Thai Herbal Pharmacopoeia 2020

Volume II

กรมวิทยาศาสตร์การแพทย์
DEPARTMENT OF MEDICAL SCIENCES

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DEPARTMENT OF MEDICAL SCIENCES

THAI HERBAL PHARMACOPOEIA 2020

VOLUME II

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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.

---

[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

Unless otherwise indicated, the test procedures are provided in the “Limit Tests for Heavy Metals in Herbal Drugs and Herbal Drug Preparations” (Appendix 5.2).

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

ปัญจขันธ์ (PANCHA KHAN)

เจียวกู่หลาน (CHIAOKULAN)
Gynostemmae Pentaphylli Herba
Five-Leaf Ginseng

Synonyms Jiaogulan, Southern Ginseng

Category Tonic.

Five-Leaf Ginseng is the dried aerial part of *Gynostemma pentaphyllum* (Thunb.) Makino (Family Cucurbitaceae), Herbarium Specimen Number: BKF 194898, Crude Drug Number: DMSc 0440.

Constituents Five-Leaf Ginseng contains triterpenoid saponins (e.g., gypenoside III or ginsenoside Rb1). It also contains flavonoids (e.g., quercetin, rutin), etc.

Description of the plant (Fig. 1) Herbaceous climber, dioecious or monoecious, 2 to 8 m long; stem slender, angular-sulcate, glabrous or sparsely pubescent; tendril 2-branched at apex. Leaves alternate, palmately compound, usually 5- to 7-foliolate or 5- to 7-pedate; leaflet ovate-oblong to narrowly elliptic, 3 to 10 cm long, 1 to 5 cm wide, outer leaflet smallest, apex acute or shortly acuminate, base attenuate, margin usually finely dentate, membranous or papery, upper surface hairy or subglabrous, lower surface puberulent or glabrous, lateral veins usually 3 to 6 pairs; petiole 3 to 6 cm long, petiolule 2 to 7 mm long. Inflorescence raceme-like or broadly panicle, 5 to 15 cm long, rarely to 30 cm long; peduncle 1 to 7 cm long; female inflorescence smaller. Flower greenish white or white, rotate, 2 to 5 mm in diameter; sepals 5, free; petals 5, free, long triangular. Male flower with pedicel 1 to 4 mm long; sepal narrowly linear-elliptic, about 1 mm long; petal 2 to 5 mm long, about 2 mm wide, 1-veined, apex acuminate. Female flower with pedicel 2 to 4 mm long; calyx and corolla as in male flower; ovary globose, about 1 mm in diameter, glabrous or hairy, 3- or 5-loculed; styles 3, each 0.5 to 1 mm long; stigma 2-lobed. Fruit dry berry, globose, 5 to 6 mm long, hairy, blackish when ripe; fruiting pedicel 2 to 5 mm long. Seeds 2, ovate-cordate, about 4 mm long, apex obtuse, base cordate, moderately compressed, irregularly verrucose or wrinkled, brown.

Description Odour, grassy; taste, slightly bitter.

Macroscopical (Fig. 1) Stems and leaves, greenish brown. Stem slender, angular-sulcate, glabrous or sparsely pubescent, some with tendrils. Leaves, irregularly broken pieces, curled.

Microscopical (Figs. 2a, 2b, 2c, 2d) Transverse sections of the leaf through the midrib and lamina show upper epidermis, mesophyll, vascular bundles, and lower epidermis. Upper epidermis, a layer of rectangular epidermal cells, with numerous large multicellular uniseriate trichomes with cuticularized striation and few glandular trichomes. Mesophyll, a layer of large palisade cells and several layers of spongy parenchyma. Vascular bundle, composed of phloem and xylem; vessels, spiral. Collenchyma cells, located beneath upper and lower epidermises of the midrib. Lower epidermis, rectangular cells with stomata; trichomes, multicellular uniseriate and glandular.

Transverse sections of the stem show epidermis, cortex and stele. Epidermis, a layer of epidermal cells, some with glandular trichomes. Cortex, collenchyma located in the angles of the stem and thin-walled parenchyma. Stele, layers of pericyclic fibre, collateral vascular bundles, vascular cambium, and pith parenchyma.

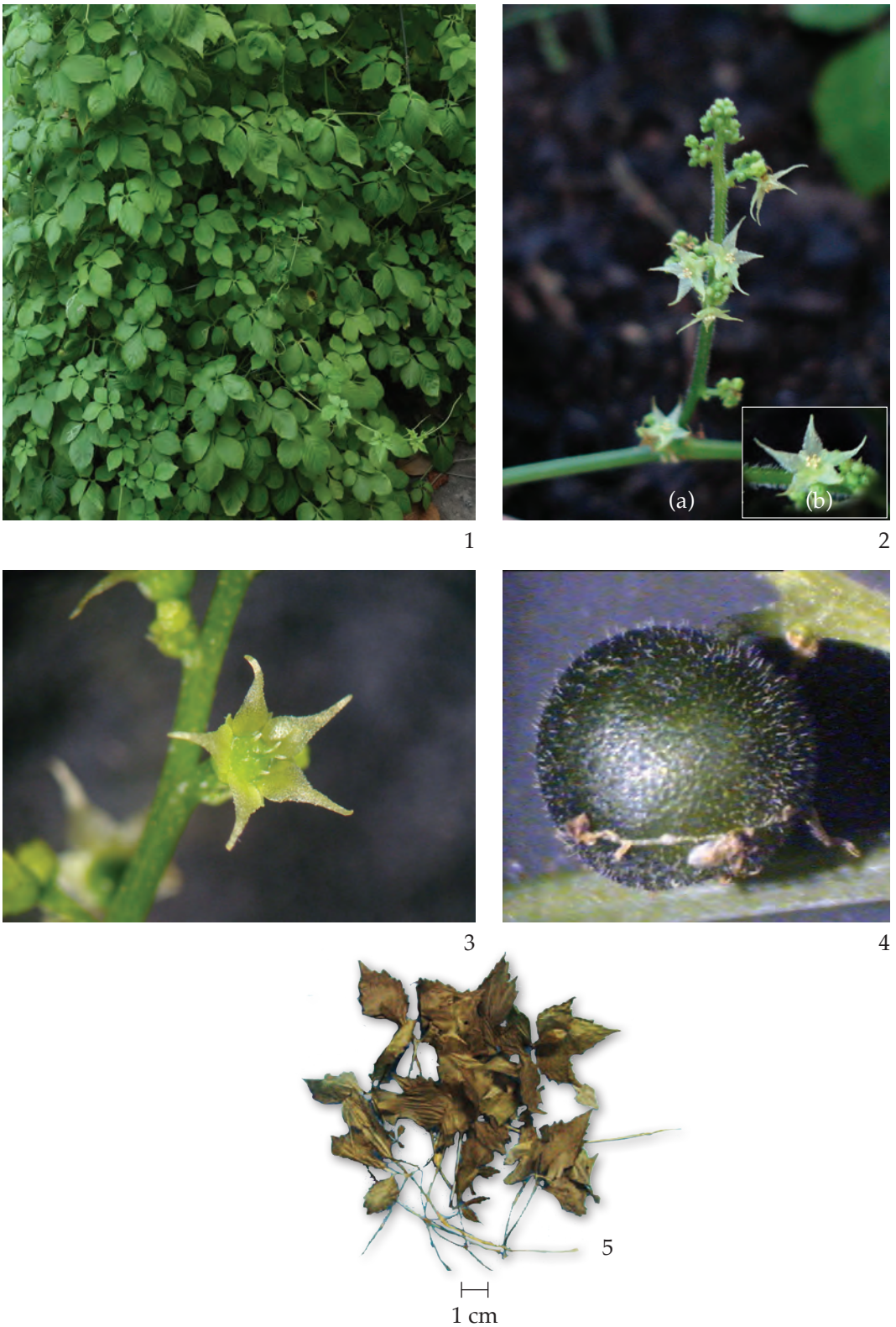
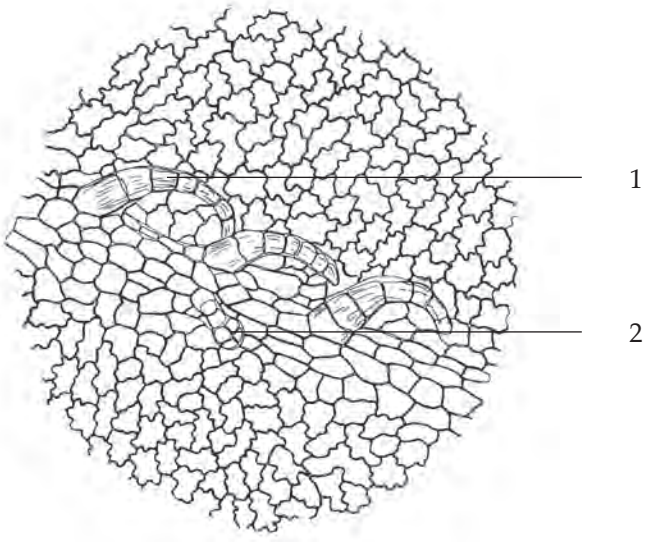
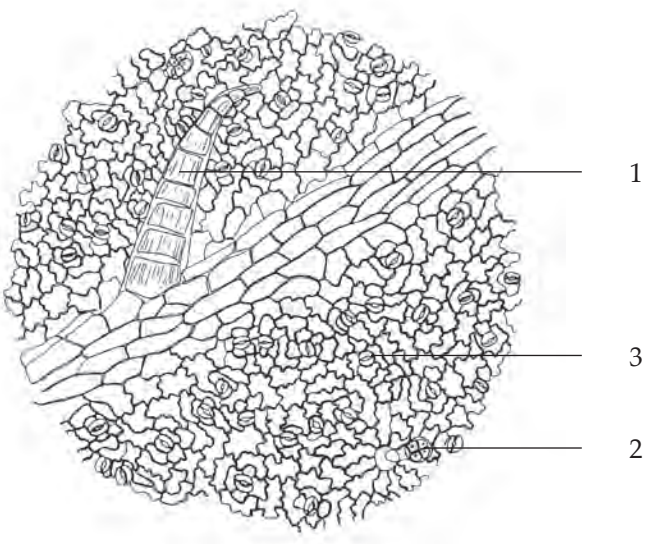


Fig. 1 *Gynostemma pentaphyllum* (Thunb.) Makino
1. habit 2. male inflorescence (a), male flower (b) 3. female flower
4. ripe fruit 5. crude drug



100 μm

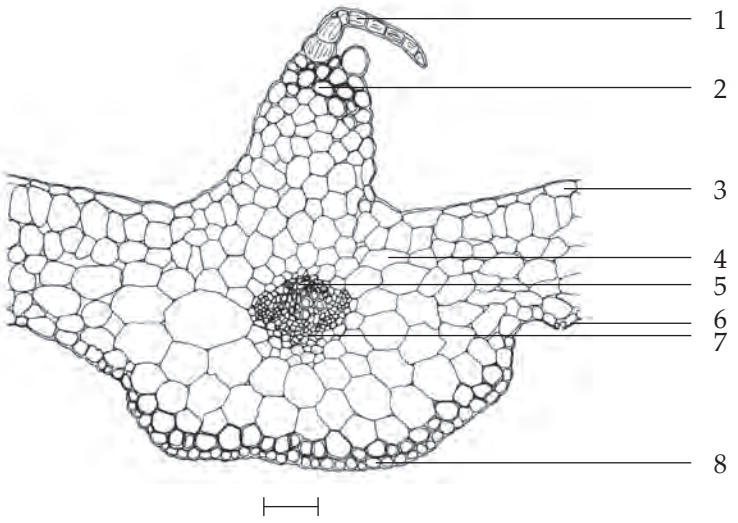
Upper Epidermis of the Lamina



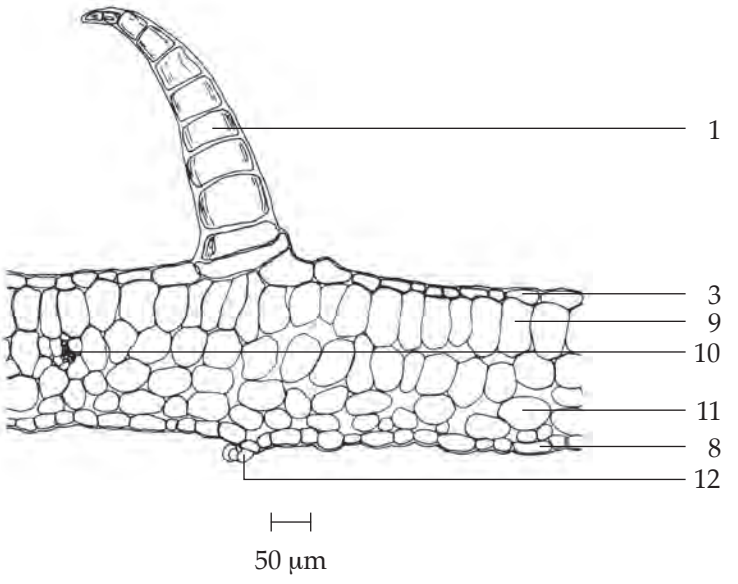
100 μm

Lower Epidermis of the Lamina

Fig. 2a Epidermises of the Leaf of *Gynostemma pentaphyllum* (Thunb.) Makino
1. multicellular uniseriate trichome
2. glandular trichome
3. anomocytic stoma



Transverse Section of the Midrib



Transverse Section of the Lamina

Fig. 2b Transverse Section of the Leaf of *Gynostemma pentaphyllum* (Thunb.) Makino

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

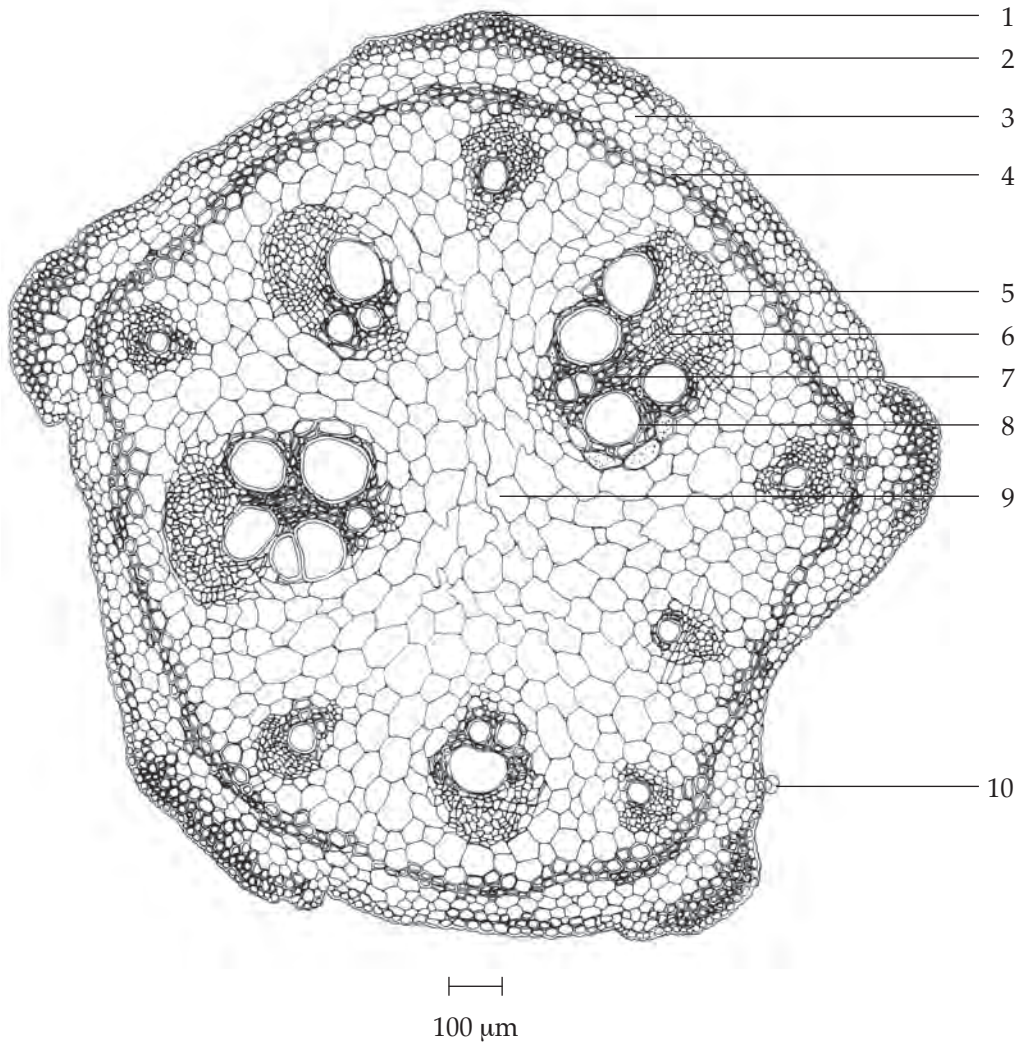


Fig. 2c Transverse Section of the Stem of *Gynostemma pentaphyllum* (Thunb.) Makino

1. epidermis
2. collenchyma
3. parenchyma
4. pericyclic fibre
5. phloem
6. vascular cambium
7. xylem
8. vessel
9. pith
10. glandular trichome

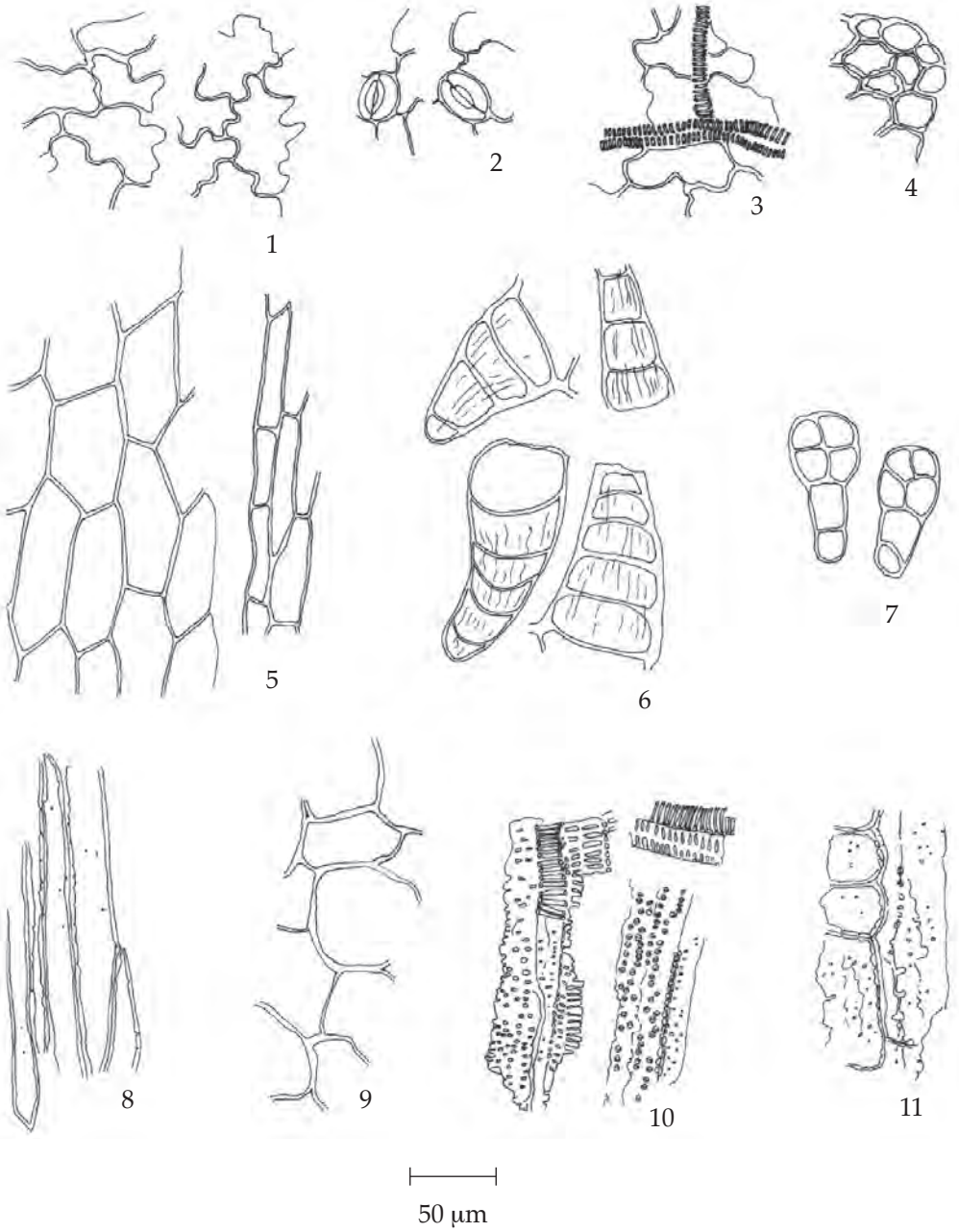


Fig. 2d Powdered Drug of the Aerial Parts of *Gynostemma pentaphyllum* (Thunb.) Makino

1. epidermis of lamina
2. lower epidermal cells with stomata
3. epidermis of lamina underlying with spiral vessels
4. epidermal cells and collenchyma in sectional view
5. epidermal cells in surface view
6. multicellular uniseriate trichomes with striation
7. glandular trichomes with stalk
8. fibres
9. parenchyma
10. simple-pitted, bordered-pitted spiral, reticulate, and scalariform vessels
11. porous parenchyma

Five-Leaf Gingseng in powder possesses the diagnostic microscopical characters of the unground drug. Glandular trichomes with stalk and multicellular uniseriate trichomes with cuticularized striation are characteristic.

Packaging and storage Five-Leaf Gingseng 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 50 mL of *water* on a water-bath for 30 minutes and filter (solution 1). Transfer 10 mL of solution 1 to a separator and extract with the equal volume of *1-butanol*. To the 1-butanol extract, add 100 mg of *decolorizing charcoal*, swirl for a few minutes and filter. Evaporate 2 mL of the filtrate to dryness and add a few drops of *sulfuric acid*: a red colour is produced.

B. Transfer 1 mL of solution 1 to a stoppered test-tube and shake for about 15 seconds: a persistent foam is produced for over 30 minutes.

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 lower layer of a mixture of 65 volumes of *chloroform*, 35 volumes of *methanol* and 10 volumes of *water* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Apply separately to the plate, 10 μL each of the following two solutions. Prepare solution (A) by transferring 10 mL of solution 1 and extracting with two 10-mL portions of *1-butanol.* Combine the 1-butanol extracts and evaporate to dryness under reduced pressure. Dissolve the residue in 1 mL of *methanol*. For solution (B), dissolve 1 mg of *gypenoside III* in 1 mL of *methanol*. After removal of the plate, allow it to dry in air. Spray the plate with a 20 per cent v/v solution of *sulfuric acid* in *methanol* and heat at 105° for 5 minutes. The chromatogram obtained from solution (A) shows a violet spot (hR_f value 26 to 27), corresponding to the gypenoside III spot obtained from solution (B). Several spots of different colours appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in the Extract of the Aerial Parts of *Gynostemma pentaphyllum* (Thunb.) Makino

Spot	hR_f Value	Detection
		20 Per Cent V/V Solution of *Sulfuric Acid* in *Methanol*
1	5–6	brownish red
2	10–11	violet
3	13–14	violet
4	15–17	purple
5	18–19	purple
6	21–22	violet
7	24–25	violet
8*	26–27	violet
9	29–31	violet
10	32–33	yellow
11	34–35	violet
12	39–41	purple
13	44–45	purple
14	49–50	yellowish green
15	53–54	violet
16	55–56	violet
17	58–59	violet
18	62–64	yellowish green
19	68–69	violet
20	70–72	violet
21	75–76	purple
22	90–94	brownish red
23	97–98	yellowish green

*gypenoside III

Loss on drying Not more than 8.0 per cent w/w after drying 5 g 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 14.0 per cent w/w (Appendix 7.7).

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

Content of total crude saponins Not less than 8.0 per cent w/w when determined by the following method.

Place about 500 mg of Five-Leaf Gingseng, in *fine powder*, accurately weighed, in a 250-mL round-bottomed flask, reflux with 50 mL of *water* for 2 hours and filter. Wash the marc with two 20-mL portions of hot *water*. Combine the washings and the filtrate, transfer to a 100-mL volumetric flask and adjust to volume. Transfer 20.0 mL of this solution to a separator and extract with three 10-mL portions of *1-butanol*. Combine the 1-butanol extracts, wash with two 10-mL portions of *water* and discard the washings. Evaporate the combined 1-butanol extract to dryness and dry the residue at 105° to constant weight. Weigh the residue and calculate the content of total crude saponins on the dried basis.

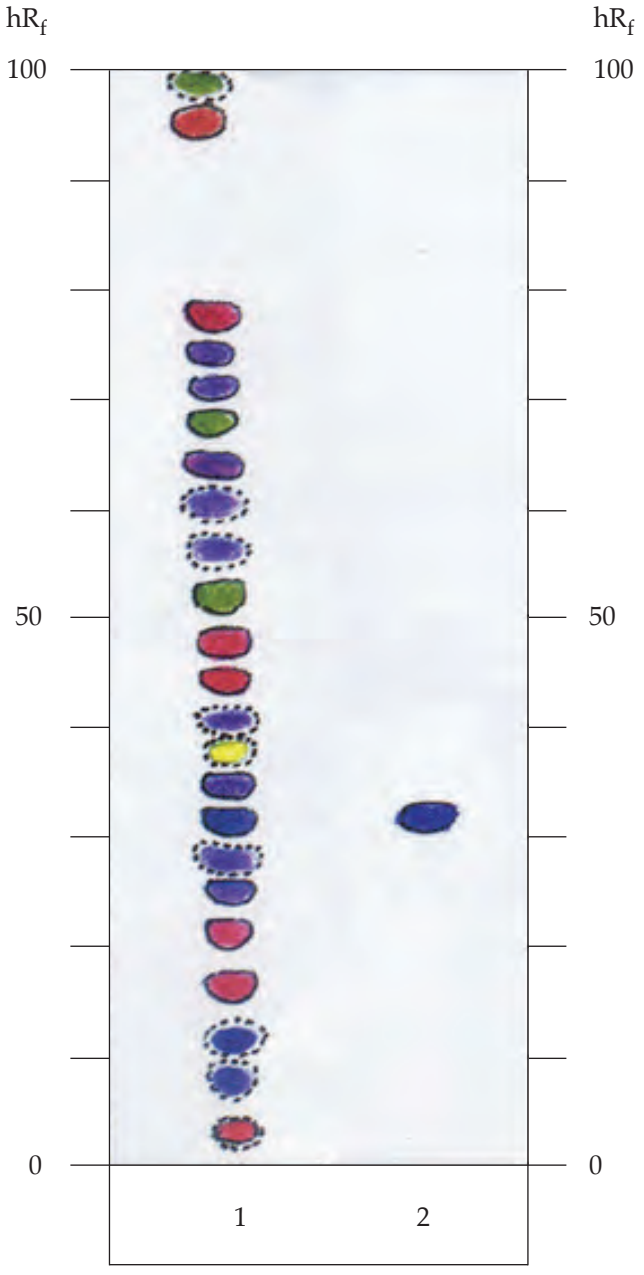


Fig. 3 Thin-Layer Chromatogram of the Extract of the Aerial Parts of *Gynostemma pentaphyllum* (Thunb.) Makino, Detected with a 20 Per Cent V/V Solution of *Sulfuric Acid* in *Methanol*

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

ยาแคปซูลปัญจขันธ์ (PANCHA KHAN CAPSULES)

Five-Leaf Ginseng Capsules

Synonym Jiaogulan Capsules

Category Tonic.

Five-Leaf Ginseng Capsules contain an amount of powdered Five-Leaf Ginseng equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the labelled amount of total crude saponins, calculated on the dried basis.

Strengths available 500 mg (powder).

Dose One to two capsules three times a day, before meals

Warning

1. Caution should be exercised if it is to be used in pregnant or nursing women.
2. It may cause mild adverse effects including nausea/vomiting, abdominal tension, diarrhoea or constipation, dizziness, blurred vision and tinnitus.

Precaution Because its bioactive components can inhibit platelet aggregation, lower blood glucose level and enhance immune system function; therefore, caution should be exercised when it is to be used concomitantly with antiplatelets, anticoagulants, antihyperglycemic agents, or immunomodulators.

Additional information The safety data of the Five-Leaf Ginseng powder have not yet been established.

Packaging and storage Five-Leaf Ginseng Capsules shall be kept in well-closed containers, protected from light, and stored in a dry place and at a temperature not exceeding 30°.

Labelling The label on the container states (1) the equivalent amount of total crude saponins; (2) the expiration date.

Identification

A. The capsule contents exhibit diagnostic structures of the powdered drug described under *Five-Leaf Ginseng*.

B. The capsule contents comply with the tests for Identification A, B and C described under *Five-Leaf Ginseng*.

Loss on drying Of the capsule contents, not more than 8.0 per cent w/w after drying 5 g 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).

Assay Weigh and mix the contents of not less than 20 capsules of Five-Leaf Ginseng Capsules. Place about 500 mg, accurately weighed, in a 250-mL round-bottomed flask, reflux with 50 mL of *water* for 2 hours, and filter. Wash the marc with two 20-mL portions of hot *water*. Combine the washings and the filtrate, transfer to a 100-mL volumetric flask and adjust to volume. Transfer 20.0 mL of this solution to a separator and extract with three 10-mL portions of *1-butanol*. Combine the 1-butanol extracts, wash with two 10-mL portions of *water* and discard the washings. Evaporate the 1-butanol extract to dryness and dry the residue at 105° to constant weight. Weigh the residue and calculate the content of total crude saponins on the dried basis.

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

สารสกัดแห้งปัญจขันธ์ (PANCHA KHAN DRY EXTRACT)

Five-Leaf Ginseng Dry Extract

Category Tonic.

Five-Leaf Ginseng Dry Extract is prepared from the powdered Five-Leaf Ginseng by extraction with *water*. It contains not less than 90.0 per cent and not more than 110.0 per cent of the labelled amount of total crude saponins; the labelled amount of total crude saponins is not less than 15.0 per cent, calculated on the dried basis. It may contains other added substances.

Description Yellowish green fine powder; odour, characteristic.

Packaging and storage Five-Leaf Ginseng 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 (1) the amount of total crude saponins; (2) the expiration date.

Identification

A. To 200 mg of the sample, in powder, add 10 mL of *water* and heat on a water-bath for 15 minutes (solution 1). Transfer solution 1 to a separator and extract with the equal volume of 1-*butanol*. To the 1-butanol extract, add 100 mg of *decolorizing charcoal*, swirl for a few minutes and filter. Evaporate 2 mL of the filtrate to dryness and add a few drops of *sulfuric acid*: a red colour is produced.

B. Transfer 1 mL of solution 1 to a stoppered test-tube and shake for about 15 seconds: a persistent foam is produced for over 30 minutes.

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 lower layer of a mixture of 65 volumes of *chloroform*, 35 volumes of *methanol* and 10 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, 5 μL each of the following two solutions. For solution (A), transfer 500 mg of the sample, in powder, to a 100-mL volumetric flask, dissolve in 50 mL of *water*, mix and adjust to volume. Transfer 10 mL of the solution to a separator, add 15 mL of *water* and extract the mixture with three 15-mL portions of 1-*butanol*. Evaporate the combined 1-butanol extract to dryness. Dissolve the residue in 1 mL of *methanol*. For solution (B), dissolve 1 mg of *gypenoside III* in 1 mL of *methanol*. After removal of the plate, allow it to dry in air. Spray the plate with a 20 per cent v/v solution of *sulfuric acid* in *methanol* and heat at 105° for 5 minutes. The chromatogram obtained from solution (A) shows a violet spot corresponding to the gypenoside III spot obtained from solution (B). Several spots of different colours appear.

Loss on drying Not more than 9.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Assay Transfer about 400 mg of Five-Leaf Ginseng Dry Extract, in powder, accurately weighed, to a 100-mL volumetric flask, add 50 mL of *water*, mix, and adjust to volume. Transfer 10.0 mL of this solution to a separator and extract with three 15-mL portions of *1-butanol*. Combine the 1-butanol extracts, wash with two 10-mL portions of *water* and discard the washings. Evaporate the combined 1-butanol extract to dryness and dry the residue at 105° to constant weight. Weigh the residue and calculate the content of total crude saponins on the dried basis.

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

ยาแคปซูลสารสกัดแห้งปัญจขันธ์ (PANCHA KHAN DRY EXTRACT CAPSULES)

Five-Leaf Ginseng Dry Extract Capsules

Synonym Jiaogulan Dry Extract Capsules

Category Tonic.

Five-Leaf Ginseng Capsules are prepared from Five-Leaf Ginseng Dry Extract and contain not less than 90.0 per cent and not more than 110.0 per cent of the labelled amount of total crude saponins, calculated on the dried basis.

Strengths available 250 and 300 mg (dry extract).

Dose 250 to 300 mg twice a day before meals.

Packaging and storage Five-Leaf Ginseng Capsules shall be kept in well-closed containers, protected from light, and stored in a dry place at a temperature not exceeding 30°.

Labelling The label on the container states (1) the amount of total crude saponins; (2) the expiration date.

Identification The capsule contents comply with the tests for Identification A, B, and C described under *Five-Leaf Ginseng Dry Extract.*

Loss on drying Of the capsule contents, not more than 9.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

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

Assay Weigh and mix the contents of not less than 20 capsules of Five-Leaf Ginseng Dry Extract Capsules. Transfer about 400 mg, in powder, accurately weighed, to a 100-mL volumetric flask, add 50 mL of *water*, mix, and adjust to volume. Transfer 10.0 mL of this solution to a separator and extract with three 15-mL portions of *1-butanol*. Combine the 1-butanol extracts, wash with two 10-mL portions of *water* and discard the washings. Evaporate the combined 1-butanol extract to dryness and dry the residue at 105° to constant weight. Weigh the residue and calculate the content of total crude saponins on the dried basis.

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

ยาชงปัญจขันธ์ (YA CHONG PANCHA KHAN)

Five-Leaf Ginseng Tea

Synonym Jiaogulan Tea

Category Tonic.

Five-Leaf Ginseng Tea contains an amount of powdered Five-Leaf Ginseng equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the labelled amount of total crude saponins, calculated on the dried basis.

Strengths available 1.5 g (powder), supplied in a sachet.

Dose One sachet, prepared as an infusion by soaking each with 200 mL of boiling water for 5 minutes, three times a day.

Packaging and storage Five-Leaf Ginseng Tea shall be kept in well-closed containers, protected from light, and stored at a temperature not exceeding 30°.

Labelling The label on the container states (1) the equivalent amount of total crude saponins; (2) expiration date.

Identification

A. The tea contents exhibit diagnostic structures of the powdered drug described under *Five-Leaf Ginseng*.

B. The tea contents comply with the tests for Identification A, B, and C described under *Five-Leaf Ginseng*.

Loss on drying Of the tea contents, not more than 8.0 per cent v/w after drying at 105° to constant weight (Appendix 4.15).

Microbial limit Complies with the requirements for Category 2 in the "Limits for Microbial Contamination" (Appendix 10.5).

Assay Grind the contents of not less than 20 sachets of Five-Leaf Ginseng Tea to *fine powder*. Place about 500 mg, accurately weighed, in a 250-mL round-bottomed flask, reflux with 50 mL of *water* for 2 hours, and filter. Wash the marc with two 20-mL portions of hot *water*. Combine the washings and the filtrate, transfer to a 100-mL volumetric flask and adjust to volume. Transfer 20.0 mL of this solution to a separator and extract with three 10-mL portions of *1-butanol*. Combine the 1-butanol extracts and wash with two 10-mL portions of *water*. Evaporate the 1-butanol extract to dryness and dry the residue at 105° to constant weight. Calculate the content of total crude saponins on the dried basis.

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

พญายอ (PHAYA YO)

ผักลิ้นเขียด (PHAK LINKHIAT), ผักมันไก่ (PHAK MAN KAI), พญาปล้องทอง (PHAYA PLONG THONG), เสลดพังพอนตัวเมีย (SALET PANGPHON TUAMIA)

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 tall, 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; petiole 3 to 15 mm long. Inflorescence dense cyme, 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, anther 1-celled; ovary compressed, style 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 trichome, multicellular uniseriate, 1- to 12-celled with warted walls. Glandular trichome, unicellular stalk, 6- to 8-celled head. Lower epidermis, almost similar to those of upper epidermis, slightly wavy-walled 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.

