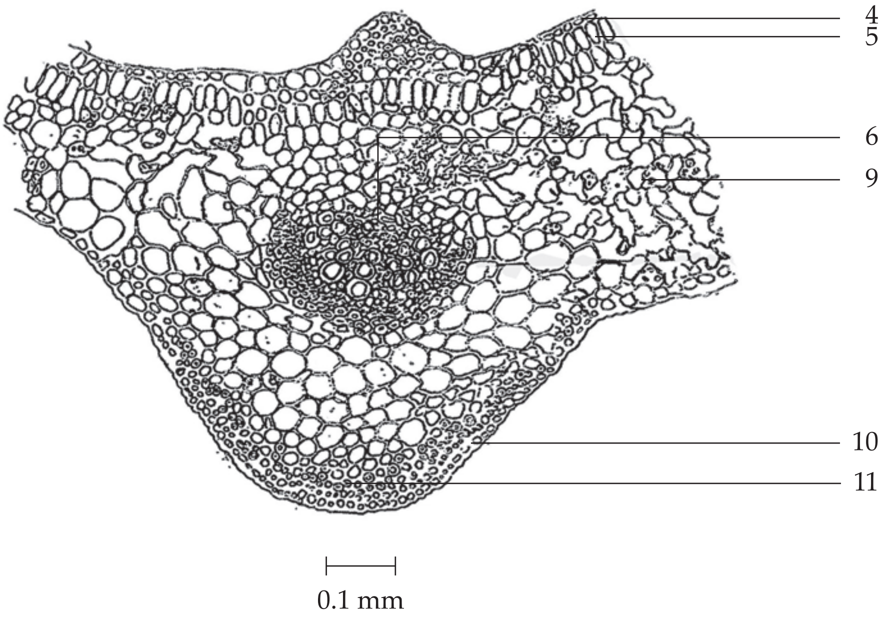


Fig. 1a *Thunbergia laurifolia* Lindl.
1. habit 2. inflorescence 3. infructescence 4. crude drug

Fig. 1b *Thunbergia laurifolia* Lindl.
1. and 2. flowering twig 3. fruiting twig



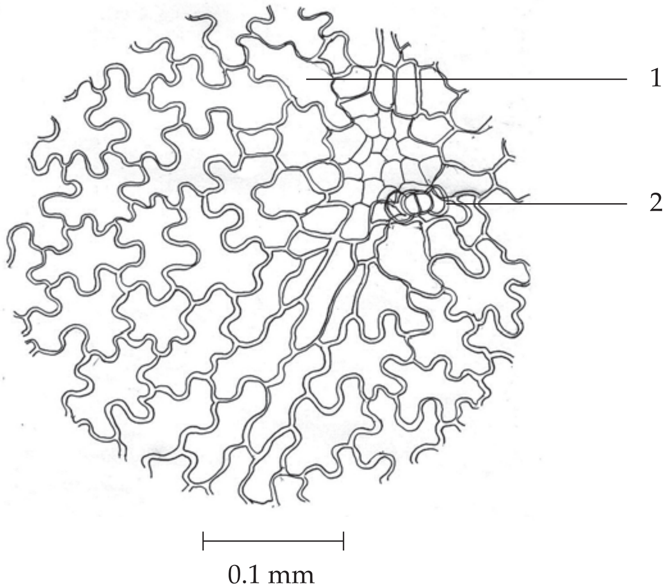
Transverse Section of the Lamina



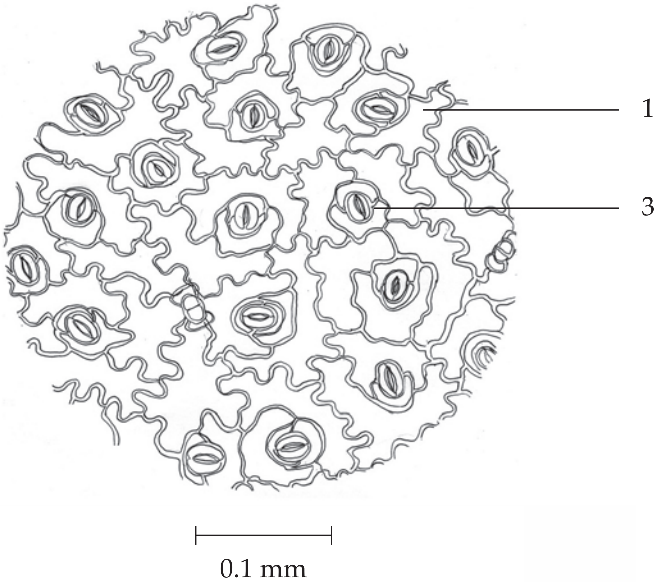
Transverse Section of the Midrib

Fig. 2a Transverse Section of the Leaf of *Thunbergia laurifolia* Lindl.

1. unicellular trichome
2. stoma
3. glandular trichome
4. upper epidermis
5. palisade cell
6. vascular bundle
7. bundle sheath
8. acicular crystals
9. spongy cell
10. lower epidermis
11. collenchyma



Upper Epidermis of the Lamina



Lower Epidermis of the Lamina

Fig. 2b Epidermises of the Leaf of *Thunbergia laurifolia* Lindl.
1. epidermal cell
2. glandular trichome
3. diacytic stoma

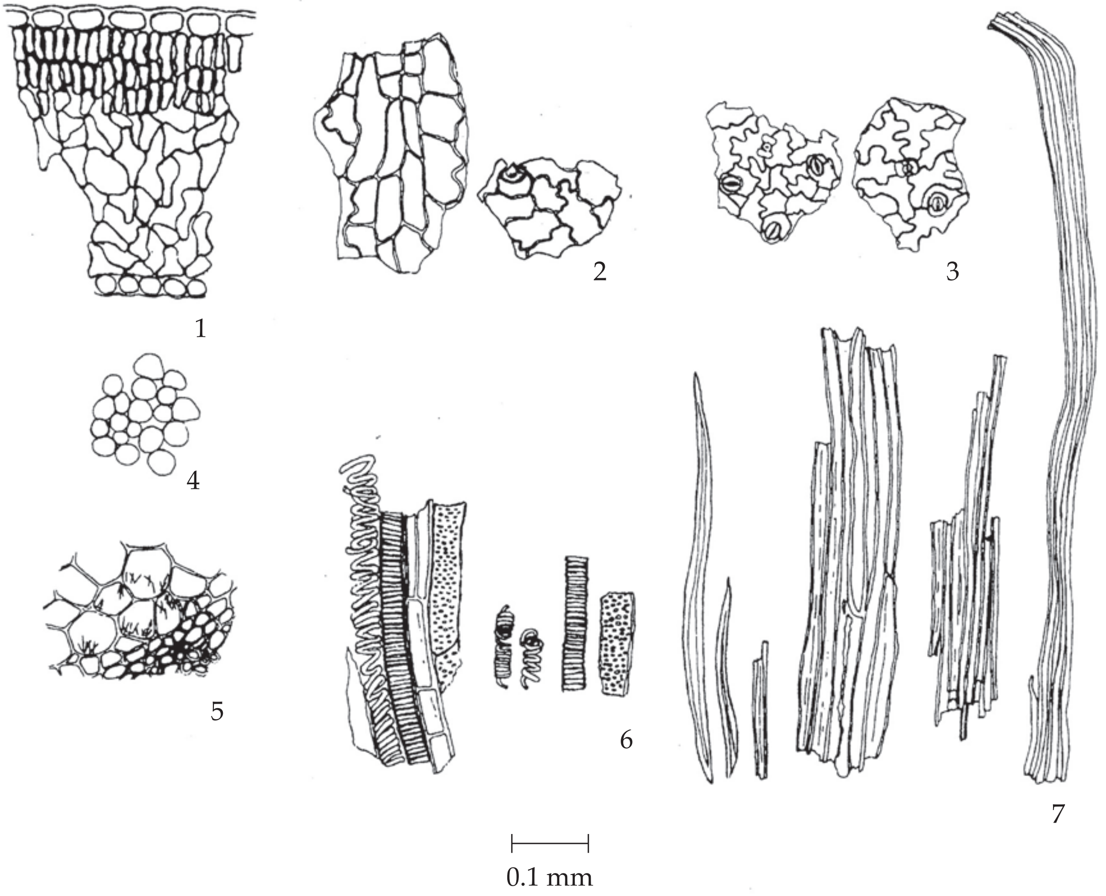


Fig. 2c Powdered Drug of the Leaves of *Thunbergia laurifolia* Lindl.

1. lamina in sectional view
2. upper epidermis with unicellular trichome
3. lower epidermis with diacytic stomata
4. palisade cells in surface view
5. collenchyma and parenchyma containing acicular crystals
6. spiral and pitted vessels
7. fibres

Warning Concomitant use with other drugs should be avoided since it may increase the excretion of the other drugs.

Additional information Ethnomedically, it has been used orally as a first-aid detoxificant. A use of 10 to 15 fresh or dried partially mature leaves, ground with sufficient amount of Nam Sao Khao (water washings from uncooked rice) or water, and squeezed to obtain about one tablespoonful of the extract, is suggested.

Packging and storage Blue Thunbergia Leaf shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 200 mg of the sample, in powder, with 10 ml of *chloroform* for 15 minutes and filter. Evaporate 2 ml of the filtrate to dryness in a test-tube. Dissolve the residue in 1 ml of *acetic anhydride*, cautiously add 1 ml of *sulfuric acid* to form two layers: a reddish brown colour forms at the zone of contact and the upper layer is green.

B. Heat 200 mg of the sample, in powder, with 10 ml of *water* in a water-bath for 10 minutes and filter. To 2 ml of the filtrate, add a few drops of *iron(III) chloride TS*: a greenish blue colour with precipitate is produced.

C. Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 70 volumes of *chloroform*, 30 volumes of *methanol* and 5 volumes of *formic acid* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate as bands of 8 mm, 8 µl of solution (A) and 3 µl of solution (B). Prepare solution (A) by refluxing 500 mg of the sample, in powder, with 25 ml of *water* for 30 minutes, filtering and evaporating to dryness. Dissolve the residue in 5 ml of a 50 per cent v/v solution of *methanol*. For solution (B), dissolve 1 mg of *caffeic acid* in 5 ml of a 50 per cent v/v solution of *methanol*. After removal of the plate, allow it to dry in air. Heat the plate at 80° for 10 minutes and then spray with *natural products (NP) TS* while the plate is still warm. Subsequently spray the plate with *polyethyleneglycol (PEG) TS* and observe the colours of the bands under ultraviolet light (366 nm) through the cut-off filter within 5 to 15 minutes. The chromatogram obtained from solution (A) shows an intense blue fluorescent band (hR_f value 83 to 90) corresponding to the caffeic acid band from solution (B). Several fluorescent bands of different colours also appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Water Extract of the Leaves of *Thunbergia laurifolia* Lindl.

Band	hR_f Value	Detection
		NP/PEG TS and UV 366
1	6-8	light blue
2	10-13	orangish brown
3	16-19	light blue
4	23-28	light blue
5	30-33	orangish brown
6	33-40	orangish brown
7	49-57	blue
8	69-78	intense blue
9*	83-90	intense blue
10	91-93	blue

*caffeic acid

Loss on drying Not more than 9.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 11.0 per cent w/w (Appendix 7.6).

Total ash Not more than 23.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 14.0 per cent w/w (Appendix 7.12).

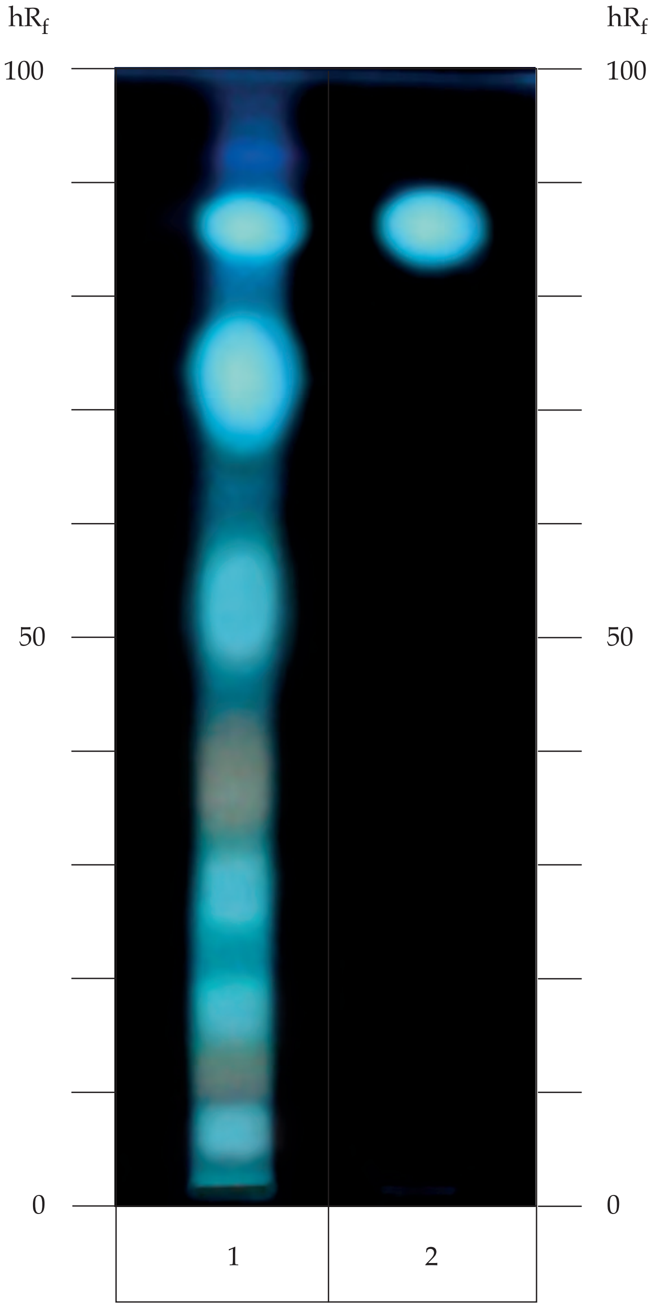


Fig. 3 Thin-layer Chromatogram of Water Extract of the Leaves of *Thunbergia laurifolia* Lindl., Detection under UV Light (366 nm) after Spraying with *NP/PEG TS*

1 = solution (A)
2 = solution (B)

สะค้าน (SAKHAN)

จะค่าน (CHAKHAN), จะค่านจิ้น (CHAKHAN CHIN), สะค้านเนื้อ (SAKHAN NEUA)
Piperis Wallichii Caulis
Piper Wallichii Stem

Category Carminative, stomachic, anti-inflammatory.

Piper Wallichii Stem is the dried stem of *Piper wallichii* (Miq.) Hand.-Mazz. (Family Piperaceae), Herbarium Specimen Number: DMSC 5175, 5176, Crude Drug Number: DMSc 0931, 0932.

Constituents Piper Wallichii Stem contains amide alkaloids (e.g., pellitorine), lignans, phenylpropanoids, aromatic glycosides, etc.

Description of the plant (Figs. 1a, 1b) Dioecious woody climber; stem black when dry, ridged, usually hispidulous; node with climbing roots. Leaves simple, alternate, ovate-lanceolate or narrowly elliptic, 5 to 26 cm long, 4 to 17.5 cm wide, apex acute to acuminate, base cuneate or cordate, slightly oblique, margin undulate, blade papery, upper surface dark green, lower surface glaucous, grayish when dry, veins 5 to 7, pinnipalmate; petiole 0.4 to 1 cm long; stipule hairy, oblong-lanceolate. Inflorescence spike, terminal or leaf-opposed, pendulous, yellow or green-yellow, cylindrical; bract spirally arranged, orbicular, about 1 mm in diameter, abaxially pubescent. Male spike 5 to 15 cm long, 3 to 5 mm wide; peduncle up to 2 cm long, puberulous; rachis sparsely pubescent; bract orbicular, 1 to 1.2 mm wide, peltate; male flower with 3 to 4 stamens, filament about 0.4 mm long, anther reniform, about 0.5 mm long; female spike 5 to 7 cm long, 2 to 5 mm wide; peduncle 2 to 4.2 cm long, pubescent; bract as in male spike; female flower with distinct ovary, subglobose, apex pointed, stigma 3- to 4-lobed, 1 to 1.5 mm in diameter. Infructescence pendulous, cylindrical, 10 to 30 cm long, 1 to 2 cm wide; stipe 2 to 4 cm long, puberulous. Fruit drupe, sessile, more or less globose, 3 to 4 mm in diameter, tuberculate, orange to red when ripe, stigma and bract persistent.

Description Odour, aromatic and pungent; taste, pungent and spicy.

Macroscopical (Fig. 1a) Sliced vine, varied in shape, length, width, and thickness; bark, fragile and breakable, externally wrinkled, brown, with numerous raised lenticels; a part of roots may be found in some pieces; internally light brown, with radial lines originated from dark brown ring encircled light brown core.

Microscopical (Figs. 2a, 2b) Transverse section of the stem shows cork layer, cortex and stele. Periderm, rectangular cork cells and scattered large raised-lenticels. Cortex, round parenchyma cells (some containing starch grains), scattered groups of thick-walled sclereids (with distinct pore canals), and circular layers of thick-walled sclereids, respectively. Stele, anomalous with two regions of vascular tissues, round parenchyma cells with scattered mucilage canals; outer region of vascular tissue, several large strips of vascular bundles and broad bands medullary rays; inner region, small strips of vascular bundles and small bands medullary rays; vascular bundles, ectophloic type; pith, round thin-walled parenchyma cells some containing starch grains and mucilage canals.

Piper Wallichii Stem in powder possesses the diagnostic microscopical characters of the unground drug. Parenchyma containing simple and compound starch grains, numerous large bordered-pitted vessels, and mostly rectangular thick-walled sclereids with distinct pore canals are characteristic.

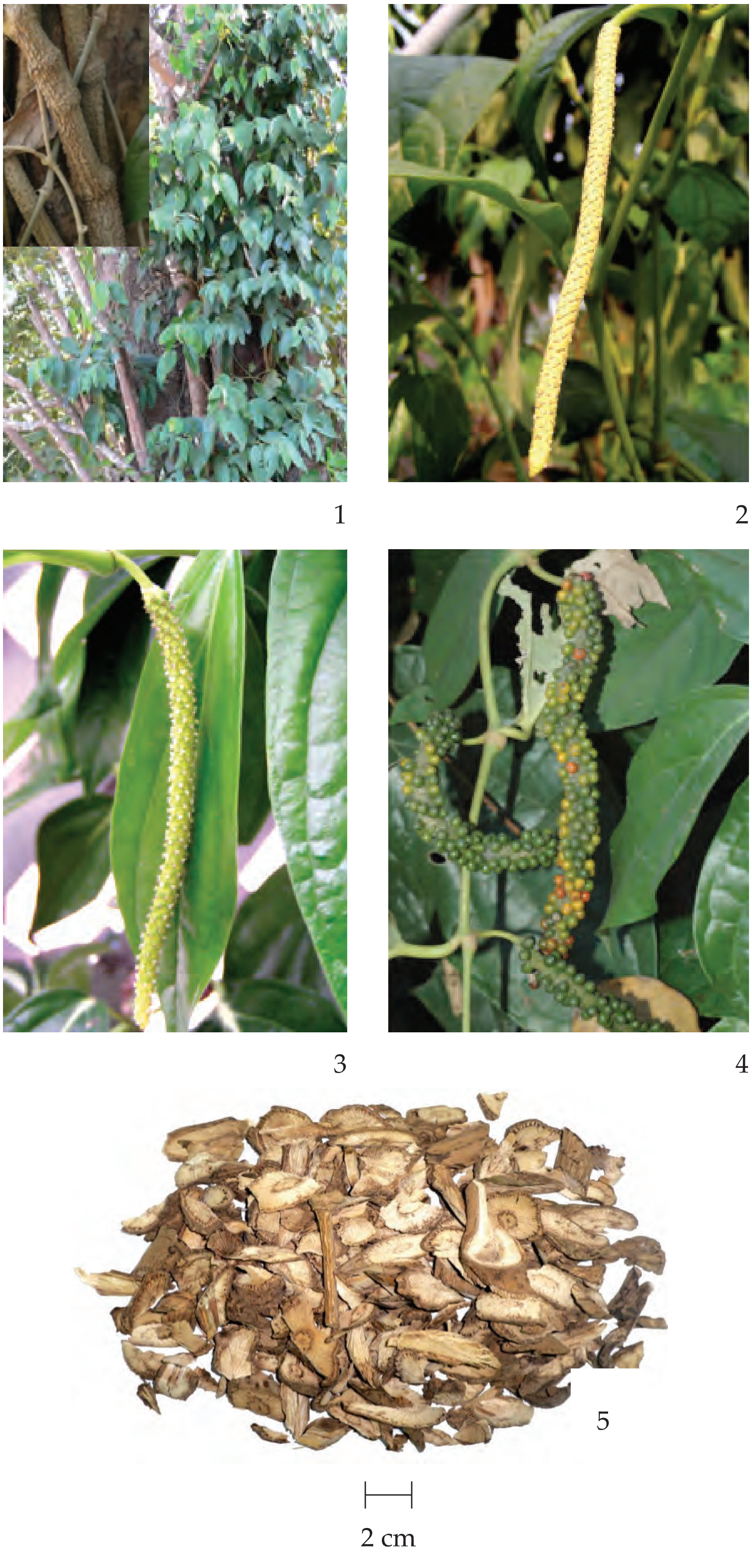


Fig. 1a *Piper wallichii* (Miq.) Hand.-Mazz.
1. habit and stem 2. male inflorescence 3. female inflorescence
4. infructescences 5. crude drug

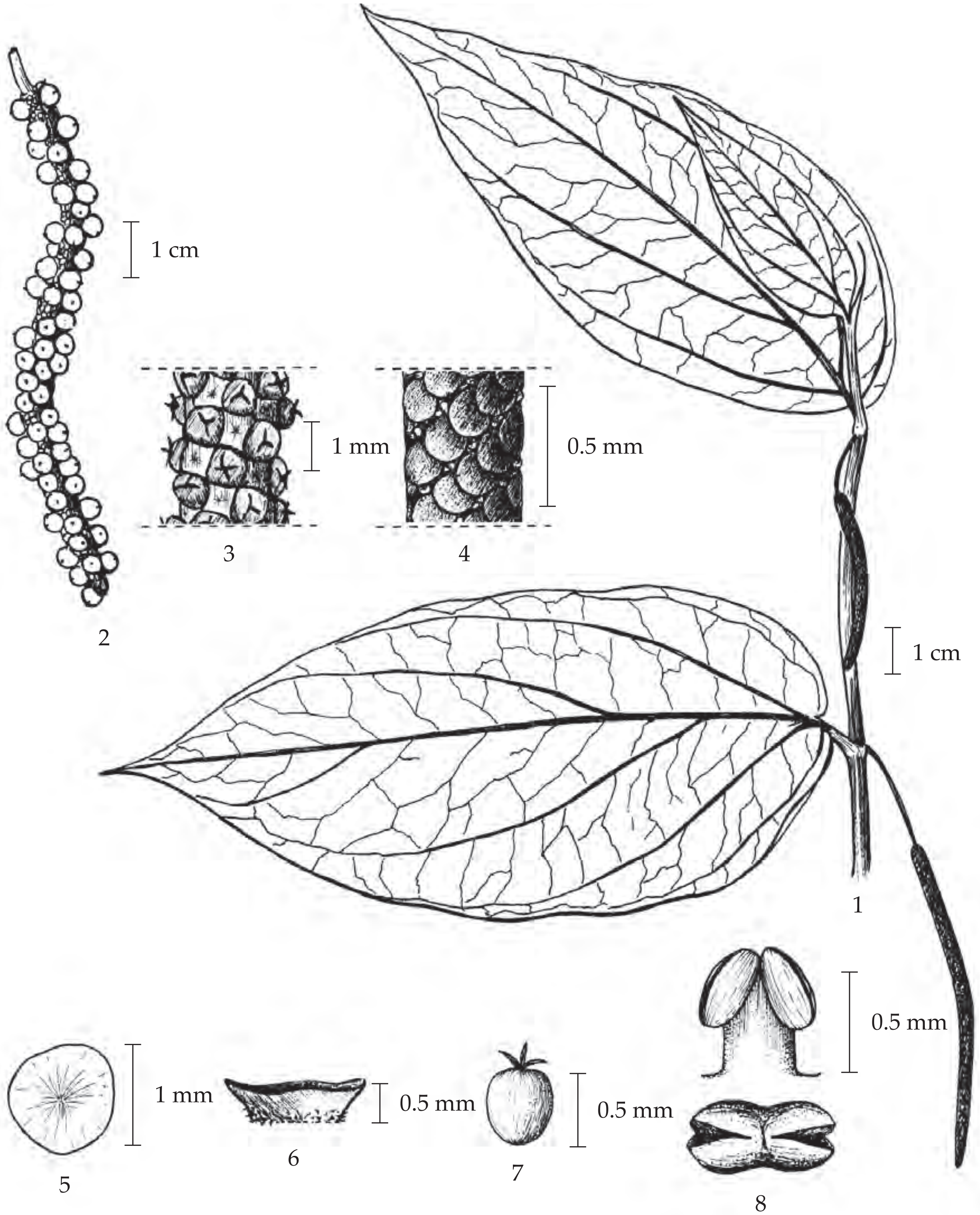


Fig. 1b *Piper wallichii* (Miq.) Hand.-Mazz.
1. twig showing leaves and male inflorescences 2. infructescence
3. part of female inflorescence 4. part of male inflorescence 5. top view of bract
6. side view of bract 7. ovary 8. side view of stamen and top view of anther sacs

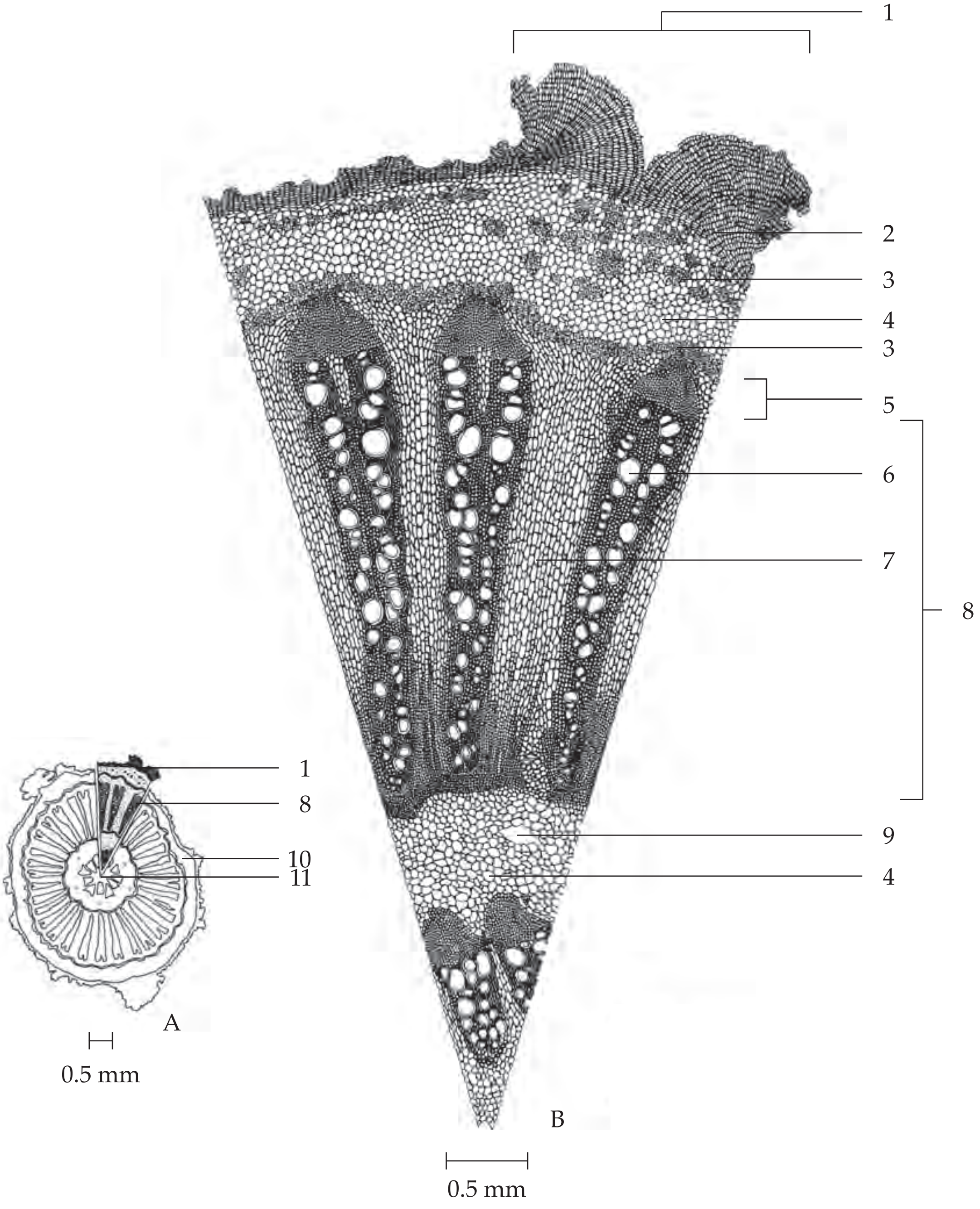


Fig. 2a Transverse Section of the Stem of *Piper wallichii* (Miq.) Hand.-Mazz.

A. Diagram
B. Part of Sectional View
1. lenticel
2. cork
3. sclereids
4. parenchyma with starch grains
5. phloem
6. vessel
7. medullary ray
8. xylem
9. mucilage canal
10. cortex
11. pith

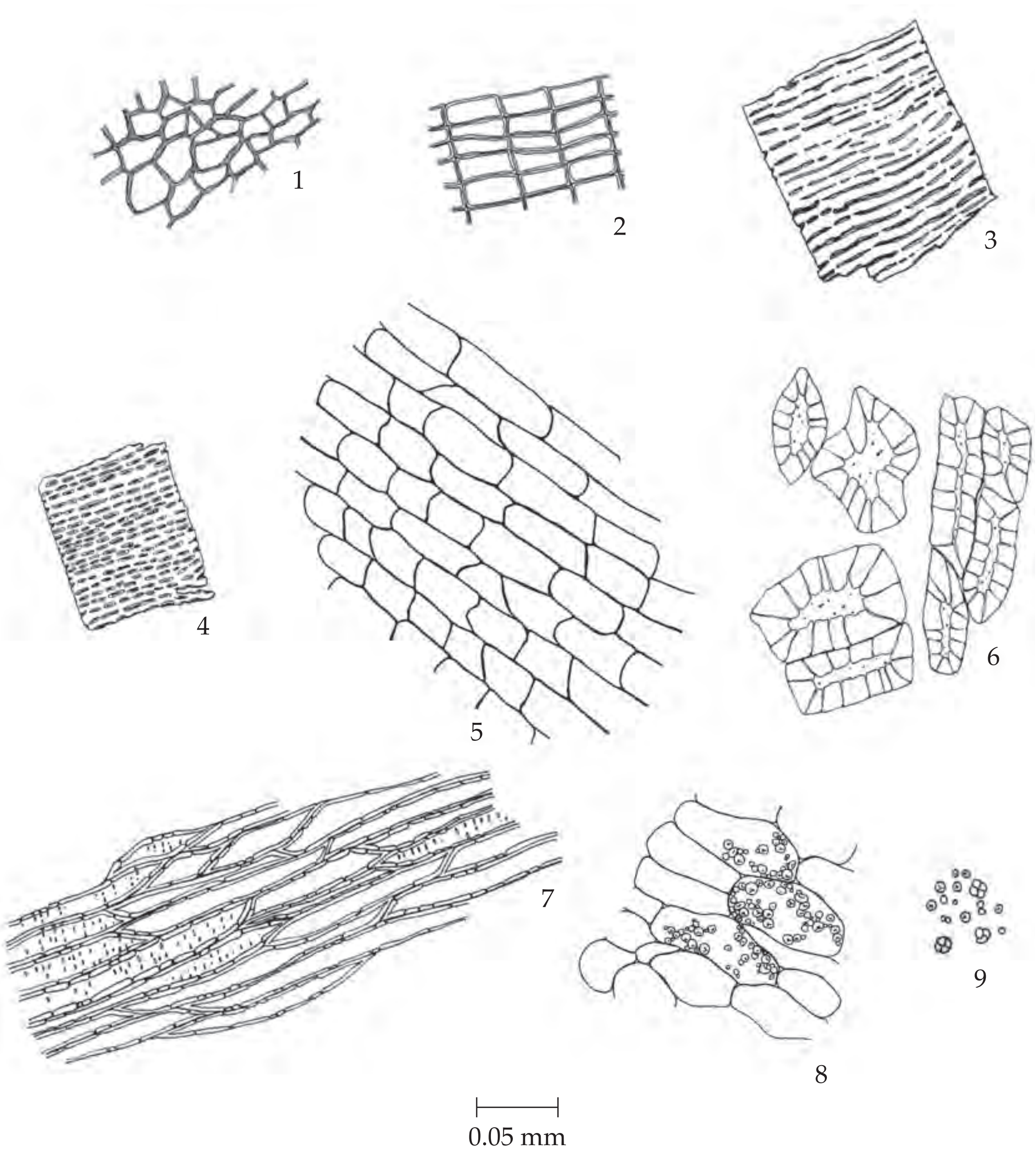


Fig. 2b Powdered Drug of the Stems of *Piper wallichii* (Miq.) Hand.-Mazz.

1. cork in surface view
2. cork in sectional view
3. fragment of large elongated-pitted vessel
4. fragment of large bordered-pitted vessel
5. parenchyma in longitudinal section
6. sclereids
7. fibres
8. parenchyma with starch grains
9. starch grains

Additional information

1. The crude drug entitled "Sakhan" sold in herbal markets in Thailand may refer to other *Piper* species. However, *Piper wallichii* (Miq.) Hand.-Mazz. is cultivated and commercially available in the markets throughout northern Thailand.
2. It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Piper Wallichii Stem shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 1 g of the sample, in powder, with 20 ml of *ethanol* on a water-bath for 15 minutes and filter (solution 1). To 1 ml of solution 1, add a few drops of *modified Dragendorff TS2*: an orange precipitate is produced.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 95 volumes of *dichloromethane*, 5 volumes of *methanol* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply to the plate, 2 µl of the test solution prepared by evaporating 10 ml of solution 1 to dryness and dissolving the residue in 2 ml of *dichloromethane*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter; several fluorescent spots of different colours are observed. Spray the plate with *modified Dragendorff TS2*. Two orange spots are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Stems of *Piper wallichii* (Miq.) Hand.-Mazz.

Spot	hR_f Value	Detection		
		UV 254	UV 366	*Modified Dragendorff TS2*
1	5-9	weak quenching	green-blue	–
2	13-21	–	blue	–
3	17-24	–	red	–
4	20-30	–	light red	–
5	23-33	quenching	–	–
6	25-35	–	blue	–
7	27-40	–	green	–
8	35-48	–	blue	–
9	45-57	–	blue	–
10	50-61	weak quenching	light blue	–
11	53-64	–	light blue	–
12	58-70	quenching	blue	orange
13	64-73	quenching	blue	orange
14	69-76	–	light blue	–
15	82-83	–	red	–

Loss on drying Not more than 8.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Total ash Not more than 9.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 6.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 25.0 per cent w/w (Appendix 7.12).

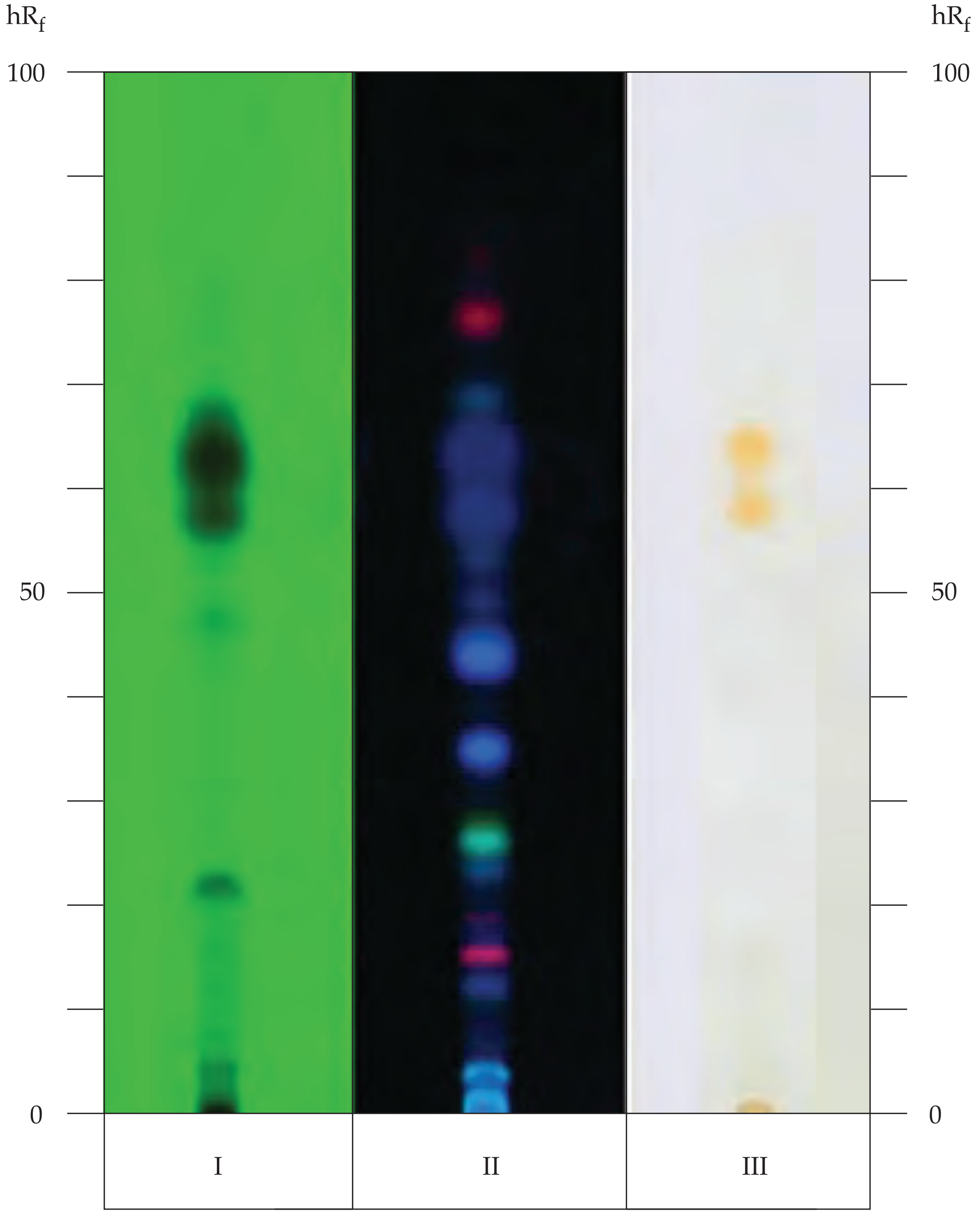


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Stems of *Piper wallichii* (Miq.) Hand.-Mazz.

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *modified Dragendorff TS2*

สมอพิเภก (SAMO PHIPHEK)

Terminaliae Belliricae Fructus
Belleric Myrobalan

Category Laxative, carminative, astringent, expectorant.

Belleric Myrobalan is the dried mature fruit of *Terminalia bellirica* (Gaertn.) Roxb. (*T. belerica* Roxb., *Myrobalanus bellirica* Gaertn.) (Family Combretaceae), Herbarium Specimen Number: DMSC 92, 171.

Constituents Belleric Myrobalan contains tannins which are chebulagic acid, ellagic acid, gallic acid, etc. It also contains β-sitosterol and green fixed oil.

Description of the plant (Figs. 1a, 1b) Large tree, up to 50 m high, deciduous, up to 2 m in girth, usually with large buttresses; bark blackish, brittle, longitudinally fissured and cracked, thick, cut yellow. Leaves coriaceous, obovate, 4 to 20 cm by 2 to 15 cm, glabrous, nerves widely spaced, 6 to 8 pairs; petiole glabrous, 3 to 9 cm long, usually with a pair of dotted glands at about the middle or near leaf-base, occasionally inconspicuous or hardly observed when dry. Inflorescences spike or raceme, 3 to 15 cm long, often crowded at ends of branchlets without leaves so as to form terminal panicles; flowers andromonoecious, male on the upper part, tomentose; calyx 1 to 2 mm long, 4 to 5 mm in diameter; calyx-segments recurved, deltoid, 1.5 mm long; stamens 3 to 3.5 mm long; ovary ellipsoid, 2 to 3.5 mm by 1.5 to 3 mm; style 4 mm long; disc densely, rusty villous. Fruits drupe, subglobose to broadly ellipsoid, 2 to 3.5 cm by 1.5 to 3 cm, slightly 5-ridged, densely velvety pubescent, very hard when dry. Seed 1, ellipsoid, rough, 1.2 cm by 0.5 cm.

Description Odour, indistinct; taste, bitter, acrid and astringent.

Macroscopical (Fig. 1a) Subglobose to broadly ellipsoid, 2 to 3.5 cm long, 1.5 to 3 cm wide, slightly 5-ridged, the base extended as a short stalk, densely velvety brownish pubescent, very hard. One seed about 1.5 cm long and 1 cm wide, one edge more acute than the other, in the central part of hard endocarp.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the fruit shows epicarp composed of a layer of thick-walled epidermal cells with unicellular trichomes. Mesocarp, 2 to 3 layers of collenchyma followed by a broad zone of parenchyma in which sclereids and stone cells, in group, and vascular bundles scattered, porous parenchyma; sclereids, various shapes and sizes, mostly elongated; tannins; prism and aggregate crystals of calcium oxalate in parenchyma. Endocarp consists of thick-walled sclereids of various shapes and sizes, mostly elongated. Sclereids, stone cells and vessels, lignified. Testa, one layer of large cubical cells, followed by a zone of reticulate parenchyma and vessels; tegmen consists of a zone of parenchyma and collapse parenchyma. Cotyledon folded and containing aleurone grains, oil globules and rosette aggregate crystals.

Belleric Myrobalan in powder possesses the diagnostic microscopical characters of the unground drug.

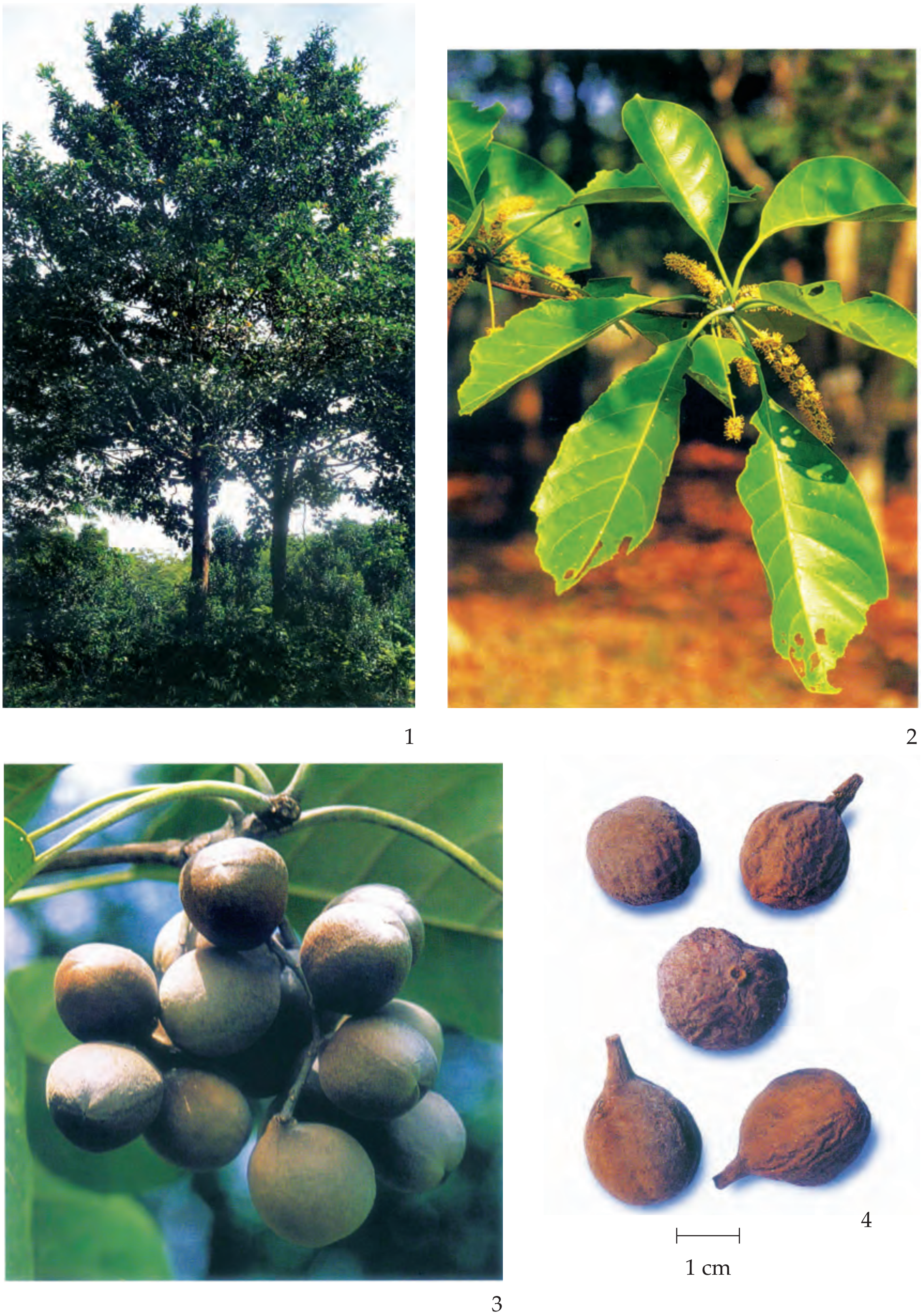


Fig. 1a *Terminalia bellirica* (Gaertn.) Roxb.
1. habit 2. flowering twig 3. fruiting twig 4. crude drug

Fig. 1b *Terminalia bellirica* (Gaertn.) Roxb.
1. flowering twig 2. flower 3. fruits

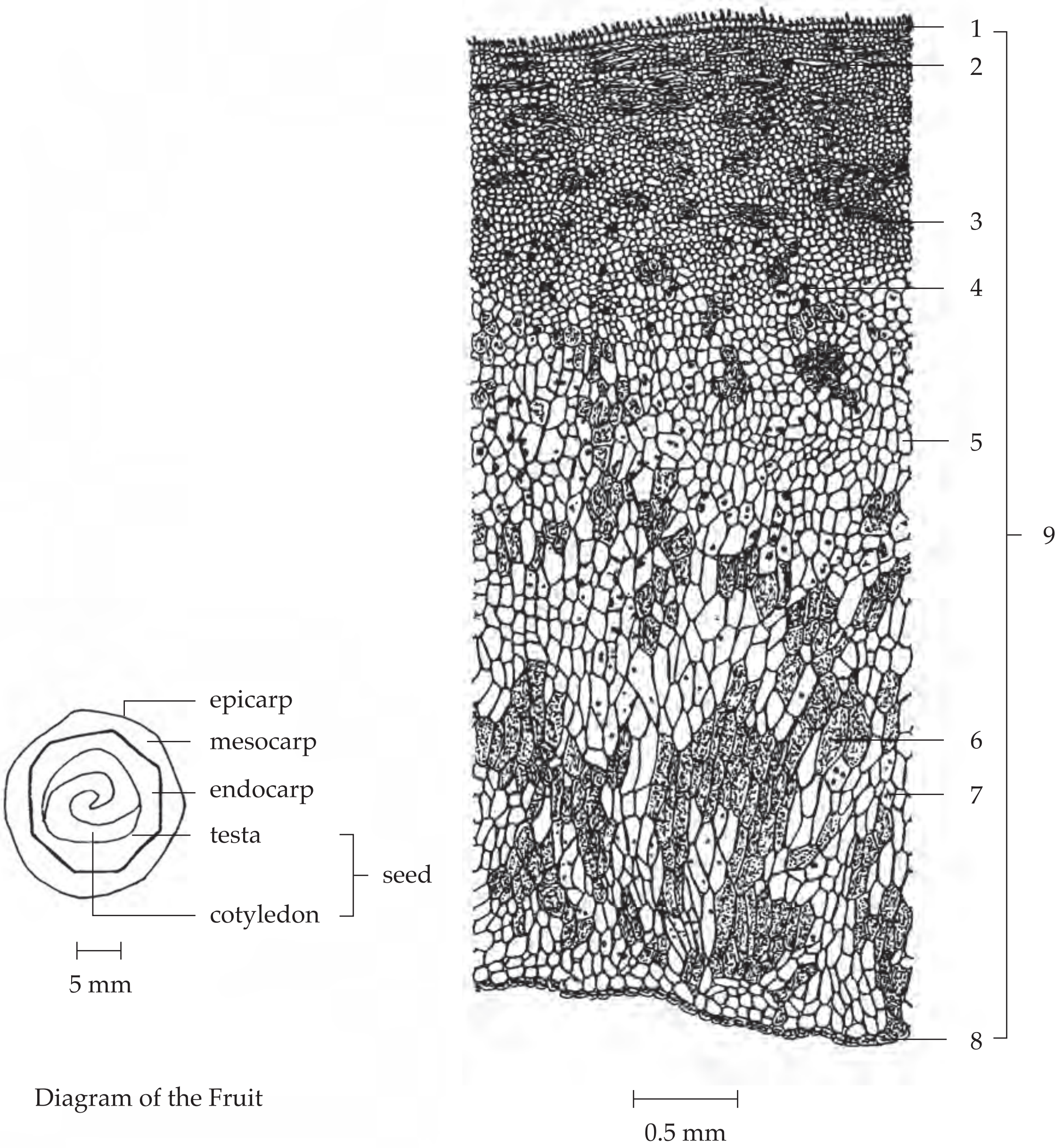
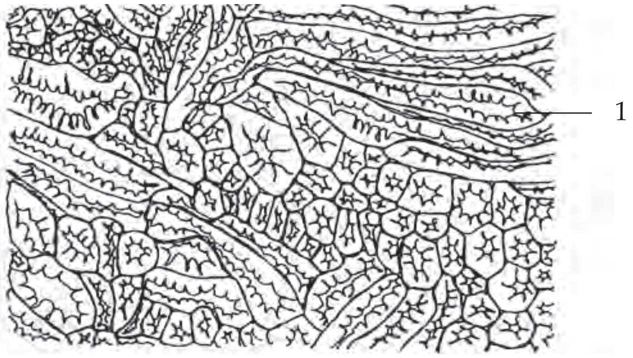


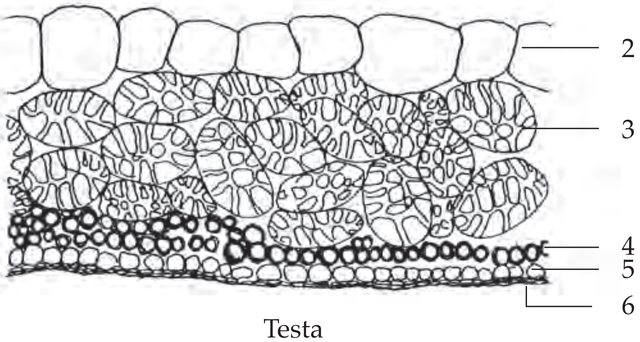
Diagram of the Fruit

Fig. 2a Transverse Section of the Fruit Pulp of *Terminalia bellirica* (Gaertn.) Roxb.

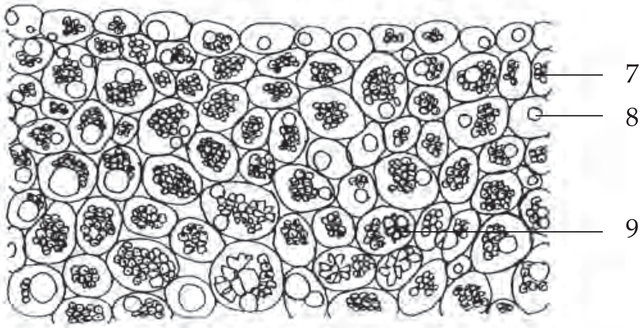
1. epidermis of epicarp with unicellular trichomes
2. sclereids
3. vascular bundle
4. brownish black mass
5. ground parenchyma
6. porous parenchyma
7. prism of calcium oxalate crystal
8. sclerenchymatous endocarp
9. mesocarp



Endocarp



Testa



Cotyledon

0.05 mm

Fig. 2b Transverse Section of the Endocarp, Testa and Cotyledon of the Fruit of *Terminalia bellirica* (Gaertn.) Roxb.

1. sclereid
2. cubical cell
3. reticulate parenchyma
4. vessels
5. parenchyma
6. collapse parenchyma
7. aleurone grains
8. oil globule
9. rosette aggregate crystals

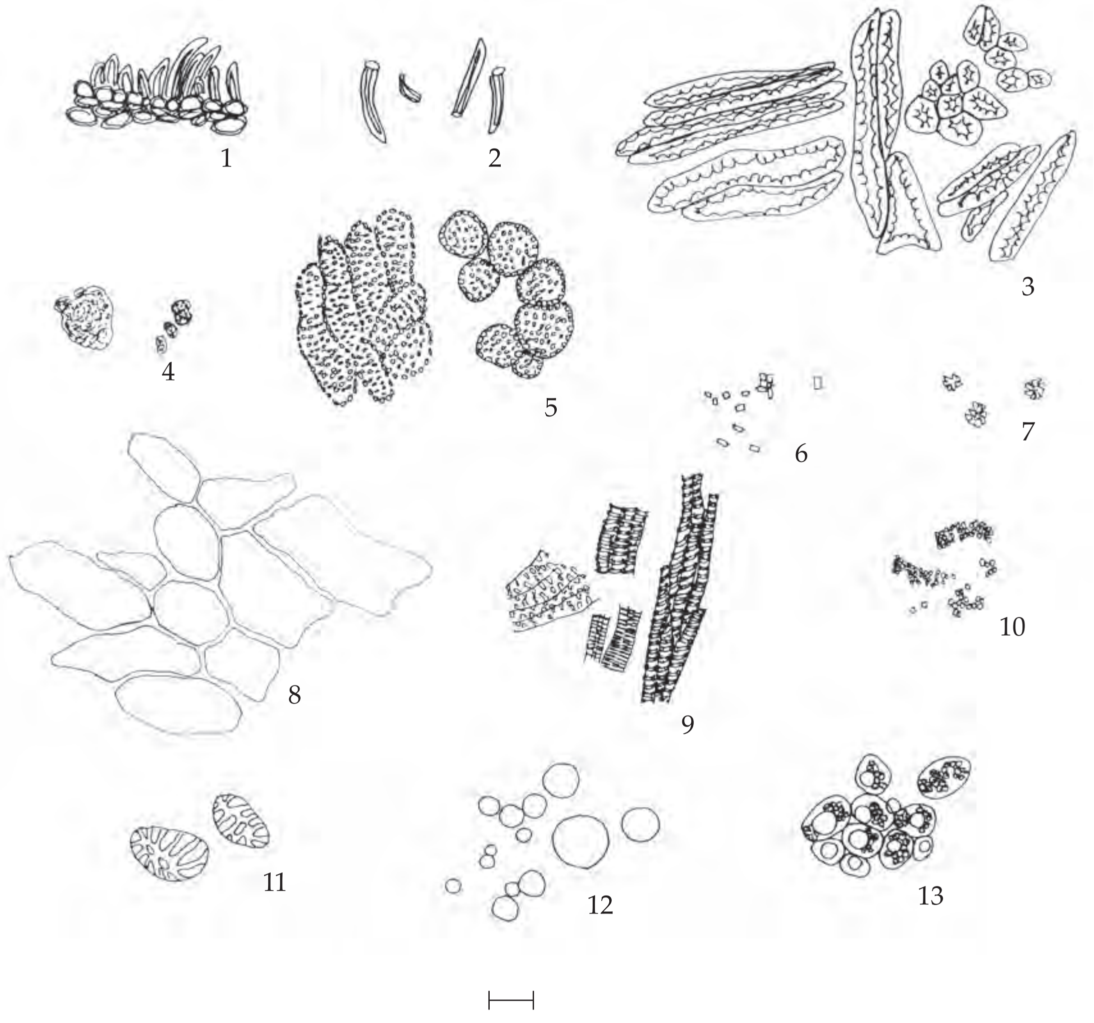


Fig. 2c Powdered Drug of the Fruits of *Terminalia bellirica* (Gaertn.) Roxb.

1. epidermis with unicellular trichomes and thick-walled cell in sectional view
2. unicellular trichomes
3. sclereids
4. masses of brownish black material
5. porous parenchyma
6. prismatic crystals
7. rosette aggregate crystals
8. ground parenchyma
9. vessels
10. starch grains
11. reticulate parenchyma
12. oil globules
13. parenchyma containing aleurone grains, oil globules and rosette aggregate crystals

Additional information A half-ripe fruit is used as a laxative.

Packaging and storage Belleric Myrobalan shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 500 mg of the sample, in powder, with 15 ml of *chloroform* for 15 minutes, filter, and evaporate the filtrate to dryness. Dissolve the residue in 2 ml of *acetic anhydride*, and then slowly add 1 ml of *sulfuric acid* to form two layers: a brown ring with a green upper layer forms.

B. To 200 mg of the sample, in powder, add 10 ml of *water*, boil in a water-bath for 5 minutes and filter. To 1 ml of the filtrate, add a few drops of *iron(III) chloride TS*: a deep blue colour develops.

C. To 500 mg of the sample, in powder, add 10 ml of *ethanol*, boil in a water-bath for 20 minutes and filter. To 1 ml of the filtrate, add 0.5 ml of *ninhydrin TS*, and warm in a water-bath for a few minutes: a purple colour is produced.

D. Reflux 200 mg of the sample, in powder, with 10 ml of *water* for 5 minutes and filter. Transfer 1 ml of the filtrate to a test-tube and shake for a few minutes: no foam is produced.

E. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 10 volumes of *toluene*, 9 volumes of *ethyl acetate* and 2 volumes of *anhydrous formic acid* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate, 4 µl of solution (A) and 2 µl of solution (B). Prepare solution (A) by refluxing 100 mg of the sample, in powder, with 5 ml of *water* for 15 minutes, filtering and evaporating to dryness. Dissolve the residue in 1 ml of *water*. For solution (B), dissolve 2 mg of *gallic acid* in 1 ml of *ethanol (50 per cent)*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 47 to 50), corresponding to the gallic acid spot from solution (B), and several spots of higher and lower hR_f values. Spray the plate with *anisaldehyde TS*, heat at 105° for 5 minutes and examine under ultraviolet light (366 nm); the spot due to gallic acid is purple and other two purple and one yellow fluorescent spots are observed (Table 1); see also Fig. 3.

Repeat the same procedure on another plate but spray with excess amount of *potassium hexacyanoferrate(III) TS* and then *iron(III) chloride TS*. The chromatogram obtained from solution (A) shows a blue spot (hR_f value 47 to 50), corresponding to the gallic acid spot from solution (B), and other two to three blue spots (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Water Extract of the Fruits of *Terminalia bellirica* (Gaertn.) Roxb.

Spot	hR_f Value	Detection		
		UV 254	*Anisaldehyde TS* and UV 366	*Potassium Hexacyanoferrate(III) TS* and *Iron(III) Chloride TS*
1	2-4	–	purple	–
2	4-5	quenching	–	–
3	5-7	–	–	blue
4	31-33	–	yellow	–
5	36-38	quenching	–	–
6	43-44	quenching	–	–
7	50-51	quenching	–	–
8	58-59	–	purple	blue
9*	60-61	quenching	purple	blue
10	66-69	quenching	–	blue
11	78-88	quenching	–	–

*gallic acid

Loss on drying Not more than 11.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Acid-insoluble ash Not more than 0.6 per cent w/w (Appendix 7.6).

Total ash Not more than 5.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 17.0 per cent w/w (Appendix 7.12).

Ethanol (70 per cent)-soluble extractive Not less than 29.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 24.0 per cent w/w (Appendix 7.12).

Tannins content Not less than 16.0 per cent w/w (Appendix 7.21H). Use 4 g of Belleric Myrobalan, in powder, accurately weighed.

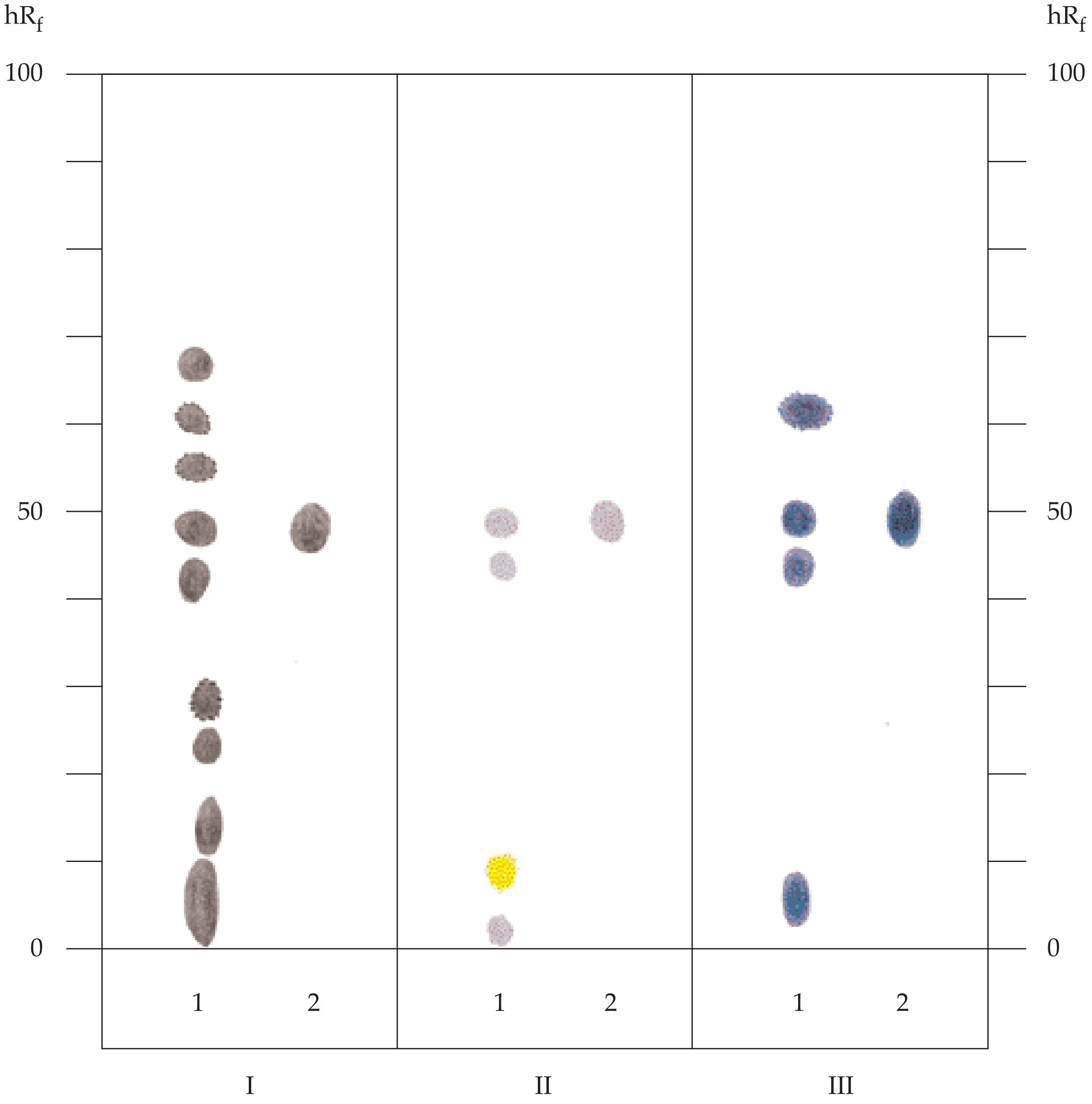


Fig. 3 Thin-layer Chromatogram of Water Extract of the Fruits of *Terminalia bellirica* (Gaertn.) Roxb.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm) after spraying with *anisaldehyde TS*
III = detection with *potassium hexacyanoferrate(III) TS* and *iron(III) chloride TS*
◌ = spots developed in some samples

สมอไทย (SAMO THAI)

สมออัพยา (SAMO APPHAYA)
Terminaliae Chebulae Fructus
Chebulic Myrobalan

Category Laxative, carminative, astringent, expectorant.

Chebulic Myrobalan is the dried mature or nearly mature fruit of *Terminalia chebula* Retz. (Family Combretaceae), Herbarium Specimen Number: DMSC 899.

Constituents Chebulic Myrobalan contains tannins which are chebulinic acid, chebulic acid, tannic acid, gallic acid, etc. It also contains β-sitosterol, saponins, and fixed oil.

Description of the plant (Figs. 1a, 1b) Medium-sized or large tree, up to 30 m high, up to 1.3 m in girth; bark rough, scaly; shoots and young leaves usually rusty villous. Leaves simple, opposite, coriaceous, broadly ovate to ovate-elliptic, 8 to 15 cm by 6 to 10 cm, glabrescent, nerves obscure above, slightly raised and usually brownish pubescent beneath, apex acute or abruptly acuminate, base cuneate, slightly cordate or rounded; petiole 1 to 3 cm long, glabrous or sparsely pubescent with a pair of nodular glands near leaf-base. Inflorescence axillary or terminal panicles, usually with 3 to 6 spikes; spikes 3 to 6 cm long; rachis pubescent; flowers 2 mm long, 3 to 4 mm in diameter; bracts nearly glabrous, 1.5 to 2 mm long ; calyx outside glabrous, inside densely villous, calyx-segments triangular; stamens 3 to 4 mm long; ovary glabrous, ovoid, 1 mm long; style glabrous, 2.5 to 3 mm long; disc lobed, densely villous. Fruit drupe, glabrous, subglobose to ellipsoid, 2.5 to 5.0 cm by 1.5 to 2.5 cm, usually smooth or frequently 5-angulate ridged, wrinkled, turning blackish when dry. Seed 1, rough, ellipsoid, 1.5 to 2.0 cm by 0.5 to 0.7 cm, without ridges.

Description Odour, indistinct; taste, sour, slightly bitter and astringent.

Macroscopical (Fig. 1a) Long ovoid to ovoid, 2.5 to 5 cm long, 1.5 to 2.5 cm wide, externally yellowish brown to dark brown, slightly gross, five to six longitudinal ridges, irregular arranged wrinkles in the inter ridge. The base marked by a circular scar, about 2 mm in diameter. One seed, about 6 mm in diameter in the central part of hard endocarp.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the fruit shows epicarp composed of a layer of epidermal cells, the outer tangential wall and upper portion of the radial walls, thick. Mesocarp, 2 to 3 layers of collenchyma followed by a broad zone of parenchyma in which fibres and sclereids, in group, and vascular bundles, scattered; fibres, simple-pitted walls, porous parenchyma; sclereids, various shapes and sizes, mostly elongated; tannins and aggregate crystals of calcium oxalate in parenchyma. Endocarp consists of thick-walled sclereids of various shapes and sizes, mostly elongated. Fibres, sclereids and vessels, lignified. Testa, one layer of large cubical cells, followed by a zone of reticulate parenchyma and vessels; tegmen consists of collapse parenchyma. Cotyledon folded and containing aleurone grains, oil globules and some rosette aggregate crystals.

Chebulic Myrobalan in powder possesses the diagnostic microscopical characters of the unground drug.

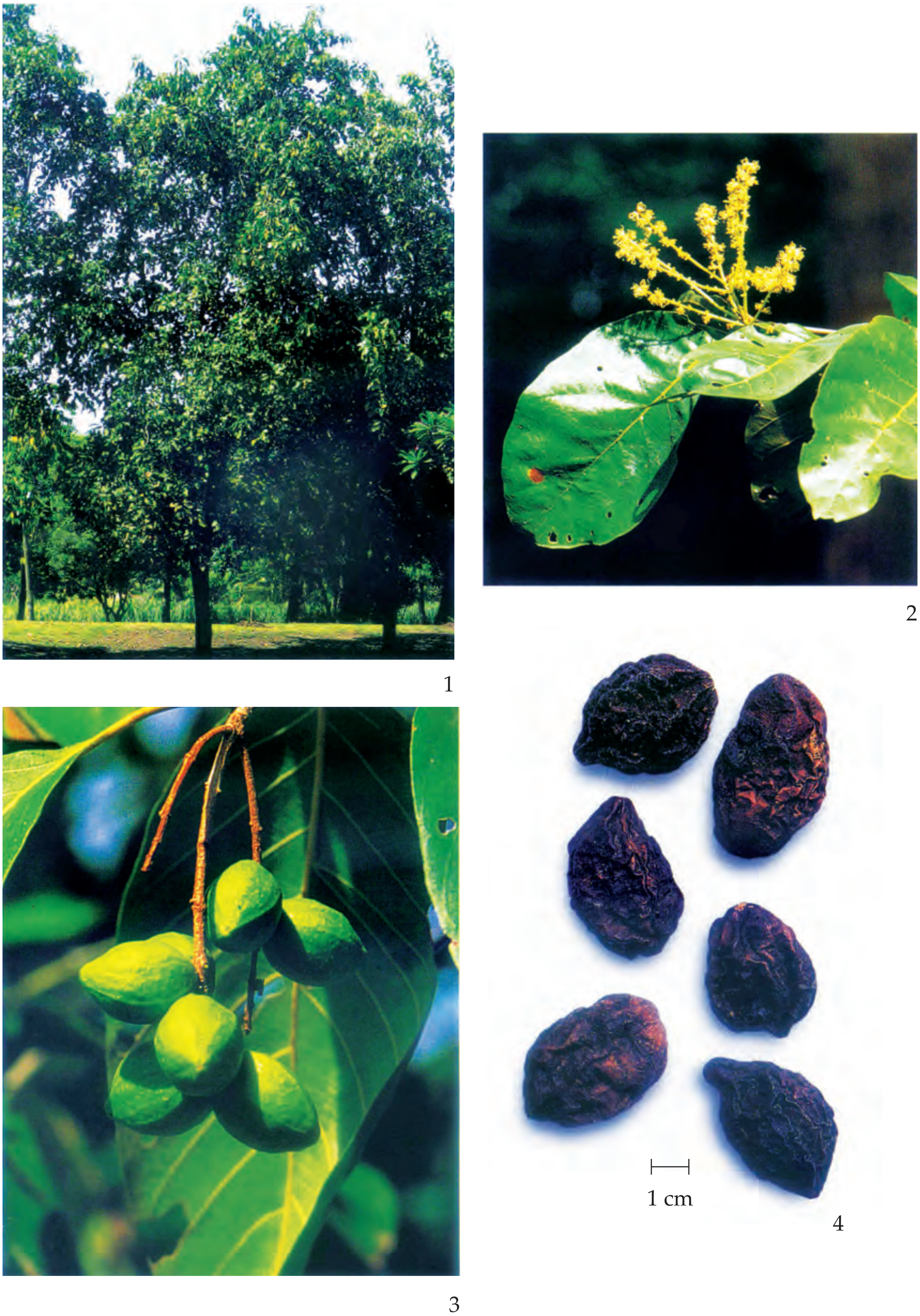


Fig. 1a *Terminalia chebula* Retz.
1. habit 2. flowering twig 3. fruiting twig 4. crude drug

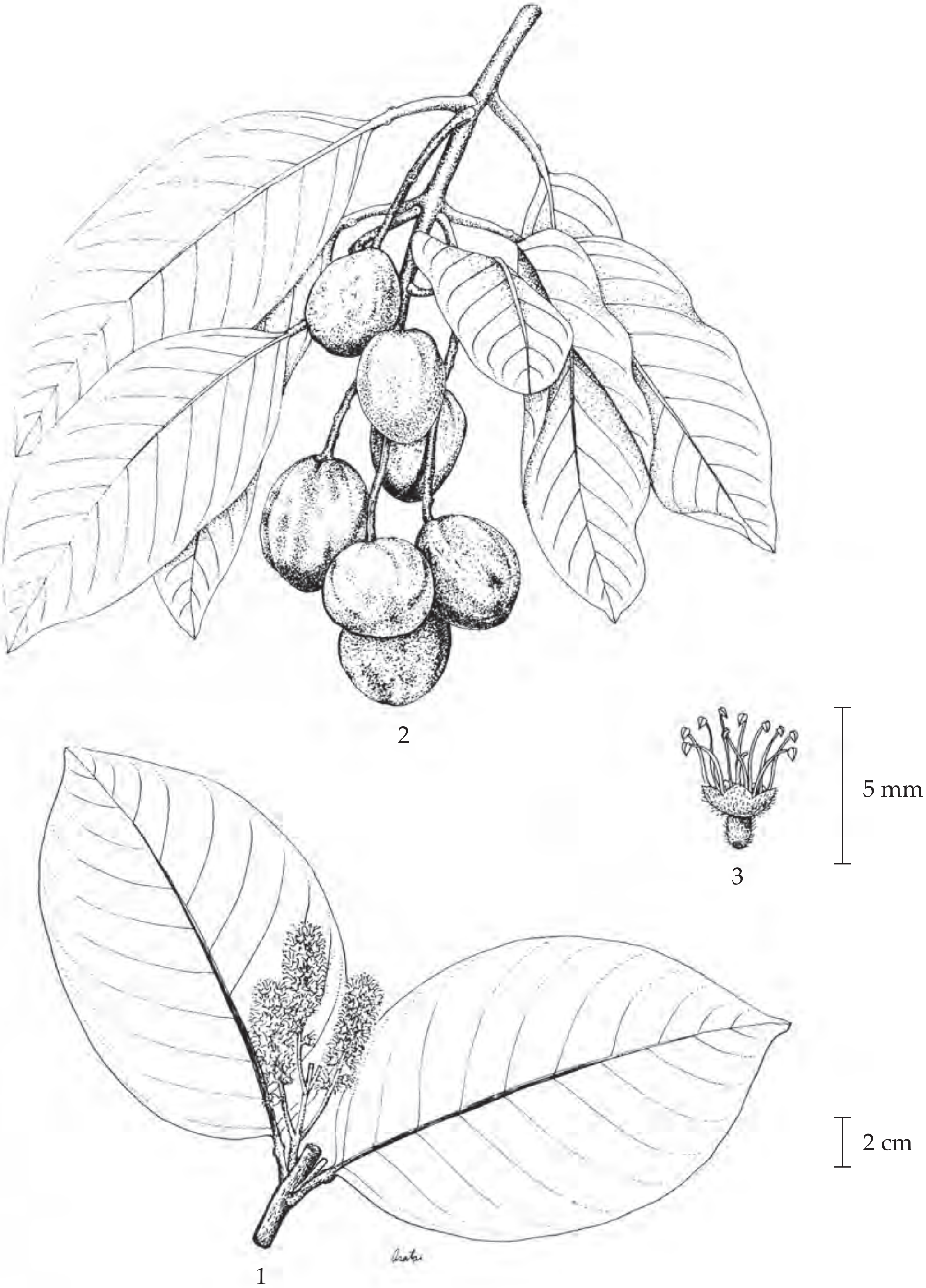


Fig. 1b *Terminalia chebula* Retz.
1. flowering twig 2. fruiting twig 3. flower

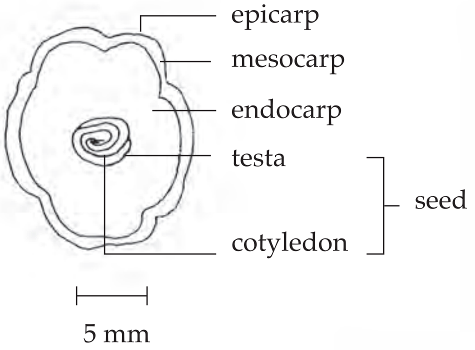


Diagram of the Fruit

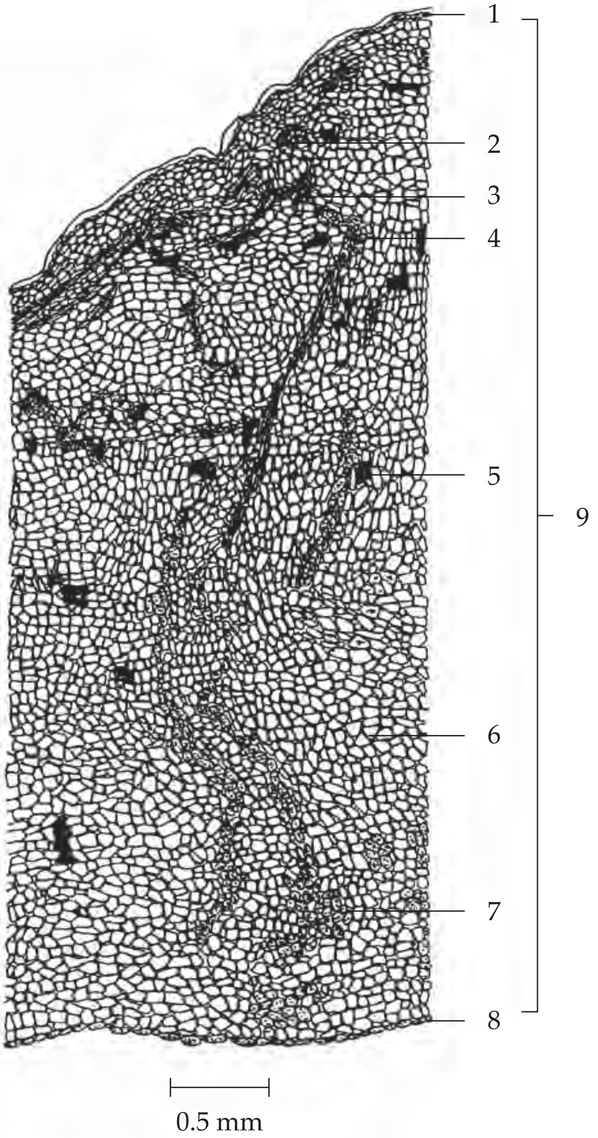


Fig. 2a Transverse Section of the Fruit Pulp of *Terminalia chebula* Retz.

1. epidermis of epicarp
2. fibres
3. sclereids
4. vascular bundle
5. brownish black mass
6. ground parenchyma
7. porous parenchyma
8. sclerenchymatous endocarp
9. mesocarp

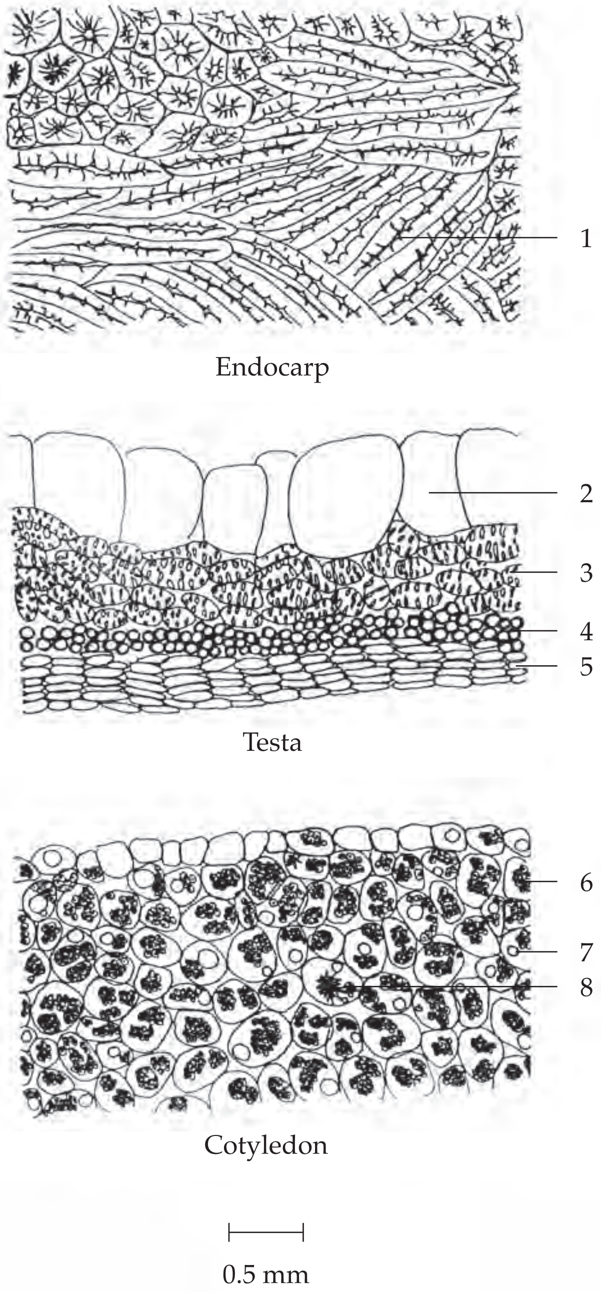


Fig. 2b Transverse Section of the Endocarp, Testa and Cotyledon of the Fruit of *Terminalia chebula* Retz.

1. sclereid
2. cubical cell
3. reticulate parenchyma
4. vessels
5. collapse parenchyma
6. aleurone grains
7. oil globule
8. rosette aggregate crystals

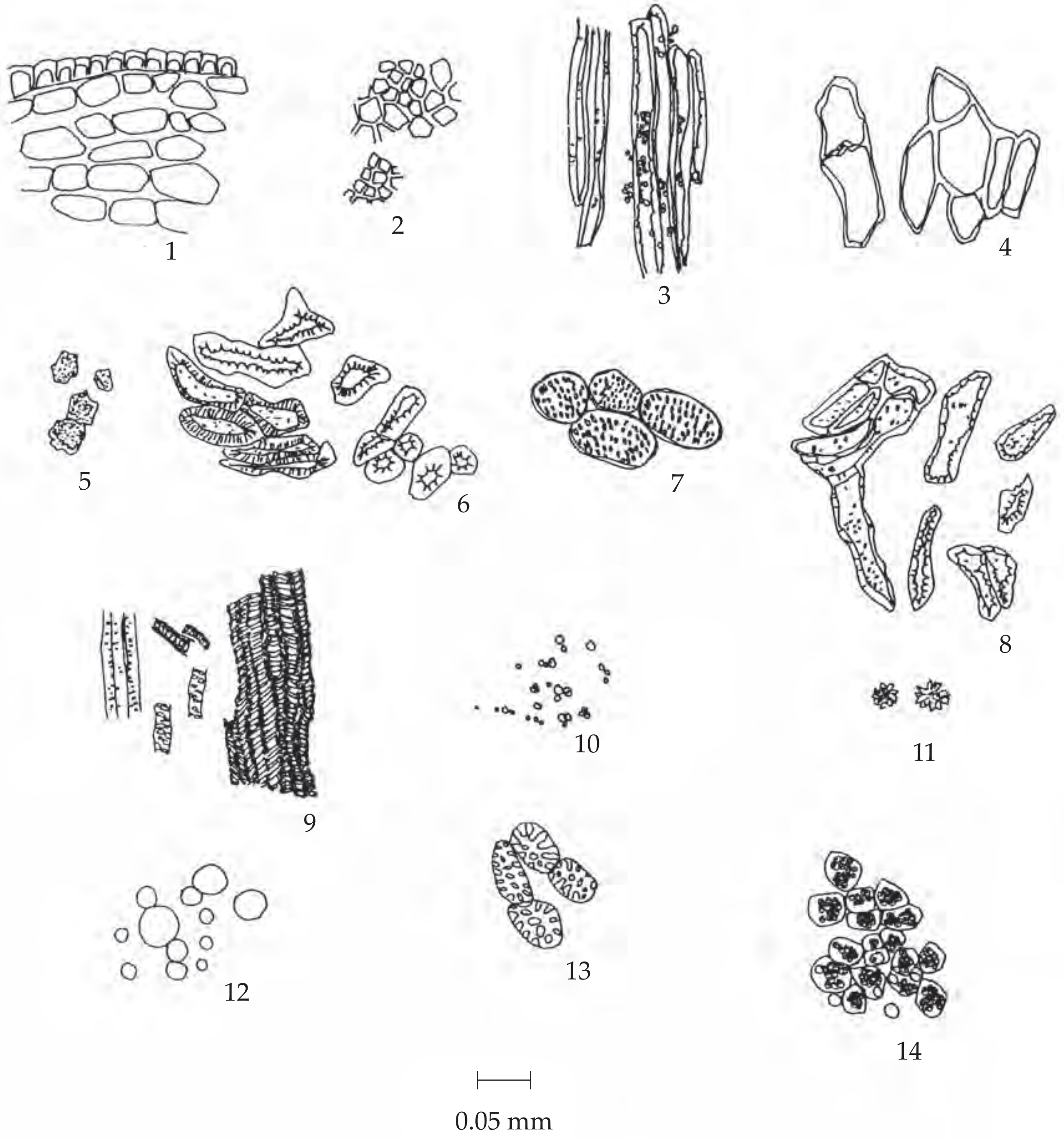


Fig. 2c Powdered Drug of the Fruits of *Terminalia chebula* Retz.