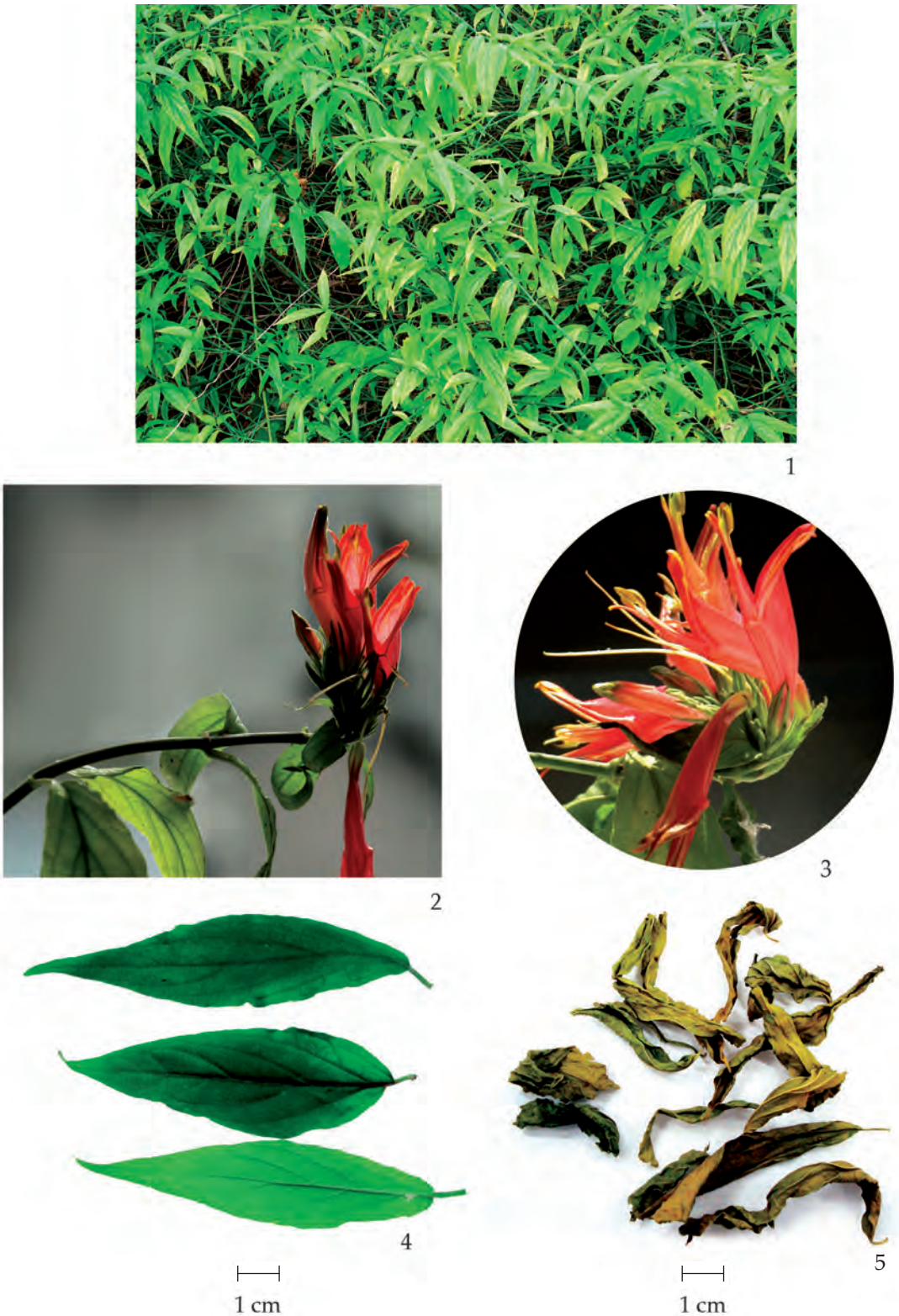
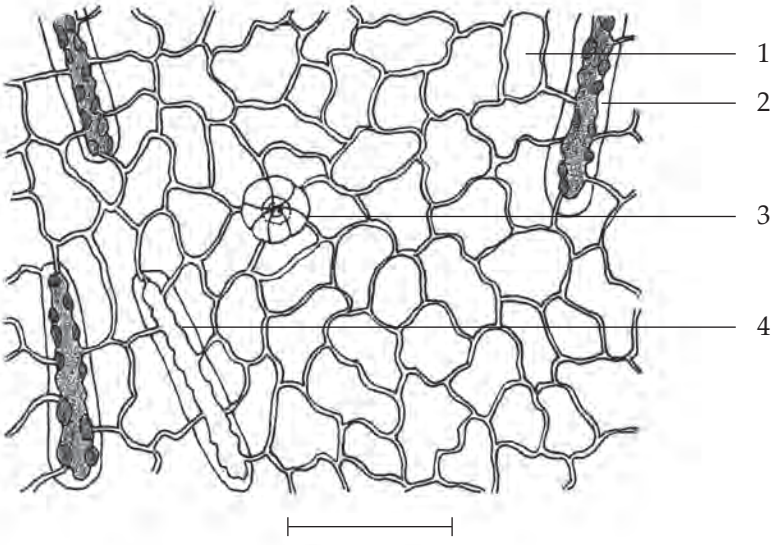
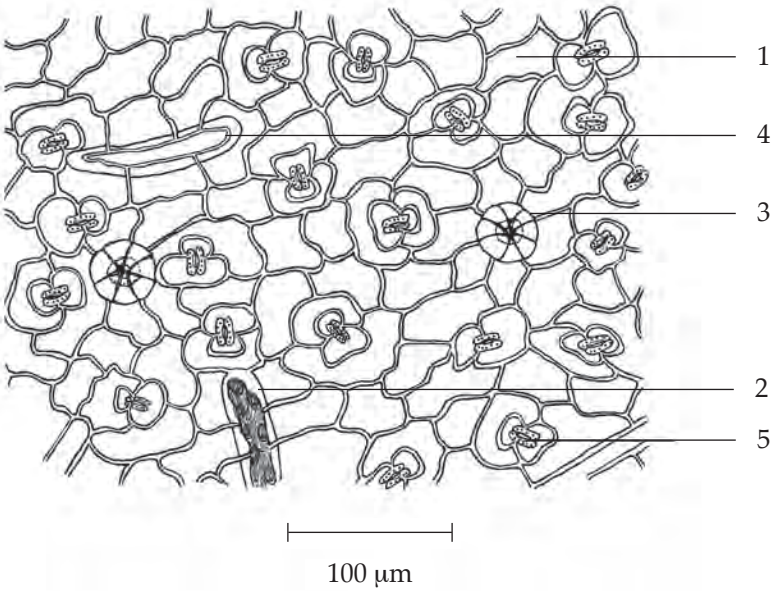


Fig. 1a *Clinacanthus nutans* (Burm. f.) Lindau
1. habit 2. flowering twig 3. inflorescence 4. fresh leaves 5. crude drug

Fig. 1b *Clinacanthus nutans* (Burm. f.) Lindau
1. flowering twigs 2. flower



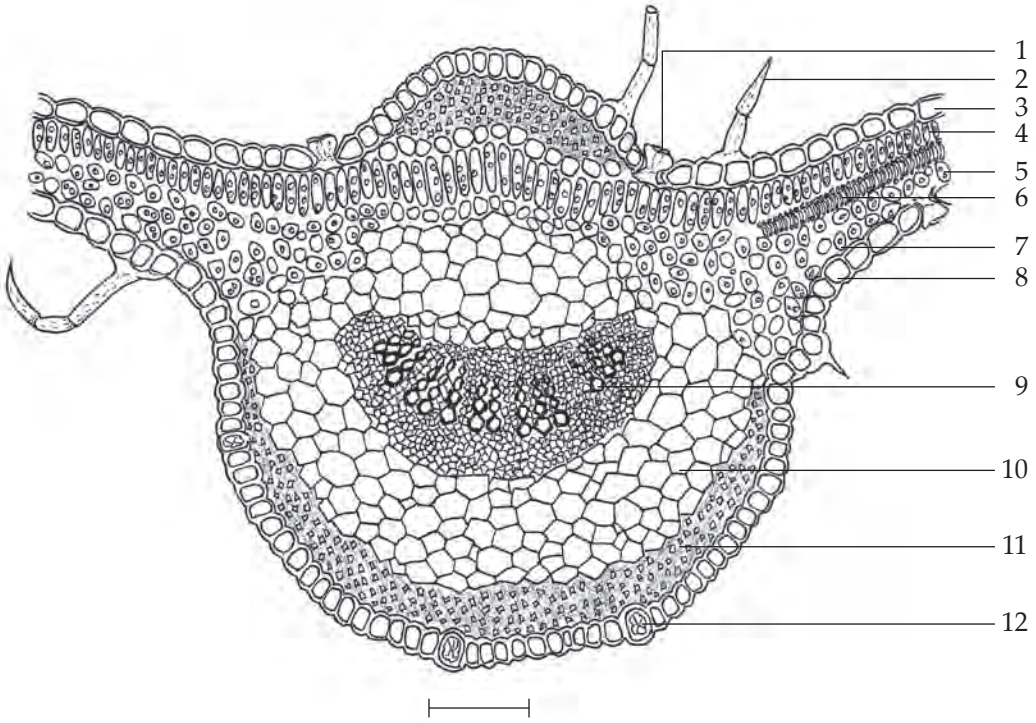
Upper Epidermis of the Lamina



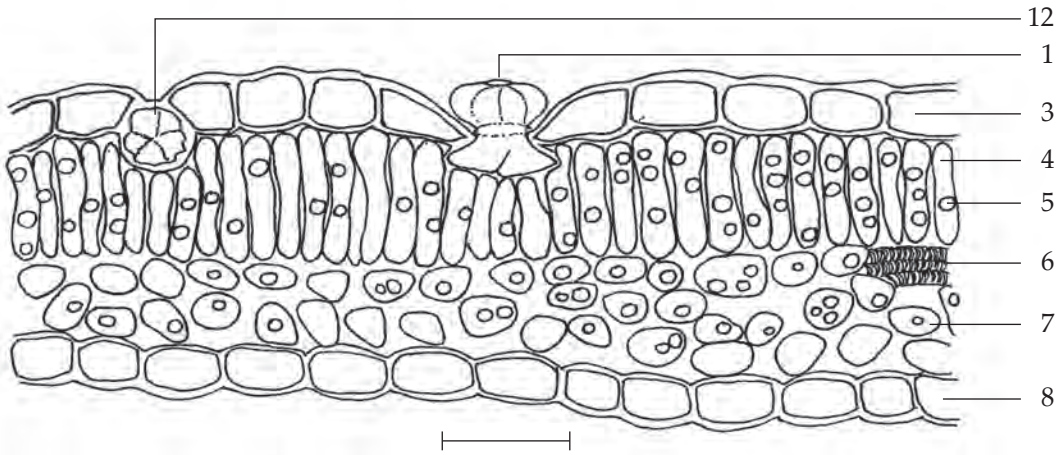
Lower Epidermis of the Lamina

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 Sections 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

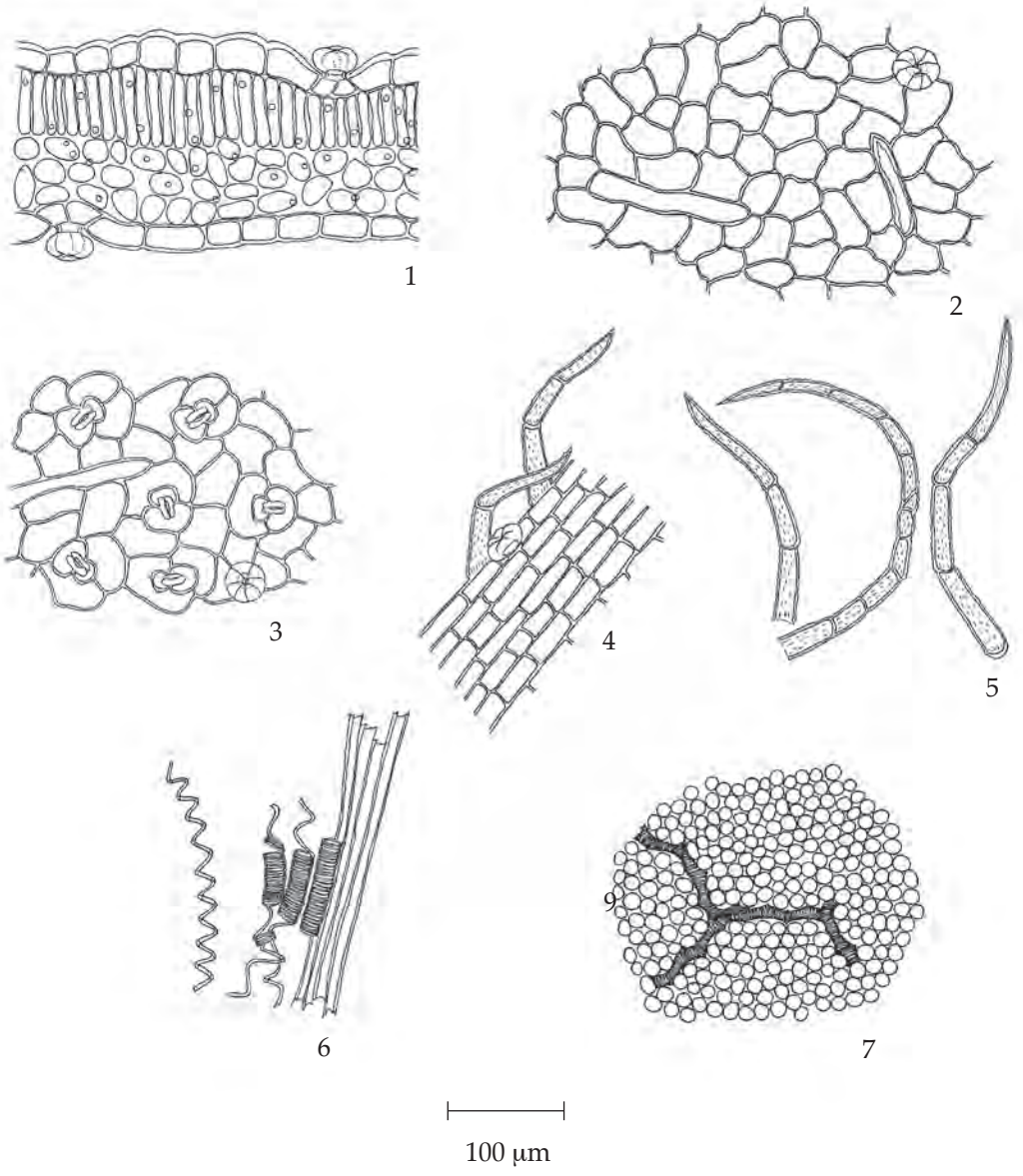


Fig. 2c Powdered Drug of the Leaves of *Clinacanthus nutans* (Burm. f.) Lindau

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).

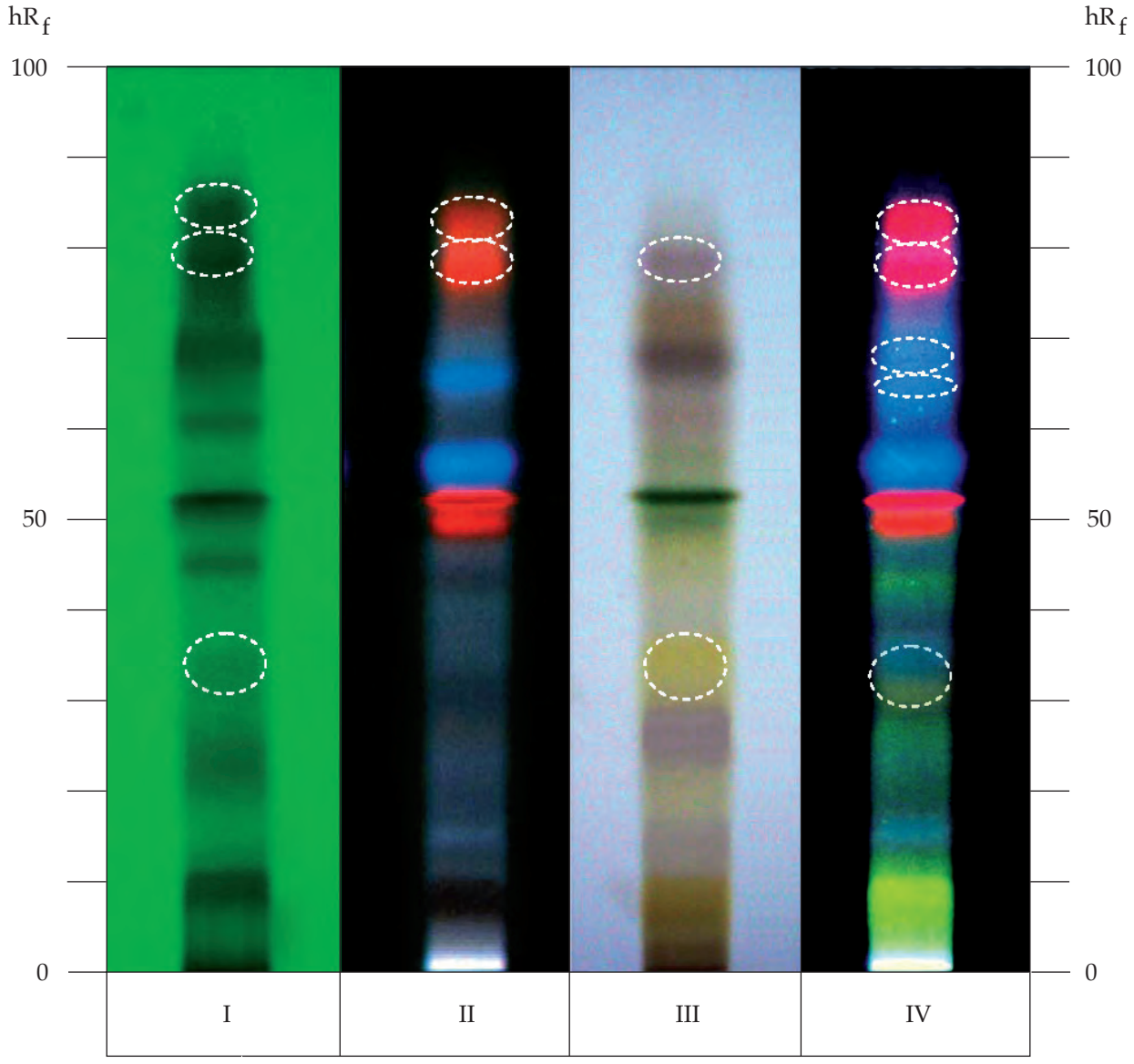


Fig. 3 Thin-Layer Chromatogram of the Extract of the Leaves of *Clinacanthus nutans* (Burm. f.) Lindau

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), เถาสามร้อยต่อ (THAO SAMROI TO), เถาสันชะควด (THAO SAN CHA KHUAT)
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., kaempferol, quercetin), 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 inconspicuously 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.

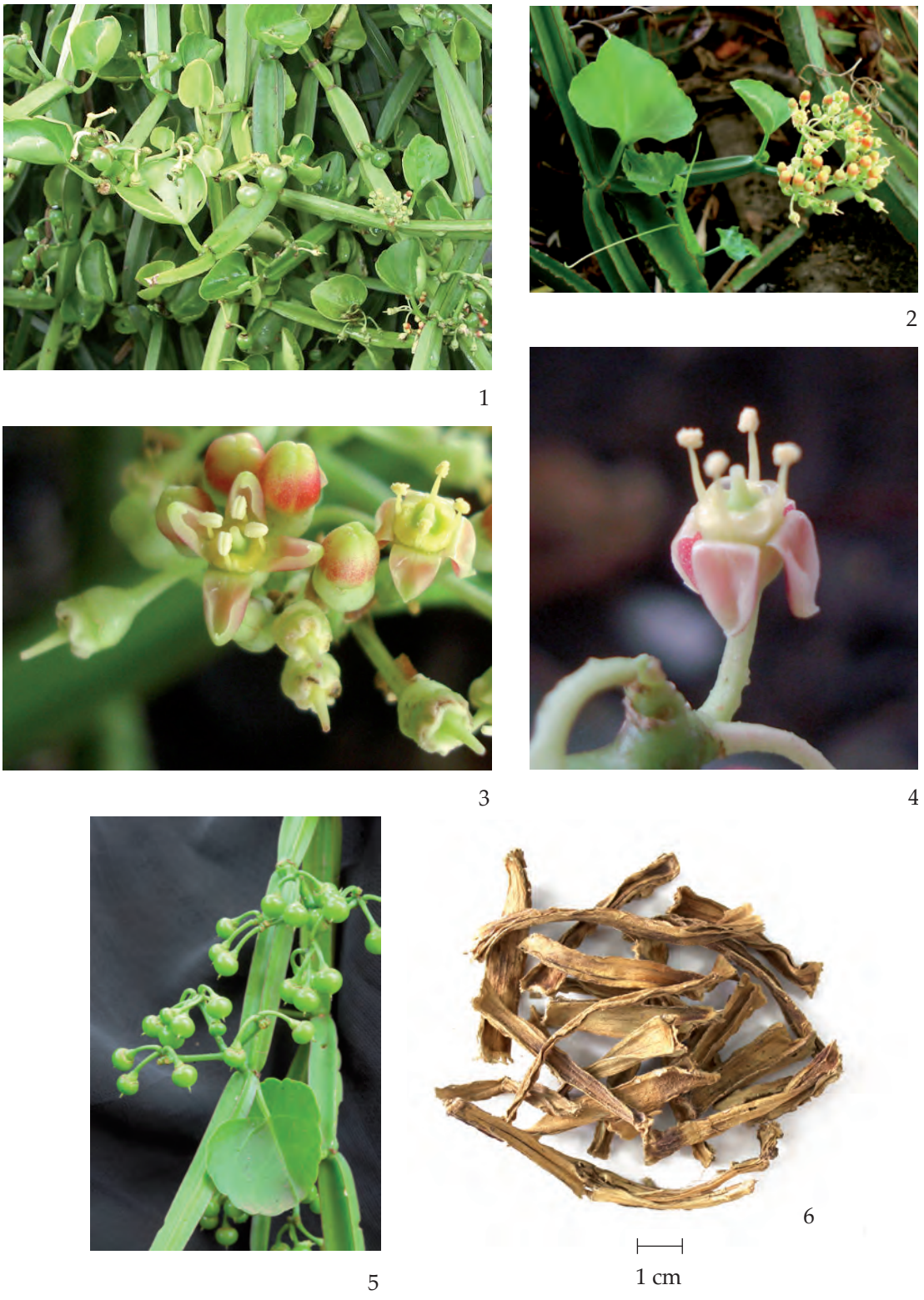


Fig. 1a *Cissus quadrangularis* L.
1. habit 2. flowering twig 3. top view of inflorescence 4. flower
5. infructescences 6. crude drug

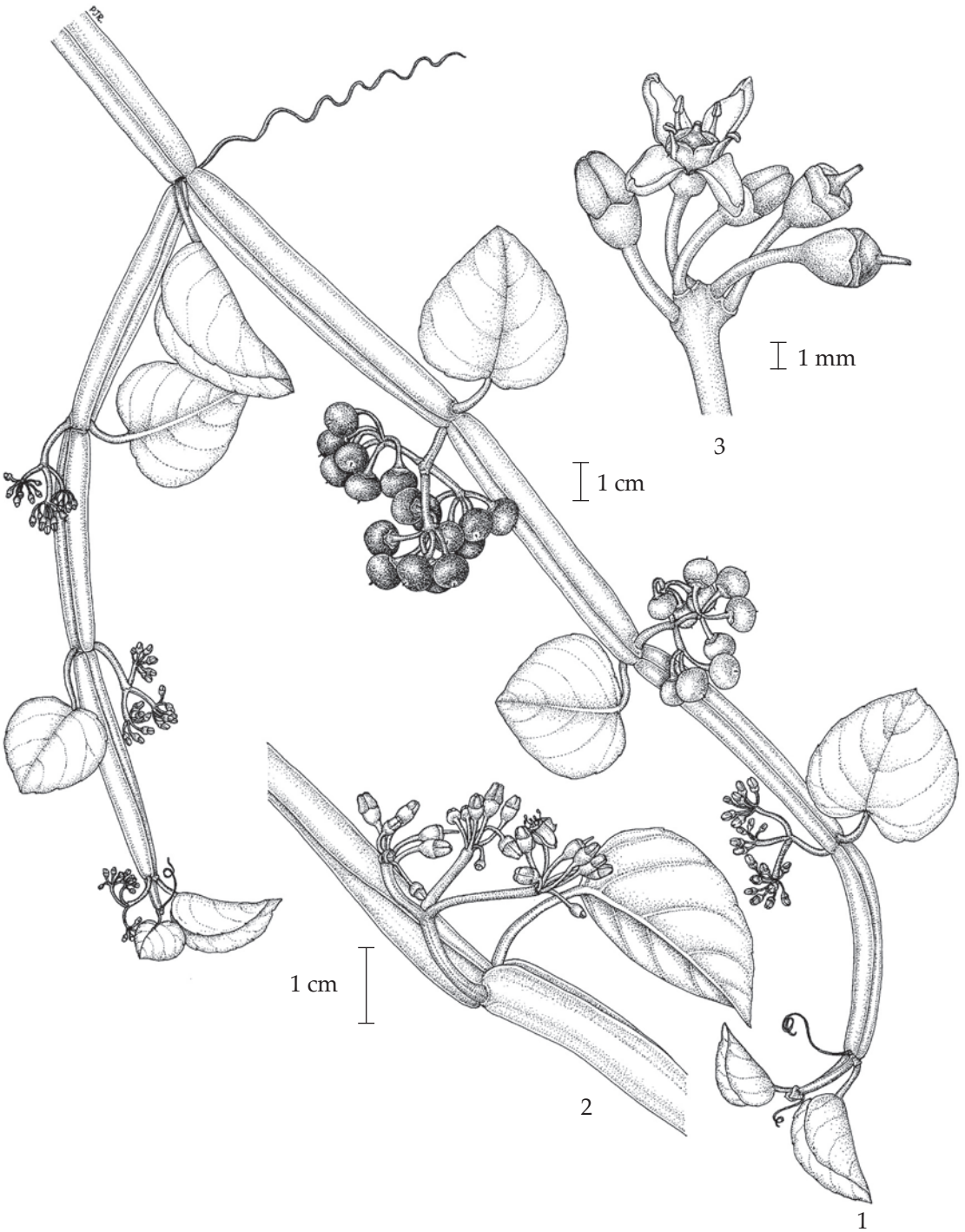


Fig. 1b *Cissus quadrangularis* L.
1. twig showing leaves, tendrils, inflorescences, and infructescences
2. inflorescence 3. flowers and young fruit

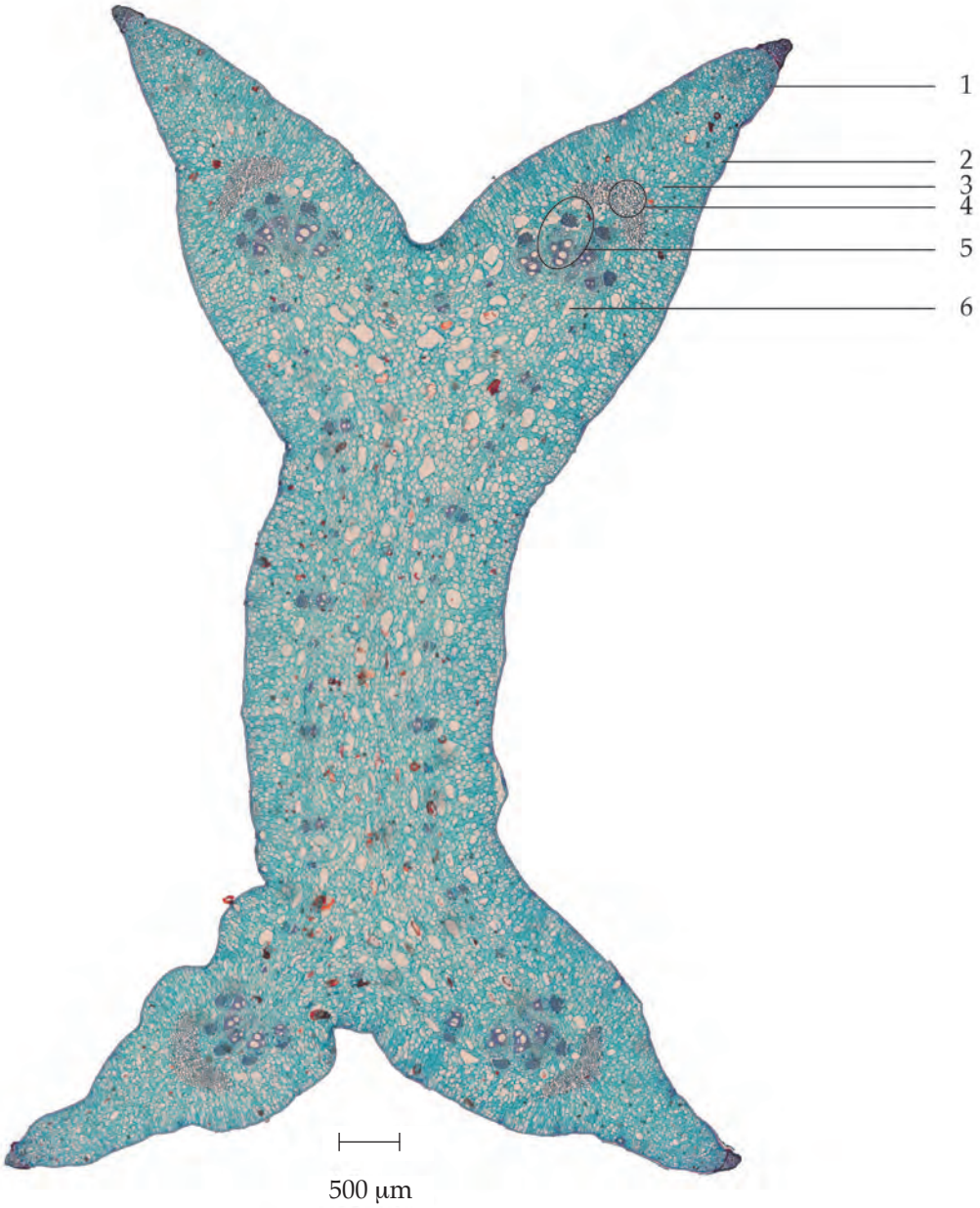


Fig. 2a Photomicrograph of Transverse Section of the Fresh Stem of *Cissus quadrangularis* L., Stained with Safranin-Fast Green

1. epidermis
2. raised stomata
3. parenchyma
4. group of angular collenchyma
5. vascular bundle
6. air space

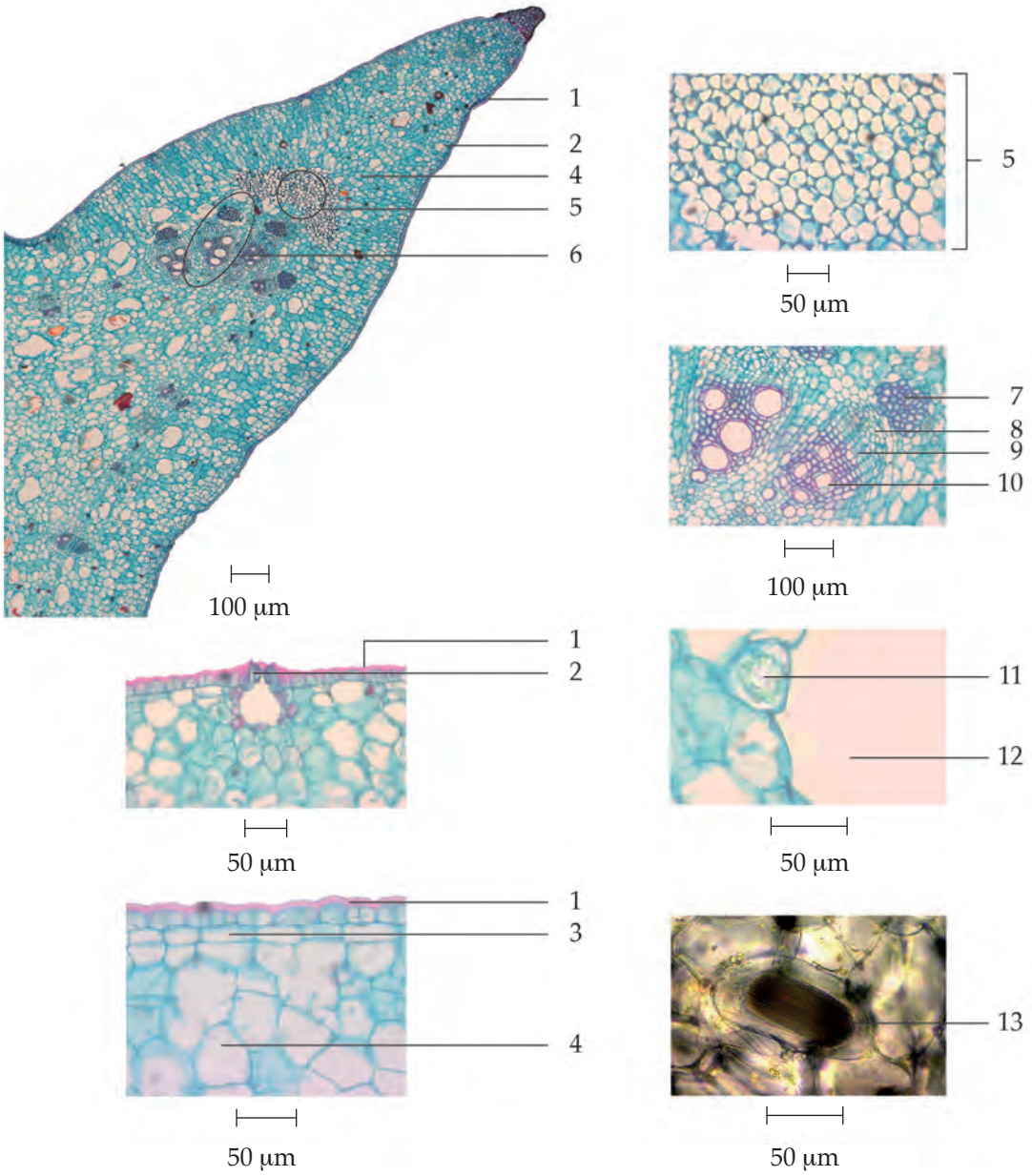


Fig. 2b Photomicrographs of Transverse Section of the Fresh Stem of *Cissus quadrangularis* L., Stained with Safranin-Fast Green

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

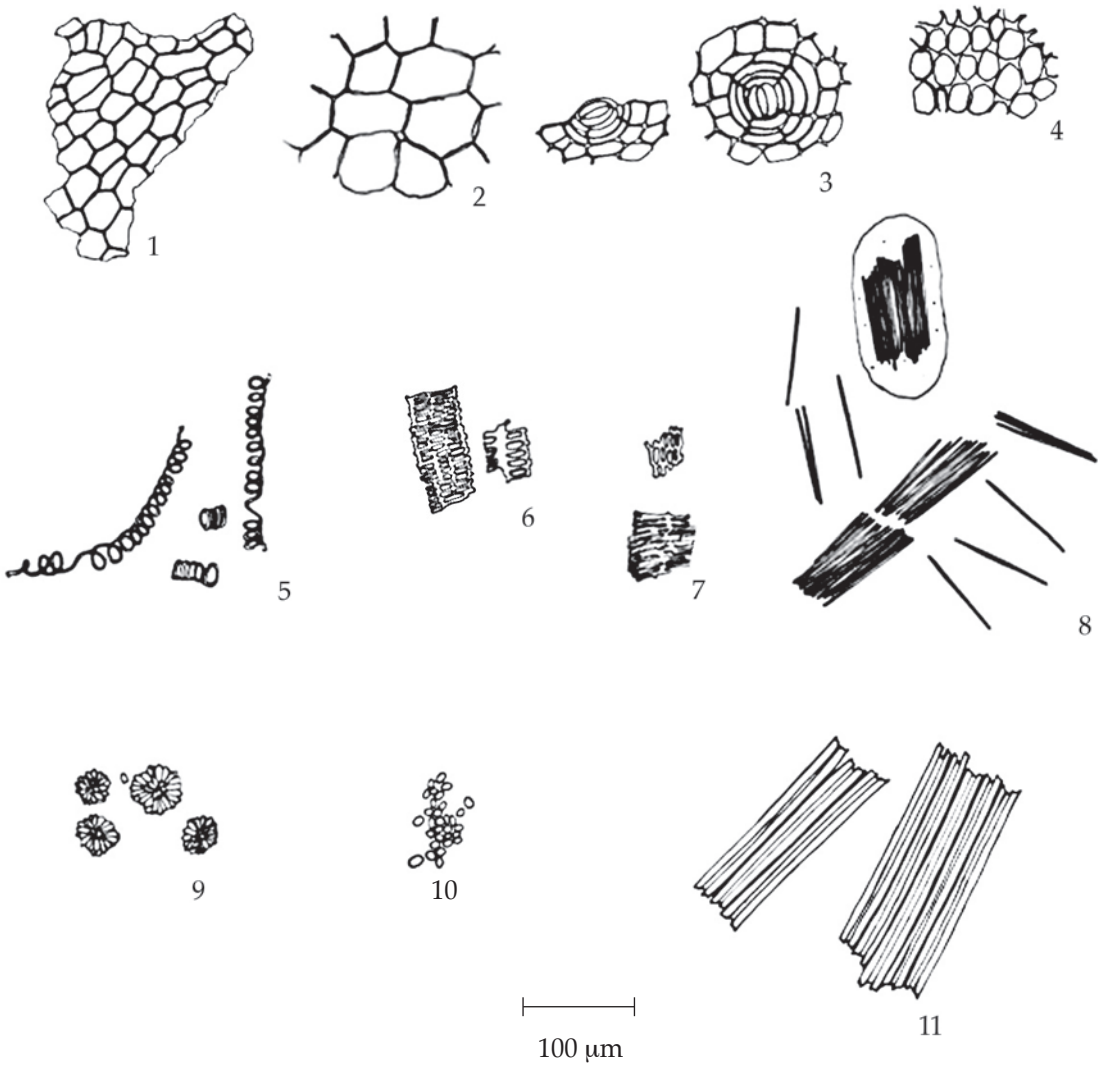


Fig. 2c Powdered Drug of the Stems of *Cissus quadrangularis* L.

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 12.5 per cent w/w (Appendix 7.12).

Water-soluble extractive Not less than 25.0 per cent w/w (Appendix 7.12).

Dose 3 to 6 g a day.

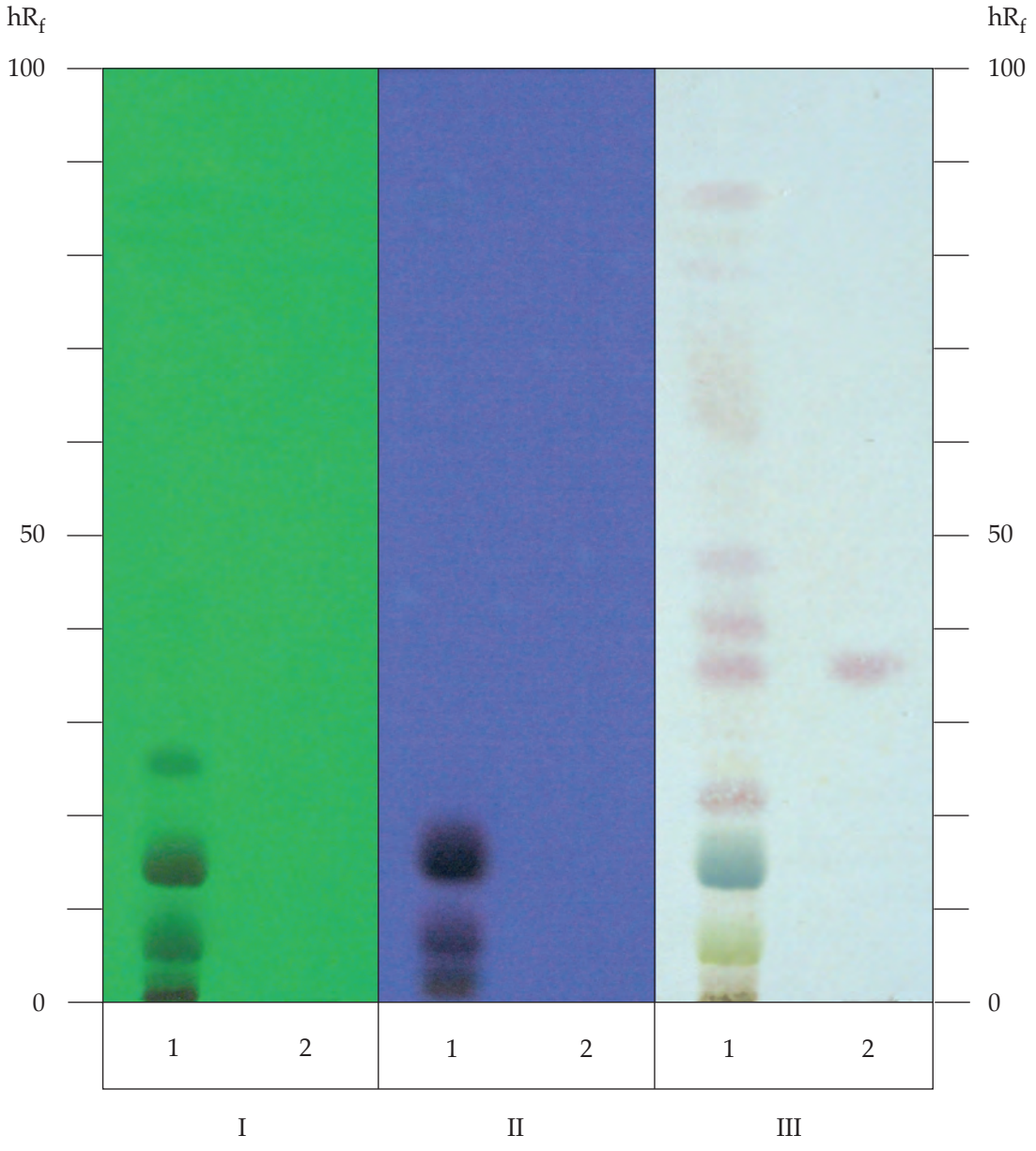


Fig. 3 Thin-Layer Chromatogram of Chloroform Extract of the Stems of *Cissus quadrangularis* L.

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)

ดอกแก้ว (DOK KAEO), ดอกกุน (DOK KUN), 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(s) 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 ones greenish brown, ovate-lanceolate, 7 to 8 mm long, about 4 mm wide, pubescent, inner ones 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. Seeds 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.

1

2

3

4

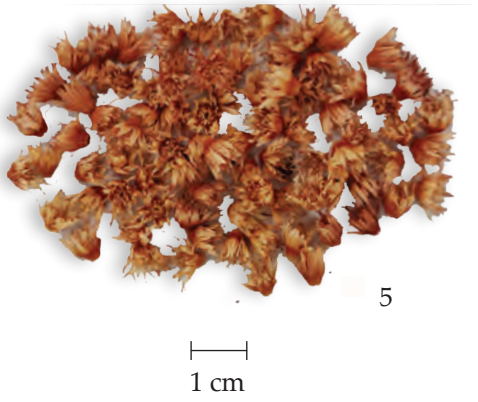


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Fig. 1a *Mimusops elengi* L.
1. habit 2. flowering twig 3. flower 4. fruiting twig 5. crude drug

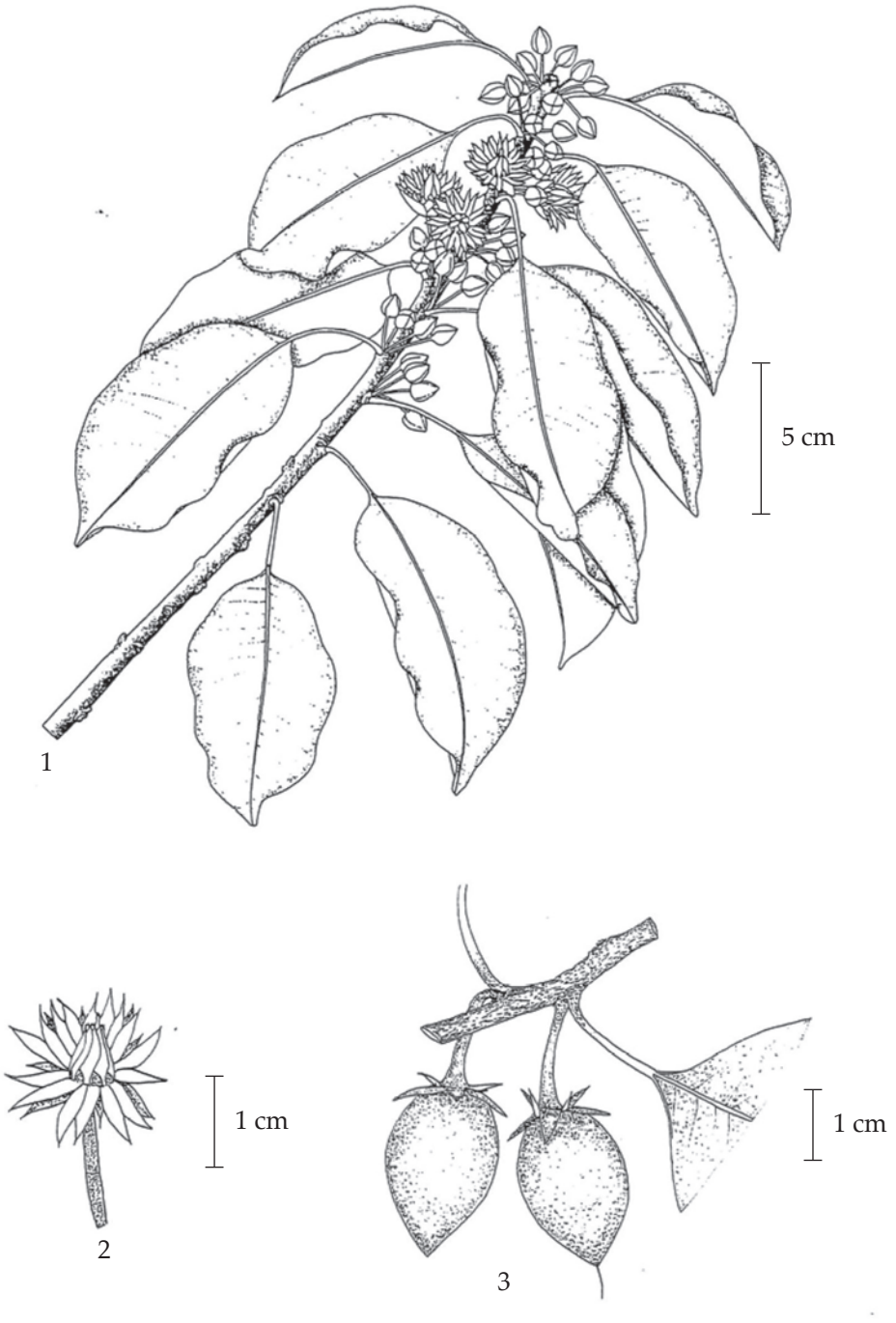


Fig. 1b *Mimusops elengi* L.
1. flowering twig 2. flower 3. fruits

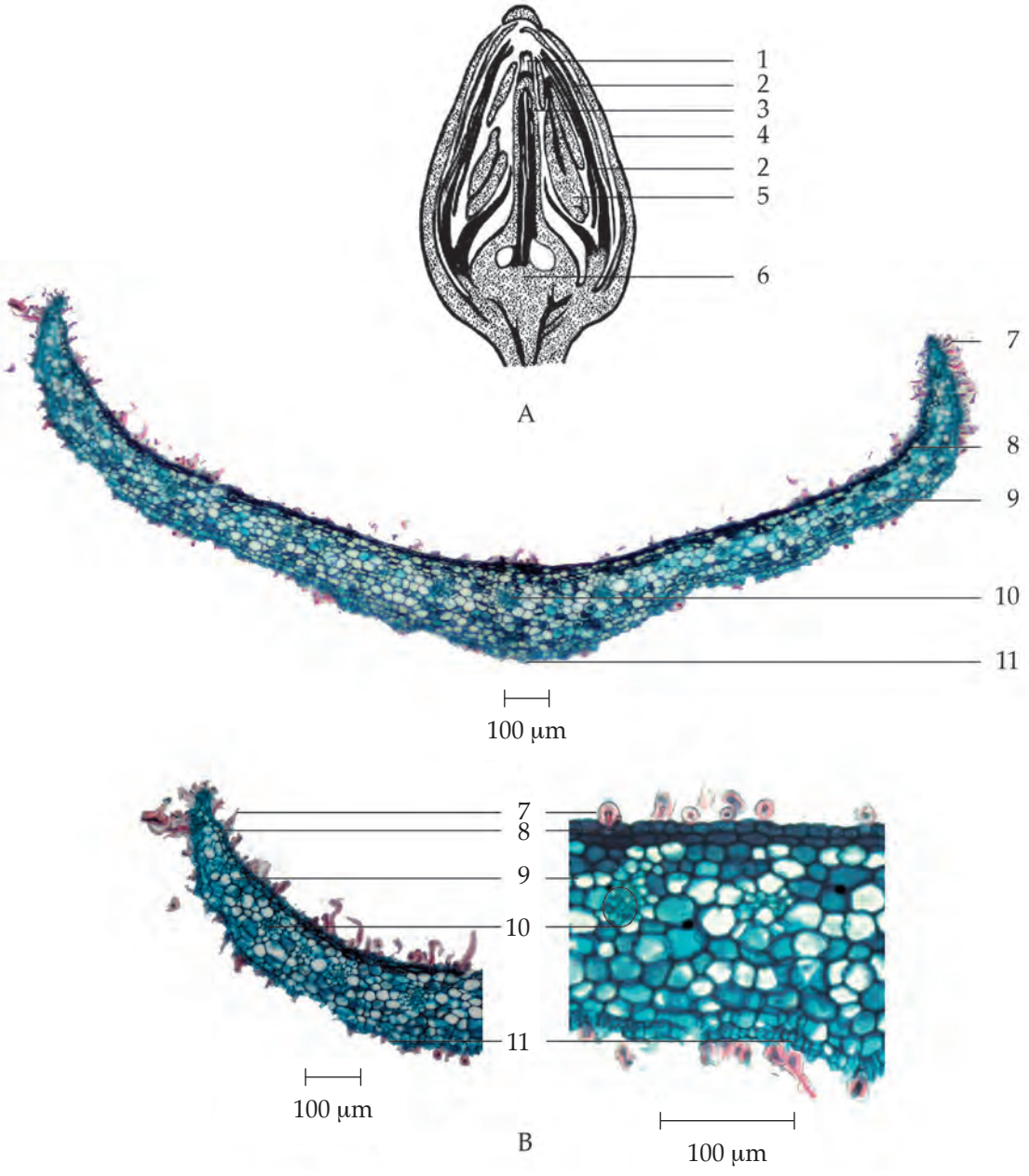


Fig. 2a Photomicrographs of Sections of the Flower of *Mimusops elengi* L.
A. Longitudinal Section of a Young Flower
B. Transverse Section of a Sepal, Stained with Safranin-Fast Green

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

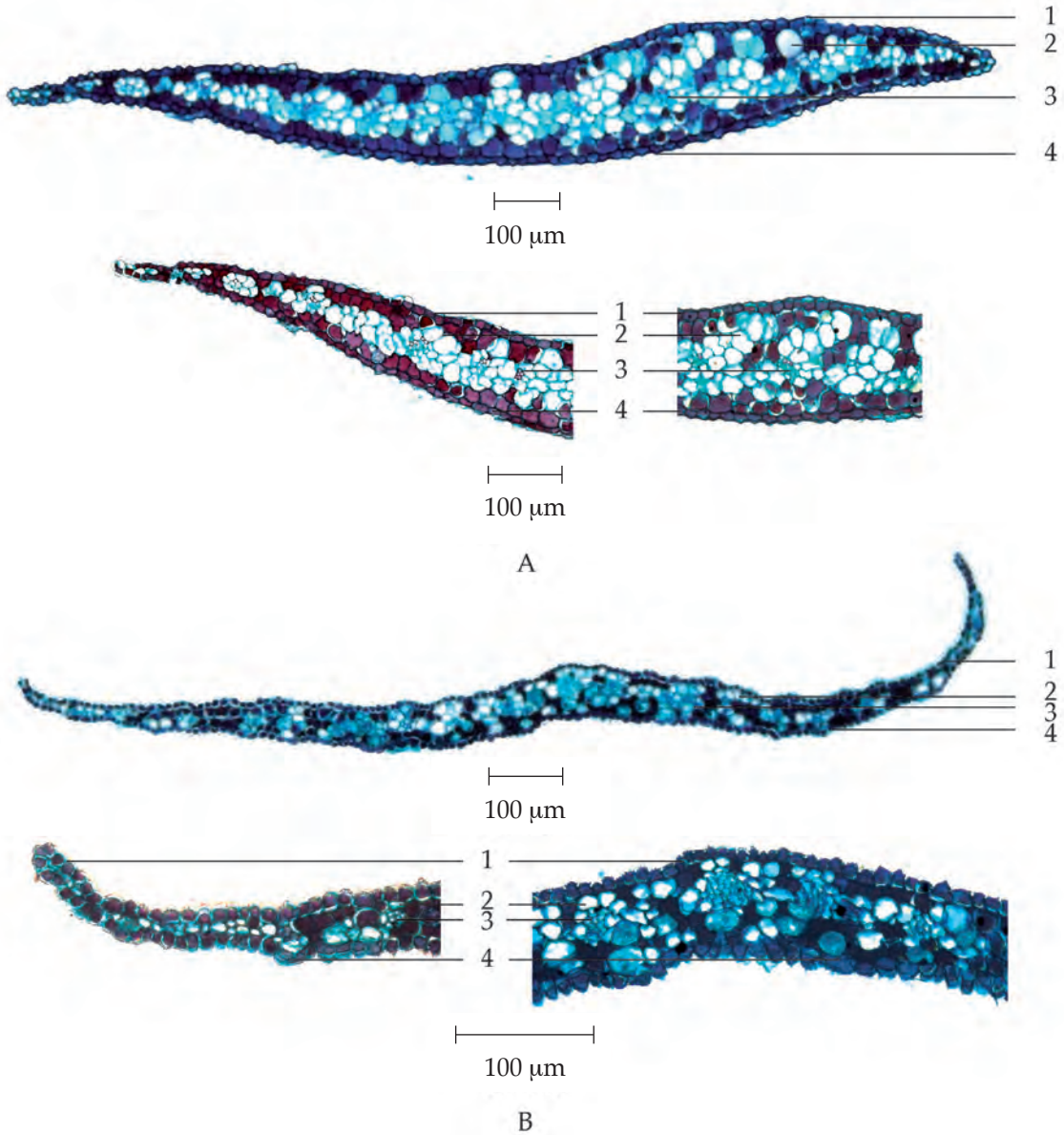


Fig. 2b Photomicrographs of Sections of the Flower of *Mimusops elengi* L., Stained with Safranin-Fast Green

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

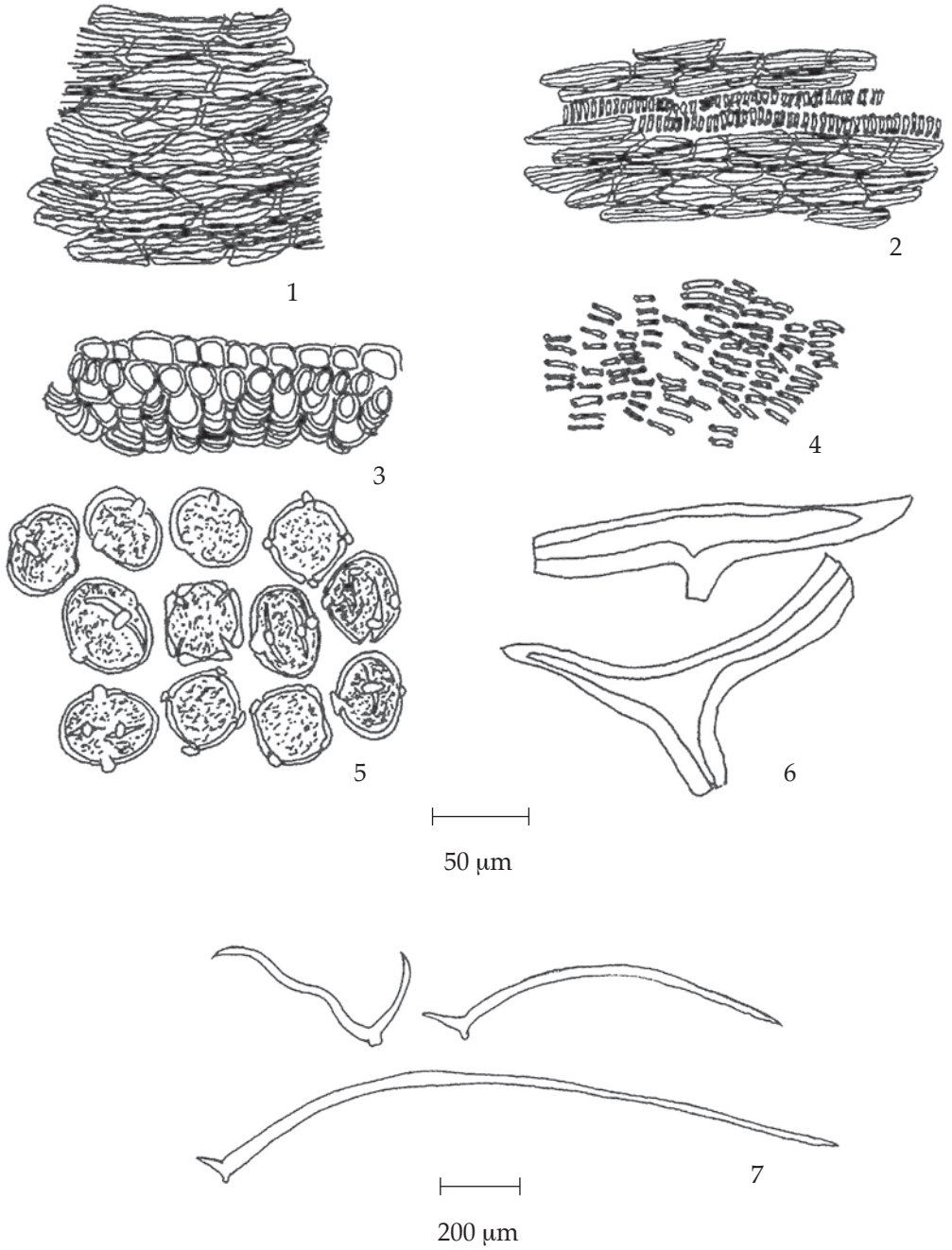


Fig. 2c Powdered Drug of the Flowers of *Mimusops elengi* L.

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).

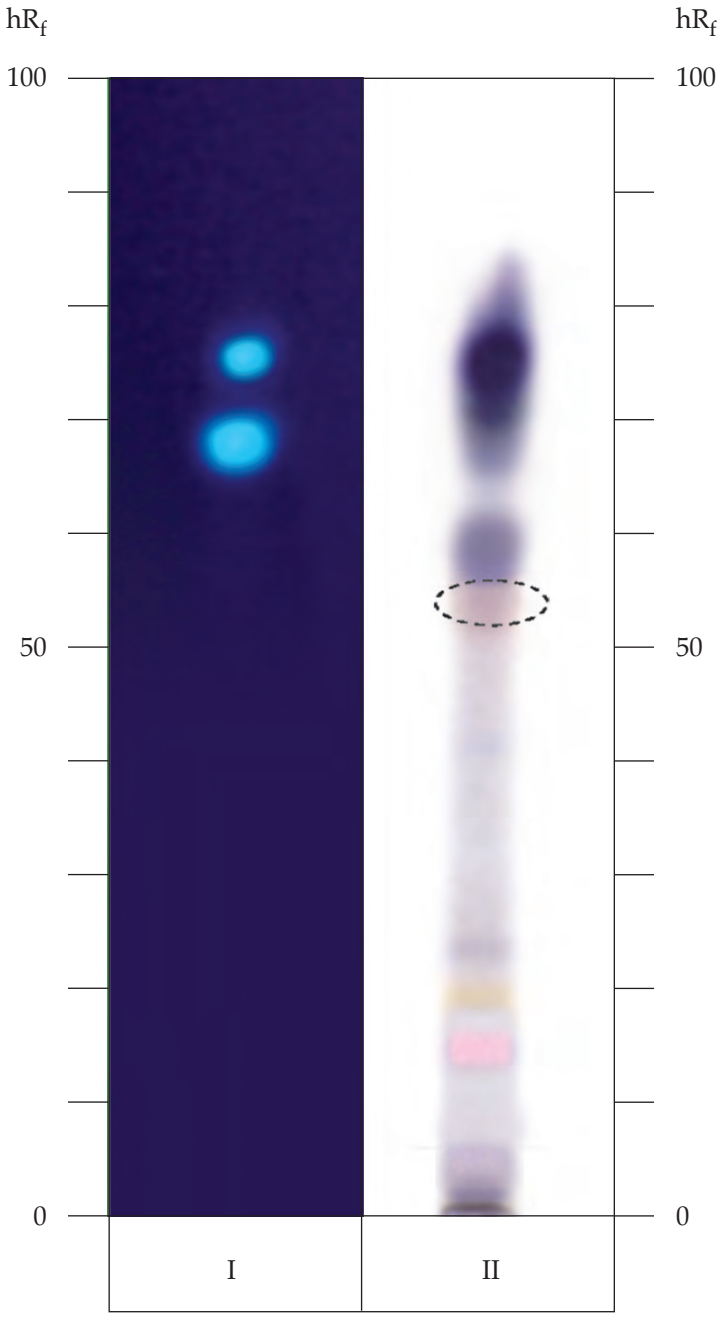


Fig. 3 Thin-Layer Chromatogram of Methanolic Extract of the Flowers of *Mimusops elengi* L.

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, Crude Drug Number: DMSc 0308.
(**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 (Figs. 1a, 1b) Herb, rhizome fleshy thick, greenish yellow to yellow inside, horizontal with many sessile tubers. Leafy stem 1 to 1.5 m tall. Leaves distichous, oblong-lanceolate, 20 to 30 cm long, 2 to 8 cm wide, pubescent below; ligule very short, bilobed, pubescent; sheath glabrous or hairy. Inflorescence scapose; peduncle 8 to 30 cm long, clothed with pubescent sheaths. Spike ovoid-ellipsoid; bract 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, pleasantly aromatic; taste, pungent.

Macroscopical (Fig. 1a) 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 the rhizome shows epidermis, outer cortex, cork, inner cortex, endodermis, stele, and fibrovascular bundle. Epidermis, a layer of rectangular cells. Outer cortex, parenchyma cells. Cork, several layers of thin-walled, rectangular cells. Inner cortex, thin-walled parenchyma cells containing numerous starch granules and oleoresin; starch granules mostly simple, 30 to 75 μm long, 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 parenchyma 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.

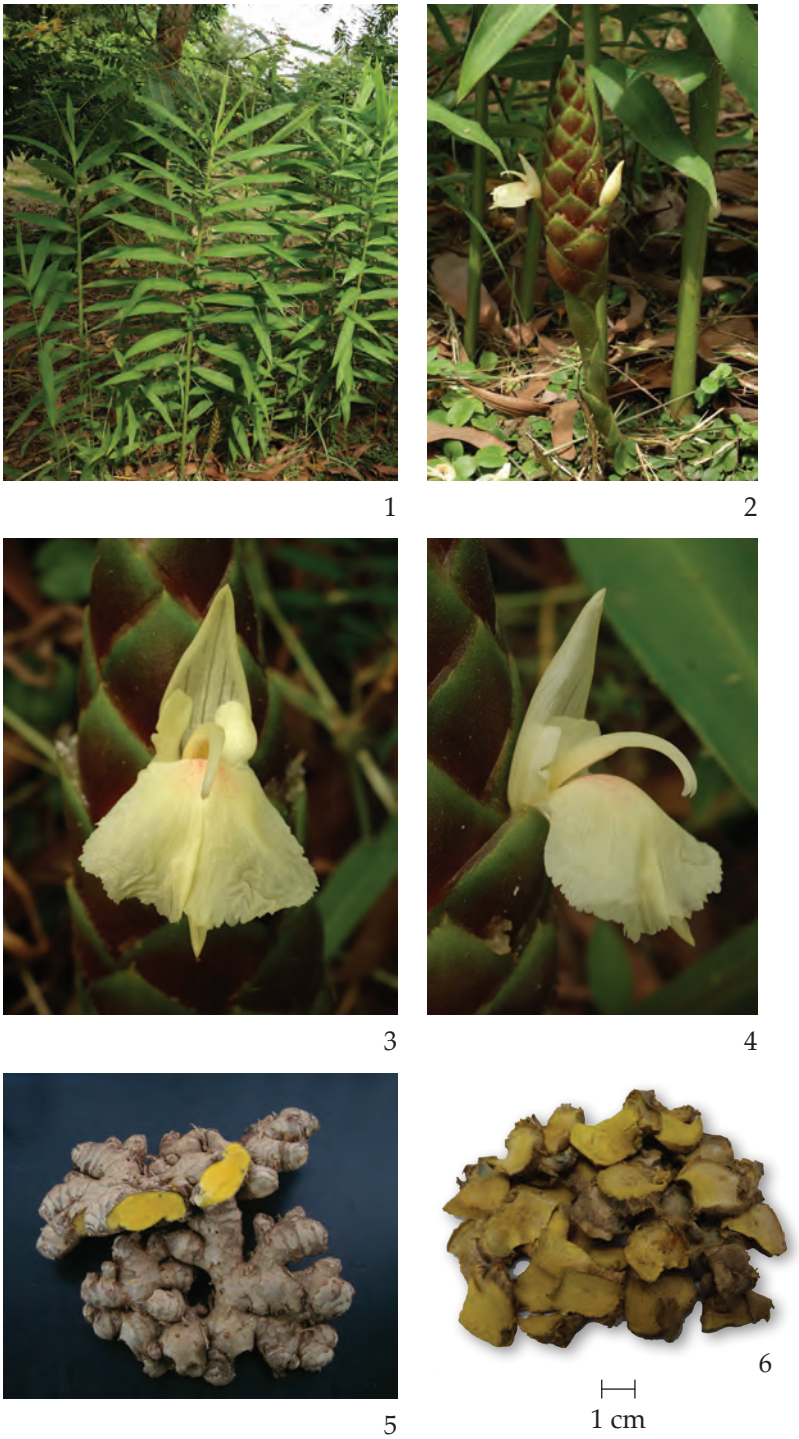


Fig. 1a *Zingiber montanum* (J. König) Link ex A. Dietr.
1. habit 2. inflorescence 3. flower (front view) 4. flower (oblique view)
5. fresh rhizomes 6. crude drug

Fig. 1b *Zingiber montanum* (J. König) Link ex A. Dietr.
1. habit 2. rhizome 3. crude drug

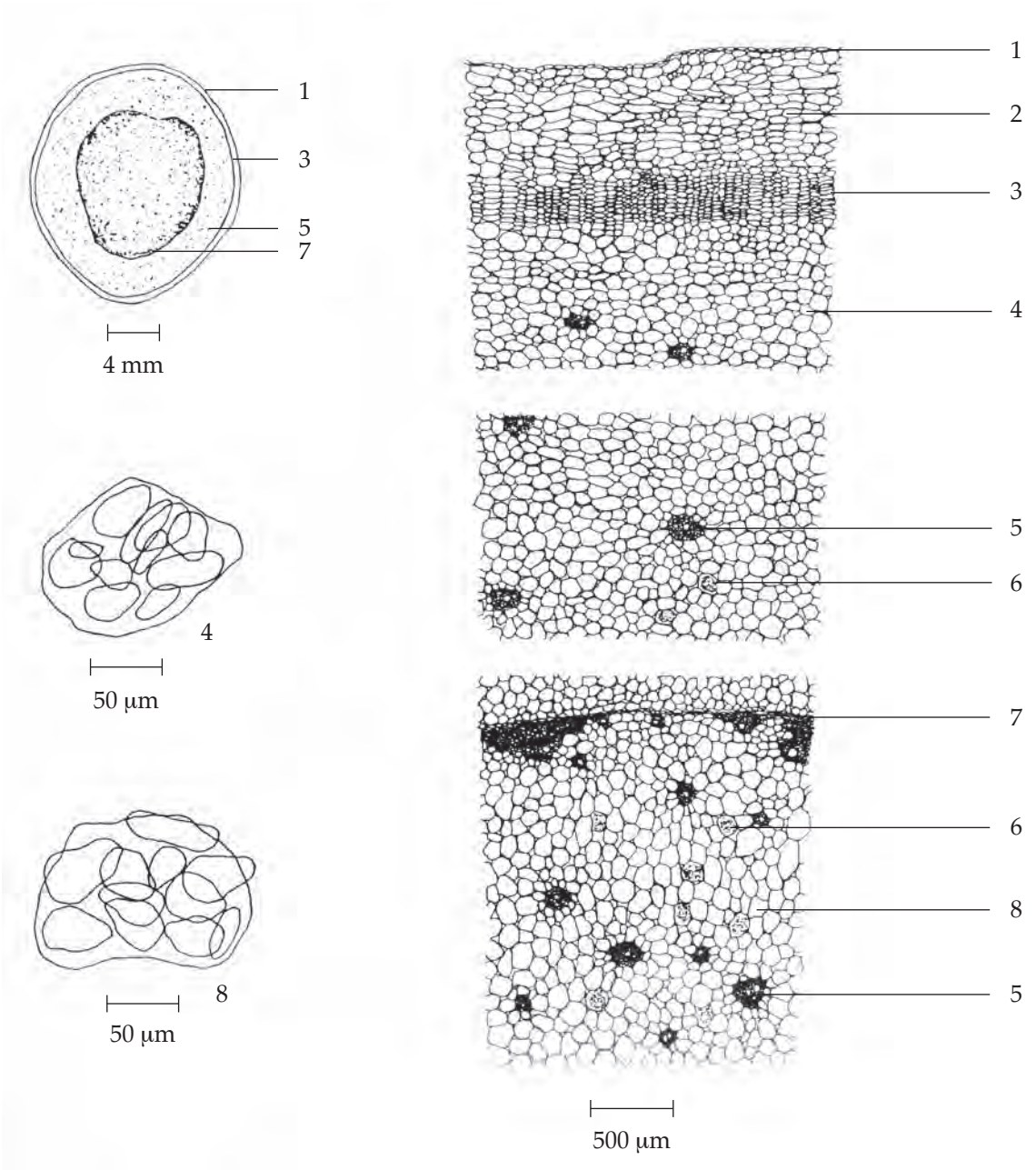


Fig. 2a Transverse Section of the Rhizome of *Zingiber montanum* (J. König) Link ex A. Dietr.

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

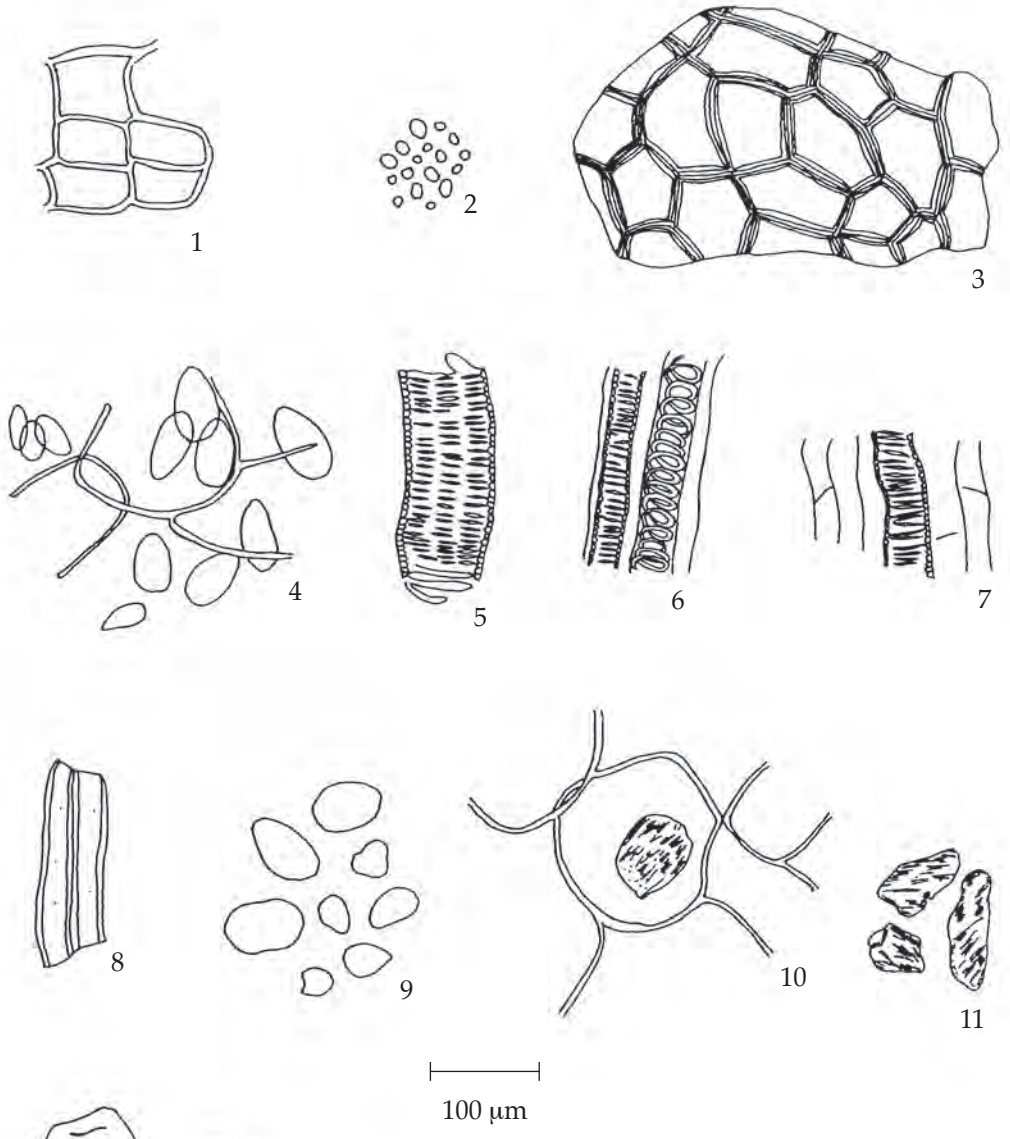


Fig. 2b Powdered Drug of the Rhizomes of *Zingiber montanum* (J. König) Link ex A. Dietr.

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. A 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 pale orange, respectively. A red-brown spot due to curcumin (hR_f values 10 to 13) 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 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 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.

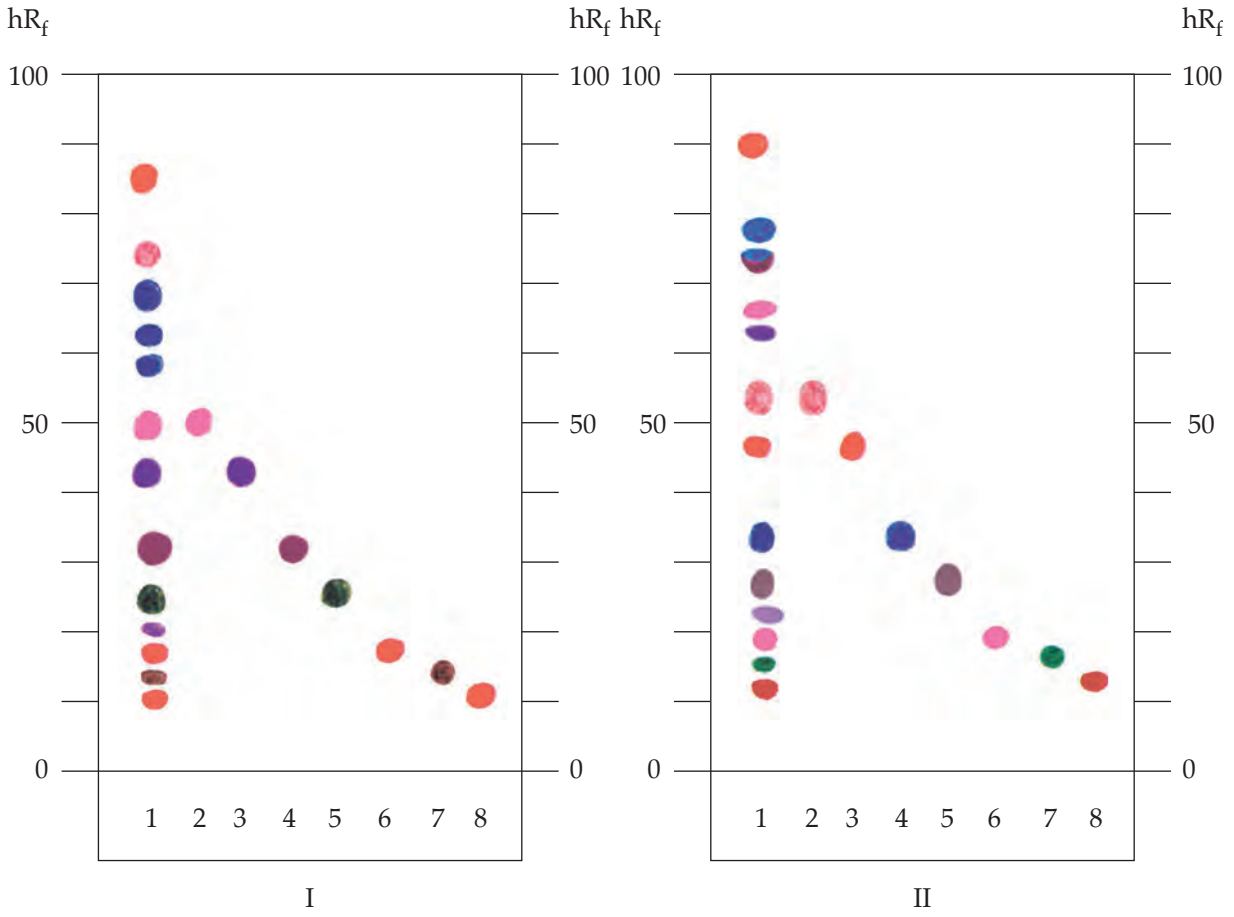


Fig. 3 Thin-Layer Chromatogram of Hexane Extract of the Rhizomes of *Zingiber montanum* (J. König) Link ex A. Dietr.

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 tall, 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) Leaves, simple, circular, ovate or ovate-oblong, 5 to 18 cm long, 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 layers of collenchyma beneath upper and lower epidermises, 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.

1

2

3

4

1 cm

Fig. 1a *Piper betle* L.
1. habit 2. flowering twig 3. leaves 4. crude drug

Fig. 1b *Piper betle* L.
1. young twig 2. inflorescence

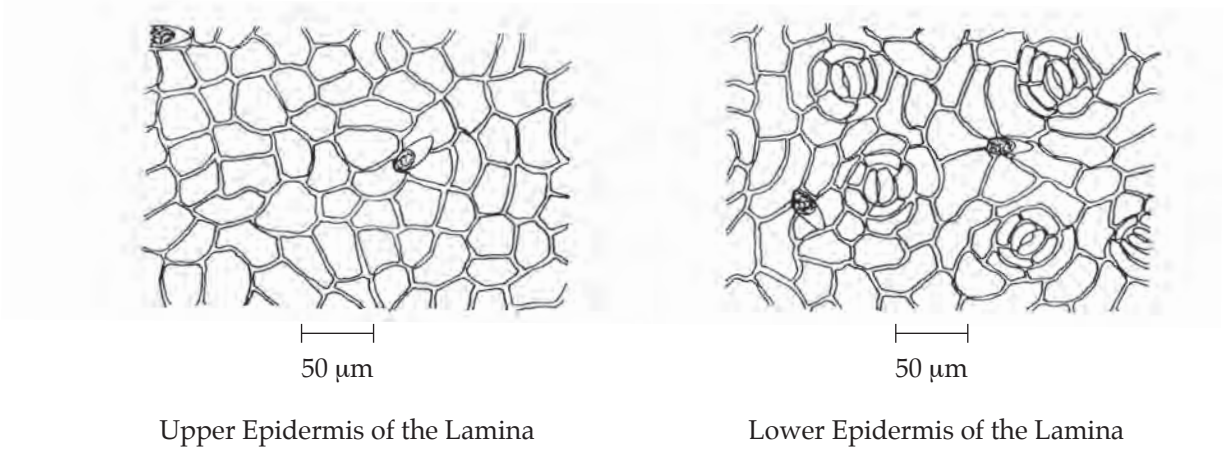


Fig. 2a Epidermises of the Leaf of *Piper betle* L.

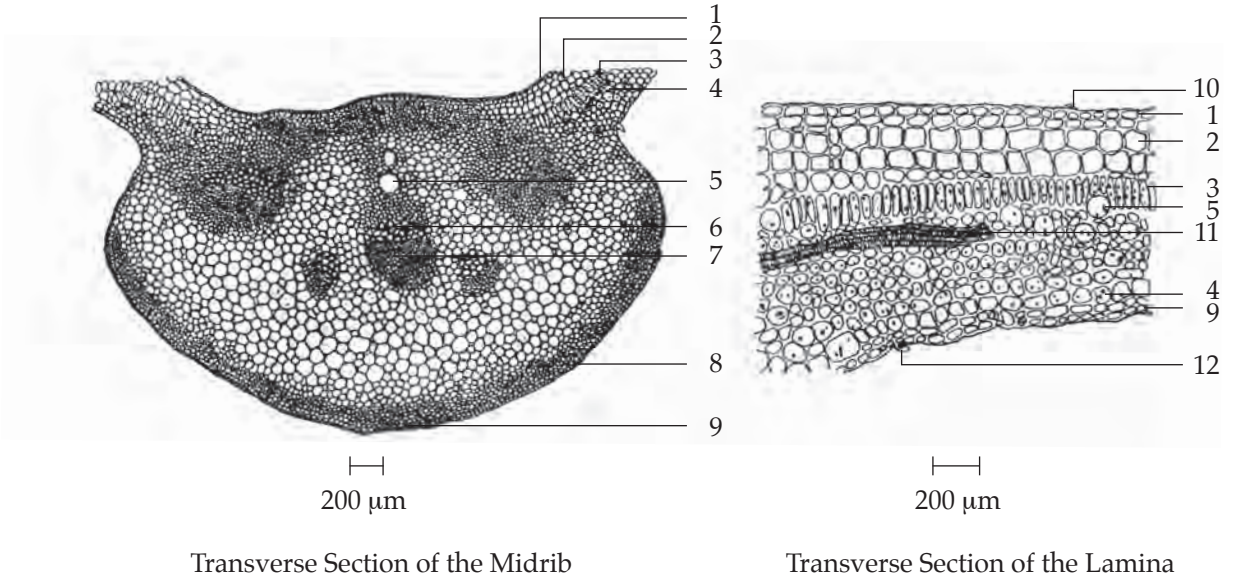


Fig. 2b Transverse Sections of the Leaf of *Piper betle* L.

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

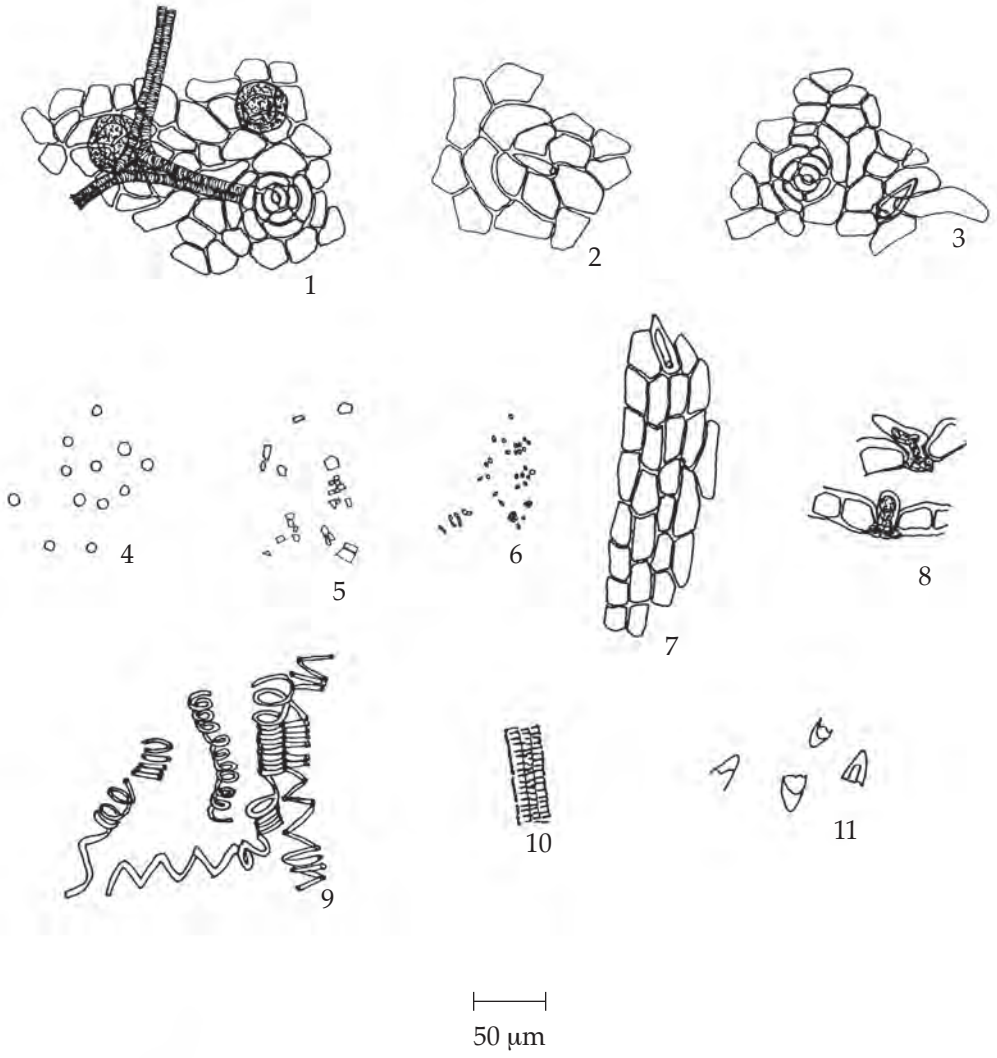


Fig. 2c Powdered Drug of the Leaves of *Piper betle* L.

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).

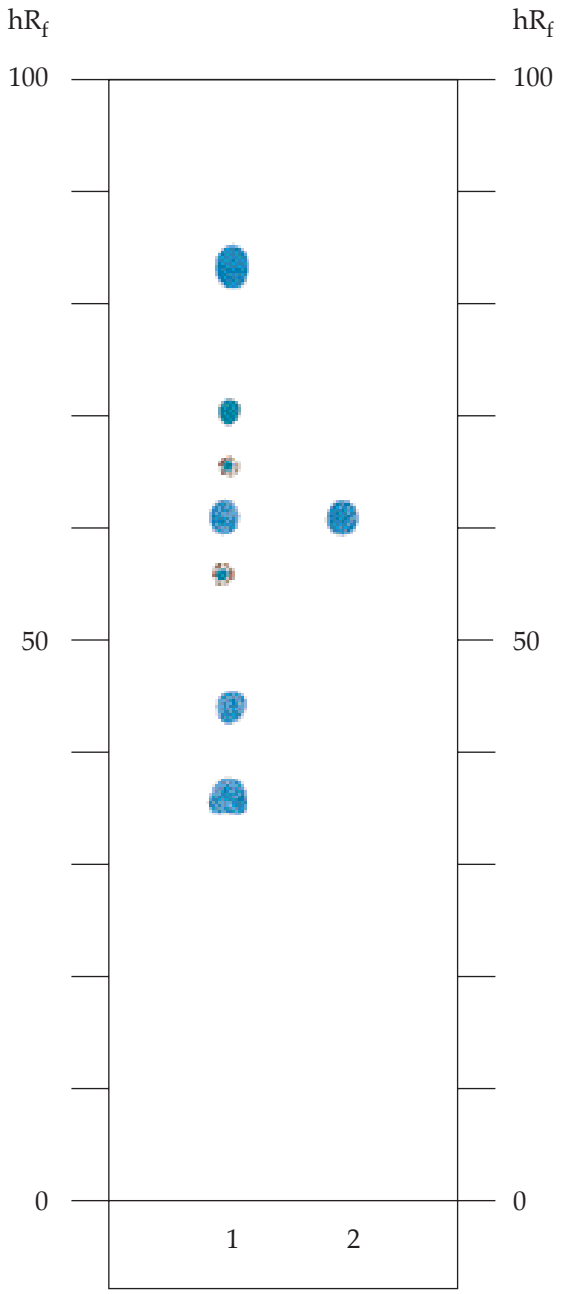


Fig. 3 Thin-Layer Chromatogram of Chloroform Extract of the Leaves of *Piper betle* L., Detected with a 20 Per Cent W/V Solution of *Phosphomolybdic Acid* in *Ethanol*

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

พริกขี้หนู (PHRIK KHINU)

พริกแด้ (PHRIK DAE), พริกแกว (PHRIK KAEO), พริกกะเหรี่ยง (PHRIK KARIANG), พริกขี้หนูสวน (PHRIK KHINU SUAN)

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 red mature 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. 1) Annual or perennial herb up to 2 m tall; 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(s) 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. 1) 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.