1. epidermis and thick-walled cell in sectional view
2. epidermis in surface view
3. fibres and starch grains
4. ground parenchyma
5. masses of brownish black material
6. sclerenchyma of endocarp
7. porous parenchyma
8. sclereids
9. vessels
10. starch grains
11. rosette aggregate crystals
12. oil globules
13. reticulate parenchyma
14. parenchyma containing aleurone grains, oil globules and rosette aggregate crystals

Packaging and storage Chebulic Myrobalan shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 500 mg of the sample, in powder, with 15 ml of *chloroform* for 15 minutes, filter, and evaporate the filtrate to dryness. Dissolve the residue in 2 ml of *acetic anhydride*, and then slowly add 1 ml of *sulfuric acid* to form two layers: a brownish red ring with green upper layer forms.

B. Complies with the tests for Identification B and C described under *Belleric Myrobalan*.

C. Reflux 200 mg of the sample, in powder, with 10 ml of *water* for 5 minutes and filter. Transfer 1 ml of the filtrate to a test-tube and shake for a few minutes: a persisting foam is produced for over 30 minutes.

D. Complies with the test for Identification E described under *Belleric Myrobalan*.
After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 47 to 50), corresponding to the gallic acid spot from solution (B), and several spots of higher and lower hR_f value. Spray the plate with *anisaldehyde TS*, heat at 105° for 5 minutes, and examine under ultraviolet light (366 nm); the spot due to gallic acid is purple and other two purple and one red fluorescent spots are observed (Table 1); see also Fig. 3.

Repeat the same procedure on another plate but spray with excess amount of *potassium hexacyanoferrate(III) TS* and then *iron(III) chloride TS*. Observe immediately, the chromatogram obtained from solution (A) shows a blue spot (hR_f value 47 to 50), corresponding to the gallic acid spot from solution (B), other three blue and one white spots. After standing overnight, another blue spot appears (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Water Extract of the Fruits of *Terminalia chebula* Retz.

Spot	hR_f Value	Detection		
		UV 254	*Anisaldehyde TS* and UV 366	*Potassium Hexacyanoferrate(III) TS* and *Iron(III) Chloride TS*
1	2-4	–	purple	–
2	4-5	quenching	–	–
3	5-7	–	–	blue
4*	10-17	quenching	red	blue
5	17-22	quenching	–	–
6	41-46	quenching	purple	blue
7**	47-50	quenching	purple	blue
8	50-53	–	–	white
9	54-58	quenching	–	–
10	59-64	quenching	–	blue

*spot appears after standing overnight

**gallic acid

Loss on drying Not more than 11.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Acid-insoluble ash Not more than 0.6 per cent w/w (Appendix 7.6).

Total ash Not more than 3.5 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 20.0 per cent w/w (Appendix 7.12).

Ethanol (70 per cent)-soluble extractive Not less than 29.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 28.0 per cent w/w (Appendix 7.12).

Tannins content Not less than 14.0 per cent w/w (Appendix 7.21H). Use 4 g of Chebulic Myrobalan, in powder, accurately weighed.

Foaming index Not less than 170, when determined by the following method.

Sample preparation Transfer about 1 g of Chebulic Myrobalan, in *coarse powder*, accurately weighed, into a 500-ml conical flask containing 100 ml of boiling *water*. Maintain at moderate boiling for 30 minutes. Cool, filter and dilute the filtrate with *water* to 100.0 ml.

Procedure Transfer *Sample preparation* into 10 stoppered test-tubes (16 mm x 16 cm) in a series of successive portions of 1, 2, 3, up to 10 ml and dilute each tube, if necessary, with *water* to 10 ml. Stopper and shake in a lengthwise motion for 15 seconds, 2 frequencies per second. Allow to stand for 15 minutes and measure the height of the foam.

If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.

If in any tube a height of foam of 1 cm is measured, the dilution in this tube (a) is the index sought. If this tube is the first or second tube in a series, it is necessary to have an intermediate dilution prepared in a similar manner to obtain a more precise result.

If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case the determination needs to be made on a new series of dilutions of the Sample preparation in order to obtain a result.

Calculation

$$\text{Foaming index} = 1000/a,$$

where a is the volume in ml of the Sample preparation used for preparing the dilution in the tube where foaming is observed.

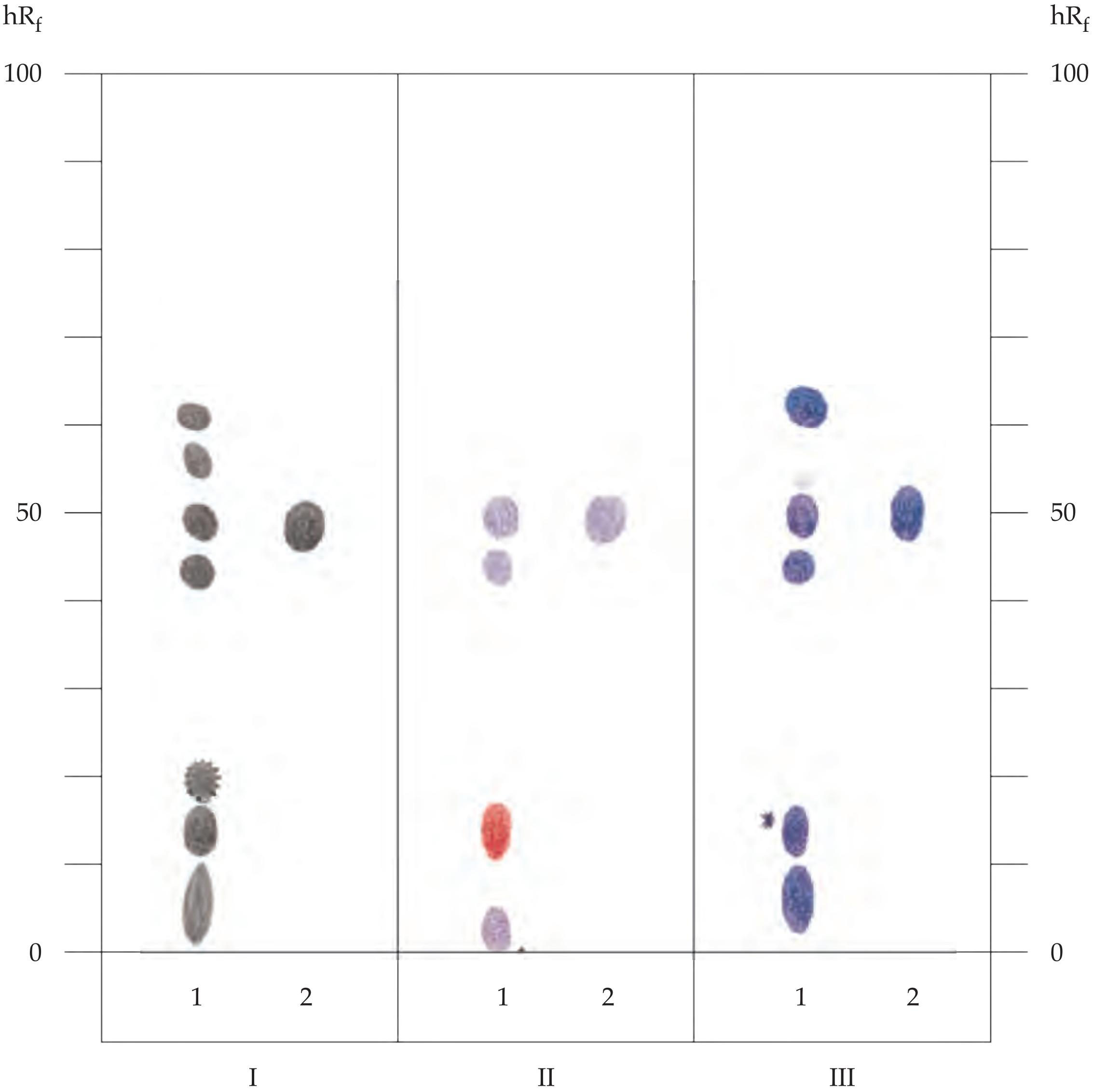


Fig. 3 Thin-layer Chromatogram of Water Extract of the Fruits of *Terminalia chebula* Retz.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm) after spraying with *anisaldehyde TS*
III = detection with *potassium hexacyanoferrate(III) TS* and *iron(III) chloride TS*
◌ = spot developed in some samples
* = spot appears after standing overnight.

สวาด (SAWAT)

Caesalpiniae Bonducis Folium
Nicker-nut Leaf

Category Carminative, antiflatulent.

Nicker-nut Leaf consists of the dried full-grown, leaflets of *Caesalpinia bonduc* (L.) Roxb. [*Guilandina bonduc* Griseb., *G. bonduccella* L., *Caesalpinia bonduccella* (L.) Fleming] (Family Leguminosae), Herbarium Specimen Number: DMSC 1090.

Constituents Nicker-nut Leaf contains a bitter substance, waxy, and alcoholic substances.

Description of the plant (Fig. 1) Climber armed with straight or recurved prickles. Stipules, pinnate consisting of 3 to 5 leaflets up to 2 cm long. Leaves bipinnately compounds, rachis 30 to 50 cm long; pinnate 3 to 9 pairs, with a pair of hooked stipulary spines at the base; leaflets, dark green, 8 to 12 pairs, opposite or subopposite, ovate-lanceolate, 2 to 4 cm long, 1 to 2 cm wide, acute or rounded and mucronulate apex, margin entire, base unequal; petiolules 0.8 mm long, stipels of short hooked spines. Inflorescences supra-axillary racemes about 6 cm long, sometimes branched; flowers yellow; bracts linear, 6 to 12 mm long, recurved, lately caducous; pedicels 4 to 6 mm long, pubescent, faintly jointed near the top; calyx tube short, segments 5, pubescent, subequal; petals 5, yellow, the upper one with a long claw and hairy inside; stamens 10, free, filaments hairy; ovary about 1 mm in diameter, stalked, hairy and bristly. Pods stalked above the receptacle (0.5 to 1 cm long), elliptic or oblong in outline, 5 to 8 cm long, 3 to 5 cm wide, set with hairy 7 to 9 mm long bristles; seeds 2, subglobular, 15 to 20 mm in diameter, greyish.

Description

Macroscopical Nicker-nut Leaf occurs as a mixture of entire and broken brownish green leaflets and frequently rachillae of the compound leaf. Leaflet short petiolule, lamina inequilaterally ovate-lanceolate, from 2 to 4 cm long and from 1 to 2 cm wide, acute or rounded and mucronulate apex, margin entire and base unequal, the upper surface brownish green, the lower surface pale greyish green and showing prominent midrib.

Microscopical (Figs. 2a, 2b) Transverse section of the leaflet shows upper epidermis, a single layer of cuticularized rectangular cells. Mesophyll consisting of a single layer of palisade parenchyma associated with oil cells and several layers of irregularly shaped spongy parenchyma rich in chloroplastids and some oil cells near the lower epidermis. Small vascular bundles with calcium oxalate prism sheaths scatter in this parenchyma region. Lower epidermis, a single layer of cuticularized rectangular cells. Transverse section through the midrib of the lamina shows several layers of collenchyma underneath the epidermis, collateral vascular bundle in the centre and the long unicellular non-glandular trichomes on upper and lower epidermides. In surface view, epidermides of the upper and lower surfaces are wavy-walled polygonal cells with anomocytic stomata in the lower epidermis.

Nicker-nut Leaf in powder possesses the diagnostic microscopical characters of the unground drug.

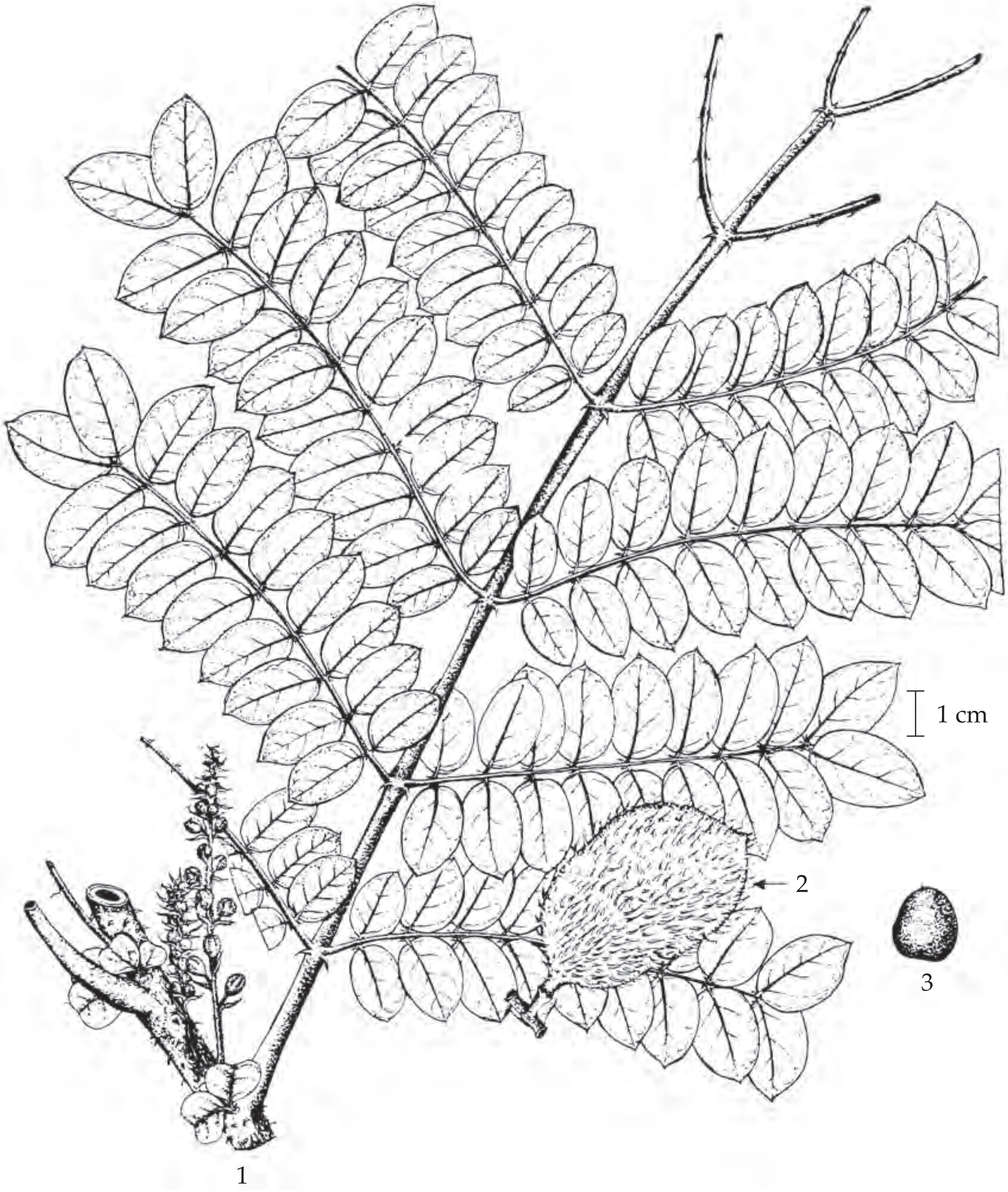


Fig. 1 *Caesalpinia bonduc* (L.) Roxb.
1. twig with inflorescence 2. pod 3. seed

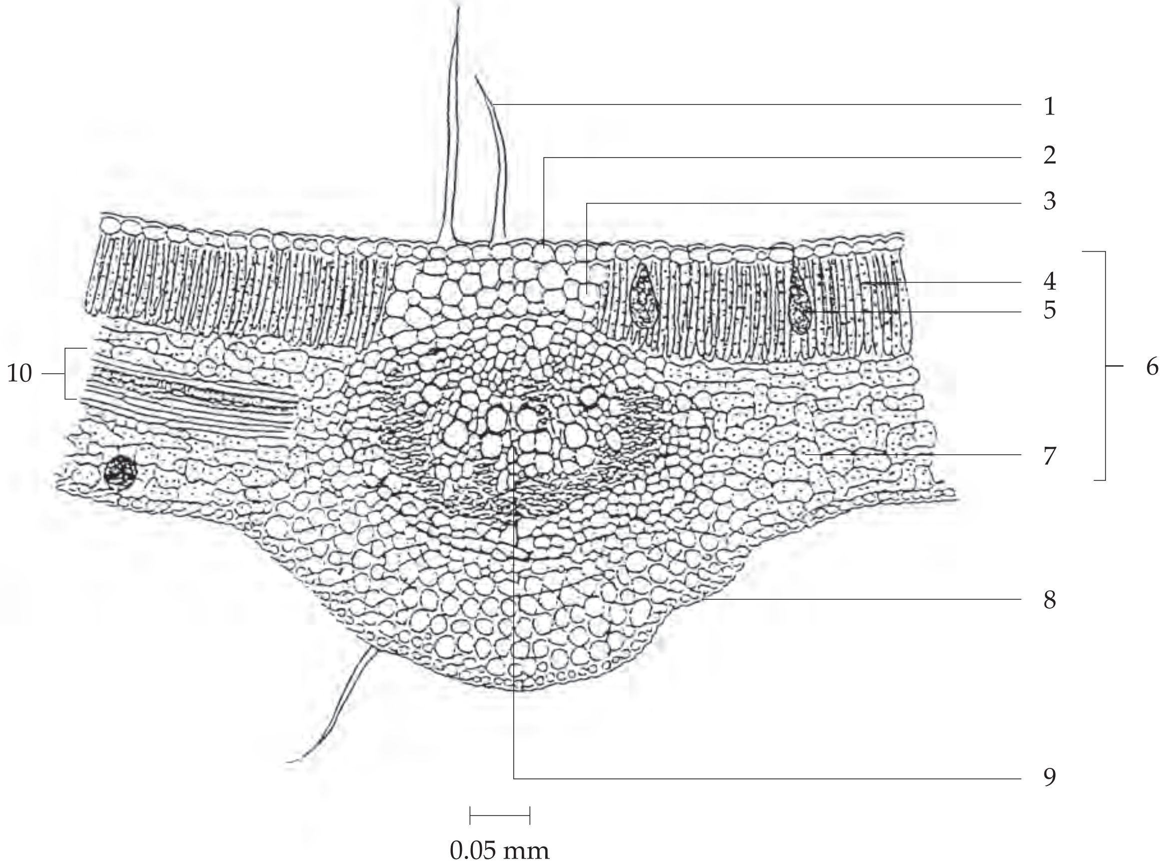


Fig. 2a Transverse Section of the Leaflet of *Caesalpinia bonduc* (L.) Roxb.

1. unicellular trichome	6. mesophyll
2. upper epidermis	7. spongy cells
3. collenchyma	8. lower epidermis
4. palisade cell	9. vascular bundle (cross section)
5. oil cells	10. vascular bundle (longitudinal section)

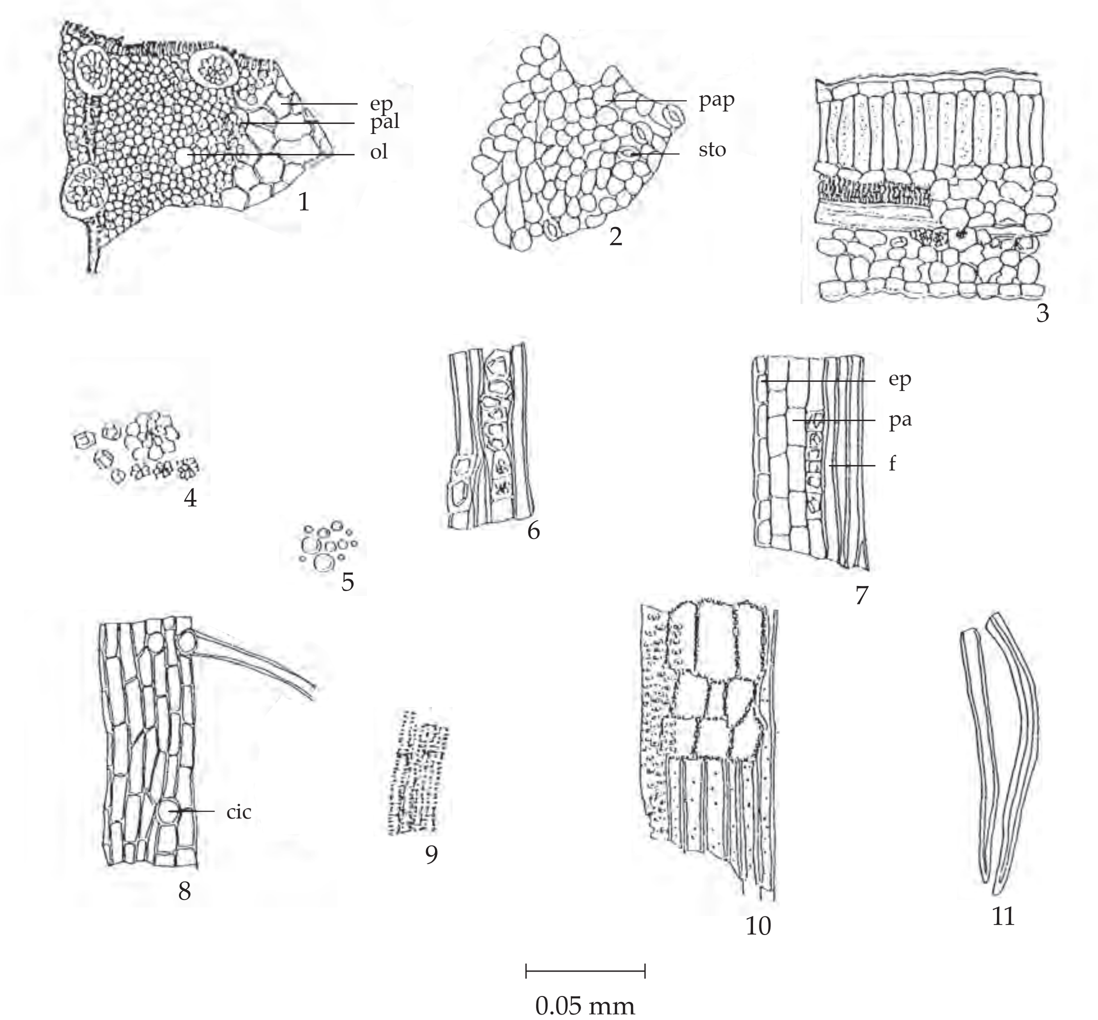


Fig. 2b Powdered Drug of the Leaflets of *Caesalpinia bonduc* (L.) Roxb.

1. upper epidermis in surface view with underlying palisade cells, oil cells and part of veinlet
2. lower epidermis in surface view showing stomata and, in some cases, papillae
3. transverse section of lamina
4. calcium oxalate crystals
5. oil droplets
6. group of fibres with part of calcium oxalate prism sheath
7. part of petiole showing epidermis, parenchyma and group of lignified fibres with part of calcium oxalate prism sheath
8. epidermis from over the midrib in surface view, with unicellular trichome attached, cicatrix
9. small xylem vessel
10. xylem element showing fibres, xylem parenchyma, bordered-pitted vessel
11. unicellular trichomes

(pal = palisade cell; ol = oil cell; pap = papilla; sto = stoma; ep = epidermis; pa = parenchyma; f = fibre; cic = cicatrix)

Packaging and storage Nicker-nut Leaf shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. To 500 mg of the sample, in powder, add 2 ml of *acetic anhydride*, warm on a water-bath for about 2 minutes, shake, and filter. Slowly add 1 ml of *sulfuric acid* to the filtrate to form a layer: a red ring forms at the zone of contact.

B. Boil 500 mg of the sample, in powder, with 10 ml of *water* and filter. To 2 ml of the filtrate, add 1 drop of a 5 per cent w/v solution of *iron(III) chloride*: a brownish green precipitate is produced.

C. Reflux 500 mg of the sample, in powder, with 10 ml of *methanol* on a water-bath for 20 minutes and filter. To 2 ml of the filtrate, add 2 to 3 pieces of *magnesium ribbon* and 2 to 3 drops of *hydrochloric acid*, and warm on a water-bath: a brownish red colour develops.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 85 volumes of *hexane* and 15 volumes of *ethyl acetate* as the mobile phase. Apply to the plate, 10 µl of the test solution prepared by refluxing 2 g of the sample, in powder, with 20 ml of *methanol* on a water-bath for about 30 minutes and filtering. After removal of the plate, allow it to dry in air and examine under ultraviolet light (366 nm); three red and one blue fluorescent spots appear. Spray the plate with *vanillin-sulfuric acid TS* and heat at 120° for 5 to 10 minutes; several spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Leaflets of *Caesalpinia bonduc* (L.) Roxb.

Spot	hR_f Value	Detection	
		UV 366	*Vanillin-Sulfuric Acid TS*
1	3-6	red	greenish grey
2	7-11	red	greenish grey
3	9-12	–	violet
4	13-18	red	greenish grey
5	17- 19	–	violet
6	24-30	–	violet
7	27-29	blue	–
8	32-39	–	violet
9	38-45	–	violet
10	67-77	–	violet
11	77-91	–	violet

Loss on drying Not more than 11.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Sulfated ash Not more than 15.0 per cent w/w (Appendix 5.3).

Ethanol-soluble extractive Not less than 10.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 25.0 per cent w/w (Appendix 7.12).

Chloroform-soluble extractive Not less than 6.0 per cent w/w (Appendix 7.12H).

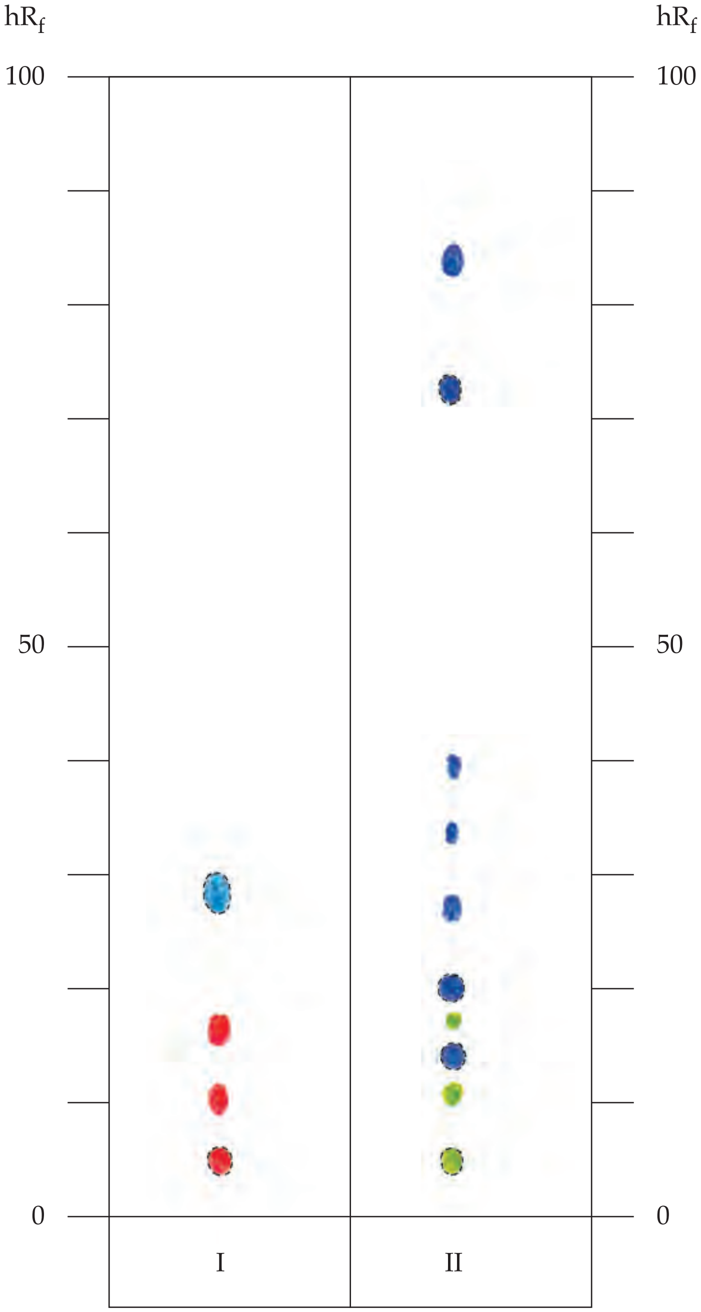


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Leaflets of *Caesalpinia bonduc* (L.) Roxb.

I = detection under UV light (366 nm)
II = detection with *vanillin-sulfuric acid TS*
◌ = spots developed in some samples

ตานหม่อน (TANMON)

Tarlmouniae Ellipticum Folium
Tarlmounia Elliptica Leaf

Category Demulcent.

Tarlmounia Elliptica Leaf is the dried full-grown leaf of *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan (*Vernonia elliptica* DC., *V. elaeagnifolia* DC.) (Family Compositae), Herbarium Specimen Number: DMSC 230.

Constituents Tarlmounia Elliptica Leaf contains glaucolides A and B and their acetates, along with lupeol, taraxasterol, sitosterol, and stigmasterol.

Description of the plant (Fig. 1) Scandent shrub; branches slender with longitudinal ridge, densely silvery hairs. Leaves simple, alternate, ovate, obovate, elliptic or oblong, 6 to 10 cm long, 3 to 4.5 cm wide, apex acute or obtuse, base obtuse, margin entire or remotely serrate, coriaceous, upper surface glabrous, lower surface densely covered with silvery hairs; veins 7 to 9 pairs; petiole 2 to 10 mm long. Inflorescence paniculate, corymbose heads of 3- to 5-flowered, 10 to 40 cm long, at end of branches or on upper axil of the leaves, flower-heads numerous, purple at first, becoming whitish on fading, sweet smelling; involucral bracts elliptic, 1 to 4 mm long, apex obtuse, shortly pubescent; calyx pappus; corolla-tube with 5-oblong toothed at the apex; stamens 5, anthers sagittate at base; style dividing into 2 branches. Fruits achenes, terete, about 2 mm long, 1 mm wide, terete, compressed, longitudinal ribbed, covered with light brown translucent glands, crowned by the pappus, 4 to 8 mm long, base pointed.

Description

Macroscopical Dried leaves more or less crumpled, blade ovate, obovate, elliptic or oblong from 6 to 9 cm long, and 2 to 4 cm wide, coriaceous; upper surface pale greenish brown to greyish brown, lower surface pale greyish brown and showing prominent midrib, petiole 4 to 10 mm long.

Microscopical (Figs. 2a, 2b) Transverse section of the leaf shows upper epidermis, a single layer of cuticularized rectangular cells, with few uniseriate multicellular trichomes and multicellular glandular trichomes. Mesophyll consisting of a single- or double-layer palisade parenchyma, some containing a small rosette aggregate crystal of calcium oxalate and several layers of irregular-shaped spongy parenchyma rich in chloroplastids. Through this region scattered small vascular bundles. Lower epidermis, a single layer of rectangular cells, with multicellular glandular trichomes and numerous T-shaped nonglandular trichomes, each with one horizontal, tapering end cell on short, 1-2 celled stalk.

Transverse section through the midrib of lamina shows several layers of collenchyma underneath the epidermis, parenchyma and collateral vascular bundles.

In surface view, epidermides of upper and lower surfaces are polygonal cells, with much more anomocytic stomata in the lower epidermis than in the upper one.

Tarlmounia Elliptica Leaf in powder possesses the diagnostic microscopical characters of the unground drug.

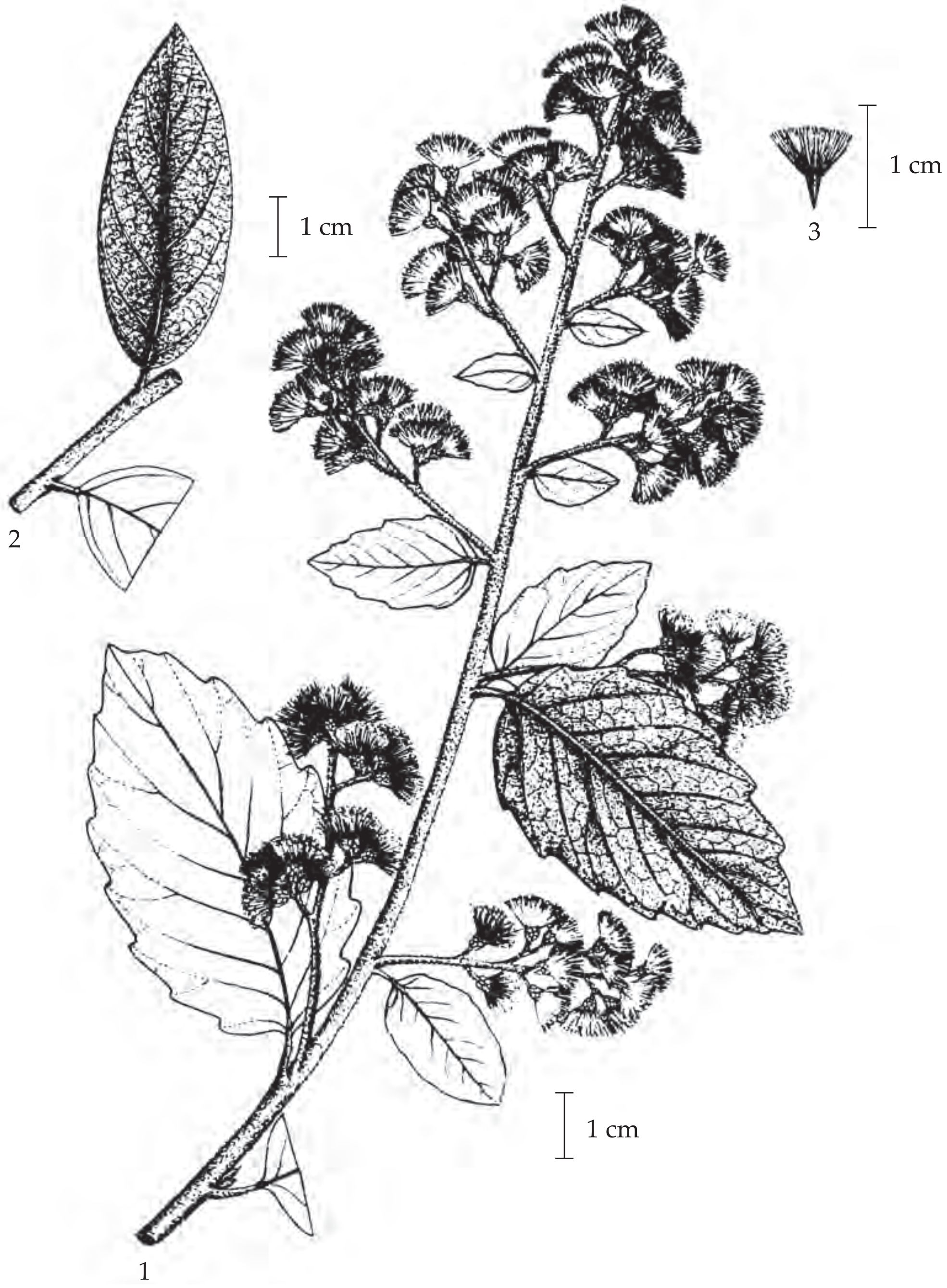


Fig. 1 *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan
1. flowering twig 2. leaf 3. fruit

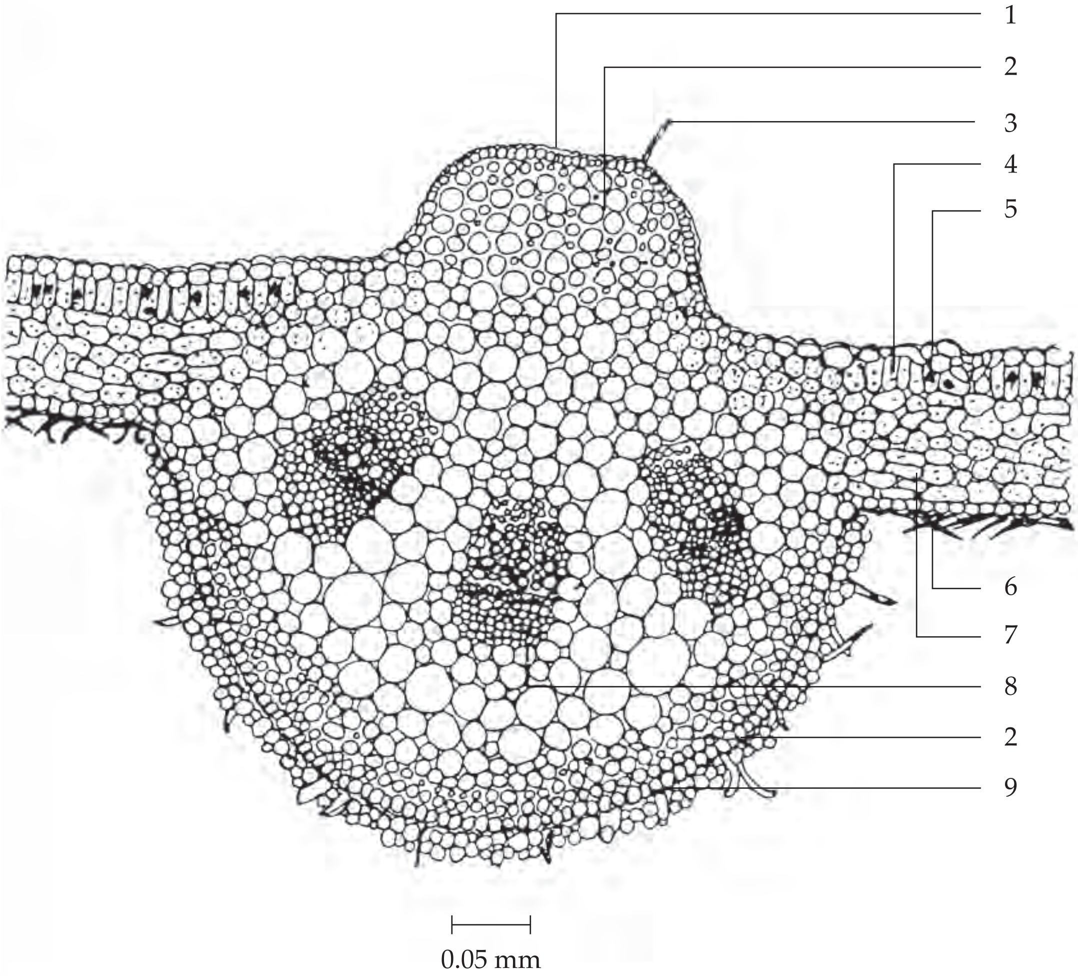


Fig. 2a Transverse Section of the Leaf of *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan

1. upper epidermis	6. T-shaped trichome
2. collenchyma	7. spongy cells
3. uniseriate multicellular trichome	8. vascular bundle
4. palisade cell	9. lower epidermis
5. rosette aggregate crystal of calcium oxalate	

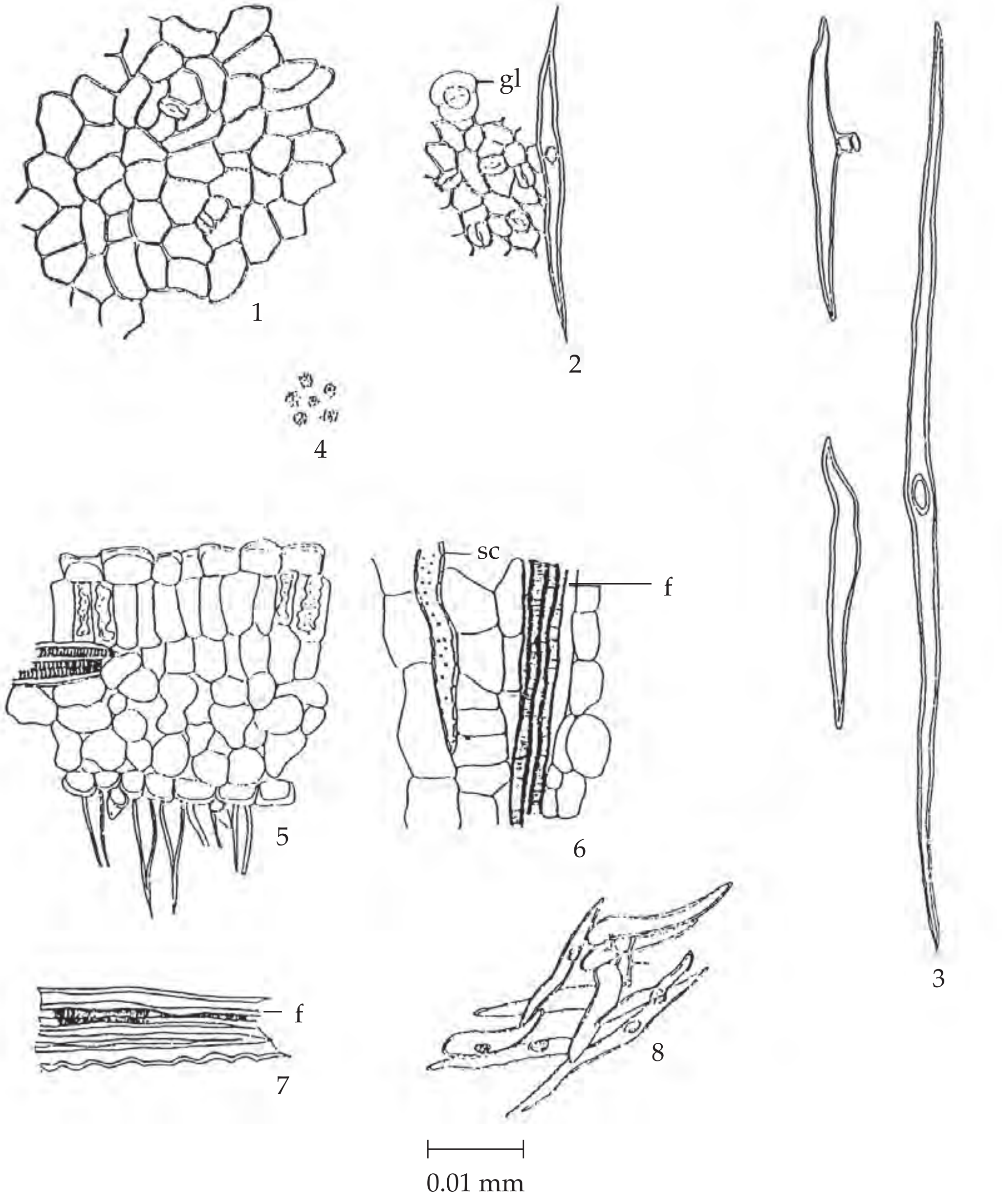


Fig. 2b Powdered Drug of the Leaves of *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan

1. upper epidermis with anomocytic stomata
2. lower epidermis with anomocytic stomata, a glandular trichome, and a T-shaped trichome
3. T-shaped trichomes
4. rosette aggregate crystals of calcium oxalate
5. transverse section of the leaf
6. cells from midrib, showing sclereid, parenchyma and small vessel
7. group of fibres and vessels
8. group of T-shaped trichomes

(gl = glandular trichome; f = fibre; sc = sclereid)

Packaging and storage Tarlmounia Elliptica Leaf shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. To 500 mg of the sample, in powder, add 2 ml of *acetic anhydride*, warm on a water-bath for about 2 minutes, shake, and filter. Slowly add 1 ml of *sulfuric acid* to the filtrate to form a layer: a brownish red ring forms at the zone of contact.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 70 volumes of *hexane*, 29 volumes of *ethyl acetate* and 1 volume of *glacial acetic acid* as the mobile phase. Apply to the plate, 10 µl of the test solution prepared by refluxing 500 mg to 1 g of the sample, in powder, with 10 ml of *methanol* on a water-bath for about 20 minutes and filtering. After removal of the plate, allow it to dry in air and spray with a 1 per cent w/v solution of *diphenylboric acid β-aminoethyl ester* in *methanol*. Examine the plate under ultraviolet light (366 nm); five orange spots appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan

Spot	hR_f Value	Detection
		Diphenylboric Acid β-Aminoethyl Ester in *Methanol* and UV 366
1	6-7	orange
2	22-28	orange
3	33-39	orange
4	45-55	orange
5	55-66	orange

Loss on drying Not more than 11.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Sulfated ash Not more than 20.0 per cent w/w (Appendix 5.3).

Ethanol-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 14.0 per cent w/w (Appendix 7.12).

Chloroform-soluble extractive Not less than 4.0 per cent w/w (Appendix 7.12H).

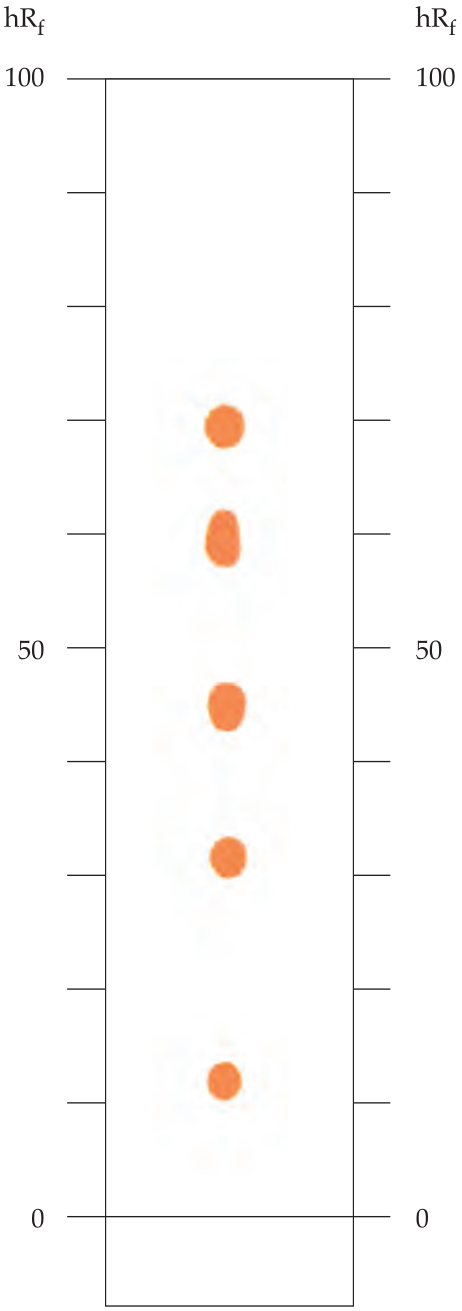


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Leaves of *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan, Detected by Spraying with a 1 Per Cent W/V Solution of *Diphenylboric Acid β-aminoethyl Ester* in *Methanol* and Examining under UV light (366 nm)

เถาวัลย์เปรียง (THAOWAN PRIANG)

เถาตาปลา (THAO TA PLA), เครือเขาหนัง (KHRUEA KHAO NANG)
Solarii Scandenidis Caulis
Hog Creeper Vine

Category Analgesic, anti-inflammatory.

Hog Creeper Vine is the dried stem of *Solori scandens* (Roxb.) Sirich. & Adema [*Derris scandens* (Roxb.) Benth., *Brachypterum scandens* (Roxb.) Benth., *Dalbergia scandens* Roxb., *Derris timoriensis* (DC.) Pittier] (Family Leguminosae), Crude Drug Number: DMSc 0438.

Constituents Hog Creeper Vine contains isoflavone glycosides such as genistein-7-*O*-[α-rhamnopyranosyl-(1→6)-β-glucopyranoside]. It also contains isoflavones (e.g., derrisisoflavones A-E, osajin, scandenone, scandinone and 5,7,4′-trihydroxy-6,3′-diprenylisoflavone), coumarins (e.g., scandenins A and B), sterols, etc.

Description of the plant (Figs. 1a, 1b) Large woody climber or scandent shrub, up to 30 m long, up to 40 cm in diameter; branches finely pubescent then glabrous. Leaves imparipinnate, spirally arranged, 7 to 15 cm long, petiole 3 to 7 cm long; leaflets 7 to 13(–19), oblong, obovate-oblong or elliptic, 2 to 9 cm long, 1 to 2.5(–5) cm wide, apex obtuse to emarginate or acute, base cuneate or obtuse, margin entire, upper surface glabrous, lower surface pubescent; stipule small, caducous, petiolule short. Inflorescence raceme or racemose panicle, axillary, 8 to 45 cm long; pedicels 4 to 6.5 mm long. Flower papilionaceous, white to pale pink or purple; calyx cup-shaped, 3.5 mm long, purple, minutely dentate, silky pubescent; petals 5, much longer than the calyx, standard pink or whitish, with a greenish yellow basal blotch, obovate, 7 to 11 mm long, glabrous or pubescent on the lower surface, keel adhearing to the wings; stamens 10; ovary superior, 1-loculed, ovule (6–)9 to 10 per locule. Fruit flat, lanceolate or oblong-lanceolate, 2.5 to 8 cm long, 1 to 2 cm wide, narrowed at both ends, with narrow wings along the dorsal suture. Seed(s) 1 to 5, reniform, dark brown.

Description Odour mild; taste bland.

Macroscopical (Fig. 1a) Dried full or fragmented pieces of obliquely sliced stems, bark yellowish to brownish, rough; sectional view lighter colour, yellowish brown, with few to several alternately light- and dark-coloured rings of vascular tissue, finely porous.

Microscopical (Figs. 2a, 2b) Transverse section of the stem shows cork layer, cortex, vascular tissue and pith. Cork, several layers of rectangular cells, some thick-walled, some containing prismatic crystals or reddish to brownish substances. Cortex, few layers of parenchyma cells, some containing simple and compound starch grains, groups of fibres and sclereids. Vascular tissue, anomalous type of phloem and xylem. Phloem, small thin-walled parenchyma cells, groups of fibres and phloem rays. Xylem consisting of younger xylem and older xylem. Younger xylem composed of large vessels, xylem parenchyma cells and xylem rays. Older xylem composed of small vessels, xylem parenchyma cells and small bands of xylem rays. Pith, round, thin-walled parenchyma cells, some containing brownish substances.

1

2

3

4

5

Fig. 1a *Solori scandens* (Roxb.) Sirich. & Adema
1. habit 2. inflorescences 3. flower 4. flowering and fruiting branches 5. crude drug

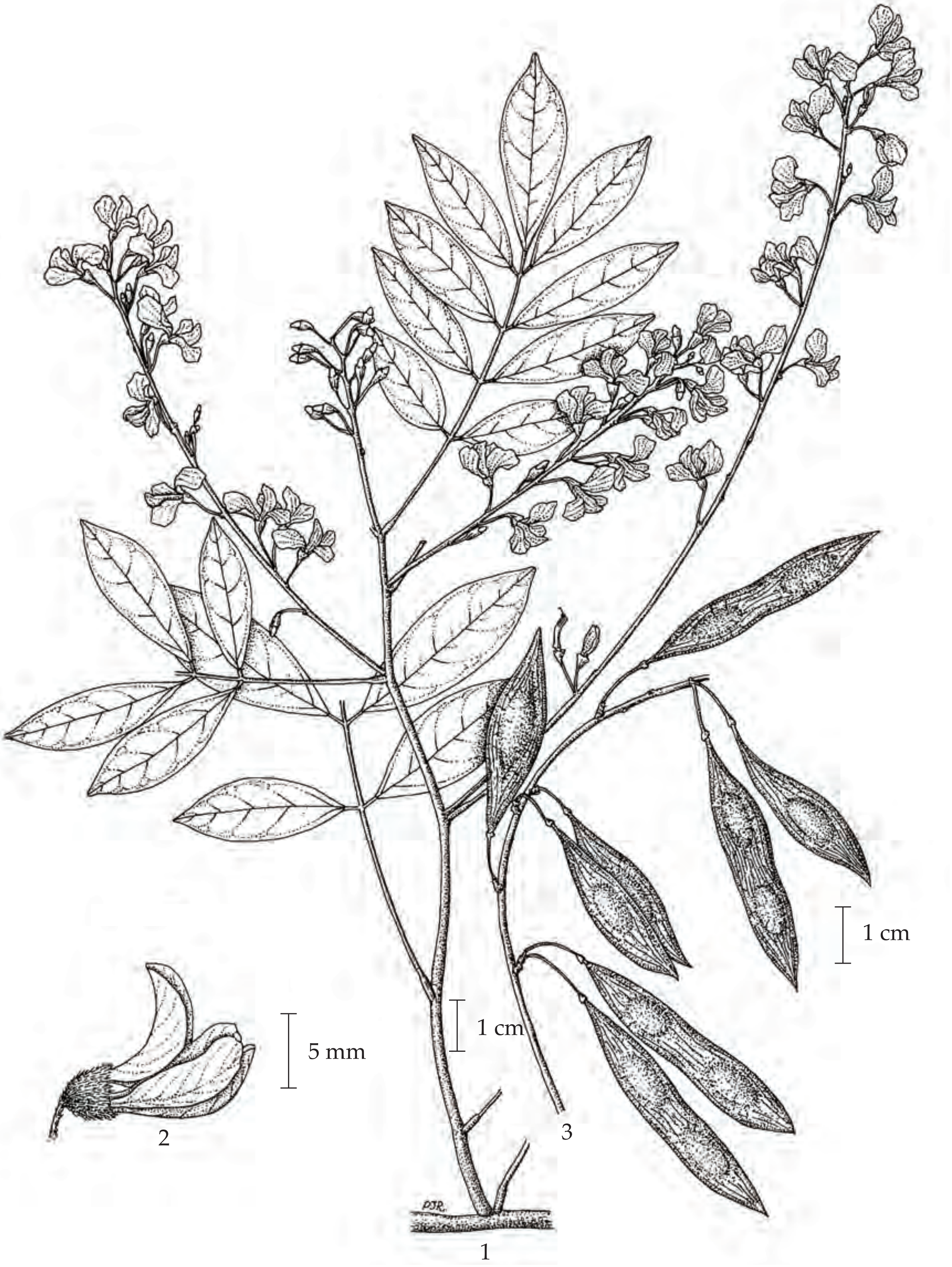


Fig. 1b *Solori scandens* (Roxb.) Sirich. & Adema
1. flowering twig 2. flower 3. fruits

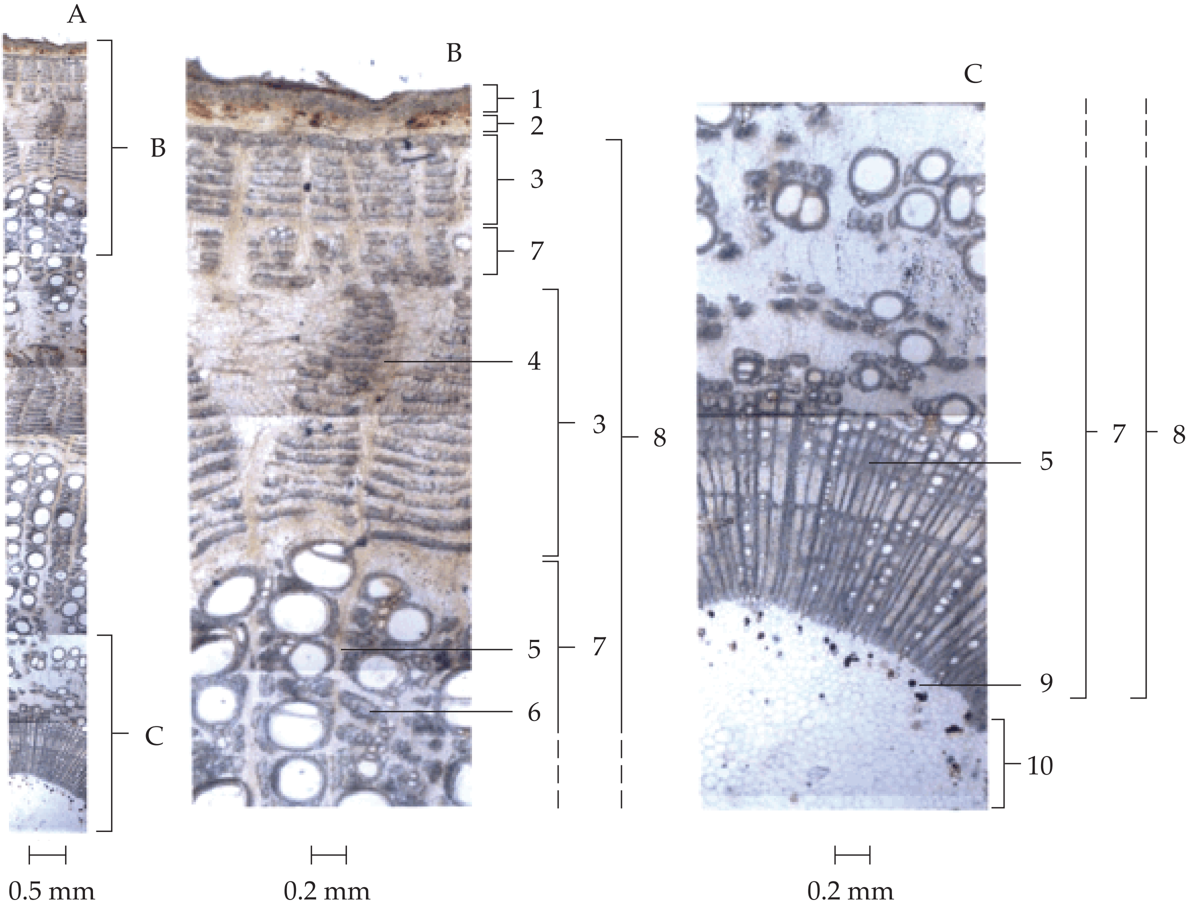


Fig. 2a Photomicrographs of Transverse Section of the Stem of *Solori scandens* (Roxb.) Sirich. & Adema

A. Overview of the Stem
B. Outermost Part
C. Innermost part

1. cork	6. xylem fibre
2. cortex	7. xylem
3. phloem	8. vascular tissue
4. phloem ray	9. reddish to brownish substances
5. xylem ray	10. pith

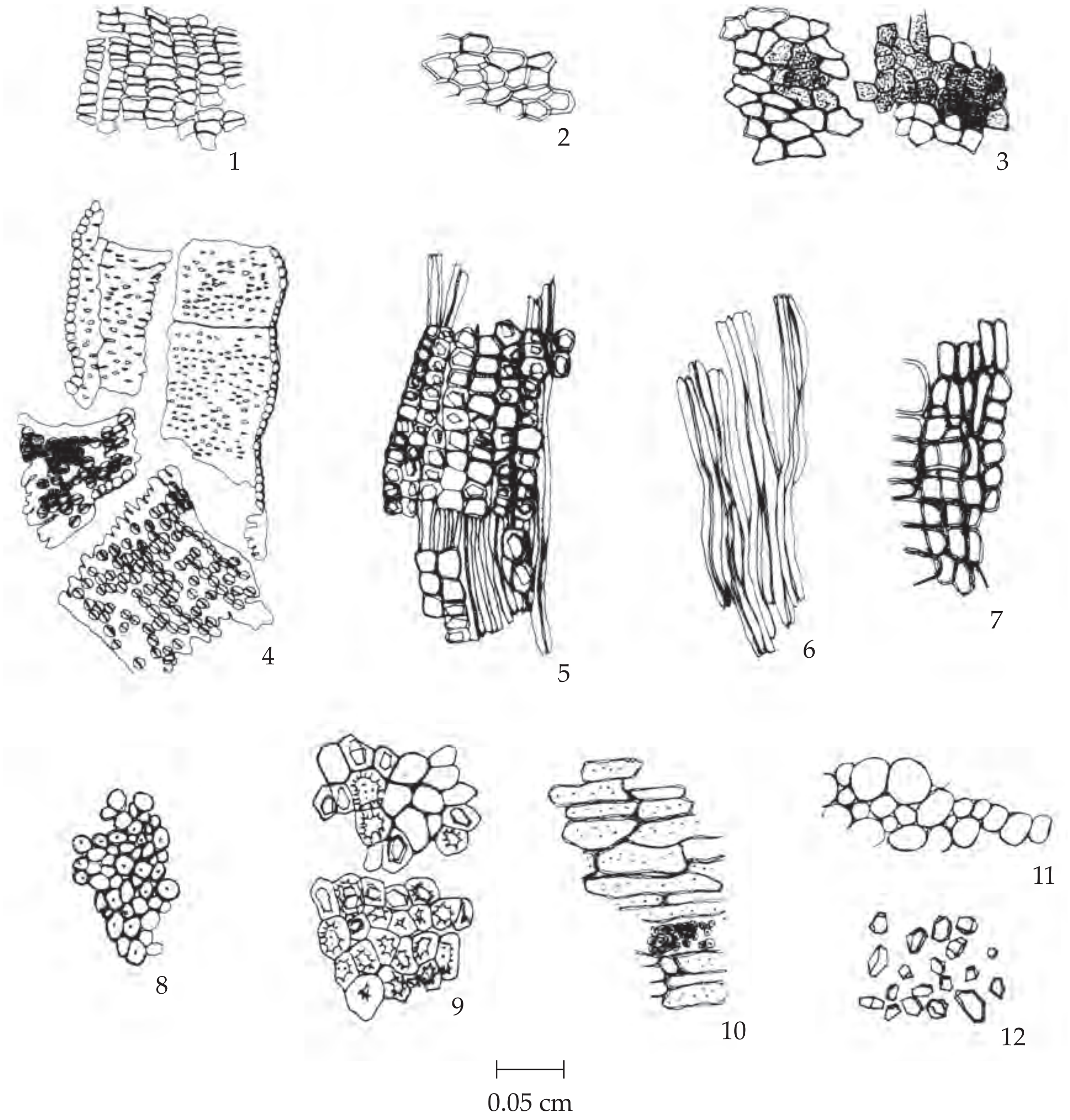


Fig. 2b Powdered Drug of the Stems of *Solori scandens* (Roxb.) Sirich. & Adema

1. cork in sectional view
2. cork in surface view
3. parenchyma containing reddish to brownish substances
4. simple-pitted and bordered-pitted vessels
5. fibres associated with xylem parenchyma containing prismatic crystals in longitudinal view
6. fibres in longitudinal view
7. xylem parenchyma associated with medullary rays
8. fibres in sectional view
9. sclereids associated with parenchyma some containing prismatic crystals
10. porous xylem parenchyma and small bordered-pitted vessel
11. parenchyma of pith
12. prismatic crystals

Hog Creeper Vine in powder possesses the diagnostic microscopical characters of the unground drug. Parenchyma with brownish substances, large bordered-pitted vessels, and numerous sclereids (some containing prismatic crystals) are characteristic.

Contra-indication It is contra-indicated in pregnant women.

Warning

1. It should be used with caution in patients with peptic ulcer.
2. It may cause gastro-intestinal irritation, stomachache, constipation, frequent urination, thirst, or palpitation.
3. Long-term use should be avoided.

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Hog Creeper Vine shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Reflux 1 g of the sample, in *fine powder*, with 10 ml of *methanol* for 5 minutes and filter. Evaporate the filtrate to 1 ml, add 1 to 2 pieces of *magnesium ribbon*, shake well and mix with a few drops of *hydrochloric acid*. Warm in a water-bath: a reddish brown colour develops.

B. To about 500 mg of the sample, in *fine powder*, in a tube with screw-capped add 10 ml of *water* and shake for a few minutes: a persisting foam is produced for over 15 minutes.

C. Carry out the test as described in the "Thin-layer chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 70 volumes of *chloroform*, 40 volumes of *methanol* and 10 volumes of *water*. Apply to the plate, 5 µl of the test solution prepared by refluxing 1 g of the sample, in fine powder, with 20 ml of *ethanol* on a water-bath for 20 minutes, filtering immediately, and evaporating the filtrate under reduced pressure at 50°. Dissolve the residue in 3 ml of *ethanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. Spray the plate with a 20 per cent v/v solution of *sulfuric acid* in *ethanol* and heat at 105° for 5 minutes. Several spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 **hR_f Values of Components in Ethanolic Extract of the Stems of *Solori scandens* (Roxb.) Sirich. & Adema**

Spot	hR_f Value	Detection	
		UV 254	20 Per Cent V/V Solution of *Sulfuric Acid* in *Ethanol*
1	7-8	–	pale brown
2	10-11	–	pale brown
3	16-18	–	dark brown
4	18-20	–	dark yellow
5	22-24	–	yellowish brown
6	27-30	–	yellowish brown
7	35-36	–	brown
8	37-40	–	yellowish brown
9	40-42	–	yellowish brown
10	44-45	–	yellowish brown
11	53-55	quenching	yellowish brown
12	58-60	–	pale brown
13	78-80	–	pale brown
14	81-83	–	pale yellow
15	92-95	quenching	yellowish brown

Loss on drying Not more than 7.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Total ash Not more than 8.0 per cent w/w (Appendix 7.7).

Ethanol (50 per cent)-soluble extractive Not less than 14.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 14.0 per cent w/w (Appendix 7.12).

Dose 500 mg to 1 g three times a day after meals.

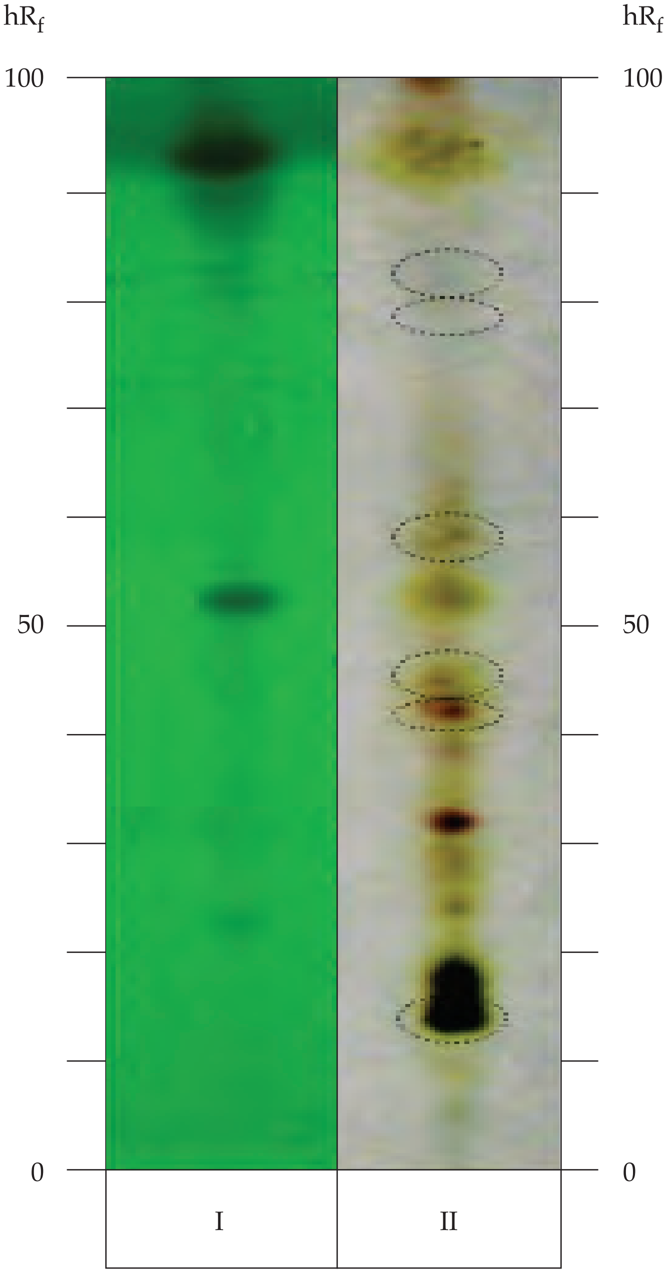


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Stems of *Solori scandens* (Roxb.) Sirich. & Adema

I = detection under UV light (254 nm)
II = detection with a 20 per cent v/v solution of *sulfuric acid* in *ethanol*
⬭ = spots developed in some samples

สารสกัดแห้งเถาวัลย์เปรียง (THAOWAN PRIANG DRY EXTRACT)

Hog Creeper Vine Dry Extract

Category Analgesic, anti-inflammatory.

Hog Creeper Vine Dry Extract is prepared from the powdered Hog Creeper Vine by extraction with *ethanol (50 per cent)*. It contains not less than 90.0 per cent and not more than 110.0 per cent of the labelled amount of genistein-7-*O*-[*α*-rhamnopyranosyl-(1→6)-*β*-glucopyranoside]; the labelled amount of genistein 7-*O*-[*α*-rhamnopyranosyl-(1→6)-*β*-glucopyranoside] is not less than 0.5 per cent, calculated as genistein ($C_{15}H_{10}O_5$) and on the dried basis.

Description Yellowish to yellow-brown powder.

Packaging and storage Hog Creeper Vine Dry Extract shall be kept in well-closed containers, protected from light, and stored in a cool and dry place.

Labelling The label on the container states the equivalent amount of genistein-7-*O*-[*α*-rhamnopyranosyl-(1→6)-*β*-glucopyranoside], calculated as genistein.

Identification

A. To 500 mg of the sample, add 10 ml of *methanol*, boil in a water-bath for 5 minutes and filter. To the filtrate, add 1 or 2 pieces of *magnesium ribbon*, shake well, mix with a few drops of *hydrochloric acid*, and warm in a water-bath: a reddish brown colour develops.

B. To 500 mg of the sample in a test-tube with screw-capped, add 10 ml of *water* and shake for 30 seconds: a persisting foam is produced for over 15 minutes.

Loss on drying Not more than 6.0 per cent w/w after drying at 105° to constant weight; use 1.0 g (Appendix 4.15).

Assay Carry out the test as described in the “High-pressure Liquid Chromatography” (Appendix 3.5).

Mobile phase A Use *acetonitrile*.

Mobile phase B Prepare a 1 per cent v/v solution of *acetic acid*.

Standard preparation Dissolve about 5 mg of Genistein RS, accurately weighed, in *ethanol (50 per cent)*, dilute to 50.0 ml and mix. Transfer 2.0 ml of this solution to a 20-ml volumetric flask, dilute with the same solvent to volume, mix and filter.

Assay preparation Transfer about 500 mg of Hog Creeper Vine Dry Extract, accurately weighed, to a 200-ml volumetric flask, add about 150 ml of *ethanol (50 per cent)*, sonicate for 30 minutes, dilute to volume, and mix. Transfer 5.0 ml of this solution to a 10-ml volumetric flask, dilute with the same solvent to volume, mix, and filter.

Chromatographic system The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm x 4.6 mm) packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (3 to 10 µm) equipped with the similarly packed guard column, (b) *Mobile phase* at a flow rate of about 1 ml per minute (the retention time is about 32 minutes for genistein.) and (c) an ultraviolet photometer set at 260 nm. The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile phase A* (Per Cent V/V)	*Mobile phase B* (Per Cent V/V)
0	15	85
15	22	78
30	45	55
35	15	85
40	15	85

To determine the suitability of the chromatographic system, chromatograph *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0 per cent, the symmetry factor is not more than 2.0 per cent and the relative retention times are about 0.5 for genistein 7-*O*-[*α*-rhamnopyranosyl-(1→6)-*β*-glucopyranoside] and 1.0 for genistein.

Procedure Separately inject equal volumes (about 20 µl) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms and measure the responses for the major peaks.

Calculation Calculate the content of genistein-7-*O*-[*α*-rhamnopyranosyl-(1→6)-*β*-glucopyranoside], calculated as genistein, in the portion of the Extract taken, using the declared content of $C_{15}H_{10}O_5$ in Genistein RS.

Other requirements Complies with the requirements described under “Extracts” (Appendix 1.16H).

ยาแคปซูลเถาวัลย์เปรียง (THAOWAN PRIANG CAPSULES)

Hog Creeper Vine Capsules

Category Analgesic, anti-inflammatory.

Hog Creeper Vine Capsules are prepared from Hog Creeper Vine Dry Extract and contain not less than 90.0 per cent and not more than 110.0 per cent of the labelled amount of genistein-7-*O*-[α-rhamnopyranosyl-(1→6)-β-glucopyranoside], calculated as genistein ($C_{15}H_{10}O_5$).

Strength available 400 mg of hog creeper vine dry extract.

Dose Low back pain and knee osteoarthritis: 400 mg twice a day immediately after meals.

Contra-indication It is contra-indicated in pregnant women.

Warning

1. It should be used with caution in patients with peptic ulcer.
2. It may cause gastro-intestinal irritation, stomachache, constipation, frequent urination, thirst, or palpitation.
3. Long-term use should be avoided.

Packaging and storage Hog Creeper Vine Capsules shall be kept in tightly closed containers, protected from light, and stored at the temperature not exceeding 30°.

Labelling The label on the container states (1) the equivalent amount of genistein -7-*O*-[α-rhamnopyranosyl-(1→6)-β-glucopyranoside], calculated as genistein; (2) expiration date.

Identification

A. The chromatogram of the Assay preparation shows several peaks, one of which corresponds to that of the Standard preparation, as obtained in the *Assay*.

B. The capsules contents comply with the tests for Identification A and B described under *Hog Creeper Vine Dry Extract*.

Microbial limit Comply with the requirements for Category 1A in the “Limits for Microbial Contamination” (Appendix 10.5).

Assay Carry out the test as described in the “High-pressure Liquid Chromatography” (Appendix 3.5).

Mobile phase A, **Mobile phase B, Standard preparation, Chromatographic system,** and **Procedure** Proceed as directed in the Assay under *Hog Creeper Vine Dry Extract.*

Assay preparation Remove, as completely as possible, the contents of not less than 20 Hog Creeper Vine Capsules. Transfer an accurately weighed portion of the capsule contents, containing about 500 mg of hog creeper vine dry extract, to a 200-ml volumetric flask. Add 150 ml of *ethanol* (*50 per cent*), sonicate for 30 minutes, dilute to volume, and mix. Transfer 5.0 ml of this solution to a 10-ml volumetric flask, dilute with the same solvent to volume, mix, and filter.

Calculation Calculate the content of genistein-7-*O*-[α-rhamnopyranosyl-(1→6)-β-glucopyranoside], calculated as genistein, in the portion of the Capsules taken, using the declared content of $C_{15}H_{10}O_5$ in Genistein RS.

Other requirements Comply with the requirements described under "Capsules" (Appendix 1.16H).

เทียนแดง (THIAN DAENG)

Lepidii Sativi Semen
Garden Cress Seed

Synonym Cress Seed

Category Expectorant, stomachic.

Garden Cress Seed is the dried mature seed of *Lepidium sativum* L. (Family Cruciferae), Herbarium Specimen Number: DMSC 4533, QSBG 22819, Crude Drug Number: DMSc 431.

Constituents Garden Cress Seed contains sinapic acid, sinapine and imidazole alkaloids. It also contains volatile oil, fatty acids, amino acids, isothiocyanate glycosides, mucilage, etc.

Description of the plant (Figs. 1a, 1b) Annual herb, 20 to 80 cm high; stem erect, branched, nearly glabrous, glaucous. Basal leaves, pinnatisect or lyrate-pinnate, 2 to 10 cm long, 1 to 5 cm wide, segments toothed or finely divided, crispate, stalked to subsessile; stem leaves becoming successively simple, linear and entire in the upper part. Inflorescence raceme, terminal or axillary. Flowers small, 2 to 3 mm in diameter, bisexual, regular; sepals 4, oblong, 1 to 1.3 mm long, 0.5 to 0.8 mm wide; petals 4, white or lavender, spatulate or obovate, 2.5 to 4 mm long, 0.7 to 1.4 mm wide; stamens typically 6, tetradynamous; ovary superior, 2-carpeled, 1 ovule each, ripening into a thin partitioned pod. Fruits silique, elliptic or oblong-ovate, 4 to 6 mm long, 3 to 5 mm wide, retuse, narrowly winged, with deep apical notch, style not projecting beyond notch; dehiscent by 2 valves; stalk more than half as long as fruit. Seeds obliquely ovate to elliptic or oblong, 2 to 3 mm long, 1 to 1.5 mm wide, red to reddish brown.

Description Odourless; taste, aromatic, slightly bitter.

Macroscopical (Figs. 1a, 1b) Obliquely ovate to elliptic or oblong, 2 to 3 mm long, 1 to 1.5 mm wide. Externally reddish brown, glabrous, smooth, with a short longitudinal groove on one side at the narrow end. Seed coat swollen when moistened.

Microscopical (Figs. 2a, 2b) Transverse section of the seed shows seed coat composed of outer integument and inner integument. Outer integument, a mucilaginous layer and a layer of reddish thick-walled cells; inner integument, colourless rectangular cells with aleurone grains and oil globules. Embryo, cotyledons, thin-walled cells, oil globules, small aleurone grains and vascular tissue.

Garden Cress Seed in powder possesses the diagnostic microscopical characters of the unground drug.

Contra-indication It is contra-indicated in pregnant women.

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Garden Cress Seed shall be kept in well-closed containers, protected from light, and stored in a dry place.

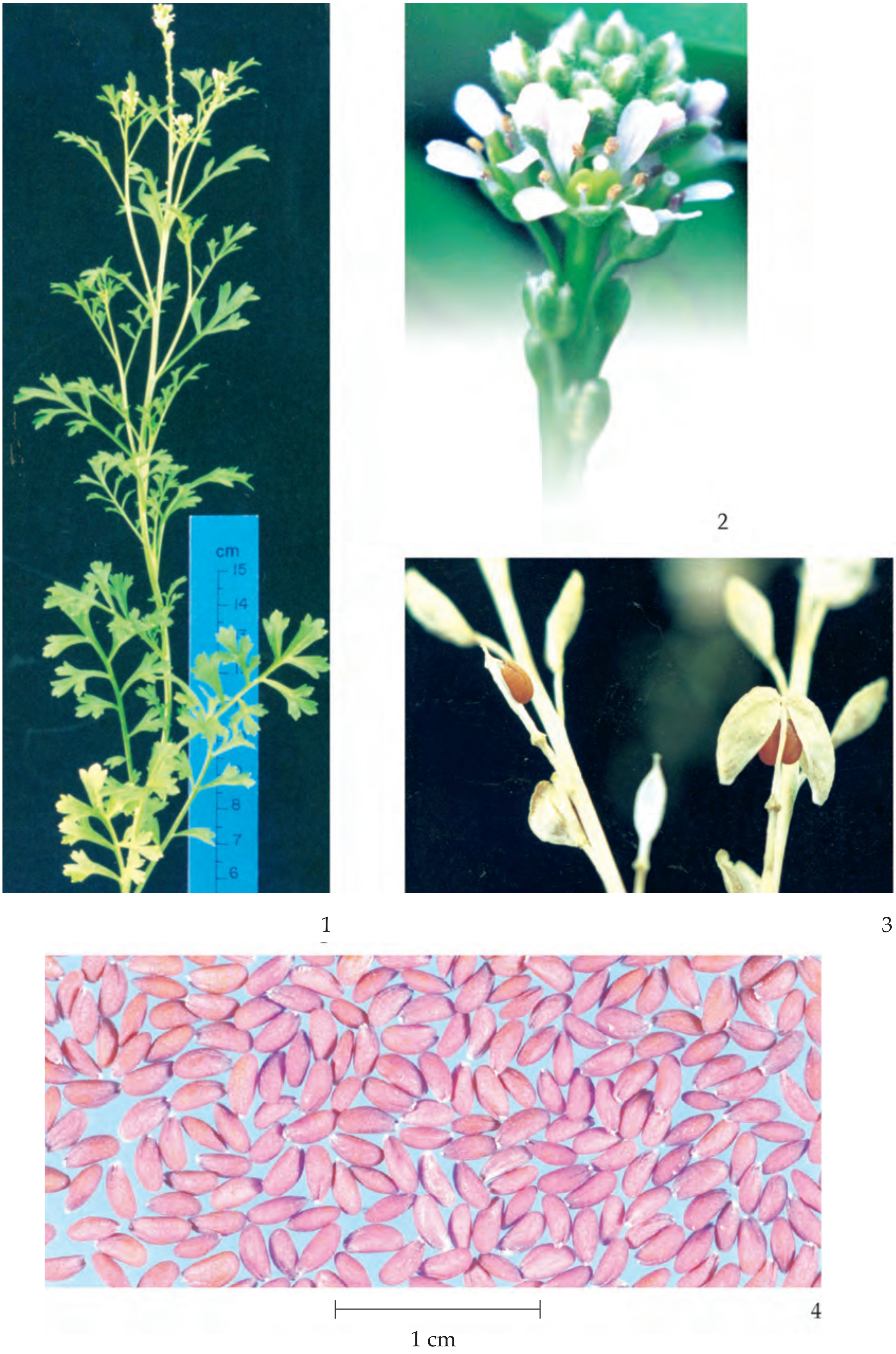


Fig. 1a *Lepidium sativum* L.
1. habit 2. flowers 3. fruits 4. crude drug

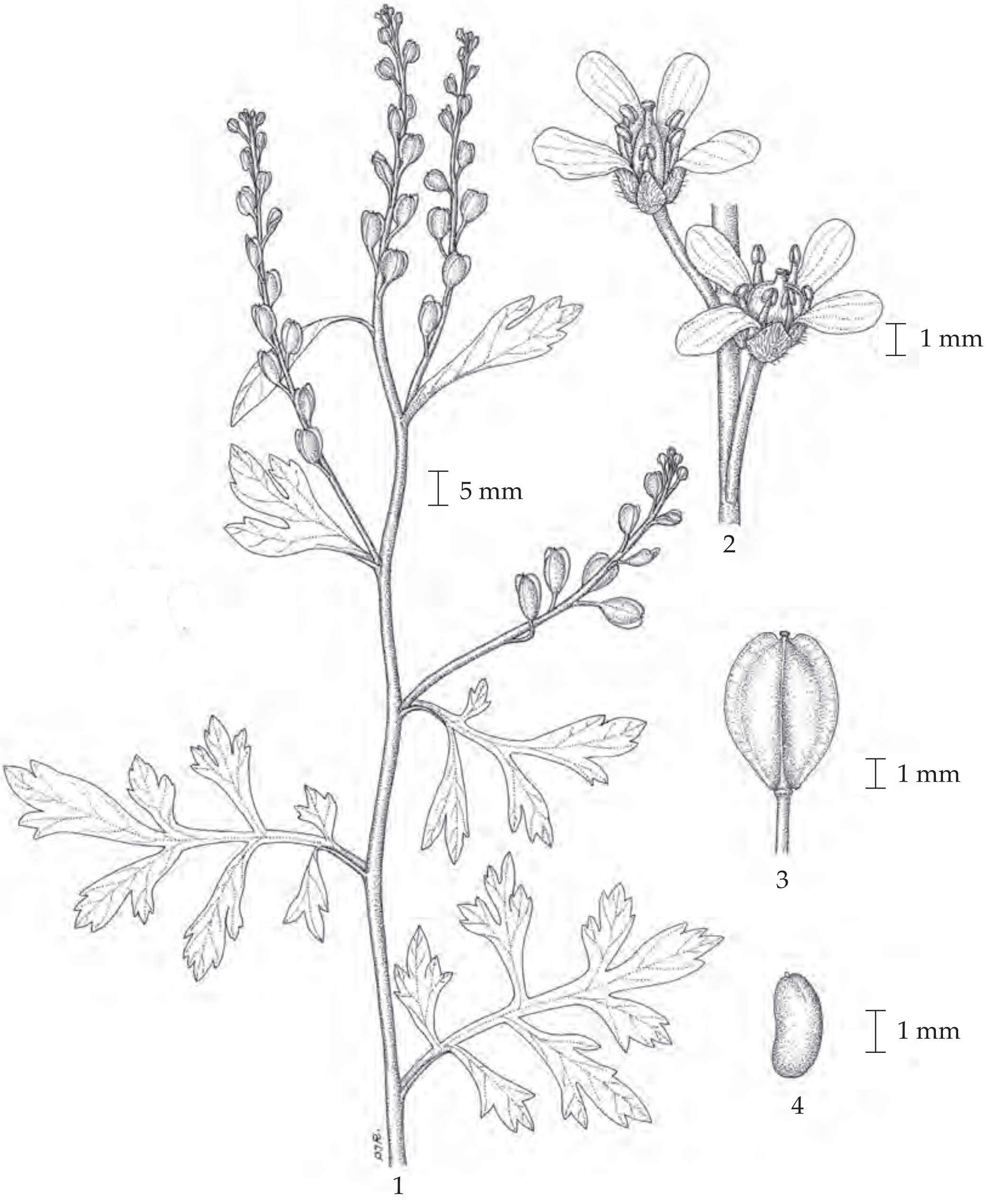


Fig. 1b *Lepidium sativum* L.
1. fruiting top 2. flowers 3. fruit 4. seed

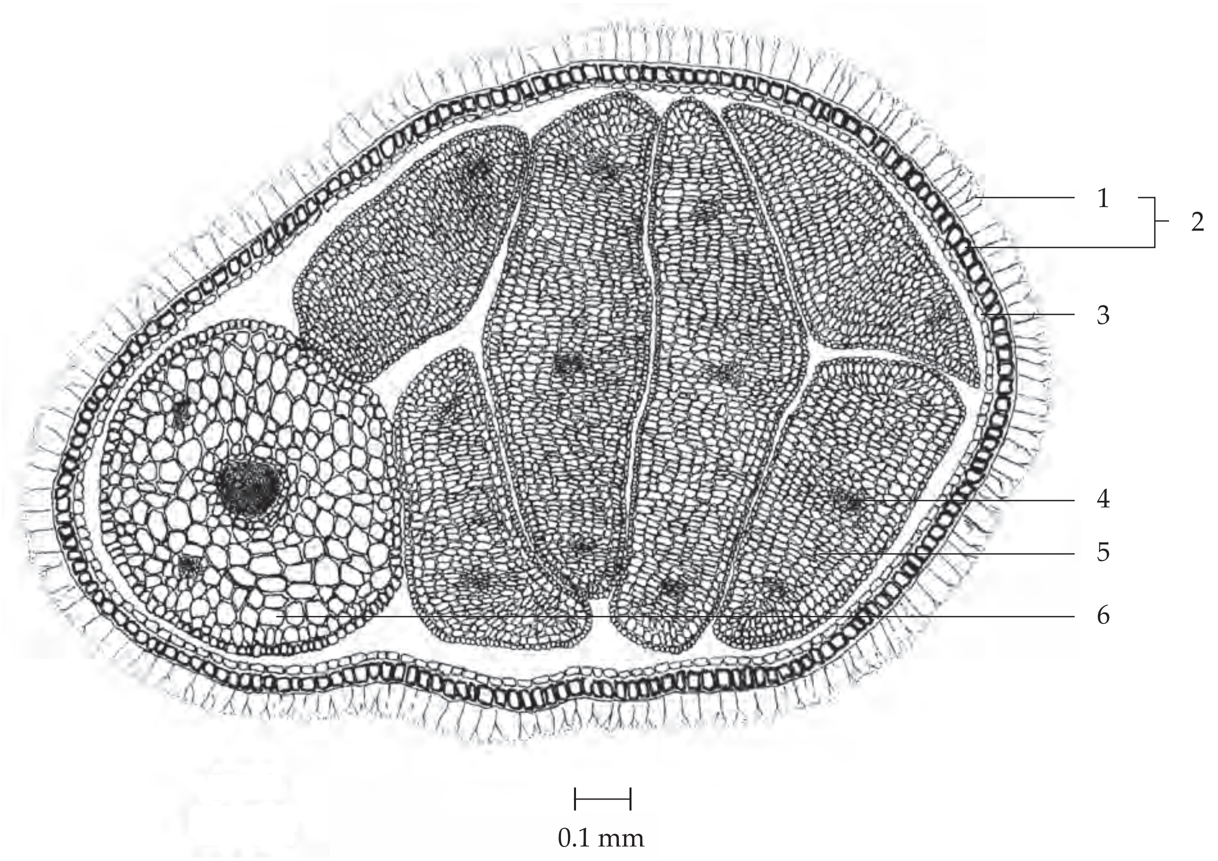


Fig. 2a Transverse Section of the Seed of *Lepidium sativum* L.

1. mucilage layer
2. outer integument
3. inner integument
4. vascular bundle
5. cotyledon
6. part of an embryo

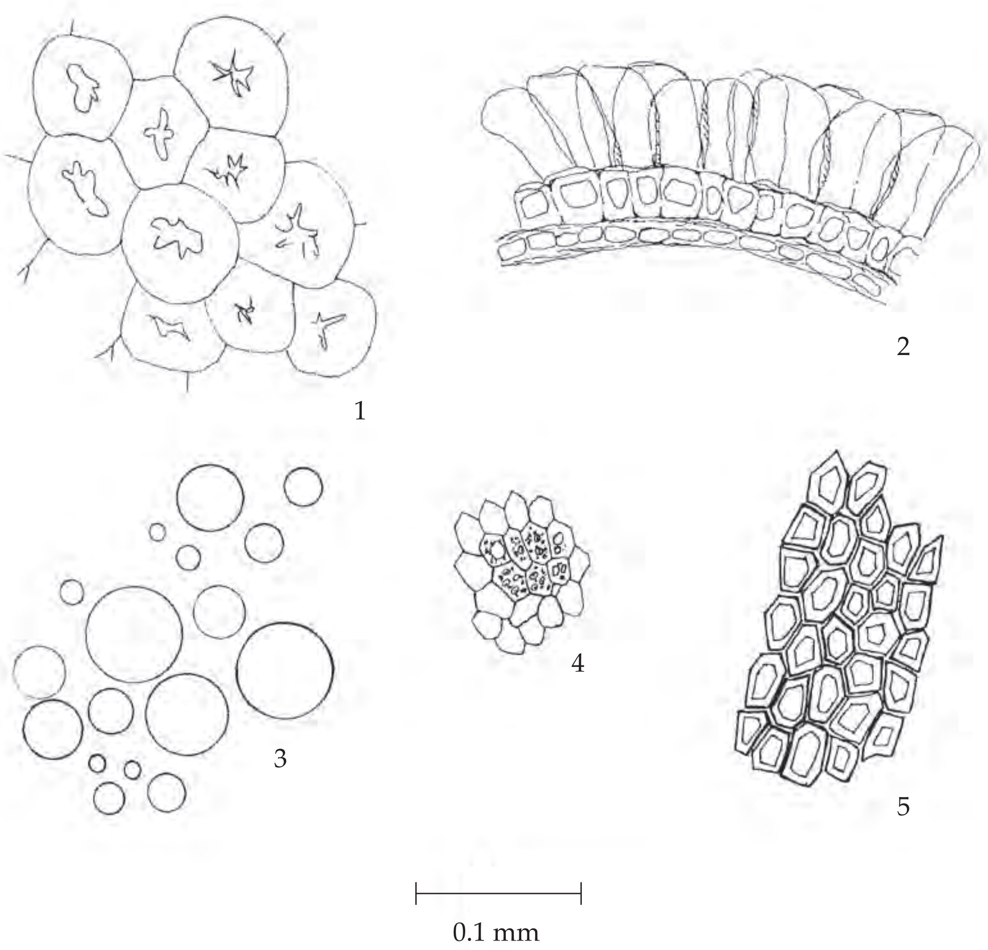


Fig. 2b Powdered Drug of the Seeds of *Lepidium sativum* L.

1. mucilage from outer surface
2. outer integument with protruding mucilage and associated inner integument
3. agglomerated oil globules
4. parenchyma of cotyledon containing oil globules and small aleurone grains
5. outer integument in surface view

Identification

A. Transfer 1 g of the sample, in powder, to a 25-ml ground-glass stoppered cylinder. Moisten the sample with 1 ml of *ethanol*, add *water* to 25 ml, stopper and shake vigorously for 5 minutes. Allow to stand for 1 hour and shake intermittently. Add 0.5 ml of *ruthenium red TS* and mix well: a pinkish swollen mucilage is observed.

B. To 1 g of the sample in a stoppered centrifuge tube, add 3 ml of *ethanol*, stopper, shake for 3 minutes, and filter. To the filtrate, add 2 or 3 drops of *acetic potassium iodobismuthate TS*: an orange precipitate is produced.

C. To 100 mg of the sample, in *fine powder*, add 2 ml of *sodium hydroxide TS* and shake well: a yellow colour is produced immediately.

D. Prepare iron(II) sulfate strip by impregnating a 2 to 3 mm wide paper strip in *iron(II) sulfate TS*. Allow it to dry in a current of air and impregnate it in a 10 per cent w/v solution of *sodium hydroxide* and dry in the same manner. Place the strip in a glass tube passing through a rubber bung of a suitable vessel. Place 5 g of the sample, in *fine powder*, in the vessel, add 3 g of *zinc powder* and 30 ml of *dilute sulfuric acid*. Quickly place the bung in position and heat on a water-bath for about 45 minutes: a black stain on the strip is produced. When the strip is treated with a mixture of 1 ml of 6 M *hydrochloric acid* and a few drops of *iron(III) chloride TS*: the black stain is decolorized with the evolution of hydrogen sulfide and a blue stain is produced.

E. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 9 volumes of *chloroform* and 1 volume of *methanol* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, 2 µl of solution (A) and 5 µl of solution (B). Prepare solution (A), by macerating 5 g of the sample, in *fine powder*, with 30 ml of *petroleum ether* (*boiling range, 40° to 60°*) for 15 minutes and filtering. Macerate the marc with two successive 25-ml portions of *methanol*, filter, and combine the filtrates. Evaporate the filtrate on a water-bath to dryness and dissolve the residue in 30 ml of *dilute hydrochloric acid*. Transfer to a separator, extract with two successive 30-ml portions of *ethyl acetate*, combine the ethyl acetate extracts, evaporate to dryness on a water-bath, and dissolve the residue in 0.5 ml of *methanol*. For solution (B) dissolve 10 mg of *sinapic acid* in 0.5 ml of *water*, add 1 ml of *methanol* and mix well. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows one quenching spot (hR_f value 27 to 29), corresponding to the sinapic acid spot from solution (B), and several spots of different hR_f values. Expose the plate to iodine vapour; the spot due to sinapic acid is pinkish brown. Other spots of different colours are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in the Extract of the Seeds of *Lepidium sativum* L.

Spot	hR_f Value	Detection	
		UV 254	Iodine Vapour
1	7-9	quenching	brown
2	12-13	quenching	pale brown
3*	27-29	quenching	pinkish brown
4	42-44	–	pale yellow
5	45-47	quenching	–
6	75-77	–	brown
7	86-88	quenching	pale yellow
8	94-97	quenching	brown

*sinapic acid

Loss on drying Not more than 10.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 0.4 per cent w/w (Appendix 7.6).

Total ash Not more than 6.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 15.0 per cent w/w (Appendix 7.12).

Swelling index Not less than 20, when determined by the following method.

In a 25-ml ground-glass stoppered cylinder graduated over a height of 125±5 mm in 0.5-ml divisions, place 1 g of Garden Cress Seed, accurately weighed, moisten it with 1.0 ml of *ethanol*, add 25 ml of *water* and close the cylinder. Shake vigorously every 10 minutes for 1 hour. Allow to stand for 3 hours. At 90 minutes after the beginning of the test, release any large volume of liquid retained in the layer of the Seed and any particles of the Seed floating at the surface of the liquid by rotating the cylinder about a vertical axis. Measure the volume occupied by the Seed, including any adhering mucilage. Carry out three tests at the same time. The swelling index is given by the mean of the three tests.

Dose 3 g.

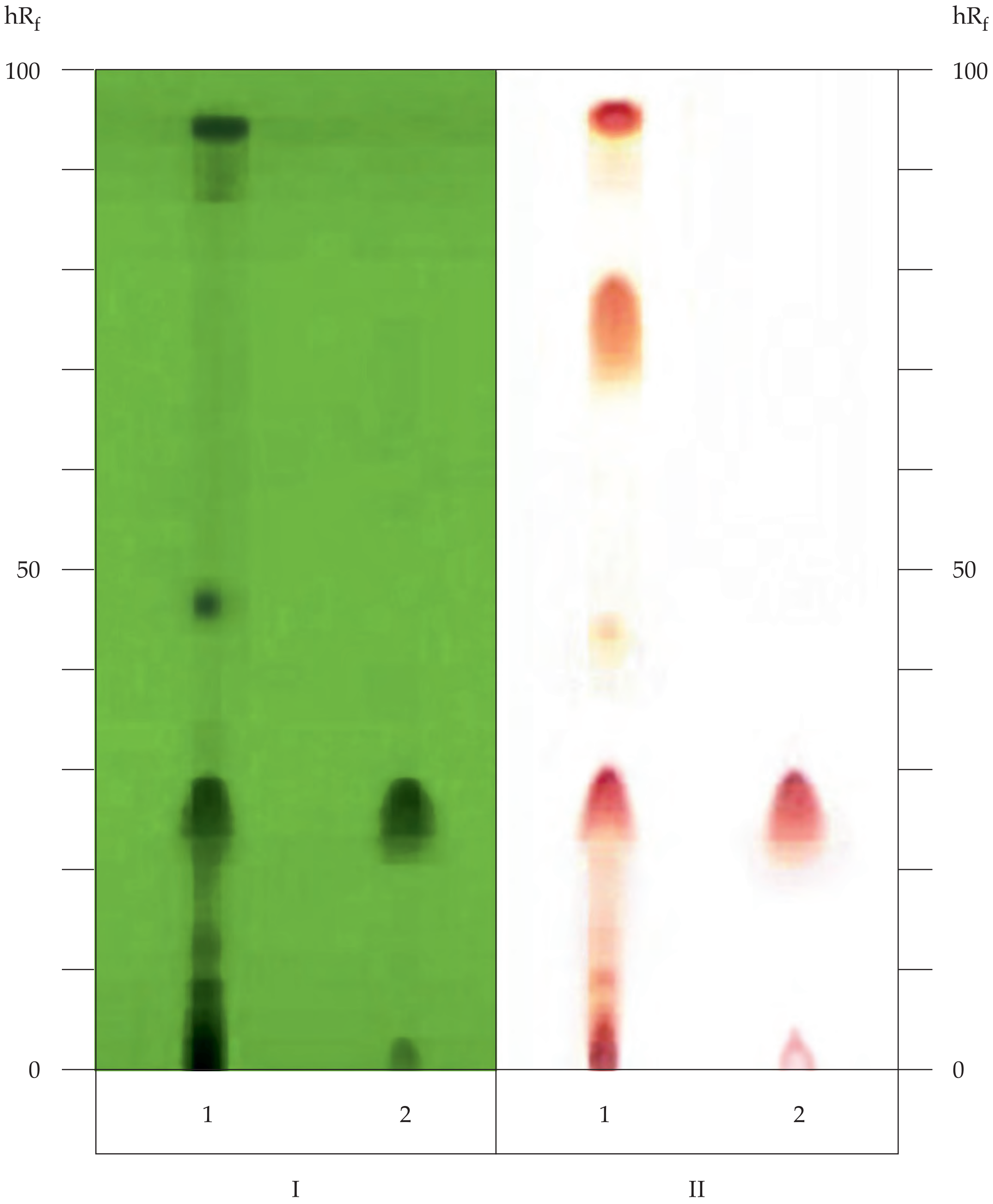


Fig. 3 Thin-layer Chromatogram of the Extract of the Seeds of *Lepidium sativum* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with iodine vapour

เทียนดำ (THIAN DAM)

Nigellae Sativae Semen
Black Cumin

Synonyms Black Caraway, Black Seed

Category Carminative, diuretic.

Black Cumin is the dried ripe seed of *Nigella sativa* L. (Family Ranunculaceae), Herbarium Specimen Number: DMSC 4536, QSBG 22815; Crude Drug Number: DMSc 418.

Constituents Black Cumin contains volatile oil, of which *p*-cymene and thymoquinone are its major components. It also contains fixed oil, alkaloids, flavonoids, sterols, etc.

Description of the plant (Fig. 1a) Annual herb, 30 to 60 cm high, branching at the top. stem green, round, hairy, 2 to 5 mm in diameter, internodes 2 to 5 cm long. Leaves alternate, 1 to 3 pinnately dissected into linear, linear-lanceolate, capillary or irregular lobes, lower leaves small, petioled, upper leaves sessile, 6 to 10 cm long, glabrous on the upper surface, hairy beneath and on the rachis. Flower regular, bisexual, terminal or axillary on branches, white, greenish white or pale blue, about 3 cm in diameter, long-stalked, pedicels 1.5 to 5.5 cm long becoming longer as the fruit matures. Sepals 5, free, greenish white to pale purple, petaloid, caducous, lanceolate or ovate, 1.2 to 1.5 cm long, 0.4 to 0.5 cm wide, longer than petals. Petals 8, about 5 mm long, about 2.5 mm wide, 3-lobed, anterior lobe, small ovate, acuminate, blue, flapped over the fused concave hairy base of the pair of posterior lobes, posterior lobes ovate, greenish white, apex blue with a blue line across the body, each carrying a shining green mass, scantily ciliated. Stamens numerous, outer one longer than the inner ones, basifixed; filaments 2.5 to 5.2 mm long, slender; anthers 1.5 to 2 mm long; ovary superior, about 5 mm long, smooth, carpels 2 to 4, styles and stigma about 7 mm long. Fruits united follicles forming a capsule, ultimately inflated with persistent horn-like styles. Seeds ovate to lanceolate, trigonal, black, numerous.

Description Odourless; taste, bitter, spicy and aromatic.

Macroscopical (Fig. 1a) Trigonal to nearly pentagonal, 2.0 to 3.2 mm long, 1.3 to 1.8 mm wide; surface black, rough, glabrous; apex narrow and sharp; base obtuse.

Microscopical (Fig. 2a, 2b) Transverse section of the seed through the cotyledon shows seed coat composed of the three layers; the outer dark brown, thick-walled cells with irregular papillae; the middle, rectangular, brown, thin-walled cells, more or less collapsed; and the inner, dark brown, thick-walled, tangentially-rectangular cells. Endosperm, thick-walled polygonal cells containing aleurone grains and oil globules. Cotyledons, thin-walled cells containing aleurone grains and oil globules.

Black Cumin in powder possesses the diagnostic microscopical characters of the unground drug; in surface view, the inner epidermis shows striated wall.

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

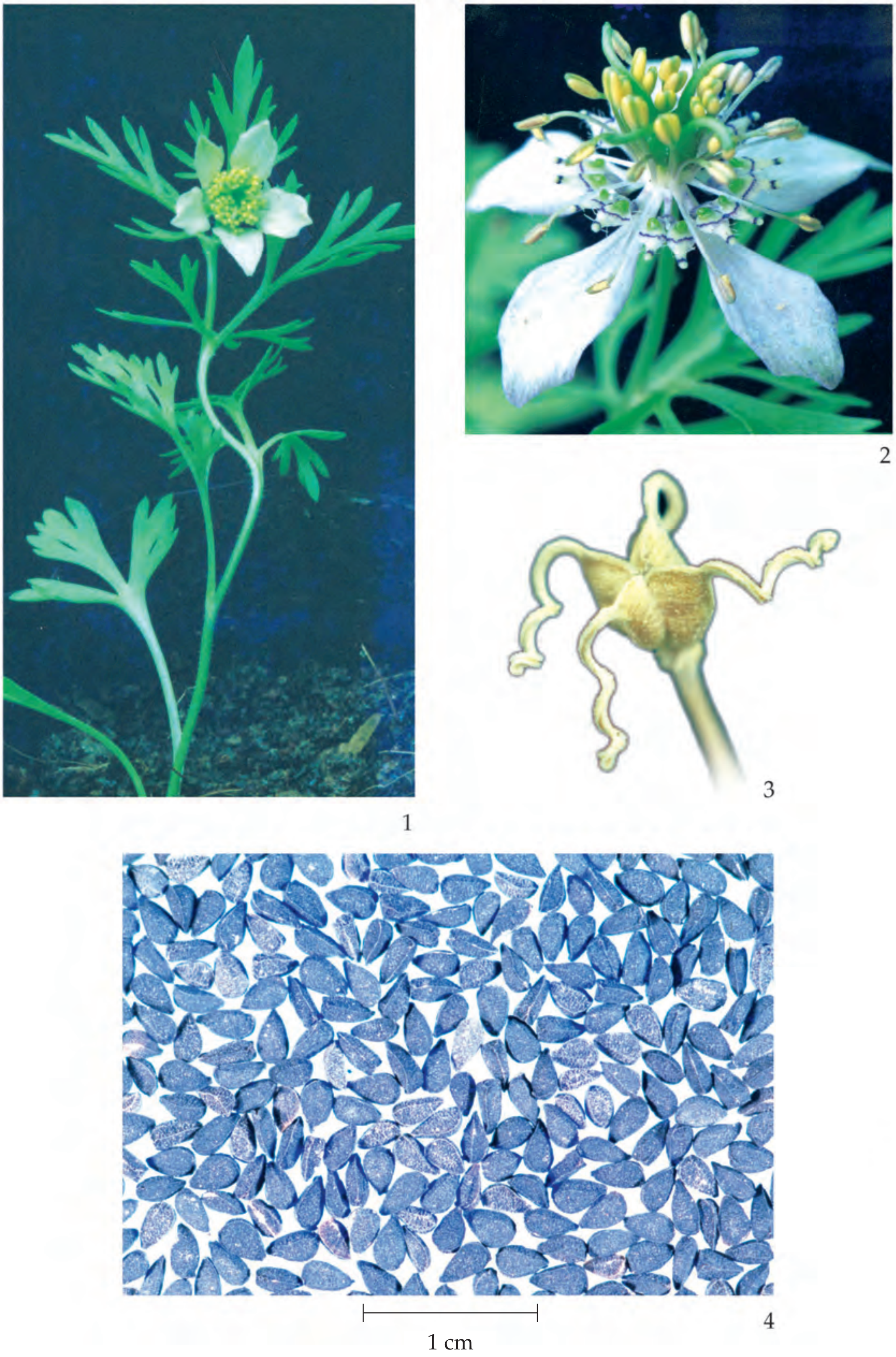


Fig. 1a *Nigella sativa* L.
1. flowering plant 2. flower 3. fruit 4. crude drug

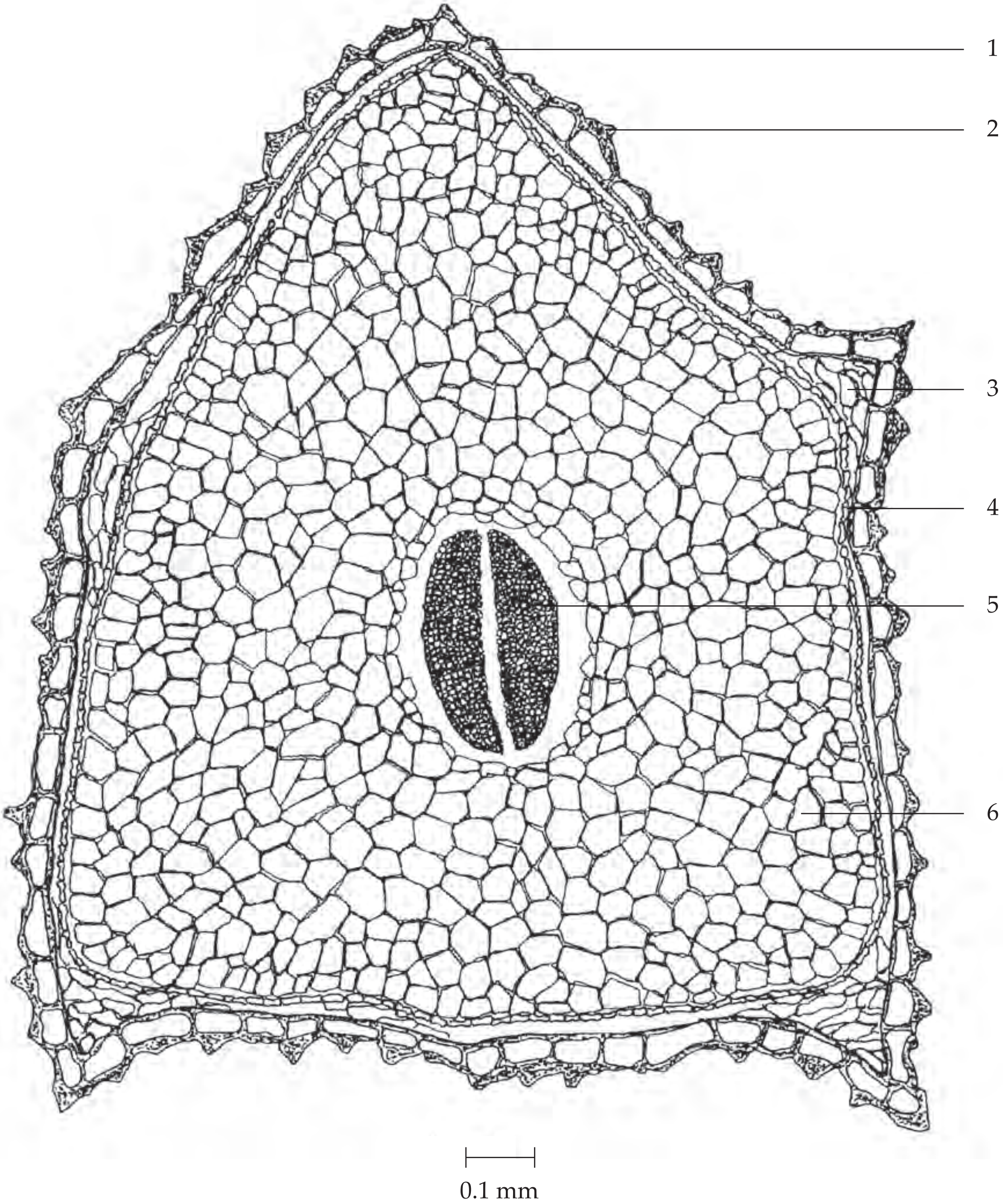


Fig. 2a Transverse Section of the Seed of *Nigella sativa* L.

1. epidermis with irregular papillae
2. papilla
3. layer of collapsed cells
4. inner layer of seed coat
5. cotyledon
6. endosperm

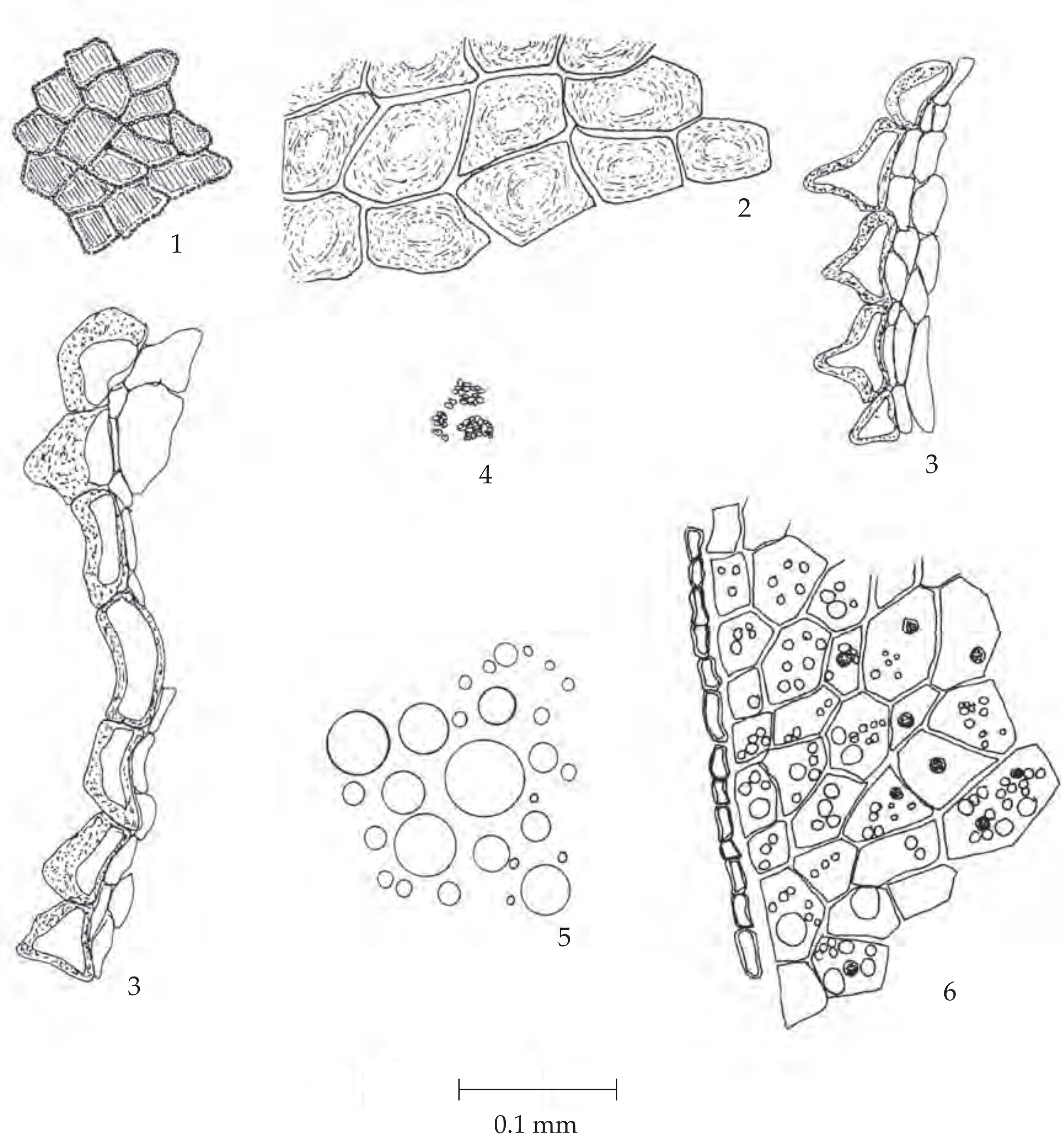


Fig. 2b Powdered Drug of the Seeds of *Nigella sativa* L.

1. inner layer of seed coat in surface view with striated wall
2. epidermis in surface view with papillae
3. epidermis in sectional view with papillae and associated collapsed cells
4. aleurone grains
5. oil globules
6. endosperm cells containing aleurone grains and oil globules with associated inner layer of seed coat

Packaging and storage Black Cumin shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Shake 1 g of the sample, in powder, with 20 ml of *ethanol* for 10 minutes and filter. To 1 ml of the filtrate, add 2 ml of *ethanol*, 2 drops of *ninhydrin TS* and place in a water-bath for a few minutes: a violet-blue colour appears.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 90 volumes of *toluene* and 10 volumes of *ethyl acetate* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate, 2 µl each of solutions (A), (B) and (C). Prepare solution (A) by shaking 3 g of the sample, in powder, with 10 ml of *methanol* for 2 minutes and filtering. For solution (B), dissolve 2 mg of *thymoquinone* in 1 ml of *methanol*. For solution (C), add 10 µl of *olive oil* to 1 ml of *ethyl acetate* and mix. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 74 to 76), corresponding to the thymoquinone spot from solution (B), and three spots of lower hR_f values. Spray the plate with *anisaldehyde TS* and heat at 105° for 15 minutes; the spot due to thymoquinone is yellowish brown. One violet spot (hR_f value 94 to 96) corresponds to a spot of triglycerides of olive oil from solution (C). Other two pale violet and five violet spots are also observed (Table 1); see also Fig. 3.

Table 1 hR$_f$ Values of Components in Methanolic Extract of the Seeds of *Nigella sativa* L.

Spot	hR$_f$ Value	Detection	
		UV 254	*Anisaldehyde TS*
1	2-4	–	violet
2	22-25	weak quenching	–
3	27-33	–	violet
4	34-37	weak quenching	violet
5	42-45	–	violet
6	46-48	weak quenching	–
7	49-50	–	pale violet
8	61-63	–	violet
9*	74-76	quenching	yellowish brown
10	86-88	–	pale violet
11**	94-96	–	violet

*thymoquinone

**triglycerides of olive oil

Water Not more than 7.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 4.0 per cent w/w (Appendix 7.2).

Total ash Not more than 7.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 24.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 0.15 per cent v/w (Appendix 7.3H). Use 20 g, in *coarse powder*, freshly prepared and accurately weighed. Use 200 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Dis til at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

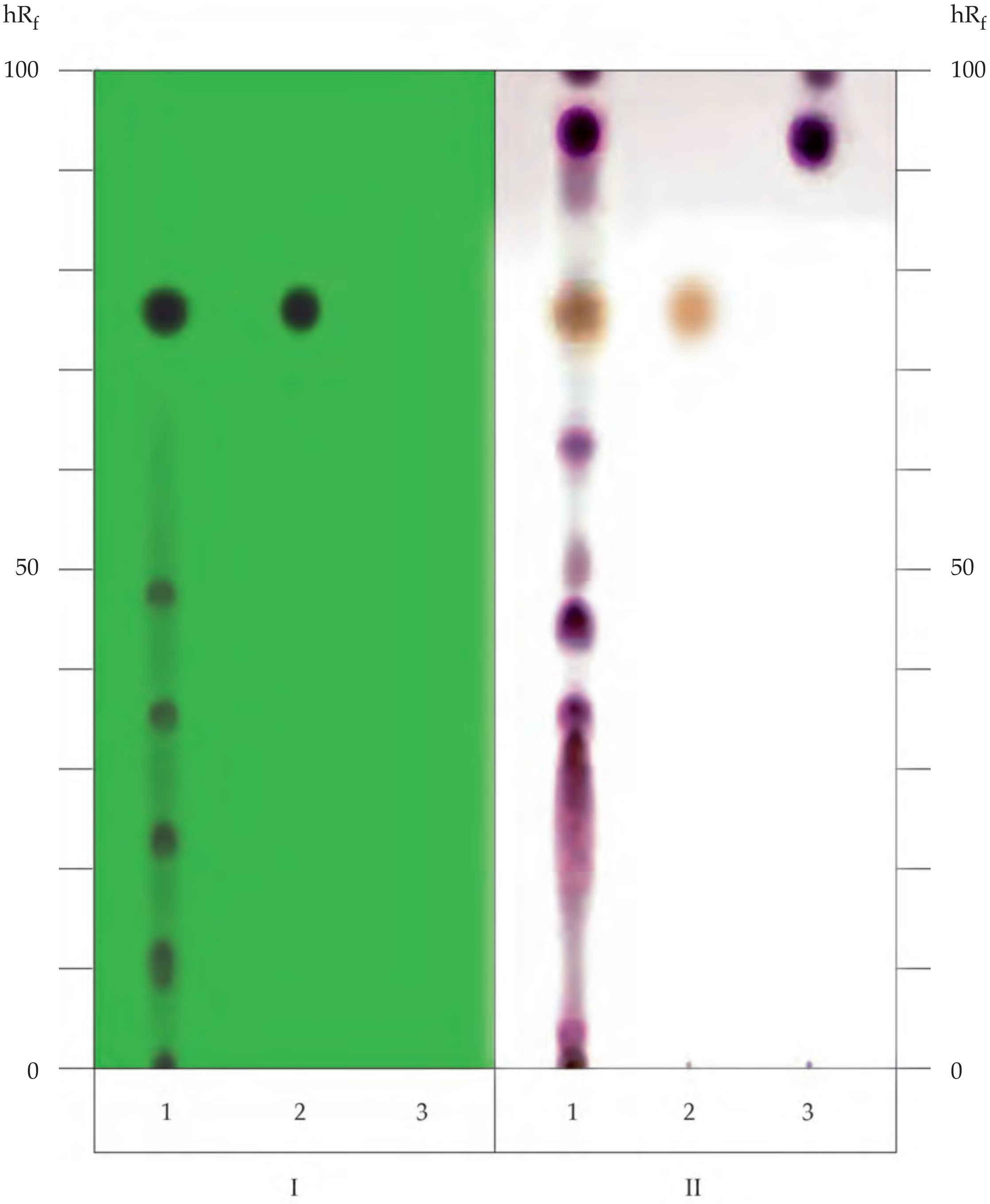


Fig. 3 Thin-layer Chromatogram of Methanolic Extract of the Seeds of *Nigella sativa* L.

1 = solution (A)
2 = solution (B)
3 = solution (C)
I = detection under UV light (254 nm)
II = detection with *anisaldehyde TS*

เทียนขาว (THIAN KHAO)

ยี่หร่า (YIRA)
Cumini Cymini Fructus
Cumin

Category Carminative, expectorant, alterative.

Cumin is the dried ripe fruit of *Cuminum cyminum* L. (Family Umbelliferae), Crude Drug Number: DMSc 330.

Constituents Cumin contains volatile oil, of which cuminaldehyde and *p*-cymene are its major components. It also contains proteins, lipids, flavonoid glycosides, mucilage, etc.

Description of the plant (Fig. 1a) Annual herb, slender, erect, herbaceous, caulescent, glabrous, 20 to 40 cm high. Basal leaves ovate in general outline, excluding the petioles 5 to 10 cm long, ternately dissected, the ultimate divisions linear-filiform, entire, 1 to 5 cm long; petioles sheathing, 1 to 2 cm long. Inflorescence of loose compound umbels; peduncle 2 to 10 cm long; involucre of 5 to 7 linear and entire or ternate bracts with linear divisions, unequal, 1 to 5 cm long; rays 2 to 6, 5 to 25 mm long, unequal; pedicels 2 to 8 mm long. Flowers small; sepals minute or wanting; petals 5, oblong with a narrower inflexed apex or emarginate, white or rose; stamens 5, alternating with the petals, inserted around an epigynous disc; ovary inferior, 2-celled, each cell 1-ovuled, styles 2, short stylopodium conic, attenuate into the rigid styles; carpophore 2-cleft to the base. Fruits mericarps, ovoid-oblong, 4 to 5 mm long, glabrous or setulose

Description Odour, aromatic; taste, spicy.

Macroscopical (Fig. 1a) Entire cremocarps with pedicels attached, about 4.5 to 7 mm long and 1.2 to 1.8 mm wide, oblong; mericarp, narrowly elliptical but slightly curved, covered with tiny bristly hair. Dorsal surface convex, brown, with 5 lighter-coloured filiform primary ridges, and at the summit with an acute conical stylopodium; secondary ridges occurred between each pair of primary ridges, covered with prominent bristles; commissural surface concave, brown.

Microscopical (Figs. 2a, 2b) Transverse section of the mericarp through the cotyledon shows epicarp covered with striated cuticle; a layer of sinuous walled epidermal cells; trichomes, multicellular, multiseriate, rounded at the apex. Mesocarp, several layers of more or less collapsed, tangentially-elongated parenchymatous cells; each of the ridge, sclerenchymatous cells and a lignified fibrovascular bundle; the dorsal side, 4 large vittae located between the vascular bundles; the commissural side, 2 large vittae; each vitta, elliptical, brown, lined by small epithelial secretory cells. Endocarp, a layer of tangentially-elongated cells. Spermoderm, a layer of brownish, tangentially-elongated cell united with the endocarp except in the region of the raphe along the commissural side where separated by collapsed thin-walled cells. Endosperm, thick-walled polygonal cells containing oil globules and aleurone grains with microcrystals. Cotyledons, thin-walled cells containing aleurone grains and oil globules.

Cumin in powder possesses the diagnostic microscopical characters of the unground drug.

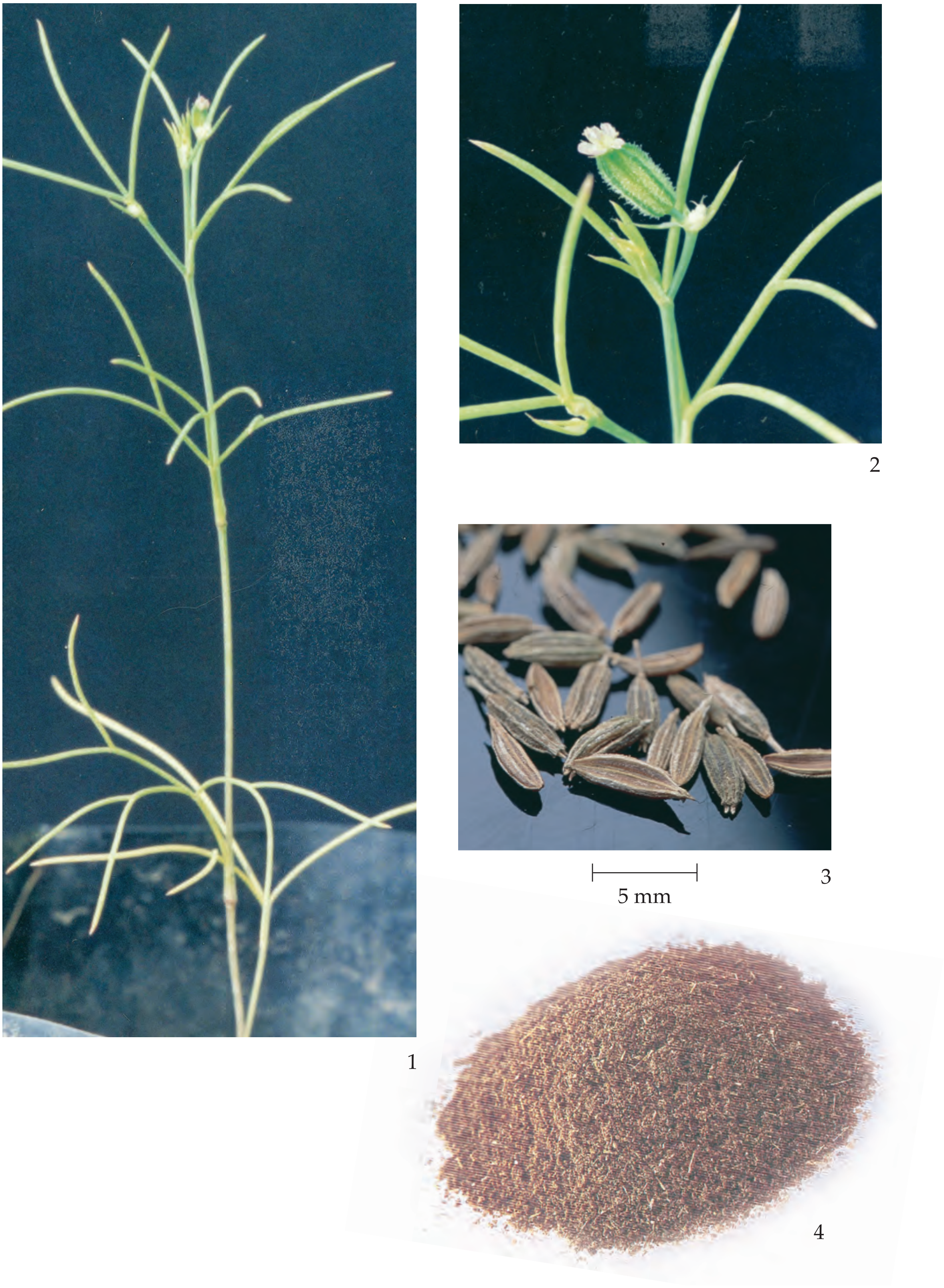


Fig. 1a *Cuminum cyminum* L.
1. habit 2. young fruit 3. crude drug 4. ground crude drug

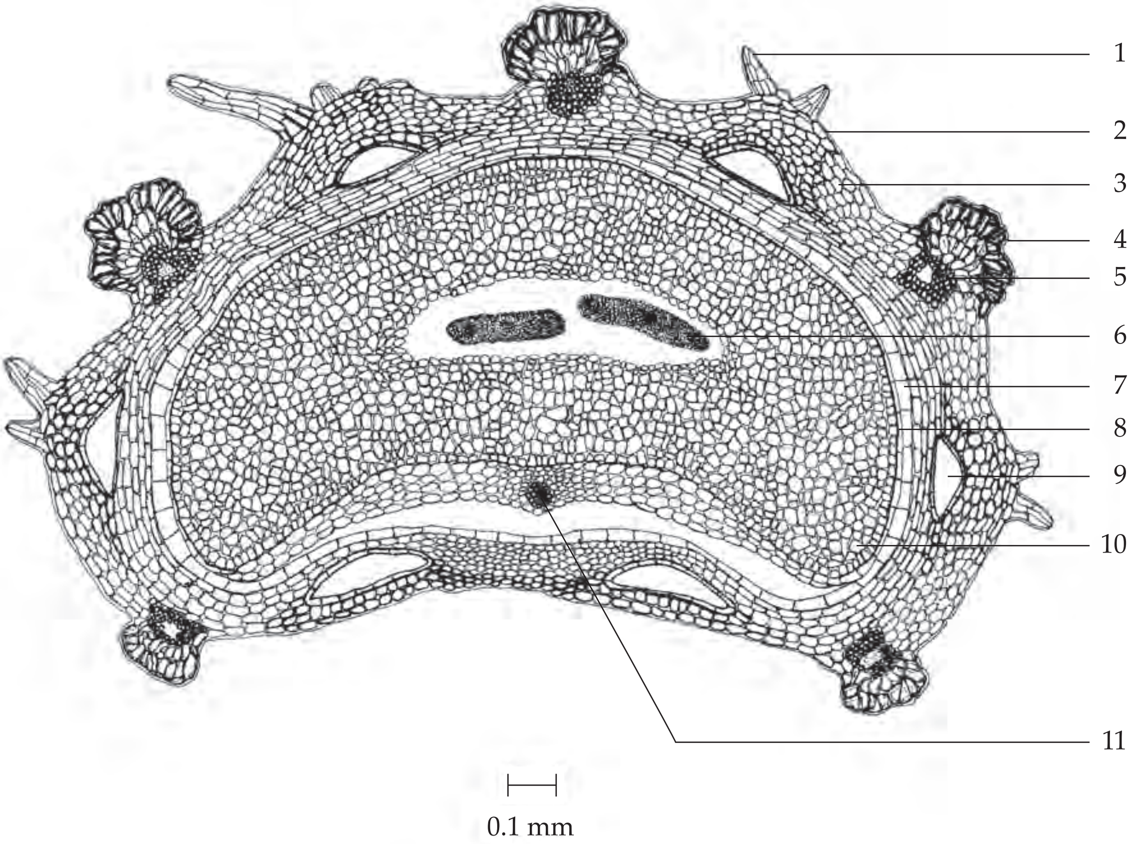


Fig. 2a Transverse Section of the Mericarp of *Cuminum cyminum* L.

1. trichome	7. endocarp
2. epicarp	8. spermoderm
3. parenchyma of mesocarp	9. vitta
4. sclerenchymatous cell	10. endosperm
5. vascular bundle	11. raphe
6. cotyledon	

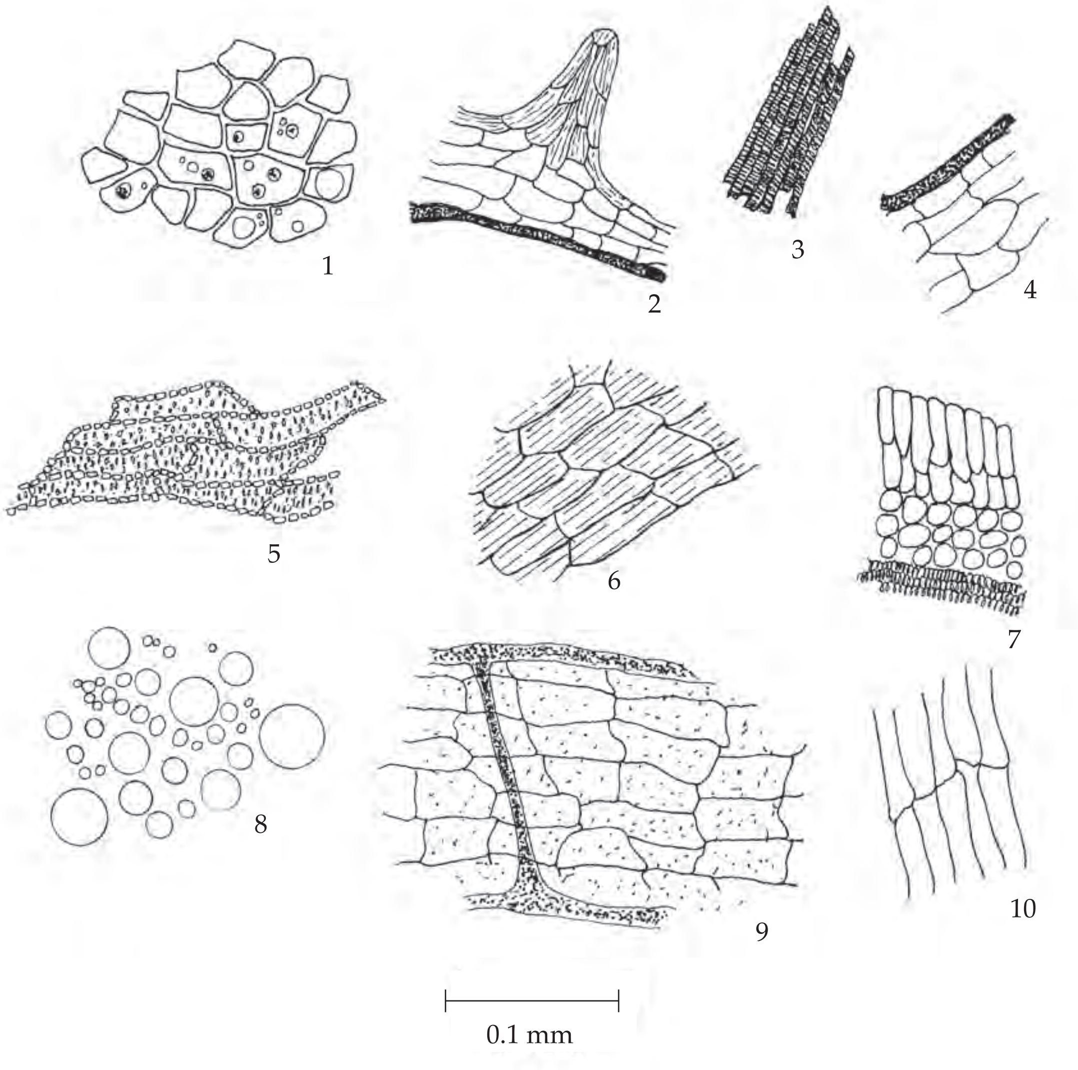


Fig. 2b Powdered Drug of the Fruits of *Cuminum cyminum* L.

1. endosperm containing oil globules and aleurone grains with microcrystals
2. epicarp with multicellular trichome in sectional view associated with mesocarp and part of vitta
3. scalariform vessels
4. mesocarp in sectional view associated with part of vitta
5. sclereids
6. striated epicarp in surface view
7. cotyledon in sectional view
8. oil globules
9. vitta in surface view
10. endocarp in surface view

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Cumin shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Mix 1 g of the sample, in powder, with 3 ml of *ethanol*, shake for 5 minutes and filter. To 1 ml of the filtrate, add 1 ml of *dinitrophenylhydrazine TS1*: a turbid, orange-yellow solution is produced.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 95 volumes of *toluene* and 5 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, 10 µl each of solution (A) and solution (B). Prepare solution (A) by shaking 1 g of the sample, in powder, with 5 ml of *ethyl acetate* for 2 to 3 minutes and filtering through 2 g of *anhydrous sodium sulfate*. For solution (B), dissolve 2 µl of *cuminaldehyde* and 30 µl of *olive oil* in 5 ml of *ethyl acetate*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm). The main quenching spot (hR_f value 49 to 52) obtained from solution (A) corresponds to that of cuminaldehyde from solution (B). Three other quenching spots, one above and two below the main spot, are also observed. Spray the plate with *dinitrophenylhydrazine TS1*; the spot corresponding to cuminaldehyde appears orange while the others appear yellow (Table 1); see also Fig. 3.

Repeat the same procedure on another plate but spray with *anisaldehyde TS* and heat at 110° for 10 minutes. The chromatogram obtained from solution (A) shows a main violet spot (hR_f value 58 to 63), corresponding to triglycerides of olive oil from solution (B); other violet spots are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethyl Acetate Extract of the Fruits of *Cuminum cyminum* L.

Spot	hR_f Value	Detection		
		UV 254	*Dinitrophenylhydrazine TS1*	*Anisaldehyde TS*
1	3-5	–	–	violet
2	11-13	–	–	violet
3	13-17	–	–	violet
4	19-22	–	–	violet
5	23-26	–	–	violet
6	33-38	–	–	violet
7	38-42	weak quenching	yellow	violet
8	42-46	weak quenching	yellow	red-violet
9*	49-52	quenching	orange	–
10**	58-63	weak quenching	pale yellow	violet

*cuminaldehyde

**triglycerides of olive oil

Water Not more than 10.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 3.0 per cent w/w of foreign matter including separated pedicels (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 9.0 per cent w/w (Appendix 7.7).

Volatile oil Not less than 2.5 per cent v/w (Appendix 7.3H). Use 20 g, in *coarse powder*, freshly prepared and accurately weighed. Use 200 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

Dose 0.5 to 2 g.

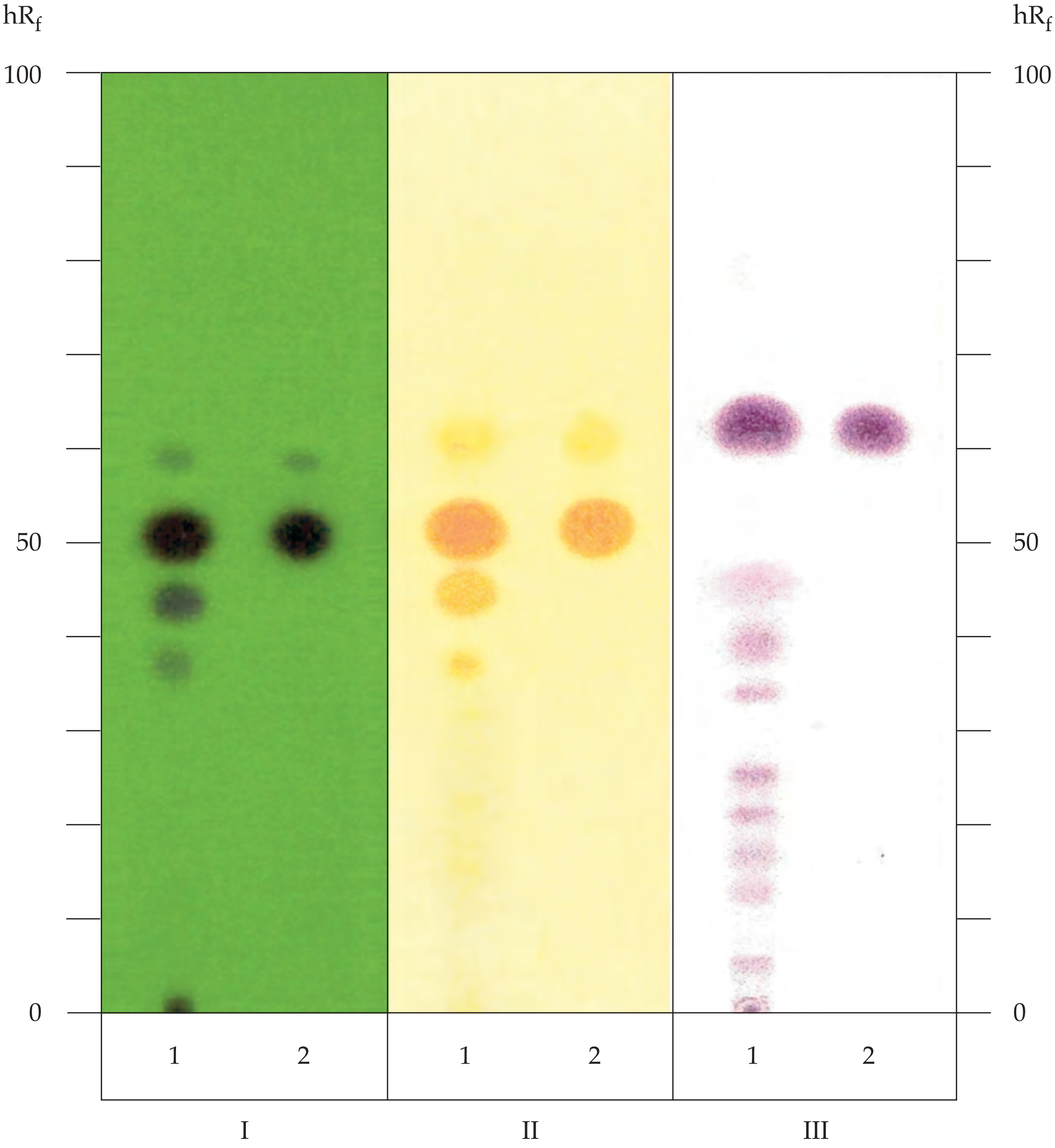


Fig. 3 Thin-layer Chromatogram of Ethyl Acetate Extract of the Fruits of *Cuminum cyminum* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with *dinitrophenylhydrazine TS1*
III = detection with *anisaldehyde TS*

เทียนข้าวเปลือก (THIAN KHAO PLUEAK)

Foeniculi Dulcis Fructus
Sweet Fennel

Category Carminative, spasmolytic.

Sweet Fennel is the dried cremocarp and mericarp of *Foeniculum vulgare* Mill. [*F. vulgare* var. *dulce* (Mill.) Batt. & Trab.] (Family Umbelliferae), Herbarium Specimen Number: DMSC 4534, QSBG 22811, Crude Drug Number: DMSc 432.

Constituents Sweet Fennel contains volatile oil of which anethole, estragole and fenchone are its major components. It also contains flavonoids.

Description of the plant (Figs. 1a, 1b) Herbs, annual or perennial, 0.4 to 2 m high; all parts strongly aromatic; stem erect, soft and hollow, terete, grey-green or lurid green, glabrous. Leaves broadly triangular in outline, 4 to 30 cm long, 5 to 40 cm wide, pinnately decompound, ultimate segment linear, 1 to 6 mm long, about 0.1 mm wide; lower petiole 5 to 15 cm long, sheaths membranous-margined. Inflorescence compound umbel, terminal and lateral, 5 to 9 cm across; bracts and bracteoles absent; rays numerous, upwards-spreading, unequal; peduncles 2 to 25 cm long; umbellules 14- to 39-flowered; pedicel thin, 2 to 10 mm long, unequal. Flower: calyx teeth obsolete; petals 5, yellow, obovate, midrib conspicuous, apex with narrowly inflexed lobule; stamens 5; ovary inferior, stylopodium conic, styles 2, very short, reflexed. Fruits cremocarp, oblong, 4 to 6 mm long, terete, glabrous, each mericarp 5 ridges, acute or round-obtuse. Seeds face, plane or slightly concave, carpophore 2-cleft to base.

Description Odour, aromatic; taste, slightly sweet and spicy.

Macroscopical (Figs. 1a, 1b) Cremocarps, pale green or pale yellowish brown, 3.6 to 8.4 mm long and 1.1 to 2.5 mm wide, oblong, laterally compressed. Mericarps frequently separate, each being broadly elliptical, more or less curved; dorsal surface convex with 5 prominent, longitudinal ridges, and at the summit a short, conical s tylopodium; commissural surface flat, with 3 narrow, light-coloured areas separated by 2 darker coloured areas.

Microscopical (Figs. 2a, 2b) Transverse section of the mericarp through the cotyledon shows epicarp layer, epidermal cells covered with smooth cuticle layer; stomata, occasional, various types; a carpophore on the commissural region, composing of lignified fibres. Mesocarp, several layers of thin-walled parenchyma cells; the ridge portions, a lignified fibrovascular bundle, surrounded by reticulate and porous, thickened, lignified parenchyma cells; vittae, large, brown, lined by small epithelial secretory cells, 4 dorsal between the vascular bundles, 2 commissural. Endocarp, a layer of tangentially-elongated cells. Spermoderm, a layer of brownish, tangentially-elongated cells attached to the endocarp, except where separated in the region of raphe. Endosperm, thick-walled, polygonal cells containing oil globules, aleurone grains with micro-rosette crystals of calcium oxalate. Two cotyledons located at the centre containing thin-walled cells with aleurone grains and oil globules.

Sweet Fennel in powder possesses the diagnostic microscopical characters of the unground drug.

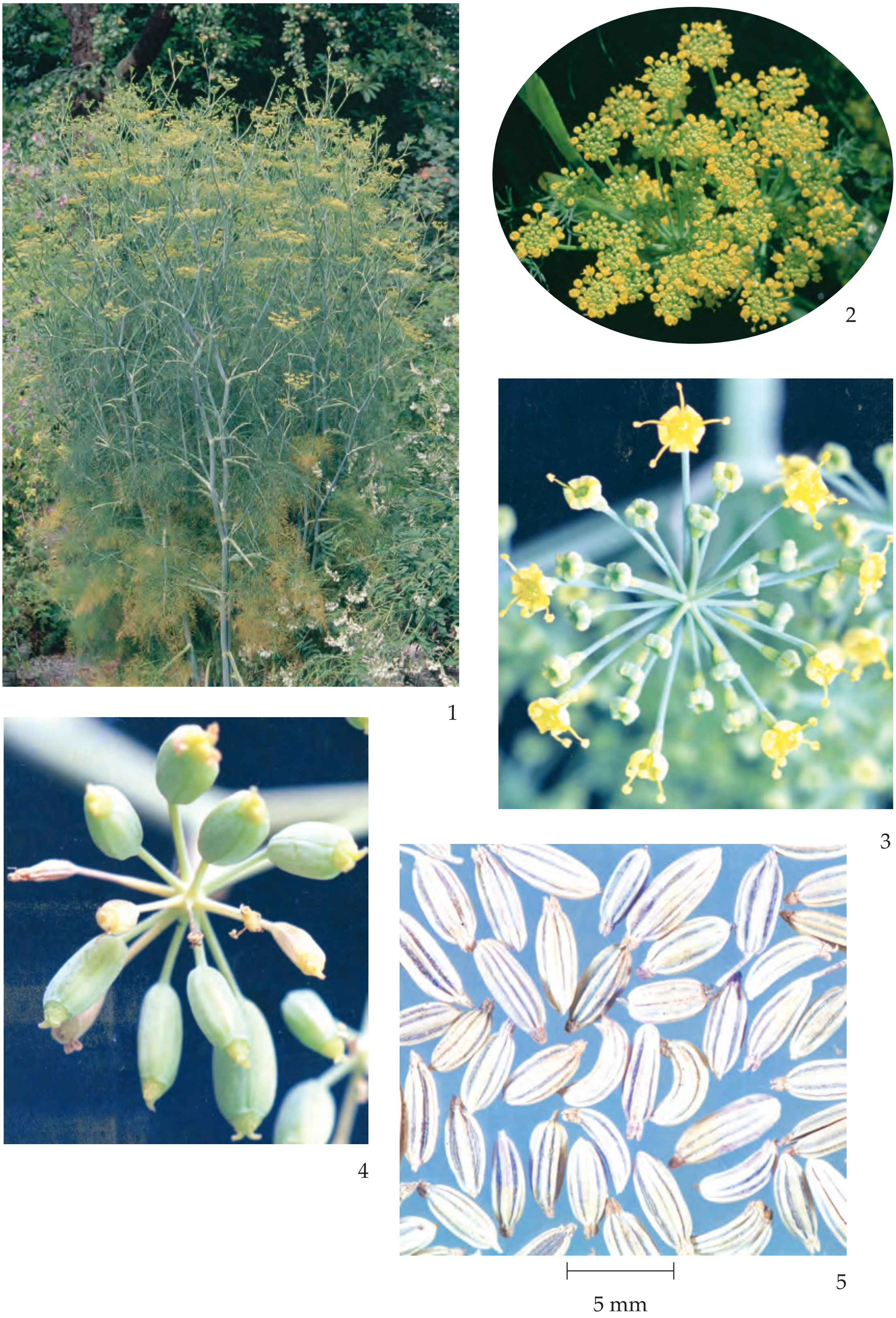


Fig. 1a *Foeniculum vulgare* Mill.
1. habit 2. inflorescence 3. umbellet 4. young infructescence 5. crude drug

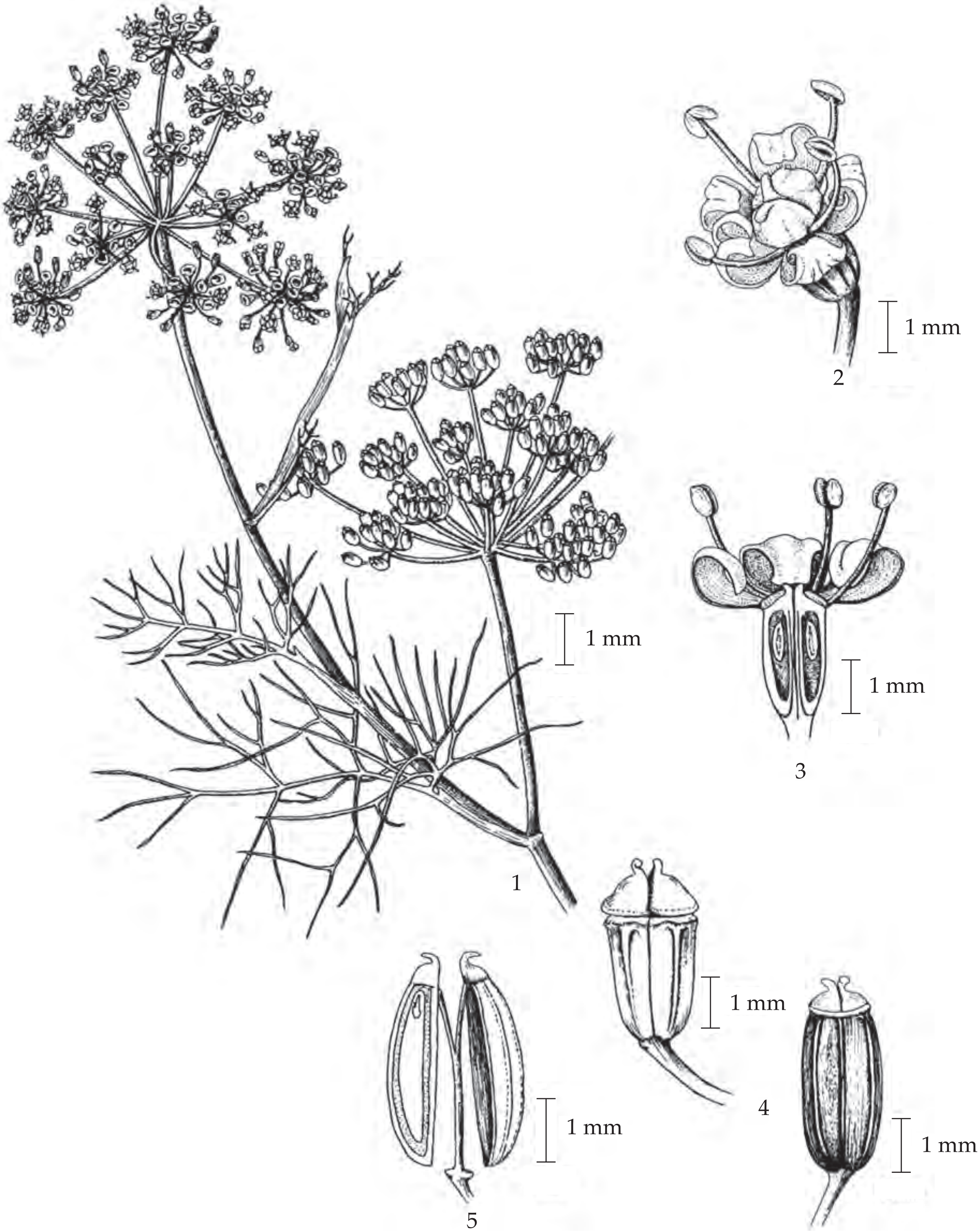


Fig. 1b *Foeniculum vulgare* Mill.
1. part of plant showing inflorescence and infructescence 2. flower
3. flower with longitudinal section of ovary 4. young fruit 5. schizocarp

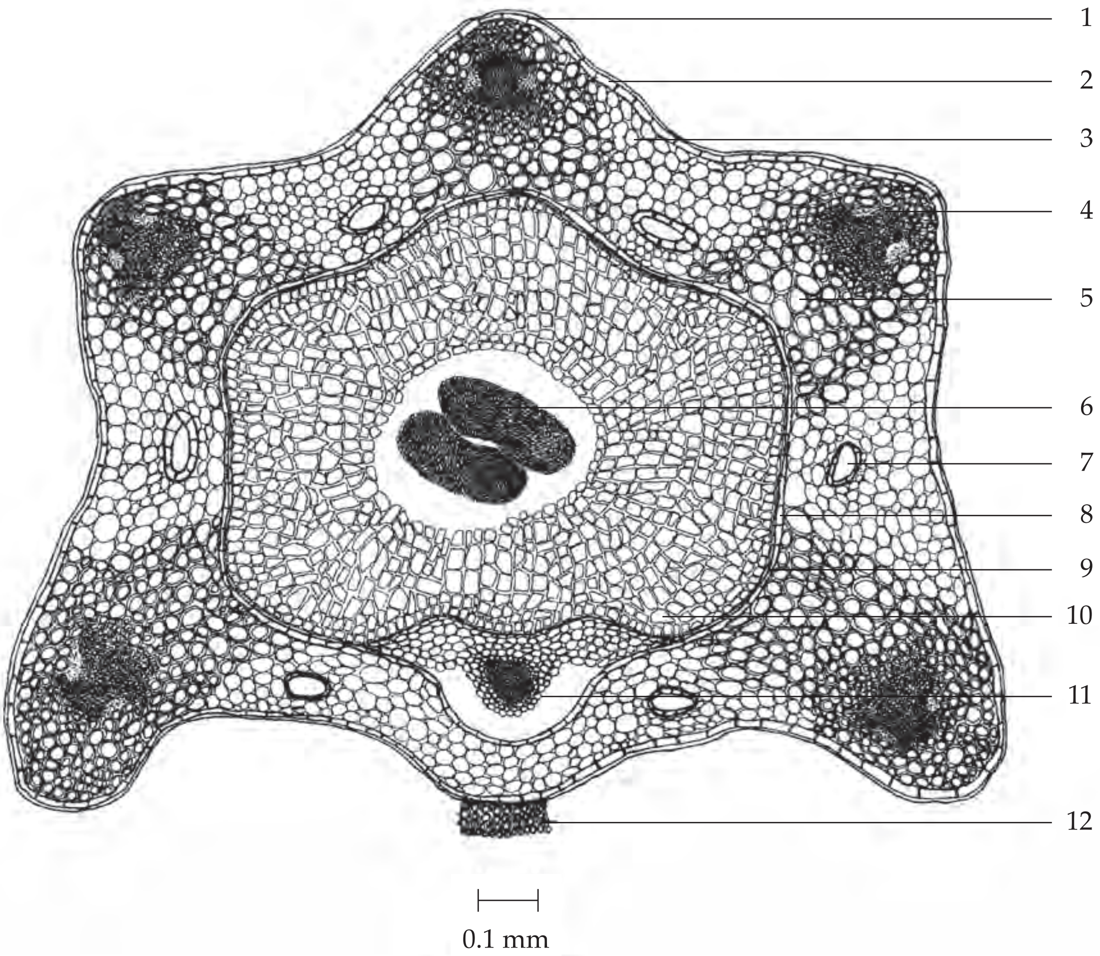


Fig. 2a Transverse Section of the Mericarp of *Foeniculum vulgare* Mill.

1. cuticle layer	7. vitta
2. epicarp	8. endocarp
3. parenchyma of mesocarp	9. spermoderm
4. vascular bundle	10. endosperm
5. thick-walled parenchyma	11. raphe
6. cotyledon	12. carpophore

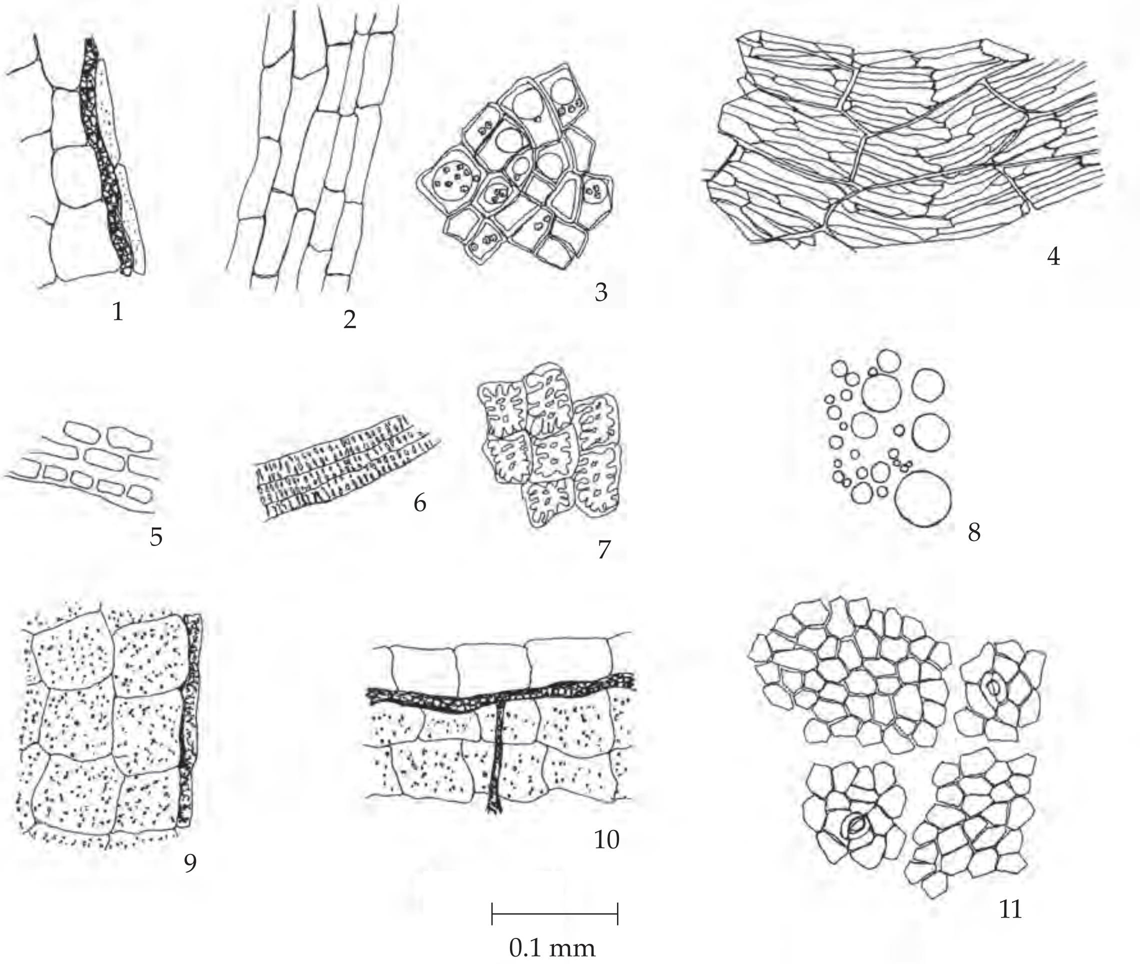


Fig. 2b Powdered Drug of the Fruits of *Foeniculum vulgare* Mill.

1. mesocarp in sectional view associated with vitta
2. spermoderm in surface view
3. endosperm containing oil globules and aleurone grains containing micro-rosette crystals of calcium oxalate
4. endocarp with overlying cells of the mesocarp, in surface view
5. epicarp associated with mesocarp in sectional view
6. reticulate vessels
7. porous, thickened lignified parenchyma
8. oil globules
9. vitta in surface view showing septa
10. vitta in surface view showing septa and mesocarp
11. epicarp in surface view showing stomata

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Sweet Fennel shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Macerate 3 g of the sample, in powder, with 10 ml of *ethanol* (*80 per cent*) for 3 hours and filter (solution 1). To 3 ml of solution 1, add 0.5 ml of *water*: the solution becomes milky turbid.

B. To 3 ml of solution 1, add 3 drops of a 5 per cent w/v solution of *iron(III) chloride*: a dark green precipitate forms.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 90 volumes of *toluene*, 10 volumes of *hexane* and 2 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate as bands of 10 mm, 10 µl each of solutions (A) and (B). Prepare solution (A) by shaking 300 mg of the freshly prepared sample, in *No. 1400 powder*, with 5.0 ml of *dichloromethane* for 15 minutes. Filter and carefully evaporate the filtrate to dryness on a water-bath at 60°. Dissolve the residue in 0.5 ml of *toluene*. For solution (B), dissolve 50 µl of *anethole*, 10 µl of *fenchone* and 25 µl of *olive oil* in 5.0 ml of *hexane*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching bands. The chromatogram obtained from solution (A) shows a quenching band (hR_f value 85 to 88), corresponding to the anethole band from solution (B) and one band of lower hR_f value. Spray the plate with *phosphomolybdic acid TS* and heat at 105° for 5 to 10 minutes. Subsequently spray with a 3.3 per cent w/v solution of *potassium permanganate* in *sulfuric acid*; the band due to anethole is dark blue. One blue band (hR_f value 43 to 45) and one dark blue band (hR_f value 60 to 63), corresponding to the fenchone band and the band of triglycerides of olive oil from solution (B), are observed, respectively. Other seven blue bands are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Dichloromethane Extract of the Fruits of *Foeniculum vulgare* Mill.

Spot	hR_f Value	Detection	
		UV 254	*Phosphomolybdic Acid TS/ Potassium Permanganate-Sulfuric Acid*
1	4-6	–	blue
2	8-9	–	blue
3	12-14	–	blue
4	17-18	–	blue
5	21-22	–	blue
6	32-34	weak quenching	blue
7*	43-45	–	blue
8**	60-63	–	dark blue
9***	85-88	quenching	dark blue
10	95-97	–	blue

*fenchone

**triglycerides of olive oil

***anethole

Water Not more than 10.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 1.5 per cent w/w of peduncles and not more than 1.5 per cent w/w of other foreign matter (Appendix 7.2).

Acid-insoluble ash Not more than 0.7 per cent w/w (Appendix 7.6).

Total ash Not more than 9.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 7.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 2.0 per cent v/w, calculated on the anhydrous basis (Appendix 7.3H). Use 20 g, in *coarse powder*, freshly prepared and accurately weighed. Use 200 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 2 hours. Use 2.0 ml of *xylene* in the graduated tube.

Anethole Not less than 80.0 per cent. Carry out the determination as described in the “Gas Chromatography” (Appendix 3.4).

Standard solution Dissolve about 5 mg of *anethole* in 0.5 ml of *xylene*.

Test solution Dilute the mixture of volatile oil and xylene obtained in the determination of *Volatile oil* and rinse the apparatus with *xylene* to obtain 5.0 ml.

Chromatographic system The chromatographic procedure may be carried out using (a) a capillary column (30 to 60 m × 0.3 mm) coated with *macrogol 20,000* and (b) *nitrogen* as the carrier gas at a flow rate of about 0.40 ml per minute and split at a ratio of 1 to 200. The column is maintained at a temperature of 60° for 4 minutes, then raising the temperature linearly at a rate of 5° per minute to 170° and maintained at 170° for 15 minutes. The injection port is maintained at 220° and the detector at 270°.

Procedure Separately inject equal volumes (about 1 µl) of *Standard solution* and *Test solution* into the chromatograph, record the chromatograms and calculate the content of anethole by normalization.

Estragole and fenchone Not more than 10.0 per cent of estragole and not more than 7.5 per cent of fenchone. Carry out the determination as described in the "Gas Chromatography" (Appendix 3.4).

Standard solution Dissolve about 5 mg of *estragole* and about 5 mg of *fenchone* in 0.5 ml of *xylene*.

Test solution, Chromatographic system and **Procedure** Proceed as directed in the determination of *Anethole*. Calculate the contents of estragole and fenchone by normalization.

Dose 0.3 to 0.6 g three times a day.

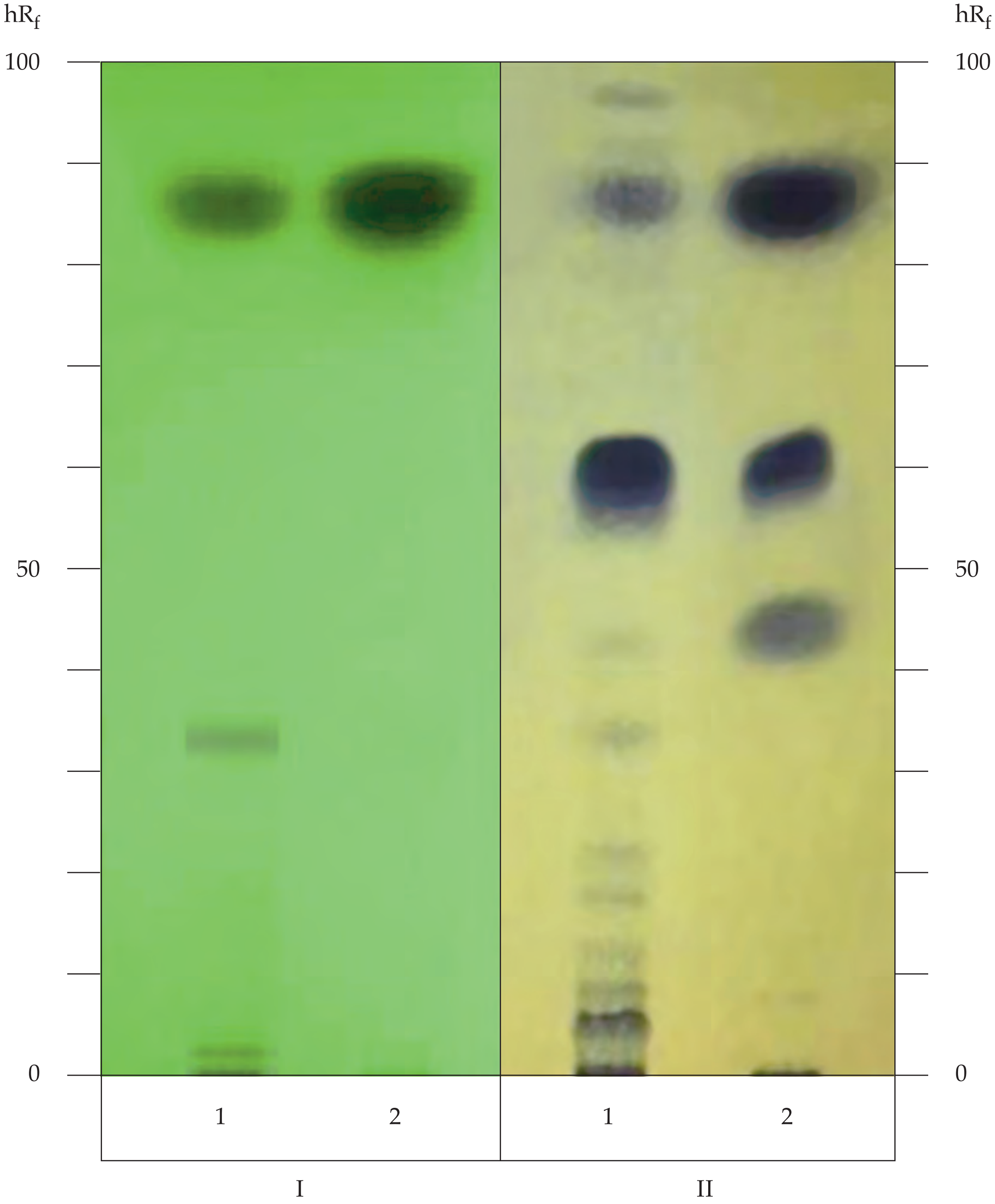


Fig. 3 Thin-layer Chromatogram of Dichloromethane Extract of the Fruits of *Foeniculum vulgare* Mill.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with *phosphomolybdic acid TS* and *potassium permanganate* in *sulfuric acid*

เทียนเกล็ดหอย (THIAN KLET HOI)

Plantaginis Ovatae Semen
Ispaghula Seed

Synonyms Blond Psyllium Seed, Indian Plantago Seed, Pale Psyllium Seed, Spogel Seed

Category Bulk-forming laxative.

Ispaghula Seed is the dried ripe seed of *Plantago ovata* Forssk. (*P. ispaghula* Roxb. ex Fleming) (Family Plantaginaceae), Herbarium Specimen Number: BK 50463, Crude Drug Number: DMSc 435.

Constituents Ispaghula Seed contains mucilage consisting mainly of arabinoxylans. It also contains aucubin, triterpenes, sterols, fixed oil, etc.

Description of the plant (Figs. 1a, 1b) Herb, annual or biennial, stemless or subcaulescent, sparsely or thickly villous, up to 30 cm high. Leaves radical, narrow-linear or filiform finely acuminate, 7 to 22 cm long, usually 3-nerved, entire or distantly toothed. Scapes axillary, longer or shorter than the leaves, glabrous or pubescent; spikes ovoid or cylindrical, up to 4 cm long; bracts with broad scarious margins, ovate-oblong, obtuse, glabrous. Flowers small, greenish to white; sepals 4, imbricate in bud, persistent, ovate to elliptic, glabrous or pubescent; corolla, salver-shaped, scarious, lobes 4, imbricate in bud, corolla-lobes rounded concave obtuse; stamens 4, exserted, filaments, persistent, anthers large, pendulous, versatile; ovary superior, 2-celled, ovule 1, basal in each cell. Fruit capsule, ovoid, membranous, 2-celled circumscissile; cell, 1-seeded.

Description Odour, slight; taste, bland and very mucilaginous.

Macroscopical (Figs. 1a, 1b) Boat-shaped, 2.5 to 3.2 mm long and 1.2 to 1.6 mm wide, colour variable, but usually pale, greyish brown with a pinkish tinge, dull and hard; on the convex surface a central, glossy reddish brown, elongated oval spot; the concave surface deeply grooved with the hilum a brown spot in the centre of the hollow, more or less covered with a whitish membrane. The seed coat swells in water and the seed becomes enveloped with a thick layer of translucent and colourless mucilage.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the seed cut through the cotyledon region shows seed coat, endosperm and cotyledon. The outer layer of seed coat, large, rectangular, mucilaginous epidermal cells; the cells rapidly swelling in water, the outer walls bursting and, liberating numerous finger-like processes of mucilage, occasionally containing starch granules. The inner layer of seed coat, brownish tangentially-elongated cells. Endosperm, thick-walled, polygonal cells with numerous large, very conspicuous pits, containing aleurone grains and oil globules. Cotyledons, thin-walled cells containing aleurone grains and oil globules.