1

2

3

4

1 cm

Fig. 1 *Capsicum annuum* L.
1. habit 2. flower 3. ripe fruits 4. crude drug

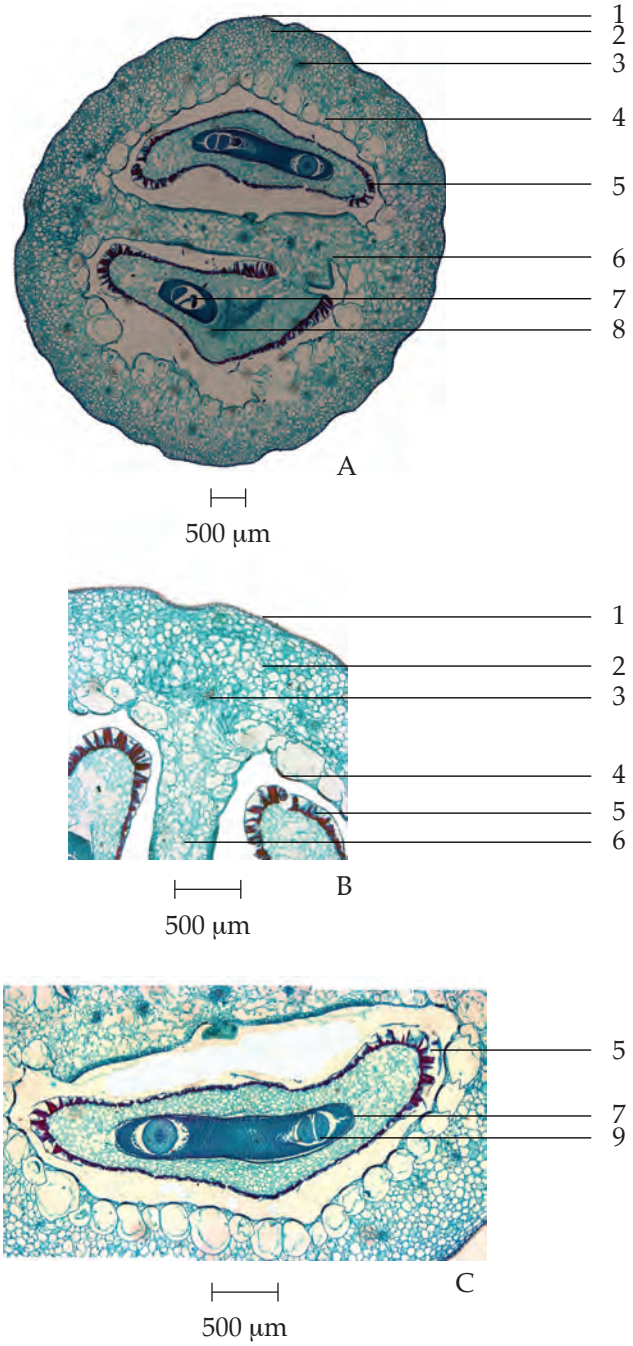


Fig. 2a Photomicrographs of Transverse Sections of the Fruit of *Capsicum annuum* L., Stained with Safranin-Fast Green

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

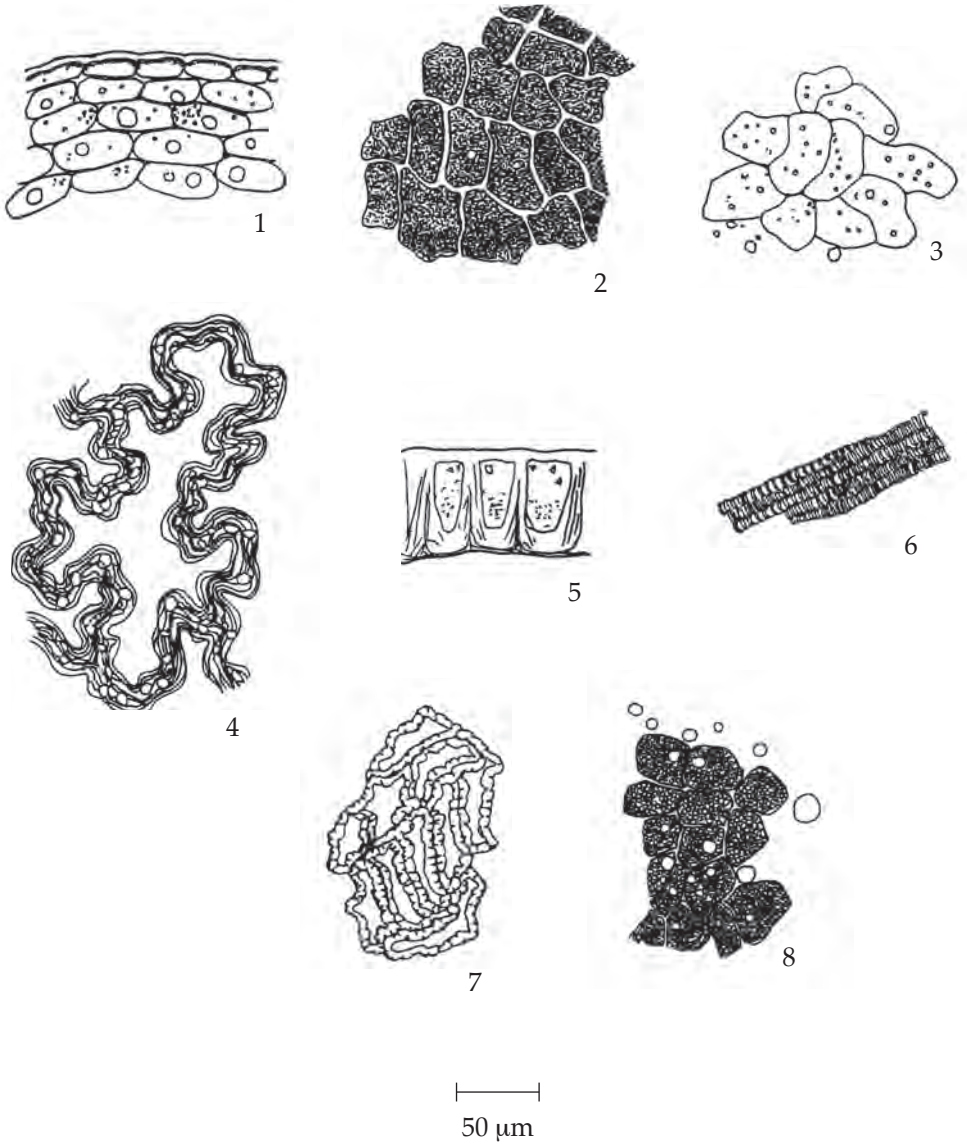


Fig. 2b Line Drawings of Powdered Drug of the Fruits of *Capsicum annuum* L.

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

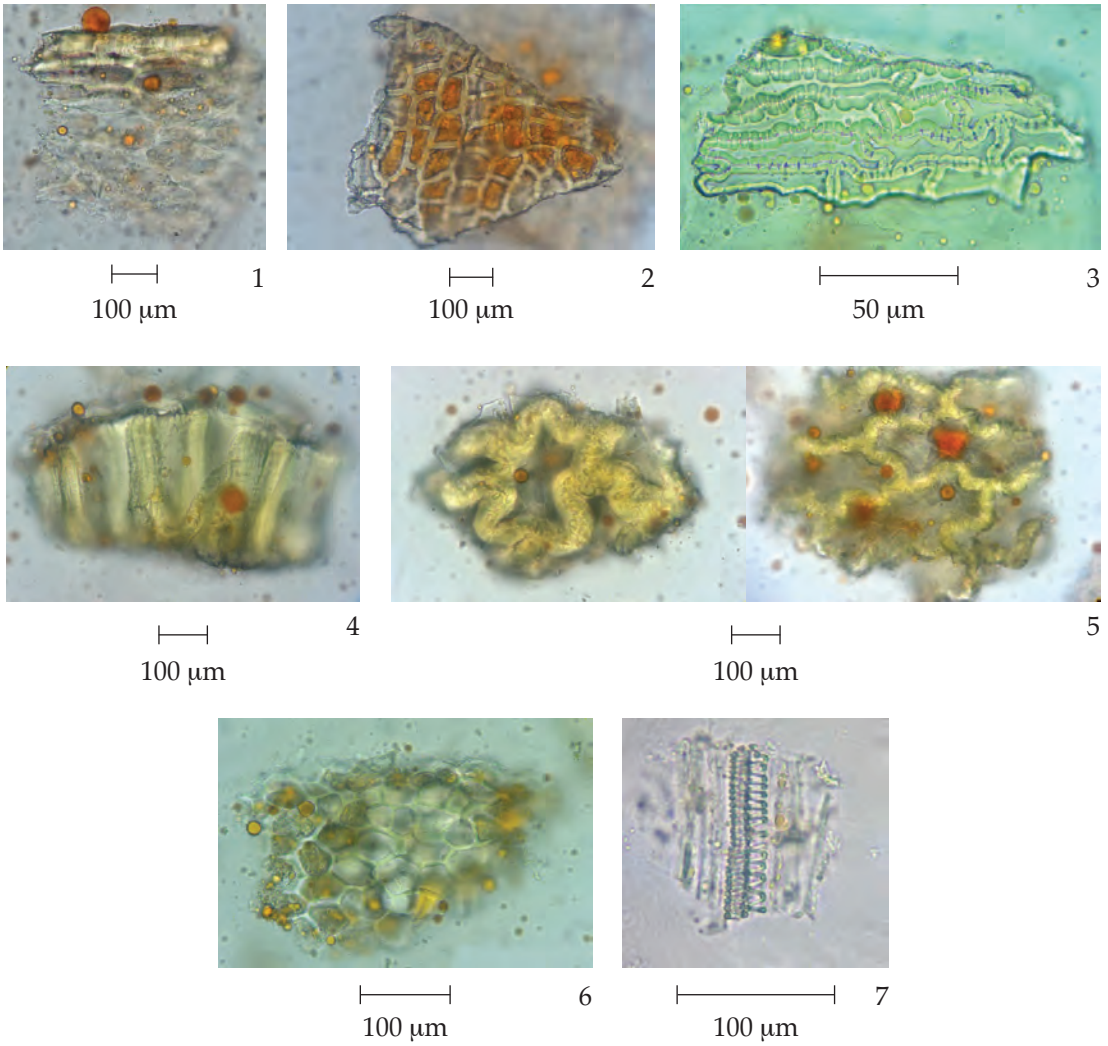


Fig. 2c Photomicrographs of Powdered Drug of the Fruits of *Capsicum annuum* L.

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, lips, 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 200 mg of the sample, in *coarse powder*, with 5 mL of *methanol* for 30 minutes and filter. To 0.1 ml of the filtrate, add a few drops of a 1 per cent w/v solution of *vanillin*, mix and add a few drops of *sulfuric acid*: a green colour slowly develops. After 15 minutes, add a few drops of *distilled water*: a bright blue colour 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 "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.

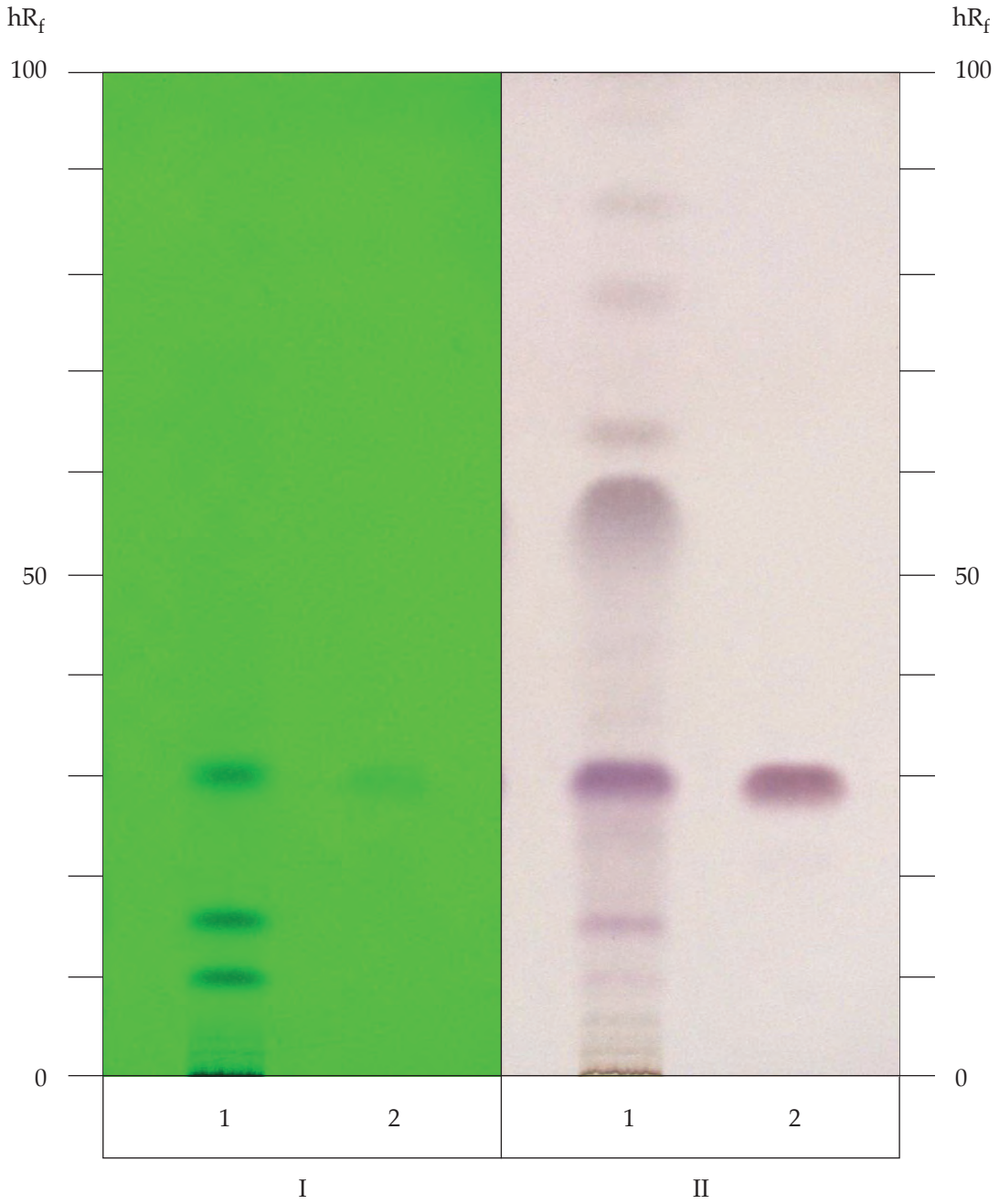


Fig. 3 Thin-Layer Chromatogram of Methanolic Extract of the Fruits of *Capsicum annuum* L.

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 states 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 "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, lips, 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) the 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 1B in the "Limits for Microbial Contamination" (Appendix 10.5).

Assay Carry out the determination as described in the "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 1.16H).

พริกไทยดำ (PHRIK THAI DAM)

Piperis Nigri Fructus
Black Pepper

Synonyms Pepper, Pepper Corn

Category Aromatic, stomachic, carminative.

Black Pepper is the dried green mature fruit of *Piper nigrum* L. (Family Piperaceae), Herbarium Specimen Number: DMSC 667, Crude Drug Number: DMSc 0275.

Constituents Black Pepper contains volatile oil, alkaloids, of which piperine and piperettine, are its major components.

Description of the plant (Figs. 1a, 1b) 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 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 long , stout. Flower usually dioecious, but often the female bears 2 anthers, and the male a pistillode, anther 2-celled. Fruiting spike, 10 to 12 cm or more long. Fruit drupe, globose or ovoid, sessile, 1-seeded, 4 to 6 mm in diameter, orange-red to reddish when ripe. Seed usually globose; testa thin.

Description Odour, aromatic; taste, pungent.

Macroscopical (Fig. 1a) Subspherical, greyish brown to brownish black, superior, 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 of 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, mesocarp, endocarp, spermoderm, and perisperm. 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 parenchyma cells. Mesocarp composed of several layers of polygonal parenchyma 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.

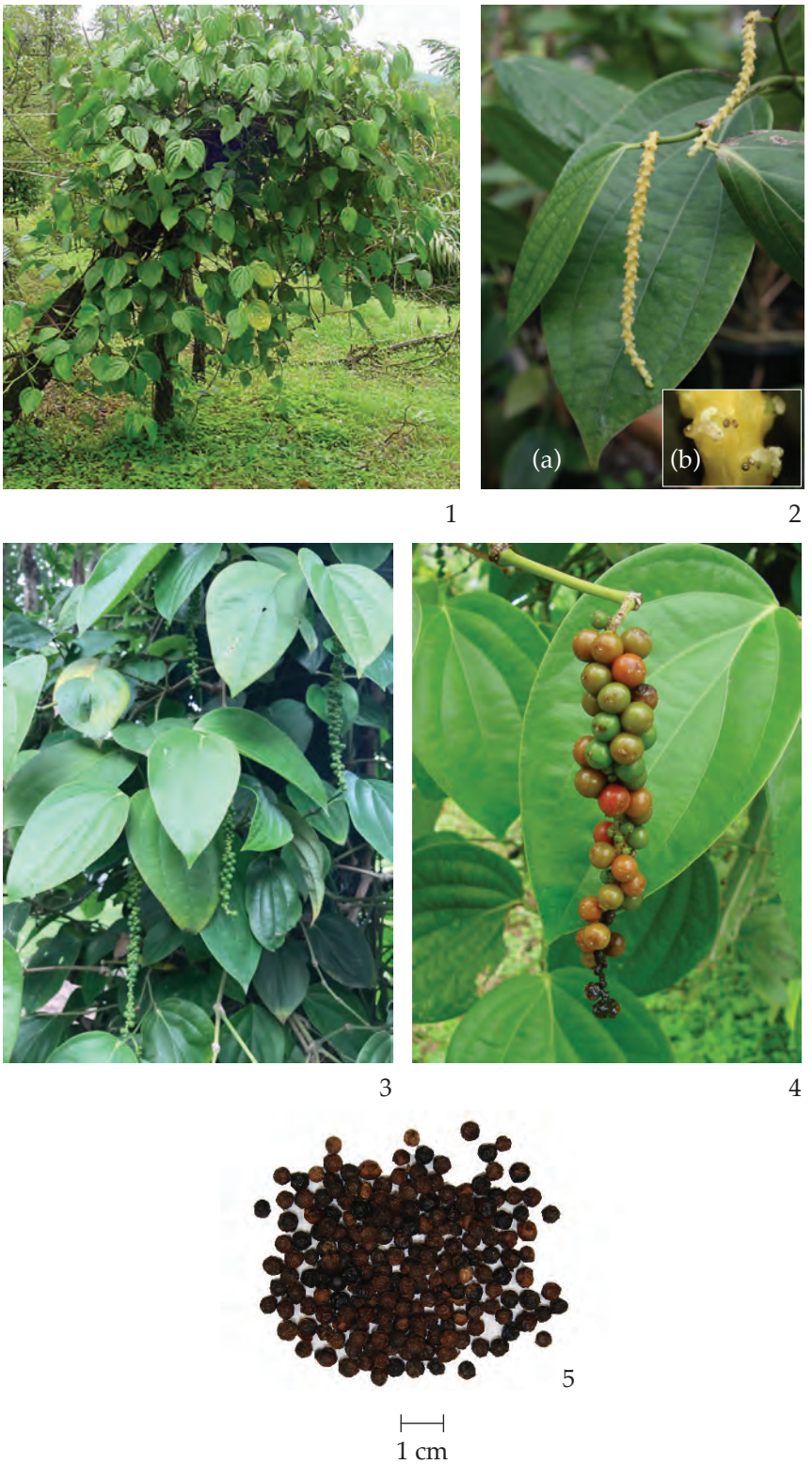


Fig. 1a *Piper nigrum* L.
1. habit 2. inflorescences (a), part of inflorescence (b) 3. leaves and inflorescences
4. infructescence showing different stages of fruits 5. crude drug

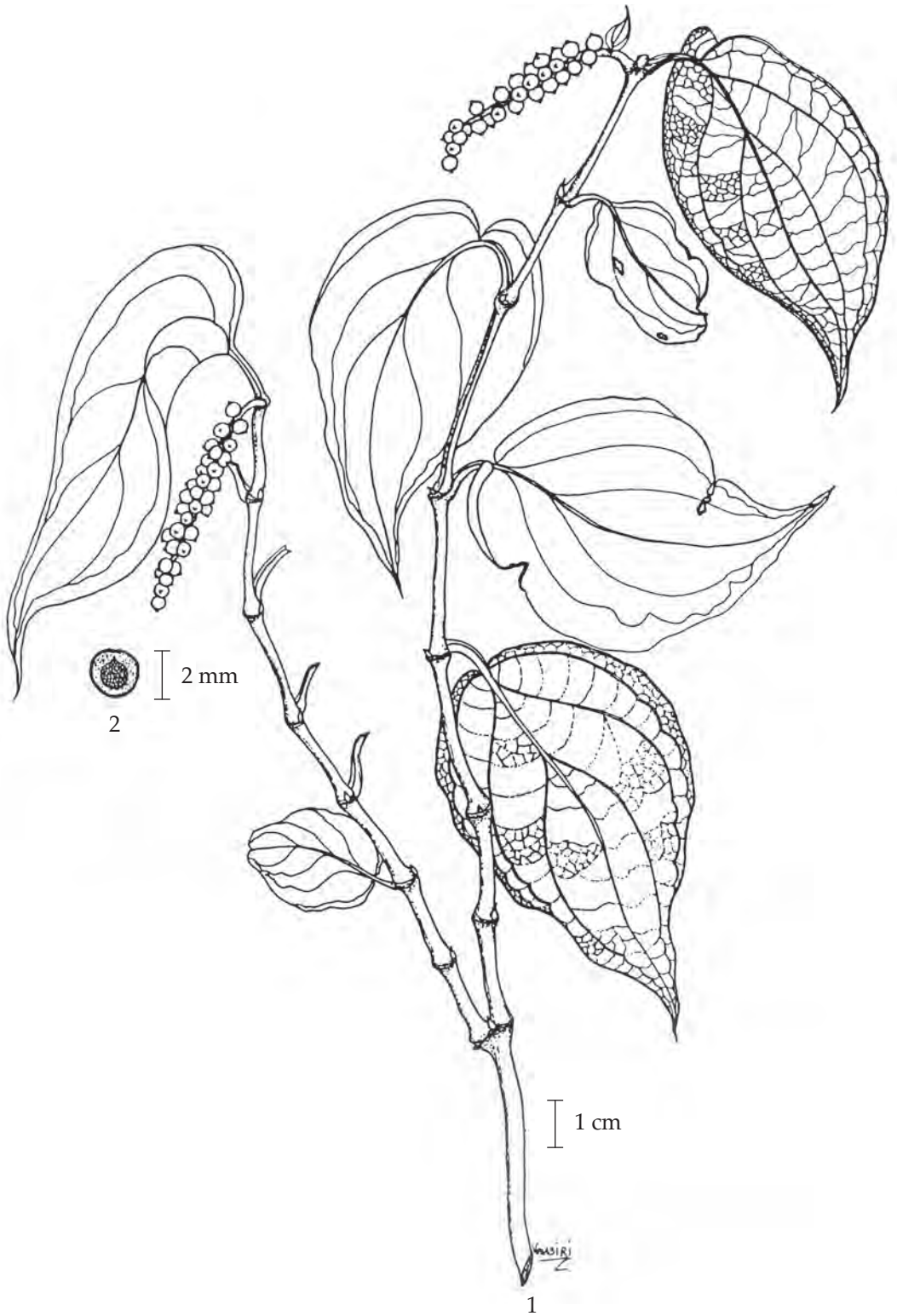


Fig. 1b *Piper nigrum* L.
1. twig 2. fruit

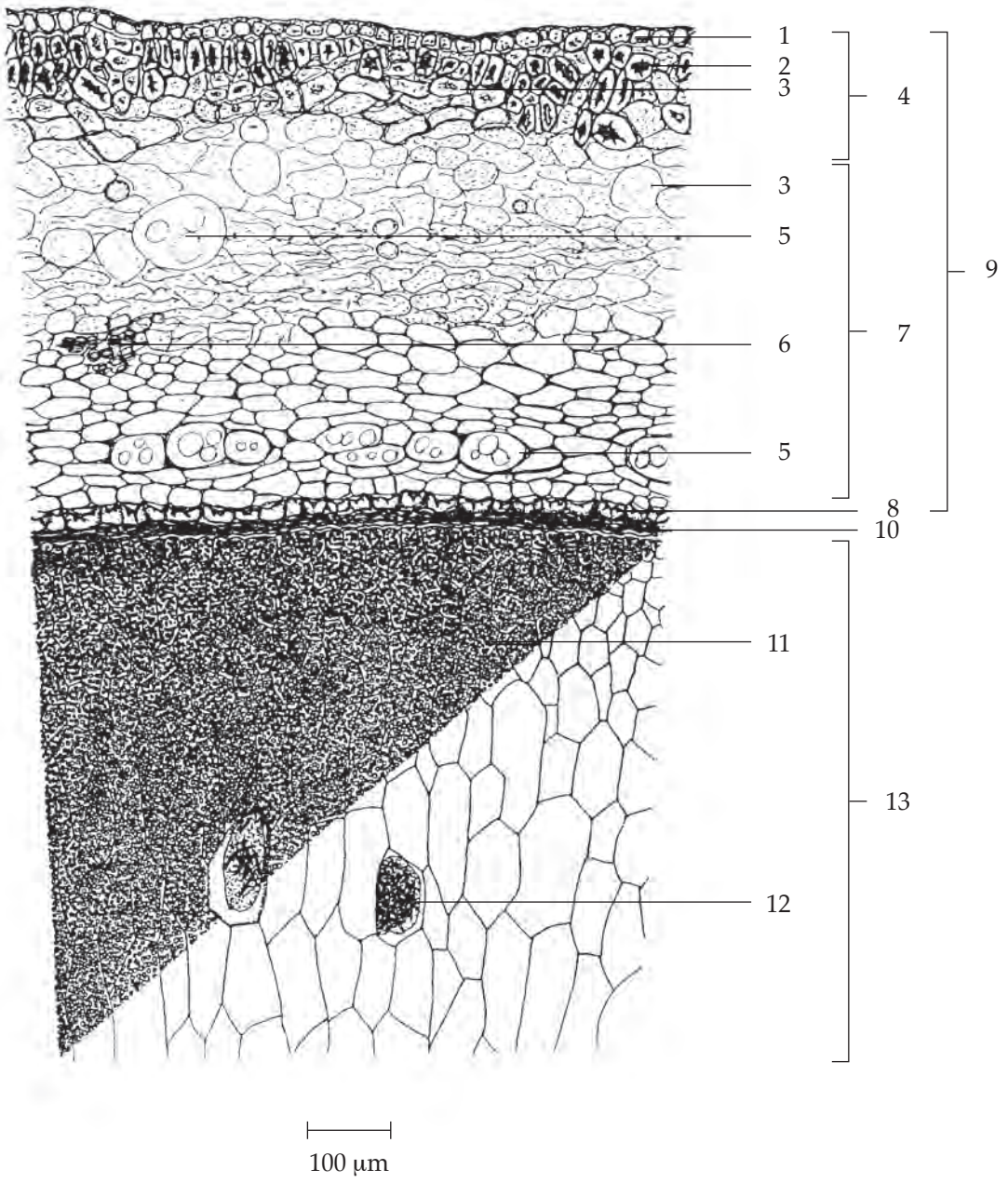


Fig. 2a Transverse Section of the Fruit of *Piper nigrum* L.

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

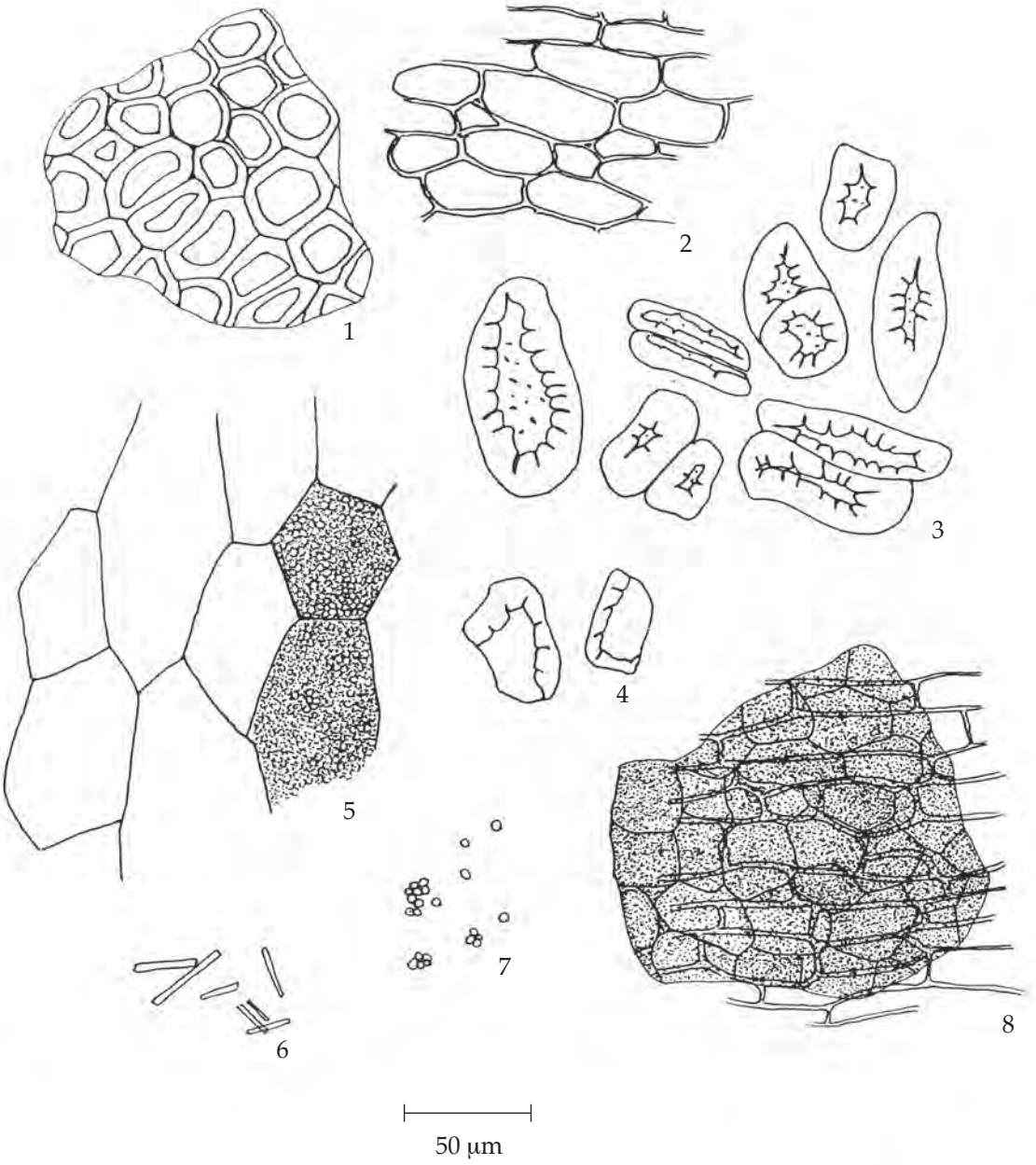


Fig. 2b Powdered Drug of the Fruits of *Piper nigrum* L.

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*	50 Per Cent V/V Solution of *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.

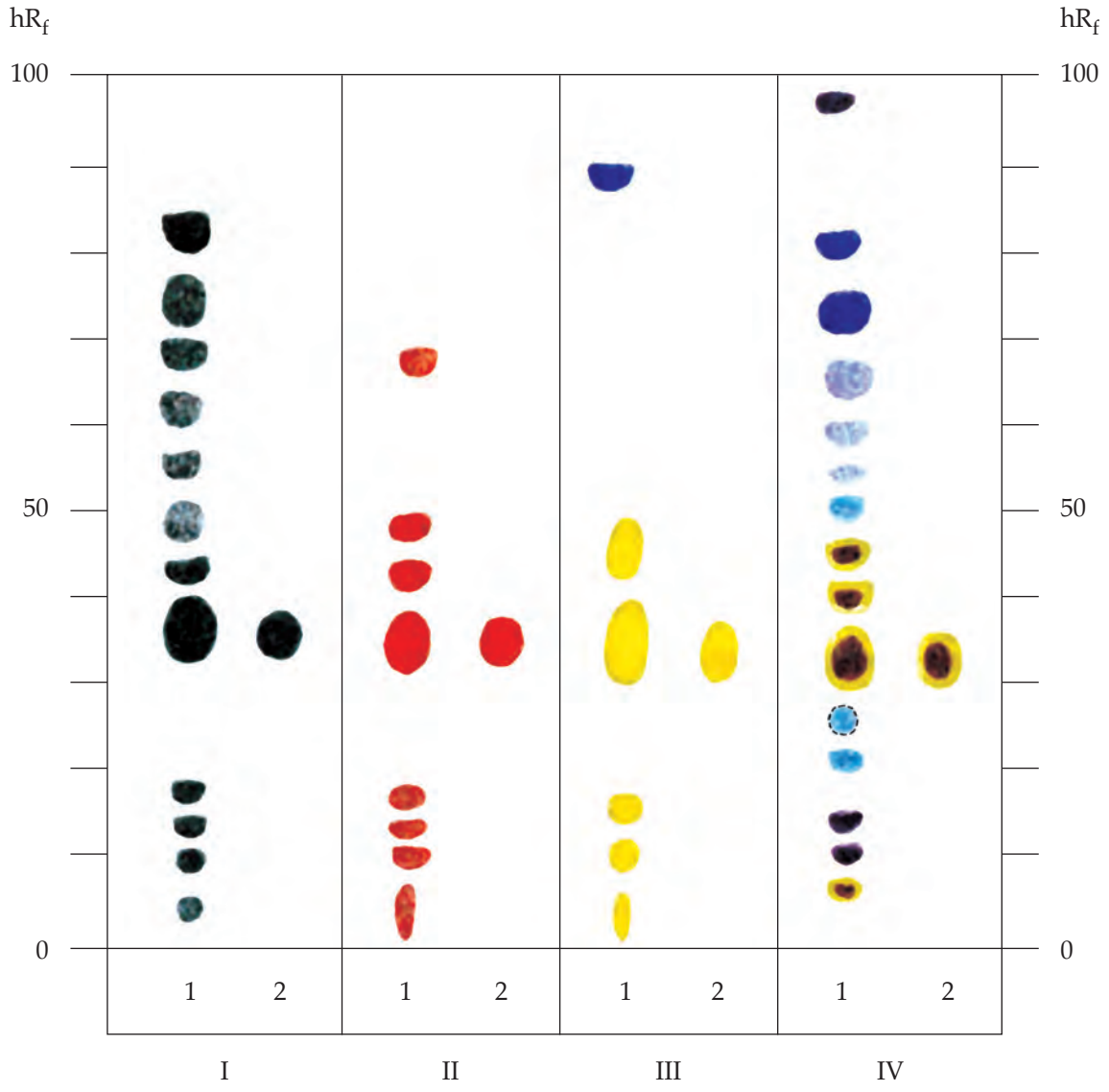


Fig. 3 Thin-Layer Chromatogram of Chloroform Extract of the Fruits of *Piper nigrum* L.

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
◌ = spot developed in some samples

พริกไทยล่อน (PHRIK THAI LON)

Piperis Nigri Semen
White Pepper

Synonyms Pepper, Pepper Corn

Category Aromatic, stomachic, carminative.

White Pepper is the dried seed of the ripe fruit, deprived of the outer part of the pericarp of *Piper nigrum* L. (Family Piperaceae), Herbarium Specimen Number: DMSC 667, Crude Drug Number: DMSc 0276.

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, 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 of 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.

ปลาไหลเผือก (PLA LAI PHUEAK)

เอียนด่อน (IAN DON), ไหลเผือก (LAI PHUEAK), ตรึงบาดาล (TREUNG BADAN), หยิกบ่อถอง (YIK BO THONG)
Eurycomae Longifoliae Radix
Longjack Root

Synonyms Ali's Umbrella Root, Ali's Walking Stick Root, Tongkat Ali Root

Category Antipyretic.

Longjack Root is the dried root of *Eurycoma longifolia* Jack (*E. latifolia* Ridl., *E. longifolia* var. *cochinchinensis* Pierre, *E. merguensis* Planch.) (Family Simaroubaceae), Herbarium Specimen Number: DMSC 5238, Crude Drug Number: DMSc 0536.

Constituents Longjack Root contains quassinoids (e.g., eurycomanone, eurycomanol, eurycomalactone, and their derivatives). It also contains β-carboline and canthin-6-one alkaloids, volatile compounds (e.g., curcumene, massoilactone), etc.

Description of the plant (Fig. 1) Shrub to treelet, 2 to 5(−10) m tall; monoecious or dioecious; stem erect, tubular, smooth; young shoot puberulous with brownish red glandular hairs. Leaves pinnately compound, spirally arranged, up to 1 m long, crowded at tip; leaflet sessile, opposite or subopposite, in one plane, lanceolate to obovate-lanceolate, rarely ovate-oblong, 5 to 10 cm long, 1.5 to 6 cm wide, apex acute or acuminate, base slightly cuneate, oblique, margin entire, subcoriaceous, dark green, nerves inconspicuous on both surfaces. Inflorescence in large panicle, axillary or terminal. Flower bisexual or unisexual, reddish; pedicel rather thick, up to 7 mm long; bract triangular, very small, about 1 mm long, caducous; calyx small, 5(−6)-lobed, about 1 mm long; petals 5(−6), lanceolate to ovate or obovate-oblong, 4 to 6 mm long, 2 to 3 mm wide, puberulous on both surfaces; stamens 5(−6), 1.5 to 2.5 mm long, anther about 0.3 mm long, staminode about 0.5 mm long in female flower, up to 2 mm long in male flower; ovary superior, carpels 5(−6), free, style up to 3 mm long, stigma peltate, 5(−6)-lobed. Fruit up to 5 drupelets; drupelet ellipsoid or ovoid, 1 to 2 cm long, 0.5 to 1.2 cm wide, red to blackish purple when ripe; drupelet stalk about 3 mm long. Seed 1 per drupelet.

Description Odour, mild; taste, bitter.

Macroscopical (Fig. 1) Obliquely or transversely sliced root, varying in shape, length, width, and thickness, hard, light; externally, pale brown; internally, ivory with radial lines.

Microscopical (Figs. 2a, 2b, 2c, 2d) Transverse section of the root shows cork, cortex, phloem, xylem, and medullary ray. Cork, several layers of suberized rectangular cells, some containing reddish brown resinous substance. Cortex, polygonal parenchyma cells, some containing starch grains, reddish brown resinous substance or prismatic crystals, sclereids scattering throughout the cortical layer. Phloem, small thin-walled parenchyma containing small starch grains and fibres. Xylem, lignified bordered-pitted vessels, slightly lignified pitted parenchyma containing large starch grains, and fibres. Medullary ray, rectangular parenchyma of phloem rays and xylem rays with varying sizes of starch grains.