Ispaghula Seed in powder possesses the diagnostic microscopical characters of the unground drug.

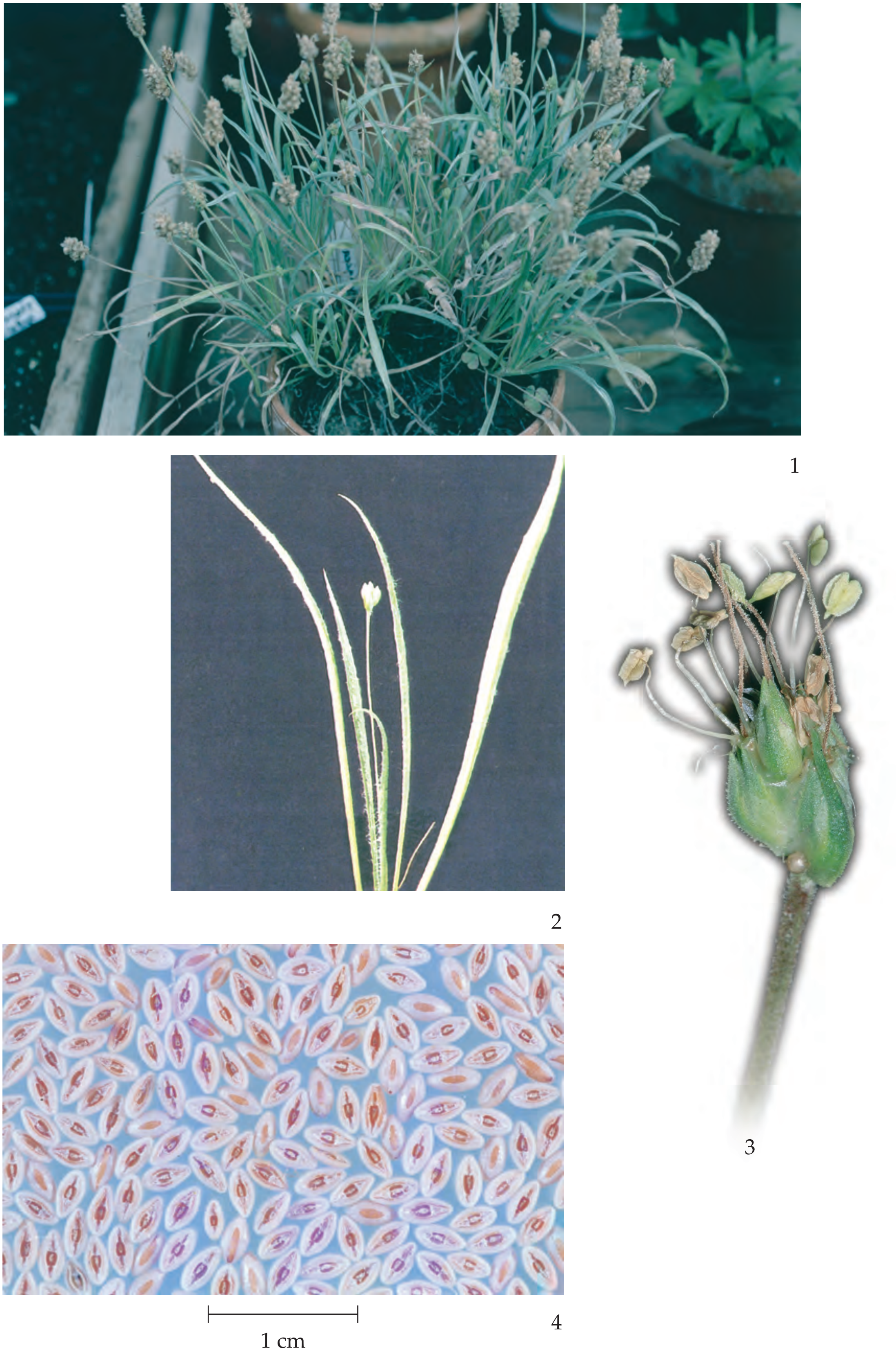


Fig. 1a *Plantago ovata* Forssk.
1. habit 2. flowering plant 3. inflorescence 4. crude drug

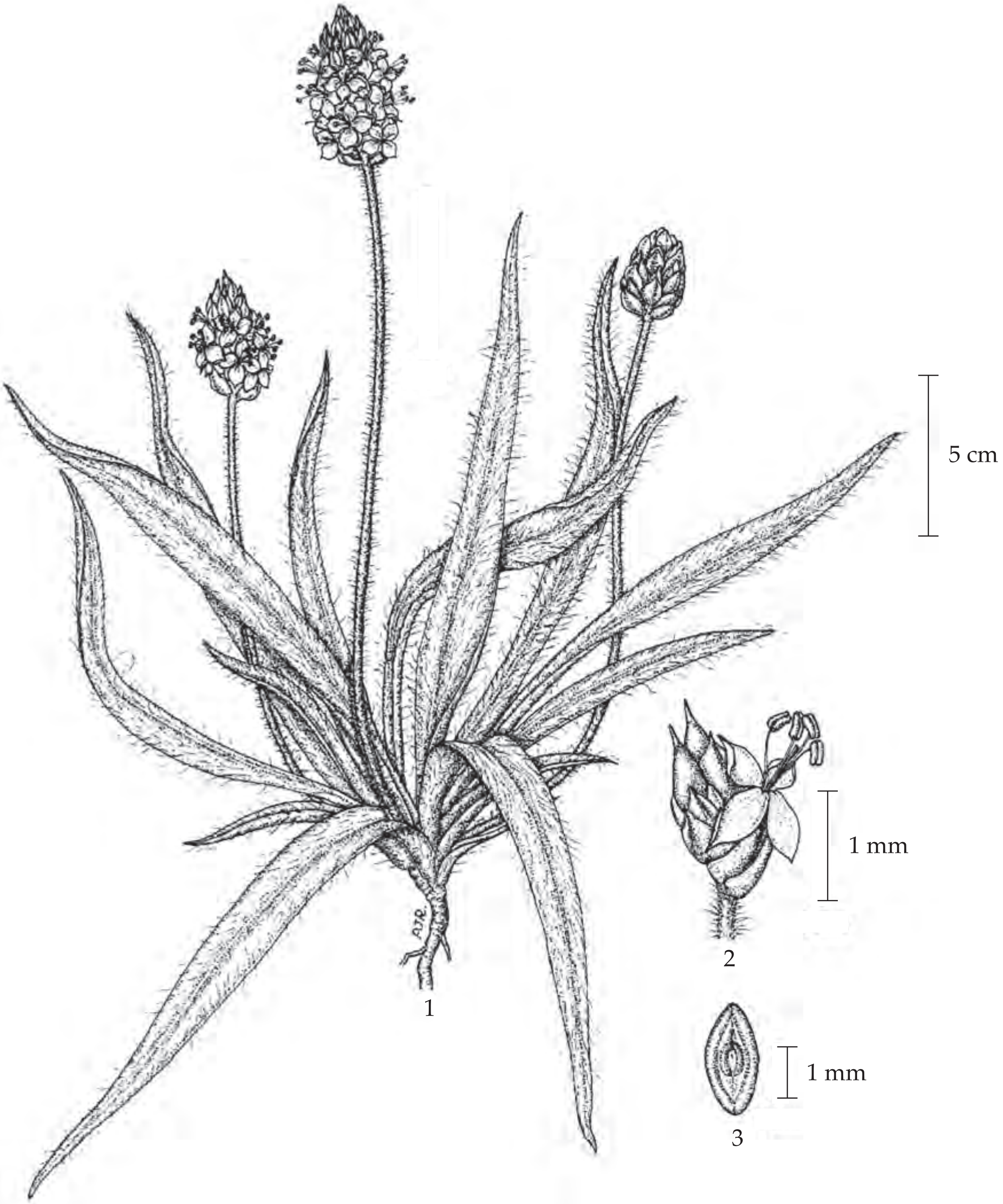


Fig. 1b *Plantago ovata* Forssk.
1. habit 2. inflorescence 3. seed

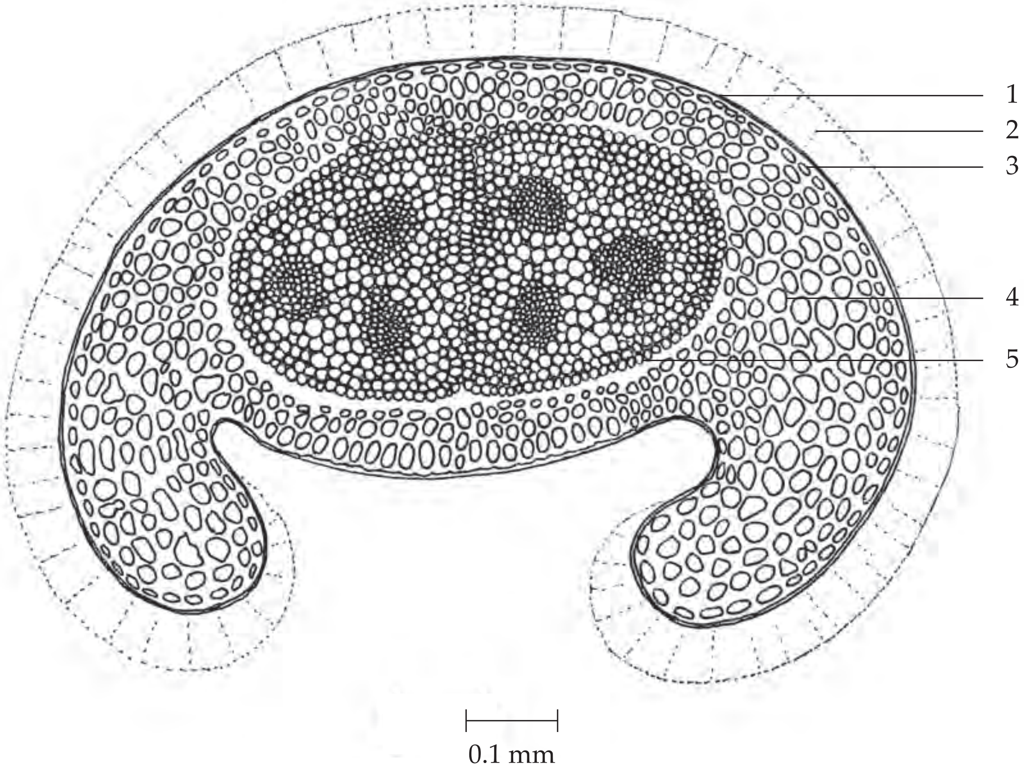


Fig. 2a Transverse Section of the Seed of *Plantago ovata* Forssk., when Mounted in a 50 Per Cent V/V Solution of *Glycerol*

1. outer layer of seed coat
2. mucilage of outer layer of seed coat
3. inner layer of seed coat (pigment layer)
4. endosperm
5. cotyledon

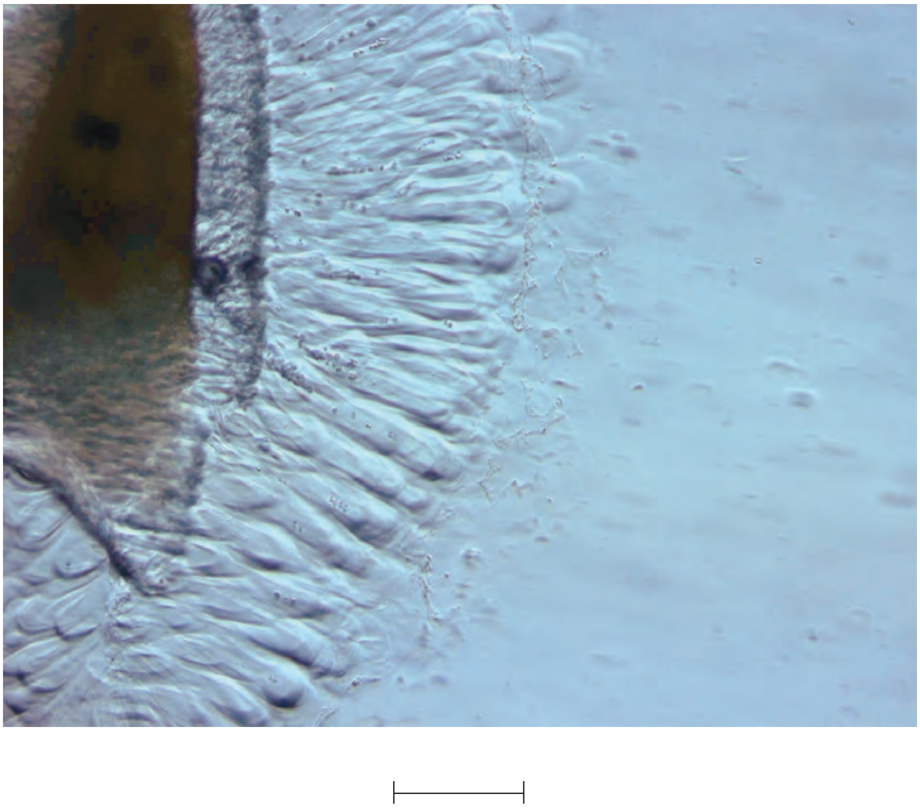


Fig. 2b Outer Layer with Swollen Mucilage of Seed Coat of *Plantago ovata* Forssk., Mounted in a 50 Per Cent V/V Solution of *Glycerol*

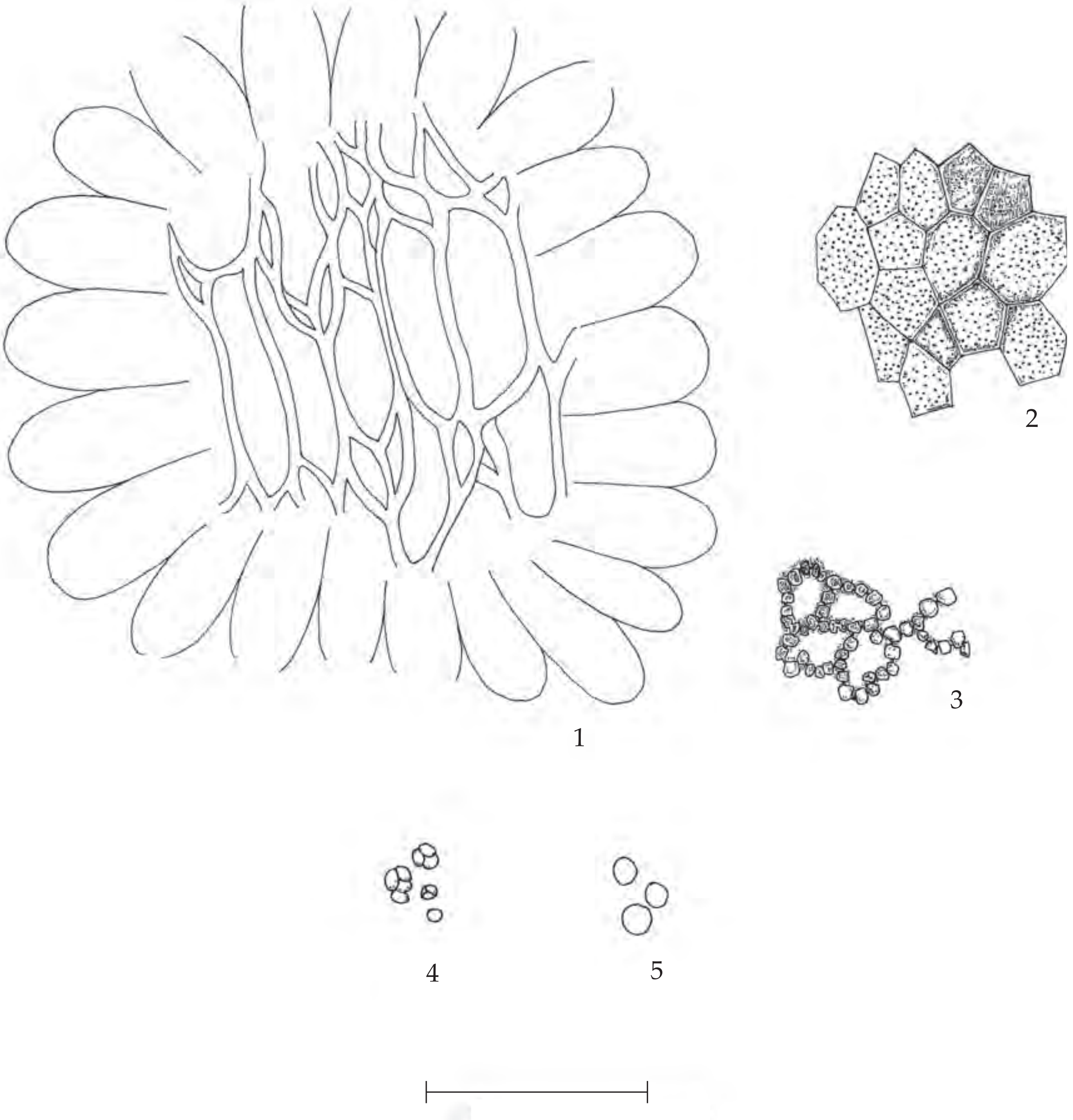


Fig. 2c Powdered Drug of the Seeds of *Plantago ovata* Forssk., when Mounted in *Water*

1. epidermal cells of the outer layer of seed coat showing swollen mucilage
2. thin-walled parenchyma cells containing brown matter
3. pitted, thick-walled endosperm cells
4. starch granules
5. oil globules

Contra-indication It is contra-indicated in cases of intestinal obstruction.

Warning Pre-swollen seeds should always be taken with plenty of fluid to avoid esophageal obstruction or fecal impaction.

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Ispaghula Seed shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Shake 100 mg of the sample, in *No. 355 powder*, with 3 ml of *water*, allow to stand for 30 minutes and filter. To the filtrate, add 3 ml of *dilute hydrochloric acid*, boil for 1 minute, cool, and neutralize with *sodium hydroxide TS*. Add 1 ml of *potassium cupri-tartrate TS* and heat on a water-bath: a brick-red precipitate is produced.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel G* as the coating substance and a mixture of 85 volumes of *acetonitrile* and 15 volumes of *water* as the mobile phase. Apply separately to the plate, 2 µl each of the following solutions. Prepare solution (A) by transferring 50 mg of the sample, in *No. 355 powder*, to a thick-walled centrifuge tube. Add 2 ml of a 23.0 per cent w/v solution of *trifluoroacetic acid* and shake vigorously. Stopper the test-tube and heat the mixture at 100° for about 1 hour. Centrifuge for 5 minutes, transfer the clear supernatant liquid into a 50-ml round-bottomed flask, add 10 ml of *water*, and evaporate again to dryness under reduced pressure at a temperature not exceeding 60°. Dissolve the residue in 10 ml of *water* and evaporate again to dryness under reduced pressure at a temperature not exceeding 60°. Dissolve the residue in 2 ml of *methanol*. For solution (B), dissolve 10 mg of *galactose* in a few drops of *water* and dilute with *methanol* to 5.0 ml. For solution (C), dissolve 10 mg of *arabinose* in a few drops of *water* and dilute with *methanol* to 5.0 ml. For solution (D), dissolve 10 mg of *xylose* in a few drops of *water* and dilute with *methanol* to 5.0 ml. After removal of the plate, allow it to dry in air. Spray the plate with a 0.5 per cent w/v solution of *thymol* in a mixture of 5 ml of *sulfuric acid* and 95 ml of *ethanol*, and heat at 130° for about 10 minutes. The chromatogram obtained from solution (A) shows a pink spot (hR_f value 18 to 21), a pinkish brown spot (hR_f value 30 to 32) and a brown spot (hR_f value 34 to 38), corresponding to the galactose, the arabinose and the xylose spots from solutions (B), (C) and (D), respectively. Other spots are brownish yellow (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Aqueous Extract of the Seeds of *Plantago ovata* Forssk.

Spot	hR_f Value	Detection
		Thymol in a Mixture of *Sulfuric Acid* and *Ethanol*
1	5-7	brownish yellow
2*	18-21	pink
3**	30-32	pinkish brown
4***	34-38	brown
5	41-44	brownish yellow
6	51-54	brownish yellow

*galactose
**arabinose
***xylose

Loss on drying Not more than 12.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 3.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Of the unground Ispaghula Seed, not more than 3.0 per cent w/w (Appendix 7.7).

Swelling index Not less than 9, when determined by the following method.

In a 25-ml ground-glass stoppered cylinder graduated over a height of 125±5 mm in 0.5-ml divisions, place 1 g of Ispaghula Seed, accurately weighed, moisten it with 1.0 ml of *ethanol*, add 25 ml of *water* and close the cylinder. Shake vigorously every 10 minutes for 1 hour. Allow to stand for 3 hours. At 90 minutes after the beginning of the test, release any large volume of liquid retained in the layer of the Seed and any particles of the Seed floating at the surface of the liquid by rotating the cylinder about a vertical axis. Measure the volume occupied by the Seed, including any adhering mucilage. Carry out three tests at the same time. The swelling index is given by the mean of the three tests.

Dose Adults: 5 to 10 g three times a day.
Children: 3 g three times a day.

Seeds shall be soaked in warm water for about 3 hours or until completely swollen before taking.

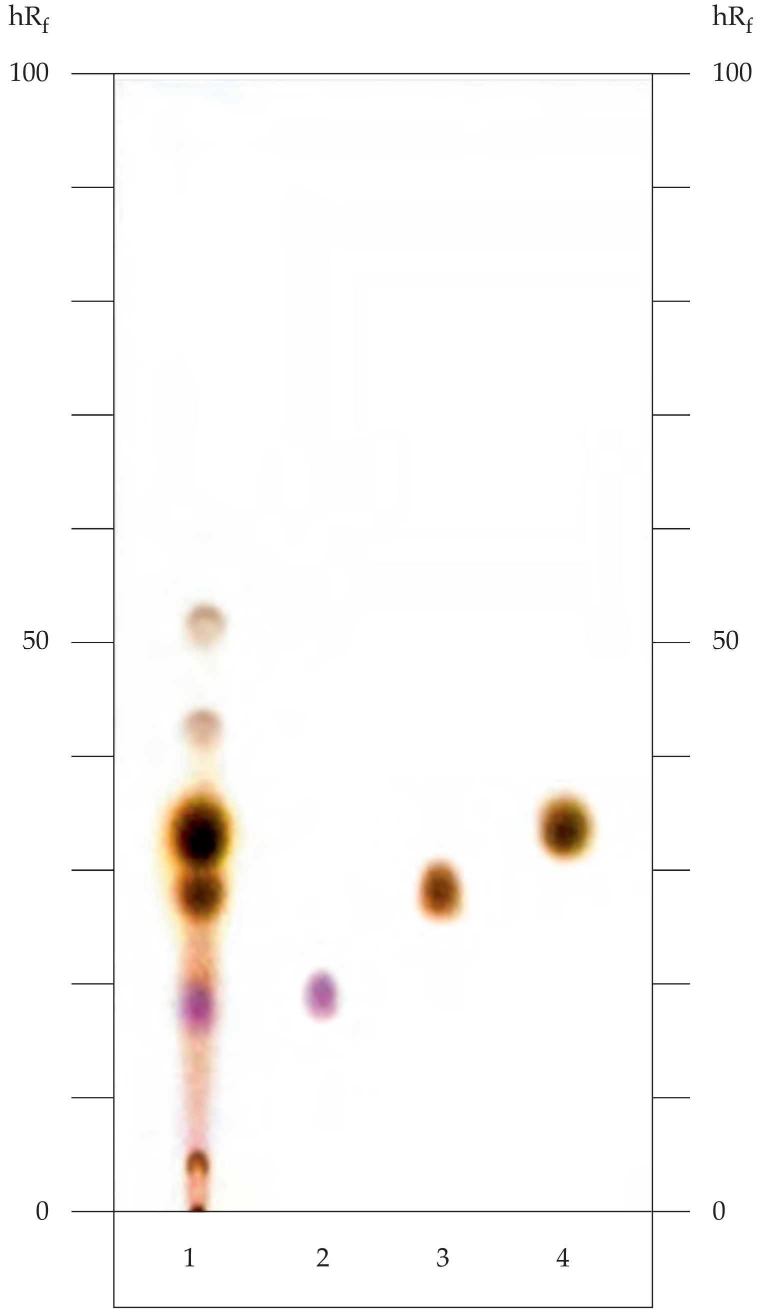


Fig. 3 Thin-layer Chromatogram of Aqueous Extract of the Seeds of *Plantago ovata* Forssk., Detected with *Thymol* in a Mixture of *Sulfuric Acid* and *Ethanol*

1 = solution (A)
2 = solution (B)
3 = solution (C)
4 = solution (D)

เทียนสัตตบุษย์ (THIAN SATTABUT)

Pimpinellae Anisi Fructus
Anise

Synonyms Aniseed, Anisum, Sweet Cumin

Category Carminative, expectorant, pharmaceutic aid (flavouring agent).

Anise is the dried ripe fruit of *Pimpinella anisum* L. (Family Umbelliferae), Herbarium Specimen Number: see *Additional information 1*, Crude Drug Number: DMSc 0433.

Constituents Anise contains volatile oil, of which *trans*-anethole is its major component with a small amount of estragole. It also contains coumarins, flavonoids, fixed oils, etc.

Description of the plant (Figs. 1a, 1b) Annual herb, 30 to 75 cm high; stem erect, cylindrical, striated, pubescent. Leaves heteromorphic, alternate below, opposite above; petiole 2 to 5 cm long, sheathing; basal leaves simple, reniform or broad-ovate, 1 to 3 cm long, 1.2 to 2.8 cm wide, margin coarse irregular teeth, puberulent along veins; stem leaves 1 to 2 pinnate, ultimate segments ovate or ovate-lanceolate, 6 to 7 mm long, 2 to 7 mm wide, 3-lobed, margin serrate or lacerate, leaves reduced upwards, becoming 3-lobed, lobe lanceolate or linear-lanceolate. Inflorescence loose compound umbels, terminal and leaf-opposite, 1.6 to 6 cm across; peduncle 2.5 to 7 cm long. Flower small; pedicel 1 to 5 mm long; sepal minute or wanting; petals 5, yellowish white, obcordate, abaxially pubescent; stamens 5, alternating with petals, inserted around an epigynous disk; ovary inferior, 2-loculed, 1-ovuled per locule, stylopodium conic, styles 2. Fruit cremocarp, oblong-ovoid, 3 to 6 mm long, 1 to 2.5 mm wide, greyish brown, densely appressed, short-hairy.

Description Odour, reminiscent of that of anethole; taste, sweet and aromatic.

Macroscopical (Fig. 1a) Cremocarp ovoid or pyriform, slightly compressed laterally, greyish brown, 3 to 6 mm long, 1 to 2.5 mm wide; mericarp frequently separated, crescent-shaped, slightly pubescent. Dorsal side convex, brown, with 5 longitudinal ridges and at the apex with a short conical stylopodium; commissural side concave, brown, with persisting carpophore and pedicel.

Microscopical (Figs. 2a, 2b) Transverse section of the mericarp through the cotyledon shows epicarp, mesocarp, endocarp, spermoderm, endosperm, and cotyledon. Epicarp, a layer of epidermal cells with numerous papillae and usually unicellular trichomes, covered with striated cuticle, generally 6 ridges. Mesocarp, 3 or 4 layers of elongated parenchyma cells, with a small lignified fibrovascular bundle under the ridge; vittae, elliptical, brown, surrounded by small epithelial secretory cells, (15-)30 to 35(-45) small vittae forming a line along the dorsal side and 2 large vittae in the commissural side. Endocarp, a layer of elongated thin-walled cells. Spermoderm, attached to the endocarp, consisting of a layer of elongated cells with some collapsed cells. Endosperm, thick-walled polygonal cells containing oil globules and aleurone grains with microcrystals. Cotyledon, thin-walled cells containing oil globules and aleurone grains.

Anise in powder possesses the diagnostic microscopical characters of the unground drug.

Fig. 1a *Pimpinella anisum* L.
1. habit 2. and 3. inflorescence 4. crude drug

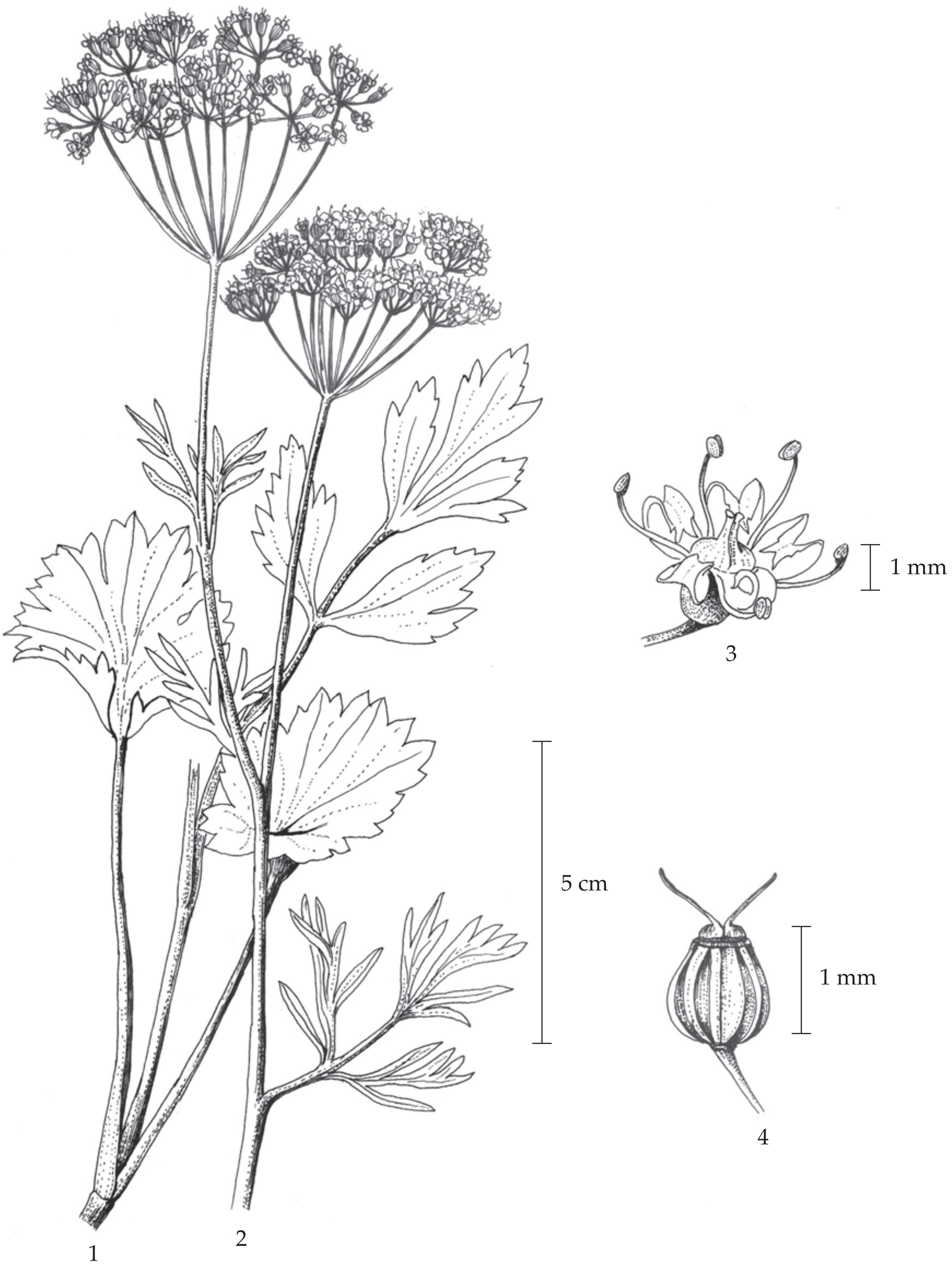


Fig. 1b *Pimpinella anisum* L.
1. leaves on lower stem
2. flowering top with leaves on upper stem and two compound umbels
3. flower 4. fruit (schizocarp)

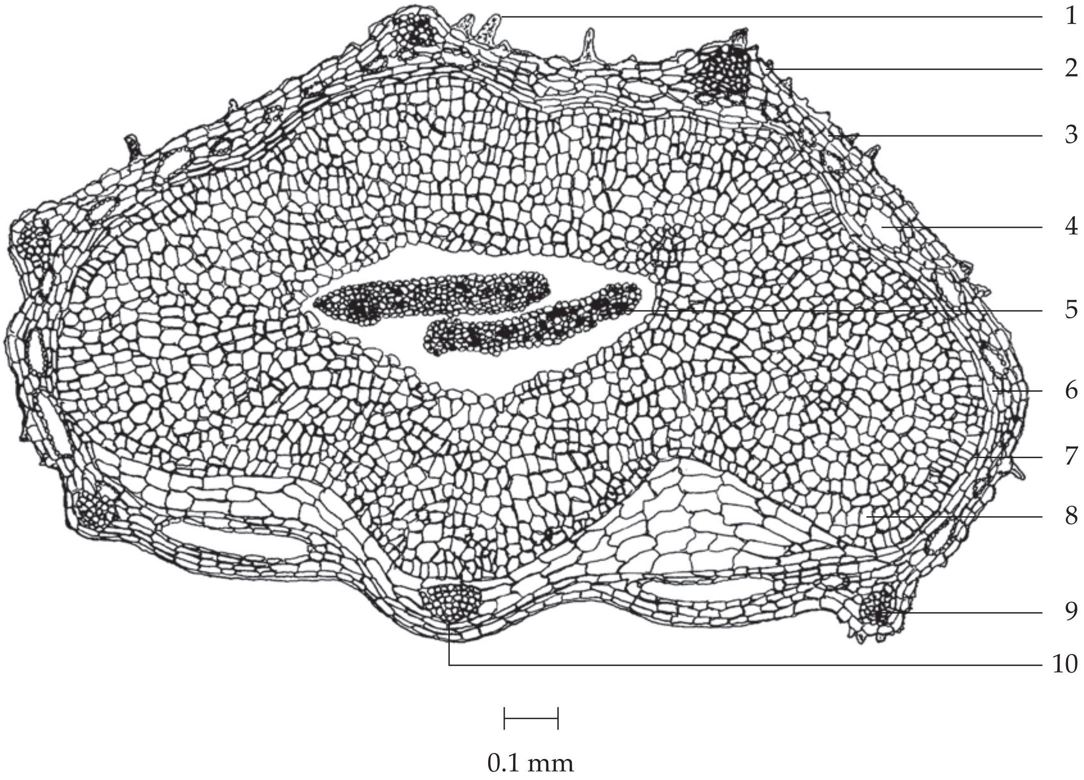


Fig. 2a Transverse Section of the Mericarp of *Pimpinella anisum* L.

1. trichome	6. endocarp
2. papilla of the epicarp	7. spermoderm
3. parenchyma of mesocarp	8. endosperm
4. vitta	9. vascular bundle
5. cotyledon	10. raphe

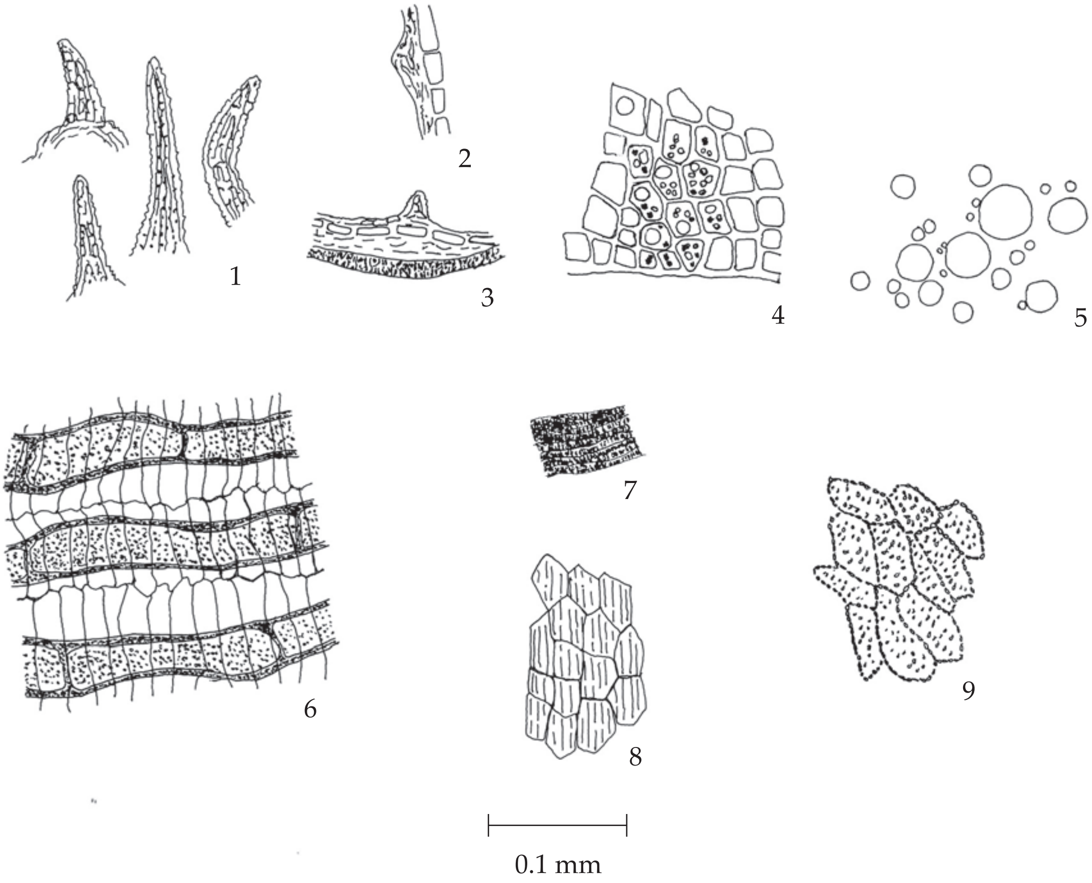


Fig. 2b Powdered Drug of the Fruits of *Pimpinella anisum* L.

1. trichomes
2. epicarp in sectional view with papilla
3. epicarp in longitudinal section associated with mesocarp and vitta
4. endosperm containing oil globules, aleurone grains and microcrystals
5. oil globules
6. vittae in longitudinal section associated with endocarp
7. scalariform vessels
8. epicarp in surface view with striated cuticle
9. porous parenchyma

Warning It should be used with caution in pregnant and nursing women.

Additional information

1. Anise plant is not native to nor commercially cultivated in Thailand. The plant yielding anise fruit is here referred to the herbarium specimen, collector's number Kruijt 245, deposited at the National Herbarium of the Netherlands (L), Leiden, the Netherlands. The photographic illustration of which can be seen at the Department of Medical Sciences Herbarium (DMSC), Nonthaburi, Thailand.
2. It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Anise shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Boil 500 mg of the sample, in powder, with 10 ml of *water* in a water-bath for 10 minutes and filter. To 2 ml of the filtrate, add 1 or 2 drops of a 1 per cent w/v solution of *iron(III) chloride*: a greenish brown colour develops.

B. Mix 1 g of the sample, in powder, with 3 ml of *ethanol*, shake for 5 minutes and filter. To 1 ml of the filtrate, add 1 ml of *dinitrophenylhydrazine TS1*: an orange-yellow solution is produced.

C. Add 5 ml of *chloroform* to 500 mg of the sample, in powder, shake well, set aside for 30 minutes, and filter. Allow 0.1 ml of the filtrate to dry and add a few drops of a 5 per cent w/v solution of *vanillin* in *sulfuric acid*: a reddish brown colour develops and changes to red and to purple, consecutively.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 90 volumes of *toluene* and 10 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, 2 µl each of solutions (A), (B) and (C). Prepare solution (A) by shaking 2 g of sample, in *fine powder*, with 5 ml of *ethyl acetate* for 3 minutes and filtering. For solution (B), add 5 µl of *anethole* to 1 ml of *ethyl acetate* and mix. For solution (C), add 2 µl of *olive oil* to 1 ml of *ethyl acetate* and mix. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 65 to 66), corresponding to the anethole spot from solution (B). Spray the plate with *vanillin-sulfuric acid TS* and heat at 110° for 10 minutes; the spot due to anethole is brownish purple. One violet spot (hR_f value 74 to 76) corresponds to the triglycerides of olive oil from solution (C). Several other spots are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethyl Acetate Extract of the Fruits of *Pimpinella anisum* L.

Spot	hR_f Value	Detection	
		UV 254	*Vanillin-Sulfuric Acid TS*
1	1-3	–	violet
2	10-12	quenching	pale violet
3	15-17	weak quenching	violet
4	19-20	–	pale violet
5	23-24	–	pale violet
6	30-31	–	pale violet
7	32-33	–	pale violet
8	41-42	weak quenching	–
9	47-49	–	violet
10	57-58	weak quenching	pale violet
11*	65-66	quenching	brownish purple
12**	74-76	–	violet

*anethole

**triglycerides of olive oil

Water Not more than 11.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 6.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 2.5 per cent w/w (Appendix 7.6).

Total ash Not more than 12.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 10.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 1.0 per cent v/w, calculated on the anhydrous basis (Appendix 7.3H). Use 20 g, in *coarse powder*, freshly prepared and accurately weighed. Use 200 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 4 hours. Use 1.0 ml of *xylene* in the graduated tube.

Dose 0.5 to 1.0 g three times a day.

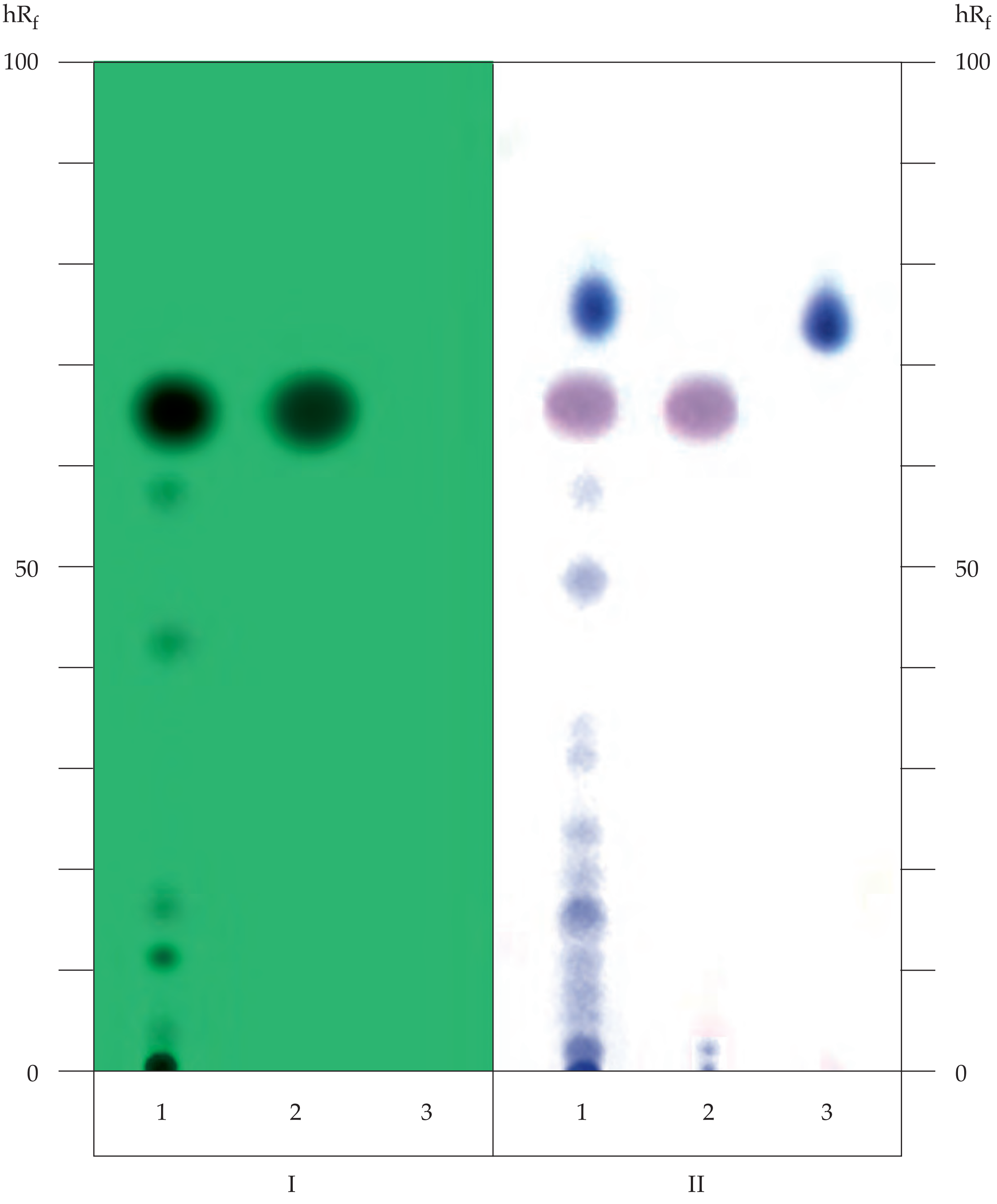


Fig. 3 Thin-layer Chromatogram of Ethyl Acetate Extract of the Fruits of *Pimpinella anisum* L.

1 = solution (A)
2 = solution (B)
3 = solution (C)
I = detection under UV light (254 nm)
II = detection with *vanillin-sulfuric acid TS*

เทียนตากบ (THIAN TA KOP)

Cari Carvi Fructus
Caraway

Synonyms Caraway Fruit, Persian Cumin

Category Carminative, antiflatulent (colic), pharmaceutic aid (flavouring agent).

Caraway is the dried ripe fruit of *Carum carvi* L. (Family Umbelliferae), Herbarium Specimen Number: see *Additional information 1*, Crude Drug Number: DMSc 0434.

Constituents Caraway contains volatile oil, of which (+)-carvone and (+)-limonene are its major components. It also contains fixed oils, flavonoids, etc.

Description of the plant (Figs. 1a, 1b) Perennial herb, 15 to 100 cm high; taproot cylindrical, up to 25 cm long; stem solitary, rarely 2 to 8, base without remnant sheaths, glabrous. Leaves 2- to 3-pinnated, alternate, basal and lower leaves oblong-lanceolate in outline, 8 to 15 cm long, 3 to 8 cm wide, gradually reduced upwards, ultimate segments linear or linear-lanceolate, 3 to 5 mm long, 1 to 2 mm wide; petiole sheathing, 5 to 8 cm long. Inflorescence loose compound umbels, terminal. Flower small; pedicel extremely unequal; sepal minute or wanting; petals 5, pinkish or white, obovate with a narrower, inflexed apex; stamens 5, inserted around epigynous disk, alternating with the petals; ovary inferior, 2-loculed, ovule 1 per locule, stylopodium short conic, styles 2, short, spreading. Fruit cremocarp, brown to dark brown, oblong to ovate, 3 to 4 mm long, consisting of 2 dry, 1-seeded, indehiscent mericarps, ridges prominent, carpophore 2-fid.

Description Odour and taste, aromatic and characteristic.

Macroscopical (Fig. 1a) Cremocarp ovoid, 3 to 6 mm long, 1 to 2 mm wide; mericarp frequently separated, each being crescent shaped, slightly pubescent. Dorsal side convex, brown, with 5 longitudinal ridges and at the summit with a short conical stylopodium; commissural side concave, brown, with persisting carpophore and pedicel.

Microscopical (Figs. 2a, 2b) Transverse section of the mericarp through the cotyledon shows epicarp, mesocarp, endocarp, spermoderm, endosperm, and cotyledon. Epicarp, slightly elongated epidermal cells, covered with striated cuticle. Mesocarp, several layers of elongated parenchyma cells with more or less collapsed cells; the ridge portions, small lignified fibrovascular bundles; vittae, large, elliptical, brown, lined by small epithelial secretory cells, 4 on dorsal side between the vascular bundles and 2 on commissural side. Endocarp, a layer of broad elongated cells. Spermoderm, attached to the endocarp, consisting of a layer of brownish, elongated cells with some collapsed cells. Endosperm, thick-walled, polygonal cells containing oil globules, aleurone grains with rosette crystals. Cotyledon, small thin-walled cells containing oil globules and aleurone grains.

Caraway in powder possesses the diagnostic microscopical characters of the unground drug.

Fig. 1a *Carum carvi* L.
1. inflorescence 2. infructescence 3. crude drug

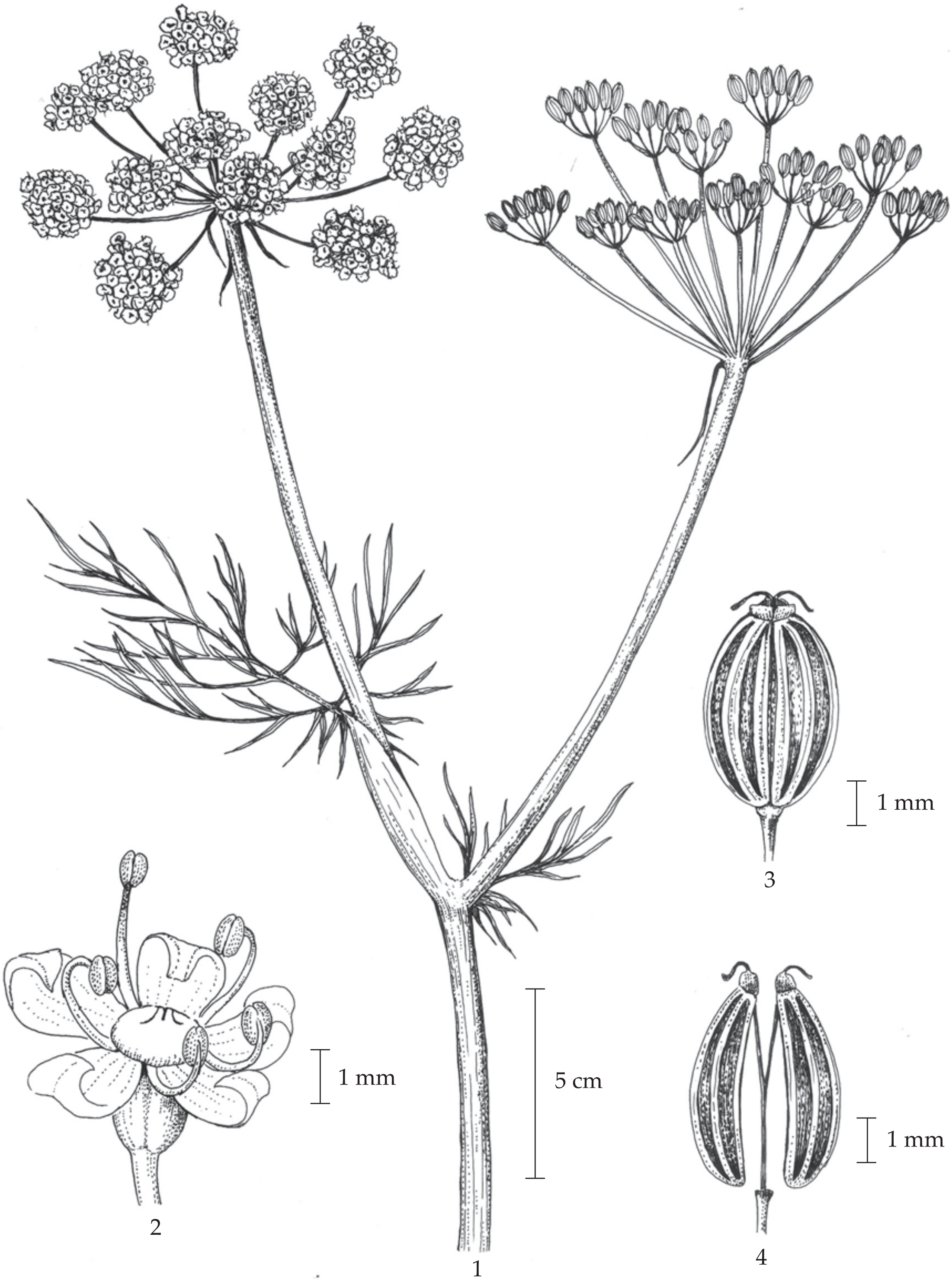


Fig. 1b *Carum carvi* L.
1. flowering top with leaves on upper stem, compound umbels and infructescence
2. flower 3. and 4. fruit (schizocarp)

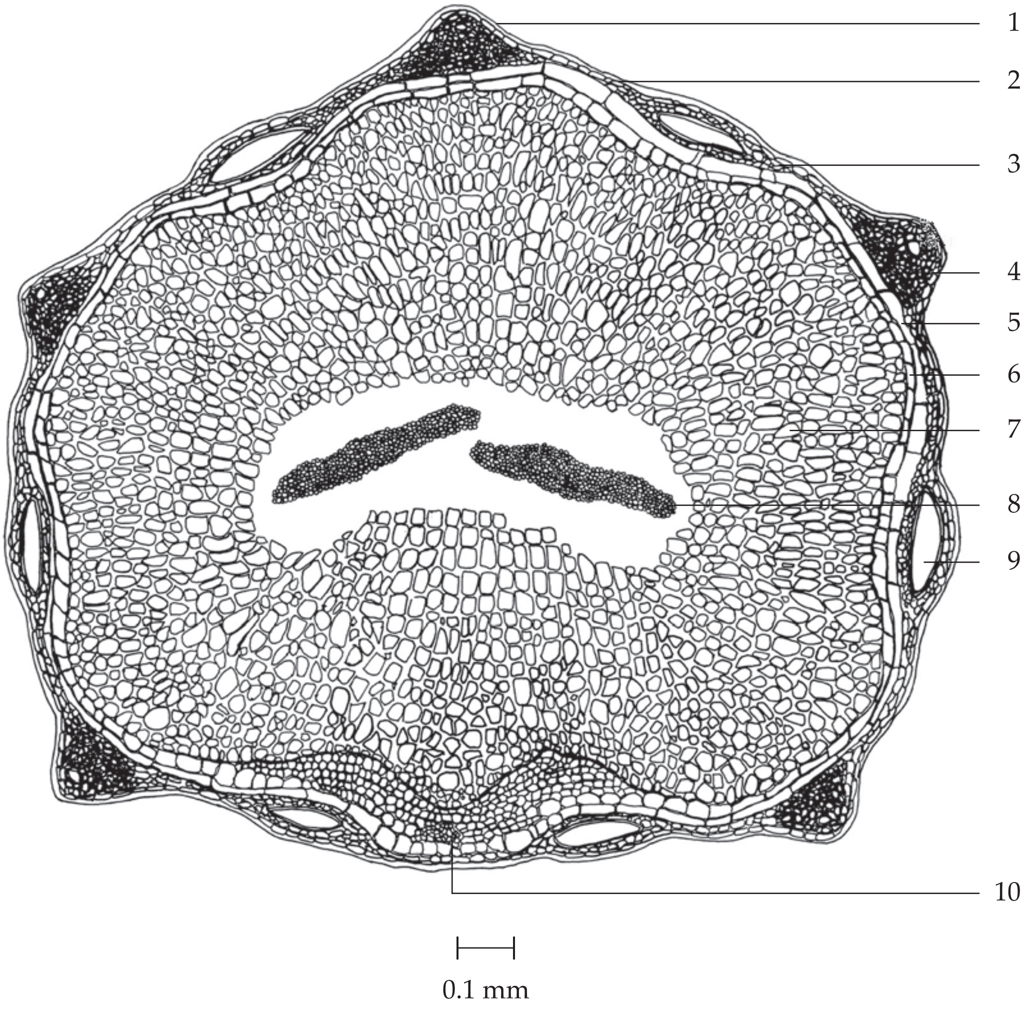


Fig. 2a Transverse Section of the Mericarp of *Carum carvi* L.

1. cuticle layer
2. epicarp
3. mesocarp
4. vascular bundle
5. endocarp
6. spermoderm
7. endosperm
8. cotyledon
9. vitta
10. raphe

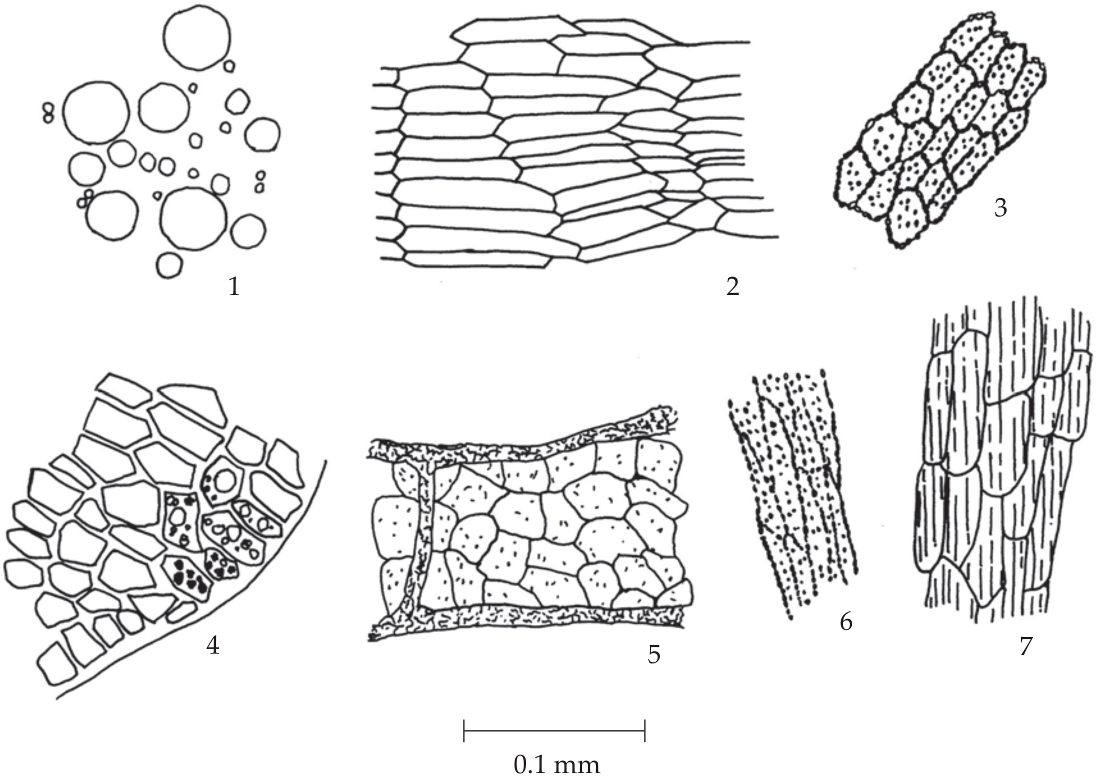


Fig. 2b Powdered Drug of the Fruits of *Carum carvi* L.

1. oil globules
2. endocarp in surface view
3. sclereids
4. endosperm containing oil globules and aleurone grains with rosette crystals
5. vitta in surface view
6. pitted vessels
7. epicarp with striated cuticle, in surface view

Additional information

1. Caraway plant is not native to nor commercially cultivated in Thailand. The plant yielding caraway fruit is here referred to the herbarium specimen, collector's number J.F. Rock 14117, deposited at the Kew Herbarium (K), London, United Kingdom. The photographic illustration of which can be seen at the Department of Medical Sciences Herbarium (DMSC), Nonthaburi, Thailand.
2. It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Caraway shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Add 5 ml of *chloroform* to 1 g of the sample, in powder, shake well, set aside for 30 minutes, and filter. Allow 0.1 ml of the filtrate to dry and add a few drops of a 5 per cent w/v solution of *vanillin* in *sulfuric acid*: a reddish brown colour develops and changes to red and to purple, consecutively.

B. Mix 1 g of the sample, in powder, with 3 ml of *ethanol*, shake for 5 minutes and filter. To 1 ml of the filtrate, add 1 ml of *dinitrophenylhydrazine TS1*: a turbid, orange-yellow solution is produced.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 90 volumes of *toluene* and 10 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, 2 µl each of solutions (A), (B) and (C). Prepare solution (A) by shaking 500 mg of sample, in *fine powder*, with 5 ml of *ethyl acetate* for 3 minutes and filtering. For solution (B), add 2 µl of *carvone* to 1 ml of *ethyl acetate* and mix. For solution (C), add 40 µl of *olive oil* to 1 ml of *ethyl acetate* and mix. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 55 to 58), corresponding to the carvone spot from solution (B). Spray the plate with *vanillin-sulfuric acid TS* and heat at 110° for 10 minutes; the spot due to carvone is purple. One violet spot (hR_f value 83 to 85) corresponds to the triglycerides of olive oil from solution (C). Other several spots are also observed (Table 1); see also Fig. 3.

Table 1 hR$_f$ Values of Components in Ethyl Acetate Extract of the Fruits of *Carum carvi* L.

Spot	hR$_f$ Value	Detection	
		UV 254	*Vanillin-Sulfuric Acid TS*
1	5-11	–	violet
2	30-31	–	pale violet
3	39-41	–	violet
4*	55-58	quenching	purple
5**	83-85	–	violet

*carvone

**triglycerides of olive oil

Water Not more than 12.5 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Total ash Not more than 7.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 5.5 per cent w/w (Appendix 7.12).

Volatile oil Not less than 2.5 per cent v/w, calculated on the anhydrous basis (Appendix 7.3H). Use 20 g, in *coarse powder*, freshly prepared and accurately weighed. Use 200 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 4 hours. Use 1.0 ml of *xylene* in the graduated tube.

Dose 0.5 to 2.0 g three times a day.

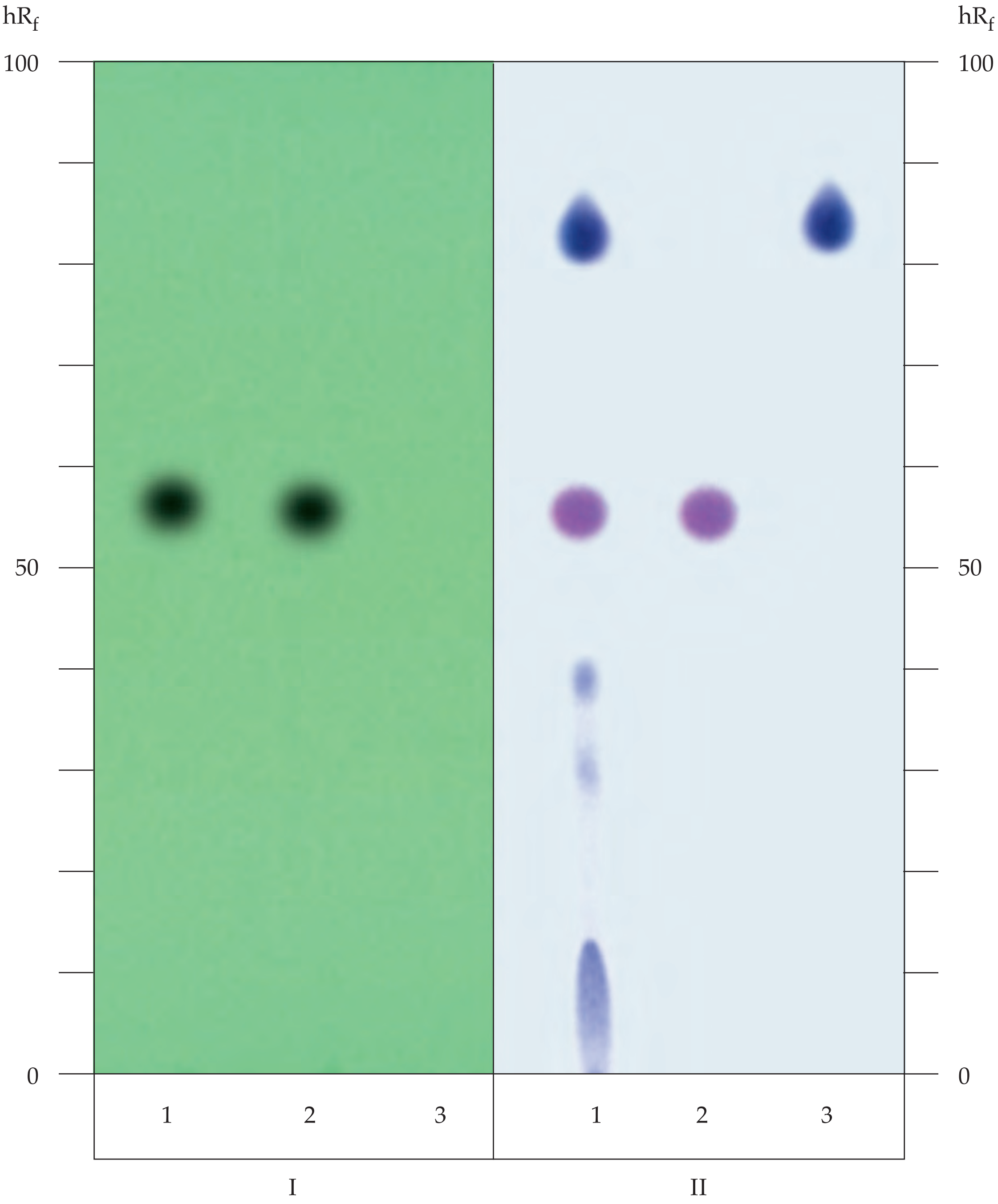


Fig. 3 Thin-layer Chromatogram of Ethyl Acetate Extract of the Fruits of *Carum carvi* L.

1 = solution (A)
2 = solution (B)
3 = solution (C)
I = detection under UV light (254 nm)
II = detection with *vanillin-sulfuric acid TS*

เทียนตาตั๊กแตน (THIAN TA TAKKATAN)

Anethi Graveolens Fructus
Dill

Synonyms Anethum, Dill Fruit

Category Carminative, pharmaceutic aid (flavouring agent).

Dill is the dried ripe fruit of *Anethum graveolens* L. (*Peucedanum graveolens* Benth. & Hook. f.) (Family Umbelliferae), Herbarium Specimen Number: DMSC 4535, QSBG 23272, Crude Drug Number: DMSc 420.

Constituents Dill contains volatile oil, of which (+)-carvone, (+)-limonene and α-phellandrene are its major components. It also contains coumarins, phenylpropanoids, xanthones, flavonoids, etc.

Description of the plant (Figs. 1a, 1b) Annual or biennial glabrous aromatic herb, up to 1.7 m high, terete, striate, with subfusiform or fusiform tap-root; main stem, hollow, glaucous, cylindrical, dichotomously branched with 5 to 8 branches. Leaves bi- or tripinnately compound with linear-filiform ultimate segments, 4 to 20 mm long; petioles 1 to 3.5 cm long; petioles sheathing, 5 to 6 cm long; upper cauline leaves greatly reduced. Inflorescence of loose compound umbels; peduncles 7 to 20 cm long; rays 10 to 45, 3 to 15 cm in spread, bearing 10 to 45 umbellules; pedicels 6 to 16 mm long. Flowers small, about 2 mm in diameter; sepals minute or wanting; petals 5, yellow, suborbicular with a narrower, obtuse, inflexed apex; stamens 5, alternating with petals, inserted around an epigynous disc; ovary inferior, 2-celled, each cell 1-seeded; styles 2, short, reflexed, the stylopodium conic, carpophore 2-cleft to the base. Fruits ovoid, flattened dorsally, consisting of 2 dry, 1-seeded, indehiscent mericaps, up to about 5 mm long, 2 to 3 mm broad and 1 mm thick.

Description Odour and taste, aromatic, pleasant and characteristic.

Macroscopical (Figs. 1a, 1b) Mericarps normally separate and free of pedicels, brown to yellow-brown, oval and glabrous, 3 to 4 mm long, 2 to 3 mm wide and 1 mm thick, compressed dorsally and almost flat. Each mericarp exhibiting 3 pale brown dorsal ridges, 2 yellowish lateral ridges being extended to form membranous wings. The commissural side, flat, attached with carpophore as a pale line.

Microscopical (Figs. 2a, 2b) Transverse section of the mericarp through the cotyledon shows epicarp covered with striated cuticle, a layer of slightly tangentially-elongated epidermal cells. Mesocarp, several layers of more or less collapsed, tangentially-elongated parenchymatous cells; each of the ridge, a lignified fibrovascular bundle, surrounded by reticulate and porous, thickened, lignified parenchymatous cells in the lateral ridges, the dorsal side, 4 large vittae located between the vascular bundles, the commissural side, 2 large vittae; each vitta, elliptical, brown, lined by small epithelial secretory cells. Endocarp, a layer of broad tangentially-elongated cells. Spermoderm, a layer of brownish, tangentially-elongated cells united with the endocarp except in the region of the raphe along the commissural side where separated by collapsed thin-walled cells. Endosperm, thick-walled polygonal cells containing oil globules and aleurone grains with microcrystals. Cotyledons, thin-walled cells containing aleurone grains and oil globules.

Dill in powder possesses the diagnostic microscopical characters of the unground drug.

1

2

3

4

Fig. 1a *Anethum graveolens* L.
1. habit 2. inflorescence 3. infructescence 4. crude drug

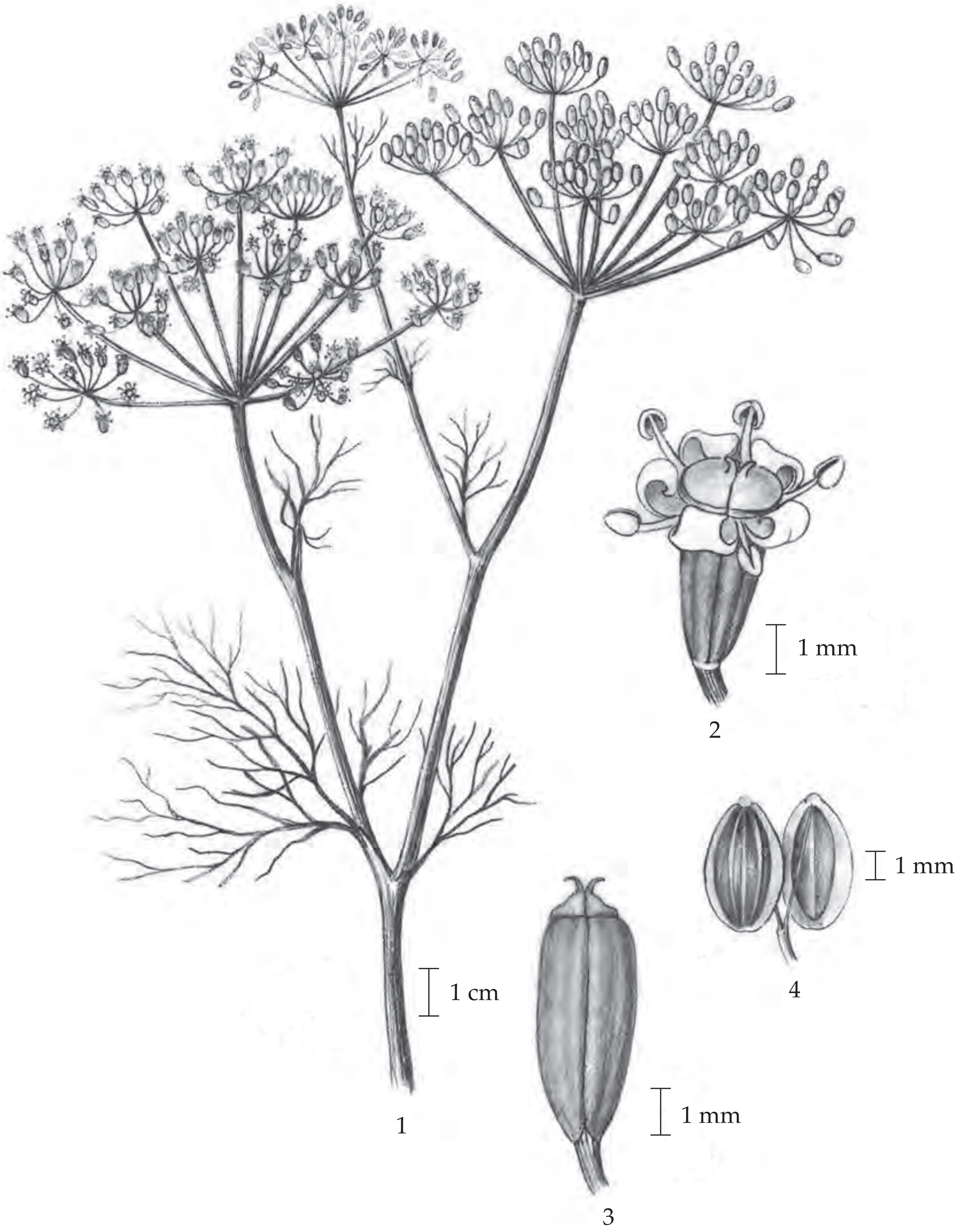


Fig. 1b *Anethum graveolens* L.
1. part of plant showing inflorescences and infructescence
2. flower 3. young fruit 4. schizocarp

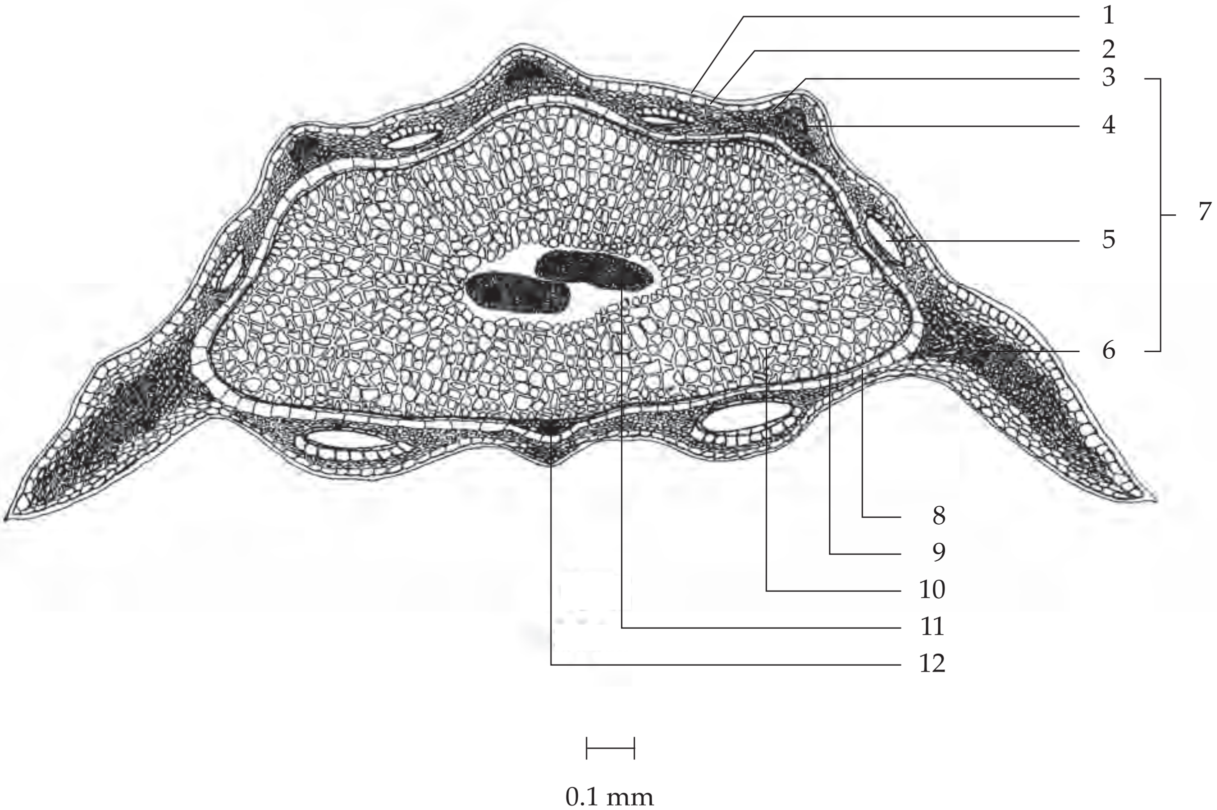


Fig. 2a Transverse Section of the Mericarp of *Anethum graveolens* L.

1. cuticle layer
2. epicarp
3. parenchyma of mesocarp
4. vascular bundle
5. vitta
6. porous parenchyma
7. mesocarp
8. endocarp
9. spermoderm
10. endosperm
11. cotyledon
12. raphe

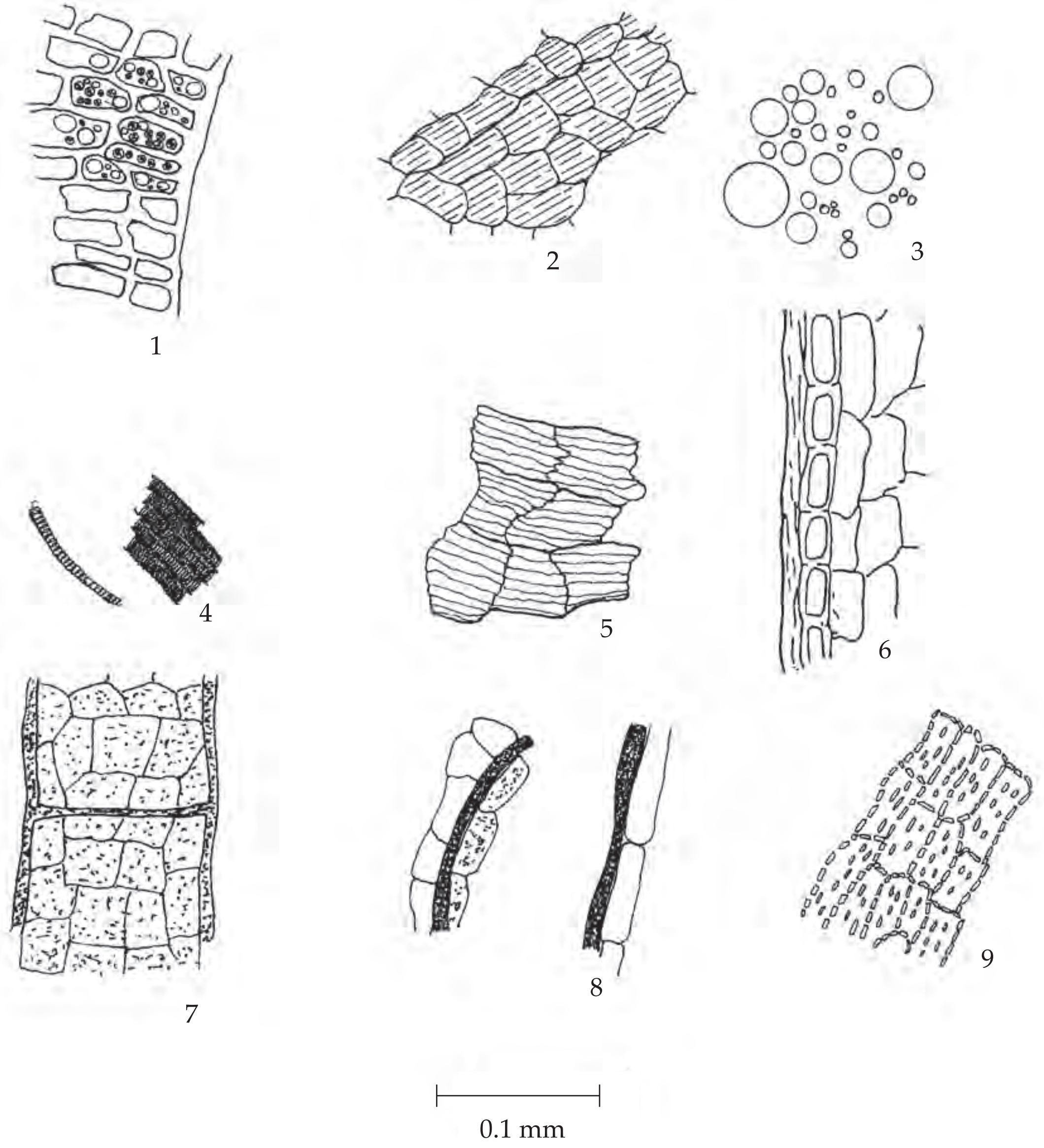


Fig. 2b Powdered Drug of the Fruits of *Anethum graveolens* L.

1. endosperm containing oil globules and aleurone grains with microcrystals
2. striated epicarp in surface view
3. oil globules
4. spiral and scalariform vessels
5. endocarp in surface view
6. epicarp and mesocarp in sectional view
7. vitta in surface view
8. vitta in sectional view associated with mesocarp cells
9. porous parenchyma

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Dill shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Add 5 ml of *chloroform* to 1 g of the sample, in powder, shake well, set aside for 30 minutes, and filter. Evaporate the filtrate to about 1 ml. Evaporate 0.1 ml of this solution to dryness and add a few drops of a 5 per cent w/v solution of *vanillin* in *sulfuric acid*: a reddish brown colour develops and changes to red and finally to purple.

B. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 95 volumes of *toluene* and 5 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, 4 µl each of the following two solutions. Prepare solution (A) by shaking 1 g of the sample, in *coarse powder*, with 2 ml of *ethanol*, for about 2 minutes and filtering. For solution (B), dissolve 10 µl of *carvone* in 2 ml of *ethanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 50 to 52), corresponding to the carvone spot from solution (B), and one spot of higher hR_f value. Spray the plate with *anisaldehyde TS* and heat at 110° for 10 minutes; the spot due to carvone is salmon-pink. Other several spots of different colours are observed (Table 1); see also Fig. 3.

Repeat the same procedure on another plate but spray with *vanillin-sulfuric acid TS* and heat at 120° for 20 minutes; the spot due to carvone is dark violet. One greenish blue and one yellowish brown spots are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Fruits of *Anethum graveolens* L.

Spot	hR_f Value	Detection		
		UV 254	*Anisaldehyde TS*	*Vanillin-Sulfuric Acid TS*
1	9-11	–	violet	–
2	24-26	–	violet	greenish blue
3*	50-52	quenching	salmon-pink	dark violet
4	68-70	quenching	brown	yellowish brown
5	74-77	–	violet	–

*carvone

Water Not more than 9.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 4.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Total ash Not more than 10.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 4.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 2.0 per cent v/w (Appendix 7.3H). Use 20 g, in *coarse powder*, freshly prepared and accurately weighed. Use 200 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 4 hours. Use 1.0 ml of *xylene* in the graduated tube.

Dose 1 to 4 g.

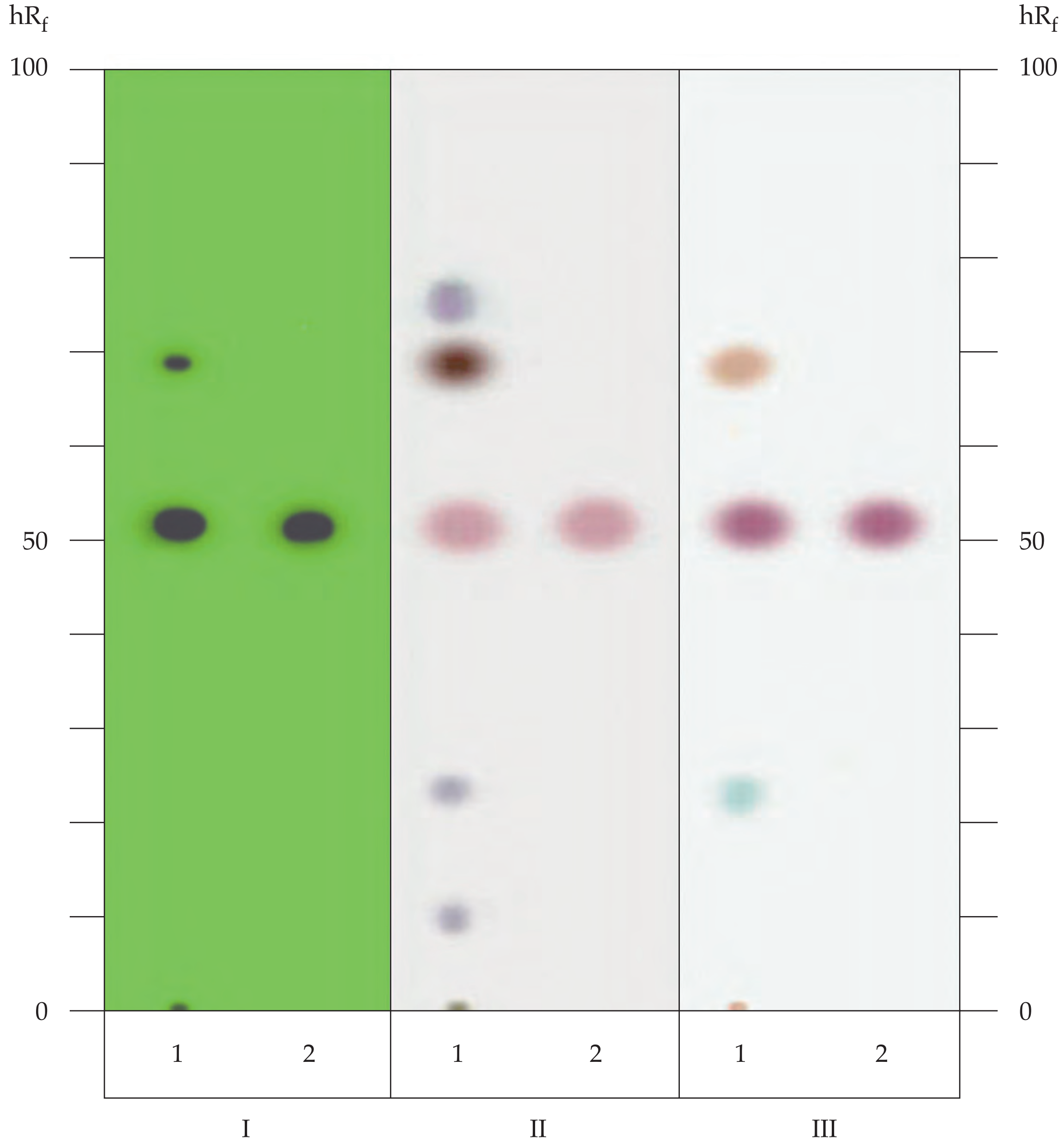


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Fruits of *Anethum graveolens* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with *anisaldehyde TS*
III = detection with *vanillin-sulfuric acid TS*

เทียนเยาวพาณี (THIAN YAOWAPHANI)

Trachyspermi Ammi Fructus
Ajowan

Synonyms Ajowan Fruit, Ajwain, King's Caraway

Category Carminative, pharmaceutic aid (flavouring agent).

Ajowan is the dried ripe fruit of *Trachyspermum ammi* (L.) Sprague [*Sison ammi* L., *Carum copticum* Benth. & Hook. f., *Trachyspermum copticum* (L.) Link] (Family Umbelliferae), Herbarium Specimen Number: DMSC 4537, 4538, 4539, QSBG 22814, 23820, 23819, Crude Drug Number: DMSc 428.

Constituents Ajowan contains volatile oil, of which thymol, γ-terpinene and p-cymene are its major components. It also contains flavonoids, coumarins, proteins, etc.

Description of the plant (Figs. 1a, 1b) Annual herb, 25 to 45 cm high; tap root narrow; stem erect, terete, much-branched, glabrous, aromatic. Leaves, triangular-ovate in outline, 2 to 8 cm long, 2 to 6 cm wide, 2 to 3-pinnate or pinnatisect; ultimate segments linear-filiform up to 15 mm long, 0.2 to 0.5 mm wide; petiole 1 to 5 cm long. Inflorescence compound umbels, 2.5 to 5 cm across; bracts 3 to 8, linear-subulate, 5 to 7 mm long; rays 6 to 20, 1 to 3 cm long; bracteoles 5 to 10, linear, 2 to 3 mm long; umbellules about 1 cm across, about 20-flowered; pedicels 0.5 to 4 mm long, unequal; calyx teeth conspicuous, minute, ovate or obsolete; petals 5, white, obovate to obcordate, about 1.3 mm long and wide, apex, inflexed; s tamens 5; stylopodium conical, style short, divaricate. Fruit cremocarp, ovoid-globose, 1.2 to 3.1 mm long, 1 to 1.8 mm wide; carpophore 2-cleft to base.

Description Odour and taste, aromatic and characteristic.

Macroscopical (Figs. 1a, 1b) Cremocarp, brown, broadly ovoid with short conical stylopodium, 2 mericarps frequently separated, crescent shaped, 1.2 to 3.1 mm long, 1 to 1.8 mm wide. Dorsal surface convex, with 5 lighter-coloured prominent longitudinal ridges alternating with roughened furrows; commissural surface concave, with a carpophore and a pedicel.

Microscopical (Figs. 2a, 2b) Transverse section of the mericarp through the cotyledon shows an epicarp layer, epidermal cells covered with striated cuticle layer, numerous thick-walled papillae of small and large sizes. Large papilla, surrounded by about 6 small papillae. Mesocarp, several layers of elongated parenchymatous cells, some collapsed, and 5 vascular bundles alternated with the epithelium tissues of elliptical brown vittae. Endocarp and seed coat united, with triangular raphe at the ventral side. Endosperm, thick-walled, polygonal parenchyma cells containing oil globules, aleurone grains and rosette aggregate crystals. Cotyledons, parenchymatous cells with aleurone grains and oil globules.

Ajowan in powder possesses the diagnostic microscopical characters of the unground drug.

Fig. 1a *Trachyspermum ammi* (L.) Sprague
1. habit 2. inflorescences 3. closed-up of flowers 4. crude drug

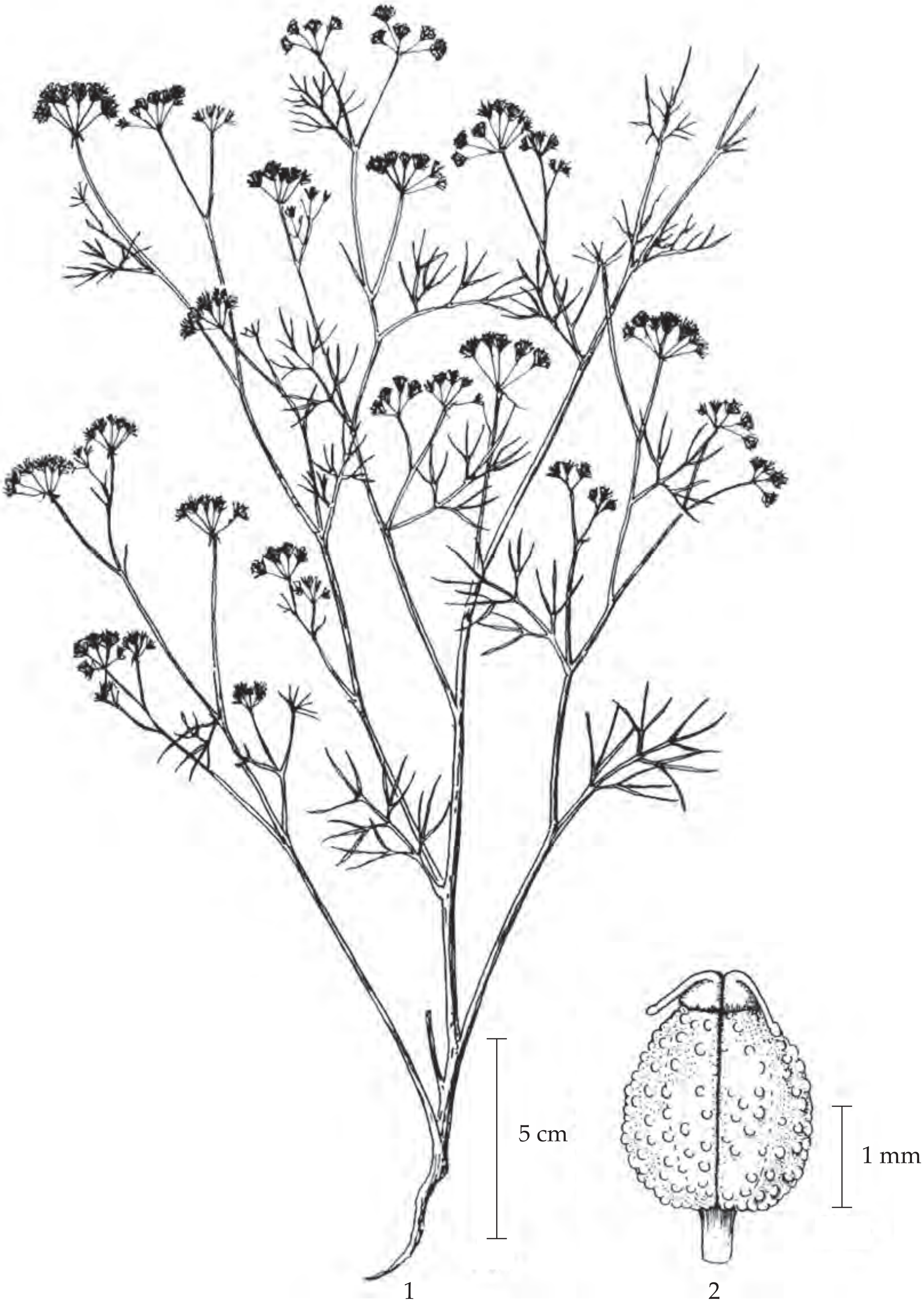


Fig. 1b *Trachyspermum ammi* (L.) Sprague
1. part of plant showing inflorescences and infructescences 2. fruit

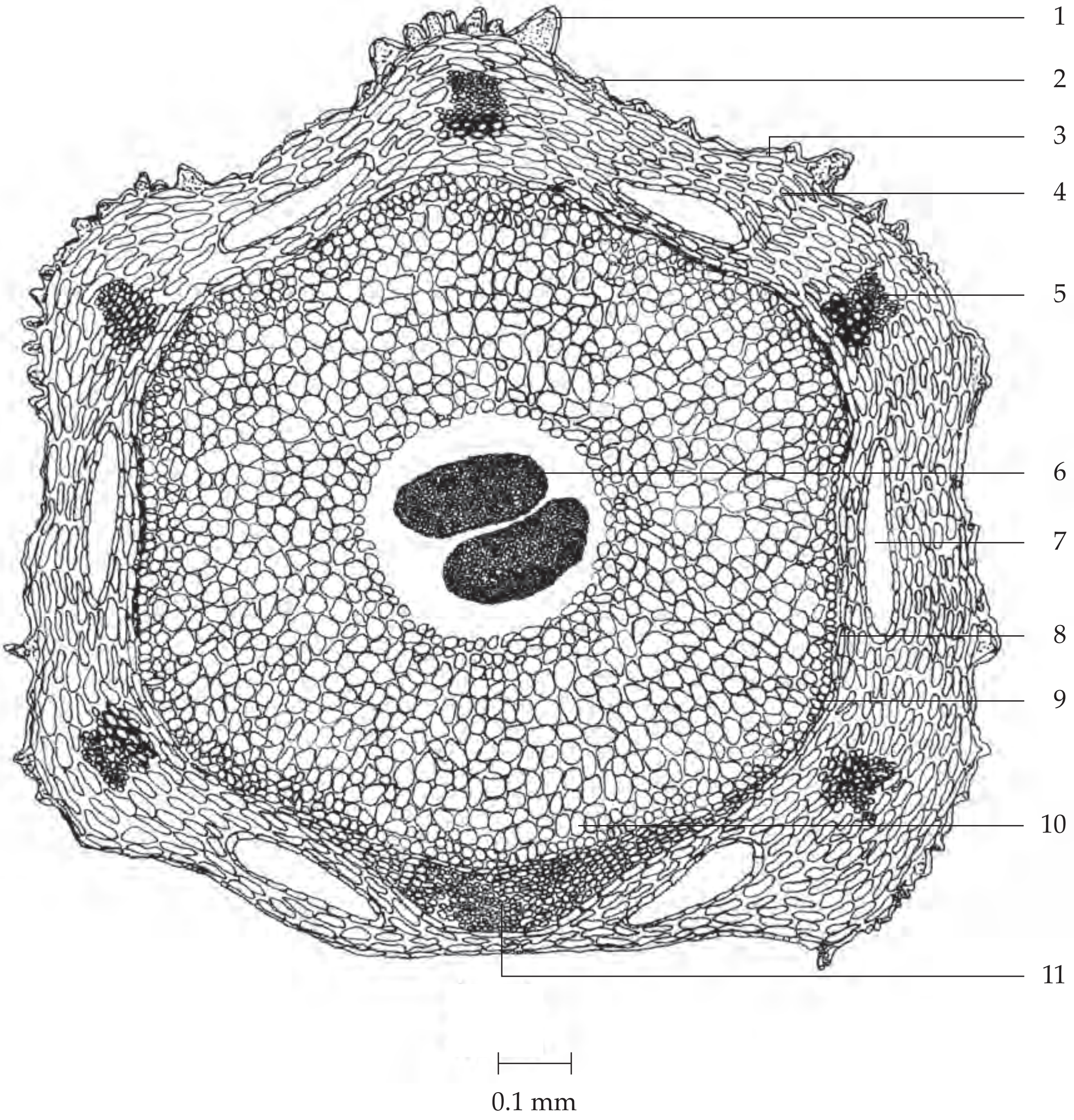


Fig. 2a Transverse Section of the Mericarp of *Trachyspermum ammi* (L.) Sprague

1. large papilla	7. vitta
2. small papilla	8. endocarp
3. epicarp	9. seed coat
4. parenchyma of mesocarp	10. endosperm
5. vascular bundle	11. raphe
6. cotyledon	

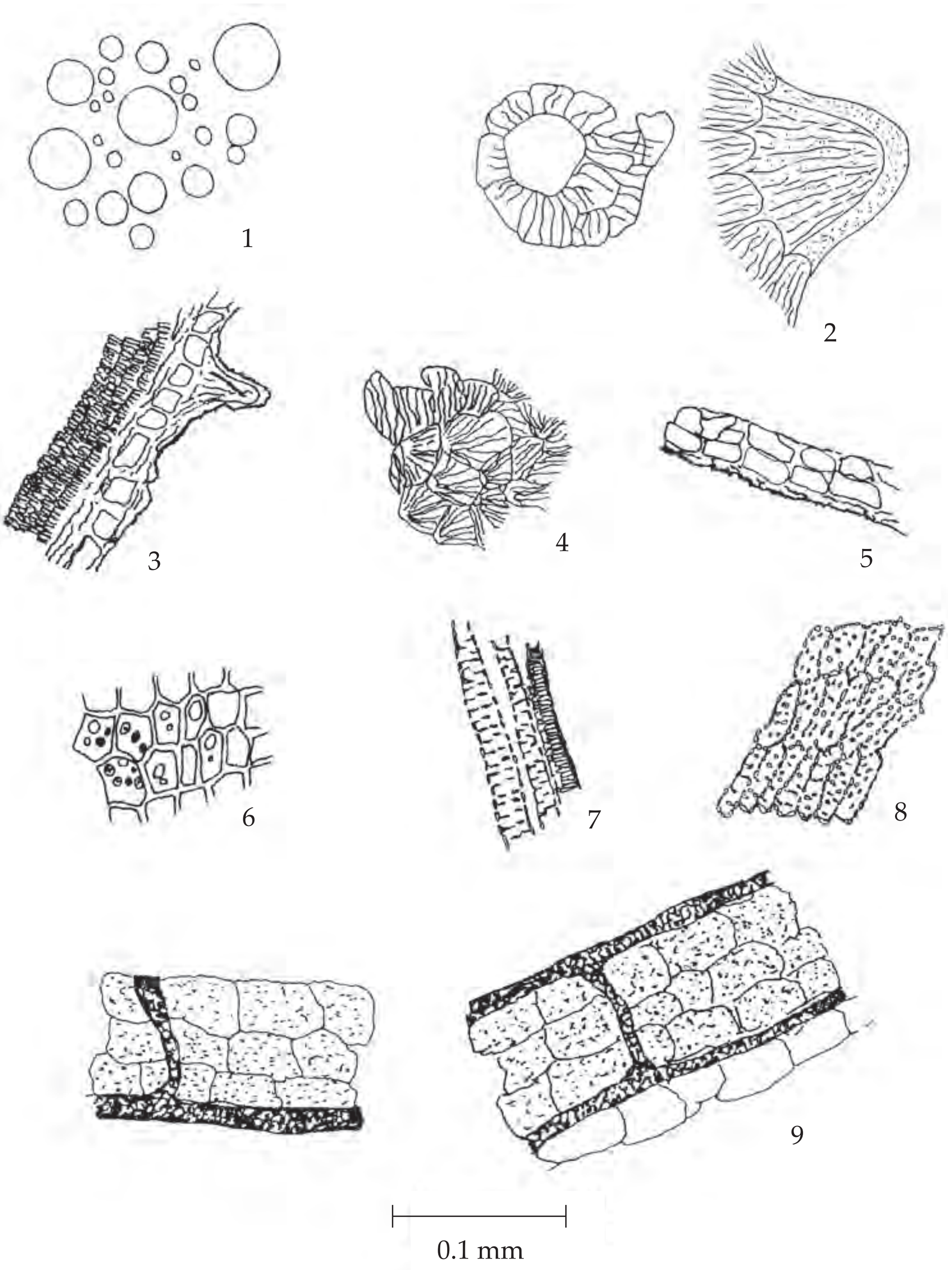


Fig. 2b Powdered Drug of the Fruits of *Trachyspermum ammi* (L.) Sprague

1. oil globules
2. large papilla surrounded by small papillae
3. epicarp in sectional view with a papilla and vascular bundle
4. epicarp showing small papillae in surface view
5. epicarp in sectional view
6. endosperm containing oil globules and aleurone grains
7. vessels
8. lignified, pitted parenchyma
9. vitta in longitudinal view

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Ajowan shall be kept in well-closed containers, preferably of metal or glass, protected from light and stored in a cool and dry place.

Identification

A. Extract 1 g of the sample, in *No. 850 powder*, with 5 ml of *petroleum ether* (*boiling range, 40° to 60°*) and filter. Evaporate the filtrate to dryness under the current of air, dissolve the residue in 1 ml of *glacial acetic acid*, add 6 drops of *sulfuric acid*, and then 1 drop of *nitric acid*: a deep bluish green colour develops.

B. Extract 2 g of the sample, in *No. 850 powder*, with 5 ml of *petroleum ether* (*boiling range, 40° to 60°*) and filter. Evaporate the filtrate to dryness under the current of air, dissolve the residue in 2 ml of a 10 per cent w/v solution of *sodium hydroxide* and heat on a water-bath for a few minutes. Cool the solution, add a few drops of *chloroform* and shake: a violet colour is produced.

C. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, 4 µl each of the following solutions. Prepare solution (A) by extracting 1 g of the sample, in *No. 850 powder*, with 2 ml of *ethanol* and filtering. For solution (B), dissolve 3 mg of *thymol* in 1 ml of *ethanol*. For solution (C), dissolve 4 µl of *olive oil* in 1 ml of *ethyl acetate*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 38 to 40), corresponding to the thymol spot from solution (B) and one spot of higher hR_f value. Spray the plate with *vanillin-sulfuric acid TS1*; the spot due to thymol is pink. One violet spot (hR_f value 65 to 68) corresponds to triglycerides of olive oil from solution (C). Other several spots are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Fruits of *Trachyspermum ammi* (L.) Sprague

Spot	hR_f Value	Detection	
		UV 254	*Vanillin-Sulfuric Acid TS1*
1	8-11	–	violet
2	13-15	–	violet
3	15-17	–	violet
4	19-21	–	violet
5	25-26	–	violet
6	30-32	–	violet
7*	38-40	quenching	pink
8	47-48	quenching	–
9**	65-68	–	violet

*thymol

**triglycerides of olive oil

Water Not more than 11.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 4.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Total ash Not more than 12.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 8.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 5.0 per cent v/w (Appendix 7.3 H). Use 20 g, in *No. 850 powder*, freshly prepared and accurately weighed. Use 200 ml of *water* as the distillation liquid and a 500-ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 4 hours. Use 1.0 ml of *xylene* in the graduated tube.

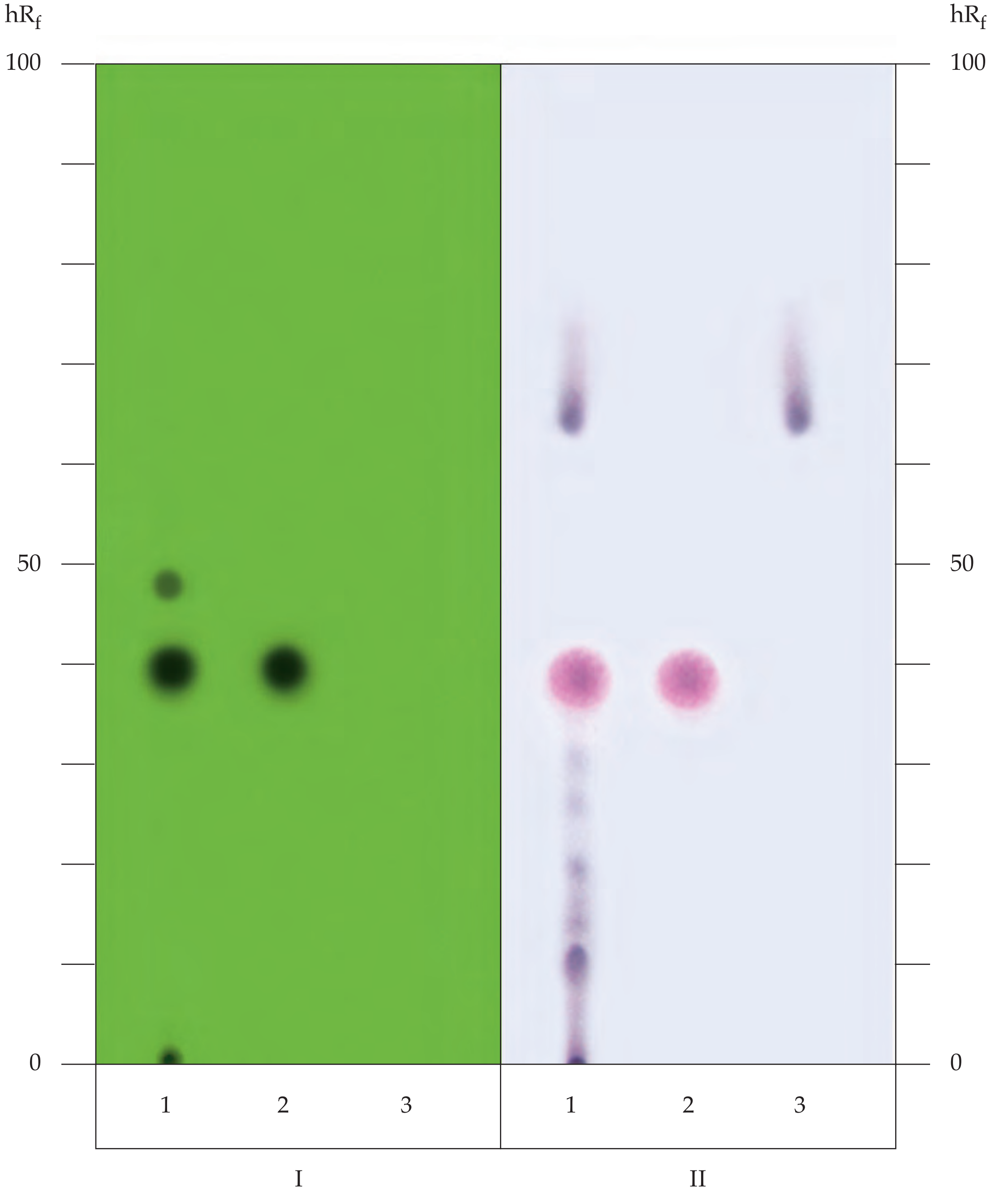


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Fruits of *Trachyspermum ammi* (L.) Sprague

1 = solution (A)
2 = solution (B)
3 = solution (C)
I = detection under UV light (254 nm)
II = detection with *vanillin-sulfuric acid TS1*

ว่านน้ำ (WANNAM)

Acori Calami Rhizoma
Sweet Flag Root

Synonyms Sweet Flag, Calamus, Acorus

Category Carminative.

Sweet Flag Root is the dried rhizome of *Acorus calamus* L. (Family Acoraceae), Herbarium Specimen Number: DMSC 890.

Constituents Sweel Flag Root contains volatile oil comprising *β*-asarone, *cis*-methyl isoeugenol, *α*-and *r*-asarone, asarylaldehyde, acorone, acoroxide, acorin, calamene, linalool, calamol, calameone, eugenol, methyl eugenol, azulene, pinene, cineole, camphor, and others.

Other constituents include sesquiterpenoid (e.g., acoragermacrone, acolamone and isoacolamone), acoric acid, tannin, and resin.

Description of the plant (Figs. 1a, 1b) Aquatic perennial herb with long creeping horizontal rhizome, growing in shallow water, marshes, or swamps. Leaves equitant, grass-like or sword-shaped, up to 2 m long, 1 to 3.5 cm wide, long and slender without distinction of petiole and finely parallel-veined. Inflorescence naked spike-like; spadix up to 10 cm long, projecting out and upwards from the leaf-like scape and spathe. Flower tiny, densely arranged, bisexual with perianth of 6 concave segments; stamens 6; ovary 2- to 4-celled with 2 to several ovules. Fruits baccate.

Description Odour, sweet and aromatic; taste, bitter and pungent.

Macroscopical (Fig. 1a) The rhizome is longitudinally split into subcylindrical pieces, 5 to 20 cm in length and 0.5 to 1.5 cm in diameter. The unpeeled drug is covered in a thin brown cork and is much shrunken and deeply wrinkled longitudinally. It bears on the upper surface triangular leaf scars and hair like fibres of fibrovascular tissue, and on the under surface many small root scars. The peeled drug is cream-yellow in colour and exhibits fewer root scars. The rhizome breaks with a sharp fracture producing a granular, white, spongy surface internally. The section has a large stele separated by a yellowish line from the cortex. Both the stele and cortex are covered with small oval fibrovascular bundles.

Microscopical (Figs. 2a, 2b) Transverse section of rhizome shows epidermis, a single layer of small thin-walled cells; outer region of the cortex, several layers of collenchyma; inner region of the cortex, chain of starchy parenchyma surrounding large air spaces with oil cells in each chain, fibrovascular bundles scattered throughout, surrounding with calcium oxalate prism sheath. Endodermis, a single layer of thin-walled elliptical cells. Stele, chain of starchy parenchyma surrounding large air spaces with yellowish brown oil cells, vascular bundles without fibre, scattered throughout, more numerously beneath the endodermis.

Sweet Flag Root in powder possesses the diagnostic microscopical characters of the unground drug.

Fig. 1a *Acorus calamus* L.
1. plant 2. juvenile spadix 3. flowering spadix 4. fruit-spadix 5. crude drug

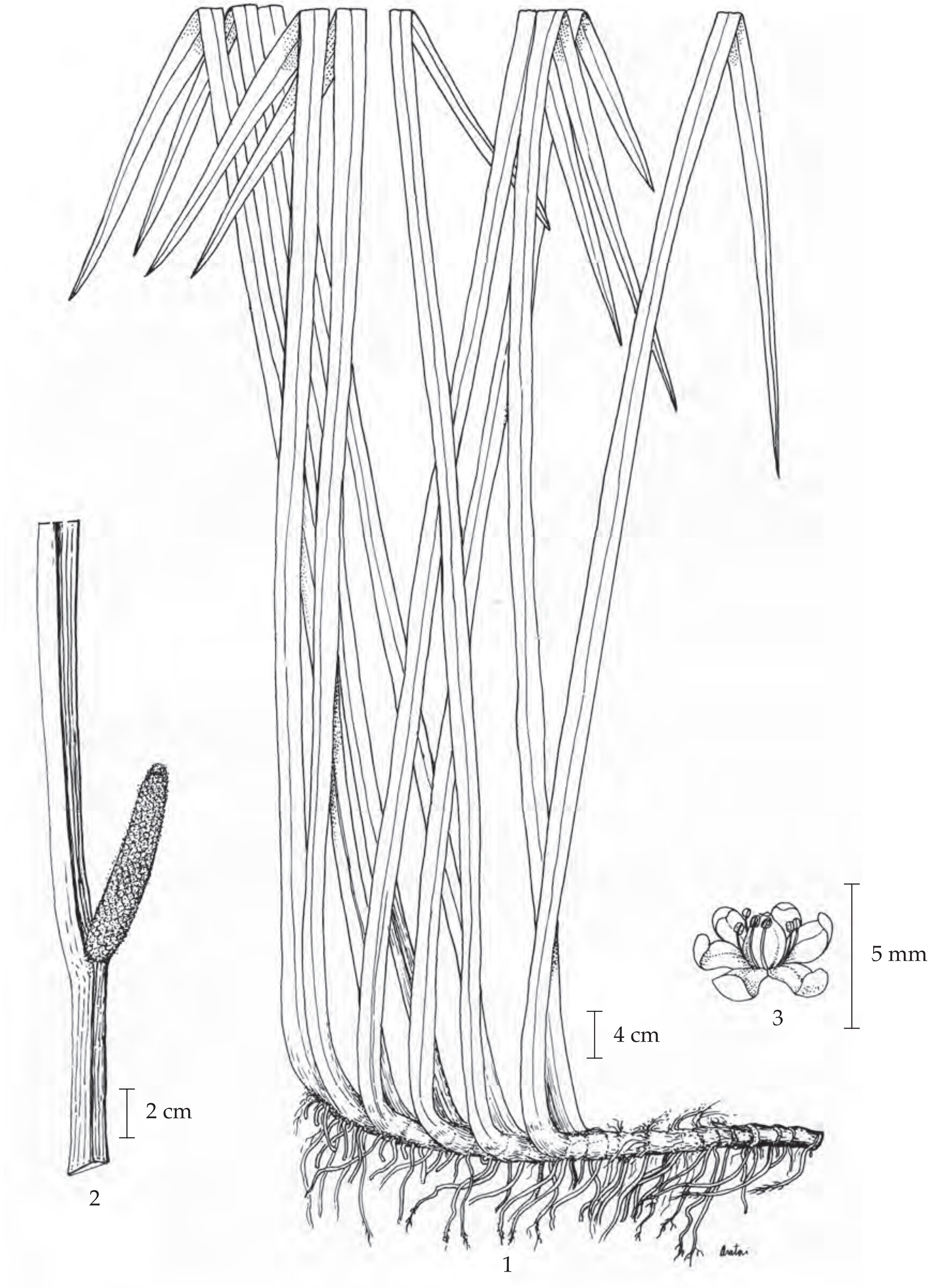


Fig. 1b *Acorus calamus* L.
1. plant 2. flowering spadix 3. flower

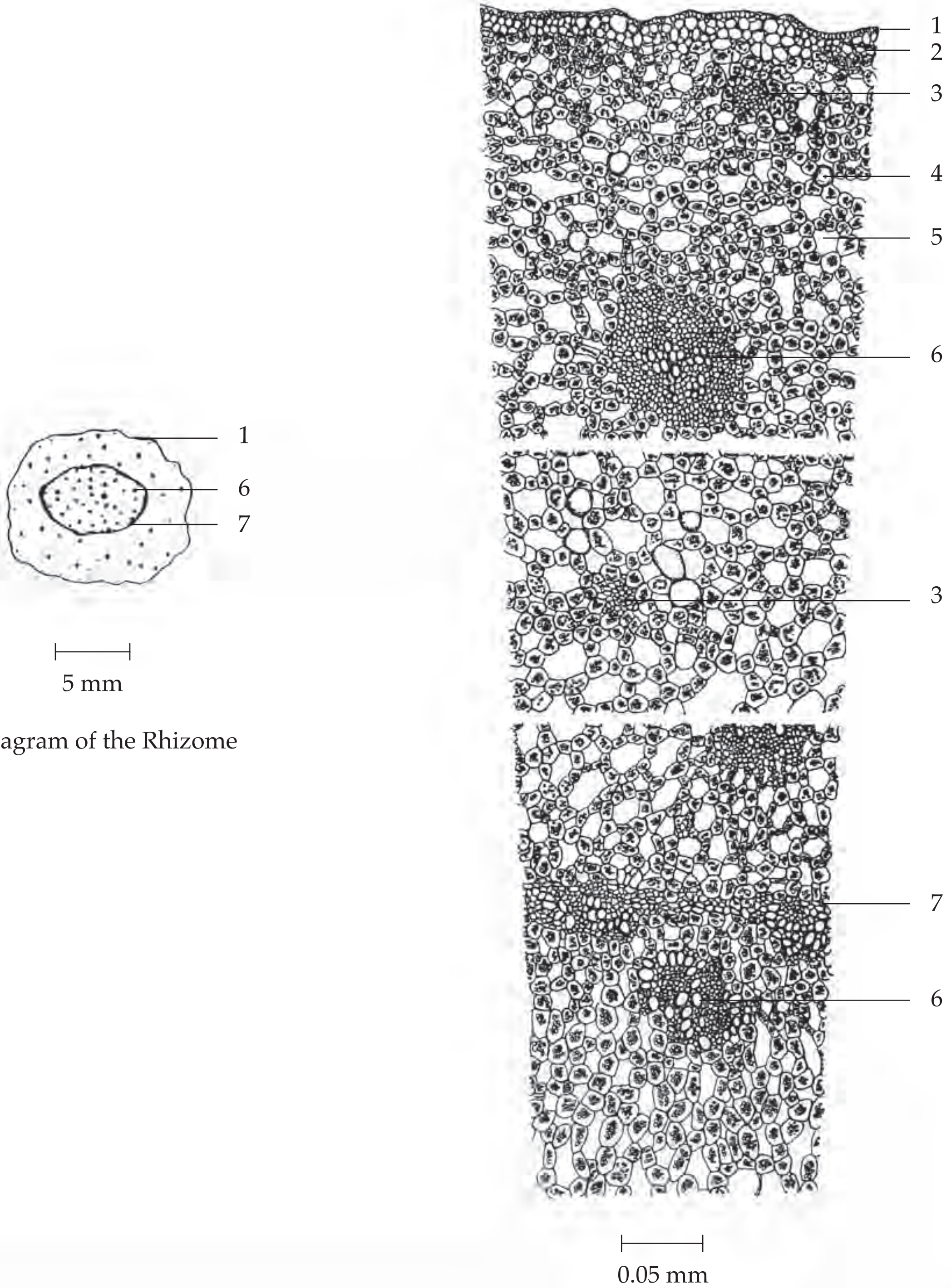


Diagram of the Rhizome

Fig. 2a Transverse Section of the Rhizome of *Acorus calamus* L.

1. epidermis
2. collenchyma
3. fibre group
4. oil cell
5. intercellular space
6. vascular bundle
7. endodermis

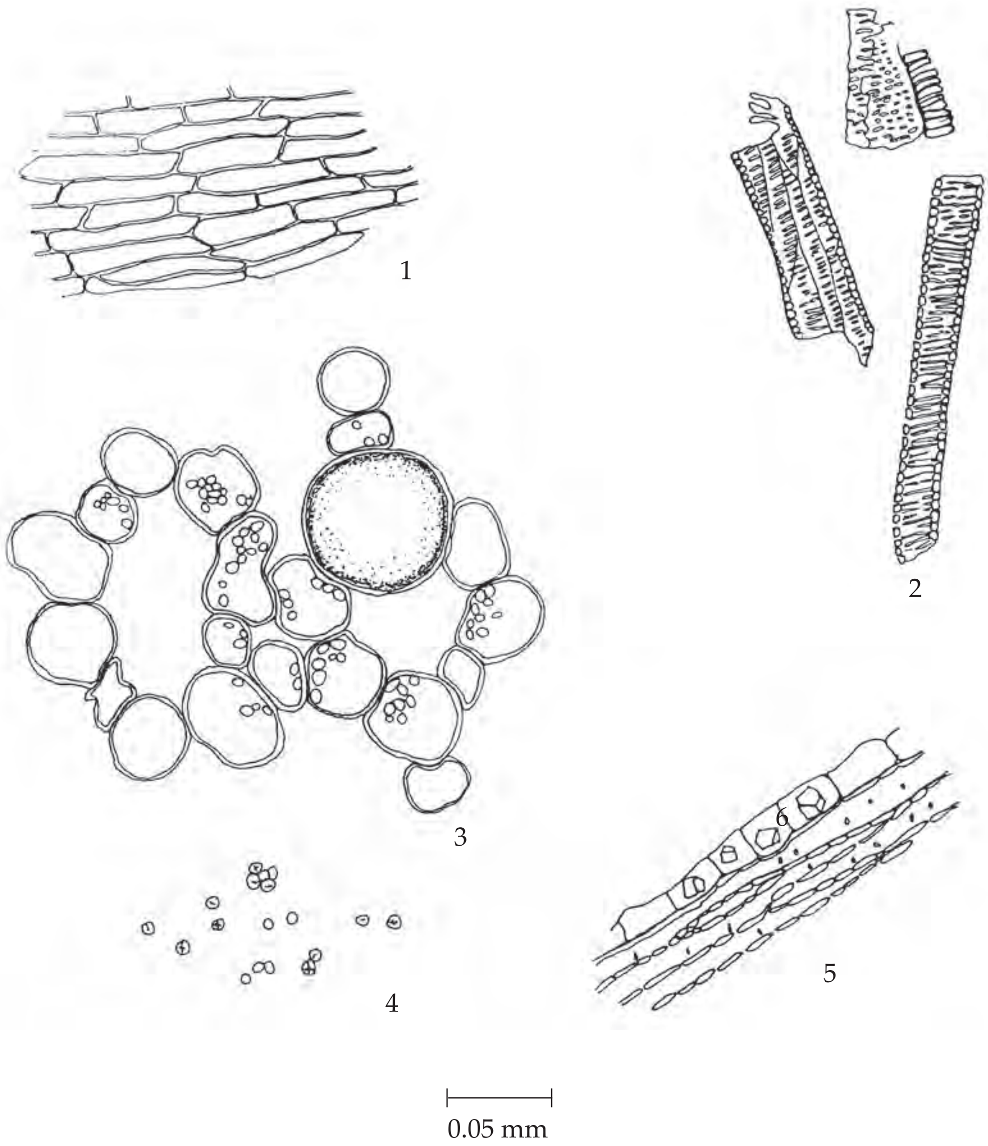


Fig. 2b Powdered Drug of the Rhizomes of *Acorus calamus* L.

1. epidermis
2. vessels
3. chain of starchy parenchyma surrounding large air space associated with large oil cell
4. starch granules
5. fibres with calcium oxalate prism sheath

Warning For reasons of optimal drug safety the use of *β*-asarone-rich races shall be avoided.

Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

Packaging and storage Sweet Flag Root shall be kept in well-closed containers, protected from light, and stored in a cool and dry place.

Identification

A. Reflux 1 g of the sample, in powder, with 25 ml of *ethanol* for 10 minutes and filter (solution 1). To 2 ml of solution 1, add a few drops of *ninhydrin TS* and heat in a water-bath: a violet colour is produced.

B. To 2 ml of solution 1, add a few drops of *basic lead acetate TS*: a white precipitate is produced.

C. Evaporate 2 ml of solution 1 to dryness, dissolve the residue in 2 ml of *acetic anhydride*, and add carefully 1 ml of *sulfuric acid* to form a layer: a brown ring forms at the zone of contact; a yellow colour develops in the upper layer and then gradually changes to dirty green.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 85 volumes of *petroleum ether* (*boiling range, 40° to 60°*) and 15 volumes of *ethyl acetate* as the mobile phase. Apply separately to the plate, 5 µl each of the following solutions. Prepare solution (A) by refluxing 1 g of the sample, in powder, with 25 ml of *ethanol* for 10 minutes, filtering, and evaporating to a volume of 2 ml. For solution (B), dissolve 2 mg of *β-asarone* in 1 ml of *ethanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the quenching spots. The chromatogram obtained from solution (A) shows a quenching spot (hR_f value 47 to 49), corresponding to the *β*-asarone spot from solution (B), and several spots of higher hR_f values (Table 1); see also Fig. 3. Subsequently examine the plate under ultraviolet light (366 nm); the spot due to *β*-asarone shows a light blue-violet fluorescence. Other two spots of different colours are also observed (Table 1); see also Fig. 3. Expose the plate to iodine vapour; the spot corresponding to *β*-asarone is yellow. Other several spots are also observed (Table 1); see also Fig. 3.

Table 1 hR$_f$ Values of Components in Ethanolic Extract of the Fruits of *Acorus calamus* L.

Spot	hR$_f$ Value	Detection		
		UV 254	UV 366	Iodine Vapour
1	8-9	–	light blue-violet	yellow
2	23-24	–	–	yellow
3	30-31	–	–	yellow
4	38-39	–	moss green	–
5*	47-49	quenching	light blue-violet	yellow
6	54-55	–	–	yellow
7	62-63	quenching	–	yellow
8	83-85	quenching	–	yellow
9	90-92	quenching	–	–
10	94-95	quenching	–	yellow

*β-asarone

Water Not more than 12.0 per cent w/w (Azeotropic Distillation Method, Appendix 4.12).

Foreign matter Not more than 2.0 per cent w/ w (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 7.0 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 5.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 11.0 per cent w/w (Appendix 7.12).

Volatile oil Not less than 1.2 per cent v/w (Appendix 7.3H). Use 20 g, in *coarse powder*, freshly prepared and accurately weighed. Use 150 ml of *water* as the distillation liquid and a 500 ml round-bottomed flask. Distil at a rate of 2 to 3 ml per minute for 5 hours. Use 2.0 ml of *xylene* in the graduated tube.

Dose 1 to 3 g.

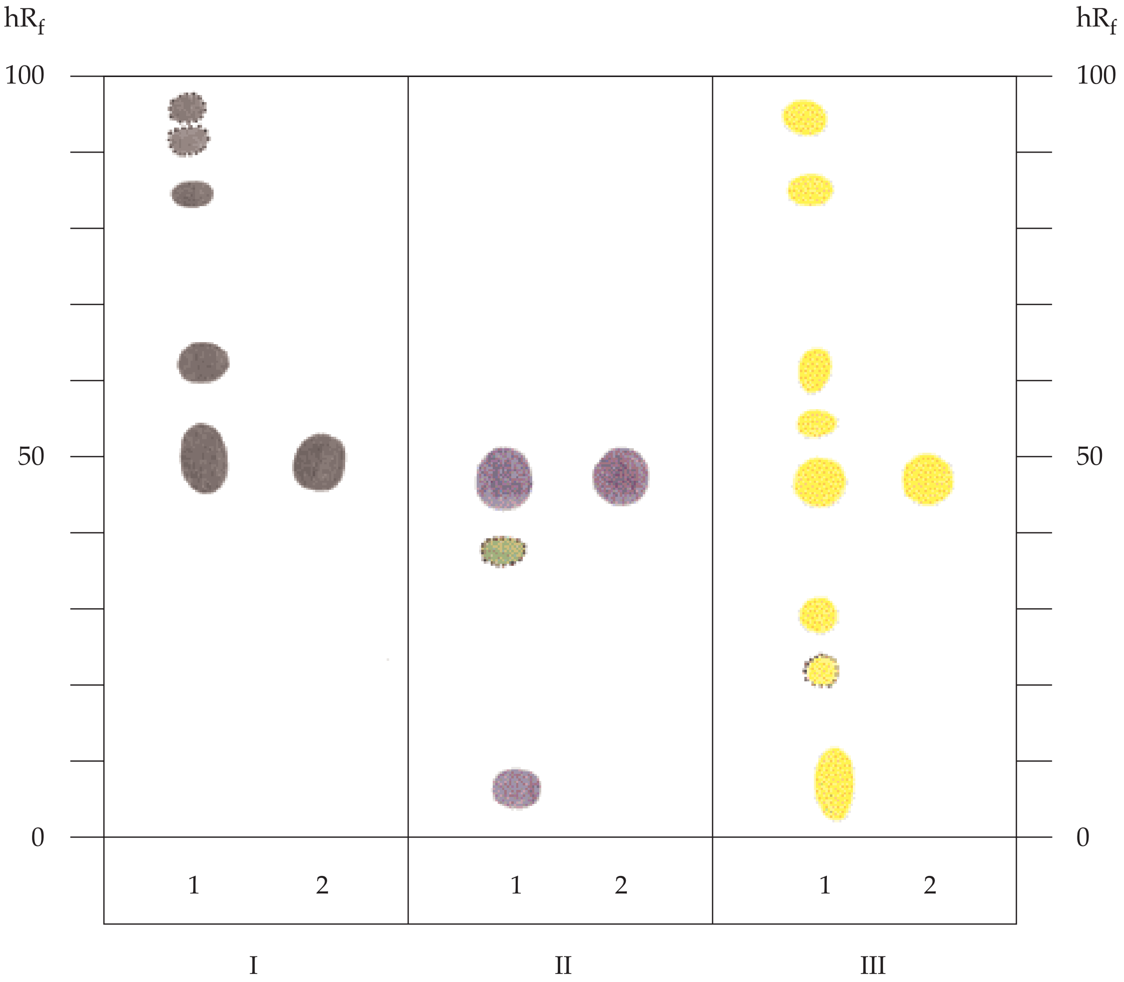


Fig. 3 Thin-layer Chromatogram of Ethanolic Extract of the Rhizomes of *Acorus calamus* L.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with iodine vapour
◌ = spots developed in some samples

หญ้าหนวดแมว (YA NUAT MAEO)

พยับเมฆ (PHAYAP MEK)
Orthosiphonis Herba
Java Tea

Synonym Orthosiphon

Category Diuretic.

Java Tea is the dried leaf and stem tip of *Orthosiphon aristatus* (Blume) Miq. [*O. spicatus* (Thunb.) Backer, Bakh. f. & Steenis, *O. stamineus* Benth.] (Family Labiatae), Herbarium Specimen Number: DMSC 1738; Crude Drug Number: DMSc 398
(**Note** Java Tea should be harvested shortly before the flowering period.)

Constituents Java Tea contains terpenoids (e.g., ursolic acid), caffeic acid and its derivatives (e.g., rosmarinic acid) and flavones (e.g., sinensetin). It also contains volatile oil, potassium, etc.

Description of the plant (Figs. 1a, 1b) Perennial herb, up to 2 m high, usually branching at base; stem quadrangular, covered with short hairs. Leaves simple, opposite, ovate, or ovate-lanceolate, 1.5 to 10 cm long, 1 to 5 cm wide, apex acuminate, base cuneate, margin coarsely serrate from about the middle upwards, minutely hairy on the nerves or glabrous, glandular punctate beneath; petiole up to 3 cm long. Flowers arranged in whorl, distantly along an elongate rachis, terminal, 7 to 30 cm long, white to violet; pedicel hairy, 1 to 6 mm long; calyx campanulate, 2.5 to 4.5 mm long (to 10 mm in fruit), curved, with longitudinal veins, 2-lipped, the upper lip broad, the lower 4-teethed; corolla much longer than calyx, about 3 cm long, the tube slender, 1 to 1.8 cm long, straight, 2-lipped, upper lip shallowly 4-lobed, recurved, the lower straight, concave; stamens 4, in 2 pairs, filament glabrous, filiform, very long, anthers 2-celled; the tip of the style club-shaped. Fruit 4 nutlets; nutlet ovoid-oblong, about 2 mm long, rather flattened, rugose.

Description Odour, slightly aromatic; taste, somewhat bitter and salty, later astringent.

Macroscopical (Figs. 1a, 1b) A mixture of entire and broken, crisp, mainly dark green ovate or ovate-lanceolate leaves and quadrangularly stems with a deep longitudinal groove down two opposite sides.

Microscopical (Figs. 2a, 2b, 2c, 2d) Transverse section of the leaf through the midrib and surface view of the lamina show upper epidermis, a single layer of rectangular cells covered with thick cuticle, wavy-walled in surface view; diacytic and anomocytic stomata; covering trichome, unicellular, multicellular up to 6 cells, non-stalk glandular and unicellular-stalk glandular radiated head with 4 cells or more; mesophyll, a layer of columnar palisade cells and several layers of spongy parenchyma; vascular bundle collateral; collenchyma occuring in the midrib, beneath upper and lower epidermises; lower epidermis, a single layer of rectangular cells covered with thick cuticle, wavy-walled in surface view; diacytic and anomocytic stomata; covering trichomes similar to those of upper epidermis.

Transverse section of the stem shows epidermal cells polygonal to elongated in surface view with scattered anomocytic and diacytic stomata; covering trichome multicellular, multicellular glandular trichome, unicellular, non-stalk similar to those of the leaf; underlying groups of collenchyma in the ridge; endodermis, small parenchymatous cells associated with fibre group. Vascular bundle, phloem and xylem; vessel, spiral, scalariform, reticulate and pitted. Pith, thin-walled and polygonal large parenchymatous cells.

Fig. 1a *Orthosiphon aristatus* (Blume) Miq.
1. and 2. inflorescences 3. flower 4. crude drug

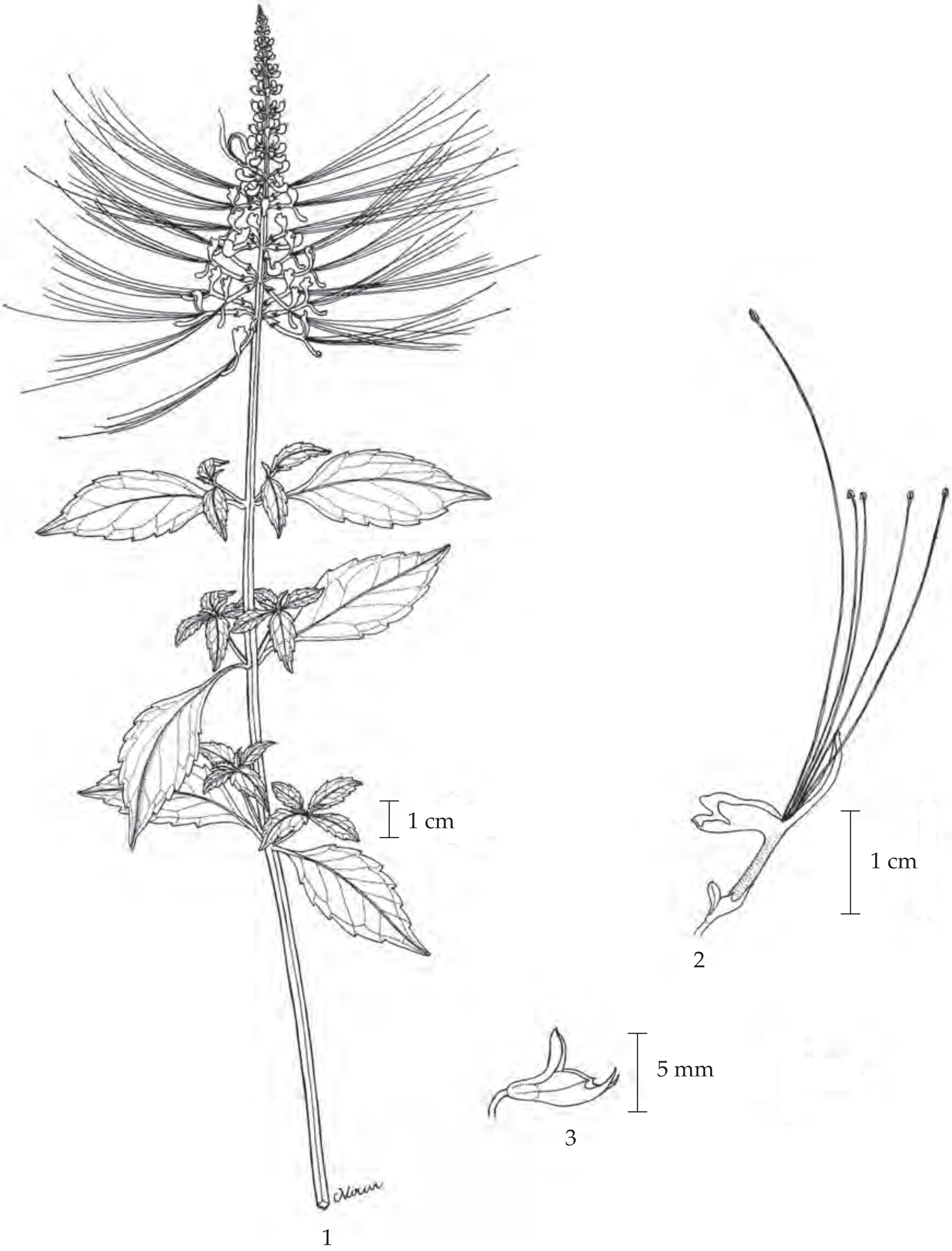


Fig. 1b *Orthosiphon aristatus* (Blume) Miq.
1. flowering top 2. flower 3. fruit with persistent calyx

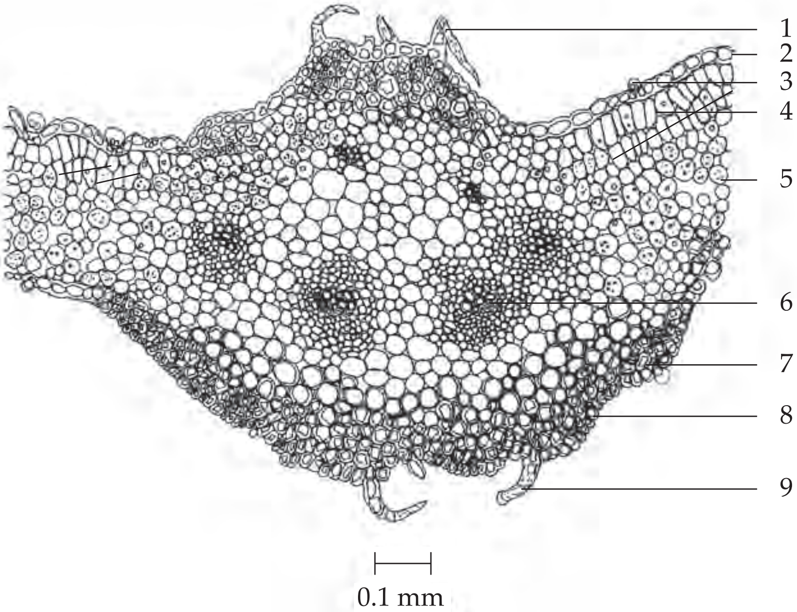
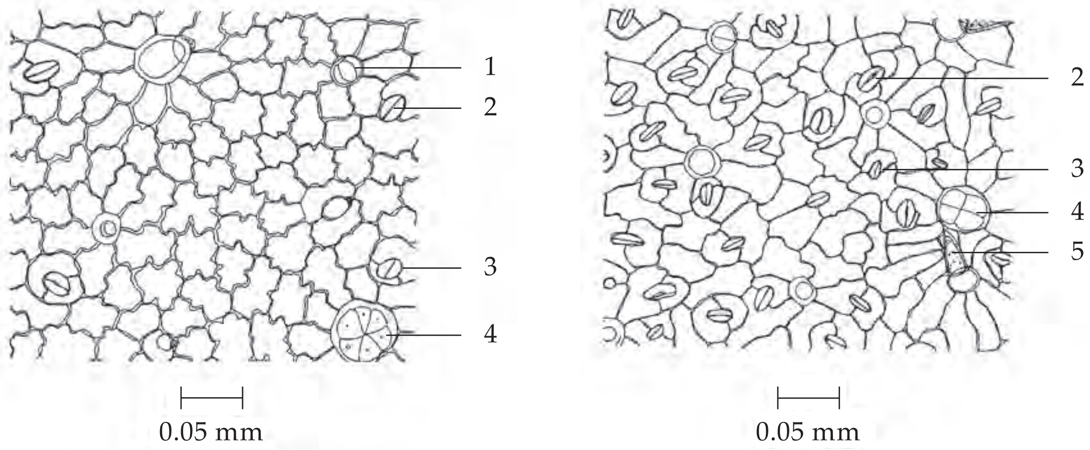


Fig. 2a Transverse Section of the Leaf through the Midrib of *Orthosiphon aristatus* (Blume) Miq.

1. multicellular trichome with acute apex
2. upper epidermis
3. glandular trichome
4. palisade cell
5. spongy parenchyma
6. vascular bundle
7. collenchyma
8. lower epidermis
9. multicellular trichome with obtuse apex



Upper Epidermis of the Lamina

Lower Epidermis of the Lamina

Fig. 2b Epidermises of the Leaf of *Orthosiphon aristatus* (Blume) Miq. in Surface View

1. cicatrice
2. anomocytic stomata
3. diacytic stomata
4. glandular trichome with radiated head
5. covering trichome

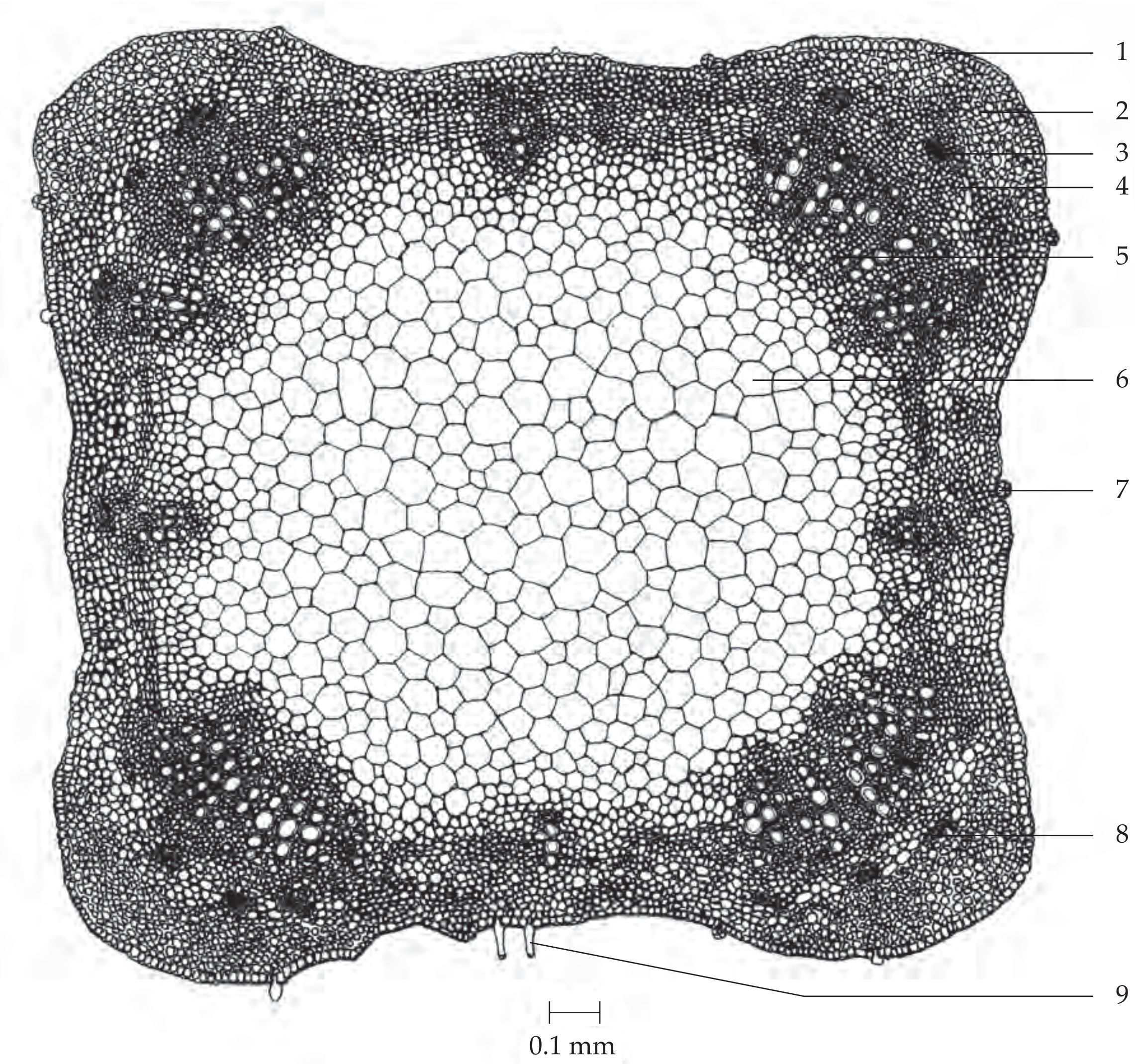


Fig. 2c Transverse Section of the Stem of *Orthosiphon aristatus* (Blume) Miq.

1. epidermis
2. collenchyma
3. parenchyma
4. phloem
5. xylem
6. thin-walled parenchyma
7. glandular trichome
8. fibre
9. covering trichome

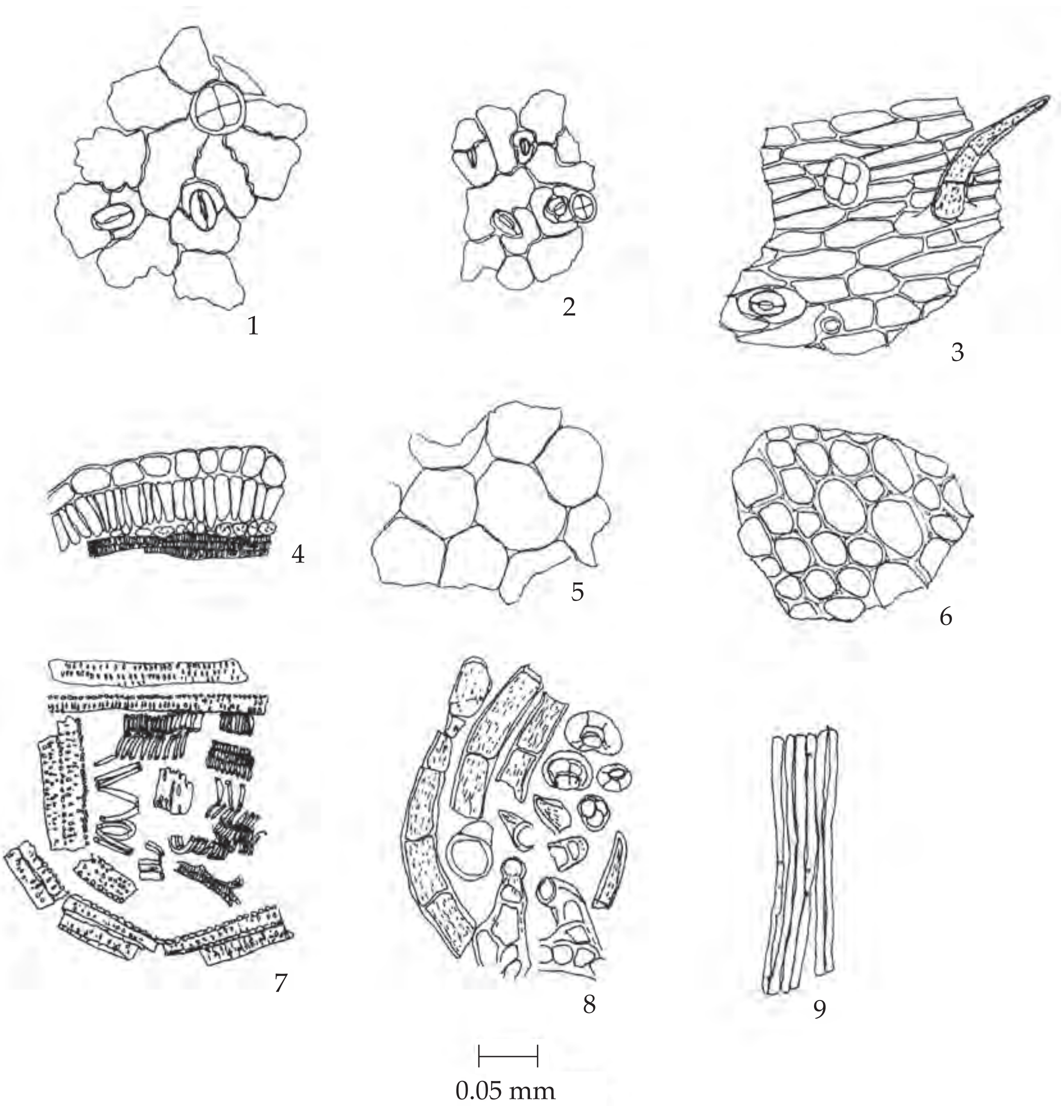


Fig. 2d Powdered Drug of the Leaves and Stem Tips of *Orthosiphon aristatus* (Blume) Miq.

1. upper epidermis of lamina in surface view with glandular trichome
2. lower epidermis of lamina in surface view with glandular trichome
3. epidermis of stem in surface view with covering and glandular trichome
4. lamina in transverse section
5. thin-walled parenchyma
6. collenchyma
7. vessels
8. covering and glandular trichomes
9. fibres

Java Tea in powder possesses the diagnostic microscopical characters of the unground drug.

Contra-indication It is contra-indicated in patients with impaired cardiac and/or renal function.

Storage Java Tea shall be stored in a dry place, protected from light.

Identification

A. Reflux 1 g of the sample, in *fine powder*, with 50 ml of *water* for 30 minutes, filter and evaporate the filtrate to 25 ml (solution 1). To 2 ml of solution 1, add a few drops of 0.02 M *potassium permanganate* and shake well: the colour of permanganate solution disappears.

B. To 1 ml of solution 1, add a few drops of a 1 per cent w/v solution of *iron(III) chloride*: a greyish green colour develops.

C. Evaporate 0.5 ml of solution 1 to dryness, moisten the residue with 1 drop of 6 M *hydrochloric acid*: it imparts a reddish violet colour to a non-luminous flame.

D. Carry out the test as described in the "Thin-layer Chromatography" (Appendix 3.1), using *silica gel GF254* as the coating substance and a mixture of 45 volumes of *toluene*, 45 volumes of *ethyl acetate* and 5 volumes of *formic acid* as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, 5 µl of solution (A), 3 µl of solution (B), 1 µl of solution (C), and 2 µl of solution (D). Prepare solution (A) by evaporating 20 ml of solution 1 to dryness. Reflux the residue with 25 ml of *methanol* for 10 minutes, filter and evaporate the filtrate to dryness. Dissolve the residue in 5 ml of a 50 per cent v/v solution of *methanol*. For solution (B), dissolve 400 µg of *rosmarinic acid* in 1 ml of *methanol*. For solution (C), dissolve 1 mg of *sinensetin* in 20 ml of *methanol*. For solution (D), dissolve 200 µg of *caffeic acid* in 1 ml of *methanol*. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm), marking the spots. The chromatogram obtained from solution (A) shows one blue fluorescent spot (hR_f value 44 to 48), corresponding to the sinensetin spot from solution (C) and two quenching spots (hR_f values 30 to 37 and 52 to 60), corresponding to the rosmarinic acid and caffeic acid spots from solutions (B) and (D), respectively. Examine the same plate under ultraviolet light (366 nm), one pale blue and two dark blue fluorescent spots corresponding to sinensetin, rosmarinic acid and caffeic acid are observed. Heat the plate at 80° for at least 10 minutes and then spray with *natural products (NP) TS* while the plate is still warm. Subsequently spray the plate with *polyethyleneglycol (PEG) TS* and observe the colours of the spots under ultraviolet light (366 nm) within 5 to 15 minutes; the spot due to sinensetin is dark blue fluorescent, the ones due to rosmarinic acid and caffeic acid are pale blue fluorescent. The other spot of lower hR_f value appears as a pale blue fluorescent spot (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in the Extract of the Leaves and Stem Tips of *Orthosiphon aristatus* (Blume) Miq.

Spot	hR_f Value	Detection		
		UV 254	UV 366	*NP/PEG TS* and UV 366
1	11-20	–	–	light blue
2*	30-37	quenching	intense blue	light blue
3**	44-48	blue	light blue	intense blue
4***	52-60	quenching	intense blue	light blue

*rosmarinic acid

**sinensetin

***caffeic acid

Loss on drying Not more than 11.0 per cent w/w after drying at 105° for 5 hours (Appendix 4.15).

Foreign matter Not more than 5 per cent w/w of dried stems with a diameter greater than 2 mm; not more than 2 per cent w/w of other foreign matter (Appendix 7.2).

Acid-insoluble ash Not more than 1.0 per cent w/w (Appendix 7.6).

Total ash Not more than 12.5 per cent w/w (Appendix 7.7).

Ethanol-soluble extractive Not less than 10.0 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 20.0 per cent w/w (Appendix 7.12).

Dose 2 to 4 g, prepared as an infusion by soaking dried material with 150 ml of boiling water for 5 to 20 minutes, two to three times a day before meals.

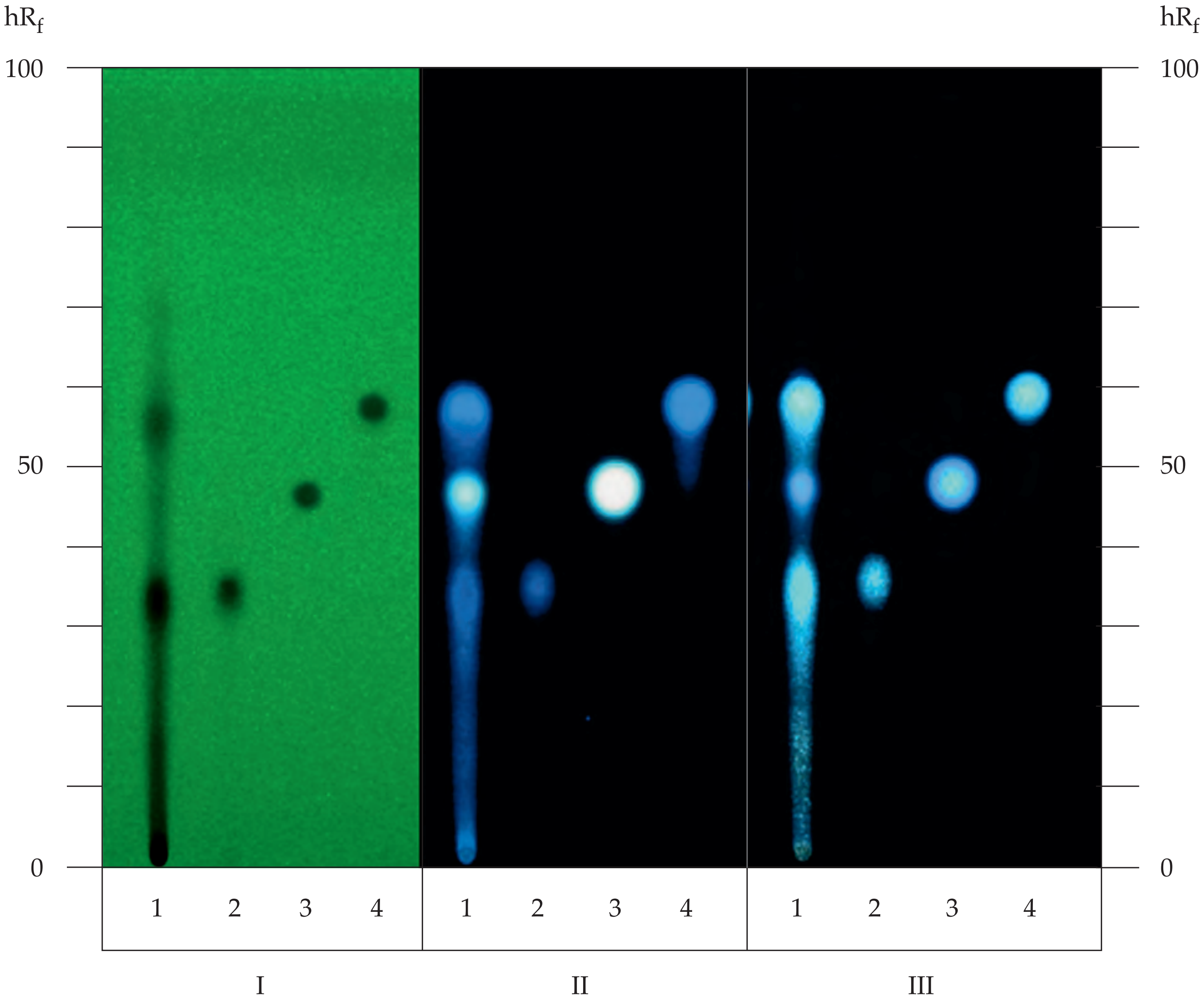


Fig. 3 Thin-layer Chromatogram of the Extract of the Leaves and Stem Tips of *Orthosiphon aristatus* (Blume) Miq.

1 = solution (A)
2 = solution (B)
3 = solution (C)
4 = solution (D)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection under UV light (366 nm) after spraying with *NP/PEG TS*

APPENDICES

APPENDICES

The majority of appendices indicated in the Thai Herbal Pharmacopoeia are those of the Thai Pharmacopoeia. With the kind permission of the Thai Pharmacopoeia Committee, the relevant texts of appendices are reproduced. Nevertheless, the appendices bearing “H” after their numerical numbers are produced only for the Thai Herbal Pharmacopoeia. For further information concerning methods of analysis and reagents, users of this Pharmacopoeia may refer to the Thai Pharmacopoeia. In cases of doubt or dispute, the text published in the Thai Pharmacopoeia is authoritative.

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APPENDIX 1 GENERAL INFORMATION

The specifications given below are strictly for the use of the materials as reagents. The inclusion of a material in this Appendix does not imply that it is suitable for use in medicines. Exceptionally, a trademark or supplier may be indicated for certain reagents whose availability is limited. It is however acceptable to use reagents from another source provided that they comply with the standards of the Pharmacopoeia.

1.1 REAGENTS

The name of a substance or solution indicates a reagent included in the following list. The specifications given for reagents do not necessarily guarantee their quality for use in medicines.

Some of the reagents included may be injurious to health. Important cautions have been stated for these reagents. They should be handled in accordance with good laboratory practice and any relevant regulations.

Reagents in aqueous solution are prepared using *water.* Where the name of the solvent is not stated, an aqueous solution is intended.

Unless otherwise specified, the reagents and reagent solutions are to be stored in well-closed containers. The labelling should comply with the relevant national legislation.

Absorbent Cotton

Use a suitable grade.

Acetic Acid To *glacial acetic acid*, add sufficient *water* to produce a solution containing 33.0 per cent w/w of $C_2H_4O_2$.

Acetic Acid, Glacial $C_2H_4O_2 = 60.05$

Contains not less than 99.0 per cent w/w of $C_2H_4O_2$.

DESCRIPTION Clear, colourless liquid; odour, pungent. Crystallizes at about 10° and does not completely remelt until warmed to about 15°.

FREEZING TEMPERATURE Not lower than 14.8° (Appendix 4.4).

WEIGHT PER MILLILITRE 1.048 to 1.051 g (Appendix 3.4).

IRON Not more than 1 ppm. To 9.50 ml add 0.1 ml of *sodium carbonate TS* and evaporate to dryness on a water-bath. Dissolve the residue by heating to boiling with a mixture of *dilute hydrochloric acid* and 10 ml of *water*, cool, and dilute to 50 ml with *water*. The solution complies with the "Limits Test for Iron" (Appendix 5.2).

CHLORIDE Not more than 67 ppm. A 5.0-ml sample shows no more chloride than that corresponds to 1.0 ml of 0.020 M *hydrochloric acid*.

SULFATE Not more than 0.023 per cent w/w. A 4.0-ml sample shows no more sulfate than that corresponds to 1.0 ml of 0.010 M *sulfuric acid*.

CERTAIN ALDEHYDIC SUBSTANCES To 10.0 g add 50 ml of *water* and 10.0 ml of a 1.25 per cent w/v solution of *sodium metabisulfite*, allow to stand for 30 minutes, and titrate the excess of *sodium metabisulfite* with 1.05 M *iodine VS*. Repeat the operation without the glacial acetic acid; the difference between the titrations does not exceed 2.3 ml.

FORMIC ACID AND OXIDIZABLE IMPURITIES Mix 5.0 ml with 10.0 ml of *water*. To 5.0 ml of the solution add 6 ml of *sulfuric acid* and cool at 20°. Add 2.0 ml of 0.0167 M *potassium dichromate* and allow to stand for 1 minute. Dilute with 25 ml of *water*, add 1 ml of *potassium iodide TS,* and titrate the liberated iodine with 0.10 M *sodium thiosulfate,* using *starch TS* as indicator. Not less than 1.0 ml of 1.0 M *sodium thiosulfate* is required.

ODOROUS IMPURITIES Dilute 1.5 ml to 5 ml with *water* and neutralize to *litmus paper* with 5 M *sodium hydroxide*: not more than a faint acetous odour is discernible.

NON-VOLATILE MATTER When volatized, leaves not more than 0.01 per cent of residue.

ASSAY Weigh accurately about 2 g into a stoppered flask containing 50 ml of *water* and titrate with 1 M *sodium hydroxide VS*, using *phenolphthalein TS* as indicator. Each ml of 1 M *sodium hydroxide VS* is equivalent to 60.05 mg of $C_2H_4O_2$.

Acetic Anhydride $C_4H_6O_3 = 102.09$

Use analytical reagent grade of commerce containing not less than 97.0 per cent w/v of $C_4H_6O_3$.

DESCRIPTION Colourless liquid.

BOILING RANGE 136° to 142° (Appendix 4.5).

Acetone $C_3H_6O = 58.08$

DESCRITION Clear, colourless, mobile, volatile liquid; odour, characteristic. Flammable.

SOLUBILITY Miscible with *water*, with *chloroform*, with *ethanol*, and with *ether*.

BOILING RANGE Not less than 95 per cent distils between 55.5° and 57.0° (Appendix 4.5).

WEIGHT PER MILLILITRE 0.790 to 0.792 g (Appendix 4.9).

ACIDITY Dilute 10.0 ml with 10.0 ml of *carbon dioxide-free water.* The solution requires for neutralization not more than 0.20 ml of 0.10 M *sodium hydroxide*, using *phenolphthalein TS* as indicator.

ALKALINITY Dilute 10 ml with 10 ml of *carbon dioxide-free water.* The solution is not alkaline to *litmus paper*.

WATER Shake 10.0 ml with 40.0 ml of *carbon disulfide*: a clear solution is produced.

OXIDIZABLE SUBSTANCES To 20.0 ml add 0.10 ml of 0.020 M *potassium permanganate*, and allow to stand for 15 minutes: the solution is not completely decolorized.

NON-VOLATILE MATTER When evaporated on a water-bath and dried at 105° to constant weight, leaves not more than 0.01 per cent w/v of residue.

Acetonitrile (Methyl Cyanide) $C_2H_3N = 41.05$

DESCRIPTION Clear, colourless liquid.

SOLUBILITY Miscible with *water*.

ACIDITY OR ALKALINITY A 10 per cent v/v solution is neutral to *litmus*.

BOILING RANGE Not less than 95 per cent distils between 80° and 82° (Appendix 4.5).

WEIGHT PER MILLILITRE 0.780 to 0.783 g (Appendix 4.9).

Aluminium Oxide, Neutral Al_2O_3 = 102. 0

Use chromatographic grade of neutral activated alumina designed for column chromatography.

Ammonia Solution, Strong NH_3 = 17.03

Contains not less than 25.0 per cent w/w and not more than 30.0 per cent w/w of NH_3 and about 13.6 M in strength.

DESCRIPTION Clear, colourless liquid, very caustic.

SOLUBILITY Miscible with *water* and with *methanol*.

IDENTIFICATION

A. Relative density: 0.892 to 0.910.

B. It is strongly alkaline.

C. To 0.5 ml add 5 ml of *water*. Bubble air through the solution and lead the gaseous mixture obtained over the surface of a solution containing 1 ml of 0.1 M *hydrochloric acid* and 0.05 ml of *methyl red TS.* The colour changes from red to yellow. Add 1 ml of *sodium cobaltinitrite TS*: a yellow precipitate is formed.

TESTS Solution S for testing chloride, sulfate iron and heavy metals is prepared by evaporating 220 ml almost to dryness on a water-bath. Cool, add 1 ml of *dilute acetic acid* and dilute to 20 ml with *distilled water*.

APPEARANCE OF SOLUTION To 2 ml add 8 ml of *water*. The solution is clear and colourless.

OXIDIZABLE SUBSTANCES Cautiously add, whilst cooling, 8.8 ml to 100 ml of *dilute sulfuric acid.* Add 0.75 ml of 0.002 M *potassium permanganate.* Allow to stand for 5 minutes. The solution remains faintly pink.

PYRIDINE AND RELATED SUBSTANCES Not more than 2 ppm, calculated as pyridine. Measure the absorbance (Appendix 2.2) at 252 nm using *water* as the compensation liquid. The absorbance is not greater than 0.06.

CARBONATES Not more than 60 ppm. To 10 ml in a test-tube with a ground-glass neck add 10 ml of *calcium hydroxide TS.* Stopper immediately and mix. Any opalescence in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of a 0.01 per cent w/v solution of *anhydrous sodium carbonate*.

CHLORIDE Not more than 1 ppm. Dilute 5 ml of *Solution S* to 15 ml with *water*. The solution complies with the “Limit Test for Chloride” (Appendix 5.2).

SULFATE Not more than 5 ppm. Dilute 3 ml of *Solution S* to 15 ml with *distilled water*. The solution complies with the “Limit Test for Sulfate” (Appendix 5.2).

IRON Not more than 0.25 ppm. Dilute 4 ml of *Solution S* to 10 ml with *water*. The solution complies with the “Limit Test for Iron” (Appendix 5.2).

HEAVY METALS Not more than 1 ppm. Dilute 4 ml of *Solution S* to 20 ml with *water*. The 12-ml solution complies with the “Limit Test for Heavy Metals” (Method I, Appendix 5.2). For the Standard Preparation, use *lead standard solution (2 ppm Pb)*.

NON-VOLATILE MATTER Not more than 0.002 per cent w/v. A 50-ml sample, when evaporated and dried at 100° to 105° to constant weight, leaves not more than 1.0 mg of residue.

ASSAY Weigh accurately a flask with a ground-glass neck containing 50.0 ml of 1 M *hydrochloric acid VS.* Add 2 ml of the substance to be examined and reweigh. Add 0.1 ml of *methyl red TS* as indicator. Titrate with 1 M *sodium hydroxide VS* until the colour changes from red to yellow. Each ml of 1 M *hydrochloric acid* is equivalent to 17.03 mg of NH_3.

Store protected from air, at a temperature not exceeding 20°.

Andrographolide $C_{20}H_{30}O_5 = 350.46$

Use analytical reagent grade of commerce containing not less than 98.0 per cent w/w of $C_{20}H_{30}O_5$.

DESCRIPTION Colourless or white rhombic prisms or plates.

SOLUBILITY Sparingly soluble in *water*; soluble in *chloroform*, in *ether* and in *methanol*.

Anethole $C_{10}H_{12}O = 148.20$

DESCRIPTION Colourless or faintly yellow liquid at or above 23°; odour aromatic. Affected by light.

SOLUBILITY Very slightly soluble in *water*; freely soluble in *ethanol*; readily miscible with *chloroform* and with *ether*.

FREEZING TEMPERATURE Not lower than 20° (Appendix 4.4).

BOILING RANGE 231° to 237° (Appendix 4.5).

REFRACTIVE INDEX 1.557 to 1.561 (Appendix 4.7).

OPTICAL ROTATION –0.15° to +0.15° (Appendix 4.8).

SPECIFIC GRAVITY 0.983 to 0.988 (Appendix 4.9).

HEAVY METALS Not more than 40 ppm (Method II, Appendix 5.2). Use 1.0 g; for the Standard Preparation; use *lead acetate solution (2 ppm Pb)*.

ALDEHYDES AND KETONES Shake 10 ml with 50 ml of saturated solution of *sodium hydrogensulfite* in a graduated cylinder, and allow the mixture to stand for 6 hours: no appreciable diminution in the volume of anethole occurs, and no crystalline deposit separates.

PHENOLS Shake 1 ml with 20 ml of *water*, and allow the liquids to separate. Filter the water layer through a filter paper previously moistened with *water* and to 10 ml of the filtrate add 3 drops of *iron(III) chloride TS*: no purple or purplish colour is produced.

Anisaldehyde (4-Methoxybenzaldehyde) $C_8H_8O_2 = 136.15$

Use general reagent grade of commerce.

DESCRIPTION Colourless or pale yellow, oily liquid; odour, aromatic.

SOLUBILITY Slightly soluble in *water*; miscible with *ethanol* and with *ether*.

BOILING TEMPERATURE About 248° (Appendix 4.6).

WEIGHT PER MILLILITRE About 1.125 g (Appendix 4.9).

Antimony Trichloride $SbCl_3 = 228.11$

Contains not less than 97.0 per cent of $SbCl_3$.

DESCRIPTION Colourless crystals, fuming in moist air.

SOLUBILITY Very soluble in *absolute ethanol* and in *chloroform*, forming solutions which are not more than slightly turbid.

ASSAY Dissolve about 500 mg, accurately weighed, in 30 ml of *water* containing 4 g of *potassium sodium tartrate*, add 2 g of *sodium hydrogencarbonate* and titrate with 0.05 M *iodine VS*, using *starch TS* as indicator near the end of the titration. Each ml of 0.05 M *iodine VS* is equivalent to 11.41 mg of $SbCl_3$.

L-Arabinose $C_5H_{10}O_5 = 150.13$

DESCRIPTION White, crystalline powder.

SPECIFIC ROTATION About +104° at 20°, determined in a 5 per cent w/v solution (Appendix 4.8).

Artemisinin $C_{15}H_{22}O_5 = 282.33$

Use analytical reagent grade of commerce containing not less than 98 per cent of $C_{15}H_{22}O_5$.

Store in a cool place.

***β*-Asarone** (1, 2, 4-Trimethoxy-5-(1-propenyl) benzene; 2, 4, 5-Trimethoxy-1-propenylbenzene) $C_{12}H_{16}O_3 = 208.26$

Use a suitable grade.

Asiatic Acid $C_{30}H_{48}O_5 = 488.70$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{30}H_{48}O_5$.

Asiaticoside $C_{48}H_{78}O_{19} = 959.12$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{48}H_{78}O_{19}$.

Benzene $C_6H_6 = 78.11$

DESCRIPTION Colourless, transparent liquid. Flammable.

BOILING RANGE Not less than 95 per cent distils between 79.5° and 81° (Appendix 4.5).

WEIGHT PER MILLILITRE 0.876 to 0.881 g (Appendix 4.9).