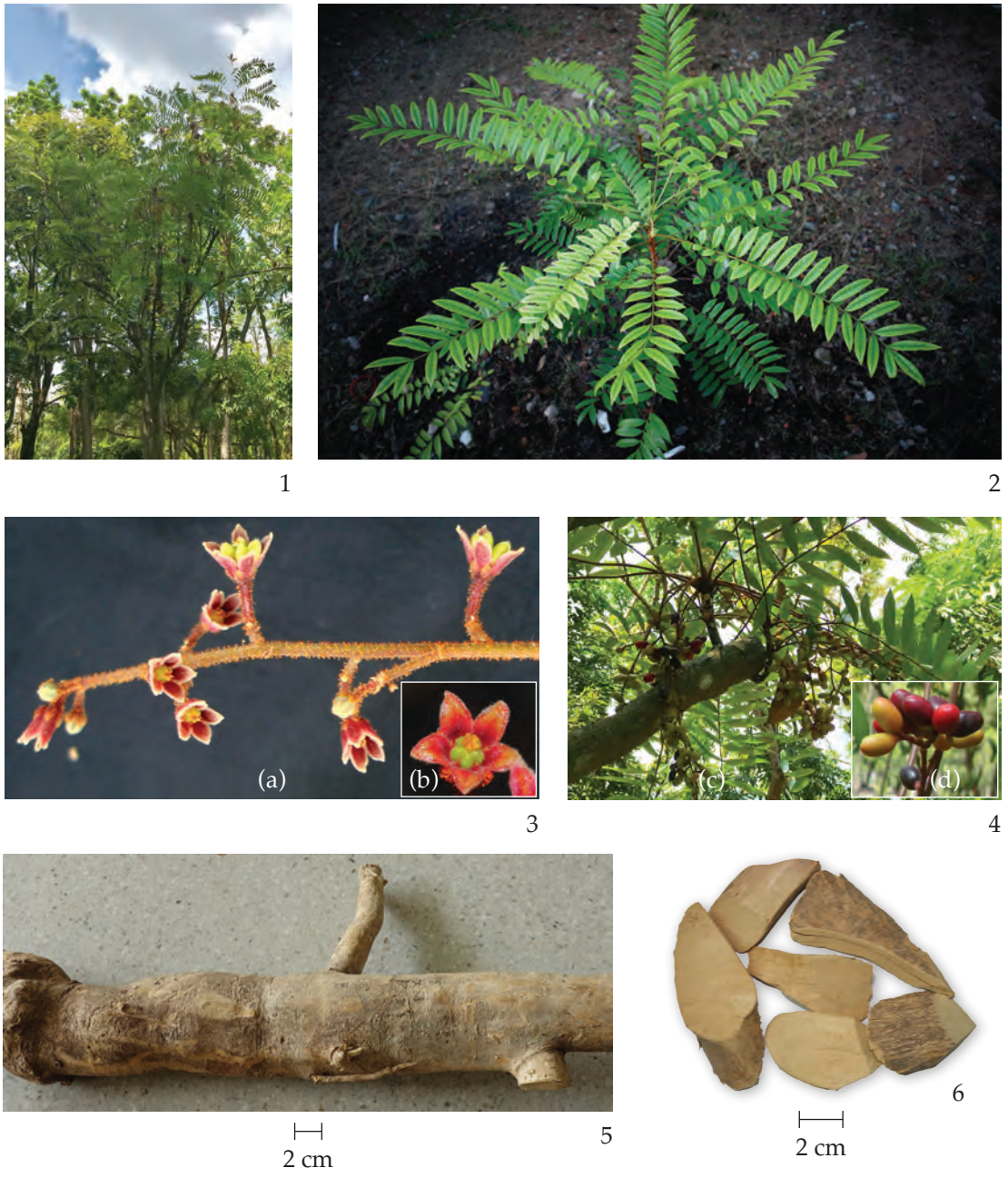
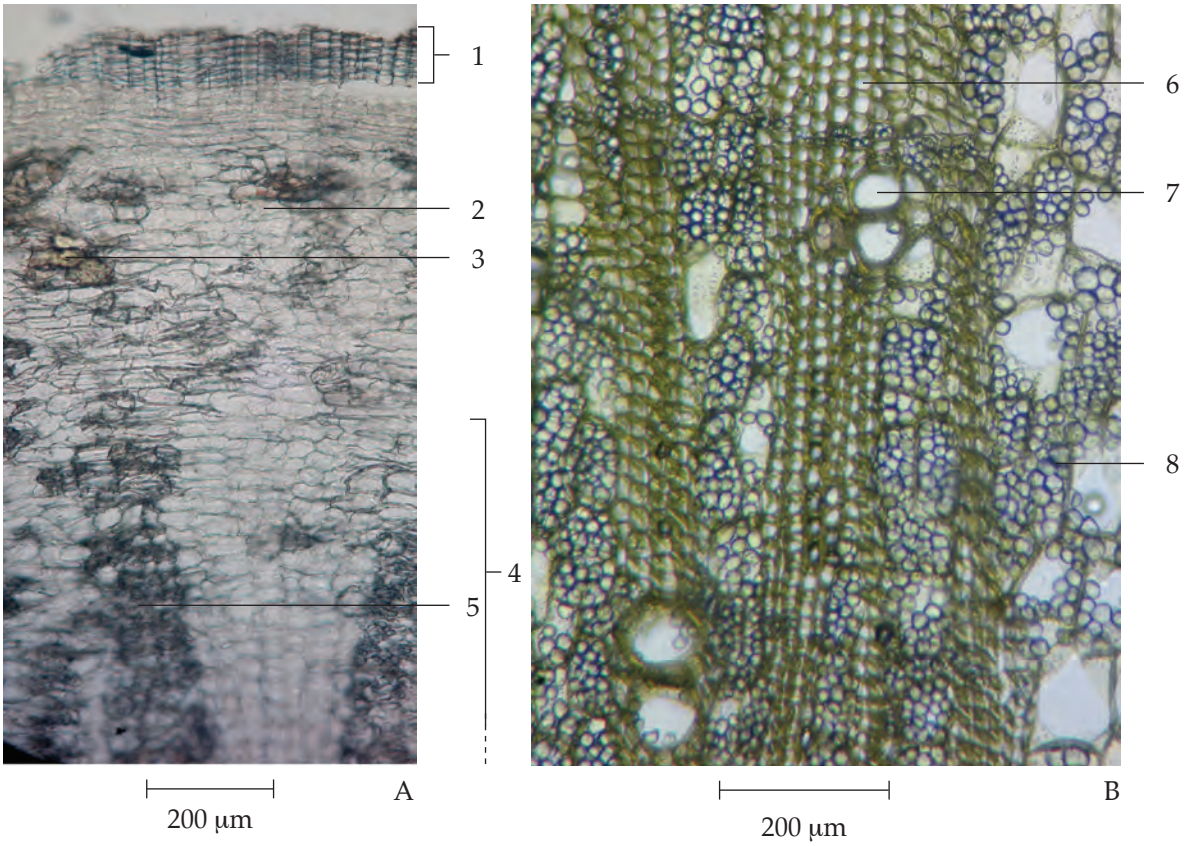


Fig. 1 *Eurycoma longifolia* Jack
1. habit 2. leaves (top view) 3. part of female inflorescence with young fruits (a), female flower (b) 4. infructescences (c), different stages of fruits (d) 5. part of dried root 6. crude drug



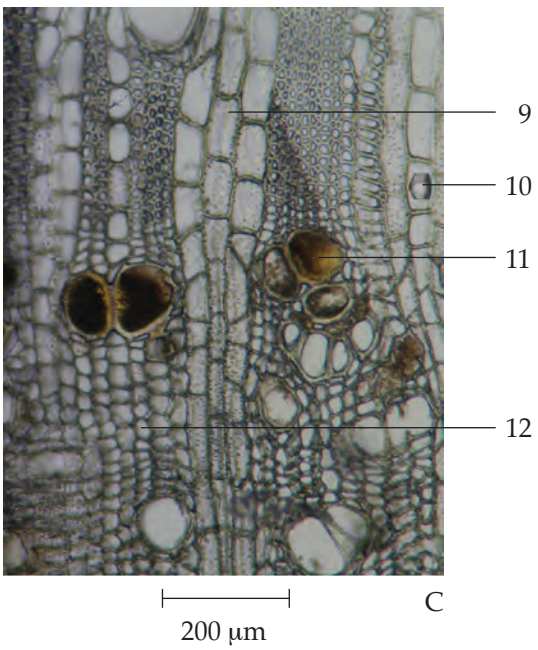
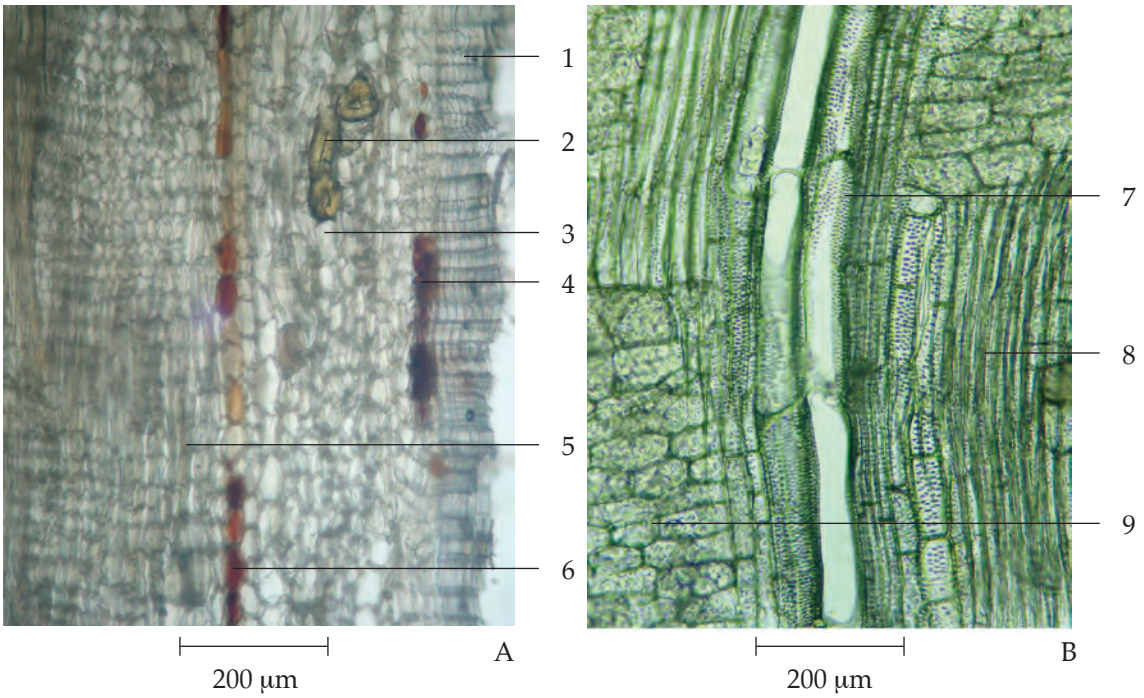


Fig. 2a Photomicrographs of Transverse Sections of the Root of *Eurycoma longifolia* Jack

A. Bark
B. Wood, Stained with Aniline Sulfate
C. Wood

1. cork
2. cortical parenchyma
3. sclereid
4. phloem
5. phloem fibre
6. xylem fibre
7. vessel
8. xylem ray with starch grains
9. xylem ray
10. prismatic crystal
11. vessel containing resinous substance
12. xylem parenchyma



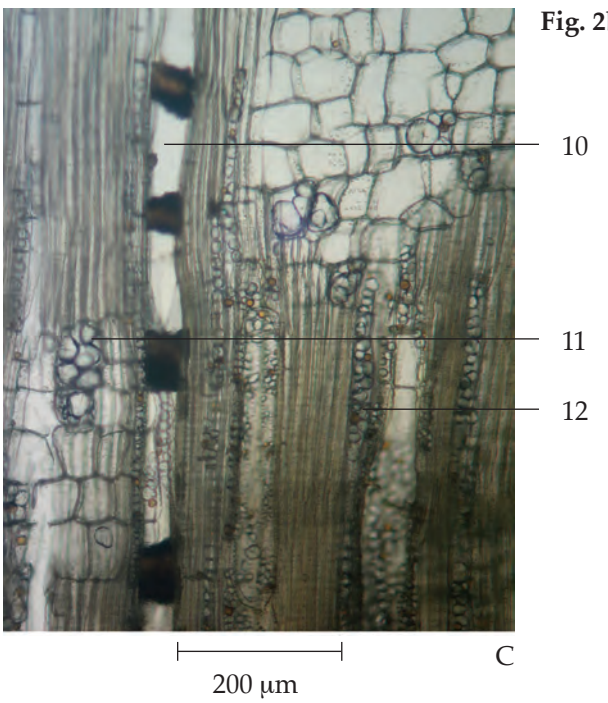
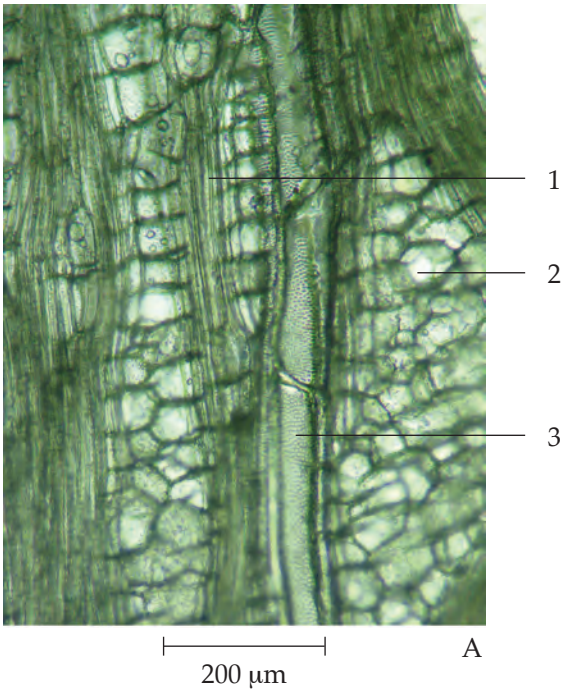


Fig. 2b Photomicrographs of Radial Longitudinal Sections of the Root of *Eurycoma longifolia* Jack

A. Bark
B. Wood, Stained with Aniline Sulfate
C. Wood

1. cork
2. sclereid
3. cortical parenchyma
4. cork cell with resin
5. phloem fibre
6. cortical parenchyma with resin
7. bordered-pitted vessel
8. xylem fibre
9. xylem ray
10. vessel containing resin
11. ray parenchyma with starch grains
12. xylem parenchyma with starch grains



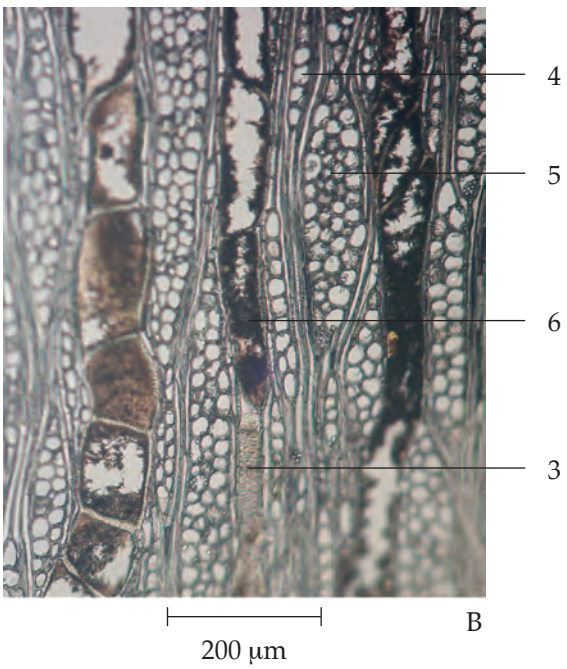


Fig. 2c Photomicrographs of Tangential Longitudinal Sections of the Root of *Eurycoma longifolia* Jack

A. Wood, Stained with Aniline Sulfate
B. Wood

1. xylem fibre
2. xylem ray
3. bordered-pitted vessel
4. uniseriate medullary ray
5. multiseriate medullary ray
6. vessel with resinous substance

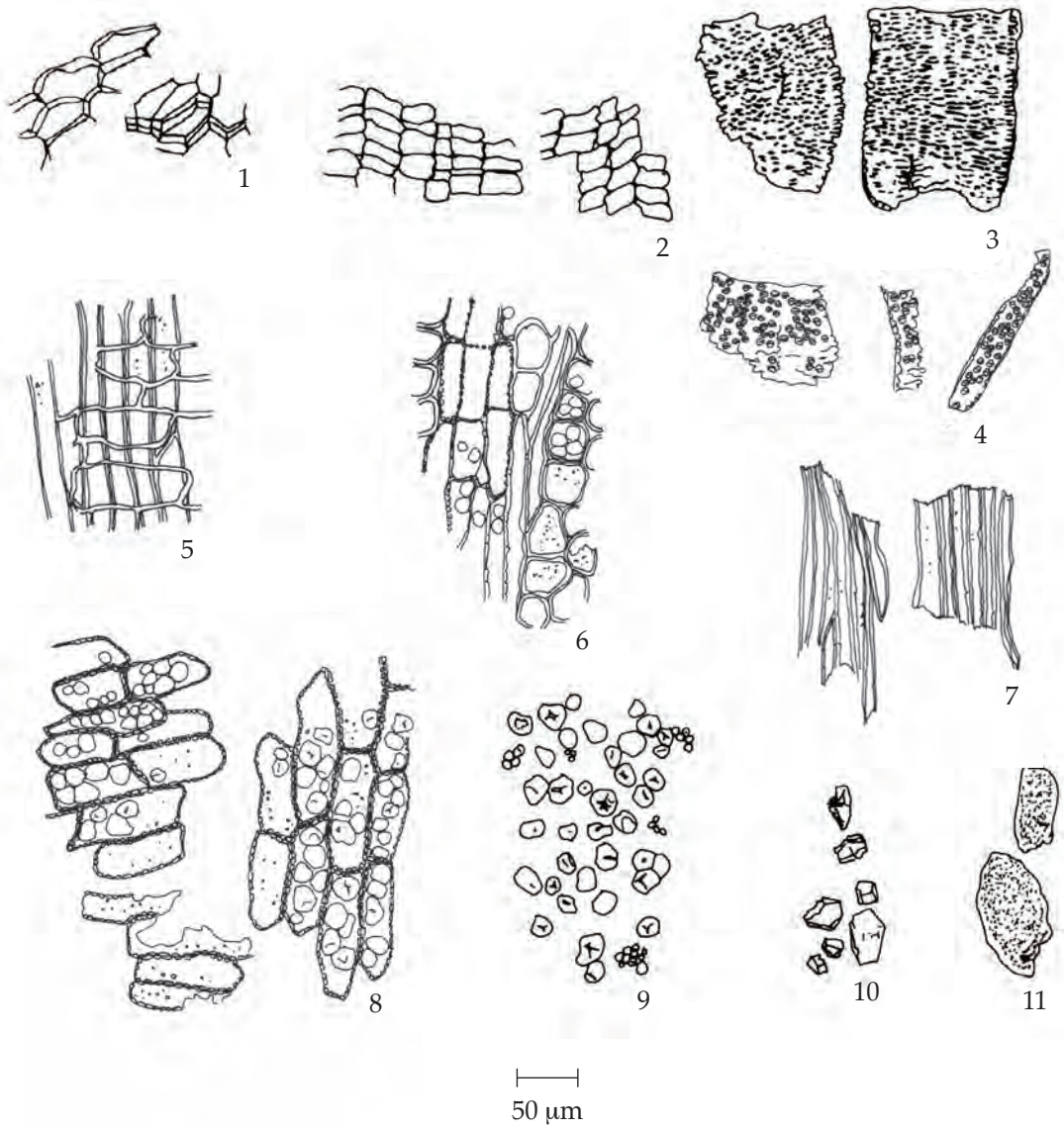


Fig. 2d Powdered Drug of the Roots of *Eurycoma longifolia* Jack

1. cork in surface view
2. cork in sectional view
3. elongated-pitted vessels
4. bordered-pitted vessels
5. xylem ray and fibres in radial longitudinal section
6. xylem rays and parenchyma, some containing starch grains in tangential longitudinal section
7. bundles of fibres
8. xylem parenchyma containing starch grains
9. starch grains
10. prismatic crystals
11. resinous substance

Radial and tangential longitudinal sections of the root show cork, cortex, phloem, xylem, and medullary ray. Cork, several layers of suberized rectangular cells, some containing reddish brown resinous substance. Cortex, thin-walled rectangular parenchyma cells, some of which with numerous starch grains, reddish brown resinous substance or prismatic crystals. Phloem, layers of rectangularly elongated parenchyma cells and fibres. Xylem, bordered-pitted vessels, some of which containing dark brown resinous substance, slightly lignified polygonal parenchyma, some of which containing starch grains, and fibres. Medullary ray in radial longitudinal view, rectangular phloem ray cells and xylem ray cells; in tangential longitudinal view, groups of round parenchyma cells, some of which containing starch grains.

Longjack Root in powder possesses the diagnostic microscopical characters of the unground drug. Various sizes of starch grains and medullary ray cells can be seen in abundant. Cork cells, large bordered-pitted vessels and prismatic crystals can also be observed.

Packaging and storage Longjack 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 *fine powder*, with 10 mL of *ethanol* on a water-bath 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 slowly add 1 mL of *sulfuric acid* to form two layers, the upper layer changes to a brownish yellow colour and a brownish red ring is formed at the zone of contact.

B. To 2 mL of solution 1, add 1 to 2 drops of a 2 per cent w/v solution of 3,5-*dinitrobenzoic acid* in *methanol*, mix and add 1 to 2 drops of a 5.7 per cent w/v solution of *potassium hydroxide* in *methanol*: a brownish purple 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 85 volumes of *ethyl acetate*, 15 volumes of *methanol* and 0.1 volume of *glacial acetic 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 10 mm, 5 μL each of the following solutions. Prepare solution (A) by refluxing 500 mg of the sample, in *fine powder*, with 20 mL of *ethanol* for 15 minutes, filtering and evaporating the filtrate to dryness under reduced pressure at 40°. Dissolve the residue in 2 mL of *ethanol*. For solution (B), dissolve 1 mg of *eurycomanone* 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 bands. The chromatogram obtained from solution (A) shows a quenching band (hR_f value 53 to 56) corresponding to the eurycomanone band from solution (B) and several quenching bands are also observed. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter; several blue fluorescent bands are observed. Spray the plate with a 20 per cent v/v solution of *sulfuric acid* in *ethanol* and heat at 110° for 5 minutes; the band due to eurycomanone is brown. Several other brown and purple bands also appear (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Roots of *Eurycoma longifolia* Jack

Band	hR_f Value	Detection		
		UV 254	UV 366	20 Per Cent V/V Solution of *Sulfuric Acid* in *Ethanol*
1	8–10	–	–	brown
2	11–16	quenching	blue	–
3	27–29	–	light blue	–
4*	53–56	quenching	–	brown
5	73–75	–	light blue	–
6	77–79	quenching	–	–
7	79–81	–	light blue	–
8	84–86	quenching	blue	pale brown
9	90–92	quenching	–	pale purple
10	95–96	quenching	–	purple
11	96–99	–	–	purple

*eurycomanone

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).

Acid-insoluble ash Not more than 2.0 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 2.0 per cent w/w (Appendix 7.12).

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

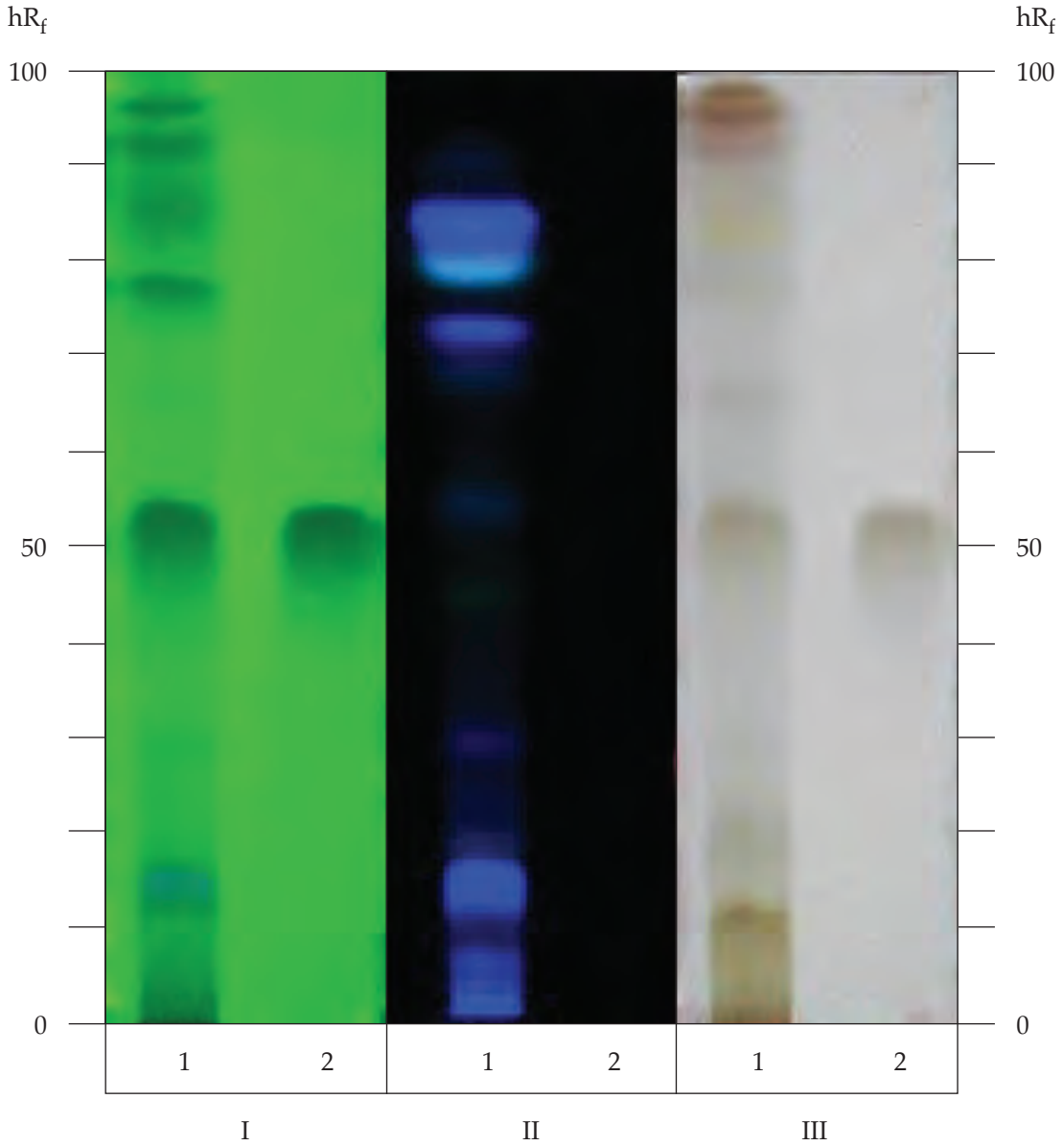


Fig. 3 Thin-Layer Chromatogram of Ethanolic Extract of the Roots of *Eurycoma longifolia* Jack

1 = solution A
2 = solution B
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with a 20 per cent v/v solution of *sulfuric acid* in *ethanol*

รางจืด, ใบ (RANGCHUET, BAI)

เครือเขาเขียว, ใบ (KHRUEA KHAO KHIAO, BAI), รางเย็น, ใบ (RANGYEN, BAI), ยาเขียว, ใบ (YAKHIAO, 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 of 2 cm long at the tip, splitting through the beak into 2 pieces. Seeds 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 trichome, unicellular stalk, 2-celled head; non-glandular trichome, unicellular. Lower epidermis, wavy-walled in surface view; diacytic stomata, numerous; glandular trichome, 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.

1

2

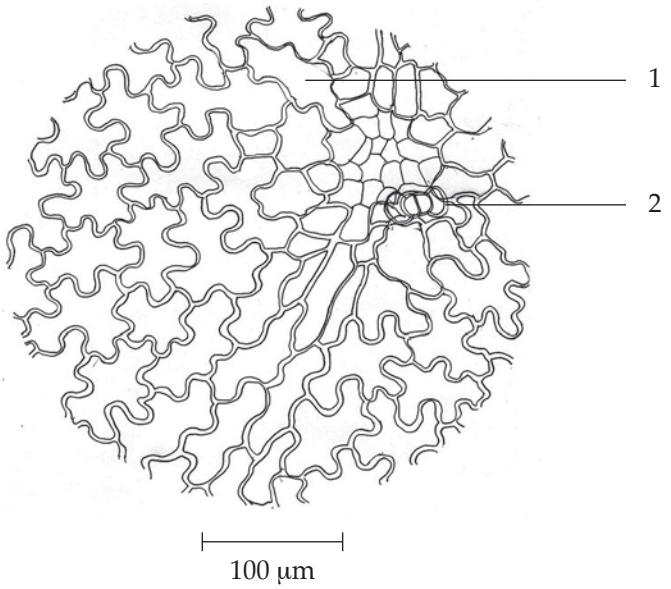
3

4

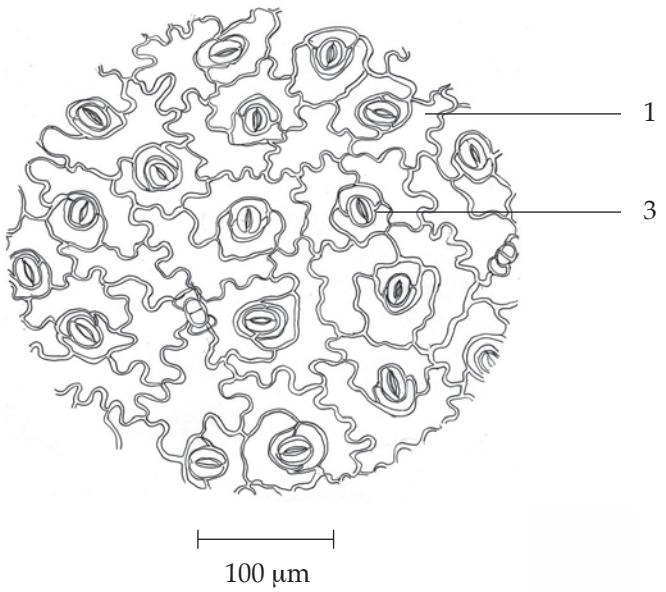
2 cm

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

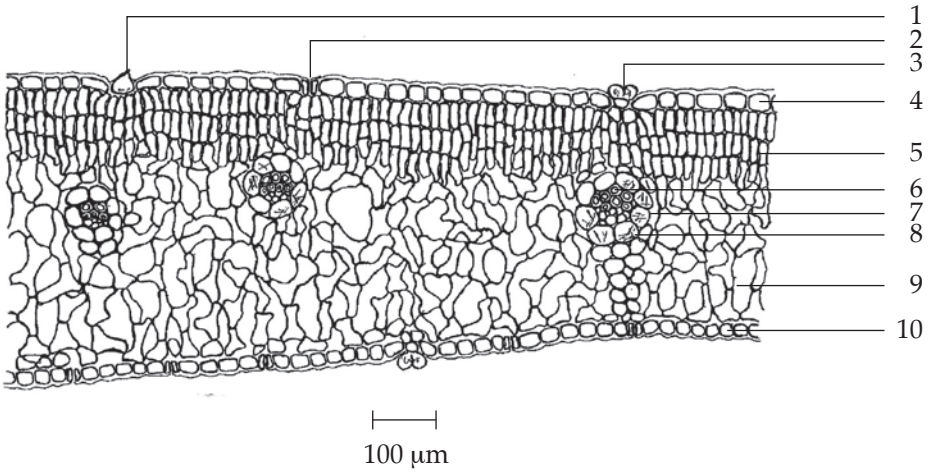


Upper Epidermis of the Lamina

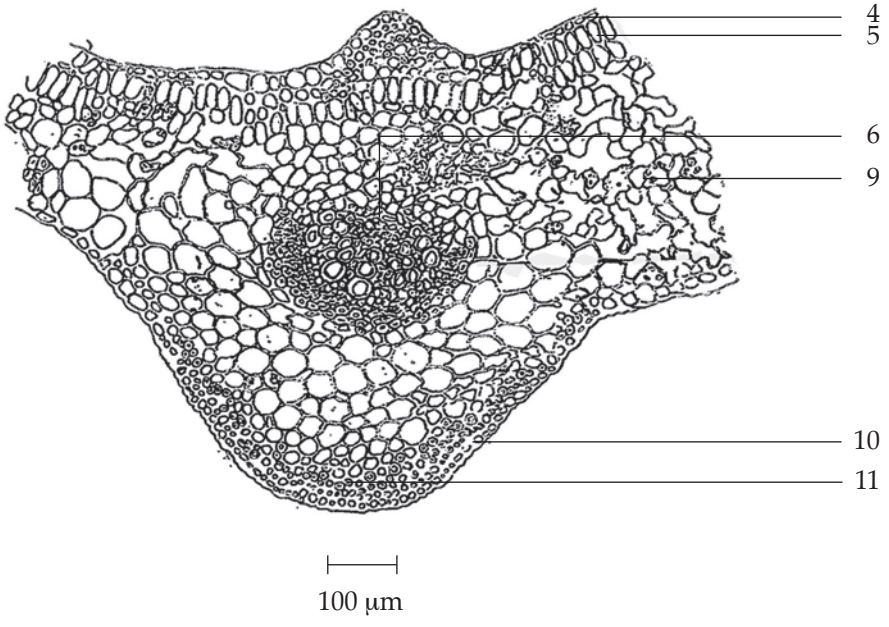


Lower Epidermis of the Lamina

Fig. 2a Epidermises of the Leaf of *Thunbergia laurifolia* Lindl.
1. epidermal cell
2. glandular trichome
3. diacytic stoma



Transverse Section of the Lamina



Transverse Section of the Midrib

Fig. 2b Transverse Sections 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

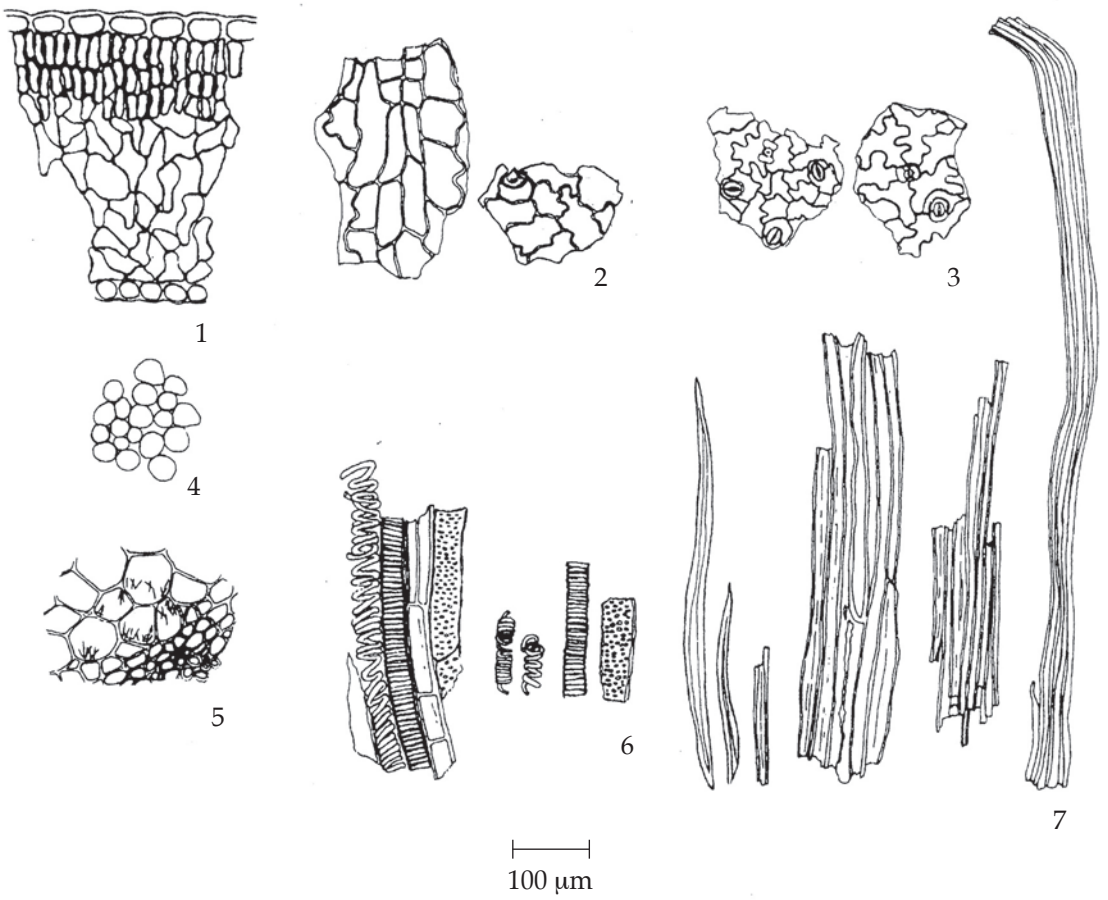


Fig. 2c Powdered Drug of the Leaves of *Thunbergia laurifolia* Lindl.

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).

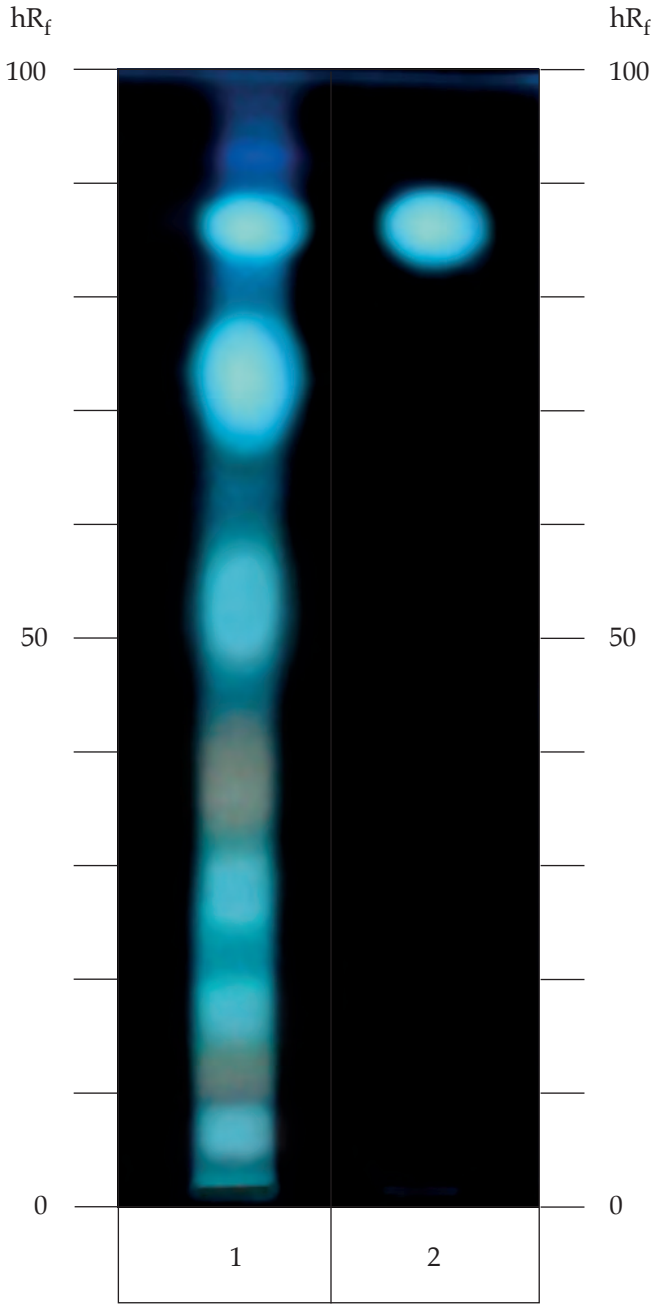


Fig. 3 Thin-Layer Chromatogram of Water Extract of the Leaves of *Thunbergia laurifolia* Lindl., Detection under UV Light (366 nm) after Spraying with *NP/PEG TS*
1 = solution (A)
2 = solution (B)

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, greyish 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, varying 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 a 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-band 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.