SULFUR COMPOUNDS Boil 10 ml with 1 ml of *absolute ethanol* and 3 ml of *potassium plumbite TS* for 15 minutes under a reflux condenser, and allow to stand for 5 minutes. The aqueous layer remains colourless.

THIOPHENE Shake 2 ml with 15 ml of *sulfuric acid* containing 3 mg of *isatin* in a stoppered tube for 5 minutes and allow to separate. No blue or green colour is produced.

NON-VOLATILE MATTER When evaporated on a water-bath and dried at 105° to constant weight, leaves not more than 0.01 per cent w/v of residue.

Berberine Chloride $C_{20}H_{18}ClNO_4 \cdot 2H_2O = 407.85$

DESCRIPTION Yellow crystals.

MELTING RANGE 204° to 206° (Appendix 4.3).

Bismuth Oxynitrate (Bismuth Subnitrate) $Bi_5O(OH)_9(NO_3)_4 = 1461.99$

DESCRIPTION White, microcrystalline powder.

SOLUBILITY Insoluble in *water* and in *ethanol*; readily soluble in *dilute nitric acid* and in *dilute hydrochloric acid*.

1-Butanol (*n*-Butyl Alcohol) $C_4H_{10}O = 74.12$

DESCRIPTION Clear, colourless liquid.

SOLUBILITY Soluble at 15.5° in 11 parts of *water*.

BOILING RANGE Not less than 95 per cent distils between 116° and 119° (Appendix 4.5).

WEIGHT PER MILLILITRE 0.807 to 0.810 g (Appendix 4.9).

FLUORESCENCE When examined under screened ultraviolet light, shows not more than the faintest trace of fluorescence.

NON-VOLATILE MATTER When evaporated on a water-bath and dried at 105° to constant weight, leaves not more than 0.01 per cent w/v of residue.

Caffeic Acid $C_9H_8O_4 = 180.16$

DESCRIPTION White or almost white, crystals or plates.

SOLUBILITY Freely soluble in hot *water* and in *ethanol*; sparingly soluble in cold *water*.

MELTING TEMPERATURE About 210°, with decomposition (Appendix 4.3).

Capsaicin $C_{18}H_{27}NO_3 = 342.85$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{18}H_{27}NO_3$.

Carbophenothion $C_{11}H_{16}ClO_2PS_3 = 305.41$

Use a suitable grade for pesticide residue analysis. A certified reference material (10 ng/ml in 2,2,4-trimethylpentane) may be used.

WEIGHT PER MILLILITRE About 1.27 g (Appendix 4.9).

Carvone $C_{10}H_{14}O = 150.22$

Use general reagent grade of commerce.

DESCRIPTION Liquid.

SOLUBILITY Practically insoluble in *water*; miscible with *ethanol*.

BOILING TEMPERATURE About 230° (Appendix 4.6).

REFRACTIVE INDEX 1.500 (Appedix 4.7).

SPECIFIC ROTATION +61° (Appendix 4.8).

RELATIVE DENSITY 0.965 (Appendix 4.9).

(+)-Catechin $C_{15}H_{14}O_6 = 290.27$

MELTING TEMPERATURE 210° (Appendix 4.3).

SPECIFIC ROTATION 0°, determined in a 1 per cent w/v solution in *ethanol* (Appendix 4.8).

Charantin

Use a suitable grade.

Charcoal, Decolorizing

DESCRIPTION Black, light powder free from grittiness.

SOLUBILITY Practically insoluble in all usual solvents.

DECOLORIZING POWER Dissolve 100 mg of *strychnine sulfate* in 50 ml of *water*, add 1 g of the test substance, shake during 5 minutes, and pass through a dry filter, rejecting the first 10 ml of the filtrate. To a 10-ml portion of the subsequent filtrate add 1 drop of *hydrochloric acid* and 5 drops of *mercuric-potassium iodide TS*: no turbidity is produced.

ACID-SOLUBLE MATTER Not more than 3 per cent w/w. To 1.0 g add 25 ml of *dilute nitric acid* and boil for 5 minutes. Filter whilst hot through a sintered-glass filter of porosity of 4 to 10 µm and wash with 10 ml of hot *water*. Evaporate the combined filtrate and washings to dryness on a water-bath, add to the residue 1 ml of *hydrochloric acid*, evaporate to dryness again and dry the residue to constant weight at 100° to 105°. The residue weighs not more than 30 mg.

SULFATED ASH Not more than 5.0 per cent w/w (Appendix 5.3).

Chloroform $CHCl_3 = 119.38$

Caution Care should be taken not to vaporize chloroform in the presence of a flame, because of the production of harmful gases.

Use analytical reagent grade of commerce containing 0.4 to 1.0 per cent w/v of *ethanol*.

DESCRIPTION Colourless, volatile liquid; odour, characteristic.

SOLUBILITY Slightly soluble in *water*; miscible with *absolute ethanol*, with *ether*, with fixed and volatile oils, and with most organic solvents.

RELATIVE DENSITY 1.475 to 1.481 (Appendix 4.9).

BOILING TEMPERATURE About 60° (Appendix 4.6).

Store protected from light.

Chloroform Water Shake 2.5 ml of *chloroform* with 900 ml of *water* until dissolved and dilute with *water* to 1000 ml.

Citral $C_{10}H_{16}O = 152.24$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{10}H_{16}O$.

Cuminaldehyde [4-(1-Methylethyl)benzaldehyde; *p*-Isopropylbenzaldehyde]
$C_{10}H_{12}O = 148.20$

DESCRIPTION Colourless to yellowish, oily liquid; odour, strong persistent; taste, acrid burning taste.

SOLUBILITY Practically insoluble in *water*; soluble in *ethanol* and in *ether*.

BOILING RANGE 235° to 236° (Appendix 4.5).

REFRACTIVE INDEX 1.5301 (Appendix 4.7).

WEIGHT PER MILLILITRE About 0.978 g (Appendix 4.9).

Curcumin $C_{21}H_{20}O_6 = 368.39$

Use general reagent grade of commerce containing a mixture of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin.

DESCRIPTION Orange-brown, crystalline powder.

SOLUBILITY Insoluble in *water*; soluble in *ethanol*.

THIN-LAYER CHROMATOGRAM

System A —— *benzene*:*chloroform*:*ethanol* 49:49:2
hR_f value —— curcumin 28-34
desmethoxycurcumin 17-20
bisdesmethoxycurcumin 11-15
System B —— *toluene*:*chloroform*:*absolute ethanol* 49:49:2
hR_f value —— curcumin 24-26
desmethoxycurcumin 12-15
bisdesmethoxycurcumin 6-8

Cyclohexane $C_6H_{12} = 84.16$

DESCRIPTION Clear, colourless liquid.

BOILING RANGE Not less than 95 per cent distils between 80° and 82° (Appendix 4.5).

REFRACTIVE INDEX 1.4262 to 1.4265, at 20° (Appendix 4.7).

WEIGHT PER MILLILITRE 0.776 to 0.780 g (Appendix 4.9).

1,2-Dichloroethane (Ethylene Chloride) $C_2H_4Cl_2 = 98.96$

DESCRIPTION Clear, colourless liquid; odour, chloroform-like.

SOLUBILITY Soluble in 2 parts of *ethanol* yielding a clear, colurless solution.

BOILING RANGE Not less than 95 per cent distils between 82° and 84° (Appendix 4.5).

WEIGHT PER MILLILITRE About 1.25 g (Appendix 4.9).

NON-VOLATILE MATTER When evaporated on a water-bath and dried at 105° to constant weight, leaves not more than 0.01 per cent w/v of residue.

Dichloromethane (Methylene Chloride) $CH_2Cl_2 = 84.93$

DESCRIPTION Clear, colourless, mobile liquid.

SOLUBILITY Soluble in 50 parts of *water*; miscible with *ethanol* and with *ether*.

BOILING RANGE Not less than 95 per cent distils between 39° and 41° (Appendix 4.5).

WEIGHT PER MILLILITRE 1.323 to 1.325 g (Appendix 4.9).

NON-VOLATILE MATTER When evaporated on a water-bath and dried at 105° to constant weight, leaves not more than 0.05 per cent w/v of residue.

Diethanolamine $C_4H_{11}NO_2 = 105.14$

DESCRIPTION Colourless or slightly tinted liquid.

SOLUBILITY Miscible with *water*, with *acetone*, with *chloroform*, with *ethanol*, and with *glycerol*; slightly soluble to insoluble in *ether* and in *petroleum ether*.

BOILING TEMPERATURE About 217° (Appendix 4.6).

WEIGHT PER MILLILITRE About 1.1 g (Appendix 4.9).

pH 10.0 to 11.5, determined in a 5.0 per cent w/v solution (Appendix 4.11).

OTHER REQUIREMENTS Diethanolamine intended for alkaline phosphatase test complies with the following additional requirement.

MONOETHANOLAMINE Not more than 1.0 per cent w/w when determined in the following manner. Dissolve 1.00 g of *3-aminopropanol* (internal standard) in sufficient *acetone* to produce 10.0 ml (solution A). Dissolve 500 mg of *monoethanolamine* in sufficient *acetone* to produce 10.0 ml (solution B). Carry out the determination as described in the "Gas Chromatography" (Appendix 3.4), using the following solutions. For solution (1) add 1.0 ml of solution A to 5.00 g of the test substance and dilute to 10 ml with *acetone*. For solution (2) dissolve 5.00 g of the test substance in sufficient *acetone* to produce 10 ml. For solution (3) add 1.0 ml of solution A to 0.5 ml of solution B and dilute to 10 ml with *acetone*. Prepare solution (4) in the same manner as solution (3) but adding 1.0 ml of solution B in place of 0.5 ml of solution B. Prepare solution (5) in the same manner as solution (3) but adding

2.0 ml of solution B in place of 0.5 ml of solution B. The chromatographic procedure may be carried out using a column (1 m × 4 mm) packed with diphenylphenylene oxide porous polymer beads (180- to 250-µm) and the carrier gas with the flow rate of 40 ml per minute. Maintain the temperature of the column at 125° for 3 minutes and then raise to 300° at a rate of 12° per minute. Maintain the temperature of the injection port at 250° and that of the detector at 280°.

Diethylamine $C_{14}H_{11}N = 73.14$

Caution May be irritating to skin and mucous membranes.

Contains not less than 99.5 per cent of $C_{14}H_{11}N$.

DESCRIPTION Clear, colourless liquid. Flammable.

WEIGHT PER MILLILITRE 0.702 to 0.704 g (Appendix 4.9).

REFRACTIVE INDEX 1.384 to 1.386 (Appendix 4.7).

ASSAY Add about 3 g, accurately weighed, to 50.0 ml of 0.5 M *sulfuric acid VS* and titrate the excess of acid with 1 M *sodium hydroxide VS*, using *methyl red TS* as indicator. Each ml of 0.5 M *sulfuric acid VS* is equivalent to 73.14 mg of $C_{14}H_{11}N$.

3,5-Dinitrobenzoic Acid $C_7H_4N_2O_6 = 212.12$

DESCRIPTION Practically colourless crystals.

SOLUBILITY Slightly soluble in *water*; very soluble in *ethanol*.

MELTING RANGE 205° to 207° (Appendix 4.3).

2,4-Dinitrophenylhydrazine $C_6H_6N_4O_4 = 198.14$

DESCRIPTION Orange-red crystals, or crystalline powder, which under the microscope appear individually to be lemon-yellow, lath-like needles.

SOLUBILITY Very slightly soluble in *water*; slightly soluble in *ethanol*; moderately soluble in dilute inorganic acids.

MELTING RANGE 197° to 200°, with decomposition (Appendix 4.3).

SOLUBILITY TEST Dissolve 500 mg in a mixture of 25 ml of *sulfuric acid* and 25 ml of *water*: the solution is clear or not more than slightly turbid.

SULFATED ASH Negligible (Appendix 5.3); use 500 mg.

Diphenylboric Acid Aminoethyl Ester (Diphenylboric Acid β-Aminoethyl Ester; Diphenylboric Acid-Ethanolamine Reagent) $C_{14}H_{16}BNO = 225.10$

Use a suitable grade.

Emodin $C_{15}H_{10}O_5 = 270.21$

DESCRIPTION Orange crystals.

SOLUBILITY Practically insoluble in *water*; soluble in *ethanol* and in solutions of *alkali hydroxides*; slightly soluble in *ether*.

MELTING TEMPERATURE About 253°, with decomposition (Appendix 4.3).

Estragole $C_{10}H_{12}O = 148.20$

Use a suitable grade.

Ethanol $C_2H_6O = 46.07$

Use Ethanol (95 Per Cent) (see under "Reagents").

Ethanol (95 Per Cent)

A mixture of ethanol and water. Contains not less than 92.3 per cent w/w and not more than 93.8 per cent w/w, corresponding to not less than 94.9 per cent v/v and not more than 96.0 per cent v/v, at 15.56°, of C_2H_6O.

DESCRIPTION Colourless, clear, mobile and volatile liquid; odour, characteristic and spirituous. Flammable, burning with a blue smokeless flame. Boils at about 78°.

SOLUBILITY Miscible with *water*, with *chloroform* and with *ether*.

IDENTIFICATION

A. Mix 5 drops in a small beaker with 1 ml of *potassium permanganate TS* and 5 drops of *dilute sulfuric acid* and cover the beaker immediately with a filter paper moistened with a solution recently prepared by dissolving 100 mg of *sodium nitroferricyanide* and 500 mg of *piperazine hydrate* in 5 ml of *water*: an intense blue colour is produced on the filter paper, the colour becoming paler after a few minutes.

B. To 5 ml of a 0.5 per cent v/v solution, add 1 ml of 0.1 M *sodium hydroxide;* then slowly add 2 ml of *iodine TS*: the odour of iodoform develops and a yellow precipitate is produced.

ACIDITY OR ALKALINITY To 20 ml add 5 drops of *phenolphthalein TS*: the solution remains colourless and requires not more than 0.20 ml of 0.10 M *sodium hydroxide* to produce a pink colour.

CLARITY OF SOLUTION Dilute 5 ml to 100 ml with *water* in a glass cylinder: the solution remains clear when examined against a black background.

ALDEHYDES AND KETONES Heat 100 ml of *hydroxylamine TS* in a loosely stoppered flask on a water-bath for 30 minutes, cool, and, if necessary, add sufficient 0.050 M *sodium hydroxide* to restore the green colour. To 50 ml of this solution add 25 ml of the sample and heat on a water-bath for 10 minutes in a loosely stoppered flask. Cool, transfer to a Nessler cylinder, and titrate with 0.050 M *sodium hydroxide* until the colour matches that of the remainder of the hydroxylamine solution contained in a similar cylinder, both solutions being viewed down the axis of the cylinder. Not more than 0.90 ml or 0.050 M *sodium hydroxide* is required.

OXIDIZABLE SUBSTANCES To 20 ml add 1 ml of 0.002 M *potassium permanganate.* Allow the solution to stand at 20° for 10 minutes protected from light: the colour is not completely discharged.

NON-VOLATILE MATTER A 100-ml sample, when evaporated and dried at 100° to 105° to constant weight, leaves not more than 2.5 mg of residue.

SPECIFIC GRAVITY 0.805 to 0.821, at 25° (Appendix 4.9), using this result to ascertain the percentage of C_2H_6O contained in the liquid examined by reference to the Alcoholometric Table.

VOLATILE IMPURITIES Carry out the test as described in the “Gas Chromatography” (Appendix 3.4).

Reference solution (a) Dilute 100 ml of *anhydrous methanol* to 50.0 ml with the test substance. Dilute 5.0 ml of the solution to 50.0 ml with the test substance.

Reference solution (b) Dilute 50 ml of *anhydrous methanol* and 50 ml of *acetaldehyde* to 50.0 ml with the test substance. Dilute 100 ml of the solution to 10.0 ml with the test substance.

Reference solution (c) Dilute 150 ml of *acetal* to 50.0 ml with the test substance. Dilute 100 ml of the solution to 10.0 ml with the test substance.

Reference solution (d) Dilute 100 ml of *benzene* to 100.0 ml with the test substance. Dilute 100 ml of the solution to 50.0 ml with the test substance.

Test solution (a) The test substance.

Test solution (b) Add 150 ml of *4-methyl-2-pentanol* to 500.0 ml of the test substance.

Chromatographic system A gas chromatograph equipped with (a) a glass (fused silica) column (30 m × 0.32 mm) packed with porous poly[(cyanopropyl)(phenyl)][dimethyl]-siloxane (1.8 mm), maintained as the following table, (b) a flame ionization detector maintained at 280°, and (c) *helium* as the carries gas.

	Time minute (s)	Temperature (°)
Column	0-12	40
	12-32	40 → 240
	32-42	240
Injection port		200

System suitability Chromatograph *Reference solution (b)* and record the peak response as directed for *Procedure*: the resolution between the first peak (acetaldehyde) and the second peak (methanol) is not less than 1.5.

Procedure Inject separately suitable volumes of each of *Reference solution (a)*, *Reference solution (b)*, *Reference solution (c)*, *Reference solution (d)*, *Test solution (a)*, and *Test solution (b).*

Limits:

- methanol in the chromatogram obtained from test solution (a): not more than half the area of the corresponding peak in the chromatogram obtained from reference solution (a) (200 ppm v/v),

- acetaldehyde + acetal: maximum 10 ppm v/v, expressed as acetaldehyde.

Calculation Calculate the sum of the contents of acetaldehyde and acetal in parts per million (v/v) using the following expression:

$$\frac{10 \times A_E}{A_T - A_E} + \frac{30 \times C_E}{C_T - C_E}$$

where A_E = area of the acetaldehyde peak in the chromatogram obtained from test solution (a),

A_T = area of the acetaldehyde peak in the chromatogram obtained from reference solution (b),

C_E = area of the acetal peak in the chromatogram obtained from test solution (a), and

C_T = area of the acetal peak in the chromatogram obtained from reference solution (c).

- benzene: maximum 2 ppm v/v.

Calculation Calculate the content of benzene in parts per million (v/v) using the following expression:

$$\frac{2B_E}{B_T - B_E}$$

where B_E = area of the benzene peak in the chromatogram obtained from the test solution (a), and

B_T = area of the benzene peak in the chromatogram obtained from reference solution (d).

If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).

- total of other impurities in the chromatogram obtained from test solution (b): not more than the area of the peak due to 4-methyl-2-pentanol in the chromatogram obtained from test solution (b) (300 ppm v/v),

- disregard limit: 0.03 times the area of the peak corresponding to 4-methyl-2-pentanol in the chromatogram obtained from test solution (b) (9 ppm v/v).

Ethanol, Absolute C_2H_6O = 46.07

Use analytical reagent grade of commerce.

DESCRIPTION Colourless, clear, mobile and volatile liquid; odour, characteristic and spirituous. Flammable, burning with a blue, smokeless flame. Hygroscopic.

SOLUBILITY Miscible with *water*, with *chloroform* and with *ether*.

BOILING RANGE 78° to 79° (Appendix 4.5).

RELATIVE DENSITY 0.791 to 0.794 (Appendix 4.9).

Store protected from light at a temperature not exceeding 30°.

Ethanols, Diluted Prepare by diluting the volumes of *ethanol* indicated in the following table with *water* to 1000 ml.

Strength (Per Cent V/V)	Volume of *Ethanol* (ml)	Weight per ml (approx.) g
90	947	0.83
85	894	0.85
80	842	0.86
70	737	0.89
60	632	0.91
50	526	0.93
45	474	0.94
25	263	0.97
20	210	0.975

Ether $C_4H_{10}O = 74.12$

Caution Ether tends to form explosive peroxides, especially when anhydrous.

Use analytical reagent grade of commerce.

DESCRIPTION Clear, colourless, volatile, very mobile liquid; odour, characteristic. Highly flammable; mixtures of its vapour with oxygen, air, or nitrous oxide in certain concentrations are explosive.

SOLUBILITY Soluble in 10 parts of *water*, miscible with *benzene*, with *chloroform*, with *dichloromethane*, with *ethanol*, with fixed oils, with *petroleum ether*, and with volatile oils.

PEROXIDES Transfer 8 ml of *potassium iodide and starch TS* to a stoppered tube of about 12-ml capacity and about 1.5 cm in diameter. Fill completely with the test substance, shake vigorously, and allow to stand in the dark for 30 minutes. No colour is produced.

Store protected from light at a temperature not exceeding 15°. The name and concentration of any added stabilizer are stated on the table.

Ethyl Acetate $C_4H_8O_2 = 88.11$

Use analytical reagent grade of commerce.

DESCRIPTION Colourless liquid; odour, fruity-like.

BOILING RANGE 76° to 78°(Appendix 4.5).

WEIGHT PER MILLILITRE 0.901 to 0.904 g (Appendix 4.9).

Ethyl Formate $C_3H_6O_2 = 74.08$

DESCRIPTION Mobile liquid.

SOLUBILITY Soluble in 10 parts of *water* with gradual decomposition into free acid and *ethanol*; miscible with *ethanol* and with *ether*.

Store in tightly closed containers and preferably in contact with calcium chloride.

Eugenol $C_{10}H_{12}O_2 = 164.20$

DESCRIPTION Colourless or pale yellow, oily liquid, darkening on exposure to air and light and becoming more viscous; odour, clove-like.

SOLUBILITY Practically insoluble in *water*; miscible with *chloroform*, with *ethanol*, with *ether*, and with fixed and volatile oils.

BOILING TEMPERATURE About 250° (Appendix 4.6).

WEIGHT PER MILLILITRE About 1.068 g (Appendix 4.9).

Fenchone $C_{10}H_{16}O = 152.23$

Use analytical reagent grade of commerce containing not less than 95.0 per cent of $C_{10}H_{16}O$.

Ferulic acid $C_{10}H_{10}O_4 = 194.18$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{10}H_{10}O_4$.

Formaldehyde Solution (Formalin) $CH_2O = 30.03$

Use analytical reagent grade of commerce containing not less than 34.0 per cent w/v and not more than 37.0 per cent w/v of CH_2O.

DESCRIPTION Colourless, aqueous solution with a lachrymatory vapour.

WEIGHT PER MILLILITRE About 1.08 g (Appendix 4.9).

Store at a temperature between 15° and 25°.

Formic Acid $CH_2O_2 = 46.03$

Use analytical reagent grade of commerce containing about 90 per cent w/w of CH_2O_2 and about 23.6 M in strength.

DESCRIPTION Colourless, corrosive liquid; odour, pungent.

WEIGHT PER MILLILITRE About 1.20 g (Appendix 4.9).

Formic Acid, Anhydrous $CH_2O_2 = 46.03$

Caution Show decomposition of this reagent may produce pressure in the bottle. Loosen cap occasionally to vent the gas.

Use analytical reagent grade of commerce containing not less than 98.0 per cent w/w of CH_2O_2.

DESCRIPTION Colourless, corrosive liquid; odour, pungent.

RELATIVE DENSITY About 1.22 (Appendix 4.9).

D-Galactose $C_6H_{12}O_6 = 180.16$

Use analytical reagent grade of commerce.

DESCRIPTION White crystalline or finely granulated powder.

SOLUBILITY Soluble in *water*; very slightly soluble in *ethanol*.

MELTING TEMPERATURE About 164°, with decomposition (Appendix 4.3).

SPECIFIC ROTATION About +80° at 20°, determined in a 10 per cent w/v solution containing about 0.05 per cent v/v of *ammonia* (Appendix 4.8).

Gallic Acid (3,4,5-Trihydroxybenzoic Acid) $C_7H_6O_5{\cdot}H_2O = 188.14$

DESCRIPTION White or almost white crystals or powder.

SOLUBILITY Sparingly soluble in cold *water*; very soluble in boiling *water* and in *ethanol*.

DISTINCTION FROM TANNIC ACID Its cold, saturated solution neither colours nor precipitates solutions of pure iron(II) salts and yields no precipitate with *gelatin TS*.

Genistein $C_{15}H_{10}O_5 = 270.24$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{15}H_{10}O_5$.

Helium He = 4.00

Use a suitable laboratory cylinder grade of commerce containing not less than 99.995 per cent v/v of He.

Helium for Chromatography

Use *Helium*.

Hexane $C_6H_{14} = 86.18$

The hexane fraction from petroleum.

DESCRIPTION Colourless, mobile, highly flammable liquid.

BOILING RANGE Not less than 95 per cent distils between 67° and 70° (Appendix 4.5).

WEIGHT PER MILLILITRE 0.670 to 0.677 g (Appendix 4.9).

NON-VOLATILE MATTER When evaporated on a water-bath and dried at 105° to constant weight, leaves not more than 0.01 per cent w/v of residue.

***n*-Hexane** $C_6H_{14} = 86.18$

Use analytical reagent grade of commerce usually containing not less than 99 per cent of the pure isomer, *n*-C_6H_{14}.

DESCRIPTION Colourless, flammable liquid.

BOILING RANGE Distils completely over a range of 1° between 67.5° and 69.5° (Appendix 4.5).

REFRACTIVE INDEX 1.374 to 1.375 (Appendix 4.7).

WEIGHT PER MILLILITRE 0.658 to 0.659 g (Appendix 4.9).

Hide Powder

Use a suitable grade.

Hydrochloric Acid HCl = 36.46

Use analytical reagent grade of commerce.

When no molarity is indicated, use analytical reagent grade of commerce with a relative density of about 1.18, containing not less than 35 per cent w/w and not more than 38 per cent w/w of HCl and about 11.5 M in strength.

DESCRIPTION Clear, colourless, fuming liquid; odour, pungent.

SOLUBILITY Miscible with *water*.

Solutions of molarity *x*M should be prepared by diluting 85*x* ml of *hydrochloric* acid to 1000 ml with *water*.

Store in a container of polyethylene or other non-reacting material at a temperature not exceeding 30°.

Hydrochloric Acid, Dilute A 10 per cent w/v solution. Prepare by mixing 226 ml of *hydrochloric acid* with sufficient *water* to produce 1000 ml.

Hydrogen Peroxide Solution, Strong $H_2O_2 = 34.01$

> ***Caution*** Strong Hydrogen Peroxide Solution decomposes vigorously in contact with oxidizable organic matter and with certain metals and if allowed to become alkaline.

Contains not less than 29.0 per cent w/w and not more than 31.0 per cent w/w H_2O_2, corresponding to about 100 times its volume of available oxygen. It may be stabilized by adding a suitable preservative.

DESCRIPTION Colourless and almost odourless liquid.

IDENTIFICATION

A. Cautiously make the solution alkaline. It decomposes with vigorous effervescence.

B. Mix 1 drop with 2 ml of *dilute sulfuric acid*, 2 ml of *ether* and 1 drop of *potassium chromate TS* and shake. The ethereal layer is coloured deep blue.

ACIDITY Dilute 10.0 ml with 100 ml of *water* and add 5 drops of *methyl red TS*. Not less than 0.05 ml and not more than 0.50 ml of 0.10 M *sodium hydroxide* is required to change the colour of the solution.

ORGANIC PRESERVATIVE Shake 20.0 ml with 10 ml of *chloroform* and then with two successive 5-ml portions of *chloroform*. Evaporate the combined chloroform solutions under reduced pressure at a temperature not exceeding 25°, and dry in a desiccator. Any residue weighs not more than 10 mg.

BARIUM Dilute 1 ml to 10 ml with *water* and add 1 ml of *dilute sulfuric acid*. The solution remains clear for not less than 15 minutes.

NON-VOLATILE MATTER When evaporated on a water-bath and dried at 105° to constant weight, leaves not more than 0.2 per cent w/v of residue.

ASSAY Dilute about 1 g, accurately weighed, to 100.0 ml with *water*. Add to 10.0 ml of this solution a cold mixture of 2.5 ml of *sulfuric acid* and 20 ml of *water*. Titrate with 0.02 M *potassium permanganate VS*. Each ml of 0.02 M *potassium permanganate VS* is equivalent to 1.701 mg of H_2O_2.

Store in a cool place, protected from light.

Imperatorin $C_{16}H_{14}O_4 = 270.28$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{16}H_{14}O_4$.

Iron(III) Chloride (Ferric Chloride) $FeCl_3·6H_2O = 270.30$

Use analytical reagent grade of commerce.

DESCRIPTION Yellowish orange or brownish, crystalline masses; deliquescent.

Store in well-closed containers.

Iron(II) Sulfate (Ferrous Sulfate) $FeCl_4{\cdot}7H_2O$ = 278.01

Use general reagent grade of commerce.

DESCRIPTION Bluish green crystals or pale, crystalline powder; odourless. Efforescent in air. Oxidizes in moist air, becoming brown.

Store in well-closed containers

Iron(III) Sulfate $Fe_2(SO_4)_3{\cdot}xH_2O$

Use general reagent grade of commerce.

DESCRIPTION White to yellow, hygroscopic powder which decomposes in air.

Store protected from light.

Lead(II) Acetate $C_4H_6O_4Pb{\cdot}3H_2O$ = 379.34

Use analytical reagent grade of commerce.

DESCRIPTION Small, white, transparent, monoclinic prisms or heavy, crystalline masses; odour, acetous. Efflorescent in warm air. Becomes basic when heated.

SOLUBILITY Soluble in 2 parts of *water* and in 63 parts of *ethanol*; freely soluble in *glycerol*.

Lead(II) Oxide (Lead Monoxide) PbO = 223.20

Use a suitable grade.

Lupeol $C_{30}H_{50}O$ = 426.72

Use general reagent grade of commerce.

Madecassoside $C_{48}H_{78}O_{20}$ = 975.12

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{48}H_{78}O_{20}$.

Store in tightly closed containers, in a cool place.

Magnesium Mg = 24.305

DESCRIPTION Silvery white ribbon. It slowly oxidizes in moist air.

Magnesium Acetate $C_4H_6MgO_4{\cdot}4H_2O$ = 214.46

Contains not less than 99.0 per cent of $C_4H_6MgO_4{\cdot}4H_2O$.

DESCRIPTION Colourless crystals.

pH 8.2 to 8.8, in a 5.0 per cent w/v solution (Appendix 4.11).

ASSAY Dissolve about 800 mg, accurately weighed, in 100 ml of *water*, add 10 ml of *strong ammonia-ammonium chloride TS* and 0.5 ml of *mordant black 11 TS*, and titrate at 40° with 0.1 M *disodium edetate VS* until the last trace of red colour disappears and the solution becomes pure blue. Each ml of 0.1 M *disodium edetate VS* is equivalent to 21.45 mg of $C_4H_6MgO_4{\cdot}4H_2O$.

Magnesium Ribbon

Use a suitable grade.

Methanol (Methyl Alcohol) CH_4O = 32.04

Use analytical reagent grade of commerce.

DESCRIPTION Colourless liquid.

SOLUBILITY Miscible with *water*, forming a clear colourless liquid.

BOILING RANGE 64° and 65° (Appendix 4.5).

RELATIVE DENSITY 0.791 to 0.793° (Appendix 4.9).

L-Methionine $C_5H_{11}NO_2S = 149.21$

DESCRIPTION White, crystalline solid.

SPECIFIC ROTATION About +23° at 20°, determined in a 5 per cent w/v solution in 1 M *hydrochloric acid* (Appendix 4.5).

Methyl *Tert*-Butyl Ether (2-Methoxy-2-methylpropane, MTBE) $C_5H_{12}O = 88.15$

Use general reagent grade of commerce.

Store in tightly closed containers, in a cool place, protected from light.

Nitric Acid $HNO_3 = 63.01$

When no molarity is indicated, use analytical reagent grade of commerce containing about 70.0 per cent w/w of HNO_3 and about 16 M in strength.

DESCRIPTION Corrosive, fuming liquid.

WEIGHT PER MILLILITRE About 1.42 g (Appendix 4.9).

When solutions of molarity *x*M are required, they should be prepared by diluting 63*x* of *nitric acid* with *water* to 1000 ml.

Store protected from light.

Nitric Acid, Fuming $HNO_3 = 63.01$

Contains not less than 95.0 per cent w/w of HNO_3.

DESCRIPTION Clear, almost colourless to yellow, fuming liquid.

WEIGHT PER MILLILITRE About 1.5 g (Appendix 4.9).

CHLORIDE Not more than 50 ppm (Appendix 5.2). A 5.0-ml portion shows no more chloride than that corresponds to 0.50 ml of 0.020 M *hydrochloric acid*.

IRON Not more than 1 ppm. Evaporate 7.0 ml on a water-bath to dryness, add 2 ml of *hydrochloric acid* and dilute to 50 ml with *water*. The solution complies with the “Limit Test for Iron” (Appendix 5.2).

SULFATE Not more than 0.16 per cent w/w (Appendix 5.2). Dilute 0.20 ml with 5 ml of *water*, add 10 mg of *sodium hydrogencarbonate*, and evaporate to dryness on a water-bath. The residue shows no more sulfate than that corresponds to 0.50 ml of 0.010 M *sulfuric acid*.

SULFATED ASH Not more than 0.01 per cent w/w (Appendix 5.3).

ASSAY Weigh accurately about 2 g into a stoppered flask containing 40 ml of *water*, and titrate with 1 M *sodium hydroxide VS*, using *methyl orange TS* as indicator. Each ml of 1 M *sodium hydroxide VS* is equivalent to 63.01 mg of HNO_3.

Nitrogen $N_2 = 28.01$

Use laboratory cylinder grade of commetce, washed with *water* and dried.

Nitrogen for Chromatography Nitrogen containing not less than 99.95 per cent v/v of N_2.

Nitrogen, Oxygen-free *Nitrogen* which has been freed from oxygen by passing through *alkaline pyrogallol TS*.

Olive Oil

Use general reagent grade of commerce.

FREEZING TEMPERATURE 110° (Appendix 4.4).

REFRACTIVE INDEX 1.4680 (Appendix 4.7).

WEIGHT PER MILLILITRE About 0.910 g (Appendix 4.9).

Oracet Blue 2R [1-Amino-4-(phenylamino) anthracene-9,10-dione; CI 61110] $C_{20}H_{14}N_2O_2$ = 314.34

Use a suitable grade.

Palmatine Iodide $C_{21}H_{22}INO_4$ = 479.31

DESCRIPTION Yellow needles.

SOLUBILITY Sparingly soluble in hot *water* and *ethanol*.

Petroleum Ether (Light Petroleum)

> ***Caution*** Petroleum Ether is dangerously flammable. Keep away from flames and store in tightly closed containers, in a cool place.

DESCRIPTION Colourless, very volatile, highly flammable liquid, obtained from petroleum, consisting of a mixture of the lower members of the paraffin series of hydrocarbons supplied in the following fractions:

boiling range, 30° to 40°; weight per ml, about 0.63 g
boiling range, 40° to 60°; weight per ml, about 0.64 g
boiling range, 50° to 70°; weight per ml, about 0.66 g
boiling range, 60° to 80°; weight per ml, about 0.67 g
boiling range, 80° to 100°; weight per ml, about 0.70 g
boiling range, 100° to 120°; weight per ml, about 0.72 g
boiling range, 120° to 160°; weight per ml, about 0.75 g.

Phosphomolybdic Acid $H_3PO_4 \cdot 12MoO_3 \cdot 24H_2O$ = 2257.62

Use analytical reagent grade of commerce.

DESCRIPTION Fine, orange-yellow crystals.

SOLUBILITY Very soluble in *water*.

Phosphorus Pentoxide Desiccant (Diphosphorus Pentoxide) P_2O_5 = 141.94

Use a grade specially supplied for use in desiccators.

DESCRIPTION White, amorphous, deliquescent powder hydrated by water with the evolution of heat.

Store in well-closed containers .

Piperine $C_{17}H_{19}NO_3 = 285.34$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{17}H_{19}NO_3$.

Polyethylene Glycol 4000 (Macrogol 4000)

DESCRIPTION White, free-flowing powder or creamy-white flakes.

SOLUBILITY Very soluble in *water*; freely soluble in *methanol* and in *pyridine*; practically insoluble in *ethanol* and in *anhydrous ether*.

SPECIFIC GRAVITY 1.212 (Appendix 4.9).

MELTING RANGE 54° to 58° (Appendix 4.3).

VISCOSITY At 100°, 76 to 110 $mm^2.s^{-1}$ (Appendix 4.10).

Potassium Hydroxide KOH = 56.11

Use analytical reagent grade of commerce.

DESCRIPTION White or practically white, fused masses, or small pellets, or flakes, or sticks, or other forms.

SOLUBILITY Freely soluble in *water*, in *ethanol* and in *glycerol*; very soluble in boiling *ethanol*.

Store in tightly closed containers.

Potassium Iodide KI = 166.0

Use analytical reagent grade of commerce.

DESCRIPTION White crystalline powder.

Potassium Permanganate $KMnO_4 = 158.03$

Use analytical grade of commerce.

1-Propanol (*n*-Propyl Alcohol) $C_3H_8O = 60.10$

DESCRIPTION Clear, colourless liquid,.

SOLUBILITY Miscible with *water* and with *ethanol*.

BOILING RANGE Not less than 95 per cent distils between 96° and 99° (Appendix 4.5).

2-Propanol (Isopropanol) $C_3H_8O = 60.10$

Use analytical reagent grade of commerce.

DESCRIPTION Colourless liquid; odour, characteristic.

BOILING RANGE 81° to 83° (Appendix 4.5).

WEIGHT PER MILLILITRE About 0.785 g (Appendix 4.9).

Pyridine $C_5H_5N = 79.10$

Use analytical reagent grade of commerce.

DESCRIPTION Clear, colourless liquid; odour, characteristic and unpleasant. Hygroscopic.

BOILING TEMPERATURE About 115° (Appendix 4.6).

Store in well-closed containers.

Resorcinol $C_6H_6O_2 = 110.10$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_6H_6O_2$.

Rhein $C_{15}H_8O_6 = 284.22$

DESCRIPTION Yellow needles.

SOLUBILITY Practically insoluble in *water*; soluble in alkalis and in *pyridine*; slightly soluble in *benzene*, in *chloroform*, in *ethanol*, in *ether*, and in *petroleum ether*.

MELTING RANGE 321° to 322°, decomposed at 330° (Appendix 4.3).

Rosmarinic Acid $C_{18}H_{16}O_8 = 360.32$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{18}H_{16}O_8$.

Ruthenium Red (Ammoniated Ruthenium Oxychloride) $C_{16}H_{42}N_{14}O_2Ru_3 = 786.35$

DESCRIPTION Dark brown powder.

SOLUBILITY Completely soluble in *water* yielding a bright crimson solution; soluble in *lead acetate TS*.

Rutin $C_{27}H_{30}O_{16} = 610.52$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{27}H_{30}O_{16}$.

Sinapic Acid (Sinapinic Acid) $C_{11}H_{12}O_5 = 224.21$

Use general reagent grade of commerce.

MELTING TEMPERATURE About 202° (Appendix 4.3).

Sinensetin $C_{20}H_{20}O_7 = 372.37$

Use general reagent grade of commerce.

Sodium Acetate $C_2H_3NaO_2 \cdot 3H_2O = 136.08$

Use analytical reagent grade of commerce.

Sodium Dodecyl Sulfate (Sodium Lauryl Sulfate) $C_{12}H_{25}NaO_4S = 288.38$

Use purified grade of commerce containing not less than 99.0 per cent w/w of $C_{12}H_{25}NaO_4S$.

DESCRIPTION White, crystalline flakes.

Sodium Hydrogencarbonate (Sodium Bicarbonate) $NaHCO_3 = 84.01$

Use analytical reagent grade of commerce.

Sodium Hydroxide $NaOH = 40.00$

Use analytical reagent grade of commerce.

DESCRIPTION White, or practically white, fused masses, in small pellets, in flakes, or sticks, and in other forms.

SOLUBILITY Freely soluble in *water* and in *ethanol*.

Store in tightly closed containers.

Sodium Sulfate (Glauber's Salt) $Na_2SO_4 \cdot 10H_2O = 322.19$

DESCRIPTION Colourless crystals or white granules; odourless. Efflorescent. Melts at 32.5°.

SOLUBILITY Soluble in 1.5 parts of *water*; soluble in *glycerol*; insoluble in *ethanol*.

INSOLUBLE MATTER Not more than 0.01 per cent w/w (Appendix 4.13); use 10.0 g.

pH 5.2 to 8.2, in a 5.0 per cent w/v solution in *ammonia*- and *carbon dioxide-free water* (Appendix 4.11).

CHLORIDE Not more than 20 ppm (Appendix 5.2). Dissolve 2.1 g in 50.0 ml of *water*, and filter if necessary. A 25.0-ml portion of the resulting solution shows no more chloride than that corresponds to 0.03 ml of 0.020 M *hydrochloric acid*.

ARSENIC Not more than 1 ppm (Appendix 5.2); use a mixture of 3.0 g and 35 ml of *water* as the Test Preparation.

CALCIUM, MAGNESIUM, AND TRIVALENT OXIDE PRECIPITATE Not more than 0.02 per cent w/w. Dissolve about 5 g, accurately weighed, in 75 ml of *water*, filter, and add 7 ml of *ammonium oxalate TS*, 2 ml of *ammonium phosphate TS,* and 10 ml of *strong ammonia solution.* Stir well, and allow to stand overnight. If any precipitate forms, filter, wash with a 25 per cent v/v solution of *dilute ammonia solution*, ignite at 800°±25° to constant weight.

HEAVY METALS Not more than 5 ppm (Appendix 5.2); use 2.0 g. For the Standard Preparation, use *lead standard solution (1 ppm Pb).*

IRON Not more than 10 ppm. Dissolve 1.0 g in 47 ml of *water*; add 2 ml of *hydrochloric acid.* The solution complies with the "Limit Test for Iron" (Appendix 5.2).

NITROGEN COMPOUNDS Not more than 5 ppm. Dissolve 2.0 g in 60 ml of *ammonia-free water* in a flask connected through a spray trap to a condenser, the end of which dips beneath the surface of 10 ml of 0.1 M *hydrochloric acid*. Add to the contents of the flask 10 ml of freshly boiled *sodium hydroxide TS* and 500 mg of *aluminium wire* in small pieces, and allow to stand for 1 hour. Distil about 35 ml and dilute the distillate with *water* to 50 ml. Add 2 ml of *sodium hydroxide TS*, mix, and add 2 ml of *alkaline mercuric-potassium iodide TS*; the colour produced is not darker than that produced by 0.1 ml of *nitrogen standard solution (100 ppm N)* when similarly treated.

Sodium Sulfate, Anhydrous $Na_2SO_4 = 142.04$

DESCRIPTION White, crystalline powder or granules. Hygroscopic.

SOLUBILITY Soluble in 6 parts of *water*, insoluble in *ethanol*.

ACIDITY OR ALKALINITY Dissolve 4 g in 100 ml of *carbon dioxide-free water*. The solution requires for neutralization to the green colour of *bromothymol blue TS* indicative of pH 7 not more than 0.50 ml of either 0.10 M *sodium hydroxide* or 0.10 M *hydrochloric acid*.

LOSS ON IGNITION Not more than 0.5 per cent w/w (Appendix 4.16). Weigh accurately about 2 g, and ignite at a low red heat in a tared dish.

CHLORIDE Not more than 0.07 per cent w/w (Appendix 5.2). A 500-mg sample shows no more chloride than that corresponds to 0.50 ml of 0.020 M *hydrochloric acid*.

IRON Not more than 20 ppm. Dissolve 500 mg in 40 ml of *water* and 2 ml of *hydrochloric acid*. The solution complies with the "Limit Test for Iron" (Appendix 5.2).

Sulfuric Acid $H_2SO_4 = 98.07$

When no molarity is indicated, use analytical reagent grade of commerce containing about 96 per cent w/w of *sulfuric acid* and about 18 M in strength.

DESCRIPTION Colourless, oily, corrosive liquid.

WEIGHT PER MILLILITRE About 1.84 g (Appendix 4.9).

When solutions of molarity xM are required, they should be prepared by carefully adding 54x ml of *sulfuric acid* to an equal volume of *water* and diluting to 1000 ml with *water*.

When "*sulfuric acid*" is followed by a percentage figure, an instruction to add, carefully, *sulfuric acid* to *water* to produce the specified percentage v/v (or, if required, w/w) proportion of sulfuric acid is implied.

Sulfuric Acid, Dilute Add 5.5 ml of *sulfuric acid* to 60 ml of *water*, allow to cool and add sufficient *water* to produce 100 ml. It contains 9.8 per cent w/v of H_2SO_4 and about 1 M in strength.

Sulfuric Acid, Ethanolic Solutions of the requisite molarity may be obtained by mixing *sulfuric acid* with *ethanol* as directed under *Sulfuric Acid*.

When "*ethanolic sulfuric acid*" is followed by a percentage figure, an instruction to use *sulfuric acid* diluted with *ethanol* to produce the specified percentage v/v proportion of *sulfuric acid* is implied. Prepare by cooling separately the required amount to about –5°, carefully adding the acid to the ethanol. Keep the solution as cool as possible and mix gently.

Talc (Purified Talc)

A native, hydrous magnesium silicate, sometimes containing a small proportion of aluminium silicate.

LOSS ON IGNITION Not more than 5.0 per cent w/w after ignition at a red heat to constant weight (Appendix 4.16).

ACID-SOLUBLE SUBSTANCES Not more than 2.0 per cent w/w. Digest 1 g with 20 ml of *dilute hydrochloric acid* at 50° for 15 minutes, add *water* to restore the original volume, mix, and filter. To 10 ml of the filtrate add 1 ml of *dilute sulfuric acid*, evaporate to dryness, and ignite to constant weight; the weight of the residue does not exceed 10.0 mg.

***n*-Tetradecane** $C_{14}H_{30} = 198.39$

DESCRIPTION Clear, colourless liquid.

SOLUBILITY Miscible with *ethanol*.

REFRACTIVE INDEX 1.428 to 1.429 (Appendix 4.7).

WEIGHT PER MILLILITRE About 0.76 g (Appendix 4.9).

Thymol $C_{10}H_{14}O = 150.22$

DESCRIPTION Colourless, often large crystals, or a white, crystalline powder.

SOLUBILITY Soluble in about 1000 parts of *water*, in 1 part of *ethanol*, in 1 part of *chloroform*, in 1.5 parts of *ether*, and in about 2 parts of *olive oil.*

MELTING RANGE 48° to 51°, when the melted substance is cooled, it remains liquid at a considerably lower temperature (Appendix 4.3).

NON-VOLATILE MATTER Volatilize 2 g on a water-bath and dry at 105° to constant weight. The residue weighs not more than 1 mg.

Store in tightly closed containers, protected from light.

Thymoquinone $C_{10}H_{12}O_2 = 164.20$

Use analytical reagent grade of commerce containing not less than 95 per cent of $C_{10}H_{12}O_2$.

Toluene (Methylbenzene) $C_7H_8 = 92.14$

Use analytical reagent grade of commerce.

DESCRIPTION Clear, colourless liquid; odour, characteristic. Flammable.

SOLUBILITY Miscible with *water* and with *ethanol*.

BOILING TEMPERATURE About 110° (Appendix 4.6).

WEIGHT PER MILLILITRE 0.865 to 0.870 g (Appendix 4.9).

Trifluoroacetic Acid $C_2HF_3O_2 = 114.02$

Use general reagent grade of commerce.

DESCRIPTION Colourless liquid.

SOLUBILITY Miscible with *acetone*, with *benzene*, with *carbon tetrachloride*, with *ethanol*, with *ether*, and with *hexane*.

BOILING TEMPERATURE About 72° (Appendix 4.6).

RELATIVE DENSITY 1.53 (Appendix 4.9).

Store in tightly closed containers.

Vanillin $C_8H_8O_3 = 152.15$

DESCRIPTION White or cream-coloured crystalline needles or powder; odour, characteristic of vanilla.

SOLUBILITY Soluble in 100 parts of *water*; more soluble in boiling *water*; freely soluble in *ethanol* and in fixed and *volatile oils*; soluble in 20 parts of *glycerol* and in solutions of the *alkali hydroxides*.

MELTING RANGE 81° to 83° (Appendix 4.3).

SULFATED ASH Not more than 0.1 per cent w/w (Appendix 5.3).

Water $H_2O = 18.02$

Use Purified Water of the Official Pharmacopoeia.

Water, Distilled Use Purified Water that has been prepared by distillation.

Xylene $C_8H_{10} = 106.17$

DESCRIPTION Clear, colourless liquid, consisting mainly of m-*xylene* with smaller proportions of o- and p-*xylenes*. Flammable.

SOLUBILITY Insoluble in *water*; miscible with *absolute ethanol*.

BOILING RANGE Not less than 90 per cent distils between 136° and 140° (Appendix 4.5).

WEIGHT PER MILLILITRE 0.85 to 0.86 g (Appendix 3.4).

SULFUR COMPOUNDS Boil 10 ml with 1 ml of *absolute ethanol* and 3 ml of *potassium plumbite TS* for 15 minutes under a reflux condenser, and allow to s tand for 5 minutes; the aqueous layer remains colourless.

REACTION WITH SULFURIC ACID Shake 5 ml with 5 ml of *sulfuric acid*; the xylene remains colourless and the acid may become yellow but not brown.

NON-VOLATILE MATTER When evaporated on a water-bath and dried at 105° to constant weight, leaves not more than 0.01 per cent w/v of residue.

Xylose $C_5H_{10}O_5 = 150.13$

For microbiological purposes, use a suitable grade.

Zinc Powder $Zn = 65.38$

Use analytical reagent grade of commerce containing not less than 90.0 per cent of Zn.

1.2 VOLUMETRIC SOLUTIONS

In this Pharmacopoeia, all concentrations of volumetric solutions are expressed in terms of molarity. The molarity of a solution is stated as the number of moles of substance contained in 1000 ml of the solution. A solution which contains x moles of substance per 1000 ml is designated as *x* molar (*x* M).

Volumetric solutions do not differ from the prescribed strength by more than 10 per cent. The molarity of the volumetric solutions is determined by an appropriate number of titrations. The repeatability does not exceed 0.2 per cent (relative standard deviation).

Throughout the Pharmacopoeia, molar solutions to be standardized before use in assays and other quantitative tests are designated by appending the letters *VS* to the name of the reagent.

Preparation and Standardization

For each solution the preparation and standardization of the most commonly used strength is described. Stronger or weaker solutions than those described are prepared and standardized using proportionate amounts of the reagents or by making an exact dilution of a stronger solution with *carbon dioxide-free water.* Solutions of molarity below 0.01 M are freshly prepared using *carbon dioxide-free water*.

The water used in preparing volumetric solutions complies with the requirements of the monograph on Purified Water. When used for the preparation of unstable solutions such as potassium permanganate and sodium thiosulfate, it should be freshly boiled and cooled. When a solution is to be used in an assay in which the end-point is determined by an electrochemical process, the solution must be standardized in the same way. All volumetric solutions, if practicable, are to be prepared, standardized and used at the same temperature.

Blank Determinations

Where it is directed that “any necessary correction” be made by a blank determination, the determination is to be conducted with the use of the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted. Appropriate blank corrections are to be made for all Pharmacopoeial titrimetric assays.

All Pharmacopoeial assays that are volumetric in nature indicate the weight of the substance being assayed to which each ml of the primary volumetric solution is equivalent. In general, these equivalents may be derived by simple calculation from the respective molecular formulae and weights.

Hydrochloric Acid, Molar (1 M)

$HCl = 36.46$

Dilute 85 ml of *hydrochloric acid* with *water* to produce 1000 ml.

Standardization: Weigh accurately about 1.5 g of *anhydrous sodium carbonate* that previously has been heated at about 270° for 1 hour. Dissolve it in 100 ml of *water*, and add 0.1 ml of *methyl red TS*. Titrate with the *hydrochloric acid*, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration. Heat again to boiling, and titrate further as necessary until the faint pink colour is no longer affected by continued boiling. Each ml of 1 M *hydrochloric acid* is equivalent to 52.99 mg of Na_2CO_3.

Sodium Hydroxide, Molar (1 M)

$NaOH = 40.00$

Dissolve 162 g of *sodium hydroxide* in 150 ml of *carbon dioxide-free water*, cool the solution to room temperature and filter through hardened filter paper. Dilute 54.5 ml of the clear filtrate with *carbon dioxide-free water* to 1000 ml.

Standardization: Weigh accurately about 5 g of *potassium hydrogenphthalate,* previously crushed lightly and dried at 120° for 2 hours, and dissolve in 75 ml of *carbon dioxide-free water*. Add 0.1 ml of *phenolphthalein TS,* and titrate with the sodium hydroxide solution to the production of a permanent pink colour. Each ml of 1 M *sodium hydroxide* is equivalent to 204.2 mg of $C_8H_5KO_4$.

Note 1. Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. They should therefore be stored in bottles with suitable non-glass, well-fitting stoppers, provided with a tube filled with soda lime.

2. Prepare solutions of lower concentration (e.g., 0.1 M, 0.01 M) by quantitatively diluting accurately measured volumes of the 1 M solution with sufficient *carbon dioxide-free water* to yield the desired concentration.

Restandardize the solution frequently.

Sulfuric Acid, Half-Molar (0.5 M)

$H_2SO_4 = 98.07$

Carefully add 28 ml of *sulfuric acid* to *water*, and dilute to 1000 ml with the same solvent.

Standardization: Standardize the solution as described under *Hydrochloric Acid, Molar (1 M).*

1.4 pH INDICATORS

Methyl Red $C_{15}H_{16}ClN_3O_2 = 305.76$

Dark red powder or violet crystals. Sparingly soluble in *water*; soluble in *ethanol*. Transition interval: from pH 4.2 to 6.3. Colour change: from red to yellow.

Methyl Red TS Dissolve 10 mg of *methyl red* in 100 ml of *ethanol*, and filter if necessary.

Phenolphthalein $C_{20}H_{14}O_4 = 318.33$

White or faintly yellowish white, crystalline powder. Insoluble in *water*; soluble in *ethanol*.

Transition interval: from pH 8.3 to 10.0. Colour change: from colourless to red.

SENSITIVITY Dissolve 100 mg in 10 ml of *neutralized ethanol*, add 0.1 ml of this solution to 200 ml of *carbon dioxide-free water*, and then add 0.05 ml of 0.10 M *sodium hydroxide*: the solution is pink in colour when compared with an equal volume of *water*.

Phenolphthalein TS Dissolve 1 g of *phenolphthalein* in 100 ml of *ethanol (50 per cent)*.

1.6 TEST SOLUTIONS

Acetic Anhydride-Sulfuric Acid TS A mixture of 9 volumes of *acetic anhydride* and 1 volume of *sulfuric acid*.

Ammonia TS It contains 9.5 to 10.5 per cent w/w of NH_3. Prepare by diluting 400 ml of *strong ammonia solution* with *water* to make 1000 ml.

Store at a temperature not exceeding 20°.

Ammonium Molybdate TS A 10.0 per cent w/v solution of *ammonium molybdate.*

Anisaldehyde TS Mix, in order, 0.5 ml of *anisaldehyde*, 10 ml of *glacial acetic acid,* 85 ml of *methanol,* and 5 ml of *sulfuric acid*.

Basic Lead Acetate TS Triturate 14 g of *lead monoxide* with 10 ml of *water*, add 10 ml of *water* and transfer into a 100-ml volumetric flask. Add 22 g of *lead acetate* dissolved in 70 ml of *water*, shake vigorously for 5 minutes, set aside for one week, filter, and add sufficient cold boiled *water* to make 100 ml.

Boric Acid-Methanol TS Dissolve 3 g of *boric acid* in 100 ml of *methanol* and 0.5 ml of diluted *hydrochloric acid* (1 in 4).

Chromotropic Acid TS Dissolve 5 mg of *chromotropic acid sodium salt* in 10 ml of a mixture of 9 ml of *sulfuric acid* and 4 ml of *water*.

Dinitrophenylhydrazine TS1 Dissolve 100 mg of *2,4-dinitrophenylhydrazine* in 100 ml of *methanol,* add 1.0 ml of *hydrochloric acid* and mix.

Dragendorff TS, Modified Dissolve 1.7 g of *bismuth oxynitrate* in a mixture of 80 ml of *water* and 20 ml of *glacial acetic acid,* warming if necessary. Cool, add 100 ml of a 50 per cent w/v solution of *potassium iodide,* and mix. Refrigerate this stock solution for prolonged storage. For use, dilute 10 ml of this stock solution with *water* to 100 ml, add 10 ml of *glacial acetic acid,* and mix. Then add 120 mg of *iodine* and shake until the iodine has completely dissolved.

Store refrigerated and discard after 2 weeks.

Dragendorff TS1, Modified Dissolve 0.4 g of *bismuth oxynitrate* in 10 ml of *hydrochloric acid*. Add this solution to 50 ml of a 10 per cent w/v solution of *potassium iodide,* mix and dilute to 100 ml with *water*.

Dragendorff TS2, Modified
Solution A: Dissolve 0.85 g of *bismuth oxynitrate* in a mixture of 40 ml of *water* and 10 ml of *acetic acid*.
Solution B: Dissolve 8 g of *potassium iodide* in 20 ml of *water*.
Mix 5 ml each of solution A and solution B with 20 ml of *glacial acetic acid* and add sufficient *water* to make 100 ml.

Hydrogen Peroxide TS (100 volumes) (Strong Hydrogen Peroxide Solution) Use *Hydrogen Peroxide Solution, Strong* (see under "Reagents").

Hydrogen Peroxide TS (20 volumes) Use analytical reagent grade of commerce containing about 6 per cent w/v H_2O_2 or dilute 1 volume of *strong hydrogen peroxide solution* with 4 volumes of *water*.

Hydrogen Peroxide TS (10 volumes) (Dilute Hydrogen Peroxide Solution) Dilute *hydrogen peroxide TS (20 volumes)* with an equal volume of *water*.

Iodoplatinate TS To 3 ml of a 10 per cent w/v solution of *chloroplatinic(IV) acid* add 97 ml of *water* and 100 ml of a 6 per cent w/v solution of *potassium iodide*.

Store in amber glass containers.

Iron(III) Chloride TS Dissolve 9 g of *iron(III) chloride* in *water* to make 100 ml.

Iron(II) Sulfate TS Dissolve 0.45 g of *iron(II) sulfate* in 50 ml of 0.1 M *hydrochloric acid* and dilute to 100 ml with *carbon dioxide-free water*.

Prepare immediately before use.

Mercuric-Potassium Iodide TS (Mayer's Reagent) Dissolve 1.358 g of *mercury(II) chloride* in 60 ml of *water*. Dissolve 5 g of *potassium iodide* in 10 ml of *water*. Mix the two solutions, and dilute with *water* to 100 ml.

Natural Products (NP) TS Dissolve 1 g of *diphenylboric acid-2-aminoethyl ester* in sufficient *methanol* to make 100 ml.

Ninhydrin TS (Triketohydrindene Hydrate TS) Dissolve 200 mg of *ninhydrin* in *water* to make 10 ml.

Ninhydrin TS, Ethanolic Dissolve 1.0 g of *ninhydrin* in 50 ml of *ethanol* and add 10 ml of *glacial acetic acid*.

Phloroglucinol TS Dissolve 500 mg of *phloroglucinol* in 25 ml of *ethanol*.

Store in tight containers, protected from light.

Phosphomolybdic Acid TS To 40 ml of a 10 per cent w/v solution of *phosphomolybdic acid* add, cautiously and with cooling, 60 ml of *sulfuric acid*.

Prepare immediately before use.

Polyethylene Glycol (PEG) TS Dissolve 5 g of *polyethylene glycol 4000* in sufficient *ethanol* to make 100 ml.

Potassium Cupri-tartrate TS (Fehling's Solution)

No.1. Dissolve 34.6 g of *copper(II) sulfate* in a mixture of 0.50 ml of *sulfuric acid* and sufficient *water* to produce 500 ml.
No.2. Dissolve 176 g of *potassium sodium tartrate* and 77 g of *sodium hydroxide* in sufficient *water* to produce 500 ml.

Mix equal volumes of solutions No.1 and No.2 immediately before use.

Potassium Hexacyanoferrate(III) TS Wash about 1 g of *potassium hexacyanoferrate(III)*, in crystals, with a little *water*, and dissolve the washed crystals in 100 ml of *water*. Gives a blue colour with solutions of iron(II) salts.

Potassium Hydroxide TS, Ethanolic Dissolve 3 g of *potassium hydroxide* in 5 ml of *water* and dilute to 100 ml with *aldehyde-free ethanol*, allow to stand for 1 hour, and decant the clear solution.

Prepare freshly before use.

Potassium Iodide and Starch Solution Dissolve 750 mg of *potassium iodide* in 100 ml of *water*, heat to boiling and add, while stirring, a solution of 500 mg of *soluble starch* in 35 ml of *water*. Boil for 2 minutes and allow to cool.

SENSITIVITY TO IODIDE To 15 ml of the solution add 0.05 ml of *glacial acetic acid* and 0.30 ml of 0.00050 M *iodine*. A blue colour is produced.

Prepare freshly before use.

Potassium Iodobismuthate TS Dissolve 10 g of *tartaric acid* in 40 ml of *water* and add 850 mg of *bismuth oxynitrate.* Shake during 1 hour, add 20 ml of a 40 per cent w/v solution of *potassium iodide*, and shake well. Allow to stand for 24 hours and filter.

Store protected from light.

Potassium Iodobismuthate TS, Acetic Dissolve 8 g of *potassium iodide* in 20 ml of *water* and add the solution to a mixture of 850 mg of *bismuth oxynitrate*, 40 ml of *water* and 10 ml of *glacial acetic acid*.

Potassium Iodobismuthate TS, Dilute Dissolve 10 g of *tartaric acid* in 50 ml of *water* and add 5 ml of *potassium iodobismuthate TS.*

Potassium Iodoplatinate TS Add 50 ml of a 5 per cent w/v solution of *chloroplatinic(IV) acid* to 45 ml of *potassium iodide TS* and dilute to 100 ml with *water*.

Store in amber glass containers.

Ruthenium Red TS Dissolve 8 mg of *ruthenium red* in 10 ml of *lead acetate TS*.

Prepare freshly before use.

Sodium Hydrogencarbonate TS Dissolve 5.0 g of *sodium hydrogencarbonate* in *water* to make 100 ml.

Sodium Hydroxide TS Dissolve 4.0 g of *sodium hydroxide* in *water* to make 100 ml.

Vanillin-Hydrochloric Acid TS Dissolve 10 mg of *vanillin* in 1 ml of *ethanol*, and then add 1 ml of *water* and 6 ml of *hydrochloric acid*.

Prepare freshly before use.

Vanillin-Sulfuric Acid TS Dissolve 3 g of *vanillin* in 100 ml of *absolute ethanol*, add 0.5 ml of *sulfuric acid* and mix.

Vanillin-Sulfuric Acid TS1 Dissolve 1 g of *vanillin* in 100 ml of *ethanol*, add 2 ml of *sulfuric acid* dropwise and mix.

Vanillin-Sulfuric Acid TS2 Dissolve 1 g of *vanillin* in 100 ml of *sulfuric acid*.

1.7 MATERIALS FOR CHROMATOGRAPHY

Gas Chromatography

SUPPORTS

Diatomaceous Support (Diatomaceous Earth; Siliceous Earth) White or almost white, fine granular powder made up of siliceous frustules of fossil diatoms or debris of fossil diatoms. It may be identified by microscopic examination with a magnification of × 500.

Diatomaceous Support, Acid-washed Diatomaceous support that has been purified by treatment with *hydrochloric acid* and washed with *water* to remove metallic impurities, and to reduce surface activity and peak-tailing.

Diatomaceous Support, Alkali-washed Diatomaceous support that has been treated with potassium hydroxide solution to reduce peak-tailing of basic compounds.

Diatomaceous Support, Silanized Diatomaceous earth for gas chromatography, silanized Acid-washed diatomaceous support that has been silanized with dimethyldichlorosilane or other suitable silanizing agents.

STATIONARY PHASES

A wide range of chemical substances is used, including polyethylene glycols, high-molecular weight esters and amides, hydrocarbons, silicone gums and fluids (polysiloxanes often substituted by methyl, phenyl, nitrilo, vinyl, or fluoroalkyl groups, or mixtures of these), and micro-porous cross-linked polyaromatic beads. Care should be taken to select grades specifically intended for use in gas chromatography. In most cases reference is made to a particular commercial brand which has been found to be suitable for the determination in question, but such statements do not imply that a different but equivalent commercial brand may not be used.

INTERNAL STANDARDS

Reagents used as internal standards should not contain any impurity which would produce a peak likely to interfere in the determination described in the monograph.

Thin-layer Chromatography

The coating substances described below are used to prepare thin-layer chromatoplates in accordance with the procedure described in Appendix 3.1. Prepare suspensions of the coating substances as recommended by the manufacturer unless otherwise prescribed. Commercial pre-coated chromatoplates may be used for Pharmacopoeial tests provided they comply with the test for chromatographic separation described for the corresponding coating substance.

Silica Gel G A fine, white, homogeneous powder of an average particle size between 10 and 40 µm containing about 13 per cent w/w of *calcium sulfate hemihydrate* and complying with the following requirements.

CONTENT OF CALCIUM SULFATE To about 250 mg, accurately weighed, add 3 ml of 2 M *hydrochloric acid* and 100 ml of *water* and shake vigorously for 30 minutes. Filter, wash the residue with *water* and carry out the “Complexometric Titration of Calcium” (Appendix 6.3) on the combined filtrate and washings. Each ml of 0.1 M *disodium edetate VS* is equivalent to 14.51 mg of $CaSO_4 \cdot \frac{1}{2}H_2O$.

SEPARATING POWER Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using *toluene* as the mobile phase. Apply to the plate, 10 µl of a solution in *dichloromethane* containing 0.1 mg per ml of each of *indophenol blue, sudan red G* and *dimethyl yellow* in *toluene*. Allow the mobile phase to ascend 10 cm. The chromatogram shows three clearly separated spots of the indophenol blue, sudan red G and dimethyl yellow in order of increasing R_f value.

ALKALINITY pH of a suspension prepared by shaking 1 g with 10 ml of *carbon dioxide-free water* for 5 minutes, about 7.

Silica Gel GF254 A fine, white, homogeneous powder of an average particle size between 10 and 40 µm containing about 13 per cent w/w of *calcium sulfate hemihydrate* and about 1.5 per cent w/w of a fluorescent indicator having a maximum intensity at 254 nm. It complies with the tests for Content of Calcium Sulfate, Alkalinity and Separating Power stated under *Silica gel G* and with the following test.

FLUORESCENCE Carry out the test as described in the “Thin-layer Chromatography” (Appendix 3.1), using a mixture of 90 volumes of *2-propanol* and 10 volumes of *anhydrous formic acid* as the mobile phase. Apply separately to the plate, increasing quantities from 1 to 10 µl of a 1 mg per ml solution of *benzoic acid* in the same solvent mixture. Develop the plate and dry in a current of warm air. Examine the chromatogram under ultraviolet light at 254 nm. The benzoic acid appears as dark spots on a fluorescent background in the upper third of the chromatogram at levels of 2 µg and greater.

1.11H POWDER FINENESS AND SIEVES

Powders

The degree of coarseness or fineness of a powder is differentiated and expressed by reference to the nominal mesh aperture size of the sieves used.

The following terms are used in the description of powders:

COARSE POWDER A powder all the particles of which pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 µm.

MODERATELY COARSE POWDER A powder all the particles of which pass through a sieve with a nominal mesh aperture of 710 µm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 250 µm.

MODERATELY FINE POWDER A powder all the particles of which pass through a sieve with a nominal mesh aperture of 355 µm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 180 µm.

FINE POWDER A powder all the particles of which pass through a sieve with a nominal mesh aperture of 180 µm.

VERY FINE POWDER A powder all the particles of which pass through a sieve with a nominal mesh aperture of 125 µm.

When the fineness of a powder is described by means of a number, it is intended that all the particles of the powder shall pass through a sieve of which the nominal mesh aperture, in µm, is equal to that number.

When a batch of a vegetable drug is being ground and sifted, no portion of the drug shall be rejected, but it is permissible, except in the case of assays, to withhold the final tailings, if an approximately equal amount of tailings from a preceding batch of the same drug has been added before grinding.

When the use of sieves is inappropriate, the definition is expressed in terms of the particle size as determined by suitable microscopical examination.

Sieves

Wire mesh sieves used in sifting powdered drugs are identified by numbers indicating the nominal mesh aperture.

The sieves should be made of wires of uniform circular cross-section. The wires may be of stainless steel or of other suitable material except that plated wire is not permitted. Sieves should conform to the specifications which are concordant with the recommended International standard ISO 3310-1:2000 (E), shown in the following table.

Calibration and recalibration of test sieves is in accordance with the most current edition of ISO 3310-1. Sieves should be carefully examined for gross distortions and fractures, especially at their screen frame joints, before use. Sieves may be calibrated optically to estimate the average opening size, and opening variability, of the sieve mesh. Alternatively, for the evaluation of the effective opening of test sieves in the size range of 212 to 850 µm, Standard Glass Spheres are available from the national or international organization, e.g. NIST[1]. Unless otherwise specified in the individual monograph, perform the sieve analysis at controlled room temperature and a relative humidity between 20 and 70 per cent.

CLEANING TEST SIEVES Ideally, test sieves should be cleaned using only an air jet or a liquid stream. If some apertures remain blocked by test particles, careful gentle brushing may be used as a last resort. Washing sieves in hot water is not recommended since the sieves can distort and rupture during heating and cooling. If it is necessary to use water, it should be used at ambient temperature and the sieve dried by first using a volatile water-miscible solvent to remove the water and then a low-pressure air jet to remove the solvent. This procedure should be carried out in a fume hood or cabinet that conforms to local regulations.

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[1] US National Institute of Standards and Technology.

Method for Determining Powder Fineness

Place the specified quantity of the test powder upon the appropriate sieve having a close-fitting receiving pan and cover. Shake the sieve in a rotary horizontal direction and vertically by tapping on a hard surface for not less than the specified time or until sifting is practically complete. Avoid prolonged shaking that would result in increasing the fineness of the powder during the testing. In the case of oily or other powders that tend to clog the openings, carefully brush the screen at intervals during the test. Breaking up lumps that form during the sifting. Weigh accurately the amount remaining on the sieve and in the receiving pan.

The fineness of a powdered drug or chemical may be determined also by screening through the sieves in mechanical sieve shaker, which reproduces the circular and tapping motion given to testing sieves in hand sifting but with a uniform mechanical action, following the directions provided by the manufacturer of the shaker.

Number of Sieve*	Nominal Mesh Aperture Size	Preferred Average Wire Diameter	Percentage Sieving Area	US Sieve No.**
µm	mm	mm		
4000	**4.00**	**1.40**	**55**	**5**
3350	3.35			6
2800	**2.80**	**1.12**	**51**	**7**
2360	2.36	1.00	49	8
2000	**2.00**	**0.90**	**48**	**10**
1700	1.70	0.80	46	12
1400	**1.40**	**0.71**	**44**	**14**
1180	1.18	0.63	43	16
1000	**1.00**	**0.56**	**41**	**18**
µm	µm	µm		
850	850			20
710	**710**	**450**	**37**	**25**
600	600	400	36	30
500	**500**	**315**	**38**	**35**
425	425	280	36	40
355	**355**	**224**	**38**	**45**
300	300	200	36	50
250	**250**	**160**	**37**	**60**
212	212	140	36	70
180	**180**	**125**	**35**	**80**
150	150	100	36	100
125	**125**	**90**	**34**	**120**
106	106	71	36	140
90	**90**	**63**	**35**	**170**
75	75	50	36	200
63	**63**	**45**	**34**	**230**
53	53	36	35	270
45	**45**	**32**	**34**	**325**

*Entries in bold are ISO “principal sizes”.

**The list of United States standard sieves is included for information purposes.

1.16H DOSAGE FORMS OF HERBAL DRUGS

HERBAL DRUGS

Herbal drugs are mainly whole, fragmented or cut, plants, parts of plants, algae, fungi, lichens, some of which are also derived from animal or mineral sources. They are usually in dried forms, but sometimes fresh, and also including certain unprocessed plant exudates. Herbal drugs are precisely defined by their scientific names (genus, species, variety and author).

Production Herbal drugs are obtained from natural or cultivated/domesticated origins. Suitable collection, cultivation, harvesting, drying, fragmentation and storage conditions are essential to guarantee the quality of herbal drugs.

Herbal drugs are, as far as possible, free from impurities such as soil, dust, dirt and other contaminants such as fungal, insect and other animal contaminations.

If a decontaminating treatment has been used, it is necessary to demonstrate that the constituents of the plant are not affected and that no harmful residues remain.

Foreign matter Where applicable, the herbal drugs comply with the limits prescribed in the monograph.

Pesticide residues Herbal drugs comply with the "Pesticide Residues" (Appendix 7.22H) taking into account the nature of the plant, where necessary the preparation in which the plant might be used, and where available the knowledge of the complete record of treatment of the batch of the plant.

Loss on drying or Water Where applicable, the herbal drugs comply with the limits prescribed in the monograph. A determination of water is carried out for herbal drugs with a high volatile oil content.

Microbial limit The products consisting of herbal drugs comply with the "Limits for Microbial Contamination" (Appendix 10.5).

Packaging and storage Herbal drugs should be kept in well-closed containers, protected from light.

CAPSULES

This appendix should be read in conjunction with Appendix 1.16 under Capsules.

Capsules are solid dosage forms with hard or soft shells. The different categories of capsules that exist include hard, soft and delayed-release capsules (enteric capsules). Hard capsules and delayed-release capsules contain crude drug powder or crude drug extract(s) or a combination of both, all with or without suitable excipient(s). Soft capsules usually contain crude drug extract(s), with or without suitable excipients(s).

Disintegration Capsules comply with the "Disintegration Test for Tablets and Capsules" (Appendix 4.23).

Weight variation Weigh individually ten capsules. Open each capsule without losing anypart of the shell and remove the contents as completely as possible. For hard capsules, clean the shell with a small brush. For soft capsules, wash the shell with ether or other suitable solvent and allow to stand until the odour of the solvent is no longer perceptible. Weigh the shell. The weight of the contents in each capsule is the difference between the weighings.

Compare the weight of the contents in each capsule with the labelled weight or with the average weight for the capsules in which assay required or no labelled weight stated. Unless otherwise directed in the monograph, not more than two of the individual weights deviate from the labelled weight or the average weight by more than the percentage deviation of 10 per cent and none deviates by more than twice that percentage.

EXTRACTS

Extracts are preparations of liquid, solid or semisolid consistency, obtained from herbal or animal matter, which is usually dried. Extracts may be subjected to purification processes that increase the content of characterized constituents with respect to the content of dry extractable matter from that which would be expected from extraction with the stated solvent: such extracts are termed “enriched”. Three types of extract can be distinguished:

Type A Type A extracts (standardized extracts) are adjusted to a defined range of therapeutically active constituents. Standardization is achieved by adjustment of the extract with inert material or by blending extracts.

Type B Type B extracts (quantified extracts) are adjusted to a defined range of active constituents. Adjustments are made either by blending batches of extracts or by blending batches of herbal or animal matter prior to extraction.

Type C Type C extracts are essentially defined by the production process (state of the matter to be extracted, solvent, extraction conditions). Constituents considered to be relevant markers may be determined.

Production Extracts are prepared by maceration, percolation or other suitable validated methods using ethanol or other suitable solvent. The matter to be extracted may undergo a preliminary treatment, for example, inactivation of enzymes, grinding or defatting. In addition, unwanted matter may be removed, if necessary, after extraction. Herbal drugs, animal matters and organic solvents used for the preparation of extracts comply with any relevant monograph of the Pharmacopoeia. For soft and dry extracts where the organic solvent is removed by evaporation, recovered or recycled solvent may be used, provided that the recovery procedures are controlled and monitored to ensure that solvents meet appropriate standards before reuse or admixture with other approved materials.

Water used for the preparation of extracts is of suitable quality. Except for the test for bacterial endotoxins, water complying with the section on Purified Water in bulk of the monograph on Purified Water is suitable. Potable water may be suitable if it complies with a defined specification that allows the consistent production of a suitable extract.

Where applicable, concentration to the intended consistency is carried out using suitable methods, usually under reduced pressure, and at a temperature at which deterioration of the constituents is reduced to a minimum. Volatile oils that have been distilled during processing may be restored to the extracts at an appropriate stage in the manufacturing process. Suitable inert excipients may be added at the various stages of the manufacturing process to improve technological qualities like homogeneity, consistency or stability of active constituents. Where applicable, as a result of analysis of the herbal or animal matter used for the production of extracts, tests for microbiological quality, heavy metals, aflatoxins, and pesticide residues in the extracts have to be carried out.

Labelling For Type A, the label on the container states (1) the herbal or animal matter used; (2) whether the extract is dry, soft or liquid; (3) the composition of the extraction solvent; (4) where applicable, that fresh herbal or animal matter has been used; (5) where applicable, that the extract is "enriched"; (6) the name and amount of any excipient used. For Types B and C, the label on the container states (1) to (6) as for type A; (7) the content of constituents (markers) used for quantification and (8) the range of starting material: final extract (Drug:Extract Ratio or DER).

Liquid Extracts

Liquid extracts are liquid preparations of which, in general, one part by mass or volume is equivalent to one part by mass of the original dried herbal or animal matter.
These preparations are adjusted, if necessary, so that they satisfy the requirements for content of solvent, and, where applicable, for constituents or dry residue.

Production Liquid extracts are prepared by using ethanol of suitable concentration or water to extract the stated herbal or animal matter or by dissolving a soft or dry extract (which has been produced using the same strength of extraction solvent as is used in preparing the liquid extract by direct extraction) of the stated herbal or animal matter in either ethanol of suitable concentration or water and filtering, if necessary. A slight sediment may form on standing, which is acceptable as long as the composition of the liquid extract is not changed significantly. Liquid extracts may contain suitable antimicrobial preservatives.

Relative density Where applicable, the liquid extract complies with the limits prescribed in the monograph.

Ethanol content For ethanolic liquid extracts, carry out the "Determination of Ethanol" (Appendix 6.5). The preparation complies with the limits prescribed in the monograph.

Methanol and 2-propanol Not more than 0.05 per cent v/v of *methanol* and not more than 0.05 per cent v/v of *2-propanol* for ethanolic liquid extracts unless otherwise prescribed.

Dry residue In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, introduce rapidly 2.0 g or 2.0 ml of the extract to be examined. Evaporate to dryness on a water-bath and dry at 105° for 3 hours. Allow to cool in a desiccator over *phosphorus pentoxide desiccant* or *self-indicating silica gel* and weigh. Calculate the result as a percentage or in grams per litre.

Packaging and storage Liquid extracts should be kept in well-closed containers, protected from light.

Labelling The label on the container states in addition to the requirements listed above (1) where applicable, the ethanol content in per cent v/v in the final extract; (2) the concentration of any added antimicrobial preservative.

Soft Extracts

Soft extracts are semi-solid preparations obtained by evaporation of the solvent used for preparation. Soft extracts generally have a dry residue of not less than 70 per cent w/w. They may contain suitable antimicrobial preservatives.

Dry residue In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, weigh rapidly 2.0 g of the extract to be examined. Heat to dryness on a water-bath and dry at 105º for 3 hours. Allow to cool in a desiccator over *phosphorus pentoxide desiccant* or *self-indicating silica gel* and weigh. Calculate the result as a percentage weight in weight. Where applicable, a monograph on a soft extract prescribes a limit test for the solvent used for extraction.

Packaging and storage Soft extracts should be kept in well-closed containers, protected from light.

Labelling The label on the container states in addition to the requirements listed above (1) where applicable, the ethanol content in per cent v/v in the final extract; (2) the concentration of any added antimicrobial preservative.

Dry Extracts

Dry extracts are solid preparations obtained by evaporation of the solvent used for their production. Dry extracts generally have a dry residue of not less than 95 per cent w/w.

Loss on drying Where applicable, the dry extract complies with the limits prescribed in the monograph. In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, weigh rapidly 500 mg of the extract to be examined, finely powdered. Dry at 105º for 3 hours. Allow to cool in a desiccator over *phosphorus pentoxide desiccant* or *self-indicating silica gel* and weigh. Calculate the result as a percentage weight in weight. Where applicable, a monograph on a drug extract prescribes a limit test for the solvent used for extraction.

Packaging and storage Dry extracts should be kept in well-closed containers, protected from light.

HERBAL TEAS

Herbal Teas consist exclusively of one or more herbal drug(s) intended for oral aqueous preparations by means of decoction, infusion or maceration. They are usually supplied in bulk form or in sachets.

Microbial limit Herbal Teas comply with the "Limits for Microbial Contamination" (Appendix 10.5), taking into account the prescribed preparation method (use of boiling or non-boiling water).

Weight variation Determine the average weight of twenty randomly chosen units as follows: weigh a single full sachet of herbal tea, open it without losing any fragments. Empty it completely using a brush. Weigh the empty sachet and calculate the weight of the contents by subtraction. Repeat the operation on the nineteen remaining sachets. Unless otherwise justified not more than two of the twenty individual weights of the contents deviate from the average weight of the contents by more than the percentage deviation shown in the table below and none deviates by more than twice that percentage.

Average Weight	Percentage Deviation
less than 1.5 g	15 per cent
1.5 g to 2.0 g	10 per cent
more than 2.0 g	7.5 per cent

Packaging and storage Herbal Teas should be kept in well-closed containers, protected from light.

TOPICAL PREPARATIONS

Topical preparations are drugs intended for topical application in a wide variety of dosage forms.

Minimum fill Topical preparations except plasters comply with the test described in the “Minimum Fill” (Appendix 4.26).

Topical Semi-solid Preparations

Topical semi-solid preparations are intended to be applied to the skin or to certain mucous surfaces for local action or percutaneous penetration of medicaments, or for their emollient or protective action. They are of homogeneous appearance. Topical semi-solid preparations consist of a simple or compound base in which, usually, one or more active substances are dissolved or dispersed. According to its composition, the base may influence the action of the preparation and the release of the active substance (s).

The bases may consist of natural or synthetic substances and may be single-phase or multi-phase systems. According to the nature of the base the preparation may have hydrophilic or hydrophobic (lipophilic) properties; it may contain suitable additives such as antimicrobial preservatives, antioxidants, stabilizers, emulsifiers and thickeners.

If a preparation is specifically intended for use on large open wounds or on severely injured skin, it should be sterile. Preparations required to be sterile must comply with the test for sterility.

If the particle size of the ingredients is of importance for the therapeutic purpose of a topical semi-solid preparation, the test to be applied should be specified.

Topical semi-solid preparations can be distinguished into four categories: (1) creams (hydrophobic or hydrophilic); (2) gels (hydrophobic or hydrophilic); (3) ointments (hydrophobic, water-emulsifying or hydrophilic); (4) pastes.

Sterility Where the preparation is labelled as sterile and unless otherwise directed in the individual mono-graph, it complies with the “Sterility Test” (Method I, Appendix 10.1).

Packaging and storage Topical semi-solid preparations should be stored in well-closed containers or, if the preparation contains water or other volatile constituents, in a tightly closed container. The containers are preferably collapsible metal tubes from which the preparation may be readily extruded. Other types of container may be used. Containers for preparations for nasal, aural, vaginal, or rectal use should be adapted to deliver the product to the site of application or should be accompanied by a suitable applicator. They should be stored at a temperature not exceeding 30° unless otherwise prescribed. For creams and gels, they shall not be frozen.

Labelling The label of topical semi-solid preparations states (1) the name and concentration of any added antimicrobial preservative(s); (2) where applicable, that the preparation is sterile.

GELS Gels are usually homogeneous, clear, semi-solid preparations consisting of a liquid phase within a three-dimensional polymeric matrix with physical or sometimes chemical cross-linkage by means of suitable gelling agents.

Hydrophobic gels Hydrophobic gel (oleogel) bases usually consist of liquid paraffin with polyethylene or fatty oils gelled with colloidal silica or aluminium or zinc soaps.

Hydrophilic gels Hydrophilic gel (hydrogel) bases usually consist of water, glycerol, or propylene glycol gelled with suitable agents such as tragacanth, starch, cellulose derivatives, carboxyvinyl polymers and magnesium aluminium silicates.

APPENDIX 2 SPECTROSCOPY

2.2 ULTRAVIOLET AND VISIBLE SPECTROPHOTOMETRY

Absorption spectroscopy in the ultraviolet and visible region is one of the most useful tools for qualitative and quantitative analysis.

When a beam of monochromatic radiation traverses a solution containing an absorbing substance, its radiant power is reduced in relation to the distance that it travels through. It also decreases in relation to the concentration of absorbing molecules or ions encountered in that medium. These two factors determine the proportion of the total incident energy that emerges. The decrease in power of monochromatic radiation is stated quantitatively by Beer's law:

$$\log (1/T) = A = abc.$$

The terms used in connection with spectrophotometric tests are defined as follows:

Absorbance (A) is the logarithm, to the base 10, of the reciprocal of the transmittance (T). (**Note** Descriptive terms used formerly include optical density, absorbancy and extinction.)

Absorptivity (a) is the quotient of the absorbance (A) per litre, of the substance (c) and the absorption path length in cm (b). (**Note** It is not to be confused with specific absorbance, specific extinction or extinction coefficient [A (1 per cent, 1 cm) or E (1 per cent, 1 cm)]. These are generally used in different pharmacopoeia, as the quotient of the absorbance divided by the product of the concentration, expressed in g per 100 ml, of the substance, and the absorption path length in cm, therefore: A (1 per cent, 1 cm) = 10 a.)

Molar absorptivity (ε) is the quotient of the absorbance (A) divided by the product of the concentration, expressed in moles per litre, of the substance (c) and the absorptivity (a) and the molecular weight of the substance. (**Note** Terms formerly used include molar absorbancy index, molar extinction coefficient and molar absorption coefficient.)

Transmittance (T) is the quotient of the radiant power transmitted by a sample (I) divided by the radiant power incident upon the sample (I_0).

Absorption spectrum is a graphic representation of absorbance, or any function of absorbance, plotted against wavelength or function of wavelength.

Where a monograph gives a single value for the position of an absorption maximum, it is understood that the value obtained may differ by not more than ±2 nm.

Apparatus

All types of spectrophotometer are designed to permit substantially monochromatic radiant energy to be passed through the test substance in a suitable form and to allow measurement of the fraction of energy that is transmitted. The spectrophotometer comprises an energy source, a dispersing device with slits for selecting the wavelength band, a cell for holding the test substance, a detector of radiant energy, associated amplifiers, and measuring and recording devices. Some instruments are manually operated, while others are equipped for automatic operation. Instruments are available for use in the visible region of the spectrum, usually 380 nm to about 700 nm, and in the ultraviolet and visible regions of the spectrum, usually 190 nm to about 700 nm.

Both double-beam and single-beam instruments are commercially available and either is suitable. Depending on the type of apparatus used, the results may be displayed on a scale, on a digital counter, or by a recorder or printer.

The apparatus should be maintained in proper working condition. The housing of the optical system should minimize any possibility of errors due to stray light; this is particularly relevant in the short-wave region of the spectrum.

CONTROL OF WAVELENGTHS Verify the wavelength scale using the absorption maxima of Holmium Perchlorate Solution, the line of a hydrogen or deuterium discharge lamp or the lines of a mercury vapour as shown below. The permitted tolerance is ±1 nm for the range 200 to 400 nm and ±3nm for the range 400 to 600 nm.

241.15 nm (Ho)
253.70 nm (Hg)
287.15 nm (Ho)
302.25 nm (Hg)
313.16 nm (Hg)
334.15 nm (Hg)
361.50 nm (Ho)
365.48 nm (Hg)
404.66 nm (Hg)
435.83 nm (Hg)
486.00 nm (Dβ)
486.10 nm (Hβ)
536.30 nm (Ho)
546.07 nm (Hg)
576.96 nm (Hg)
579.07 nm (Hg)

The wavelength scale may also be calibrated by means of suitable glass filters that have useful absorption bands through the visible and ultraviolet regions. Standard glass containing didymium (a mixture of praseodymium and neodymium) has been widely used. Glass containing holmium is considered superior. The exact values for the position of characteristic maxima in holmium glass filters are 241.5±1, 287.5±1, 360.9±1, and 536.2±3 nm. Holmium glass filters are obtainable from some national institutions and from commercial sources. The performance of an uncertified filter should be checked against one that has been properly certified.

CONTROL OF ABSORBANCE Check the absorbance using Potassium Dichromate Solution UV at the wavelengths indicated in the following table, which gives for each wavelength the exact value of *A* (1 per cent, 1 cm) and the permitted limits.

Wavelength (nm)	*A* (1 per cent, 1 cm)	Maximum Tolerance
235	124.5	122.9 to 126.2
257	144.0	142.4 to 145.7
313	48.6	47.0 to 50.3
350	106.6	104.9 to 108.2

LIMIT OF STRAY LIGHT Stray light may be detected at a given wavelength with suitable filters or solutions. For example, the absorbance of a 1.2 per cent w/v solution of *potassium chloride* at a path-length of 1 cm should be greater than 2 at 200 nm when compared with *water* as reference liquid.

SPECTRAL SLIT WIDTH When measuring the absorbance at an absorption maximum, the spectral slit width must be small compared with the half-width of the absorption band, or erroneously low absorbances will be measured. Particular care is needed for certain substances and the instrumental slit width used should always be such that further reduction does not result in an increased absorbance reading.

CELLS Cells usually in the spectral range discussed are 1-cm absorption cells with glass or silica windows. Other path lengths may also be used. The cells used for the test solution and the blank should be matched, and must have the same spectral transmittance when containing only the solvent. If this is not the case, an appropriate correction must be applied.

SOLVENTS In measuring the absorbance of a solution at a given wavelength, the absorbance of the solvent cell and its contents shall not exceed 0.4 and is preferably less than 0.2 when measured with reference to air at the same wavelength. The solvent in the solvent cell shall be of the same batch as that used to prepare the solution and must be free from fluorescence at the wavelength of measurement. *Ethanol, absolute ethanol, methanol* and *cyclohexane UV* used as solvents shall have an absorbance, measured in a 1-cm cell at 240 nm with reference to *water*, not exceeding 0.10. Statements of concentration and thickness of the solution to be used in the determination of light absorption apply to measurements made with photoelectric instruments.

SOLUTIONS FOR USE IN THE CALIBRATION OF WAVELENGTHS AND ABSORBANCE

Holmium Perchlorate Solution Dissolve 40 g of *holmium oxide* in sufficient 1.4 M *perchloric acid* to produce 1000 ml.

Potassium Dichromate Solution UV Dry a quantity of *potassium dichromate* by heating to constant weight at 130°. Weigh accurately a quantity not less than 57.0 mg and not more than 63.0 mg and dissolve it in sufficient 0.005 M *sulfuric acid* to produce 1000.0 ml.

Determination of Absorbance

Unless otherwise prescribed, measure the absorbance, *A*, at the prescribed wavelength using a path length of 1 cm, and the measurements are carried out with reference to the solvent used to prepare the solution being examined. In certain cases measurements are carried out with reference to a mixture of reagents, details of which are prescribed in the monograph.

When the absorbance is being measured for a quantitative determination, for example, an assay or a limit test, a manually-scanning instrument is used. In tests for identification, it is more convenient to use a recording instrument and the concentration of the solution and the pathlength are specified accordingly. If these conditions are not appropriate for a particular instrument, the thickness and the concentration of the solution should be varied. A statement in an assay or test of the wavelength at which maximum absorption occurs implies the maximum occurring either precisely at or in the vicinity of the given wavelength.

When an assay or test prescribes the use of a reference substance, the spectrophotometric measurements are made first with the solution prepared from the reference substance and second with the corresponding solution prepared from the substance being examined. The second measurement is carried out as quickly as possible after the first, using the matched cell and the same experimental conditions.

The requirements for light absorption in the Pharmacopoeia apply to the dried, anhydrous, or solvent-free material in all those monographs in which standards for loss on drying, water or solvent content are given. In calculating the result, the loss on drying, water, or solvent content determined by the method specified in the monograph is used.

SECOND DERIVATIVE SPECTROPHOTOMETRY

Derivative spectrophotometry involves the transformation of absorption spectra (zero order) into first, second or higher order derivative spectra. A first derivative spectrum is a plot of the gradient of the absorption curve (rate of change of the absorbance with wavelength, $dA/d\lambda$) against wavelength. A second derivative spectrum is a plot of the curvature of the absorption spectrum ($d^2A/d\lambda^2$) against wavelength.

If the absorbance follows the Beer-Lambert relationship, the second derivative at any wavelength, λ , is related to concentration by the following equation:

$$\frac{d^2 A}{d\lambda^2} = \frac{d^2 A(1\%, 1\,\text{cm})}{d\lambda^2} \times cd$$

where A = the absorbance at wavelength l,

A (1%, 1 cm) = the specific absorbance at wavelength l,

c = the concentration of the absorbing solute expressed as a percentage w/v, and

d = the thickness of the absorbing layer in cm.

Apparatus

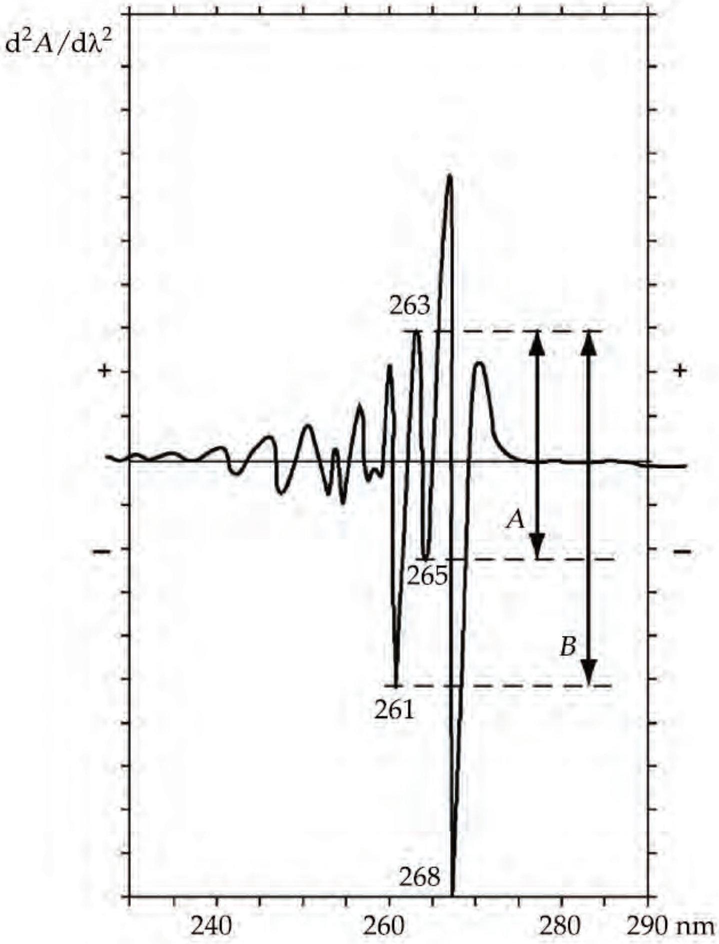
A spectrophotometer complying with the requirements prescribed above and equipped with an analogue resistance-capacitance differentiation module or a digital differentiator or another means of producing second derivative spectra should be used in accordance with the manufacturer's instructions. Some methods of producing second derivative spectra lead to a wavelength shift relative to the zero order spectrum and this should be taken into account, when necessary. Unless otherwise stated in the monograph, the spectral slit width of the spectrophotometer, where variable, should be set as described under Spectral slit width above. The cells used should comply with the statements given under the heading Cells.

Resolution

When prescribed in a monograph, record the second derivative spectrum in the range 255 to 275 nm of a 0.020 per cent v/v solution of *toluene* in *methanol* using *methanol* in the reference cell. The spectrum shows a small negative extremum (or trough) located between two large negative extreme at 261 nm and 268 nm, respectively as shown in the figure. Unless otherwise prescribed in the monograph, the ratio A/B (see the figure) is not less than 0.2.

Procedure

Prepare a solution of the substance being examined, adjust the various instrument settings according to the manufacturer's instructions and calculate the amount of the substance being determined as prescribed in the monograph.



APPENDIX 3 CHROMATOGRAPHY

3.1 THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is used for the rapid separation of compounds by means of a uniform layer of dry, finely powdered material applied to a glass, plastic, or metal sheet or plate. The coated plate can be considered as an “open chromatographic column”. Solutions of analytes are deposited on the plate prior to development. The separation is based on adsorption, partition, ion-exchange or on combinations of these mechanisms and is carried out by migration (development) of solutes (solutions of analytes) in a solvent or a suitable mixture of solvents (mobile phase) through the thin-layer (stationary phase).

The retardation factor (R_f) is defined as the ratio of the distance from the point of application to the centre of the spot and the distance travelled by the solvent front from the point of application. As R_f values may vary significantly with the experimental conditions, it is always necessary to prepare chromatograms of authentic specimens or reference substances; preferably in varied quantities, alongside the chromatogram of the sample. Positive identification may be effected by observation of two spots of identical R_f value and about equal magnitude. A visual comparison of the size of the spots may serve for semi-quantitative estimation. More accurate quantitative measurements can be made by densitometry, fluorescence, and fluorescence quenching, or careful removal of the spots from the plate, followed by elution with a suitable solvent and spectrophotometric measurement. For two-dimensional thin-layer chromatography, the chromatographed plate is turned at a right angle and again chromatographed, usually in another chamber equilibrated with a different solvent system.

Apparatus

Plate The chromatography is carried out using the TLC plate (typically 20 cm × 20 cm) of which the stationary phase has an average particle size of 10 to 15 μm, and that of high-performance thin-layer chromatography (HPTLC) plates (typically 10 cm × 10 cm) has an average particle size of 5 μm. Commercial plates with a pre-adsorbent zone can be used if they are specified in a monograph.

Spreader A spreader, which, when moved over the plate, will apply a uniform layer of adsorbant, 250 to 300 μm thick, over the entire surface of the plate. Other thicknesses might be desirable in some procedures, and an adjustable spreader would be particularly useful in such cases.

Preparation of the TLC plate Use flat glass plates of convenient size typically 20 cm × 20 cm.

The adsorbent consists of finely divided solid material, normally 10 to 15 μm in diameter, suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of plaster of Paris (hydrated calcium sulfate) at a ratio of 5 to 15 per cent, or with starch paste or other binders. The former will not yield as hard a surface as will the starch, but it is not affected by strongly oxidizing spray reagents. The adsorbent may contain fluorescing material to aid in the visualization of spots that absorb ultraviolet light. Clean the plates scrupulously, as by immersion in *chromic acid cleansing mixture*, rinsing them with copious quantities of *water* until the water runs off the plates without leaving any visible water or oily spots, and then dry. It is important that the plates be completely free from lint and dust when the adsorbent is applied.

Arrange the plate or plates on the aligning tray, place a 5-cm × 20-cm plate adjacent to the front edge of the first square plate and another 5-cm × 20-cm plate adjacent to the rear edge of the last square, and secure all of the plates so that they will not slip during the application of the adsorbant. Position the spreader on the end plate opposite to the raised end of the aligning tray. Mix 1 part of adsorbent with 2 parts of *water* (or in the ratio suggested by the supplier) by shaking vigorously for about 30 seconds in a glass-stoppered conical flask, and transfer the slurry to the spreader. Usually 30 g of adsorbent and 60 ml of water are sufficient for five 20-cm × 20-cm plates. Complete the application of adsorbents using plaster of Paris binder within 2 minutes of addition of the water, since thereafter the mixture begins to harden. Draw the spreader smoothly over the plates towards the raised end of the aligning tray, and remove the spreader when it is on the end plate next to the raised end of the aligning tray. (Wash away all traces of adsorbant from the spreader immediately after use). Allow the plates to remain undisturbed for 5 minutes, then transfer the square plates, layer side up, to the storage rack and dry at 105° for 30 minutes. Preferably place the rack at an angle in the drying oven to prevent the condensation of moisture on the back side of the plates in the rack. When the plates are dry, allow them to cool to room temperature, and inspect the uniformity of the distribution and the texture of the adsorbant layer; transmitted light will show uniformity of texture. Store the satisfactory plates over *self-indicating silica gel* in a suitable chamber.

Pre-treatment of the plate It may be necessary to wash the plates prior to separation. This can be done by migration of an appropriate solvent. The plates may also be impregnated by procedures such as development, immersion or spraying. At the time of use, the plates may be activated, if necessary, by heating in an oven at 120° for 20 minutes.

Developing chamber A developing chamber with a flat bottom or twin trough, of inert, transparent material, of a size suitable for the plates is used and provided with a tightly fitting lid. For horizontal development, the chamber is provided with a trough for the mobile phase and it additionally contains a device for directing the mobile phase to the stationary phase.

Micropipette, microsyringe, calibrated disposable capillary A micropipette, microsyringe, calibrated disposable capillary or other application devices suitable for the proper application of the solutions are used.

Template A template (generally made of plastic) is used to aid in placing the test spots at definite intervals, to mark distances as needed, and to aid in labelling the plate.

Detection/Visualization device An ultraviolet (UV) light source suitable for observations under short- (254 nm) and long- (366 nm) wavelength UV light and a variety of other spray reagents to make spots visible are used.

A device may be used to provide documentation of the visualized chromatogram, for example a photograph or a computer file.

Procedure

Sample application Apply the prescribed volume of the solutions at a distance of at least 15 mm (5 mm on HPTLC plates) from the lower edge and from the sides of the plate and on a line parallel to the lower edge; allow an interval of at least 10 mm (5 mm on HPTLC plates) between the centres of circular spots and 5 mm (2 mm on HPTLC plates) between the edges of bands.

Apply the solutions in sufficiently small portions to obtain circular spots of 2 to 5 mm in diameter (1 to 2 mm on HPTLC plates) or bands of 10 to 20 mm (5 to 10 mm on HPTLC plates) by 1 to 2 mm (0.5 to 1 mm on HPTLC plates) and allow to dry. Avoid physical disturbance of the adsorbant during the spotting procedure (by the pipette or other applicator) or when handling the plates. The template will aid in determining the spot points and the specified distance through which the solvent front should pass.

Development Line the walls of the developing chamber with filter paper. Pour into the developing chamber a sufficient quantity of the mobile phase for the size of the chamber to give after impregnation of the filter paper a layer of appropriate depth related to the dimension of the plate to be used. For saturation of the developing chamber, replace the lid and allow to stand for 1 hour. Unless otherwise indicated in the monograph, the chromatographic separation is performed in a saturated chamber. Apply the prescribed volume of solutions as described above. When the solvent has evaporated from the applied solutions, place the plate in the developing chamber, ensuring that the plate is as vertical as possible and that the spots or bands are above the surface of the mobile phase. Close the developing chamber. Remove the plate when the mobile phase has moved over 15 cm, or over three-quarters of the length of the plate, above the initial spots or bands, unless otherwise indicated in the monograph. Dry the plate and visualize the chromatograms as prescribed.

Horizontal development can be used in place of vertical development, if specified in the monograph. For two-dimensional chromatography, dry the plates after the first development and carry out a second development in a direction perpendicular to that of the first development.

Detection/Visualization Observe the principal spot or band in the chromatogram first under short-wavelength ultraviolet light (254 nm) and then under long-wavelength ultraviolet light (366 nm). Measure and record the distance of each spot or band from the point of origin, and indicate for each spot or band the wavelength under which it was observed. If further directed, spray the spots or bands with the reagent specified, observe, and compare the sample with the standard chromatogram.

3.4 GAS CHROMATOGRAPHY

Gas chromatography is a method of chromatographic separation in which the mobile phase is a gas (the carrier gas) and the stationary phase is a solid or liquid coated on a suitable solid support contained in a column. On emerging from the column the carrier gas is passed through a suitable detector.

Apparatus

The apparatus consists of an injector, a chromatographic column contained in an oven, a detector and data collection devices. The carrier gas flows through the column at a controlled rate or pressure and then through the detector. The chromatography is carried out either at a constant temperature or according to a given temperature programme.

INJECTORS Sample injection devices range from simple syringes to fully programmable automatic injectors. Direct injections of solutions are the usual mode of injection, unless otherwise prescribed in the monograph. Injection may be carried out either directly at the head of the column using a syringe or an injection valve, or into a vaporization chamber which may be equipped with a stream splitter. The amount of sample that can be injected into a capillary column without overloading is small compared to the amount that can be injected into packed columns and may be less than the smallest amount that can be manipulated satisfactorily by syringe. Capillary columns, therefore, often are used with injectors able to split samples into two fractions, a small one that enters the column and a large one that goes to waste. Such injectors may be used in a splitless mode for analyses of trace or minor components.

Purge and trap injectors are equipped with a sparging device by which volatile compounds in solution are carried into a low-temperature trap. When sparging is complete, trapped compounds are desorbed into the carrier gas by rapid heating of the temperature-programmable trap.

Headspace injectors are equipped with a thermostatically controlled sample heating chamber. Solid or liquid samples in tightly closed containers are heated in the chamber for a fixed period of time allowing the volatile components in the sample to reach an equilibrium between the nongaseous phase and the gaseous or headspace phase.

After this equilibrium has been established, the injector automatically introduces a fixed amount of the headspace in the sample container into the gas chromatograph.

COLUMNS Capillary columns which are usually made of fused silica, are typically 0.2 to 0.53 mm in internal diameter and 5 to 60 m in length. The liquid or stationary phase, which is sometimes chemically bonded to the inner surface, is 0.1 to 1.0 µm thick, although nonpolar stationary phases may be up to 5 µm thick.

Packed columns, made of glass or metal, are 1 to 3 m in length with internal diameters of 2 to 4 mm. Those used for analysis typically are porous polymers or solid supports with liquid phase loadings of about 5 per cent (w/w). High-capacity columns, with liquid phase loadings of about 20 per cent (w/w), are used for large test specimens and for the determination of low molecular weight compounds such as water. The capacity required influences the choice of solid support.

Supports for analysis of polar compounds on low-capacity, low-polarity liquid phase columns must be inert to avoid peak tailing. The reactivity of support materials can be reduced by silanizing prior to coating with liquid phase. Acid-washed, flux-calcined diatomaceous earth is often used for drug analysis. Support materials are available in various mesh sizes, with 80- to 100-mesh and 100- to 120-mesh being most commonly used with 2- to 4-mm columns.

Helium or nitrogen is usually employed as the carrier gas for packed columns, whereas commonly used carrier gases for capillary columns are nitrogen, helium and hydrogen. Retention time and the peak efficiency depend on the carrier gas flow rate; retention time is also directly proportional to column length, while resolution is proportional to the square root of the column length. For packed columns, the carrier gas flow rate is usually expressed in ml per minute at atmospheric pressure and room temperature. It is measured at the detector outlet with a flowmeter while the column is at operating temperature. The linear flow rate through a packed column is inversely proportional to the square of the column diameter for a given flow volume. Flow rates of 60 ml per minute in a 4-mm column and 15 ml per minute in a 2-mm column give identical linear flow rates and thus similar retention times. Unless otherwise specified in the monograph, flow rates for packed columns are about 30 to 60 ml per minute. For capillary columns, linear flow velocity is often used instead of flow rate. This is conveniently determined from the length of the column and the retention time of a dilute methane sample, provided a flame-ionization detector is in use. At high operating temperatures there is sufficient vapour pressure to result in a gradual loss of liquid phase, a process called bleeding.

DETECTORS Flame-ionization detectors are used for most pharmaceutical analyses, with lesser use made of thermal conductivity, electron-capture, nitrogen-phosphorous (alkali flame-ionization), mass spectrometric, Fourier transform infrared spectrophotometric detectors, and others, depending on the purpose of the analysis. For quantitative analyses, detectors must have a wide linear dynamic range: the response must be directly proportional to the amount of compound present in the detector over a wide range of concentrations. Flame-ionization detectors have a wide linear range and are sensitive to most organic compounds. Detector response depends on the structure and concentration of the compound and on the flow rates of the combustion, air, makeup, and carrier gases. Unless otherwise specified in individual monographs, flame-ionization detectors with either helium or nitrogen carrier gas are to be used for packed columns and helium or hydrogen is used for capillary columns.

DATA COLLECTION DEVICES Modern data stations receive the detector output, calculate peak areas and peak heights, and print chromatograms, complete with run parameters and peak data. Chromatographic data may be stored and reprocessed, with integration and other calculation variables being changed as required. Data stations are used also to program the chromatograph, controlling most operational variables and providing for long periods of unattended operation.

Data can also be collected for manual measurement on simple recorders or on integrators whose capabilities range from those providing a printout of peak areas and peak heights calculated and data stored for possible reprocessing.

The design of a particular chromatograph may require modification of the conditions detailed in the monograph. In such a case, the analyst should be satisfied that the modified conditions produce comparable results. If necessary, adjust the flow rate of the carrier gas to improve the quality of the chromatogram or to modify the retention times of the peaks of interest.

Performance

Criteria for assessing the suitability of the system are described in the "Chromatographic Separation Techniques" (Appendix 3.9). The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given.

Procedure

Equilibrate the column, the injector and the detector at the temperatures and the gas flow rates specified in the monograph until a stable baseline is achieved. Prepare the test solution(s) and the standard solution(s) as prescribed in the monograph. The solutions must be free from solid particles. Using standard solution determine experimentally suitable instrument settings and volumes of the solutions to be injected to produce an adequate response.

In applications where an internal standard is used, an injection of sample solution containing only the substance being examined should be made to determine whether any peak is present that will interfere with that of the internal standard. If an interfering peak is present, a suitable correction should be made.

Inject the selected volumes of the solutions prescribed in the monograph and record the resulting chromatograms. Repeat the determinations to ensure a consistent response.

For qualitative analysis, the retention time for a peak in the chromatogram obtained for a test specimen is "the same as," or "corresponding to" that obtained for a standard preparation under the conditions specified in the individual monograph.

For quantitative analysis, determine the peak areas or, alternatively, when the symmetry factor is between 0.80 and 1.20, determine the peak heights corresponding to the components of interest. From the values obtained calculate the content of the component or components being determined.

Assays require quantitative comparison of one chromatogram with another, and lack of control of the specimen size injected is a major source of error. Addition of an internal standard to the test specimen minimizes this error. The ratio of peak response of the components of interest to the internal standard is compared from one chromatogram to another. Where the internal standard is chemically similar to the substance being examined, minor variations in column and detector parameters are controlled also. In some cases, the internal standard may be carried through the assay procedure prior to gas chromatography to control other quantitative aspects of the procedure.

Materials

Supports, stationary phases and internal standards for gas chromatography are stated in the "Materials for Chromatography" (Appendix 1.7).

Solvents and reagents used in the preparation of solutions for examination should be of a quality suitable for use in gas chromatography.

3.5 HIGH-PRESSURE LIQUID CHROMATOGRAPHY

High-pressure liquid chromatography (HPLC), sometimes called high-performance liquid chromatography, is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption, or ion-exchange processes, depending upon the type of stationary phase used. HPLC has distinct advantages over gas chromatography for the analysis of organic compounds. Compounds to be analyzed are dissolved in a suitable solvent, and most separations take place at room temperature. Thus, most drugs, being nonvolatile or thermally unstable compounds, can be chromatographed without decomposition or the necessity of making volatile derivatives. Most pharmaceutical analyses are based on partition chromatography and are completed within 30 minutes.

As in gas chromatography, the elution time of a compound can be described by the capacity factor, k', which depends on the chemical nature of the analyte, the composition and flow rate of the mobile phase, and the composition and surface area of the stationary phase. Column length is an important determinant of resolution. Only compounds having different capacity factors can be separated by HPLC.

Apparatus

A liquid chromatograph consists of a reservoir containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column, a detector, and a data collection device such as a computer, integrator, or recorder. Short, small-bore columns containing densely packed particles of stationary phase provide for the rapid exchange of compounds between the mobile and stationary phases. In addition to receiving and reporting detector output, computers are used to control chromatographic settings and operations, thus providing for long periods of unattended operation.

PUMPING SYSTEM HPLC pumping systems deliver metered amounts of mobile phase from the solvent reservoirs to the column through high-pressure tubing and fittings. Modern systems consist of one or more computer-controlled metering pumps that can be programmed to vary the ratio of mobile phase components, as is required for gradient chromatography, or to mix isocratic mobile phases (i.e., mobile phases having a fixed ratio of solvents). However, the proportion of ingredients in premixed isocratic mobile phases can be more accurately controlled than in those delivered by most pumping systems. Operating pressures up to 34,474 kPa (about 5000 psi) or higher, with delivery rates up to about 10 ml per minute are typical. Pumps used for quantitative analysis should be constructed of materials inert to corrosive mobile phase components and be capable of delivering the mobile phase at a cons tant rate with minimal fluctuations over extended periods of time.

INJECTORS After dissolution in mobile phase or other suitable solution, compounds to be chromatographed are injected into the mobile phase, either manually by a syringe or loop injectors, or automatically by autosamplers. The latter consist of a carousel or rack to hold sample vials with tops that have a pierceable septum or stopper and an injection device to transfer sample from the vials to a loop from which it is loaded into the chromatograph. Some autosamplers can be programmed to control sample volume, the number of injections and loop rinse cycles, the interval between injections, and other operating variables.

A syringe can be used for manual injection of sample through a septum when column head pressures are less than 6897 kPa (about 1000 psi). At higher pressures an injection valve is essential. Some valve systems incorporate a calibrated loop that is filled with the test solution for transfer to the column in the mobile phase. In other systems, the test solution is transferred to a cavity by a syringe and then switched into the mobile phase.

COLUMNS For most pharmaceutical analyses, separation is achieved by partition of compounds in the test solution between the mobile phase and stationary phase. Systems consisting of polar stationary phases and nonpolar mobile phases are described as normal phase, while the opposite arrangement, polar mobile phases and nonpolar stationary phases, is called reverse-phase chromatography. Partition chromatography is almost always used for hydrocarbon-soluble compounds of molecular weight less than 1000. The affinity of a compound for the stationary phase, and thus its retention time on the column, is controlled by making the mobile phase more or less polar. Mobile phase polarity can be varied by the addition of a second, and sometimes a third or even a fourth, component.

Stationary phases for modern, reverse-phase liquid chromatography typically consist of an organic phase chemically bound to silica or other materials. Particles are usually 3 to 10 µm in diameter, but sizes may range up to 50 µm or more for preparative columns. Small particles thinly coated with the organic phase provide for low mass transfer resistance and, hence, rapid transfer of compounds between the stationary and mobile phases. Column polarity depends on the polarity of the bound functional groups, which range from relatively nonpolar octadecyl silane to very polar nitrile groups. Liquid, nonbound stationary phases must be largely immiscible in the mobile phase. Even so, it is usually necessary to presaturate the mobile phase with stationary phase to prevent stripping of the stationary phase from the column. Polymeric stationary phases coated on the support are more durable.

Columns used for analytical separations usually have internal diameters of 2 to 5 mm; larger diameter columns are used for preparative chromatography. Columns may be heated to give more efficient separations, but only rarely are they used at temperatures above 60° because of potential stationary phase degradation or mobile phase volatility. Unless otherwise specified in the individual monograph, columns are used at ambient temperature.

Ion-exchange chromatography is used to separate water-soluble, ionizable compounds of molecular weight less than 1500. The stationary phases are usually synthetic organic resins; cation-exchange resins contain negatively charged active sites and are used to separate basic substances such as amines, while anion-exchange resins have positively charged active sites for separation of compounds with negatively charged groups such as phosphate, sulfonate, or carboxylate groups. Water-soluble ionic or ionizable compounds are attracted to the resins, and differences in affinity bring about the chromatographic separation. The pH of the mobile phase, temperature, ion type, ionic concentration, and organic modifiers affect the equilibrium, and these variables can be adjusted to obtain the desired degree of separation.

In size-exclusion chromatography, columns are packed with a porous stationary phase. Molecules of the compounds being chromatographed are filtered according to size. Those too large to enter the pores pass unretained through the column. Smaller molecules enter the pores and are increasingly retained as molecular size decreases. These columns are typically used to measure aggregation and degradation of large molecules.

DETECTORS Many compendial HPLC methods require the use of spectrophotometric detectors. Such a detector consists of a flow-through cell mounted at the end of the column. A beam of UV radiation passes through the flow cell and into the detector. As compounds elute from the column, they pass through the cell and absorb the radiation, resulting in measurable energy level changes.

Fixed, variable-, and multi-wavelength detectors are widely available. Fixed wavelength detectors operate at a single wavelength, typically 254 nm, emitted by a low-pressure mercury lamp, Variable-wavelength detectors contain a continuous source such as a deuterium or high-pressure xenon lamp, and a monochromator or an interference filter to generate monochromatic radiation at a wavelength selected by the operator. The wavelength accuracy of a variable-wavelength detector equipped with a monochromator should be checked by the procedure recommended by its manufacturer, if the observed wavelengths differ by more than 3 nm from the correct values, recalibration of the instrument is indicated. Modern variable-wavelength detectors can be programmed to change wavelength while an analysis is in progress. Multi-wavelength detectors measure absorbance at two or more wavelengths simultaneously. In diode array multi-wavelength detectors, continuous radiation is passed through the sample cell then resolved into its constituent wavelengths, which are individually detected by the photodiode array. These detectors acquire absorbance data over the entire UV-visible range, thus providing the analyst with chromatograms at multiple, selectable wavelengths and spectra of the eluting peaks. Diode array detectors usually have lower-signal-to-noise ratios than fixed or variable wavelength detectors, and thus are less suitable for analysis of compounds present at low concentrations.

Differential refractometer detectors measure the difference between the refractive index of the mobile phase alone and that of the mobile phase containing chromatographed compounds as it emerges from the column. Refractive index detectors are used to detect non-UV absorbing compounds, but they are less sensitive than UV detectors. They are sensitive to small changes in solvent composition, flow rate and temperature so that a reference column may be required to obtain a satisfactory baseline.

Fluorometric detectors are sensitive to compounds that are inherently fluorescent or that can be converted to fluorescent derivatives either by chemical transformation of the compound or by coupling with fluorescent reagents at specific functional groups. If derivatization is required, it can be done prior to chromatographic separation or, alternatively, the reagent can be introduced into mobile phase just prior to its entering the detector.

Potentiometric, voltametric, or polarographic electrochemical detectors are useful for the quantitation of species that can be oxidized or reduced at a working electrode. These detectors are selective, sensitive, and reliable, but require conducting mobile phases free of dissolved oxygen and reducible metal ions. A pulseless pump must be used, and care must be taken to ensure that the pH, ionic strength, and temperature of the mobile phase remain constant. Working electrodes are prone to contamination by reaction products with consequent variable responses.

Electrochemical detectors with carbon-paste electrodes may be used advantageously to measure nanogram quantities of easily oxidized compounds, notably phenols and catechols.

New detectors continue to be developed in attempts to overcome the deficiencies of those being used.

DATA COLLECTION DEVICES Modern data stations receive and store detector output and print out chromatograms complete with peak heights, peak areas, sample identification, and method variables. They are also used to program the liquid chromatograph, controlling most variables and providing for long periods of unattended operation.

Data also may be collected on simple recorders for manual measurement or on stand-alone integrators, which range in complexity from those providing a printout of peak areas to those providing chromatograms with peak areas and peak heights calculated and data stored for possible subsequent reprocessing.

Performance

Criteria for assessing the suitability of the system are described in the "Chromatographic Separation Techniques" (Appendix 3.9). The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given.

Procedure

The mobile phase composition significantly influences chromatographic performance and the resolution of compounds in the mixture being chromatographed. For accurate quantitative work, high-purity reagents and "HPLC grade" organic solvents must be used. Water of suitable quality should have low conductivity and low UV absorption, appropriate to the intended use.

Reagents used with special types of detectors (e.g., electrochemical, mass spectrometer) may require the establishment of additional tolerances for potential interfering species. Composition has a much greater effect than temperature on the capacity factor, k'.

In partition chromatography, the partition coefficient, and hence the separation, can be changed by addition of another component to the mobile phase. In ion-exchange chromatography, pH and ionic strength, as well as changes in the composition of the mobile phase, affect capacity factors. The technique of continuously changing the solvent composition during the chromatographic run is called gradient elution or solvent programming. It is sometimes used to chromatograph complex mixtures of components differing greatly in their capacity factors. Detectors that are sensitive to change in solvent composition, such as the differential refractometer, are more difficult to use with the gradient elution technique.

The detector must have a broad linear dynamic range, and compounds to be measured must be resolved from any interfering substances. The linear dynamic range of a compound is the range over which the detector signal response is directly proportional to the amount of the compound. For maximum flexibility in quantitative work, this range should be about three orders of magnitude. HPLC systems are calibrated by plotting peak responses in comparison with known concentrations of a reference standard, using either an external or an internal standardization procedure.

Reliable quantitative results are obtained by external calibration if automatic injectors or autosamplers are used. This method involves direct comparison of the peak responses obtained by separately chromatographing the test and reference standard solutions. If syringe injection, which is irreproducible at the high pressures involved, must be used, better quantitative results are obtained by the internal calibration procedure where a known amount of a noninterfering compound, the internal standard, is added to the test and reference standard solutions, and the ratios of peak responses of drug and internal standard are compared.

3.6 SIZE-EXCLUSION CHROMATOGRAPHY

Size-exclusion chromatography is a chromatographic technique which separates molecules in solution according to their size. With organic mobile phases, the technique is known as gel-permeation chromatography and with aqueous mobile phases, the term gel-filtration chromatography has been used. The sample is introduced into a column, which is filled with a gel or a porous particle packing material and is carried by the mobile phase through the column. The size separation takes place by repeated exchange of the solute molecules between the solvent of the mobile phase and the same solvent in the stationary liquid phase within the pores of the packing material. The pore-size range of the packing material determines the molecular-size range within which separation can occur.

Molecules small enough to penetrate all the pore spaces elute at the total permeation volume, VT. On the other hand, molecules apparently larger than the maximum pore size of the packing material migrate along the column only through the spaces between the particles of the packing material without being retained and elute at the exclusion volume, VO (void volume). Separation according to molecular size occurs between the exclusion volume and the total permeation volume, useful separation usually occurring in the first two-thirds of this range.

Apparatus

COLUMN If necessary, the column is temperature controlled. It is packed with a separation material that is capable of fractionation in the appropriate range of molecular sizes and through which the eluent is passed at a constant rate. One end of the column is usually fitted with a suitable device for applying the sample, such as a flow adaptor, a syringe through a septum or an injection valve, and it may also be connected to a suitable pump for controlling the flow of the eluent. Alternatively, the sample may be applied directly to the drained bed surface, or, where the sample is denser than the eluent, it may be layered beneath the eluent. The packing material may be a soft support such as a swollen gel or a rigid support composed of a material such as glass, silica, or a solvent-compatible, cross-linked organic polymer. Rigid supports usually require pressurized systems giving faster separations. The mobile phase is chosen according to sample type, separation medium, and method of detection.

DETECTOR The outlet of the column is usually connected to a suitable detector fitted with an automatic recorder that enables the monitoring of the relative concentrations of separated components of the sample. Detectors are usually based on photometric, refractometric, or luminescent properties. An automatic fraction collector may be attached, if necessary.

Performance

Criteria for assessing the suitability of the system are described in the "Chromatographic Separation Techniques" (Appendix 3.9). The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given.

Procedure

Before carrying out the separation, the packing material is treated and the column is packed, as described in the individual monograph or according to the manufacturer's instructions. The elution characteristics of a compound in a particular column may be described by the distribution coefficient, KD, which is calculated by the formula:

$$(V_I - V_O)/(V_T - V_O),$$

in which V_O, V_T, and V_I are the retention volumes for the non-retained component, the component that has full access to all the pores in the support, and the compound under test, respectively. Each retntion volume is measured from the time of application to the time of the peak maximum.

DETERMINATION OF RELATIVE COMPONENT COMPOSITION OF MIXTURE Carry out the separation as directed in the individual monograph. Monitor the elution of the components continuously, and measure the corresponding peak areas. If all the components under test exhibit equivalent responses to the physicochemical property being monitored (for example, if they exhibit corresponding absorptivities), calculate the relative amount of each component by dividing the respective peak area by the sum of the peak areas of all the components under test. If the responses to the property used for the detection of the components under test are not equivalent, calculate the content either from calibration curves obtained from the calibration procedure specified in the individual monograph or by any other means stated in the individual monograph.

DETERMINATION OF MOLECULAR WEIGHTS Size exclusion chromatography is used to determine molecular weights of components under test by comparison to calibration standards specified in the individual monograph. Plot the retention volumes of the calibration standards versus the logarithm of their molecular weights. Draw the line that best fits the plotted points within the exclusion and total permeation limits for the particular separation medium. From the calibration curve, molecular weights of components under test are estimated. This calibration is valid only for the particular macromolecular solute-solvent system used under the specified experimental conditions.

DETERMINATION OF MOLECULAR WEIGHT DISTRIBUTION OF POLYMERS The material used for calibration and the methods for determination of the distribution of molecular weights of polymers are specified in the individual monograph. However, sample comparison is valid only for results obtained under identical experimental conditions.

3.9 CHROMATOGRAPHIC SEPARATION TECHNIQUES

Interpretation of Chromatograms

Fig. 1 represents a typical chromatographic separation of two substances, 1 and 2, where t_1 and t_2 are the respective retention times. h, $h/2$, and $W_{h/2}$ are the height, the half- height, and the width at half-height, respectively, for peak 1. W_1 and W_2 are the respective widths of peaks 1 and 2 at the baseline. Air peaks are a feature of gas chromatograms and correspond to the solvent front in liquid chromatography.

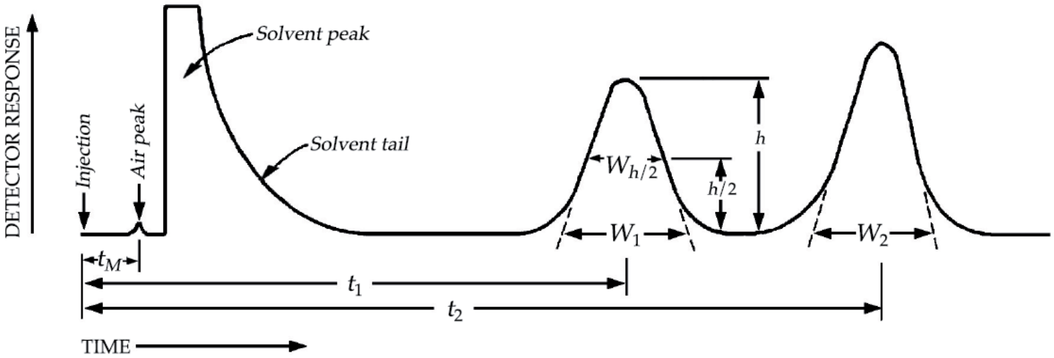


Fig. 1 Chromatographic Separation of Two Substances

Chromatographic retention times are characteristic of the compounds they represent but are not unique. Coincidence of retention times of a test and a reference substance can be used as a feature in construction of an identity profile but is insufficient on its own to establish identity. Absolute retention times of a given compound vary from one chromatogram to the next.

Because in most procedures there is no need to identify an unretained peak, comparisons are normally made in terms of relative retention times, R_r:

$$R_r = \frac{t_2}{t_1},$$

where t_2 and t_1 are retention times, measured from the point of injection, of the test and reference substances, respectively, determined under identical experimental conditions on the same column.

Other procedures may identify the peak position using the relative retention, r:

$$r = \frac{t_2 - t_M}{t_1 - t_M},$$

where t_M is the retention time of a non-retained marker, which needs to be defined in the procedure.

The number of theoretical plates, N, is a measure of column efficiency. For Gaussian peaks, it is calculated by the equation:

$$N = 16\left(\frac{t}{W}\right)^2,$$

where t is the retention time of the substance and W is the width of the peak at its base, obtained by extrapolating the relatively straight sides of the peak to the baseline. The value of N depends on the substance being chromatographed as well as the operating conditions such as mobile phase or carrier gas flow rates and temperature, the quality of the packing, the uniformity of the packing within the column and, for capillary columns, the thickness of the stationary phase film, and the internal diameter and length of the column.

The separation of two components in a mixture, the resolution, R, is determined by the equation:

$$R = \frac{2(t_2 - t_1)}{W_2 + W_1},$$

in which t_2 and t_1 are the retention times of the two components, and W_2 and W_1 are the corresponding widths at the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

Where electronic integrators are used, it may be convenient to determine the resolution, *R*, by the equation:

$$R = \frac{2(t_2 - t_1)}{1.70(W_{1,h/2} + W_{2,h/2})} ,$$

and to determine the number of theoretical plates, *N*, by the equation:

$$N = 5.54(t / W_{2,h/2})^2 ,$$

where $W_{h/2}$ is the peak width at half-height, obtained directly by electronic integrators. However, in the event of dispute, only equations based on peak width at baseline are to be used.

Peak areas and peak heights are usually proportional to the quantity of compound eluting. These are commonly measured by electronic integrators but may be determined by more classical approaches. Peak areas are generally used but may be less accurate if peak interference occurs. For manual measurements, the chart should be run faster than usual, or a comparator should be used to measure the width at half-height and the width at the base of the peak, to minimize error in these measurements. For accurate quantitative work, the components to be measured should be separated from any interfering components. Peak tailing and fronting and the measurement of peaks on solvent tails are to be avoided.

Chromatographic purity tests for drug raw materials are sometimes based on the determination of peaks due to impurities, expressed as a percentage of the area due to the drug peak. It is preferable, however, to compare impurity peaks to the chromatogram of a standard at a similar concentration. The standard may be the drug itself at a level corresponding to, for example, 0.5 per cent impurity, or in the case of toxic or signal impurities, a standard of the impurity itself.

System Suitability

System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the detection sensitivity, resolution, and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such.

The detection sensitivity is a measure used to ensure the suitability of a given chromatographic procedure for the complete detection of the impurities in the Chromatographic purity or Related compounds tests by injecting a volume of a quantitation limit solution equal to that of the Test solution. Unless otherwise specified in the individual monograph, the quantitation limit solution may be prepared by dissolving the Drug Reference Substance in the same solvent as that used for the Test solution at a 0.05 per cent concentration level relative to the amount of drug substance in the Test solution for drug substances, and a 0.1 per cent level relative to the amount of drug substance in the Test solution for drug products. The signal-to-noise ratio for the drug substance peak obtained with the quantitation limit solution should be not less than 10.

The resolution, *R*, (**Note** All terms and symbols are defined in the Glossary of Symbols) is a function of column efficiency, *N*, and is specified to ensure that closely eluting compounds are resolved from each other, to establish the general resolving power of the system, and to ensure that internal standards are resolved from the drug. Column efficiency may be specified also as a system suitability requirement, especially if there is only one peak of interest in the chromatogram; however, it is a less reliable means to ensure resolution than direct measurement. Column efficiency is a measure of peak sharpness, which is important for the detection of trace components.

Replicate injections of a standard preparation used in the assay or other standard solution are compared to ascertain whether requirements for precision are met. Unless otherwise specified in the individual monograph, data from five replicate injections of the analyte are used to calculate the relative standard deviation, RSD, if the requirement is 2.0 per cent or less; data from six replicate injections are used if the relative standard deviation requirement is more than 2.0 per cent.

The symmetry factor (or tailing factor), *T*, a measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced (Fig. 2). In some cases, values less than unity may be observed. As peak asymmetry increases, integration and hence precision, becomes less reliable.

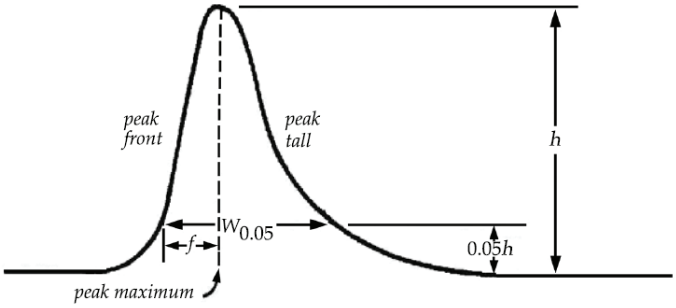


Fig. 2 Asymmetrical Chromatographic Peak

These tests are performed by collecting data from replicate injections of standard or other solutions as specified in the individual monograph. The specification of definitive parameters in a monograph does not preclude the use of other suitable operating conditions. Adjustments of operating conditions to meet system suitability requirements may be necessary.

Unless otherwise directed in the monograph, system suitability parameters are determined from the analyte peak.

Relative retention times may be provided in monographs for informational purposes only, to aid in peak identification. There are no acceptance criteria applied to relative retention times.

To ascertain the effectiveness of the final operating system, it should be subjected to suitability testing. Replicate injections of the standard preparation required to demonstrate adequate system precision may be made before the injection of samples or may be interspersed among sample injections. System suitability must be demonstrated throughout the run by injection of an appropriate control preparation at appropriate intervals.

The control preparation can be a standard preparation or a solution containing a known amount of analyte and any additional materials useful in the control of the analytical system, such as excipients or impurities. Whenever there is a significant change in equipment or in a critical reagent, suitability testing should be performed before the injection of samples. No sample analysis is acceptable unless the requirement of system suitability have been met. Sample analyses obtained while the system fails system suitability requirements are unacceptable.

Adjustment of Chromatographic Conditions

The extent to which the various parameters of a chromatographic test may be adjusted to satisfy the system suitability criteria without fundamentally modifying the methods are listed below for information. The chromatographic conditions described have been validated during the elaboration of the monograph. The system suitability tests are included to ensure the separation required for satisfactory performance of the test or assay. Nonetheless, since the stationary phases are described in a general way and there is such a variety available commercially, with differences in chromatographic behaviour, some adjustments of the chromatographic conditions may be necessary to achieve the prescribed system suitability requirements. With reverse-phase liquid chromatographic methods, in particular, adjustment of the various parameters will not always result in satisfactory chromatography. In that case, it may be necessary to replace the column with another of the same type (e.g., octadecylsilyl silica gel) which exhibits the desired chromatographic behaviour.

For critical parameters the adjustments are defined clearly in the monograph to ensure the system suitability.

Multiple adjustments which may have a cumulative effect in the performance of the system are to be avoided.

Thin-layer Chromatography and Paper Chromatography

COMPOSITION OF THE MOBILE PHASE; pH OF THE AQUEOUS COMPONENT OF THE MOBILE PHASE; CONCENTRATION OF SALTS The adjustments can be made as described under High-pressure Liquid Chromatography (Appendix 3.5).

APPLICATION VOLUME The application volume can be adjusted to 10 to 20 per cent of the prescribed volume if using fine particle size plates (2 to 10 μm).

MIGRATION DISTANCE The migration distance of the solvent front is to be not less than 50 mm or 30 mm on high-performance plates.

High-pressure Liquid Chromatography

COMPOSITION OF THE MOBILE PHASE The following adjustment limits apply to minor components of the mobile phase (specified at 50 per cent or less). The amount(s) of these component(s) can be adjusted by ±30 per cent relative. However, the change in any component cannot exceed ±10 per cent absolute (i.e., in relation to the total mobile phase), nor can the final concentration of any component be reduced to zero. Adjustment can be made to one minor component in a ternary mixture. Examples of adjustments are given below.

Specified ratio of 50:50 Thirty per cent of 50 is 15 per cent absolute, but this exceeds the maximum permitted change of ±10 per cent absolute in either component. Therefore, the mobile phase ratio may be adjusted only within the range of 40:60 to 60:40.

Specified ratio of 2:98 Thirty per cent of 2 is 0.6 per cent absolute. Therefore, the maximum allowed adjustment is within the range of 1.4:98.6 to 2.6:97.4.

Specified ratio of 60:35:5 For the second component, 30 per cent of 35 is 10.5 per cent absolute, which exceeds the maximum permitted change of ±10 per cent absolute in any component. Therefore, the second component may be adjusted only within the range of 25 per cent to 45 per cent absolute. For the third component, 30 per cent of 5 is 1.5 per cent absolute. In all cases, a sufficient quantity of the first component is used to give a total of 100 per cent. Therefore, mixture ranges of 50:45:5 to 70:25:5 or 58.5: 35:6.5 to 61.5:35:3.5 would meet the requirement.

pH OF THE AQUEOUS COMPONENT OF THE MOBILE PHASE The pH of the aqueous buffer used in the preparation of the mobile phase can be adjusted to within ±0.2 units of the value or range specified, or ±1.0 units when neutral substances are to be examined.

CONCENTRATION OF SALTS The concentration of the salts used in the preparation of the aqueous buffer used in the mobile phase can be adjusted to within ±10 per cent, provided the permitted pH variation is met.

DETECTOR WAVELENGTH No adjustment permitted.

STATIONARY PHASE
—column length: ±70 per cent,
—column internal diameter: ±25 per cent,
—particle size: maximal reduction of 50 per cent, no increase permitted.

FLOW RATE The flow rate can be adjusted by as much as ±50 per cent.

COLUMN TEMPERATURE The column temperature can be adjusted by as much as ±10°. Column thermostating is recommended to improve control and reproducibility of retention time.

INJECTION VOLUME The injection volume can be reduced as far as is consistent with accepted precision and detection limits.

Gas Chromatography

STATIONARY PHASE
—column length: ±70 per cent,
—column internal diameter: ±50 per cent,
—particle size: maximal reduction of 50 per cent, no increase permitted,
—film thickness: –50 per cent to +100 per cent.

FLOW RATE The flow rate can be adjusted by as much as ±50 per cent.

OVEN TEMPERATURE The oven temperature can be adjusted by as much as ±10 per cent.

OVEN TEMPERATURE PROGRAM Adjustment of temperatures is permitted as stated above. For the times specified for the temperature to be maintained or for the temperature to be changed from one value to another, an adjustment of up to ±20 per cent is permitted.

INJECTION VOLUME The injection volume can be reduced as far as is consistent with accepted precision and detection limits.

Glossary of Symbols

To promote uniformity of interpretation, the following symbols and definitions are employed where applicable in presenting formulas in the individual monographs. Where a different symbol or definition is used in an individual monograph, the monograph text takes precedence. (**Note** Where the terms *W* and *t* both appear in the same equation, they must be expressed in the same units.)

f distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5 per cent of the peak height from the baseline.

k' capacity factor,

$$k' = \frac{\text{amount of substance in stationary phase}}{\text{amount of substance in mobile phase}}$$

$$k' = \frac{\text{time spent by substance in stationary phase}}{\text{time spent by substance in mobile phase}} = \frac{t}{t_M} - 1$$

N number of theoretical plates in a chromatographic column,

$$N = 16\left(\frac{t}{W}\right)^2 \text{ or } N = 5.54\left(\frac{t}{W_{h/2}}\right)^2$$

r relative retention,

$$r = \frac{t_2 - t_M}{t_1 - t_M}$$

R resolution between two chromatographic peaks,

$$R = \frac{2(t_2 - t_1)}{W_1 + W_2} \text{ or } R = \frac{2(t_2 - t_1)}{1.70(W_{1,h/2} + W_{2,h/2})}$$

R_r relative retention time,

$$R_r = \frac{t_2}{t_1}$$

$RSD\ (\%)$ relative standard deviation in percentage,

$$RSD\ (\%) = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}(X_i - \overline{X})^2}{n-1}\right]^{1/2}$$

where X_i is an individual measurement in a set of n measurements and $\overline{X}$ is the arithmetic mean of the set.

T	symmetry factor (or tailing factor),
t	retention time measured from time of injection to time of elution of peak maximum.
t_M	retention time of non-retained component, air with thermal conductivity detection.
V	retention volume, $V = \text{flow rate} \times t$
V_O	retention volume of non-retained component (void volume of the column)
W	width of peak measured by extrapolating the relatively straight sides to the baseline.
$W_{h/2}$	width of peak at half height.
$W_{0.05}$	width of peak at 5 per cent height.

APPENDIX 4 PHYSICAL TESTS

4.3 DETERMINATION OF MELTING RANGE AND MELTING TEMPERATURE

For Pharmacopoeial purposes, the melting range of a substance is defined as the range between the temperature at which the substance begins to collapse or form droplets on the wall of a capillary tube and the temperature at which it is completely melted as shown by the disappearance of the solid phase; the latter temperature is also considered to be the melting temperature, except as defined otherwise for Classes II and III below. Any apparatus or method capable of equal accuracy may be used. The apparatus should be calibrated with appropriate substances of known melting point.[1]

Five procedures for the determination of melting range and melting temperature are given herein, varying in accordance with the nature of the substance. When no class is designated in the monograph, use the procedure for Class Ia. The procedure known as the mixed-melting point determination, whereby the melting range of a solid under test is compared with that of an intimate mixture of equal parts of the solid and an authentic sample of it, may be used as a confirmatory identification test. Agreement of the observations on the original and the mixture constitutes reliable evidence of chemical identity.

Apparatus I

An example of a suitable melting range Apparatus I consists of a glass container for a bath of transparent fluid, a suitable stirring device, an accurately standardized thermometer, and a controlled source of heat. The thermometers should cover the range of –10º to +360º, the length of one degree on the scale being not less than 0.8 mm. Each thermometer should preferably be of the mercury-in-glass, solid-stem type with a cylindrical bulb and made of approved thermometric glass suitable for the range covered, and should have a safety chamber. The bath fluid is selected with a view to the temperature required, but liquid paraffin is used generally and certain liquid silicones are well adapted to the higher temperature ranges. The fluid is deep enough to permit immersion of the thermometer to its specified immersion depth so that the bulb is still about 2 cm above the bottom of the bath. The heat may be supplied electrically or by an open flame. The capillary tube is about 10 cm long and 0.8 to 1.2 mm in internal diameter with walls, 0.2 to 0.3 mm in thickness.

The thermometer is selected for the desired accuracy and range of temperature.

The procedures for Classes I and Ia are applied to those substances readily reduced to fine powders.

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[1] Suitable substances are issued by the World Health Organization Collaborating Centre for Chemical Reference Substances, Apotekens Centrallaboratorium, Box 3045, 17103 SOLNA 3, Sweden.

Apparatus II

An instrument may be used in the procedures for Classes I, Ia, and Ib. An example of a suitable melting range Apparatus II consists of a block of metal that maybe heated at a controlled rate, its temperature being monitored by a sensor. The block accommodates the capillary tube containing the test substance and permits monitoring of the melting process, typically by means of a beam of light and a detector. The detector signal maybe processed by a microcomputer to determine and display the melting point or range, or the detector signal may be plotted to allow visual estimation of the melting point or range.

Procedure for Class I

Apparatus I—Reduce the substance under test to a very fine powder. Unless otherwise directed, render it anhydrous when it contains water of hydration by drying at the temperature specified in the monograph. When the substance contains no water of hydration, dry it as directed for Loss on drying in the individual monograph; or if no Loss on drying test is specified, dry it over a suitable desiccant for not less than 16 hours.

Charge a capillary glass tube, one end of which is sealed, with sufficient quantity of the dry powder to form a column in the bottom of the tube 2.5 to 3.5 mm high when packed down as closely as possible by moderate tapping on a solid surface.

Heat the bath until the temperature is about 30° below the expected melting point. Remove the thermometer, and quickly attach the capillary tube to the thermometer by wetting both with a drop of the liquid of the bath, and adjust its height so that the material in the capillary is level with the thermometer bulb. Replace the thermometer, and continue the heating, with constant stirring sufficiently to cause the temperature to rise at a rate of about 3° per minute. When the temperature is about 3° below the lowest figure of the melting range for the substance under test, reduce the heating so that the temperature rises at a rate of about 1° to 2° per minute. Continue heating until melting is complete.

The temperature at which the column of the substance under test is observed to collapse definitely against the side of the tube at any point is defined as the beginning of melting and the temperature at which the test substance becomes liquid throughout is defined as the end of melting or the "melting temperature". The two temperatures fall within the limits of the melting range.

Apparatus II—Prepare the substance under test and charge the capillary tube as directed for *Class I, Apparatus I.* Operate the apparatus according to the manufacturer's instructions: Heat the block until the temperature is about 30° below the expected melting point. Insert the capillary tube into the heating block, and continue heating at a rate of temperature increase of about 1° to 2° per minute until melting is complete.

The temperature at which the detector signal first leaves its initial value is defined as the beginning of melting, and the temperature at which the detector signal reaches its final value is defined as the end of melting, or the melting point. The two temperatures fall within the limits of the melting range.

In the event of dispute, only the melting range or temperature obtained as directed for *Class I, Apparatus I,* is definitive.

Procedure for Class Ia

Apparatus I—Prepare the substance under test and charge the capillary as directed for *Class I.*

Heat the bath until the temperature is about 10° below expected melting point and is rising at a rate of 1°±0.5° per minute. Insert the capillary as directed under *Class I* when the temperature is about 5° below the lowest figure of the melting range for the substance under test and continue heating until melting is complete. Record the melting range as directed for *Class I, Apparatus I.*

Procedure for Class Ib

Apparatus I—Place the substance under test in a closed container and cool to 10°, or lower, for at least 2 hours. Without previous powdering, charge the cooled material into the capillary tube as directed for *Class I,* immediately place the charged tube in a vacuum desiccator and dry at a pressure not exceeding 2.7 kPa (about 20 Torr) for 3 hours. Immediately upon removal from the desiccator, fire-seal the open end of the tube, and as soon as practicable proceed with the determination of the melting range as follows: heat the bath until a temperature is 10°±1° below the lowest figure of the melting range for the substance under test, introduce the charged tube, and heat at a rate of rise of 3°±0.5° per minute until melting is complete. Record the melting range as directed for *Class I, Apparatus I.*

If the particle size of the material is too large for the capillary, pre-cool the test substance as directed above, then with as little pressure as possible gently crush the particles to fit the capillary, and immediately charge the tube.

Procedure for Class II

This procedure is applied to such substances as fats, fatty acids, paraffins or waxes, that are insoluble in water and not readily reduced to powder. Carefully melt the material under test at as low a temperature as possible, and draw it into a capillary tube, which is left open at both ends, to a height of about 10 mm. Cool the charged tube at 10°, or lower, for 24 hours, or in contact with ice for at least 2 hours. Then attach the tube to the thermometer by a suitable means, adjust it in a water-bath so that the upper edge of the material is 10 mm below the water level, and heat as directed for *Class I* except, within 5° of the expected melting temperature, to regulate the rate of rise of temperature to 0.5° to 1.0° per minute. The temperature at which the material is observed to rise in the capillary tube is the melting temperature.

Procedure for Class III

This procedure is applied to petrolatums. Melt a quantity of the substance under test slowly, while stirring, until it reaches a temperature of 90° to 92°. Remove the source of the heat and allow the molten substance to cool to a temperature of 8° to 10° above the expected melting temperature. Chill the bulb of a suitable thermometer to 5°, wipe it dry, and while still cold, dip into the molten substance so that approximately the lower half of the bulb is submerged. Withdraw it immediately, and hold vertically away from the heat until the wax surface dulls. Then dip for 5 minutes into a water-bath having a temperature not higher than 16°.

Fix the thermometer securely in a test-tube so that the lower end is 1.5 cm above the bottom of the test-tube. Suspend the test-tube in a water-bath adjusted to about 16°, and raise the temperature of the bath at a rate of 2° per minute to 30°. Then change to a rate of 1° per minute, and note the temperature at which the first drop of melted substance leaves the thermometer. Repeat the determination twice on a freshly melted portion of the substance under test. If the variation of three determinations is less than 1°, take the average of the three as the melting temperature. If the variation of three determinations is more than 1°, make two additional determinations and take the average of the five.

4.12 DETERMINATION OF WATER

Karl Fischer Method

Principle The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine dissolved in pyridine and methanol. The test sample may be titrated with Karl Fischer reagent directly or the analysis may be carried out by a residual titration procedure. In the residual titration, excess Karl Fischer reagent is added to the test sample, sufficient time is allowed for the reaction to reach completion, and the unconsumed Karl Fischer reagent is titrated with a standard solution of water in methanol. The residual titration procedure is applicable generally and avoids the difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly.

The stoichiometry of the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative concentrations of the Karl Fischer reagent ingredients, the nature of the inert solvent used to dissolve the test sample, and the technique used in the particular determination. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test sample; however, other suitable solvents may be used for special or unusual test sample.

Apparatus Use a closed system consisting of a suitable titration vessel, previously dried at 105° and cooled in a desiccator, fitted with two platinum electrodes, a nitrogen inlet tube, a stopper which accommodates the burette tip, a vent tube protected by a suitable desiccant, and a magnetic stirrer, connected to a suitable electrometric apparatus. This apparatus embodies a simple electrical circuit which serves to pass 5 to 10 μA of direct current between a pair of platinum electrodes immersed in the solution to be titrated. At the endpoint of the titration a slight excess of the reagent increases the flow of current to between 50 and 150 μA for 30 seconds or longer, depending upon the solution being titrated.

Reagent

KARL FISCHER REAGENT Dissolve 125 g of *iodine* in 170 ml of *pyridine*, add 670 ml of *anhydrous methanol*, and cool. To 100 ml of *pyridine* kept in an ice-bath, add liquid *anhydrous sulfur dioxide* until the volume reaches 200 ml. Slowly add this solution to the cooled iodine mixture, and shake well to dissolve the iodine. Allow the solution to stand for 24 hours before use. Preserve the reagent under refrigeration in a suitably sealed, glass-stoppered bottle, protected from light and from moisture in the air. This methanolic reagent is somewhat unstable and requires frequent standardization. A more stable reagent may be prepared by substituting *2-methoxyethanol* for *anhydrous methanol.* A commercially available, stabilized solution of Karl Fischer reagent may be used.

METHANOL SOLUTION Add sufficient *water* (usually 2 ml per litre) to *anhydrous methanol* so that each ml of the resulting methanol solution is equivalent to about 0.5 ml of Karl Fischer reagent.

Standardization of the reagent

WATER EQUIVALENCE OF KARL FISCHER REAGENT (F)

Standardize the Karl Fischer reagent no longer than 1 hour before use by one of the following methods.

Place enough *anhydrous methanol* in the titration vessel to cover the electrodes, and add sufficient Karl Fischer reagent to give the characteristic end-point colour, or 100±50 µA of direct current at about 200 mV of applied potential.

For determination of trace amounts of water (less than 1 per cent), *sodium tartrate* may be used as a convenient water reference substance.

Quickly add 120 to 300 mg of *sodium tartrate* ($C_4H_4Na_2O_6\cdot 2H_2O$), accurately weighed by difference, and titrate to the end-point. Calculate the water equivalence of the Karl Fischer reagent as follows:

$$F = 2(18.02/230.08)(W/Vr),$$

where F = water equivalence of the Karl Fischer reagent in terms of mg of water per ml,

18.02 = molecular weight of water,

W = weight of *sodium tartrate* in mg,

230.08 = molecular weight of *sodium tartrate*, and

Vr = ml of Karl Fischer reagent consumed in the second titration.

For the precise determination of significant amounts of water (more than 1 per cent), use *water* obtained by distillation as the reference substance.

Quickly add between 25 and 250 mg of *water*, accurately weighed by difference, from a weighing pipette or from a pre-calibrated syringe or micropipette. Titrate to the end-point. Calculate the water equivalence of Karl Fischer reagent as follows:

$$F = W/Vr,$$

where F = water equivalence of the Karl Fischer reagent in terms of mg of water per ml,

W = weight of water in mg, and

Vr = ml of Karl Fischer reagent consumed in the second titration.

KARL FISCHER REAGENT EQUIVALENCE OF METHANOL SOLUTION (*f*)

Titrate a known volume of Karl Fischer reagent with methanol solution until the end-point is reached. Calculate the millilitres of Karl Fischer reagent equivalent to each ml of methanol solution as follows:

$$f = Vr / Vm,$$

where f = ml of Karl Fischer reagent equivalent to each ml of methanol solution,

Vr = ml of Karl Fischer reagent used, and

Vm = ml of methanol solution used.

Sample preparation For Pharmacopoeial articles, unless otherwise specified in the individual monograph, use an accurately weighed or measured amount of the specimen under test estimated to contain 10 to 250 mg of *water*.

TABLETS AND CAPSULES Tablets, use accurately weighed powder from not less than 4 tablets ground to a fine powder in an atmosphere of about 10 per cent relative humidity.

Capsules, use an accurately weighed portion of the mixed contents of not less than 4 capsules.

OINTMENTS AND OILS Use an accurately weighed portion of about 1 to 2 g.

AEROSOLS WITH PROPELLANT Store it in a suitable freezing unit having a temperature of not higher than 0° for at least 2 hours. After removing from the freezer, immediately open the container, and test 10.0 ml of the well-mixed sample. In titrating the sample, determine the end-point at a temperature of 10° or higher.

HYGROSCOPIC POWDERS Accurately weigh the immediate container. Using a suitable dry hypodermic needle and syringe, inject an appropriate volume of anhydrous methanol, accurately measured, into the container and shake to dissolve the contents. Using the same syringe, remove the solution from the container and transfer it to a titration vessel containing 20 ml of a mixture of *carbon tetrachloride, chloroform* and *anhydrous methanol* (2:2:1), previously titrated to the end-point as directed under Titration procedure. Repeat the procedure with a second portion of *anhydrous methanol,* accurately measured, add this washing to the titration vessel, and immediately titrate. Determine the volume, in ml, of Karl Fischer reagent equivalent to a portion of *anhydrous methanol* of the same total volume as that used to dissolve the sample and to wash the container and syringe, as directed under Titration procedure. Subtract this value from the volume, in ml, of Karl Fischer reagent obtained in the titration of the sample under test. Dry the container and its closure at 100° for 3 hours, allow to cool in a desiccator, and weigh. Determine the weight of the sample tested from the difference in weight from the initial weight of the container.

Titration procedure Determine the water by Method I, unless otherwise specified in the individual monograph. In the case of a colourless solution that is titrated directly, the end-point may be observed visually as a change in colour from canary-yellow to amber. The reverse is observed in the case of a test sample that is titrated residually. More commonly, however, the end-point is determined electrometrically.

METHOD I: DIRECT TITRATION Add 20 ml of *anhydrous methanol* to the titration vessel, and titrate with Karl Fischer reagent to the electrometric or visual end-point to consume any moisture that may be present. (Disregard the volume consumed, since it does not enter into the calculations.) Quickly add an accurately weighed or measured amount of the sample under test, mix, and again titrate with Karl Fischer reagent to the electrometric or visual end-point.

METHOD II: RESIDUAL TITRATION Where the individual monograph specifies that the water content is to be determined by Method II, the residual titration procedure, transfer 20 ml of *anhydrous methanol* to the titration vessel, and titrate with Karl Fischer reagent to the electrometric or visual end-point. Quickly add an accurately weighed or measured amount of the sample under test, mix, and add an accurately measured excess of Karl Fischer reagent. Allow sufficient time for the reaction to reach completion, and titrate the unconsumed Karl Fischer reagent with standardized methanol solution to the electrometric or visual end-point.

Calculation

Calculate the percentage of water in the sample as follows:

If titration Method I is used:

Percentage of water in weighed samples = $(Vr \times F \times 100)/Ws$

Percentage of water in aerosols = $Vr \times F/(\text{ml of sample used} \times 10)$

Percentage of water in hygroscopic powders = $[(Vr - Vb) \times F \times 100]/Ws$

If titration Method II is used:

Percentage of water in weighed samples = $[(Vr - Vm \times f) \times F \times 100]/Ws$

Percentage of water in aerosols = $[(Vr - Vm \times f) \times F]/(\text{ml of sample used} \times 10)$

Percentage of water in hygroscopic powders = $[(Vr - Vm \times f - Vb) \times F \times 100]/Ws$

where f = ml of Karl Fischer reagent equivalent to each ml of methanol solution determined as directed in Karl Fischer reagent equivalence of methanol solution,

F = water equivalence of the Karl Fischer reagent determined as directed in Water Equivalence of Karl Fischer reagent of this section,

Vb = ml of Karl Fischer reagent equivalent to the *anhydrous methanol* used as a sample solvent,

Vm = ml of methanol solution used,

Vr = ml of Karl Fischer reagent used, and

Ws = weight of the sample in mg.

Coulometric Titration

Principle The Karl Fischer reaction is used in the coulometric determination of water. Iodine, however, is not added in the form of a volumetric solution but is produced in an iodide-containing solution by anodic oxidation. The reaction cell usually consists of a large anode compartment and a small cathode compartment that are separated by a diaphragm. Other suitable types of reaction cells (e.g., without diaphragms) may also be used. Each compartment has a platinum electrode that conducts current through the cell. Iodine, which is produced at the anode electrode, immediately reacts with water present in the compartment. When all the water has been consumed, an excess of iodine occurs, which usually is detected electrometrically, thus indicating the end-point. Moisture is eliminated from the system by pre-electrolysis. Changing the Karl Fischer reagent after each determination is not necessary since individual determinations can be carried out in succession in the same reagent solution. A requirement for this method is that each component of the test sample is compatible with the other components, and no side reactions take place. Samples are usually transferred into the vessel as solutions by means of injection through a septum. Gases can be introduced into the cell by means of a suitable gas inlet tube. Precision in the method is predominantly governed by the extent to which atmospheric moisture is excluded from the system; thus, the introduction of solids into the cell is not recommended, unless elaborate precautions are taken, such as working in a glove-box in an atmosphere of dry inert gas. Control of the system may be monitored by measuring the amount of baseline drift. This method is particularly suited to chemically inert substances like hydrocarbons, alcohols, and ethers. In comparison with the volumetric Karl Fischer titration, coulometry is a micromethod.

Apparatus Any commercially available apparatus consisting of an absolutely tight system fitted with the necessary electrodes and a magnetic stirrer is appropriate. The instrument's microprocessor controls the analytical procedure and displays the results. Calibration of the instrument is not necessary, as the current consumed can be measured absolutely.

Reagent See Reagent under Karl Fischer Method.

Sample preparation Where the sample is a soluble solid, dissolve an appropriate quantity, accurately weighed, in *anhydrous methanol* or other suitable solvents. Liquids may be used as such or as accurately prepared solutions in appropriate anhydrous solvents.

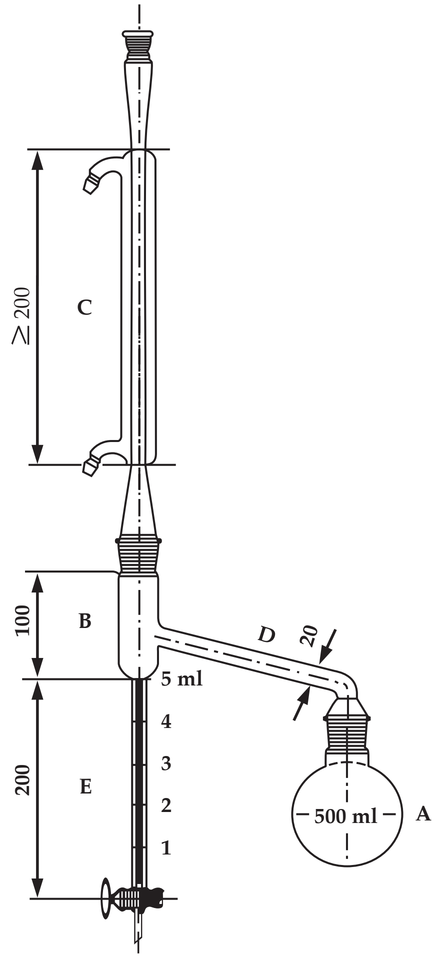
Where the sample is an insoluble solid, the water may be extracted using a suitable anhydrous solvent from which an appropriate quantity, accurately weighed, may be injected into the anolyte solution. Alternatively an evaporation technique may be used in which water is released and evaporated by heating the specimen in a tube in a stream of dry inert gas, this gas being then passed into the cell.

Procedure Using a dry syringe, quickly inject sample preparation, accurately measured and estimated to contain 0.5 to 5 mg of *water*, or as recommended by the instrument manufacturer into the anolyte, mix, and perform the coulometric titration to the electrometric end-point. Read the water content of sample preparation directly from the instrument's display, and calculate the percentage that is present in the substance. Perform a blank determination, and make any necessary corrections.

Calculation One mole of iodine corresponds to 1 mole of water, a quantity of electricity of 10.71 C corresponds to 1 mg of water.

Azeotropic Distillation Method

Apparatus The apparatus (see figure) consists of a glass flask (A) connected by a tube (D) to a cylindrical tube (B) fitted with a graduated receiving tube (E) and a reflux condenser (C). The receiving tube (E) is graduated in 0.1-ml subdivisions so that the error of reading is not greater than 0.05 ml. The source of heat is preferably an electric heater with rheostat control or an oil-bath. The upper portion of the flask and the connection tube may be insulated with asbestos.



Apparatus for Determination of Water by the Azeotropic Distillation Method
Dimensions in mm

Method Clean the receiving tube and the condenser of the apparatus by a suitable method, thoroughly rinse with *water*, and dry.

Introduce 200 ml of *toluene* and about 2 ml of *water* into the dry flask. Distil for about 2 hours, allow to cool to room temperature and read the water volume to an accuracy of 0.05 ml. Place in the flask a quantity of the substance, weighed to the nearest centigram, expected to give about 2 to 3 ml of *water*. If the substance is of a pasty character, weigh it in a boat of metal foil. Add a few pieces of porous material and heat the flask gently for 15 minutes. When the toluene begins to boil, distil at the rate of 2 drops per second until most of the water has distilled over, and then increase the rate of distillation to about 4 drops per second. When the water has all distilled over, rinse the inside of the condenser tube with toluene. Continue the distillation for 5 minutes, remove the heat, allow the receiving tube to cool to room temperature, and dislodge any droplets of water which adhere to the walls of the receiving tube. When the water and toluene have completely separated, read the volume of water and calculate the percentage present in the substance using the expression:

$$\frac{100\,(n' - n)}{p}\ ,$$

where p = the weight in g of the substance to be examined,
n = the volume in ml of water obtained in the first distillation, and
n' = the total volume in ml of water obtained in the two distillations.

4.15 LOSS ON DRYING

The procedure set forth in this section determines the amount of volatile matter of any kind that is driven off under the conditions specified. For substances appearing to contain water as the only volatile constituent, the procedure given in "Determination of Water" (Appendix 4.12), is appropriate, and is specified in the individual monograph.

Unless otherwise directed in the monograph, conduct the determination on 1 to 2 g of the substance (2 to 5 g in case of crude drugs), previously mixed and accurately weighed. If the test substance is in the form of large crystals, reduce the particle size to about 2 mm by quickly crushing. Tare a glass-stoppered, shallow weighing bottle that has been dried for 30 minutes under the same conditions to be employed in the determination. Put the test substance in the bottle, replace the cover, and accurately weigh the bottle and the contents. By gentle, sidewise shaking distribute the test substance as evenly as practicable to a depth of about 5 mm generally, and not over 10 mm in the case of bulky materials. Place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber. Dry the test substance at the temperature and for the time specified in the monograph. The temperature of heating is within the range of ±2° of the stated figure in the monograph. Upon opening the chamber, close the bottle promptly and allow it to come to room temperature in a desiccator before weighing.

If the substance melts at a lower temperature than that specified for the determination of Loss on drying, maintain the bottle with its contents for 1 to 2 hours at a temperature 5° to 10° below the melting temperature, then dry at the specified temperature.

Where the sample under test is Capsules, use a portion of the mixed contents of not less than 4 capsules.

Where the sample under test is Tablets, use powder from not less than 4 tablets ground to a fine powder.

Where the individual monograph directs that loss on drying be determined by thermogravimetric analysis, a sensitive electrobalance is to be used.

Where drying in vacuum over a desiccant is directed in the individual monograph, a vacuum desiccator or a vacuum drying pistol, or other suitable vacuum drying apparatus, is to be used.

Where drying in a desiccator is specified, exercise particular care to ensure that the desiccant is kept fully effective by frequent replacement.

Where drying in a capillary-stoppered bottle in vacuum is directed in the individual monograph, use a bottle or tube fitted with a stopper having a 225±25 μm diameter capillary, and maintain the heating chamber at a pressure not exceeding 0.7 kPa (about 5 Torr). At the end of the heating period, admit dry air to the heating chamber, remove the bottle, and with the capillary stopper still in place allow it to cool in a desiccator before weighing.

4.24H DISSOLUTION TEST FOR HERBAL DRUG PREPARATIONS

Introduction

This test is provided to determine compliance with the dissolution requirements where stated in the individual monograph for herbal drug preparations, except where the label states that tablets are to be chewed. See “Dissolution Test” (Appendix 4.24) for description of apparatus used, Apparatus Suitability Test and other related information. Of the types of apparatus described in “Dissolution Test” (Appendix 4.24), use the one specified in the individual monograph.

Compliance with dissolution requirements necessitates the testing of six dosage units individually, or testing two or more dosage units in each of the six vessels of the dissolution apparatus and measuring the dissolution of the marker compound(s) specified in the individual monograph.

Procedure

Combine equal volumes of the filtered solutions of the six or more individual samples withdrawn, and use the pooled sample as the test solution. Determine the average amount of the marker compound(s) dissolved in the pooled sample by the procedure specified in the individual monograph. Make any necessary modifications, including concentration of the analyte in the volume of the test solution taken. Use the dissolution medium for preparation of the standard solution and dilution, if necessary, of the test solution.

APPENDIX 5 CHEMICAL TESTS

5.3 DETERMINATION OF SULFATED ASH

Use Method I unless otherwise directed.

Method I

Heat a platinum dish to redness for 10 minutes, allow to cool in a desiccator and weigh. Place about 1 g of the substance, or the quantity specified in the monograph, accurately weighed, in the dish, moisten with *sulfuric acid*, heat gently until white fumes are no longer evolved, again moisten with *sulfuric acid* and ignite at 800º±25º until the carbon is consumed. Allow the crucible to cool in a desiccator over *self-indicating silica gel*, weigh it again and calculate the weight of the residue. If the weight of residue so obtained exceeds the prescribed limit, repeat the moistening with *sulfuric acid* and ignition, as previously, to constant weight, unless otherwise prescribed.

Method II

Ignite a suitable crucible (silica, platinum, porcelain or quartz) at 600º±50º for 30 minutes, allow to cool in a desiccator over *self-indicating silica gel* and weigh. Place the prescribed amount of the substance in the crucible and weigh. Moisten the substance with a small amount of *sulfuric acid* (usually 1 ml) and heat gently at temperature as low as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount of *sulfuric acid*, heat gently until white fumes are no longer evolved and ignite at 600º±50º until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Allow the crucible to cool in a desiccator over *self-indicating silica gel*, weigh it again and calculate the weight of the residue. If the weight of residue so obtained exceeds the prescribed limit, repeat the moistening with *sulfuric acid* and ignition, as previously, to constant weight, unless otherwise prescribed.

APPENDIX 6 ASSAYS

6.17 RESIDUAL TITRATIONS

Some assays require the addition of a measured volume of a volumetric solution, in excess of the amount actually needed to react with the substance being assayed, the excess of this solution then being titrated with a second volumetric solution. This constitutes a residual titration and is known also as a “back titration”. The quantity of the substance being titrated may be calculated from the difference between the volume of the volumetric solution originally added and that consumed by the titrant in the back titration, due allowance being made for the respective molarity factors of the two solutions, and the equivalence factor for the substance given in the individual monograph.

APPENDIX 7 CRUDE DRUGS

7.1 SAMPLING

Sampling of material in bulk

Unless otherwise specified, the sample should be taken by the following methods, and, if necessary, preserved in tightly closed containers.

1. Examine the uniformity and the integrity of the containers (or of the outer wrapping), the markings and the labelling in the whole batch.

2. If on external examination the batch is considered as homogeneous, take individual samples from the number of packages as indicated in the following table.

Schedule Recommended for Sampling

Number of Packages in the Batch	Number of Packages to Be Sampled (n)
1 to 5	All
6 to 50	5
> 50*	10 per cent

*Round calculated "n" to next highest whole number.

3. From each container or package selected, take three original samples, taking care to avoid fragmentation. Samples should be taken from the top, middle and bottom of the container. In the case of sacks and packages, the three samples should be taken by hand, the first from a depth of not less than 10 cm from the top and the second and third from the middle and bottom after cutting into the side of the package. Samples of seeds should be withdrawn with a grain probe. Material in boxes should first be sampled from the upper layer; then approximately half of the contents should be removed and a second sample taken. Finally after further removal of material, another sample should be taken from the bottom. Samples should be as uniform as possible in mass. The three original samples should then be combined into a pooled sample which should be mixed carefully.

The average sample is obtained by quartering. Form the pooled sample, adequately mixed, into an even and square-shaped heap, and divide it diagonally into four equal parts. Take two diagonally opposite parts and mix carefully. Repeat the process as necessary until the required quantity, to within ±10 per cent, is obtained. Any remaining material should be returned to the batch.

Using the same quartering procedure, divide the average sample into four final samples, taking care that each portion is representative of the bulk material.

Sampling of material in retail packages

From each wholesale container (boxes, cartons, etc.) selected for sampling, take at random two consumer packages. From small batches (1 to 5 boxes), take ten consumer packages. Prepare the pooled sample by mixing the contents of the selected consumer packages and proceed as described above to obtain the final sample.

7.2 FOREIGN MATTER

Vegetable drugs should be free from moulds, insects and other animal contamination. Foreign matter is material consisting of any or all of the following:

1. *Foreign organs*: matter coming from the source plant but not defined as the drug.

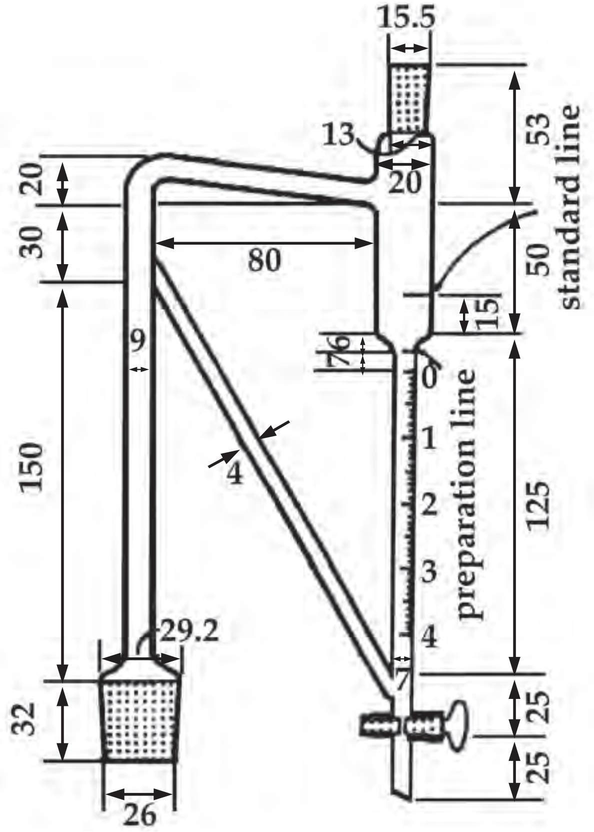
2. *Foreign elements*: matter not coming from the source plant and of either vegetable or mineral origin.