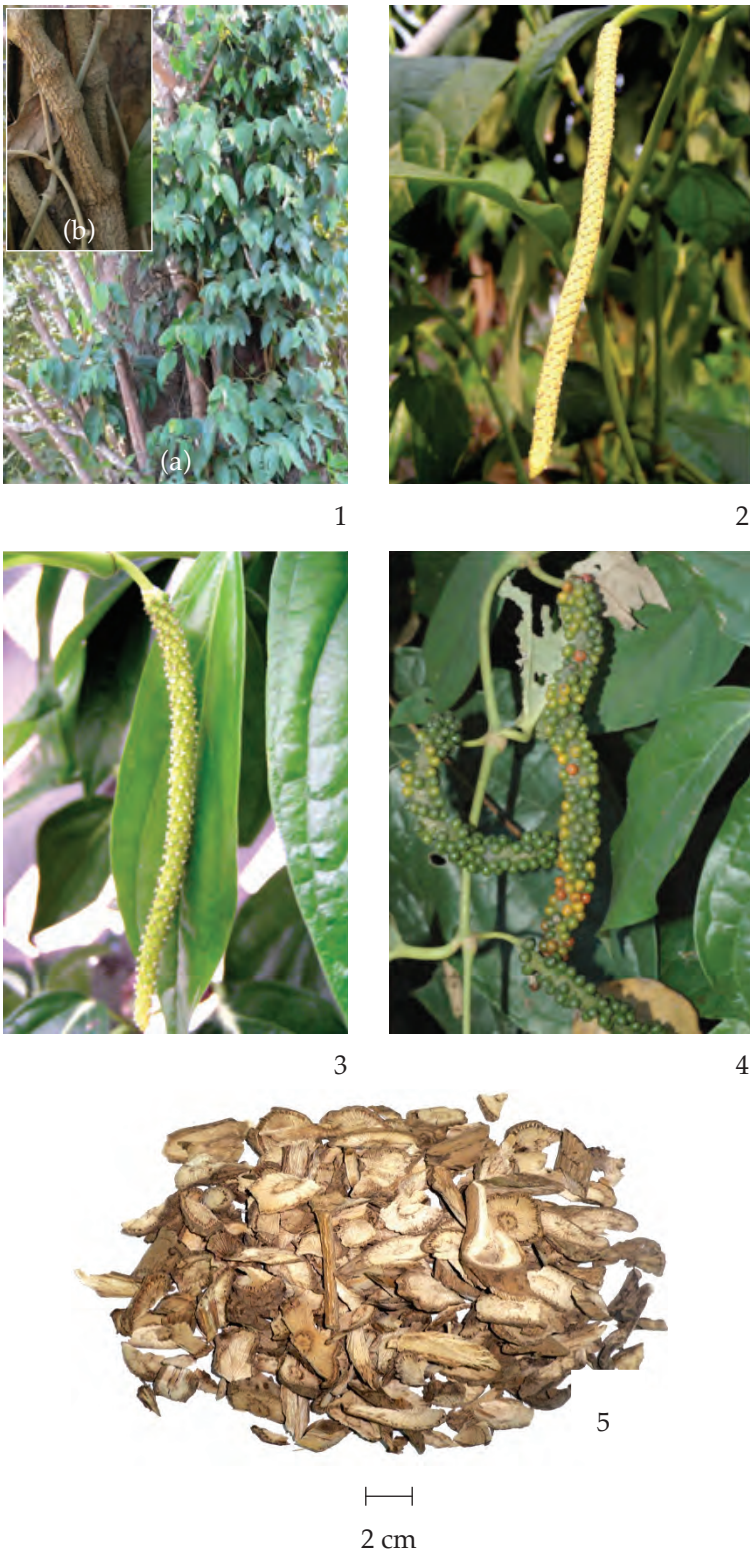


Fig. 1a *Piper wallichii* (Miq.) Hand.-Mazz.
1. habit (a), stem (b) 2. male inflorescence 3. female inflorescence
4. infructescences 5. crude drug

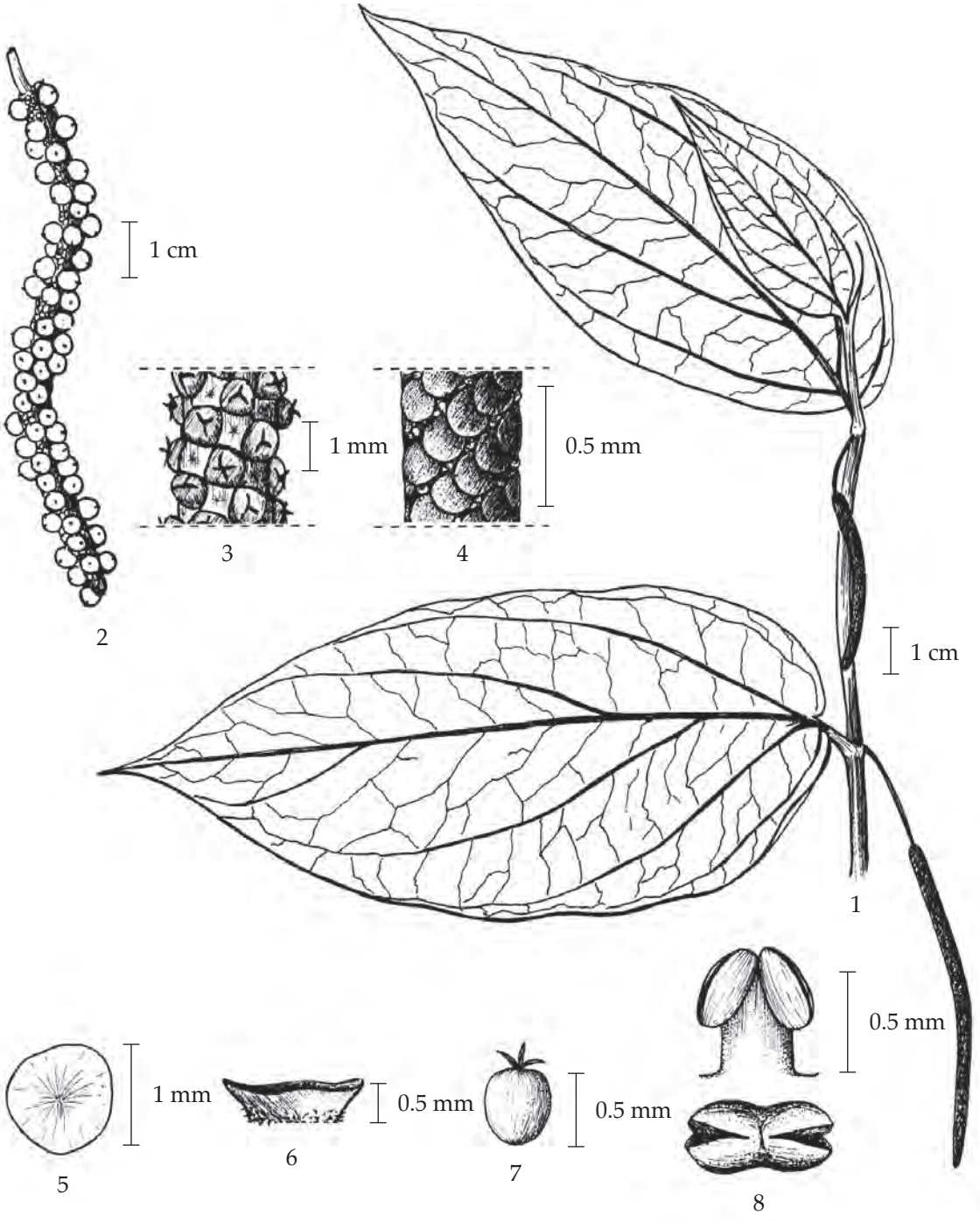


Fig. 1b *Piper wallichii* (Miq.) Hand.-Mazz.
1. twig showing leaves and male inflorescences 2. infructescence
3. part of female inflorescence 4. part of male inflorescence 5. top view of bract
6. side view of bract 7. ovary 8. side view of stamen and top view of anther sacs

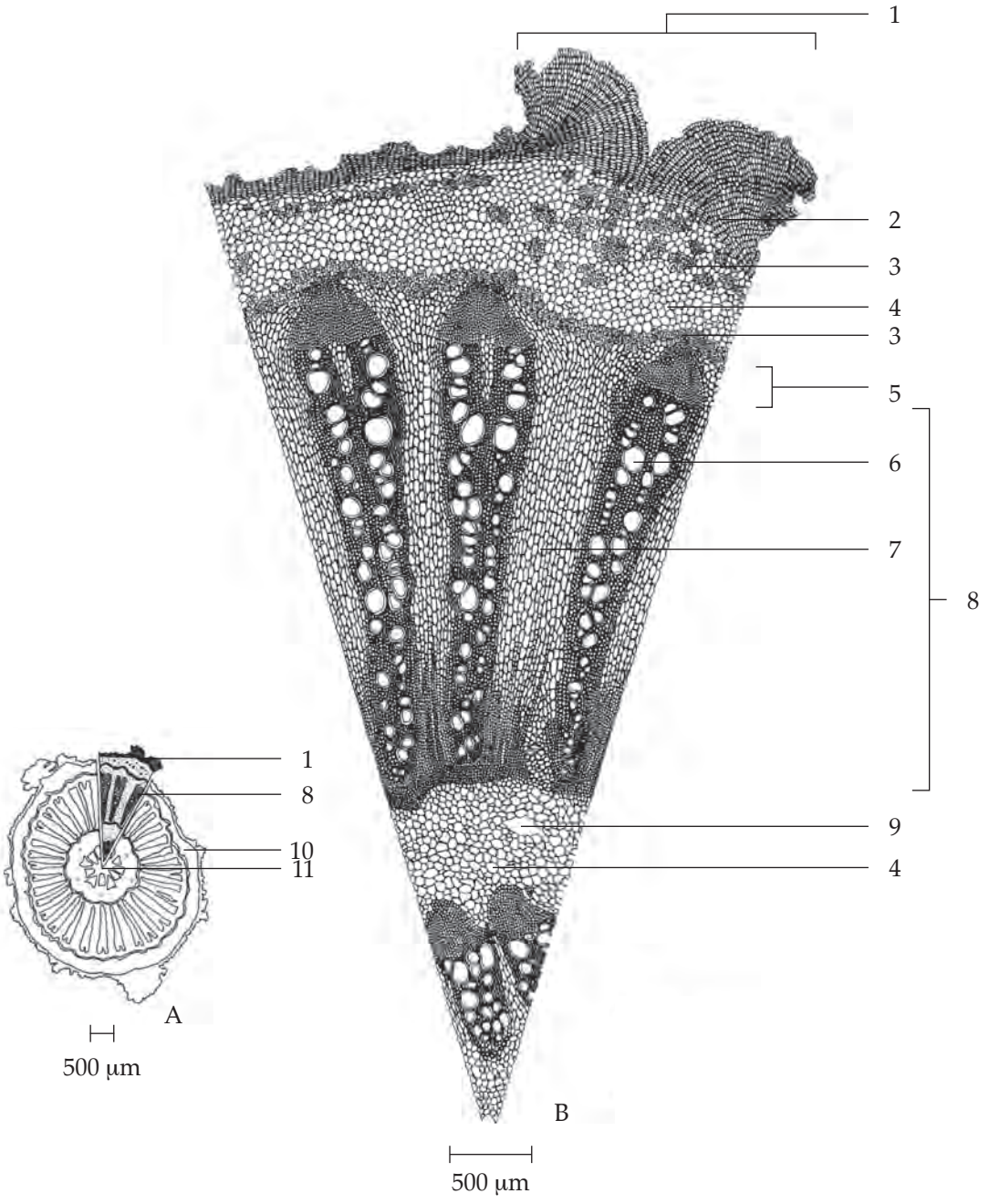


Fig. 2a Transverse Section of the Stem of *Piper wallichii* (Miq.) Hand.-Mazz.

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

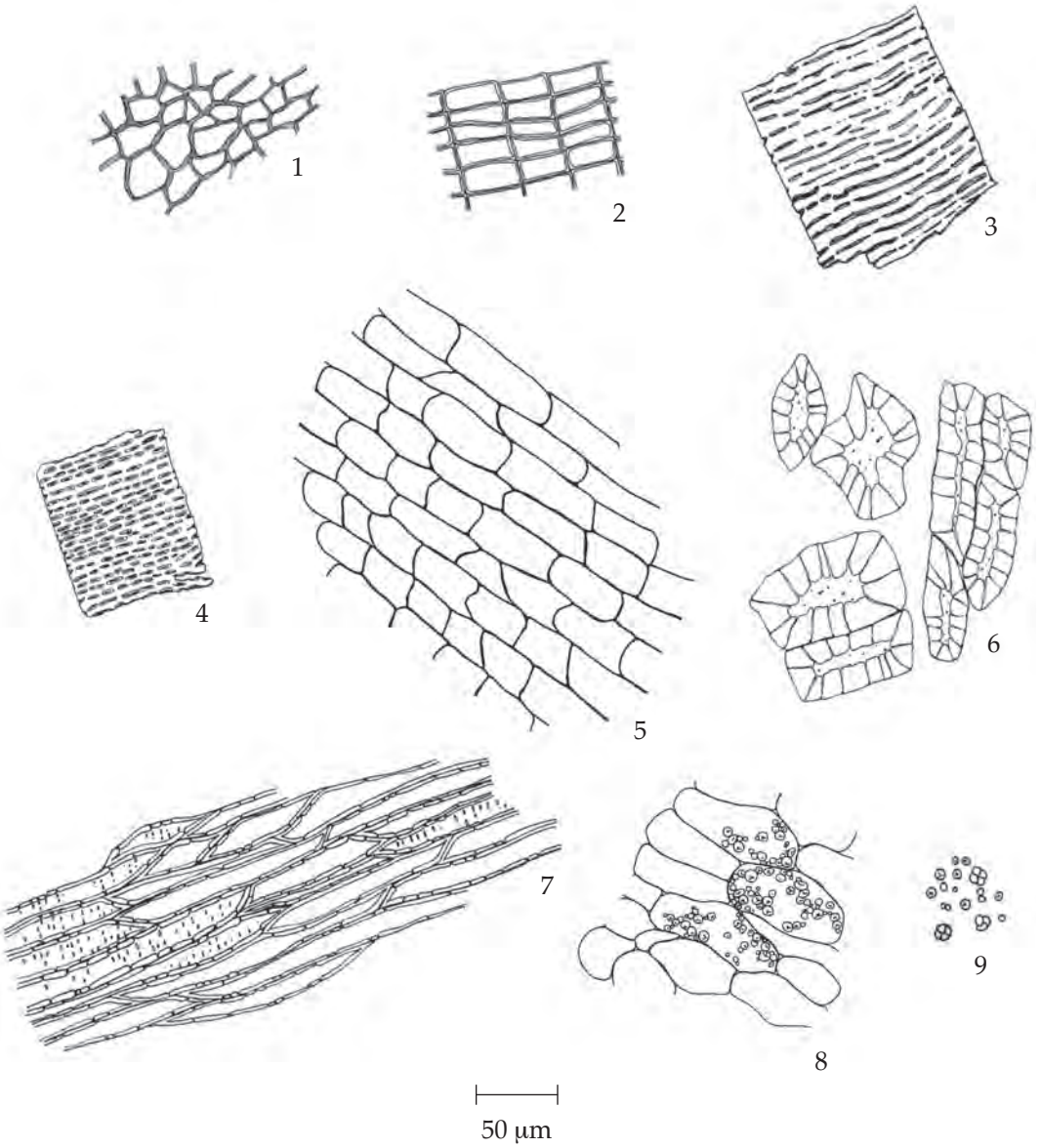


Fig. 2b Powdered Drug of the Stems of *Piper wallichii* (Miq.) Hand.-Mazz.

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* and 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).

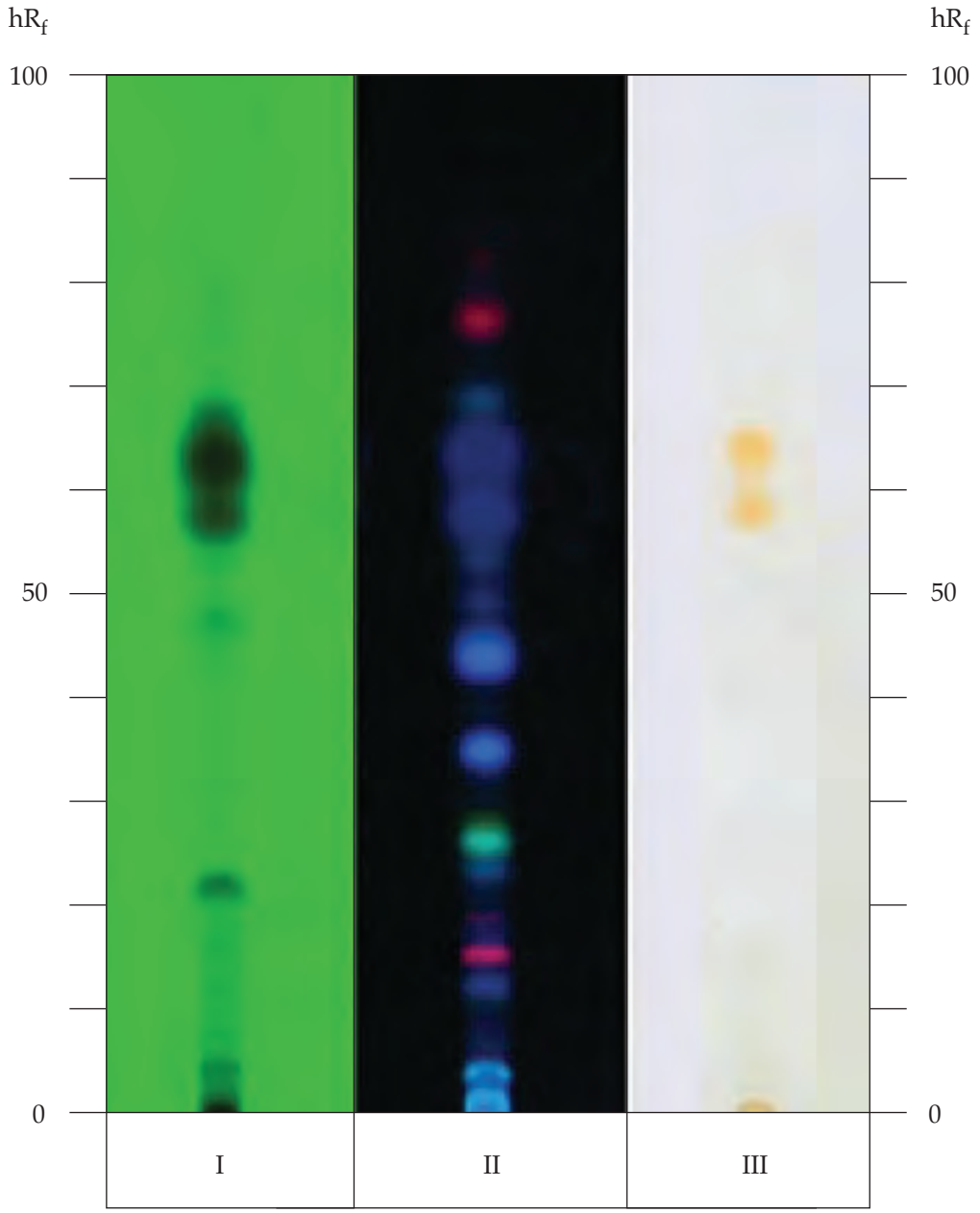


Fig. 3 Thin-Layer Chromatogram of Ethanolic Extract of the Stems of *Piper wallichii* (Miq.) Hand.-Mazz.

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *modified Dragendorff TS2*

แสมสาร (SAMAE SAN)

ขี้เหล็กโคก (KHI LEK KHOK), ขี้เหล็กป่า (KHI LEK PA), ขี้เหล็กสาน (KHI LEK SAN)
Sennae Garrettianae Lignum
Senna Garrettiana Heartwood

Category Laxative.

Senna Garrettiana Heartwood is the dried heartwood of *Senna garrettiana* (Craib) H. S. Irwin & Barneby (*Cassia garrettiana* Craib) (Family Leguminosae-Caesalpinioideae), Herbarium Specimen Number: DMSC 5225, Crude Drug Number: DMSc 1098.

Constituents Senna Garrettiana Heartwood contains anthraquinones (e.g., aloe-emodin, cassialoin, chrysophanol, emodin) and stilbenes (e.g., cassigarols, piceatannol).

Description of the plant (Fig. 1) Tree up to 10 m tall; bark greyish black, cracked. Leaves even-pinnate, alternate; rachis 10 to 20 cm long; petiole 4 to 5 cm long; stipule early caducous; leaflets 6 to 9 pairs, lanceolate to broadly ovate, 5 to 9 cm long, 2 to 5 cm wide, apex acuminate, base rounded, margin entire, glabrous to pubescent; petiolule 4 to 6 mm long. Inflorescence compound raceme, terminal, 9 to 20 cm long; bract ovate, about 4 mm long, apex acute, caducous; bracteole minute. Flower bright yellow; pedicel 2.5 to 4 cm long; sepals 5, unequal, 2 outer smaller, about 5 mm long, 3 inner twice as long, broadly elliptic, puberulous outside; petals 5, obovate or obovate-elliptic, 1 to 1.8 cm long, 0.8 to 1.2 cm wide, with claw, about 4 mm long; stamens 10, 2 longest with flattened filaments, 7 to 8 mm long, anther 7 to 9 mm long, curved, opening by apical pores, 5 shorter with smaller anthers, and 3 stamens reduced, about 3 mm long; ovary superior, glabrous or puberulous, style short, stigma indistinct. Fruit pod, flattened, 15 to 22 cm long, 2 to 4 cm wide, often slightly twisted, glabrous or puberulous; stalk 3 to 5 cm long. Seed ovoid or oblong-ovoid, 6 to 9 mm long, about 5 mm wide, brownish.

Description Odour, mild; taste, bland.

Macroscopical (Fig. 1) Longitudinally sliced heartwood, rough, dense, hard, reddish brown to dark-brown.

Microscopical (Figs. 2a, 2b, 2c, 2d) Transverse section of the heartwood shows a diffused-porous wood type consisting of vessels, axial parenchyma, xylem rays, and fibres. Vessel, large, solitary or in cluster of 2 to 4, scattered, some containing brown substance. Axial parenchyma, paratracheal type, slightly thick-walled cells, some containing brown substance. Xylem ray, 1 to 2 layers, rectangular cells, some containing brown substance. Fibre, thick-walled cells, some containing brown substance.

Radial and tangential longitudinal sections of the heartwood show vessels, axial parenchyma, xylem ray, and fibres. Vessel, large cylindrical, bordered-pitted, some containing brown substance. Axial parenchyma, rectangular, slightly thick-walled cells, some containing brown substance. Xylem ray: homocellular type, rectangular, porous, slightly thick-walled cells, some containing brown substance or prismatic crystals, in radial longitudinal view; uniseriate rays and mostly biseriate rays, in tangential longitudinal view. Fibre, thick-walled cells, some containing brown substance.

Senna Garrettiana Heartwood in powder possesses the diagnostic microscopical characters of the unground drug. Groups of fibres, some containing brown substance are unique. Large bordered-pitted vessels may also be observed.

1

2

3

4

5

1 cm

Fig. 1 *Senna garrettiana* (Craib) H. S. Irwin & Barneby
1. habit showing bark and leaves 2. inflorescences with leaves 3. flowers
4. young pods and seeds 5. crude drug

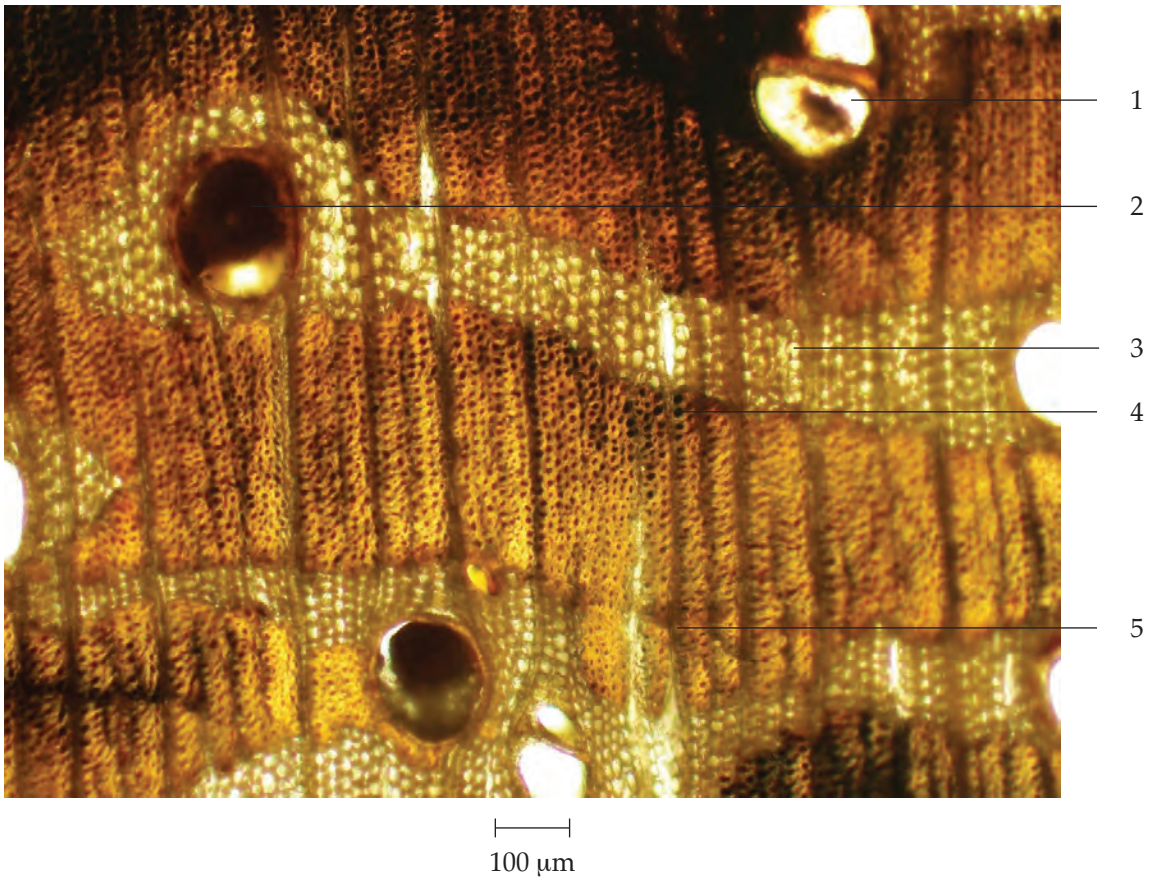


Fig. 2a Photomicrograph of Transverse Section of the Heartwood of *Senna garrettiana* (Craib) H. S. Irwin & Barneby

1. vessel
2. vessel containing brown substance
3. axial parenchyma
4. xylem fibre containing brown substance
5. xylem ray

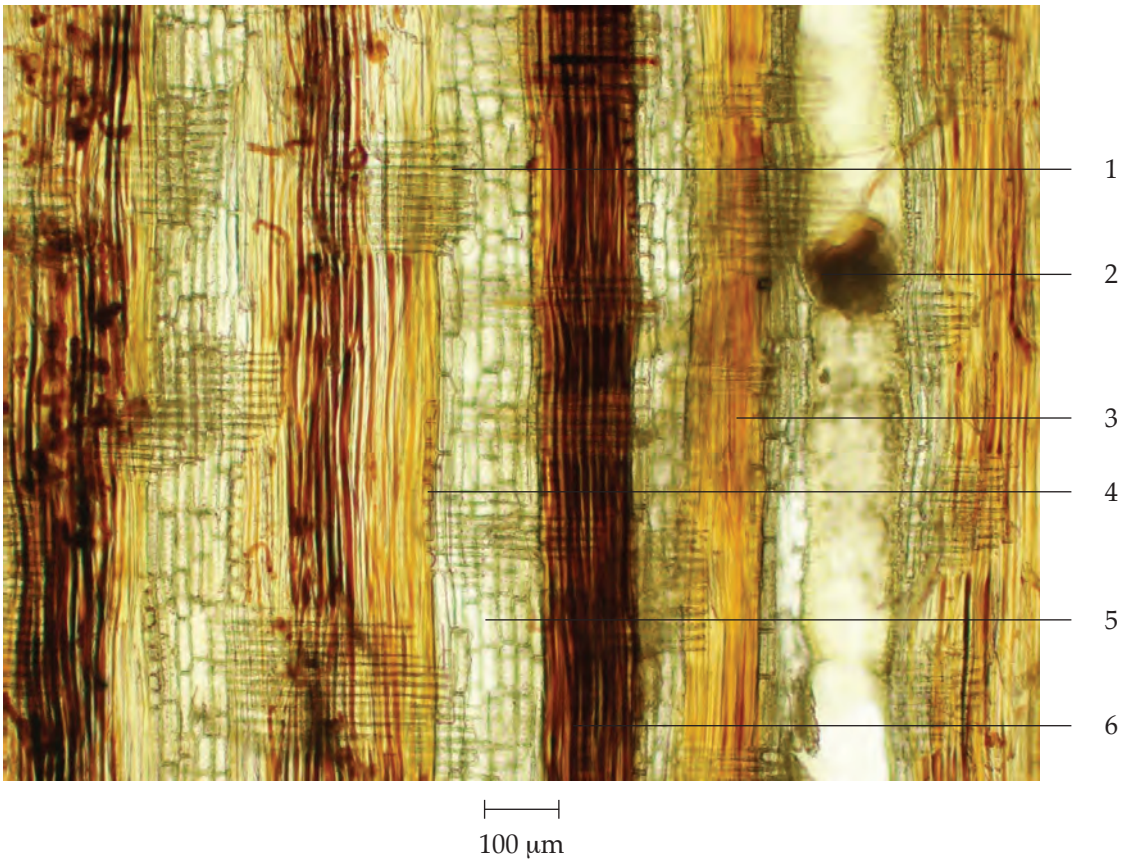


Fig. 2b Photomicrograph of Radial Longitudinal Section of the Heartwood of *Senna garrettiana* (Craib) H. S. Irwin & Barneby

1. xylem ray
2. vessel containing brown substance
3. xylem fibre
4. prismatic crystal
5. axial parenchyma
6. xylem fibre containing brown substance

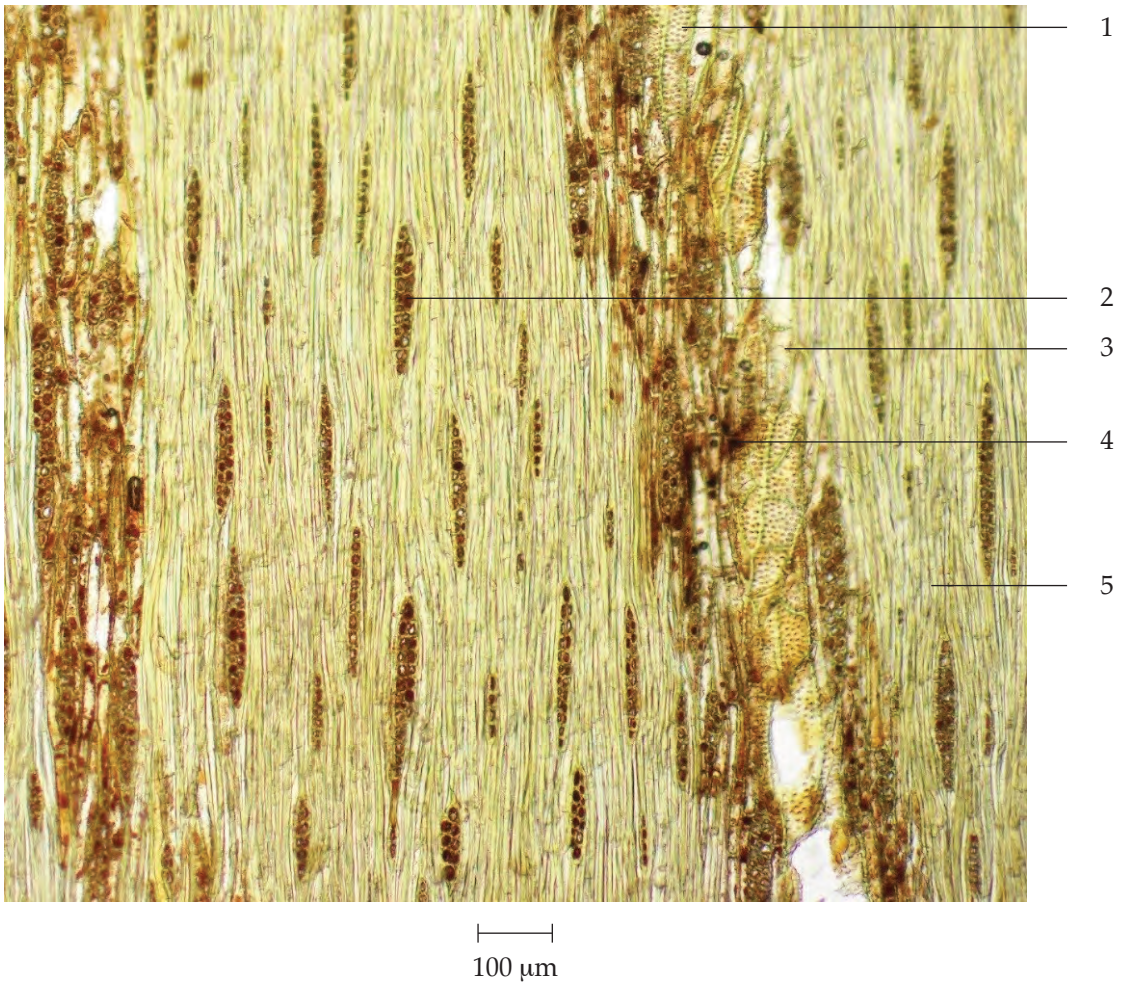


Fig. 2c Photomicrograph of Tangential Longitudinal Section of the Heartwood of *Senna garrettiana* (Craib) H. S. Irwin & Barneby

1. bordered-pitted vessel
2. xylem ray
3. axial parenchyma
4. vessel containing brown substance
5. xylem fibre

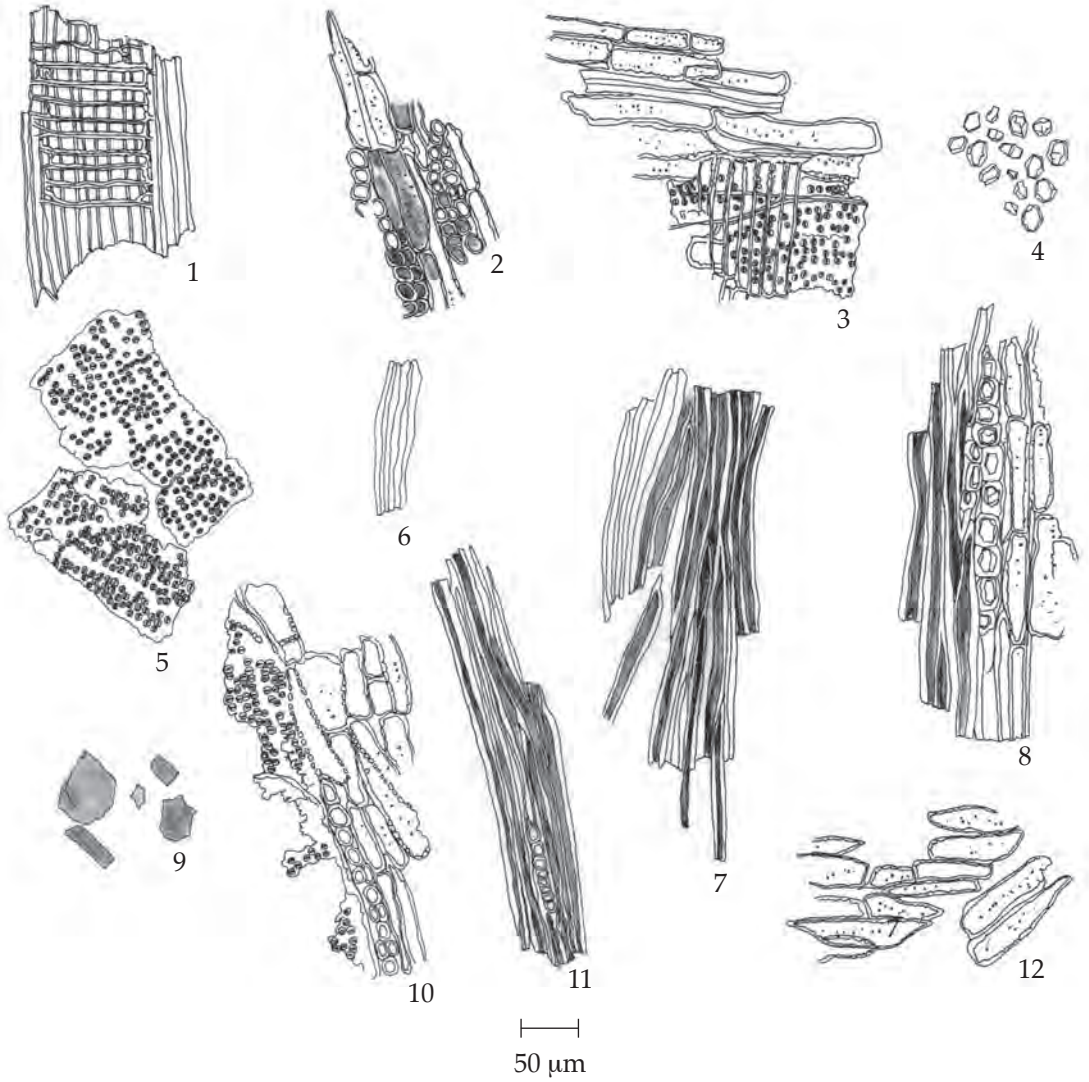


Fig. 2d Powdered Drug of the Heartwood of *Senna garrettiana* (Craib) H. S. Irwin & Barneby

1. group of fibres underlying xylem rays in radial longitudinal view
2. axial parenchyma and xylem rays, some containing brown substance in tangential longitudinal view
3. axial parenchyma, xylem ray and bordered-pitted vessels in radial longitudinal view
4. prismatic crystals
5. bordered-pitted vessels
6. group of fibres
7. group of fibres, some containing brown substance
8. group of fibres, some containing brown substance, prism sheath and axial parenchyma
9. brown substance
10. axial parenchyma associated with xylem rays and bordered-pitted vessels in tangential longitudinal view
11. group of fibres, some containing brown substance and xylem ray in tangential longitudinal view
12. axial parenchyma

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

Identification

A. To 1 g of the sample, in *fine powder*, add 10 mL of *dichloromethane*, warm on a water-bath for 5 minutes and filter. To 1 mL of the filtrate, add 1 mL of a 10 per cent w/v solution of *potassium hydroxide*: the aqueous layer becomes red.

B. Reflux 1 g of the sample, in *fine powder*, with 20 mL of *ethanol* for 15 minutes and filter. To 2 mL of the filtrate, add a few drops of *iron(III) chloride TS*: a dark blue colour is produced.

C. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1), using *silica gel F254* as the coating substance and a mixture of 60 volumes of *toluene*, 40 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 as bands of 8 mm, 5 μL each of the following solutions. Prepare solution (A) by refluxing 1 g of the sample, in *fine powder*, with 10 mL of *ethanol* for 10 minutes and filtering. Evaporate the filtrate to dryness and dissolve the residue in 2 mL of *ethanol*. For solution (B), dissolve 1 mg of *piceatannol* in 1 mL of *ethanol*. After removal of the plate, allow it to dry in air and examine the plate under ultraviolet light (254 nm), marking the quenching bands. The chromatogram obtained from solution (A) shows a quenching band (hR_f value 48 to 51) corresponding to the piceatannol band from solution (B); other quenching bands are also observed. Subsequently examine the plate under ultraviolet light (366 nm) through the cut-off filter, the band corresponding to piceatannol shows blue fluorescence. Several other fluorescent bands of different colours are also observed. Spray the plate with *anisaldehyde TS* and heat at 105° for 5 minutes; the band due to piceatannol is purple. Several other bands of different colours are observed. (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Heartwood of *Senna garrettiana* (Craib) H. S. Irwin & Barneby

Band	hR_f Value	Detection		
		UV 254	UV 366	*Anisaldehyde TS*
1	10–12	–	light red	pale brown
2	12–15	–	light blue	–
3	16–18	–	light blue	pale purple
4	28–30	–	blue	pale purple
5	38–40	weak quenching	blue	purple
6	41–42	–	light blue	–
7*	48–51	quenching	intense blue	dark purple
8	54–55	–	light blue	–
9	58–60	weak quenching	light blue	pale purple
10	78–81	–	–	purple
11	84–87	–	–	purple
12	90–95	quenching	yellow	yellow

*piceatannol

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 3.0 per cent w/w (Appendix 7.12).

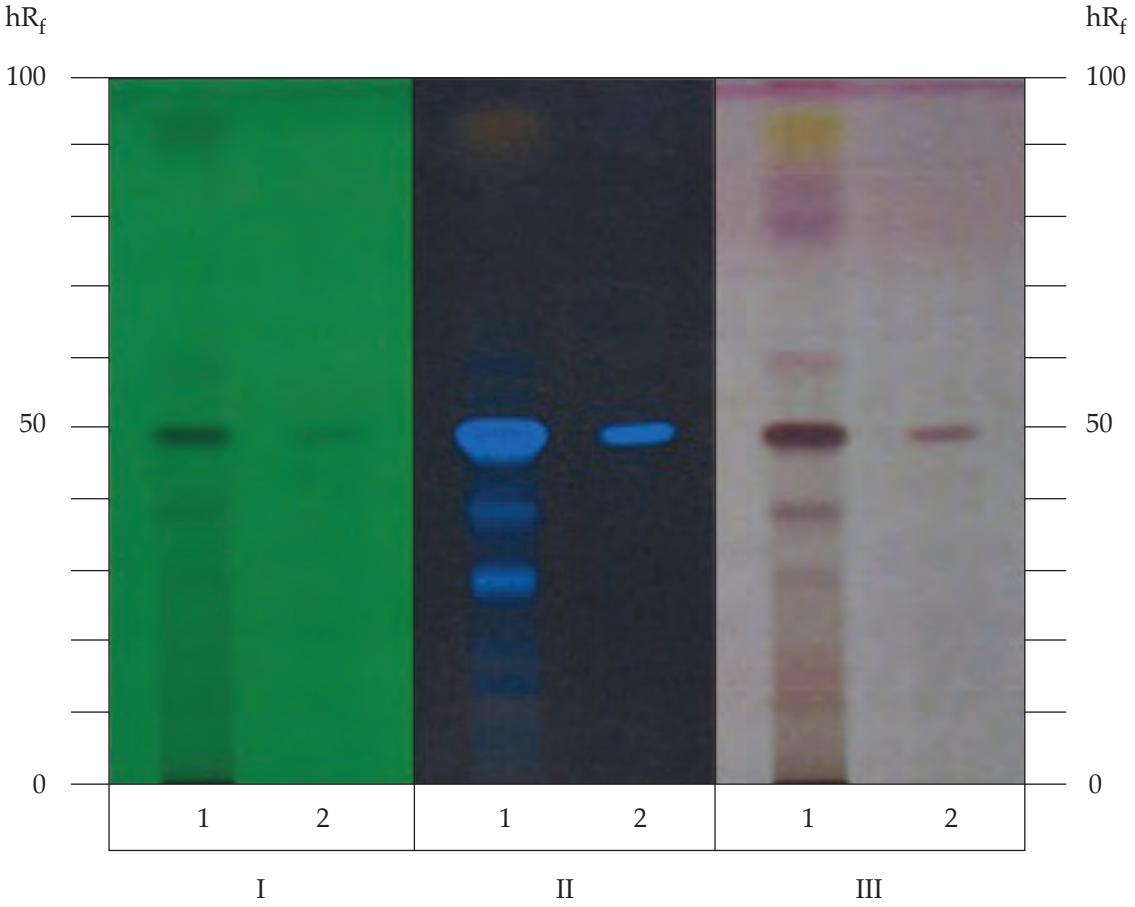


Fig. 3 Thin-Layer Chromatogram of Ethanolic Extract of the Heartwood of *Senna garrettiana* (Craib) H. S. Irwin & Barneby

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*

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 tall, 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 long, 2 to 15 cm wide, 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. Inflorescence spike or raceme, 3 to 15 cm long, often crowded at the 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; stamen 3 to 3.5 mm long; ovary ellipsoid, 2 to 3.5 mm long, 1.5 to 3 mm wide; style 4 mm long; disc densely, rusty villous. Fruit drupe, subglobose to broadly ellipsoid, 2 to 3.5 cm long, 1.5 to 3 cm wide, slightly 5-ridged, densely velvety pubescent, very hard when dry. Seed 1, ellipsoid, rough, 1.2 cm long, 0.5 cm wide.

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, a layer of large cubical cells, followed by a zone of reticulated parenchyma and vessels; tegmen consists of a zone of parenchyma and collapsed 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.

1

2

3

4

1 cm

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

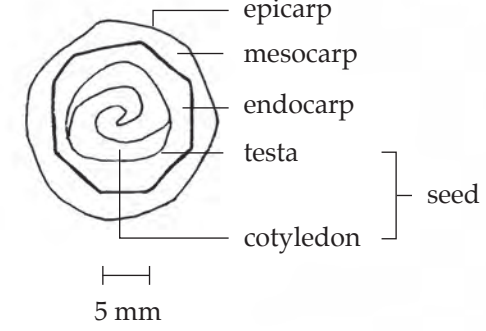


Diagram of the Fruit

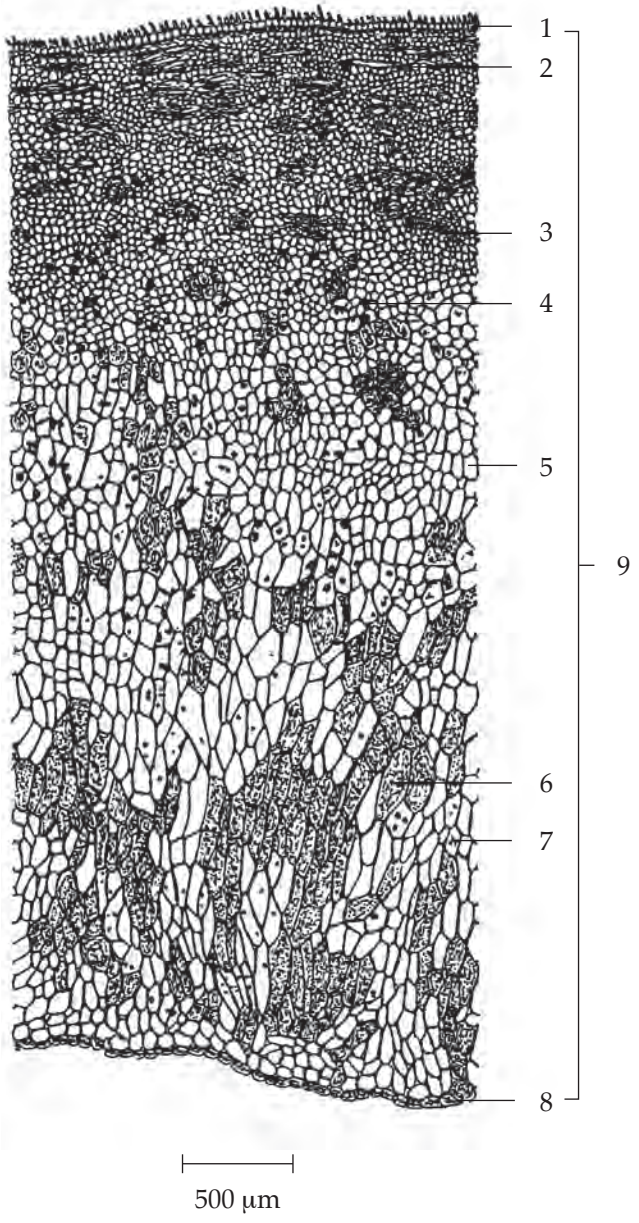


Fig. 2a Transverse Sections of the Fruit Pulp of *Terminalia bellirica* (Gaertn.) Roxb.