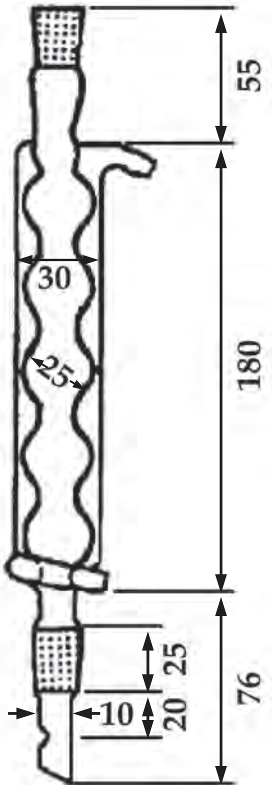
Method

Weigh 100 to 500 g of the substance being examined or the quantity specified in the monograph and spread it in a thin layer. Separate the foreign matter by hand as completely as possible, weigh it and calculate the percentage present.

7.3H DETERMINATION OF VOLATILE OIL

Carry out the determination according to the nature of the drug to be examined. Place the prescribed quantity of the drug in the round-bottomed flask of suitable capacity as specified in the monograph and add the prescribed volume of distillation liquid. Set up the apparatus (Fig. 1), inserting a reflux condenser (Fig. 2). Fill the graduated tube with *water* to the standard line and add the prescribed volume of *xylene*. Heat the liquid in the flask in an oil-bath between 130° and 150° to boiling and adjust the distillation rate as prescribed in the monograph. Unless otherwise specified, continue boiling for 5 hours at the same rate. Allow it to stand for some time, open the stopper of the apparatus and draw off the water slowly until the level of the xylene and volatile oil mixture corresponds to the preparation line, and allow it to stand for more than 1 hour at cool temperature. Then lower the level of the xylene and volatile oil mixture to the zero line, and read the volume in millilitre of the mixture of xylene and volatile oil at cool temperature. Subtract the volume in millilitre of xylene from the volume of the mixture of xylene and volatile oil and calculate the percentage of volatile oil content in the sample.





Dimensions in mm

Fig. 1

Fig. 2

7.4 TEST FOR COMPLETE EXTRACTION OF ALKALOIDS

Complete extraction is indicated by the following tests.

For an Aqueous or Alcoholic Extraction

After extracting at least three times with the liquid, add to a few drops of the next portion, after acidifying with 2 M *hydrochloric acid* if necessary, 0.05 ml of *mercuric-potassium iodide TS* or, for solanaceous alkaloids, 0.05 ml of *potassium iodobismuthate TS*: no precipitate, or turbidity, is produced.

For an Immiscible Solvent Extraction

After extracting at least three times with the solvent, add to 1 to 2 ml of the next portion 1 to 2 ml of 0.1 M *hydrochloric acid*, remove the organic solvent by evaporation, transfer the aqueous residue to a test-tube, and add 0.05 ml of *mercuric-potassium iodide TS* or, for solanaceous alkaloids, 0.05 ml of *potassium iodobismuthate TS* or for emetine, 0.05 ml of *iodine TS*: not more than a very faint opalescence is produced.

7.6 ACID-INSOLUBLE ASH

Use Method I unless otherwise indicated in the monograph.

Method I

Boil the total ash for 5 minutes with 25 ml of *dilute hydrochloric acid*, collect the insoluble matter on an ashless filter paper, wash with hot *water* until the filtrate is neutral, and ignite at about 500°. Calculate the percentage of acid-insoluble ash with reference to the air-dried substance.

Method II

Place the total ash or the sulfated ash, as directed in the monograph, in a crucible, add 15 ml of *water* and 10 ml of *hydrochloric acid*, cover with a watch glass, and boil for 10 minutes; allow to cool. Collect the insoluble matter on an ashless filter paper, wash with hot *water* until the filtrate is neutral, ignite to dull redness (550° to 700°), cool in a desiccator and weigh. Reheat until the difference between two successive weighings is not more than 1 mg. Calculate the percentage of acid-insoluble ash with reference to the air-dried substance.

7.7 TOTAL ASH

Use Method I unless otherwise directed in the monograph.

Method I

For vegetable drugs Incinerate 2 to 3 g of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450° until free from carbon, cool and weigh. If a carbon-free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450°. Calculate the percentage of total ash with reference to the air-dried drug.

For other substances Carry out the above method using 1 g, unless otherwise stated. Calculate the percentage of total ash.

Method II

Heat a silica or platinum crucible to red heat for 30 minutes, allow to cool in a desiccator and weigh. Unless otherwise specified in the monograph, evenly distribute 1 g of the substance being examined in the crucible, dry at 100° to 105° for 1 hour and ignite to constant weight in a muffle furnace at 600°±25°. Allow the crucible to cool in a desiccator after each ignition. Flames should not be produced at any time during the procedure.
If after prolonged ignition a carbon-free ash cannot be obtained, take up with hot *water*, filter through an ashless filter paper and ignite the residue and the filter paper. Combine the filtrate with the ash, carefully evaporate to dryness and ignite to constant weight.

7.12 EXTRACTIVES

Ethanol-soluble Extractive

Use Method I unless otherwise indicated in the monograph.

Method I Macerate 5 g of the air-dried drug, coarsely powdered and accurately weighed, with 100.0 ml of *ethanol* of the specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and then allowing to stand for 18 hours. Filter rapidly, taking precautions against loss of ethanol, evaporate 20.0 ml of the filtrate to dryness in a tared, flat-bottomed, shallow dish and dry at 105° to constant weight. Calculate the percentage of ethanol-soluble extractive with reference to the air-dried drug.

Method II Transfer about 4 g of the air-dried drug, coarsely powdered and accurately weighed, to a glass-stoppered conical flask. Add 100 ml of *ethanol* of the specified strength, and weigh the flask. Shake and allow to stand for 1 hour. Attach a reflux condenser to the flask. Boil gently for 1 hour, cool, and weigh. Readjust to the original weight with *ethanol*. Shake, and filter rapidly through a dry filter. Transfer 25.0 ml of the filtrate to a tared flat-bottomed dish, and evaporate on a water-bath to dryness. Dry at 105° for 6 hours, cool in a desiccator for 30 minutes, and weigh without delay. Calculate the percentage of ethanol-soluble extractive with reference to the air-dried drug.

Water-soluble Extractive

Use Method I unless otherwise indicated in the monograph.

Method I Proceed as directed in Method I under Ethanol-soluble Extractive but using *chloroform water* in place of *ethanol*.

Method II Proceed as directed in Method II under Ethanol-soluble Extractive but using *chloroform water* in place of *ethanol*.

7.12H EXTRACTIVES

Hexane-soluble Extractive

Extract completely about 2 g, in *No. 850 powder*, accurately weighed, by subjecting it to the action of hexane in an apparatus for continuous extraction of drugs (Appendix 7.18) during 20 hours. Transfer the hexane solution to a tared porcelain dish and allow it to evaporate spontaneously. Then dry it over *phosphorus pentoxide desiccant* for 18 hours and weigh. Calculate the percentage of hexane-soluble extractive from the weight of drug taken.

Chloroform-soluble Extractive

Proceed as directed in Hexane-soluble Extractive but using *chloroform* in place of *hexane*.

7.18 CONTINUOUS EXTRACTION OF DRUGS

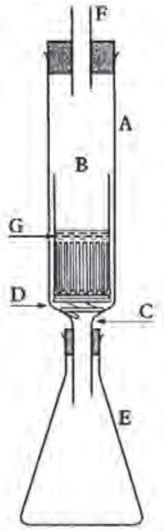
Where the process of maceration or percolation is specified in a monograph, carry out the following procedures with any modification indicated in the monograph.

Maceration Place the solid materials with the whole of the menstruum in a closed vessel and allow to stand for 7 days, shaking occasionally. Strain, press the marc and mix the liquids obtained. Clarify by subsidence or filtration.

Percolation Moisten the solid materials with a sufficient quantity of the menstruum, allow to stand for 4 hours in a well-closed vessel, pack in a percolator, and add a sufficient quantity of the menstruum to saturate the materials. When the liquid begins to drop from the percolator, close the outlet, add a sufficient quantity of the menstruum to leave a layer above the drug and allow to macerate for 24 hours. Allow percolation to proceed slowly until the percolate measures about three-quarters of the required volume. Press the marc, mix the expressed liquid with the percolate and add a sufficient quantity of the menstruum to produce the required volume. Clarify by subsidence or filtration.

Continuous extraction of a drug for the purpose of an assay consists of percolating the drug with the solvent stated in the monograph at a temperature approximately that of the boiling point of the solvent.

The apparatus described below, or any similar apparatus, may be used, provided that it permits the uniform percolation of the drug and the regular flow of the vapour of the solvent around the percolator.



Apparatus for the Continuous Extraction of Drugs

The apparatus is shown in the figure. A is an outer tube of stout glass; the wider part is about 18 cm long and has an internal diameter of 4.8 to 5 cm; the lower end C is about 5 cm long and has an external diameter of about 1.6 cm. B is a straight glass tube open at both ends, about 9 cm long and with an external diameter of about 3.8 cm; over its lower, flanged end is tied firmly a piece of calico or other suitable material. D is a glass coil which supports the margin of the tube B and prevents it from resting in contact with the outer tube A. The lower end C of the outer tube A is fitted by a cork or ground-glass joint to the flask E, in which a suitable quantity of the solvent has been placed. The drug to be extracted, previously moistened with the solvent or subjected to any preliminary treatment required, is introduced into the inner tube B, which is supported so that the percolate drops into the outer tube. A pad of absorbent cotton G is placed on the top of the drug, the inner tube is lowered into position and the outer tube connected by means of a suitable cork or ground-glass joint with the tube of a reflux condenser F. The flask is heated and the extraction continued as directed.

7.21H DETERMINATION OF TANNINS

Sample preparation Place the prescribed quantity of the substance, previously powdered and accurately weighed as specified in the monograph, in a 250-ml conical flask. Add 150 ml of *water* and heat on a water-bath for 30 minutes. Cool in running water, transfer the mixture quantitatively to a 250-ml volumetric flask with *water* and dilute to volume with *water*. Allow the solids to settle and filter.

Determination of total water-extractives Evaporate 50.0 ml of the Sample preparation and dry the residue at 105° for 4 hours and weigh (T_1).

Determination of water-extractives not bound with hide powder To 80.0 ml of the Sample preparation, add 6.00 g of *hide powder* and shake well for 60 minutes. Filter and evaporate 50.0 ml of the filtrate to dryness. Dry the residue at 105° for 4 hours and weigh (T_2).

Determination of water-solubles of hide powder To 6.00 g of *hide powder*, add 80.0 ml of *water* and shake well for 60 minutes. Filter and evaporate 50.0 ml of the filtrate to dryness. Dry the residue at 105° for 4 hours and weigh (T_0).

Calculation Calculate the percentage of tannins from the expression:

$$\frac{(T_1 - T_2 + T_0) \times 5 \times 100}{W},$$

where W is the weight in g of the substance taken, calculated on the dried basis.

7.22H PESTICIDE RESIDUES

Medicinal plant materials are liable to contain pesticide residues which accumulate from agricultural practices, such as spraying, treatment of soils during cultivation and administration of fumigants during storage. Many medicinal preparations of plant origin are taken over long periods of time, limits for residues should be therefore established.

Definition For the purposes of the Pharmacopoeia, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of medicinal plant materials. The item includes substances intended for use as growth-regulators, defoliants or desiccants and any substance applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

Limits Unless otherwise indicated in the monograph, the plant material being examined at least complies with the limits indicated in Table 1. Limits for pesticides that are not listed in Table 1 are calculated using the following formula:

$$\frac{ADI \times M}{MDD \times 100}$$

where ADI = acceptable daily intake, as published by FAO-WHO in milligrams per kilogram of body weight,

M = body weight in kilograms (60 kg), and

MDD = daily dose of the plant material in kilograms.

If the plant material is intended for the preparation of extracts, tinctures or other pharmaceutical forms the preparation method of which modifies the content of pesticides in the finished product, the limits are calculated using the following formula:

$$\frac{ADI \times M \times E}{MDD \times 100}$$

where E = extraction factor of the method of preparation, determined experimentally.

Higher limits can also be authorized, in exceptional cases, especially when a plant requires a particular cultivation method or has a metabolism or a structure that gives rise to a higher than normal content of pesticides.

The competent authority may grant total or partial exemption of the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely.

Table 1

Substance	Limit (mg/kg)
Alachlor	0.02
Aldrin and Dieldrin (sum of)	0.05
Azinphos-methyl	1.0
Bromopropylate	3.0
Chlordane (sum of *cis*-, *trans*- and Oxychlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyrifos	0.2
Chlorpyrifos-methyl	0.1
Cypermethrin (and isomers)	1.0
DDT (sum of *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE and *p,p′*-TDE)	1.0
Deltamethrin	0.5
Diazinon	0.5
Dichlorvos	1.0
Dithiocarbamates (as CS_2)	2.0
Endosulfan (sum of isomers and Endosulfan sulfate)	3.0
Endrin	0.05
Ethion	2.0
Fenitrothion	1.5
Fonofos	0.05
Heptachlor (sum of Heptachlor and Heptachlorepoxide)	0.05
Hexachlorobenzene	0.1
Hexachlorocyclohexane isomers (other than γ)	0.3
Lindane (γ-Hexachlorocyclohexane)	0.6
Malathion	1.0
Methidathion	0.2
Parathion	0.5
Parathion-methyl	0.2
Permethrin	1.0
Phosalone	0.1
Piperonyl butoxide	3.0
Pirimiphos-methyl	4.0
Pyrethrins (sum of)	3.0
Quintozene (sum of quintozene, pentachloroanaline and methyl pentachlorophenyl sulfide)	1.0

Sampling

METHOD For containers up to 1 kg, take one sample from the total content, thoroughly mixed, sufficient for the tests. For containers between 1 and 5 kg, take three samples, equal in volume, from the upper, middle and lower parts of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests. For containers of more than 5 kg, take three samples, each of at least 250 g from the upper, middle and lower parts of the container. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests.

SIZE OF SAMPLING If the number (n) of containers is three or fewer, take samples from each container as indicated above under Method. If the number of containers is more than three, take samples as indicated under Method, from $\sqrt{n} + 1$ containers, rounding up to the nearest unit if necessary.

The samples are to be analyzed immediately to avoid possible degradation of the residues. If this is not possible, the samples are stored in tightly closed containers suitable for food contact, at a temperature below 0°, protected from light.

Reagents All reagents and solvents are free from any contaminants, especially pesticides, which might interfere with the analysis. It is often necessary to use special quality solvents or if this is not possible, solvents that have recently been redistilled in an apparatus made entirely of glass. In any case, suitable blank tests must be carried out.

Apparatus Clean the apparatus and especially glassware to ensure that they are free from pesticides, for example, soak for at least 16 hours in a solution of phosphate-free detergent, rinse with large quantities of distilled water, and wash with acetone and hexane or heptane.

Qualitative and quantitative analysis of pesticide residues The analytical procedures used are validated according to the regulations in force. In particular, they satisfy the following criteria:

(1) the chosen method, especially the purification steps, is suitable for the substance being analyzed, and not susceptible to interference from co-extractives; the limits of detection and quantification are measured for each pesticide-matrix combination being analyzed;

(2) between 70 and 110 per cent of each pesticide is recovered;

(3) the repeatability of the method is not less than the values indicated in Table 2;

(4) the reproducibility of the method is not less than the values indicated in Table 2; and

(5) the concentration of test and reference solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

Table 2

Concentration of the Analyte (mg/kg)	Repeatability (difference±mg/kg)	Repeatability (difference±mg/kg)
0.010	0.005	0.01
0.100	0.025	0.05
1.000	0.125	0.25

Test for Organochlorine, Organophosphorus and Pyrethroid Insecticides

The following methods may be used, in connection with the general method above. Depending on the substance being examined, it may be necessary to modify, sometimes extensively, the procedure described hereafter. In any case, it may be necessary to use, in addition, another column with a different polarity or another detection method (for example, mass spectrometry) or a different method (for example, immunochemical methods) to confirm the results obtained.

This procedure is valid only for the analysis of samples of medicinal plant materials containing less than 15 per cent of water. Samples with a higher content of water may be dried, provided it has been shown that the drying procedure does not affect significantly the pesticide content.

Extraction

To 10 g of the substance being examined, coarsely powdered, add 100 ml of *acetone* and allow to stand for 20 minutes. Add 1 ml of a solution containing 1.8 µg per millilitre of *carbophenothion* in *toluene*. Homogenize using a high-speed blender for 3 minutes. Filter and wash the filter cake with two 25-ml portions of *acetone*. Combine the filtrate and the washings and heat using a rotary evaporator at a temperature not exceeding 40° until the solvent has almost completely evaporated. To the residue add a few millilitres of *toluene* and heat again until the acetone is completely removed. Dissolve the residue in 8 ml of *toluene*. Filter through a membrane filter having a 45-µm porosity, rinse the flask and the filter with toluene and dilute to 10 ml with the same solvent (solution A).

Purification

ORGANOCHLORINE, ORGANOPHOSPHORUS AND PYRETHROID INSECTICIDES

Carry out the determination as described in the “Size-exclusion Chromatography” (Appendix 3.6). The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 7.8 mm) packed with styrene-divinylbenzene copolymer (5 µm), (b) *toluene* as mobile phase at a flow rate of about 1 ml per minute.

Performance of the column Inject 100 µl of a solution containing 0.05 per cent w/v of *methyl red* and 0.05 per cent w/v of *oracet blue* in *toluene* and proceed with the chromatography. The column is not suitable unless the colour of the eluate changes from orange to blue at an elution volume of about 10.3 ml. If necessary calibrate the column, using a solution containing, in *toluene*, at a suitable concentration, the insecticide being analyzed with the lowest molecular weight (for example, dichlorvos) and that with the highest molecular weight (for example, deltamethrin). Determine which fraction of the eluate contains both pesticides.

Purification of the test solution Inject a suitable volume of solution A (100 to 500 µl) and proceed with the chromatography. Collect the fraction as determined above (solution B). Organophosphorus insecticides are usually eluted between 8.8 ml and 10.9 ml. Organochlorine and pyrethroid insecticides are usually eluted between 8.5 ml and 10.3 ml.

ORGANOCHLORINE AND PYRETHROID INSECTICIDES

In a chromatography column (10 cm × 5 mm) introduce a piece of defatted cotton and 0.5 g of silica gel treated as follows: heat *silica gel for chromatography* in an oven at 150° for 4 hours. Allow to cool and add dropwise a quantity of *water* corresponding to 1.5 per cent of the weight of silica gel used; shake vigorously until agglomerates have disappeared and continue shaking for 2 hours using a mechanical shaker. Condition the column using 1.5 ml of *hexane*. Prepacked columns containing about 500 mg of a suitable silica gel may also be used provided they are previously validated.

Concentrate solution B in a current of *helium* or *oxygen-free nitrogen* almost to dryness and dilute to a suitable volume with *toluene* (200 µl to 1 ml according to the volume injected in the preparation of solution B). Transfer quantitatively onto the column and proceed to the chromatography using 1.8 ml of *toluene* as the mobile phase. Collect the eluate (solution C).

Quantitative analysis

ORGANOPHOSPHORUS INSECTICIDES

Carry out the determination as described in the "Gas Chromatography" (Appendix 3.4), using *carbophenothion* as the internal standard. It may be necessary to use a second internal standard to identify possible interferences with the peak due to carbophenothion.

Test solution Concentrate solution B in a current of *helium* almost to dryness and dilute to 100 µl with *toluene*.

Standard solution Prepare at least three solutions in *toluene* containing the insecticides being determined and *carbophenothion* at concentrations suitable for plotting a calibration curve.

Chromatographic system The chromatographic procedure may be carried out using (a) a fused-silica column (30 m × 0.32 mm) the internal wall of which is covered with a layer 0.25 µm thick of poly(dimethyl)siloxane, maintained at 80° for 1 minute, then increased to 150° at a rate of 30° per minute, maintained at 150° for 3 minutes, then increased to 280° at a rate of 4° per minute, and maintained at this temperature for 1 minute; (b) the injection port maintained at 250°, and the detector at 275°; (c) *hydrogen* as the carrier gas; other gases such as *helium* or *nitrogen for chromatography* may also be used provided the chromatography is suitably validated; and (d) a phosphorus-nitrogen flame-ionization detector or a flame photometry detector.

Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table 3. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

Table 3

Substance	Relative Retention Times
Dichlorvos	0.20
Fonofos	0.50
Diazinon	0.52
Parathion-methyl	0.59
Chlorpyrifos-methyl	0.60
Pirimiphos-methyl	0.66
Malathion	0.67
Parathion	0.69
Chlorpyrifos	0.70
Methidathion	0.78
Ethion	0.96
Carbophenothion	1.00
Azinphos-methyl	1.17
Phosalone	1.18

ORGANOCHLORINE AND PYRETHROID INSECTICIDES

Carry out the determination as described in the "Gas Chromatography" (Appendix 3.4), using *carbophenothion* as internal standard. It may be necessary to use a second internal standard to identify possible interferences with the peak due to carbophenothion.

Test solution Concentrate solution C in a current of *helium* or *oxygen-free nitrogen* almost to dryness and dilute to 500 µl with *toluene*.

Standard solution Prepare at least three solutions in toluene containing the insecticides being determined and *carbophenothion* at concentrations suitable for plotting a calibration curve.

Chromatographic system The chromatographic procedure may be carried out using (a) a fused silica column (30 m × 0.32 mm) the internal wall of which is covered with a layer 0.25 µm thick of poly(dimethyl)(diphenyl)siloxane, maintained at 80° for 1 minute, then increased to 150° at a rate of 30° per minute, maintained at 150° for 3 minutes, then increased to 280° at a rate of 4° per minute, and maintained at this temperature for 1 minute; (b) the injection port maintained at 250°, and the detector at 275°; (c) *hydrogen* as the carrier gas; other gases such as *helium* or *nitrogen for chromatography* may also be used; provided the chromatography is suitably validated; (d) an electron-capture detector; and (e) a device allowing direct cold on-column injection.

Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table 4.

Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

Table 4

Substance	Relative Retention Times
α-Hexachlorocyclohexane	0.44
Hexachlorobenzene	0.45
β-Hexachlorocyclohexane	0.49
Lindane	0.49
δ-Hexachlorocyclohexane	0.54
ε-Hexachlorocyclohexane	0.56
Heptachlor	0.61
Aldrin	0.68
cis-Heptachlor-epoxide	0.76
o,p′-DDE	0.81
β-Endosulfan	0.82
Dieldrin	0.87
p,p′-DDE	0.87
o,p′-DDD	0.89
Endrin	0.91
β-Endosulfan	0.92
o,p′-DDT	0.95
Carbophenothion	1.00
p,p′-DDT	1.02
cis-Permethrin	1.29
trans-Permethrin	1.31
Cypermethrin	1.40
Fenvalerate*	1.47, 1.49
Deltamethrin	1.54

*The substance shows several peaks.

APPENDIX 10 MICROBIOLOGICAL TEST

10.2 MICROBIAL LIMIT TESTS

Introduction

The hazard of microbiological contamination in non-sterile pharmaceuticals has been well realized, especially in those products of vegetable, animal and mineral origins and in those which lack good manufacturing practices (GMP). Microbiological attributes of non-sterile pharmaceutical products are described in Appendix 10.4.

This appendix, therefore, comprises two parts of tests. Part I allows quantitative enumeration of mesophilic bacteria and fungi that may grow under aerobic condition and Part II allows determination of the absence or limited occurrence of specified micro-organisms that may be detected under the conditions described.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes, follow the instructions given below, including the number of samples to be taken, and interpret the results as stated below.

The methods are not applicable to products containing viable micro-organisms as active ingredients.

Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

Acceptance criteria for microbiological quality of non-sterile pharmaceuticals are given in the table under "Limits for Microbial Contamination" (Appendix 10.5).

In the following, the term "micro-organisms" is covering bacteria and fungi only; the term "pharmaceuticals" means pharmaceutical products of any kind, from raw materials to the finished forms; the term "growth" is used to designate the presence and presumed proliferation of micro-organisms.

Part I Microbial Enumeration Tests

PROCEDURE

In preparing for and in applying the tests, precautions are taken so as to avoid the accidental microbial contamination of the product to be examined, as well as the inadvertent suppression of the growth of any micro-organisms that are to be revealed in the test.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized. If inactivators are used for this purpose, their efficacy and their absence of toxicity for micro-organisms must be demonstrated.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated.

ENUMERATION METHODS

Use the membrane filtration method or the plate-count method, as prescribed. The most probable number (MPN) method is generally the least accurate method for microbial counts; however, for certain product groups with a very low bioburden, it may be the most appropriate method. The choice of method is based on factors such as the nature of the product and the required limit of micro-organisms. The chosen method must allow testing of a sufficient sample size to judge compliance with the specification. The suitability of the method chosen must be established.

GROWTH PROMOTION TEST, SUITABILITY OF THE COUNTING METHOD AND NEGATIVE CONTROLS

The ability of the test to detect micro-organisms in the presence of product to be tested must be established. Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

Preparation of test strains

Use standardized stable suspensions of test strains or prepare them as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than five passages removed from the original master seed-lot. Grow each of the bacterial and fungal test strains separately as described in Table 1.

Use Buffered sodium chloride-peptone solution pH 7.0 or Phosphate buffer pH 7.2 to make test suspensions; to suspend *Aspergillus niger* spores, 0.05 per cent of *polysorbate 80* may be added to the buffer. Use the suspensions within 2 hours or within 24 hours if stored at 2° to 8°. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *A. niger* or *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2° to 8° for a validated period of time.

Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described in Testing of Products. A failed negative control requires an investigation.

Table 1 Preparation and Use of Test Micro-organisms

Micro-organism*	Preparation of Test Strain	Growth Promotion		Suitability of Counting Method in the Presence of the Product	
		Total Aerobic Microbial Count (TAMC)	Total Yeasts and Moulds Count (TYMC)	Total Aerobic Microbial Count (TAMC)	Total Yeasts and Moulds Count (TYMC)
Staphylococcus aureus such as: ATCC 6538 DMST 8013 NCIMB 9518 C.I.P. 4.83 NBRC 13276	Soybean-caseins digest agar or Soybean-casein digest broth 30° to 35° 18 to 24 hours	Soybean-casein digest agar or Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days		Soybean-casein digest agar/MPN Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days	
Pseudomonas aeruginosa such as: ATCC 9027 DMST 15501 NCIMB 8626 C.I.P. 82.118 NBRC 13275	Soybean-casein digest agar or Soybean-casein digest broth 30° to 35° 18 to 24 hours	Soybean-casein digest agar or Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days		Soybean-casein digest agar/MPN Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days	
Bacillus subtilis such as: ATCC 6633 DMST 15896 NCIMB 8054 C.I.P. 52.62 NBRC 3134	Soybean-casein digest agar or Soybean-casein digest broth 30° to 35° 18 to 24 hours	Soybean-casein digest agar or Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days		Soybean-casein digest agar /MPN Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days	

Table 1 (Continued)

Micro-organism*	Preparation of Test Strain	Growth Promotion		Suitability of Counting Method in the Presence of the Product	
		Total Aerobic Microbial Count (TAMC)	Total Yeasts and Moulds Count (TYMC)	Total Aerobic Microbial Count (TAMC)	Total Yeasts and Moulds Count (TYMC)
Candida albicans such as: ATCC 10231 DMST 5815 NCPF 3179 I.P. 48.72 NBRC 1594	Sabouraud dextrose agar or Sabouraud dextrose broth 20° to 25° 2 to 3 days	Soybean-casein digest agar ≤100 CFU 30° to 35° ≤5 days	Sabouraud dextrose agar ≤100 CFU 20° to 25° ≤5 days	Soybean-casein digest agar ≤100 CFU 30° to 35° ≤5 days MPN: not applicables	Sabouraud dextrose agar ≤100 CFU 20° to 25° ≤5 days
Aspergillus niger such as: ATCC 16404 DMST 15538 IMI 149007 I.P. 1431.83 NBRC 9455	Sabouraud dextrose agar or Potato dextrose agar 20° to 25° 5 to 7 days, or until good sporulation is achieved	Soybean-casein digest agar ≤100 CFU 30° to 35° ≤5 days	Sabouraud dextrose agar ≤100 CFU 20° to 25° ≤5 days	Soybean-casein digest agar ≤100 CFU 30° to 35° ≤5 days MPN: not applicables	Sabouraud dextrose agar ≤100 CFU 20° to 25° ≤5 days

*ATCC = American Type Culture Collection, USA; DMST = Department of Medical Sciences, Thailand; NCIMB = National Collection of Industrial and Marine Bacteria Ltd., Great Britain; C.I.P. = Collection de Bactéries de l' Institut Pasteur, France; NBRC = Biological Resource Center, Japan; NCPF = National Collection of Pathogenic Fungi, London School of Hygiene and Tropical Medicine, Great Britain; I.P. = Collection Nationale de Culture de Micro-organismes (C.N.C.M.) Institut Pasteur, France; IMI = International Mycological Institute, Great Britain

Growth promotion of the media

Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described.

Inoculate portions/plates of Soybean-casein digest broth and Soybean-casein digest agar with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 1, using a separate portion/plate of medium for each. Inoculate plates of Sabouraud dextrose agar with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 1, using a separate plate of medium for each. Incubate in the conditions described in Table 1.

For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs. Liquid media are suitable if clearly visible growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Suitability of the counting method in the presence of product

PREPARATION OF THE SAMPLE The method for sample preparation depends upon the physical characteristics of the product to be tested. If none of the procedures described below can be demonstrated to be satisfactory, an alternative procedure must be developed.

Water-soluble products Dissolve or dilute (usually a 1 in 10 dilution is prepared) the product to be examined in Buffered sodium chloride-peptone solution pH 7.0, Phosphate buffer solution pH 7.2 or Soybean-casein digest broth. If necessary, adjust to pH 6 to 8. Further dilutions, where necessary, are prepared with the same diluent.

Non-fatty products insoluble in water Suspend the product to be examined (usually a 1 in 10 dilution is prepared) in Buffered sodium chloride-peptone solution pH 7.0, Phosphate buffer solution pH 7.2 or Soybean-casein digest broth. A surface-active agent such as 0.1 per cent w/v of *polysorbate 80* may be added to assist the suspension of poorly wettable substances. If necessary, adjust to pH 6 to 8. Further dilutions, where necessary, are prepared with the same diluent.

Fatty products Dissolve in *isopropyl myristate*, sterilized by filtration or mix the product to be examined with the minimum necessary quantity of sterile polysorbate 80 or another non-inhibitory sterile surface-active agent, heated if necessary to not more than 40°, or in exceptional cases to not more than 45°. Mix carefully and if necessary maintain the temperature in a water-bath. Add sufficient of the pre-warmed chosen diluent to make a 1 in 10 dilution of the original sample. Mix carefully whilst maintaining the temperature for the shortest time necessary for the formation of an emulsion. Further serial ten-fold dilutions may be prepared using the chosen diluent containing a suitable concentration of sterile *polysorbate 80* or another non-inhibitory sterile surface-active agent.

Fluids or solids in aerosol form Chill the container(s) for approximately 1 hour, cut open the container(s), and allow to reach room temperature, permitting the propellant to escape, or warming to drive off the propellant if feasible. Aseptically transfer the product into a membrane filter apparatus or a sterile container for further sampling. Use either the total contents or a defined number of metered doses from each of the containers tested.

Transdermal patches Remove the protective cover sheets ("release liners") of the transdermal patches and place them, adhesive side upwards, on sterile glass or plastic trays. Cover the adhesive surface with a sterile porous material, for example sterile gauze, to prevent the patches from sticking together, and transfer the patches to a suitable volume of the chosen diluent containing inactivators such as polysorbate 80 and/or lecithin. Shake the preparation vigorously for at least 30 minutes.

INOCULATION AND DILUTION Add to the sample prepared as described in Preparation of the Sample and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than 100 CFU. The volume of the suspension of the inoculum should not exceed 1 per cent of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution factor of the prepared sample must be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols must be developed. If inhibition of growth by the sample cannot otherwise be avoided, the aliquot of the microbial suspension may be added after neutralization, dilution or filtration.

NEUTRALIZATION / REMOVAL OF ANTIMICROBIAL ACTIVITY The number of micro-organisms recovered from the prepared sample diluted as described in Inoculation and Dilution and incubated following the procedure described in Recovery of Micro-organism in the Presence of Product, is compared to the number of micro-organisms recovered from the control preparation.

If growth is inhibited (reduction by a factor greater than 2), then modify the procedure for the particular enumeration test to ensure the validity of the results. Modification of the procedure may include, for example, (1) an increase in the volume of the diluent or culture medium, (2) incorporation of specific or general neutralizing agents into the diluent such as Casein digest-soy lecithin polysorbate 20 broth, (3) membrane filtration, or (4) a combination of the above measures.

Neutralizing agents Neutralizing agents may be used to neutralize the activity of antimicrobial agents (Table 2). They may be added to the chosen diluent or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for micro-organisms must be demonstrated by carrying out a blank with the neutralizer and without the product.

Table 2 Common Neutralizing Agents for Interfering Substances

Interfering Substance	Potential Neutralizing Method
Glutaraldehyde, mercurials	Sodium hydrogensulfite (sodium bisulfite)
Phenolics, ethanol, aldehydes, sorbate	Dilution
Aldehydes	Glycine
Quaternary Ammonium Compound (QACs), parahydroxy benzoates (parabens), bisbiguanides	Lecithin
QACs, iodine, parabens	Polysorbate
Mercurials	Thioglycolate
Mercurials, halogens, aldehydes	Thiosulfate
EDTA (edetate)	Mg^{2+} or Ca^{2+} ions

If no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This information serves to indicate that the product is not likely to be contaminated with the given species of the micro-organism. However, it is possible that the product only inhibits some of the micro-organisms specified herein, but does not inhibit others not included amongst the test strains or for which the latter are not representative. Then, perform the test with the highest dilution factor compatible with microbial growth and the specific acceptance criterion.

RECOVERY OF MICRO-ORGANISMS IN THE PRESENCE OF PRODUCT For each of the micro-organisms listed, separate tests are performed. Only micro-organisms of the added test strain are counted.

Membrane filtration Use membrane filters having a nominal pore size not greater than 0.45 µm. The type of filter material is chosen such that the bacteria-retaining efficiency is not affected by the components of the sample to be investigated. For each of the micro-organisms listed, one membrane filter is used.

Transfer a suitable amount of the sample prepared as described under Preparation of the Sample, under Inoculation and Dilution, and under Neutralization/Removal of Antimicrobial Activity (preferably representing 1 g of the sample, or less if large numbers of CFU are expected) to the membrane filter, filter immediately and rinse the membrane filter with an appropriate volume of diluent.

For the determination of total aerobic microbial count (TAMC), transfer the membrane filter to the surface of Soybean-casein digest agar. For the determination of total combined yeasts and moulds count (TYMC), transfer the membrane to the surface of Sabouraud dextrose agar. Incubate the plates as indicated in Table 1. Perform the counting.

Plate-count methods Perform plate-count methods at least in duplicate for each medium and use the mean count of the result.

Pour-plate method For Petri dishes 9 cm in diameter, add to the dish 1 ml of the sample prepared as described under Preparation of the Sample, under Inoculation and Dilution, and under Neutralization/Removal of Antimicrobial Activity and 15 to 20 ml of Soybean-casein digest agar or Sabouraud dextrose agar, both media being at not more than 45°. If larger Petri dishes are used, the amount of agar medium is increased accordingly. For each of the micro-organisms listed in Table 1, at least two Petri dishes are used. Incubate the plates as indicated in Table 1. Take the arithmetic mean of the counts per medium and calculate the number of CFU in the original inoculum.

Surface-spread method For Petri dishes 9 cm in diameter, add 15 to 20 ml of Soybean-casein digest agar or Sabouraud dextrose agar at about 45° to each Petri dish and allow to solidify. If larger Petri dishes are used, the volume of the agar is increased accordingly. Dry the plates, for example in a laminar-air-flow cabinet or an incubator. For each of the micro-organisms listed in Table 1, at least two Petri dishes are used. Spread a measured volume of not less than 0.1 ml of the sample prepared as described under Preparation of the Sample, under Inoculation and Dilution, and under Neutralization/Removal of Antimicrobial Activity over the surface of the medium. Incubate and count as prescribed under Pour-plate Method.

Most probable number (MPN) method The precision and accuracy of the MPN method is less than that of the membrane filtration method or the plate-count method. Unreliable results are obtained particularly for the enumeration of moulds. For these reason the MPN method is reserved for the enumeration of TAMC in situations where no other method is available. If the use of the method is justified, proceed as follows.

Prepare a series of at least three serial ten-fold dilutions of the product as described under Preparation of the Sample, under Inoculation and Dilution, and under Neutralization/Removal of Antimicrobial Activity. From each level of dilution, three aliquots of 1 g or 1 ml are used to inoculate three tubes with 9 to 10 ml of Soybean-casein digest broth. If necessary, a surface-active agent such as polysorbate 80 or an inactivator of antimicrobial agents may be added to the medium. Thus, if three levels of dilution are prepared, nine tubes are inoculated.

Incubate all tubes at 30° to 35° for not more than 3 days. If reading of the results is difficult or uncertain owing to the nature of the product to be examined, subculture in the same broth, or in Soybean-casein digest agar, for 1 to 2 days at the same temperature and use these results. Determine the most probable number of micro-organisms per g or per ml of the product to be examined from Table 3.

Results and interpretation

When verifying the suitability of the membrane filtration method or the plate-count method, a mean count of any of the test organisms not differing by a factor greater than 2 from the value of the control defined in Inoculation and Dilution in the absence of the product must be obtained. When verifying the suitability of the MPN method the calculated value from the inoculum must be within 95 per cent confidence limits of the results obtained with the control.

If the above criteria cannot be met for one or more of the organisms tested with any of the described methods, the method and test conditions that come closest to the criteria are used to test the product.

Table 3 Most Probable Number (MPN) Values of Micro-organisms

Observed Combinations of Numbers of Tubes Showing Growth in Each Set			MPN per g or per ml of Product	95 Per Cent Confidence Limits
Number of Grams or Millilitres of Product per Tube				
0.1	0.01	0.001		
0	0	0	<3	0-9.4
0	0	1	3	0.1-9.5
0	1	0	3	0.1-10
0	1	1	6.1	1.2-17
0	2	0	6.2	1.2-17
0	3	0	9.4	3.5-35
1	0	0	3.6	0.2-17
1	0	1	7.2	1.2-17
1	0	2	11	4-35
1	1	0	7.4	1.3-20
1	1	1	11	4-35
1	2	0	11	4-35
1	2	1	15	5-38
1	3	0	16	5-38
2	0	0	9.2	1.5-35
2	0	1	14	4-35
2	0	2	20	5-38
2	1	0	15	4-38
2	1	1	20	5-38
2	1	2	27	9-94
2	2	0	21	5-40
2	2	1	28	9-94
2	2	2	35	9-94
2	3	0	29	9-94
2	3	1	36	9-94
3	0	0	23	5-94
3	0	1	38	9-104
3	0	2	64	16-181
3	1	0	43	9-181
3	1	1	75	17-199
3	1	2	120	30-360
3	1	3	160	30-380
3	2	0	93	18-360
3	2	1	150	30-380
3	2	2	210	30-400
3	2	3	290	90-990
3	3	0	240	40-990
3	3	1	460	90-1980
3	3	2	1100	200-4000
3	3	3	>1100	

TESTING OF PRODUCTS

Amount used for the test

Unless otherwise prescribed, use 10 g or 10 ml of the product to be examined taken with the precautions referred to above. For fluids or solids in aerosol form, sample 10 containers. For transdermal patches, sample 10 patches.

The amount to be tested may be reduced for active substances that will be formulated in the following conditions: the amount per dosage unit (e.g., tablet, capsule, injection) is less than or equal to 1 mg or the amount per g or per ml (for preparations not presented in dosage units) is less than 1 mg. In these cases, the amount to be tested is not less than the amount present in 10 dosage units or 10 g or 10 ml of the product.

For materials used as active substances where sample quantity is limited or batch size is extremely small (i.e., less than 1000 ml or 1000 g), the amount tested shall be 1 per cent of the batch unless a lesser amount is prescribed or justified and authorized.

For products where the total number of entities in a batch is less than 200 (e.g., samples used in clinical trials), the sample size may be reduced to 2 units, or 1 unit if the size is less than 100.

Select the sample(s) at random from the bulk material or from the available containers of the preparation. To obtain the required quantity, mix the contents of a sufficient number of containers to provide the sample.

Examination of the product

MEMBRANE FILTRATION Use a filtration apparatus designed to allow the transfer of the filter to the medium. Prepare the sample using a method that has been shown suitable as described in Growth Promotion Test, Suitability of the Counting Method and Negative Controls. Transfer the appropriate amount to each of two membrane filters and filter immediately. Wash each filter following the procedure shown to be suitable.

For the determination of TAMC, transfer one of the membrane filters to the surface of Soybean-casein digest agar. For the determination of TYMC, transfer the other membrane to the surface of Sabouraud dextrose agar. Incubate the plate of Soybean-casein digest agar at 30° to 35° for 3 to 5 days and the plate of Sabouraud dextrose agar at 20° to 25° for 5 to 7 days. Calculate the number or CFU per g or per ml of product.

When examining transdermal patches, filter 10 per cent of the volume of the preparation described under Preparation of the Sample separately through each of two sterile filter membranes. Transfer one membrane to Soybean-casein digest agar for TAMC and the other membrane to Sabouraud dextrose agar for TYMC.

PLATE-COUNT METHODS

Pour-plate method Prepare the sample using a method that has been shown to be suitable as described in Growth Promotion Test, Suitability of the Counting Method and Negative Controls. Prepare for each medium at least two Petri dishes for each level of dilution. Incubate the plates of Soybean-casein digest agar at 30° to 35° for 3 to 5 days and the plates of Sabouraud dextrose agar at 20° to 25° for 5 to 7 days. Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC. Take the arithmetic mean per culture medium of the counts and calculate the number of CFU per g or per ml of product.

Surface-spread method Prepare the sample using a method that has been shown to be suitable as described in Growth Promotion Test, Suitability of the Counting Method and Negative Controls. Prepare at least two Petri dishes for each medium and each level of dilution. For incubation and calculation of the number of CFU proceed as described for the pour-plate method.

MOST PROBABLE NUMBER METHOD

Prepare and dilute the sample using a method that has been shown to be suitable as described in Growth Promotion Test, Suitability of the Counting Method and Negative Controls. Incubate all tube at 30° to 35° for 3 to 5 days. Subculture if necessary, using the procedure shown to be suitable. Record for each level of dilution the number of tubes showing microbial growth. Determine the most probable number of micro-organisms per g or per ml of the product to be examined from Table 3.

Interpretation of the results

The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU found using Soybean-casein digest agar; if colonies of fungi are detected on this medium, they are counted as part of the TAMC. The total combined yeasts and mould count (TYMC) is considered to be equal to the number of CFU found using Sabouraud dextrose agar; if colonies of bacteria are detected on this medium, they are counted as part of the TYMC. When the TYMC is expected to exceed the acceptance criterion due to the bacterial growth, Sabouraud dextrose agar with antibiotics may be used. If the count is carried out by the MPN method the calculated value is the TAMC.

The recommended solutions and media are described in Part II.

The limits prescribed in the Limits for Microbial Contamination (Appendix 10.5) are the maximum acceptable limits.

Part II Test for Specified Micro-organisms

PROCEDURE

The preparation of the samples is carried out as described in Part I.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized as described in Part I.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated as described in Part I.

GROWTH-PROMOTING AND INHIBITORY PROPERTIES OF THE MEDIA, SUITABILITY OF THE TEST AND NEGATIVE CONTROLS

The ability of the test to detect micro-organisms in the presence of the product to be tested must be established. Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

Preparation of test strains

Use standardized stable suspensions of test strains or prepare them as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than five passages removed from the original master seed-lot.

AEROBIC MICRO-ORGANISMS Grow each of the bacterial test strains separately in Soybean-casein digest broth or on Soybean-casein digest agar at 30° to 35° for 18 to 24 hours. Grow the test strain for *Candida albicans* separately on Sabouraud dextrose agar or in Sabouraud dextrose broth at 20° to 25° for 2 to 3 days.

- *Staphylococcus aureus* such as ATCC 6538, DMST 8013, NCIMB 9518, C.I.P. 4.83 or NBRC 13276;
- *Pseudomonas aeruginosa* such as ATCC 9027, DMST 15501, NCIMB 8626, C.I.P. 82.118 or NBRC 13275;
- *Escherichia coli* such as ATCC 8739, DMST 15537, NCIMB 8545, C.I.P. 53.126 or NBRC 3972;
- *Salmonella enterica* subsp. *enterica* serovar Typhimurium, such as ATCC 14028, DMST 13311, or, as an alternative, *Salmonella enterica* subsp. *enterica* serovar Abony such as NCTC 6017, DMST 21863, C.I.P. 80.39, or NBRC 100797;
- *Candida albicans* such as ATCC 10231, DMST 5815, NCPF 3179, I.P. 48.72 or NBRC 1594.

Use Buffered sodium chloride-peptone solution pH 7.0 or Phosphate buffer pH 7.2 to make test suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2° to 8°.

CLOSTRIDIUM SPP. Use *Clostridium sporogenes* such as ATCC 11437 (DMST 15536, NCIMB 12343, C.I.P. 100651, NBRC 14293) or ATCC 19404 (DMST 15282, NCTC 532, C.I.P. 79.03). Grow the clostridial test strain under anaerobic conditions in Reinforced medium for clostridia at 30° to 35° for 24 to 48 hours. As an alternative to preparing and then diluting down a fresh suspension of vegetative cells of *Cl. sporogenes*, a stable spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2° to 8° for a validated period.

Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described in Testing of Products. A failed negative control requires an investigation.

Growth promotion and inhibitory properties of the media

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients.

Verify suitable properties of relevant media as described in Table 4.

TEST FOR GROWTH PROMOTING PROPERTIES, LIQUID MEDIA: inoculate a portion of the appropriate medium with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

TEST FOR GROWTH PROMOTING PROPERTIES, SOLID MEDIA: perform the surfacespread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

TEST FOR INHIBITORY PROPERTIES, LIQUID OR SOLID MEDIA: inoculate the appropriate medium with at least 100 CFU of the appropriate micro-organism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the test micro-organism occurs.

TEST FOR INDICATIVE PROPERTIES: perform the surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

Suitability of the test method

For each product to be tested, perform the sample preparation as described in the following paragraph in Testing of Products. Add each test strain at the time of mixing, in the prescribed growth medium. Inoculate the test strains individually. Use a number of micro-organisms equivalent to not more than 100 CFU in the inoculated test preparation. Perform the test as described in the following paragraph in Testing of Products using the shortest incubation period prescribed.

The specified micro-organisms must be detected with the indication reactions as described in Testing of Products.

Any antimicrobial activity of the sample necessitates a modification of the test procedure described in Neutralization/Removal of Antimicrobial Activity under Part I.

If for a given product the antimicrobial activity with respect to a micro-organism for which testing is prescribed cannot be neutralized, then it is to be assumed that the inhibited micro-organism will not be present in the product.

TESTING OF PRODUCTS

Bile-tolerant gram-negative bacteria

SAMPLE PREPARATION AND PRE-INCUBATION Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in Part I, but using Soybean-casein digest broth as the chosen diluent, mix and incubate at 20° to 25° for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 hours but not more than 5 hours).

TEST FOR ABSENCE Use the volume corresponding to 1 g of the product, as prepared in Sample Preparation and Pre-incubation, to inoculate Enterobacteria enrichment broth-Mossel. Incubate at 30° to 35° for 24 to 48 hours. Subculture on plates of Violet red bile dextrose agar. Incubate at 30° to 35° for 18 to 24 hours. The product passes the test if there is no growth of colonies of Gram-negative bacteria on any plate.

Table 4 Growth Promoting, Inhibitory and Indicative Properties of Media

Test	Medium	Property	Test Strains
Test for bile-tolerant gram-negative bacteria	Enterobacteria enrichment broth-Mossel	Growth promoting Inhibitory	*E. coli* *P. aeruginosa* *S. aureus*
	Violet red bile dextrose agar	Growth promoting + indicative	*E. coli* *P. aeruginosa*
Test for *Escherichia coli*	MacConkey broth	Growth promoting Inhibitory	*E. coli* *S. aureus*
	MacConkey agar	Growth promoting + indicative	*E. coli*
Test for *Pseudomonas aeruginosa*	Cetrimide agar Pseudomonas for detection fluorescin Pseudomonas for detection pyocyanin	Growth promoting Inhibitory	*P. aeruginosa* *E. coli*

Table 4 (Continued)

Test	Medium	Property	Test Strains
Test for *Salmonella*	Rappaport-Vassiliadis broth Tetrathionate bile brilliant green broth	Growth promoting	*Salmonella enterica* subsp. *enterica* serovar Typhimurium or *Salmonella enterica* subsp. *enterica* serovar Abony
		Inhibitory	*S. aureus*
	Xylose-lysine-deoxycholate agar Brilliant green agar Bismuth sulfite agar	Growth promoting + indicative	*Salmonella enterica* subsp. *enterica* serovar Typhimurium or *Salmonella enterica* subsp. *enterica* serovar Abony
Test for *Staphylococcus aureus*	Mannitol salt agar Baird-Parker agar Vogel-Johnson agar	Growth promoting + indicative	*S. aureus*
		Inhibitory	*E. coli*
Test for *Clostridium* spp.	Reinforced medium for Clostridia	Growth promoting	*Cl. sporogenes*
	Columbia agar Defibrinated sheep blood agar	Growth promoting	*Cl. sporogenes*
Test for *Candida albicans*	Sabouraud dextrose broth	Growth promoting	*C. albicans*
	Sabouraud dextrose agar	Growth promoting + indicative	*C. albicans*

QUANTITATIVE TEST

Selection and subculture Inoculate suitable quantities of Enterobacteria enrichment broth-Mossel with the preparation as described under Sample Preparation and Pre-incubation and/or dilutions of it containing respectively 0.1 g (or 0.1 ml), 0.01 g (or 0.01 ml), and 0.001 g (or 0.001 ml) of the sample to be examined. Incubate at 30° to 35° for 24 to 48 hours. Subculture each of the cultures on a plate of Violet red bile dextrose agar to obtain selective isolation. Incubate at 30° to 35° for 18 to 24 hours.

Interpretation Growth of well-developed colonies, generally red or reddish, of Gram-negative bacteria constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result. Determine from Table 5 the probable number of bacteria.

***Salmonella* species**

SAMPLE PREPARATION AND PRE-INCUBATION Prepare the product to be examined as described in Part I, and use the portion corresponding to not less than 10 g or 10 ml to inoculate a suitable amount (determined as described under Suitability of the Test Method) of Soybean-casein digest broth, mix and incubate at 30° to 35° for 18 to 24 hours.

SELECTION AND SUBCULTURE Separately transfer 0.1 ml and 1 ml of the enrichment culture to 10 ml of Rappaport-Vassiliadis broth and Tetrathionate bile brilliant green broth, respectively, mix and incubate at 30° to 35° for 18 to 24 hours. Subculture on plates of Xylose-lysine-deoxycholate agar, Brilliant green agar, and Bismuth sulfite agar. Cover and invert the dishes, and incubate at 30° to 35° for 18 to 48 hours.

INTERPRETATION Upon examination, if none of the colonies conforms to the description given in Table 6, the product meets the requirements of the test for absence of the genus *Salmonella*. If colonies of Gram-negative rods matching the description in Table 6 are found, proceed with further identification.

IDENTIFICATION Transfer representative suspect colonies individually, by means of an inoculating wire, to a butt-slant tube of Triple sugar-iron agar by first streaking the surface of the slant and then stabbing the wire well beneath the surface, and incubate. If the examination discloses no evidence of tubes having alkaline (red) slants and acid (yellow) butts (with or without concomitant blackening of the butt from hydrogen sulfide production), the product meets the requirements of the test for absence of the genus *Salmonella*. The presence of *Salmonella* may be confirmed by other suitable cultural or biochemical and serological tests, if necessary.

Table 5 Probable Number of Bacteria

Results for Each Quantity of Product			Probable Number of Bacteria per g or per ml of Product
0.1 g (or 0.1 ml)	0.01 g (or 0.01 ml)	0.001 g (or 0.001 ml)	
+	+	+	More than 10^3
+	+	-	Less than 10^3 and more than 10^2
+	-	-	Less than 10^2 and more than 10
-	-	-	Less than 10

Table 6 Morphology Characteristics of *Salmonella* Species on Selective Agar Media

Selective Medium	Characteristic Colonial Morphology
Xylose-lysine-deoxycholate agar	Red, with or without black centres
Brilliant green agar	Small, transparent, colourless or pink to white opaque (frequently surrounded by pink to red zone)
Bismuth sulfite agar	Black or green

Escherichia coli

SAMPLE PREPARATION AND PRE-INCUBATION Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in Part I, and use 10 ml or the portion corresponding to 1 g or 1 ml to inoculate a suitable amount (determined as described under Suitability of the Test Method) of Soybean-casein digest broth, mix and incubate at 30° to 35° for 18 to 24 hours.

SELECTION AND SUBCULTURE Transfer 1 ml of the enrichment culture to 100 ml of MacConkey broth and incubate at 42° to 44° for 24 to 48 hours. Subculture on plates of MacConkey agar and incubate at 30° to 35° for 18 to 72 hours.

INTERPRETATION Upon examination, if none of the colonies conforms to the description given in Table 7, the product meets the requirements of the test for absence of *Escherichia coli*. If colonies matching the description in Table 7 are found, proceed with further identification.

IDENTIFICATION Transfer the suspect colonies individually, making subculture the suspect colonies individually on plates of Levine eosin-methylene blue agar, and incubate at 30° to 35° for 18 to 24 hours.

Upon examination, if none of the colonies exhibits both a characteristic metallic sheen under reflected light and a blue-black appearance under transmitted light, the product meets the requirements of the test for absence of *Escherichia coli*. The presence of *Escherichia coli* may be confirmed by suitable cultural and, if necessary, biochemical tests. Further serological test may be performed.

Table 7 Morphology Characteristics of *Escherichia coli* on MacConkey Agar

Gram Stain	Characteristic Colonial Morphology
Negative rods (cocco-bacilli)	Brick-red; may have surrounding zone of precipitated bile

Staphylococcus aureus and *Pseudomonas aeruginosa*

SAMPLE PREPARATION AND PRE-INCUBATION Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in Part I, and use 10 ml or the portion corresponding to 1 g or 1 ml to inoculate a suitable amount (determined as described under Suitability of the Test Method) of Soybean-casein digest broth and mix. When testing transdermal patches, filter the volume of sample corresponding to one patch of the preparation described under Preparation of the Sample in Part I through a sterile filter membrane and place in 100 ml of Soybean-casein digest broth. Incubate at 30° to 35° for 18 to 24 hours.

SELECTION AND SUBCULTURE If growth is present, use an inoculating loop to streak a portion of the culture medium on the surface of Mannitol-salt agar, or Baird-Parker agar, or Vogel-Johnson agar and of Cetrimide agar, and incubate at 30° to 35° for 18 to 72 hours.

INTERPRETATION Upon examination, if none of the plates contains colonies having the characteristics listed in Tables 8 and 9 for the media used, the product meets the requirements for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.* If colonies matching the description in Table 8 and 9 are found, proceed with further identification.

IDENTIFICATION

Coagulase test (for *Staphylococcus aureus*) With the aid of an inoculating loop, transfer representative suspect colonies from the agar surfaces of the Mannitol-salt agar (or Baird-Parker agar or Vogel-Johnson agar) to individual tubes, each containing 0.5 ml of mammalian, preferably rabbit or horse, plasma with or without suitable additives. Incubate in a water-bath at 37°, examining the tubes at 3 hours and subsequently at suitable intervals up to 24 hours. Test positive and negative controls simultaneously with the unknown products. If no coagulation in any degree is observed, the product meets the requirements of the test for absence of *Staphylococcus aureus*.

*Oxidase and pigment te*sts (for *Pseudomonas aeruginosa*) With the aid of an inoculating loop, streak representative suspect colonies from the agar surfaces of Cetrimide agar on the agar surface of Pseudomonas agar for detection of fluorescin and Pseudomonas agar for detection of pyocyanin contained in Petri dishes. Cover and invert the inoculated media, and incubate at 30° to 35° for not less than 3 days. Examine the streaked surfaces under UV light. Examine the plates to determine whether colonies having the characteristics listed in Table 9 are present.

Table 8 Morphology Characteristics of *Staphylococcus aureus* on Selective Agar Media

Selective Medium	Characteristic Colonial Morphology	Gram Stain
Mannitol-salt agar	Yellow colonies surrounded by yellow zone	Positive cocci (in clusters)
Baird-Parker agar	Black, shiny colonies surrounded by clear zones of 2 to 5 mm	Positive cocci (in clusters)
Vogel-Johnson agar	Black surrounded by yellow zones	Positive cocci (in clusters)

Table 9 Morphology and Diagnostic Characteristics of *Pseudomonas aeruginosa* on Selective Agar Media

Selective Medium	Characteristic Colonial Morphology	Fluorescence in UV Light	Oxidase Test	Gram Stain
Cetrimide agar	Generally greenish	Greenish	Positive	Negative rods
Pseudomonas agar for to detection of fluorescin	Generally colourless yellowish	Yellowish	Positive	Negative rods
Pseudomonas agar for detection of pyocyanin	Generally greenish	Blue	Positive	Negative rods

Confirm any suspect colonial growth on one or more of the media as *Pseudomonas aeruginosa* by means of the oxidase test. Upon the colonial growth, place or transfer colonies to strips or discs of filter paper that previously has been impregnated with *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride. If there is no development of a pink colour, changing to purple, the product meets the requirements of the test for the absence of *Pseudomonas aeruginosa.* The presence of *Pseudomonas aeruginosa* may be confirmed by suitable cultural and, if necessary, biochemical tests.

Candida albicans

SAMPLE PREPARATION AND PRE-INCUBATION Prepare the product to be examined as described under Preparation of the Sample and use 10 ml or the portion corresponding to 1 g or 1 ml to inoculate a suitable amount (determined as described under Suitability of the Test Method) of Sabouraud dextrose broth and mix. Incubate at 30°to 35° for 3 to 5 days.

SELECTION AND SUBCULTURE Subculture on a plate of Sabouraud dextrose agar and incubate at 30° to 35° for 24 to 48 hours.

INTERPRETATION When growth of white colonies may indicate the presence of *Candida albicans* occurs, proceed with further identification.

IDENTIFICATION Transfer the suspect colonies individually, making subculture the suspect colonies individually on plates of a suitable selective medium[1].

Upon examination, the product passes the test if there is no growth of colonies of *Candida albicans* on any plate.

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[1] Biggy agar, CHROMagar Candida, or Candida isolation agar is recommended.

Clostridium spp.

SAMPLE PREPARATION AND HEAT TREATMENT Prepare the product to be examined as described under Preparation of the Sample in Part I. Use two 10-ml portions each corresponding to 1 g or 1 ml of the product to be examined to inoculate a suitable amount (determined as described under Suitability of the Test Method) of Reinforced medium for clostridia. Heat one portion at 80° for 10 minutes and cool rapidly. Do not heat the other portion. Incubate both containers under anaerobic conditions at 30° to 35° for 48 hours.

SELECTION AND SUBCULTURE After incubation, make subcultures from each container on plates of Columbia agar to which gentamicin has been added and incubate under anaerobic conditions at 30° to 35° for 48 to 72 hours.

INTERPRETATION If no growth occurs, the product passes the test for absence of Clostridium spp. When growth of rods (with or without endospores) giving a negative catalase reaction occurs, subculture each distinct colony from on plates of Columbia agar, without gentamicin, and incubate at 30° to 35° for 48 to 72 hours, one plate anaerobically and the other aerobically, to check that the organism will not grow under aerobic condition.

Examine the appearance of only anaerobic growth of Gram-positive bacilli giving a negative catalase reaction together with the extent of hemolysis, by making subculture on a plate of Defibrinated sheep blood agar, and also examine microscopically for spore formation, using Gram stain or spore stain technique and confirmed by further suitable biochemical and biological tests. The description in Table 10 gives the characteristics of some *Clostridium* species on Defibrinated sheep blood agar.

Table 10 Characteristics of *Clostridium* Species on Defibrinated Sheep Blood Agar

Selective Species	Colonies	Hemolysis	Spores (Staining)
Clostridium botulinum	Irregular, translucent with a granular surface and indefinited fimbriated spreading edge.	+	Oval, central, subterminal distend bacilli
Clostridium perfringens	Large, circular, convex, semitranslucent, smooth with an entire edge.	Double zone	Oval and subterminal (very rare)
Clostridium tetani	Transparent with long feathery spreading projections.	+	Spherical and terminal (drumstick)

Buffer Solution and Media

Culture media may be prepared as follows, or dehydrated culture media may be used if they have similar or comparable nutritive and selective properties for the micro-organisms to be tested for.

In preparing the media according to the formulae set forth herein, dissolve the soluble solids in the water, using heat, if necessary, to effect complete solution, and add other ingredients. Add, if necessary, a solution of hydrochloric acid or sodium hydroxide in quantities sufficient to yield the desired pH in the medium when it is ready for use. Determine the pH at 25°±2°.

Where agar is called for in a formula, use agar that has a moisture content of not more than 15 per cent.

Unless otherwise indicated, the buffer solution and media should be dispensed and sterilized by heating in an autoclave at 121°±2° for not less than 15 minutes, depending on the volume to be sterilized. Store under refrigeration.

BUFFER SOLUTION

Stock buffer solution

Place 34 g of *potassium dihydrogenphosphate* in a 1000-ml volumetric flask, dissolve in 500 ml of *water*, adjust to pH 7.2±0.2 with *sodium hydroxide*, dilute to 1000.0 ml with *water* and mix. Dispense into containers and sterilize. Store at 2° to 8°.

Phosphate buffer pH 7.2

Prepare a mixture of 1 volume of stock buffer solution and 800 volumes of *water* and sterilize.

Buffered sodium chloride-peptone solution pH 7.0

Potassium dihydrogenphosphate	3.56	g
Disodium hydrogenphosphate heptahydrate	10.89	g
Sodium chloride	4.30	g
Peptone, dried	1.0	g
Water	1000	ml

Polysorbate 20 or *80* may be added to obtain a 0.1 to 1.0 per cent w/v solution.

pH after sterilization: 7.0±0.1.

MEDIA

Baird-Parker agar

Pancreatic digest of casein	10.0	g
Beef extract	5.0	g
Yeast extract	1.0	g
Lithium chloride	5.0	g
Agar	20.0	g
Glycine	12.0	g
Sodium pyruvate	10.0	g
Water	950	ml

Heat with frequent agitation, and boil for 1 minute. Sterilize, cool to between 45° and 50°, and add 10 ml of a sterile, 1 per cent w/v solution of *potassium tellurate(IV)* and 50 ml of egg-yolk emulsion. Mix intimately but gently, and pour into plates.

pH after sterilization: 6.8±0.2.

Preparation of the egg-yolk emulsion: Disinfect the surface of whole shell eggs, aseptically crack the eggs, and separate out intact yolks into a sterile graduated cylinder. Add *saline TS* to obtain a 3 to 7 ratio of egg-yolk to saline. Add to a sterile blender cup, and mix at high speed for 5 seconds.

Bismuth sulfite agar

Beef extract	5.0	g
Pancreatic digest of casein	5.0	g
Peptic digest of animal tissue	5.0	g
Dextrose monohydrate	5.0	g
Disodium hydrogenphosphate heptahydrate	4.0	g
Iron(II) sulfate	0.3	g
Bismuth sulfite indicator	8.0	g
Agar	20.0	g
Brilliant green	25.0	mg
Water	1000	ml

Heat the mixture of solids and *water*, with swirling, just to the boiling point. *Do not overheat or sterilize*. Transfer at once to a water-bath maintained at about 50°, and pour into plates as soon as the medium has cooled.

Final pH: 7.6±0.2.

Brilliant green agar

Yeast extract	3.0	g
Peptic digest of animal tissue	5.0	g
Pancreatic digest of casein	5.0	g
Lactose	10.0	g
Sodium chloride	5.0	g
Sucrose	10.0	g
Phenol red	80.0	mg
Agar	20.0	g
Brilliant green	12.5	mg
Water	1000	ml

Boil the solution of solids for 1 minute. Sterilize just prior to use. Melt the medium, pour into Petri dishes, and allow to cool.

pH after sterilization: 6.9±0.2.

Casein digest-soy lecithin polysorbate 20 broth

Pancreatic digest of casein	20.0	g
Soy lecithin	5.0	g
Polysorbate 20	40	ml
Water	960	ml

Dissolve pancreatic digest of casein and soy lecithin in 960 ml of *water*, heating in a water-bath at 48° to 50° for about 30 minutes to effect solution. Add 40 ml of *polysorbate 20*. Mix and dispense as desired.

pH after sterilization: 7.3±0.2.

Cetrimide Agar

Pancreatic digest of casein	20.0	g
Magnesium chloride	1.4	g
Potassium sulfate	10.0	g
Agar	13.6	g
Cetrimide	0.3	g
Glycerol	10.0	ml
Water	1000	ml

Dissolve all solid components in *water*, and add *glycerol*. Heat, with frequent agitation, and boil for 1 minute to effect solution.

pH after sterilization: 7.2±0.2.

Columbia agar

Pancreatic digest of casein	10.0	g
Peptic digest of animal tissue	5.0	g
Heart pancreatic digest	3.0	g
Yeast extract	5.0	g
Maize starch	1.0	g
Sodium chloride	5.0	g
Agar, according to gelling power	10.0 to 15.0	g
Water	1000	ml

Hydrate the agar, and dissolve by heating to boiling with continuous stirring. Sterilize, cool to between 45° and 50° and add, where necessary, gentamicin sulfate corresponding to 20 mg of gentamicin base. Pour into Petri dishes.

pH after sterilization: 7.3±0.2.

Defibrinated sheep blood agar (Blood agar)

Heat Soybean casein digest agar and cool to 45° to 50° in a water-bath. Add sufficient amount of defibrinated sheep blood to make 5 per cent and mix.

Enterobacteria enrichment broth-Mossel

Pancreatic digest of gelatin	10.0	g
Dextrose monohydrate	5.0	g
Dehydrated ox bile	20.0	g
Potassium dihydrogenphosphate	3.0	g
Disodium hydrogenphosphate dihydrate	8.0	g
Brilliant green	15.0	mg
Water	1000	ml

Mix and heat at 100° for 30 minutes to sterilize and cool immediately. *Do not autoclave*.

Final pH: 7.2±0.2.

Lactose broth

Beef extract	3.0	g
Pancreatic digest of gelatin	5.0	g
Lactose	5.0	g
Water	1000	ml

Cool as quickly as possible after sterilization.

pH after sterilization: 6.9±0.2.

Levine eosin-methylene blue agar

Pancreatic digest of gelatin	10.0	g
Dipotassium hydrogenphosphate	2.0	g
Agar	15.0	g
Lactose	10.0	g
Eosin Y	0.4	g
Methylene blue	65.0	mg
Water	1000	ml

Dissolve pancreatic digest of gelatin, *dipotassium hydrogenphosphate* and *agar* in *water*, with warming, and allow to cool. Just prior to use, liquefy the gelled agar solution, add the remaining ingredients, as solutions, in the following amounts, and mix: for each 100 ml of the liquefied agar solution 5 ml of a 20 per cent w/v solution of *lactose*, 2 ml of a 2 per cent w/v solution of *eosin Y*, and 2 ml of a 0.33 per cent w/v solution of *methylene blue*. The finished medium may not be clear.

pH after sterilization: 7.1±0.2.

MacConkey agar

Pancreatic digest of gelatin	17.0	g
Pancreatic digest of casein	1.5	g
Peptic digest of animal tissue	1.5	g
Lactose	10.0	g
Bile salts mixture	1.5	g
Sodium chloride	5.0	g
Agar	13.5	g
Neutral red	30.0	mg
Crystal violet	1.0	mg
Water	1000	ml

Boil the mixture of solids and water for 1 minute to effect solution.

pH after sterilization: 7.1±0.2.

MacConkey broth

Pancreatic digest of gelatin	20.0	g
Lactose	10.0	g
Dehydrated ox bile	5.0	g
Bromocresol purple	10.0	mg
Water	1000	ml

Prepare as directed under Buffer Solution and Media.

pH after sterilization: 7.3±0.2.

Mannitol-salt agar

Pancreatic digest of casein	5.0	g
Papaic digest of animal tissue	5.0	g
Beef extract	1.0	g
Mannitol	10.0	g
Sodium chloride	75.0	g
Agar	15.0	g
Phenol red	25.0	mg
Water	1000	ml

Mix, then heat with frequent agitation, and boil for 1 minute to effect solution.

pH after sterilization: 7.4±0.2.

Potato dextrose agar

Cook 300 g of peeled and diced potatoes in 500 ml of *water* prepared by distillation, filter through cheesecloth, add *water* prepared by distillation to make 1000 ml, and add the following:

Agar	15.0	g
Dextrose monohydrate	20.0	g

Dissolve by heating and sterilize.

pH after sterilization: 5.6±0.2.

For use, just prior to pouring the plates, adjust the melted and cooled to 45° medium with a sterile 10 per cent w/v solution of *tartaric acid* to a pH of 3.5±0.1. *Do not reheat the pH 3.5 medium.*

Pseudomonas agar for detection of fluorescin

Pancreatic digest of casein	10.0	g
Peptic digest of animal tissue	10.0	g
Dipotassium hydrogenphosphate	1.5	g
Magnesium sulfate	1.5	g
Agar	15.0	g
Glycerol	10.0	ml
Water	1000	ml

Dissolve the solid components in *water* before adding *glycerol*. Heat, with frequent agitation, and boil for 1 minute to effect solution.

pH after sterilization: 7.2±0.2.

Pseudomonas agar for detection of pyocyanin

Pancreatic digest of gelatin	20.0	g
Magnesium chloride	3.0	g
Potassium sulfate	10.0	g
Agar	15.0	g
Glycerol	10.0	ml
Water	1000	ml

Dissolve the solid components in *water* before adding glycerol. Heat, with frequent agitation, and boil for 1 minute to effect solution.

pH after sterilization: 7.2±0.2.

Rappaport-Vassiliadis broth

Soya peptone	4.5	g
Sodium chloride	8.0	g
Dipotassium phosphate	0.4	g
Potassium dihydrogenphosphate	0.6	g
Magnesium chloride	29.0	g
Malachite green	36.0	mg
Water	1000	ml

Mix and heat to effect solution.

pH after sterilization: 5.±0.2.

Reinforced medium for clostridia

Beef extract	10.0	g
Peptone	10.0	g
Yeast extract	3.0	g
Soluble starch	1.0	g
Dextrose monohydrate	5.0	g
Cysteine hydrochloride	0.5	g
Sodium chloride	5.0	g
Sodium acetate	3.0	g
Agar	0.5	g
Water	1000	ml

Hydrate the agar, and dissolve by heating to boiling with continuous stirring.

pH after sterilization: 6.8±0.2.

Sabouraud dextrose agar

Dextrose monohydrate	40.0	g
Mixture of equal parts of Peptic digest of animal tissue and Pancreatic digest of casein	10.0	g
Agar	15.0	g
Water	1000	ml

Mix and boil to effect solution.

pH after sterilization: 5.6±0.2.

Sabouraud dextrose agar with antibiotics

Dextrose monohydrate	40.0	g
Mixture of equal parts of Peptic digest of animal tissue and pancreatic digest of casein	10.0	g
Agar	15.0	g
Water	1000	ml

Mix and boil to effect solution. Immediately before use, add 0.10 g of *benzylpenicillin sodium* and 0.10 g of *tetracycline* per litre of medium as sterile solutions or alternatively, add 50 mg of *chloramphenicol* per litre of medium before sterilization.

pH after sterilization: 5.6±0.2.

(**Note** Other antibiotics can all be used, individually or in combination.)

Sabouraud dextrose broth

Dextrose monohydrate	20.0	g
Mixture of equal parts of Peptic digest of animal tissue and Pancreatic digest of casein	10.0	g
Water	1000	ml

Prepare as directed under *Buffer Solution and Media*.

pH after sterilization: 5.6±0.2.

Soybean-casein digest agar

Pancreatic digest of casein	15.0	g
Papaic digest of soybean meal	5.0	g
Sodium chloride	5.0	g
Agar	15.0	g
Water	1000	ml

Prepare as directed under *Buffer Solution and Media.*

pH after sterilization: 7.3±0.2.

Soybean-casein digest broth

Pancreatic digest of casein	17.0	g
Papaic digest of soybean meal	3.0	g
Sodium chloride	5.0	g
Dipotassium hydrogenphosphate	2.5	g
Dextrose monohydrate	2.5	g
Water	1000	ml

Prepare as directed under *Buffer Solution and Media*.

pH after sterilization: 7.3±0.2.

Tetrathionate bile brilliant green broth

Peptone	8.6	g
Ox bile, dried	8.0	g
Sodium chloride	6.4	g
Calcium carbonate	20.0	g
Potassium tetrathionate	20.0	g
Brilliant green	70.0	mg
Water	1000	ml

Heat the solution of solids to boiling. *Do not reheat*.

Final pH: 7.0±0.2.

Triple sugar-iron-agar

Pancreatic digest of casein	10.0	g
Pancreatic digest of animal tissue	10.0	g
Lactose	10.0	g
Sucrose	10.0	g
Dextrose monohydrate	1.0	g
Ammonium iron(II) sulfate	0.2	g
Sodium chloride	5.0	g
Sodium thiosulfate	0.2	g

Agar	13.0	g
Phenol red	25.0	mg
Water	1000	ml

Prepare as directed under *Buffer Solution and Media*.

pH after sterilization: 7.3±0.2.

Violet red bile dextrose agar

Yeast extract	3.0	g
Pancreatic digest of gelatin	7.0	g
Bile salts mixture	1.5	g
Lactose	10.0	g
Sodium chloride	5.0	g
Dextrose monohydrate	10.0	g
Agar	15.0	g
Neutral red	30.0	mg
Crystal violet	2.0	mg
Water	1000	ml

Mix and heat to boiling. *Do not overheat or sterilize.* Transfer at once to a water-bath maintained at about 50°, and pour into plates as soon as the medium has cooled.

Final pH: 7.4±0.2.

Vogel-Johnson Agar

Pancreatic digest of casein	10.0	g
Yeast extract	5.0	g
Mannitol	10.0	g
Dipotassium hydrogenphosphate	5.0	g
Lithium chloride	5.0	g
Glycine	10.0	g
Agar	16.0	g
Phenol red	25.0	mg
Water	1000	ml

Boil the solution of solids for 1 minute. Sterilize, cool to between 45° and 50°, and add 20 ml of a sterile 1 per cent w/v solution of *potassium tellurate(IV).*

pH after sterilization: 7.2±0.2.

Xylose-lysine-deoxycholate agar

Xylose	3.5	g
L-Lysine	5.0	g
Lactose	7.5	g
Sucrose	7.5	g
Sodium chloride	5.0	g
Yeast extract	3.0	g
Agar	13.5	g
Sodium desoxycholate	2.5	g
Sodium thiosulfate	6.8	g
Ammonium iron(III) citrate	0.8	g
Phenol red	80.0	mg

Water	1000	ml

Heat the mixture of solids and *water*, with swirling, just to the boiling point. *Do not overheat or sterilize.* Transfer at once to a water-bath maintained at about 50°, and pour into plates as soon as the medium has cooled.

Final pH: 7.4±0.2.

10.4 MICROBIOLOGICAL ATTRIBUTES OF NON-STERILE PHARMACEUTICAL PRODUCTS

Few raw materials used in making pharmaceutical products are sterile as received, and special treatment may be required to render them microbiologically acceptable for use. Strict adherence to effective environmental control and sanitation, equipment cleaning practices, and good personal hygiene practices in pharmaceutical manufacture is vital in minimizing both the type and the number of micro-organisms.

Monitoring, in the form of regular surveillance, should include an examination of the microbiological attributes of Pharmacopoeial articles and a determination of compliance with such microbiological standards as are set forth in the individual monographs. It maybe necessary also to monitor the early and intermediate stages of production, with emphasis being placed on raw materials, especially those of animal or botanical origin, or from natural mineral sources which may harbour objectionable micro-organisms not destroyed during subsequent processing. It is essential that ingredients and components be stored under conditions designed to deter microbial proliferation. Microbiological purity of the raw materials as well as manufacturing conditions, including water used for the production, should be such a degree that the microbiological purity requirements for the final product i.e., the pharmaceutical preparation, are observed to fulfill the requirements for good manufacturing practices (GMP).

The nature and frequency of testing vary according to the product. Monographs for some articles require freedom from one or more species of selected indicator micro-organisms such as *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. For some articles, a specific limit on the total aerobic microbial count (TAMC) and/or the total combined yeasts and moulds count (TYMC) is set forth in the individual monograph. In these cases a requirement for freedom from specified indicator micro-organisms is also included. The significance of micro-organisms in a non-s terile pharmaceutical product should be evaluated in terms of the use of the product, the nature of the product, and the potential hazard to the user. Also taken into account is the processing of the product in relation to an acceptable quality for pharmaceutical purposes.

It is suggested that certain categories of products be tested routinely for total microbial count and for specified indicator microbial contaminants, e.g., natural plant, animal, and some mineral products for *Salmonella* species; oral solutions and suspensions for *E. coli*; articles applied topically for *P. aeruginosa* and *S. aureus*; and articles intended for rectal, urethral, or vaginal administration for yeasts and moulds.

Definitive microbial limits (stipulated micro-organisms and/or counts) are incorporated into specific monographs on the basis of a major criterion, i.e., the potential of the stipulated micro-organisms and/or counts, and of any others that they may reflect, to constitute a hazard in the end product. Such considerations also take into account the processing to which the product components are subjected, the current technology for testing, and the availability of desired quality material. Any of these may preclude the items from specific requirements in the "Microbial Limit Tests" (Appendix 10.2). Regardless of such preclusion, it remains essential to apply strict good manufacturing practices to assure a lowest possible load of micro-organisms. "Limits for Microbial Contamination" (Appendix 10.5) is also set forth to control microbial purity in non-sterile pharmaceutical products. For herbal remedies or herbal drug preparation described in categories Table 2, the same criteria are applicable to both crude drugs and their preparations.

The relevant tests for determining the total aerobic microbial count and the total combined yeasts and moulds count, and for detection and identification of designated species are given in the "Microbial Limit Tests" (Appendix 10.2). For reliable results, the personnel responsible for the conduct of the test should have specialized training in microbiology and in the interpretation of microbiological data.

10.5 LIMITS FOR MICROBIAL CONTAMINATION

In the manufacture, packaging, storage and distribution of pharmaceutical preparations, suitable means must be taken to ensure their microbiological quality. Unless otherwise specified in the individual monograph, the non-sterile pharmaceutical preparations should comply with the acceptance criteria given in Table 1 and the herbal drug preparations should comply with the acceptance criteria given in Table 2.

Table 1 Acceptance Criteria for Microbiological Quality of Non-sterile Pharmaceutical Preparations

Category	Types	Requirements*
1	Topical preparations for broken skins, abscess, lesions, and mucous total membranes excluding for vaginal and rectal routes.	- Total aerobic microbial count: not more than 2×10^1 CFU per g or per ml and combined yeasts and moulds count: not more than 2×10^1 CFU per g or per ml. - Absence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* per g or per ml.
2	A. Preparations for inhalation use except where required to be sterile.	- Total aerobic microbial count: not more than 2×10^2 CFU per g or per ml and total combined yeasts and moulds count: not more than 2×10^1 CFU per g or per ml. - Absence of bile-tolerant gram-negative bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus* per g or per ml.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

Table 1 Acceptance Criteria for Microbiological Quality of Non-sterile Pharmaceutical Preparations

Category	Types	Requirements*
	B. Preparation for vaginal use.	- Total aerobic microbial count: not more than 2 × 10^2 CFU per g or per ml and total combined yeasts and moulds count: not more than 2 × 10^1 CFU per g or per ml. - Absence of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* per g or per ml.
	C. Preparation for rectal use.	- Total aerobic microbial count: not more than 2 × 10^3 CFU per g or per ml and total combined yeasts and moulds count: not more than 2 × 10^2 CFU per g or per ml.
3	Transdermal patchs and topical preparation for intact skin, e.g. creams, lotions, ointments, solutions, powders, etc.	- Total aerobic microbial count: not more than 2 × 10^2 CFU per g or per ml or per patch and total combined yeasts and moulds count: not more than 2 × 10^1 CFU per g or per ml or per patch. - Absence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* per g or per ml or per patch.
4	A. Aqueous preparations for oral use.	- Total aerobic microbial count: not more than 2 × 10^2 CFU per g or per ml and total combined yeasts and moulds count: not more than 2 × 10^1 CFU per g or per ml. - Absence of *Escherichia coli* per g or per ml.
	B. Non-aqueous preparations for oral use.	- Total aerobic microbial count: not more than 2 × 10^3 CFU per g or per ml and total combined yeasts and moulds count: not more than 2 × 10^2 CFU per g or per ml. - Absence of *Escherichia coli* per g or per ml.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

Table 1 Acceptance Criteria for Microbiological Quality of Non-sterile Pharmaceutical Preparations

Category	Types	Requirements*
5	Preparations for oral administration containing raw materials of natural origin (animal, vegetable or mineral) which cannot be treated with a process for reduction of microbial count.**	- Total aerobic microbial count: not more than 2×10^4 CFU per g or per ml and total combined yeasts and moulds count: not more than 2×10^2 CFU per g or per ml. - Bile-tolerant gram-negative bacteria: not more than 10^2 probable number of bacteria per g or per ml. - Absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* per g or per ml. - Absence of *Salmonella* spp. per 10 g or per 10 ml.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

**Specified for raw materials of natural origin that are not obtainable with the required purity including those for manufacturing drugs where an antimicrobial treatment (e.g., with ethylene oxide or ionizing radiations) is not feasible or permissible. The examples are arabic gum, tragacanth, pancreas powder, pepsin, and trypsin.

Table 2 Acceptance Criteria for Microbiological Quality of Herbal Drug Preparations

Category	Types	Requirements*
1	Preparations from crude drug extracts which underwent a process for reduction of micro-organisms.	
	A. Preparations for oral use.	- Total aerobic microbial count: not more than 5×10^3 CFU per g or per ml and total combined yeasts and moulds count: not more than 5×10^2 CFU per g or per ml. - Absence of *Escherichia coli* per g or per ml. - Absence of *Salmonella* spp. per 10 g or per 10 ml.
	B. Topical preparations for intact skin.	- Total aerobic microbial count: not more than 5×10^2 CFU per g or per ml and total combined yeasts and moulds count: not more than 5×10^1 CFU per g or per ml. - Absence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* per g or per ml.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

Table 2 Acceptance Criteria for Microbiological Quality of Herbal Drug Preparations

Category	Types	Requirements*
2	Preparations of crude drugs and mixtures of crude drugs for internal use which will undergo a process for reduction of microbial count before use (e.g., by pouring boiling water over them).	- Total aerobic microbial count: not more than 5 × 10^7 CFU per g or per ml and total combined yeasts and moulds count: not more than 5 × 10^5 CFU per g or per ml. - Absence of *Escherichia coli* and *Clostridium* spp. per g or per ml. - Absence of *Salmonella* spp. per 10 g or per 10 ml.
3	Preparations of crude drugs and mixtures of crude drugs for external use.**	- Total aerobic microbial count: not more than 5 × 10^7 CFU per g or per ml and total combined yeasts and moulds count: not more than 5 × 10^5 CFU per g or per ml. - Absence of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Clostridium* spp. per g or per ml.
4	Other preparations for internal use containing whole or ground crude drugs.	- Total aerobic microbial count: not more than 5 × 10^5 CFU per g or per ml and total combined yeasts and moulds count: not more than 5 × 10^4 CFU per g or per ml. - Bile-tolerant gram-negative bacteria: not more than 10^3 probable number of bacteria per g or per ml. - Absence of *Escherichia coli* and *Clostridium* spp. per g or per ml. - Absence of *Salmonella* spp. per 10 g or per 10 ml.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

**The examples are LUKPRAKHOP (herbal compress), YAPHOK (herbal poultice).

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