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

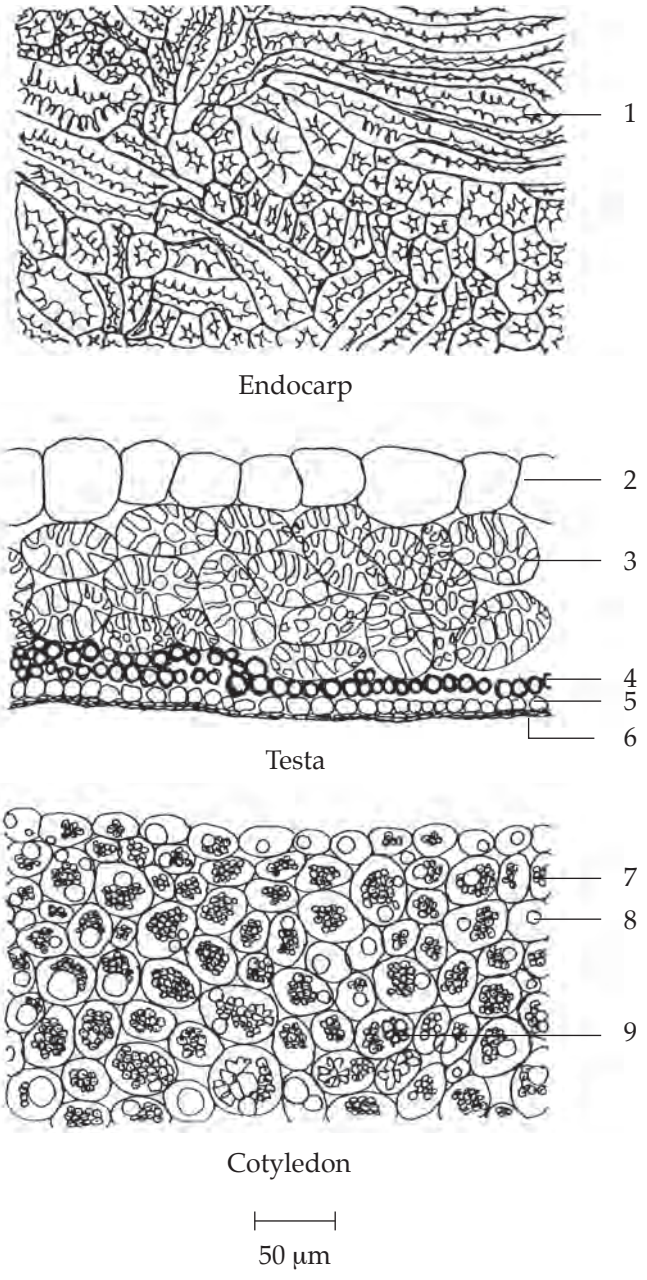


Fig. 2b Transverse Section of the Endocarp, Testa and Cotyledon of the Fruit of *Terminalia bellirica* (Gaertn.) Roxb.

1. sclereid
2. cubical cell
3. reticulate parenchyma
4. vessels
5. parenchyma
6. collapsed parenchyma
7. aleurone grains
8. oil globule
9. rosette aggregate crystals

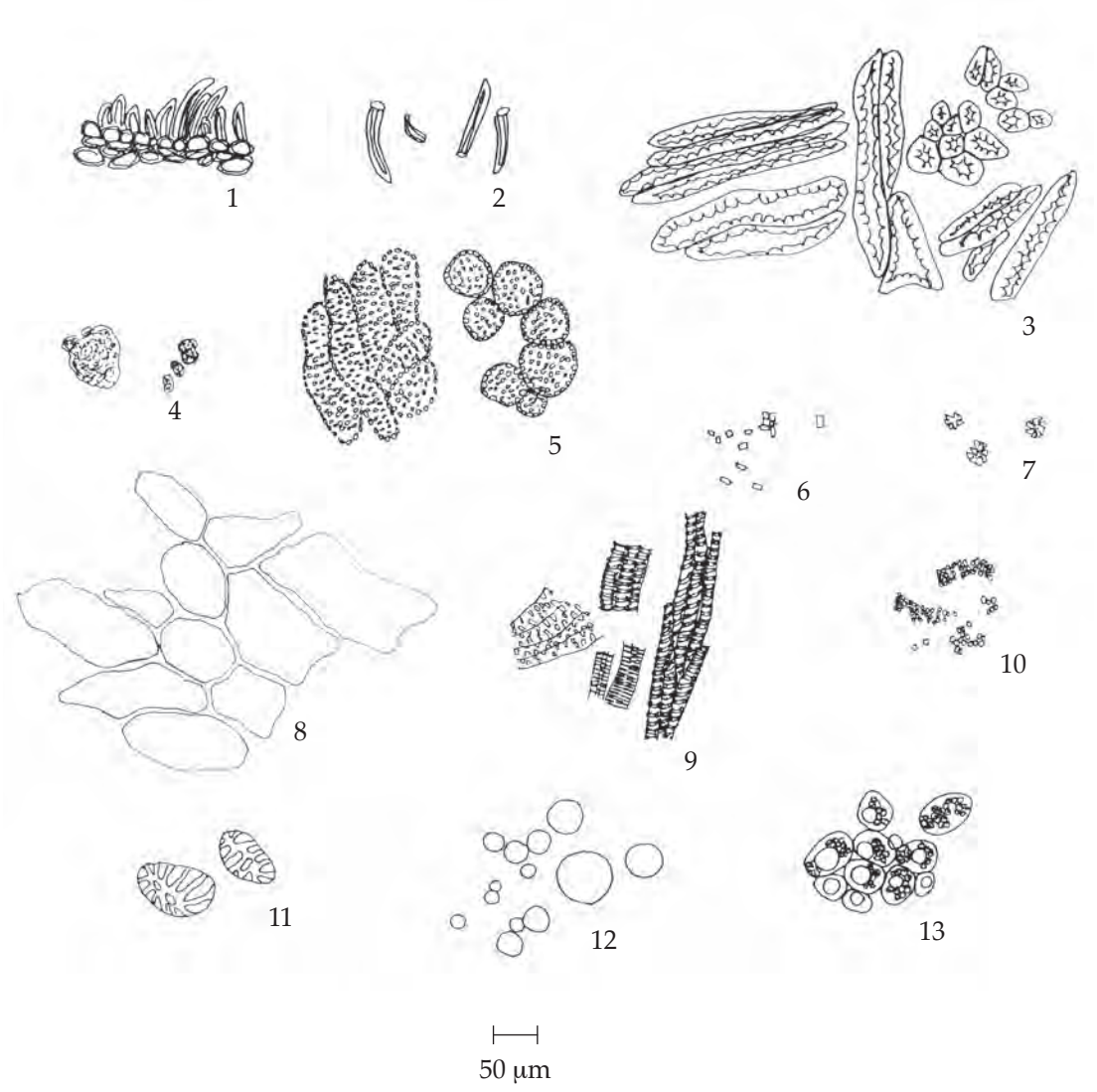


Fig. 2c Powdered Drug of the Fruits of *Terminalia bellirica* (Gaertn.) Roxb.

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 50 volumes of *toluene*, 45 volumes of *ethyl acetate* and 10 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 the filtrate 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	9–11	–	yellow	–
5	10–17	quenching	–	–
6	22–25	quenching	–	–
7	27–30	quenching	–	–
8	41–46	quenching	purple	blue
9*	47–50	quenching	purple	blue
10	52–56	quenching	–	–
11	58–62	quenching	–	blue
12	64–69	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.

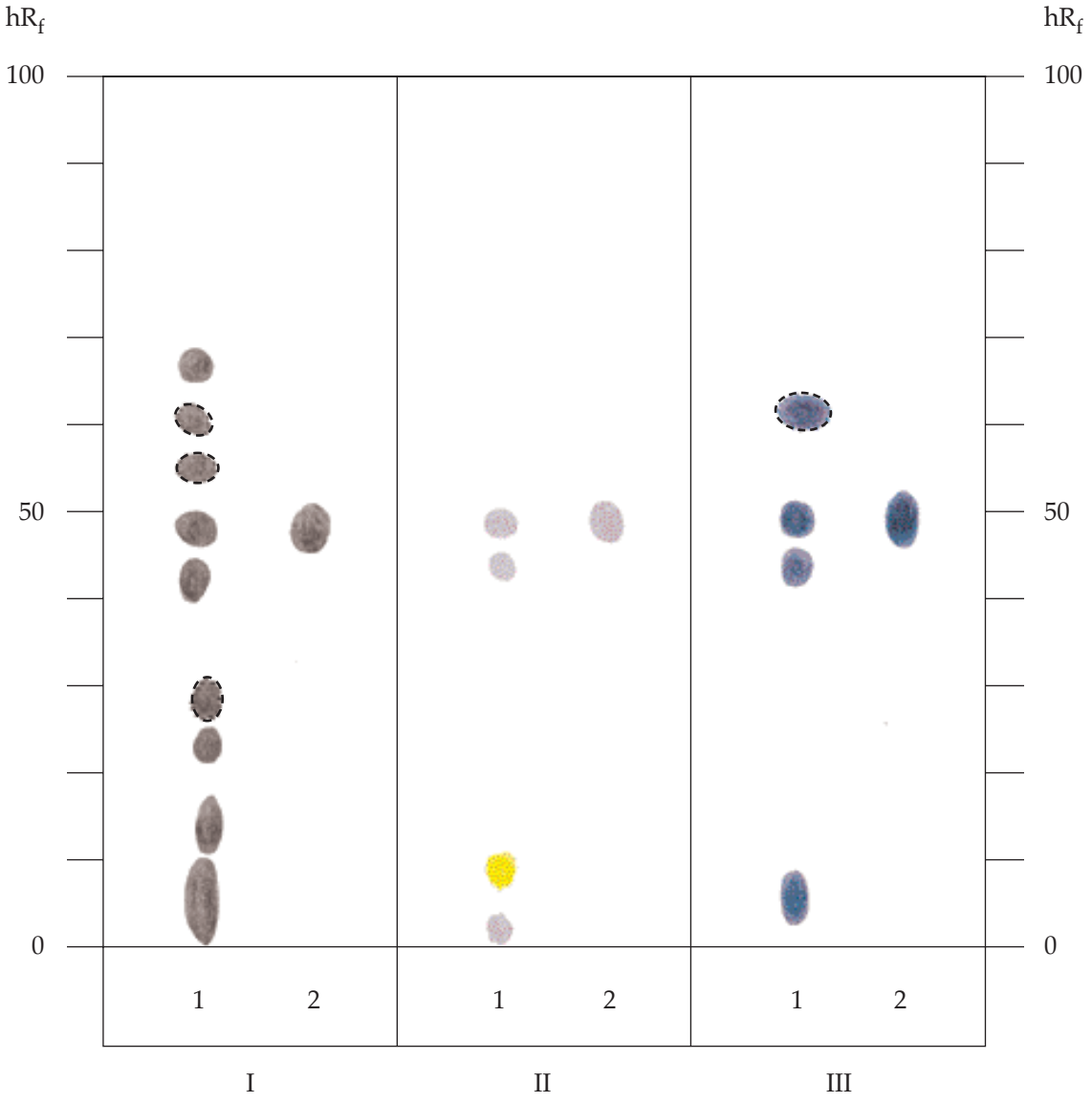


Fig. 3 Thin-Layer Chromatogram of Water Extract of the Fruits of *Terminalia bellirica* (Gaertn.) Roxb.

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 tall, 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 long, 6 to 10 cm wide, 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 panicle, usually with 3 to 6 spikes; spike 3 to 6 cm long; rachis pubescent; flower 2 mm long, 3 to 4 mm in diameter; bract nearly glabrous, 1.5 to 2 mm long; calyx outside glabrous, inside densely villous, calyx-segment triangular; stamen 3 to 4 mm long; ovary glabrous, ovoid, about 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 cm long, 1.5 to 2.5 cm wide, usually smooth or frequently 5-angulate ridged, wrinkled, turning blackish when dry. Seed 1, rough, ellipsoid, 1.5 to 2 cm long, 0.5 to 0.7 cm wide, 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, 5 to 6 longitudinal ridges, irregularly arranged wrinkles in the inter ridge. The base marked by a circular scar, about 2 mm in diameter. Seed 1, 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, a 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.

1

2

3

4

Fig. 1a *Terminalia chebula* Retz.
1. habit 2. flowering twig 3. fruiting twig 4. crude drug

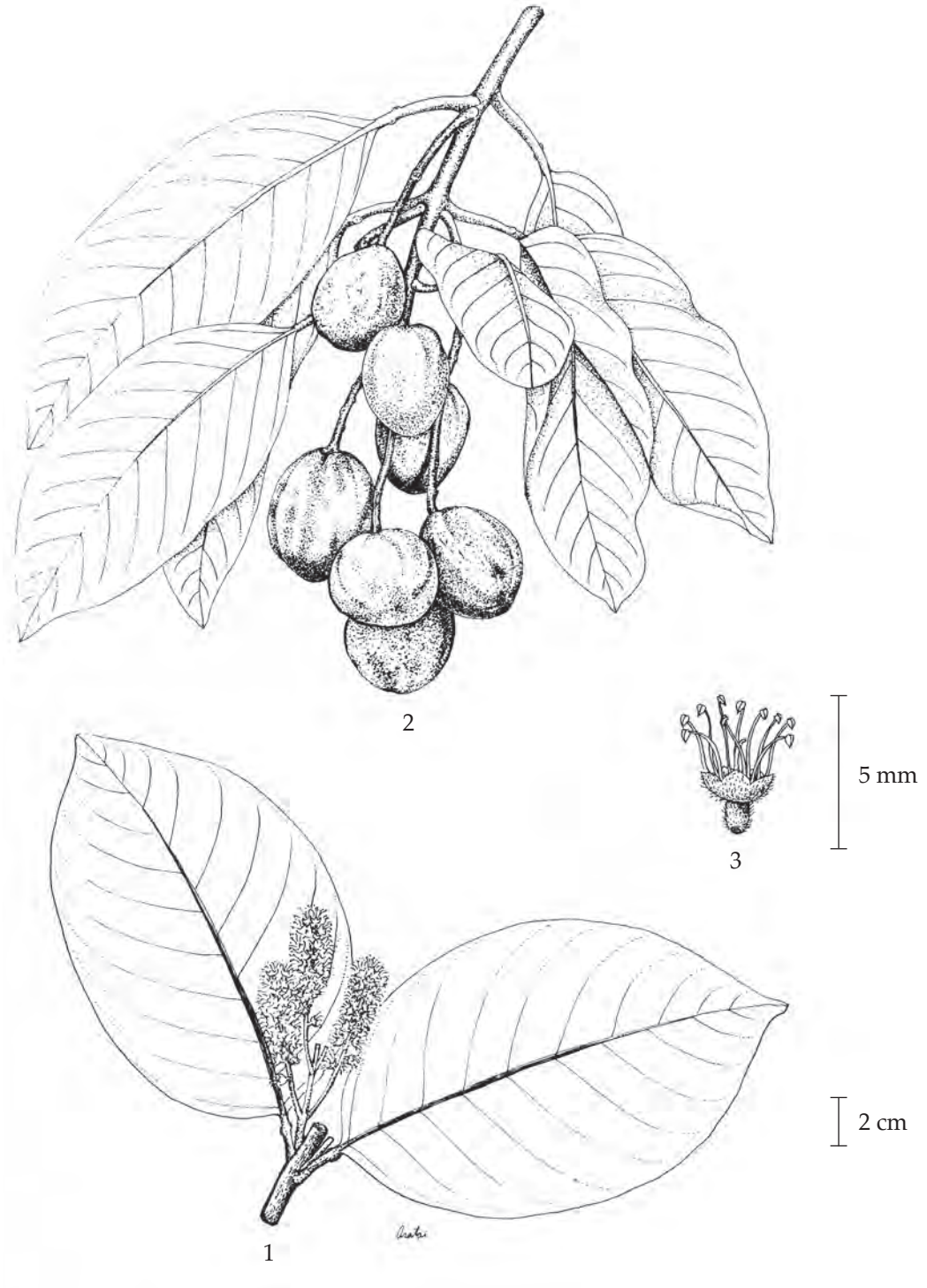


Fig. 1b *Terminalia chebula* Retz.
1. flowering twig 2. fruiting twig 3. flower

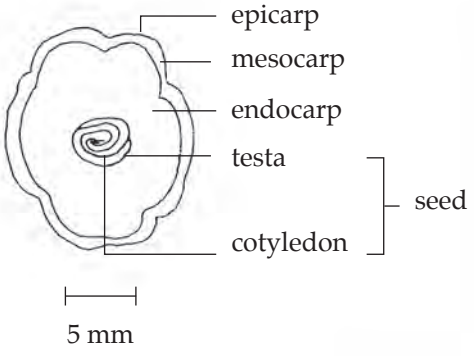


Diagram of the Fruit

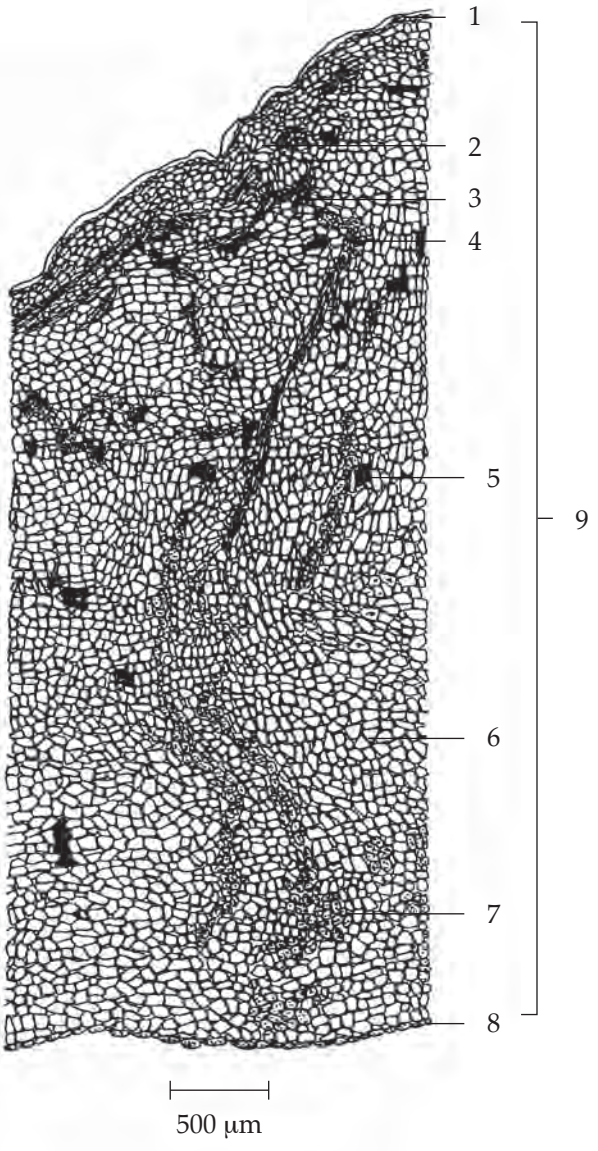


Fig. 2a Transverse Section of the Fruit Pulp of *Terminalia chebula* Retz.

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

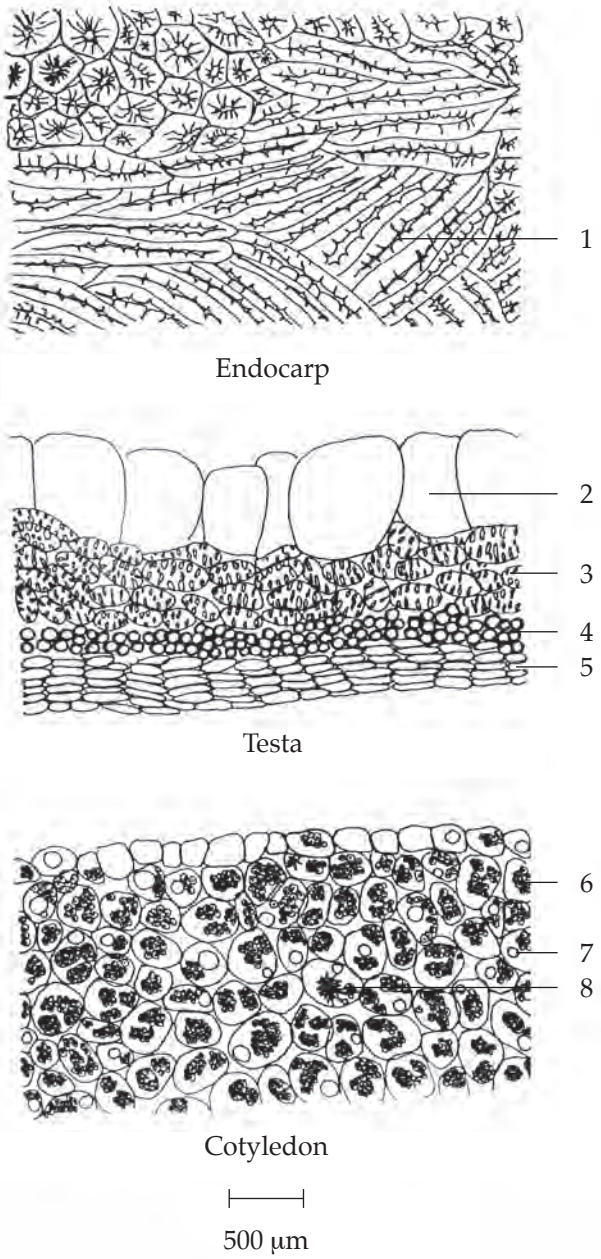


Fig. 2b Transverse Section of the Endocarp, Testa and Cotyledon of the Fruit of *Terminalia chebula* Retz.

1. sclereid
2. cubical cell
3. reticulate parenchyma
4. vessels
5. collapse parenchyma
6. aleurone grains
7. oil globule
8. rosette aggregate crystals

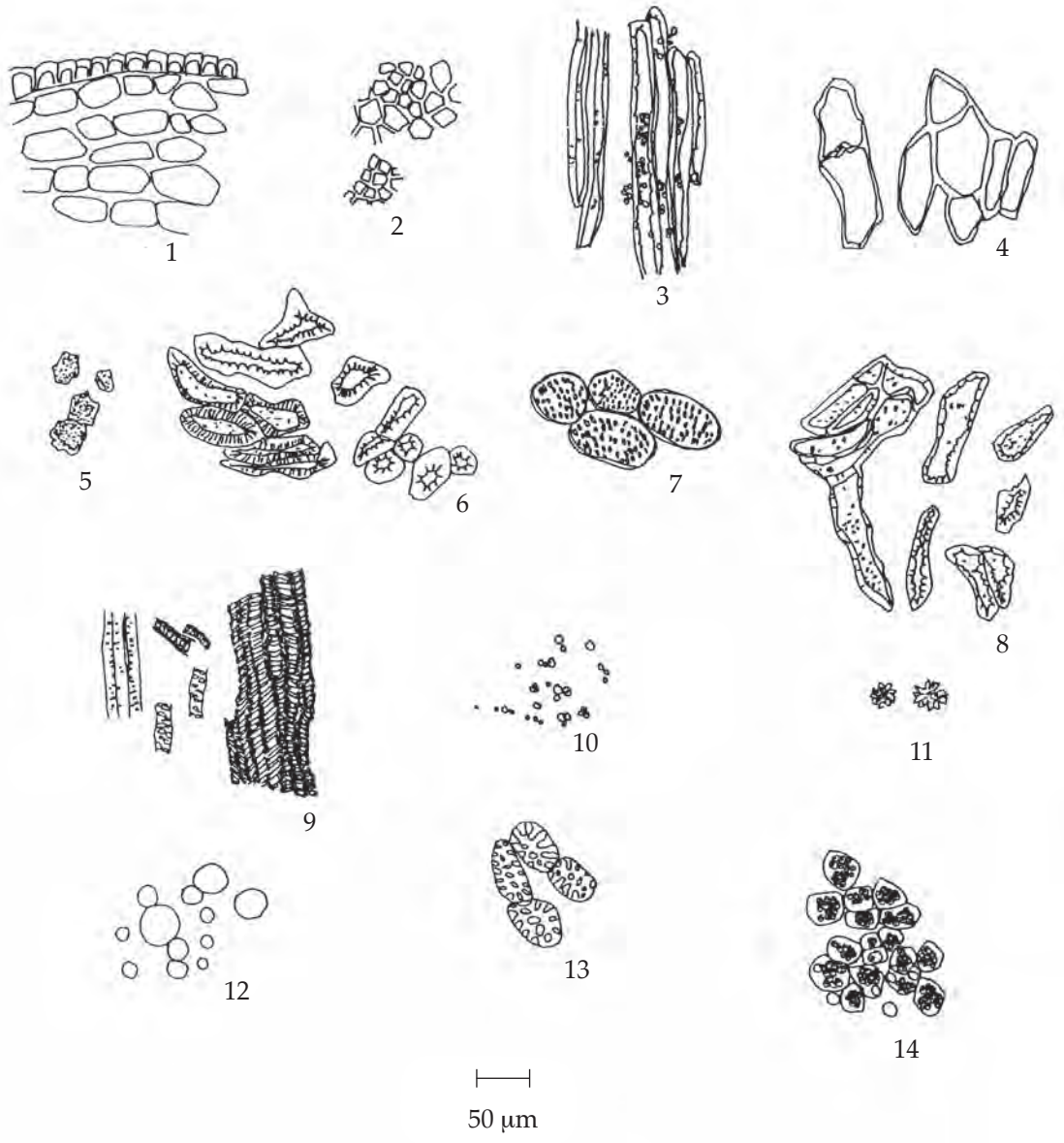


Fig. 2c Powdered Drug of the Fruits of *Terminalia chebula* Retz.

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 × 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.

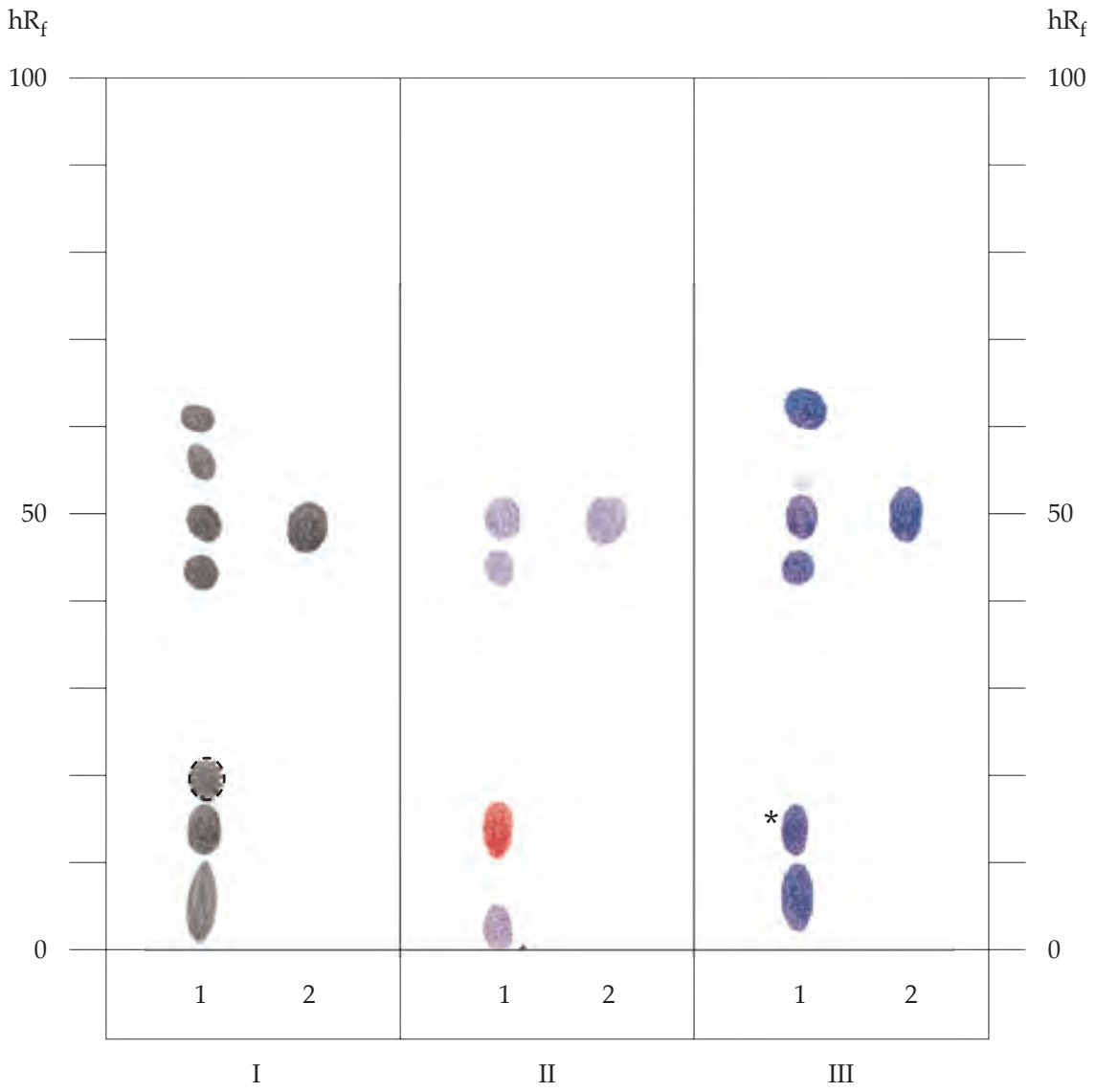


Fig. 3 Thin-Layer Chromatogram of Water Extract of the Fruits of *Terminalia chebula* Retz.

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 is 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, Crude Drug Number: DMSc 0277.

Constituents Nicker-Nut Leaf contains a bitter substance, waxy, and alcoholic substances.

Description of the plant (Figs. 1a, 1b) Climber armed with straight or recurved prickles. Stipules, pinnate, consisting of 3 to 5 leaflets, up to 2 cm long. Leaves bipinnately compound, 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 round and mucronulate apex, margin entire, base unequal; petiolule 0.8 mm long, stipels of short hooked spines. Inflorescence supra-axillary raceme, about 6 cm long, sometimes branched; flower yellow; bract linear, 6 to 12 mm long, recurved, lately caducous; pedicel 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, filament hairy; ovary about 1 mm in diameter, stalked, hairy and bristly. Pod 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 (Fig. 1a) 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, 2 to 4 cm long, 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 irregular-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 epidermises.

In surface view, epidermises 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. 1a *Caesalpinia bonduc* (L.) Roxb.
1. habit 2. inflorescence (a), flower (b) 3. fruiting twig
4. fruits and seeds 5. crude drug

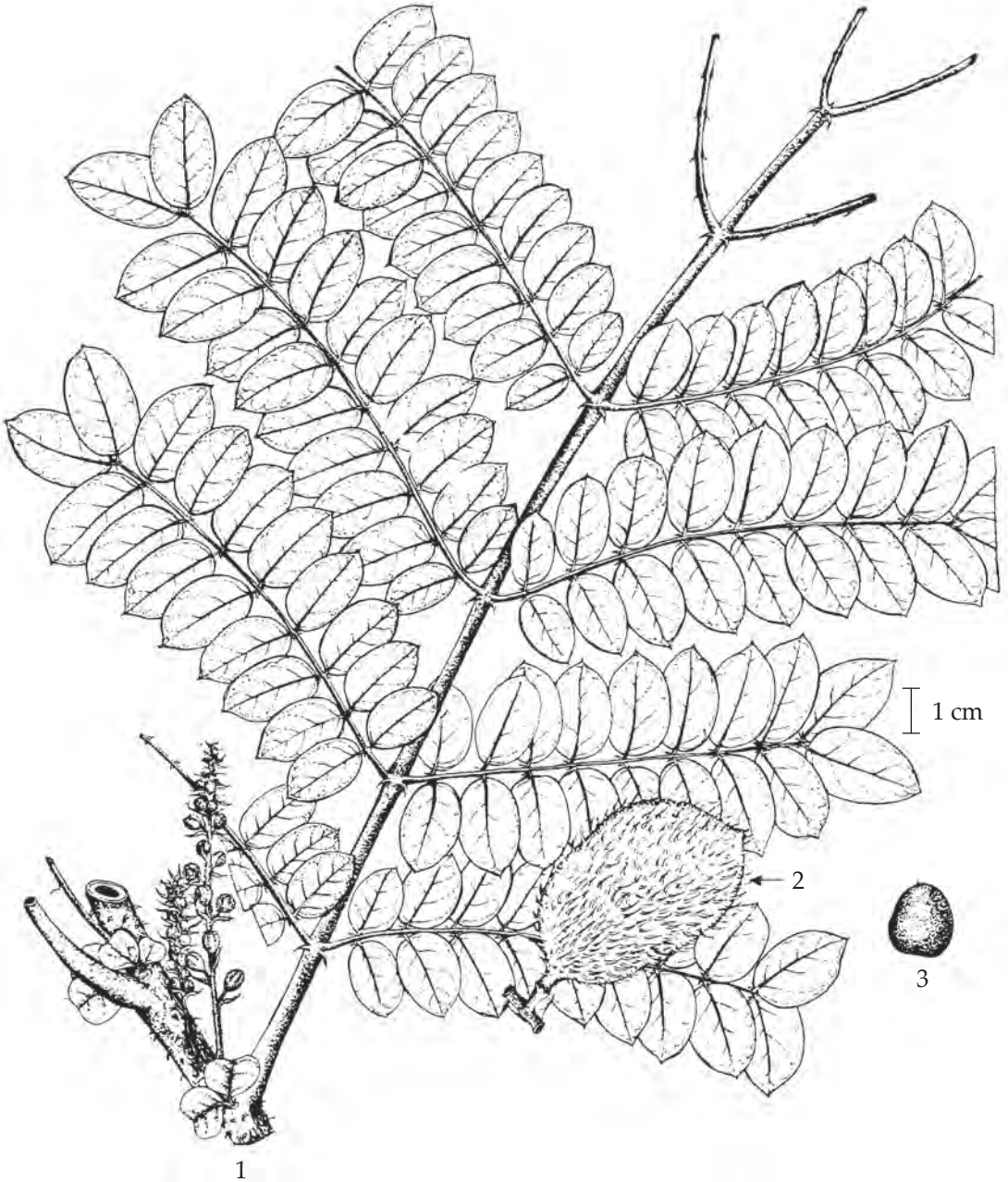


Fig. 1b *Caesalpinia bonduc* (L.) Roxb.
1. twig with inflorescence 2. pod 3. seed

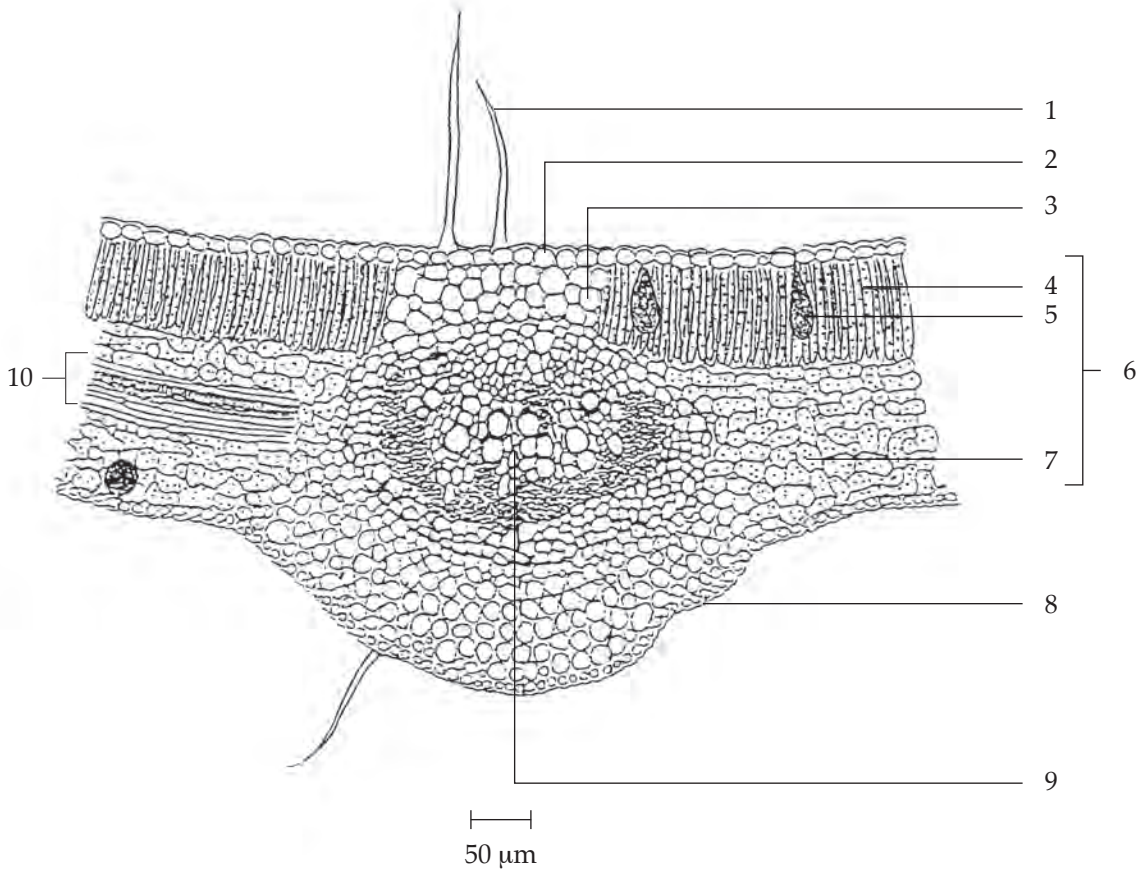


Fig. 2a Transverse Section of the Leaflet of *Caesalpinia bonduc* (L.) Roxb.

1. unicellular trichome
2. upper epidermis
3. collenchyma
4. palisade cell
5. oil cells
6. mesophyll
7. spongy cells
8. lower epidermis
9. vascular bundle in sectional view
10. vascular bundle in longitudinal view

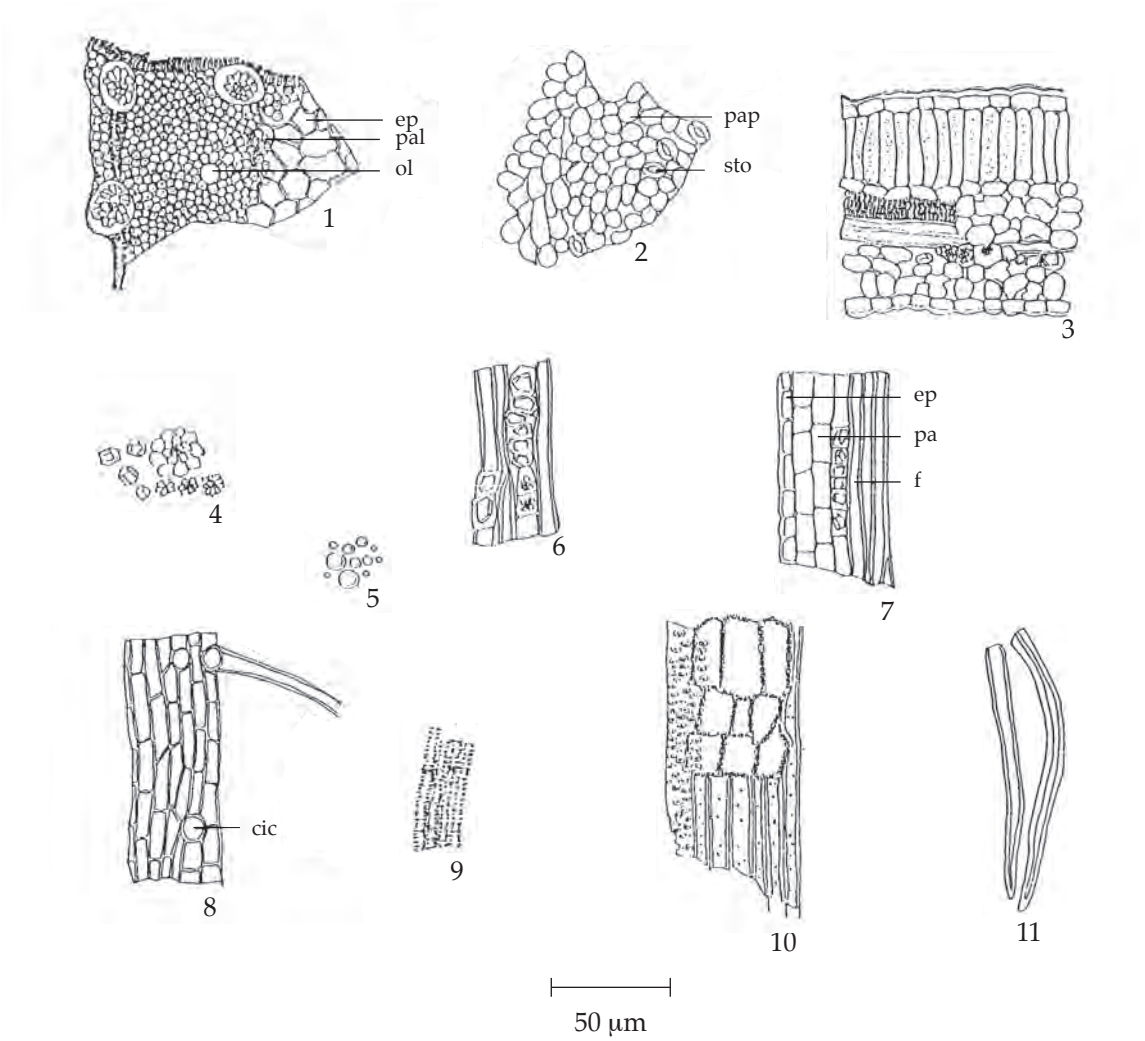


Fig. 2b Powdered Drug of the Leaflets of *Caesalpinia bonduc* (L.) Roxb.

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).

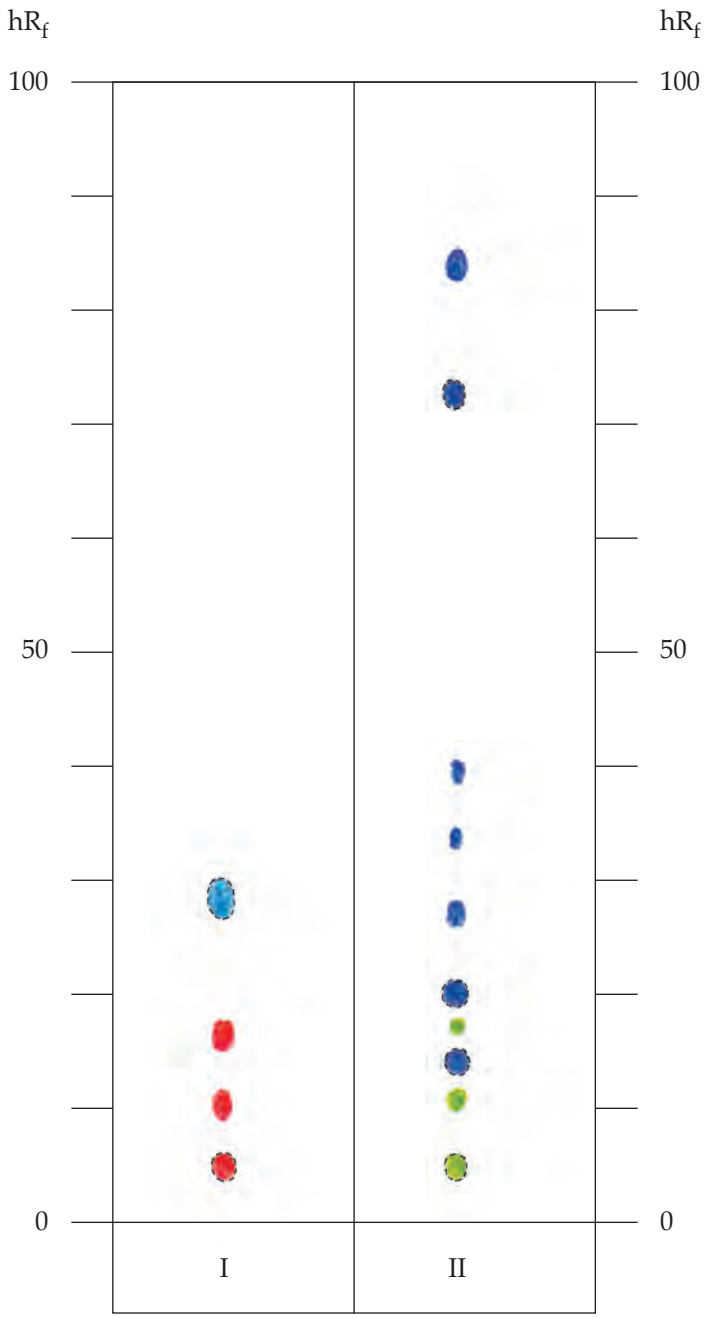


Fig. 3 Thin-Layer Chromatogram of Methanolic Extract of the Leaflets of *Caesalpinia bonduc* (L.) Roxb.

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

ตานหม่อน (TANMON)

Tarlmouniae Ellipticae 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, Crude Drug Number: DMSc 0558.

Constituents Tarlmounia Elliptica Leaf contains glaucolides A and B and their acetates, along with lupeol, taraxasterol, sitosterol, and stigmasterol.

Description of the plant (Figs. 1a, 1b) Scandent shrub; branch 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 the 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, anther sagittate at base; style divided into 2 branches. Fruit achene, terete, about 2 mm long, about 1 mm wide, terete, compressed, longitudinally ribbed, covered with light brown translucent glands, crowned by the pappus, 4 to 8 mm long, base pointed.

Description

Macroscopical (Fig. 1a) 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- to 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, epidermises 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.

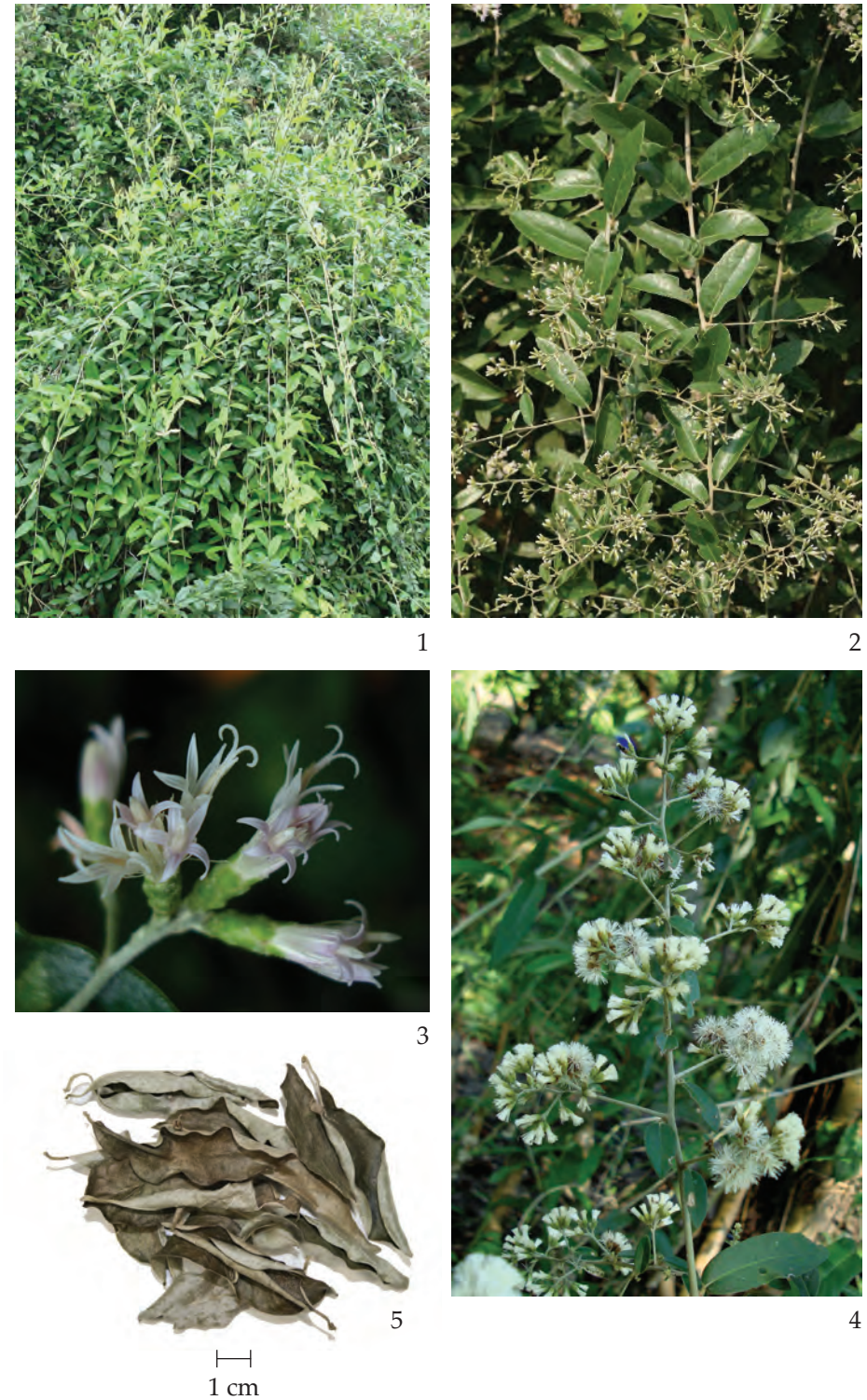


Fig. 1a *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan
1. habit 2. flowering twigs 3. flowering heads 4. fruiting twigs 5. crude drug

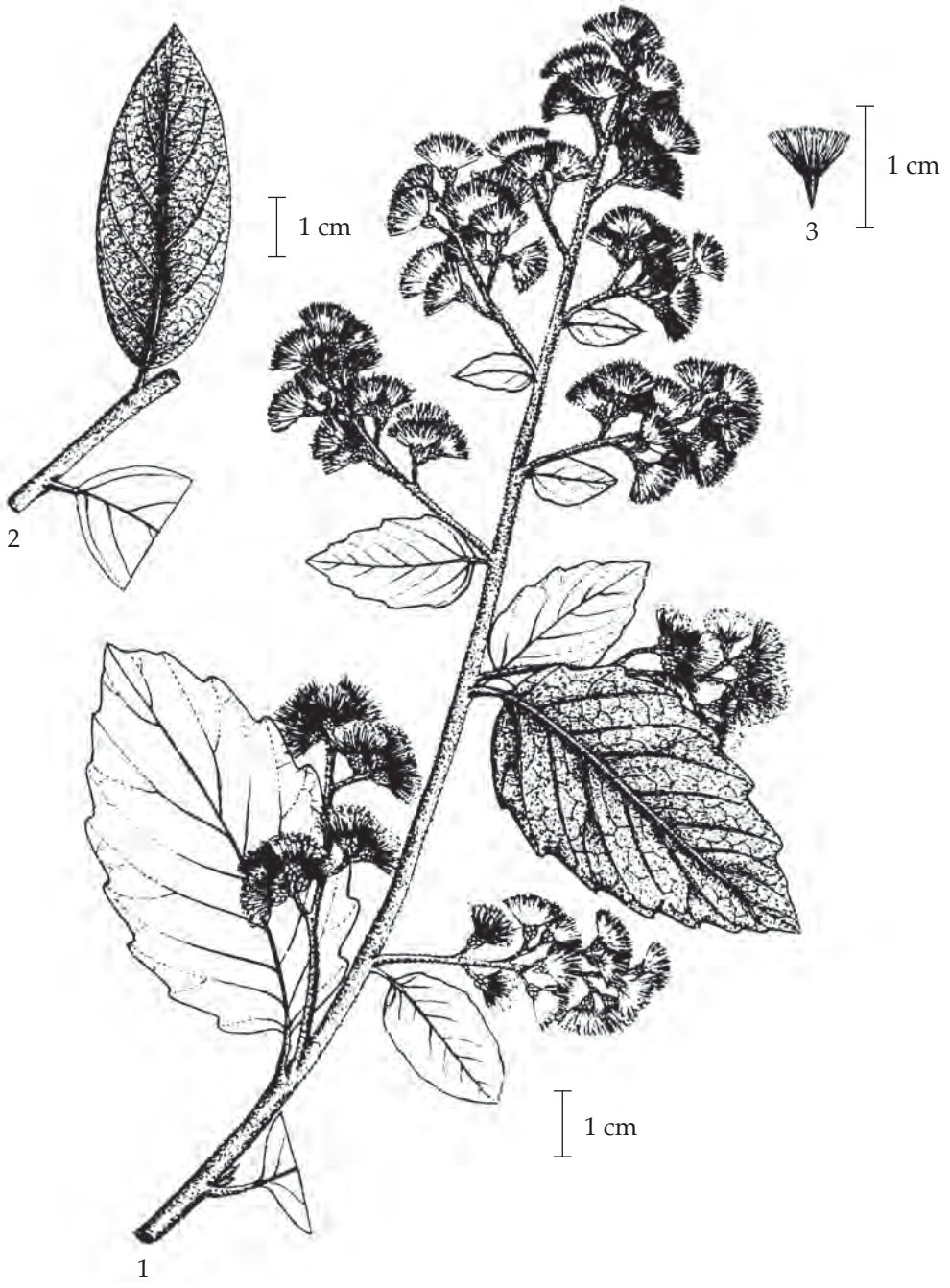


Fig. 1b *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan
1. fruiting twig 2. leaf 3. fruit

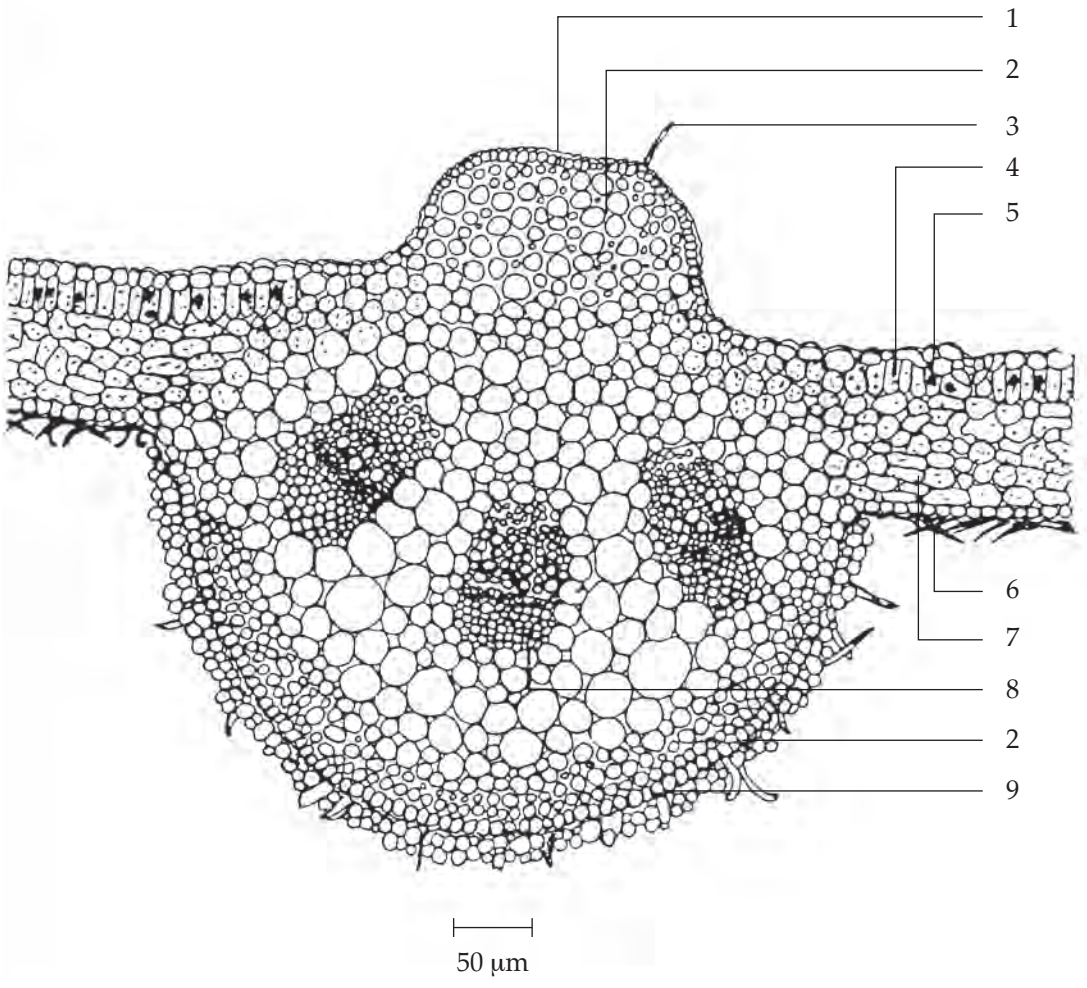


Fig. 2a Transverse Section of the Leaf of *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan

1. upper epidermis
2. collenchyma
3. uniseriate multicellular trichome
4. palisade cell
5. rosette aggregate crystal of calcium oxalate
6. T-shaped trichome
7. spongy cells
8. vascular bundle
9. lower epidermis

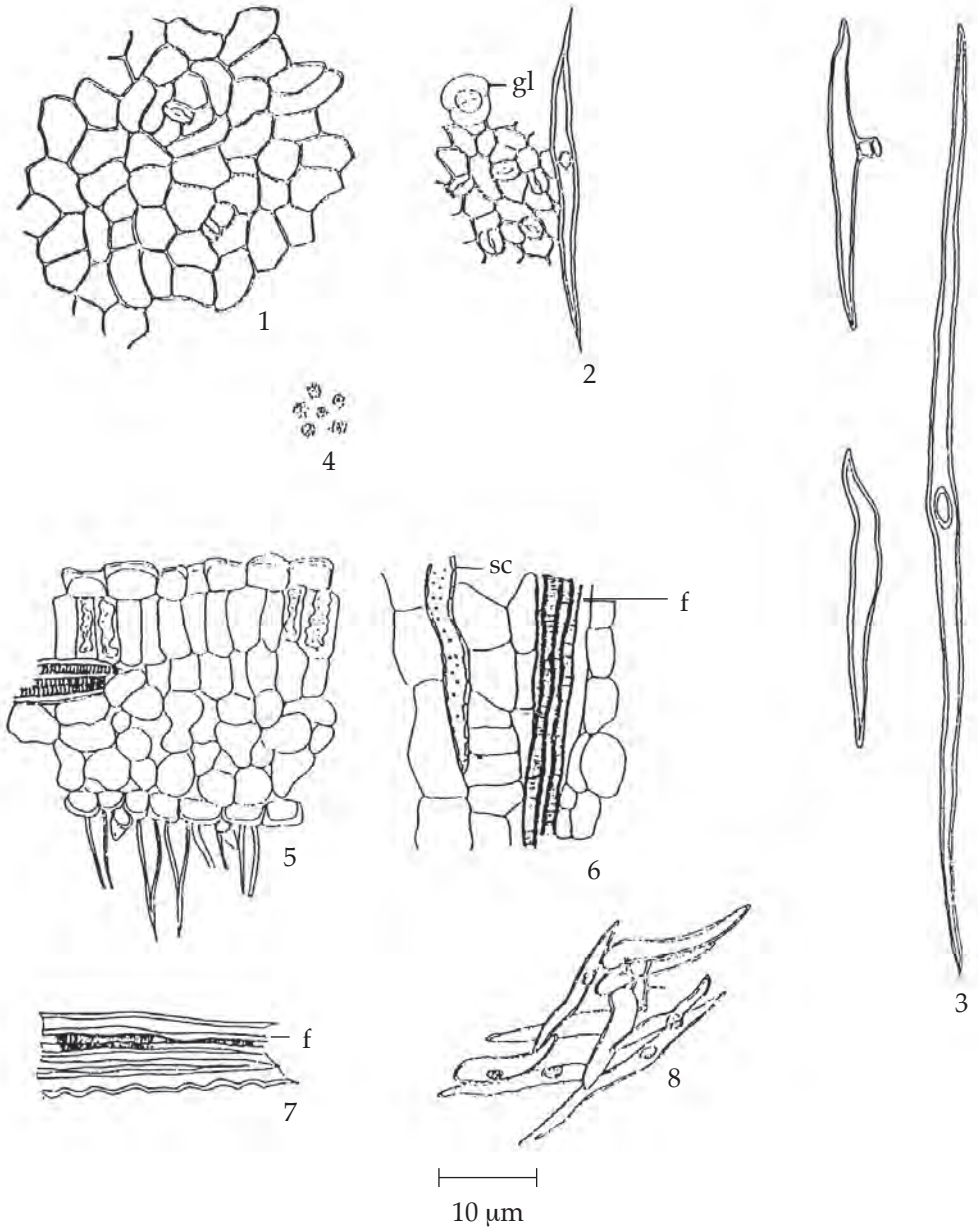


Fig. 2b Powdered Drug of the Leaves of *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan

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 the leaves of *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan

Spot	hR_f Value	Detection
		1 Per Cent W/V Solution of *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).

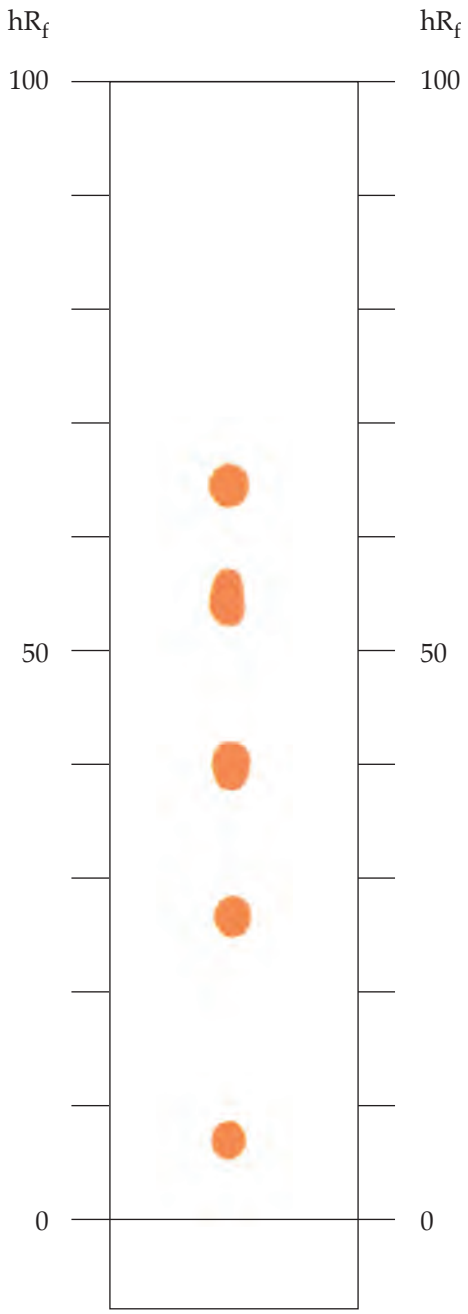


Fig. 3 Thin-Layer Chromatogram of Methanolic Extract of the Leaves of *Tarlmounia elliptica* (DC.) H. Rob., S. C. Keeley, Skvaria & R. Chan, Detected by Spraying with a 1 Per Cent W/V Solution of *Diphenylboric Acid β-aminoethyl Ester* in *Methanol* and Examining under UV light (366 nm)

เถาวัลย์เปรียง (THAOWAN PRIANG)

เครือเขาหนัง (KHRUEA KHAO NANG), เถาตาปลา (THAO TA PLA)
Solorii 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; branch 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; pedicel 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, ovules (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

1 cm

5

Fig. 1a *Solori scandens* (Roxb.) Sirich. & Adema
1. habit 2. inflorescences 3. flower 4. flowering and fruiting branches 5. crude drug

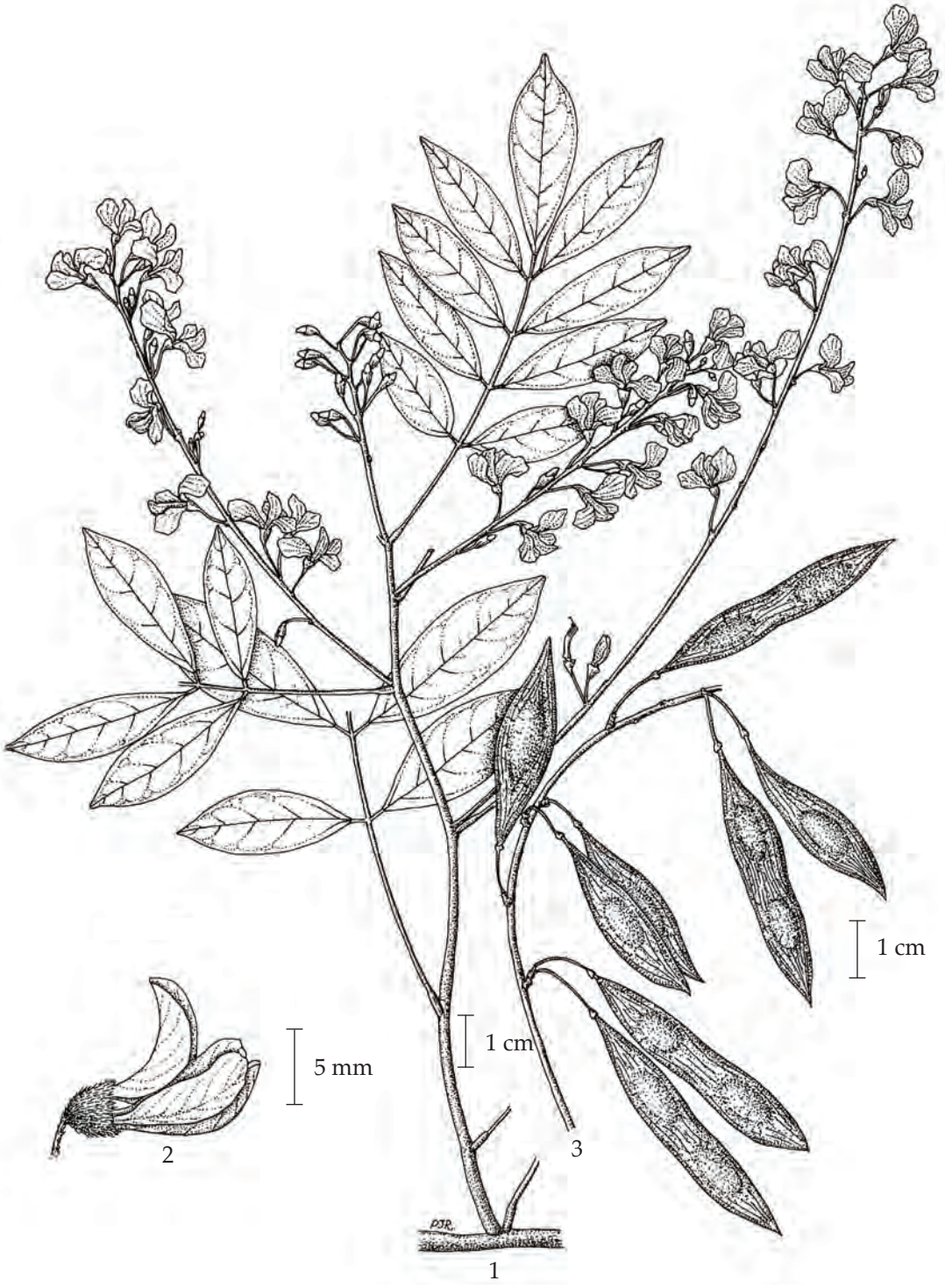


Fig. 1b *Solori scandens* (Roxb.) Sirich. & Adema
1. flowering twig 2. flower 3. fruits

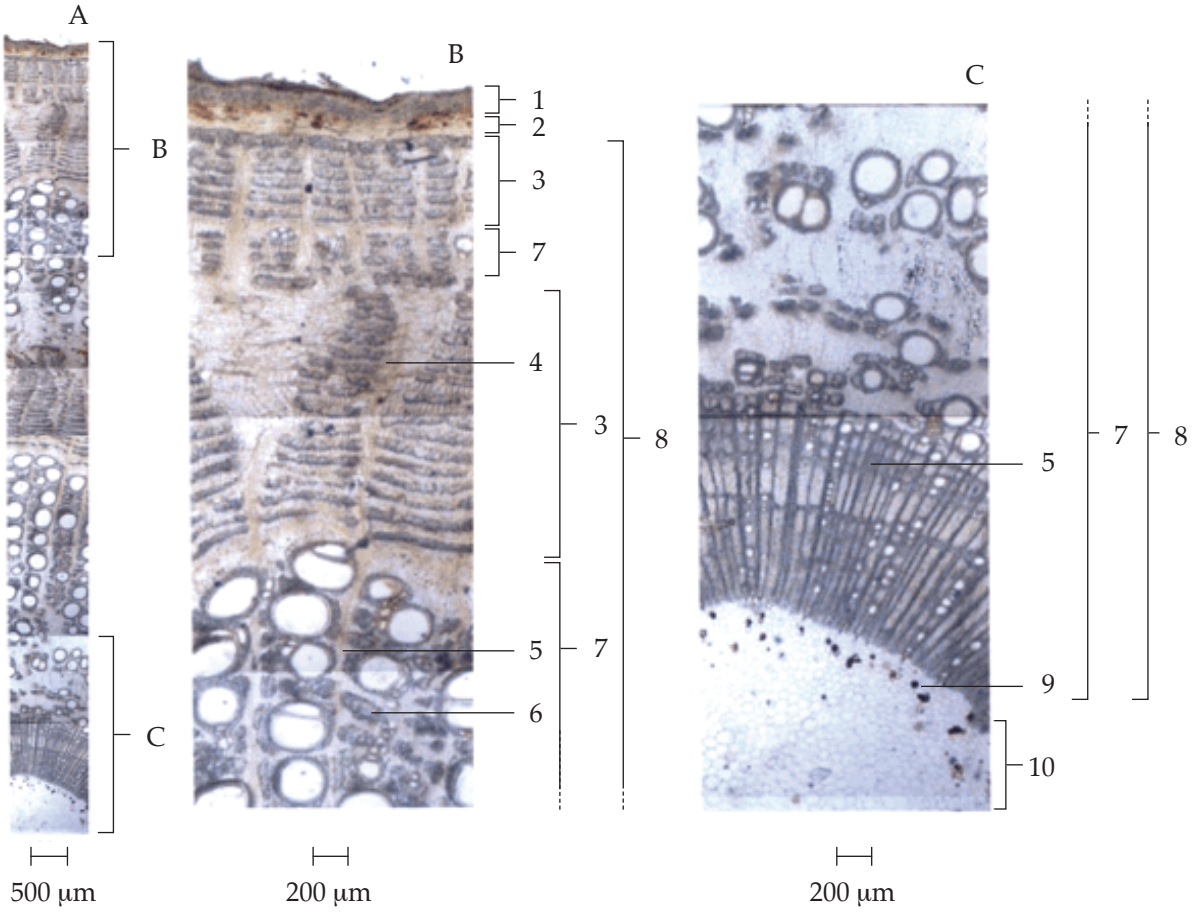


Fig. 2a Photomicrographs of Transverse Sections of the Stem of *Solori scandens* (Roxb.) Sirich. & Adema

A. Overview of the Stem
B. Outermost Part
C. Innermost Part

1. cork
2. cortex
3. phloem
4. phloem ray
5. xylem ray
6. xylem fibre
7. xylem
8. vascular tissue
9. reddish to brownish substances
10. pith

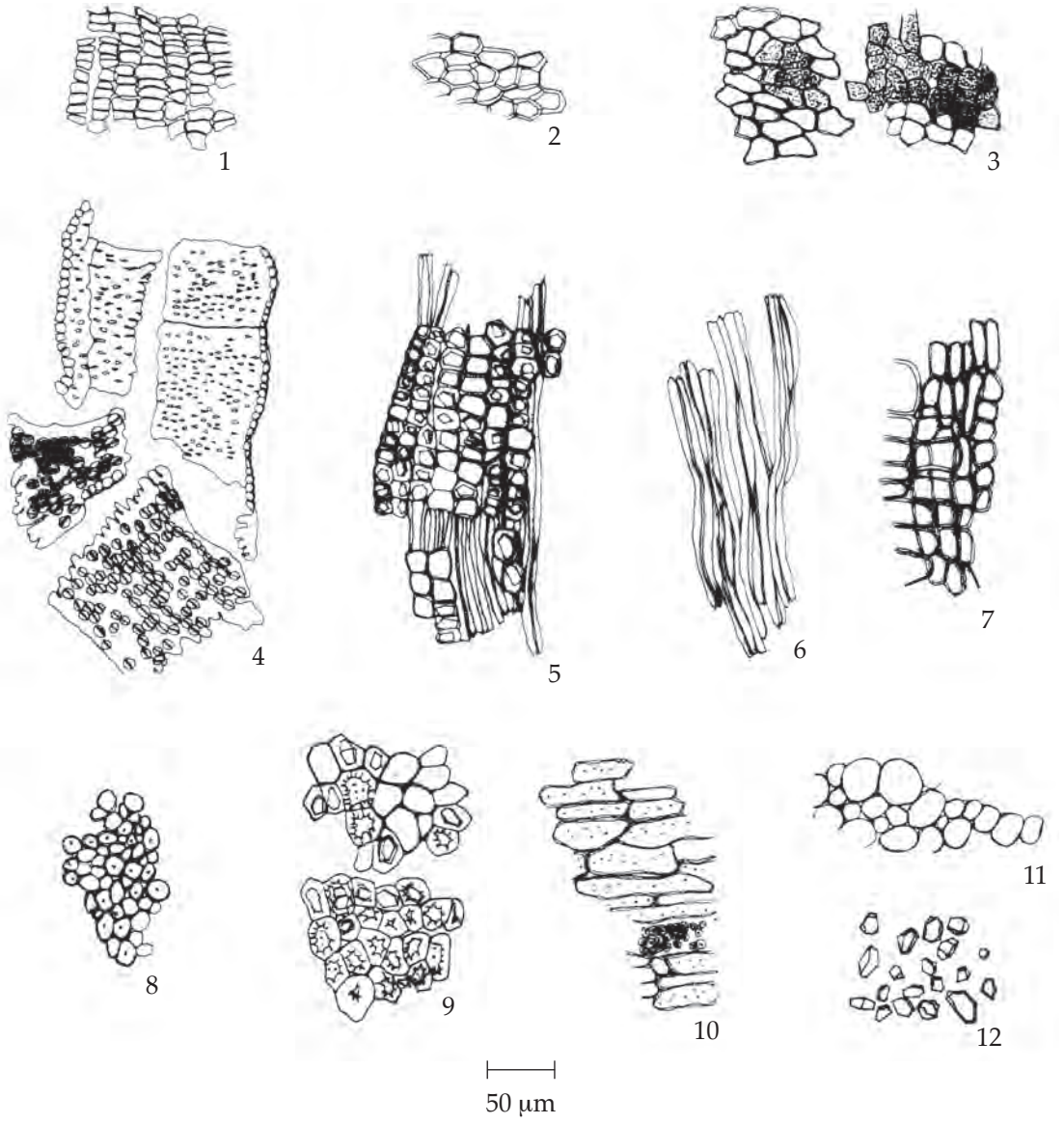


Fig. 2b Powdered Drug of the Stems of *Solori scandens* (Roxb.) Sirich. & Adema

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 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.

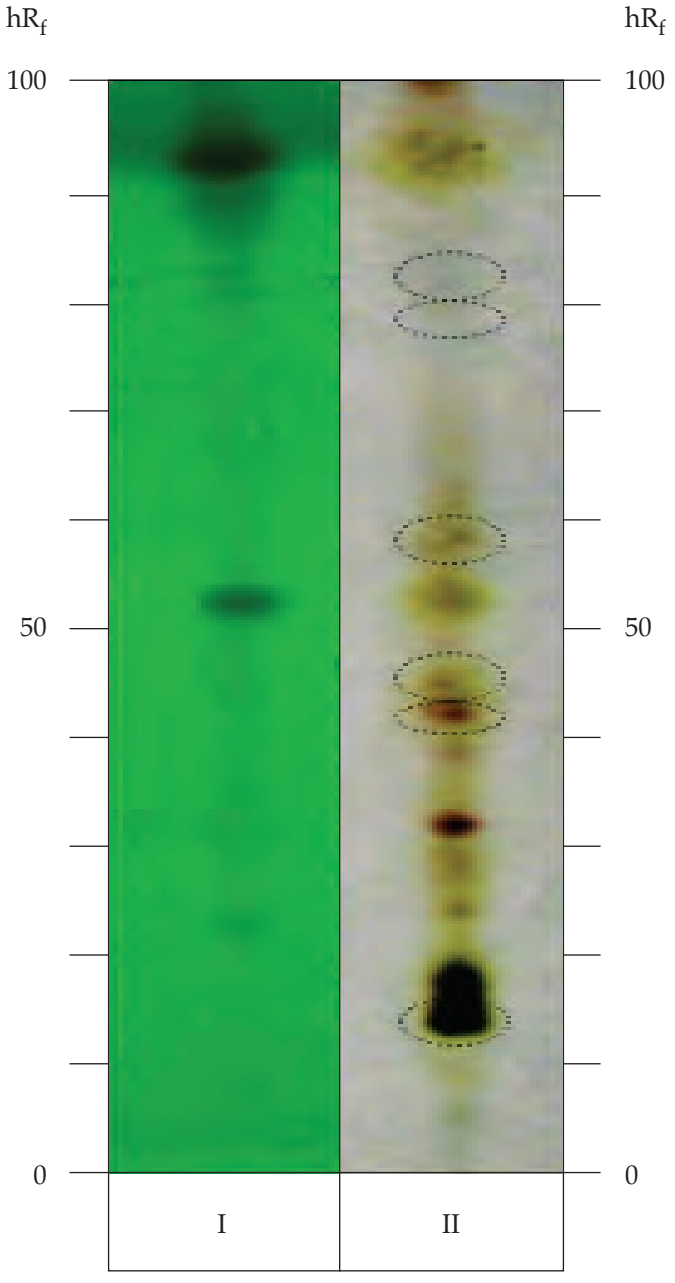


Fig. 3 Thin-Layer Chromatogram of Ethanolic Extract of the Stems of *Solori scandens* (Roxb.) Sirich. & Adema

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 (1) the equivalent amount of genistein-7-*O*-[*α*-rhamnopyranosyl-(1→6)-*β*-glucopyranoside], calculated as genistein; (2) the expiration date.

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 "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 × 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).

ยาแคปซูลสารสกัดแห้งเถาวัลย์เปรียง (THAOWAN PRIANG DRY EXTRACT CAPSULES)

Hog Creeper Vine Dry Extract Capsules

Category Analgesic, anti-inflammatory.

Hog Creeper Vine Dry Extract 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 Lower 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 Dry Extract 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) the 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 "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 tall; 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. Flower 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. Fruit 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. Seed 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.

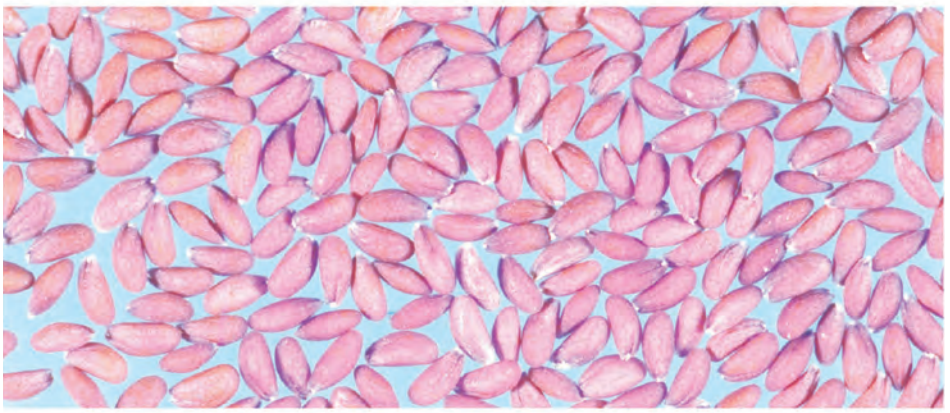
Additional information It is commonly used with other herbal drugs in Thai traditional herbal preparations.

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

1

2

3



4

1 cm

Fig. 1a *Lepidium sativum* L.
1. habit 2. flowers 3. fruits 4. crude drug

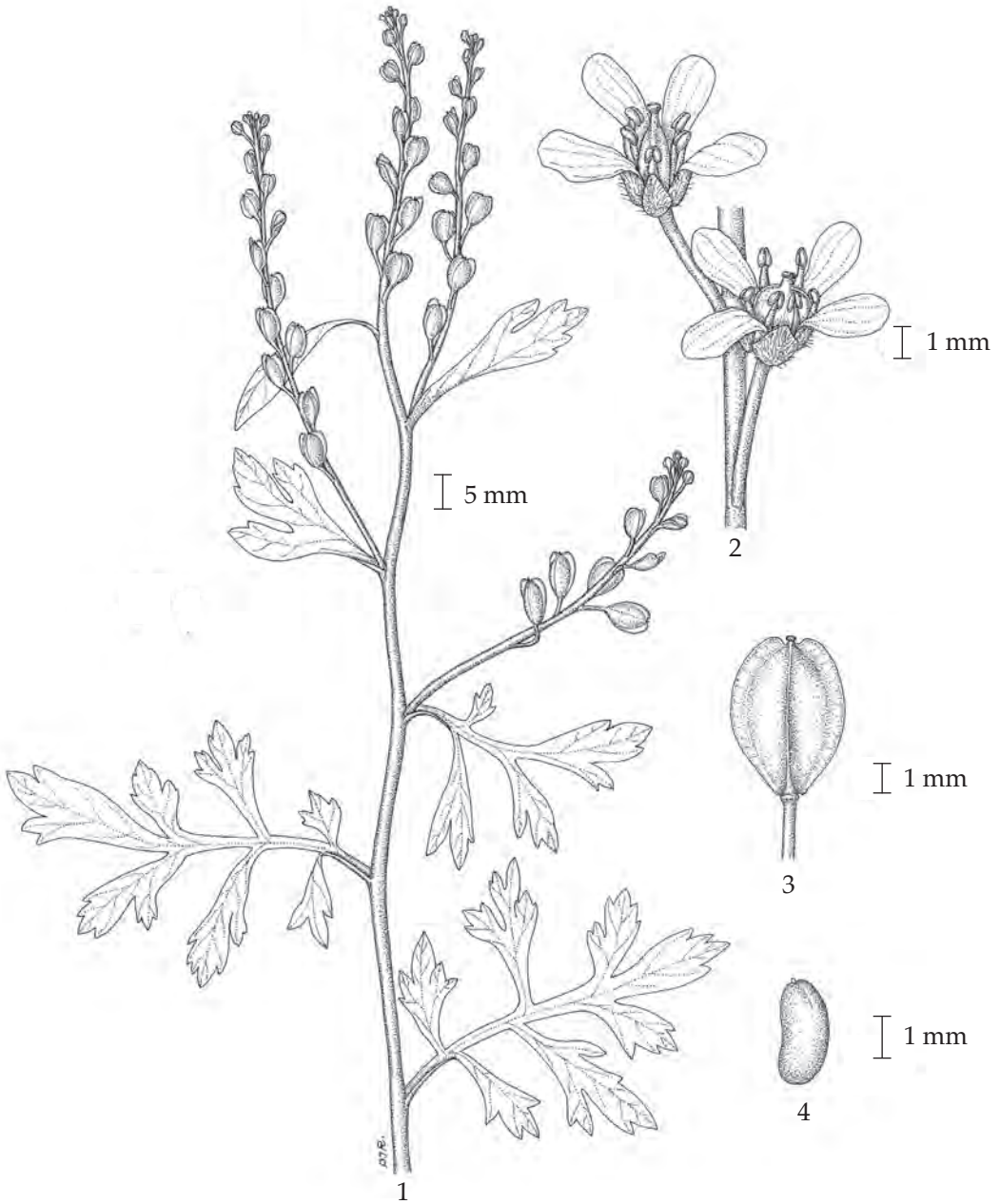


Fig. 1b *Lepidium sativum* L.
1. fruiting top 2. flowers 3. fruit 4. seed

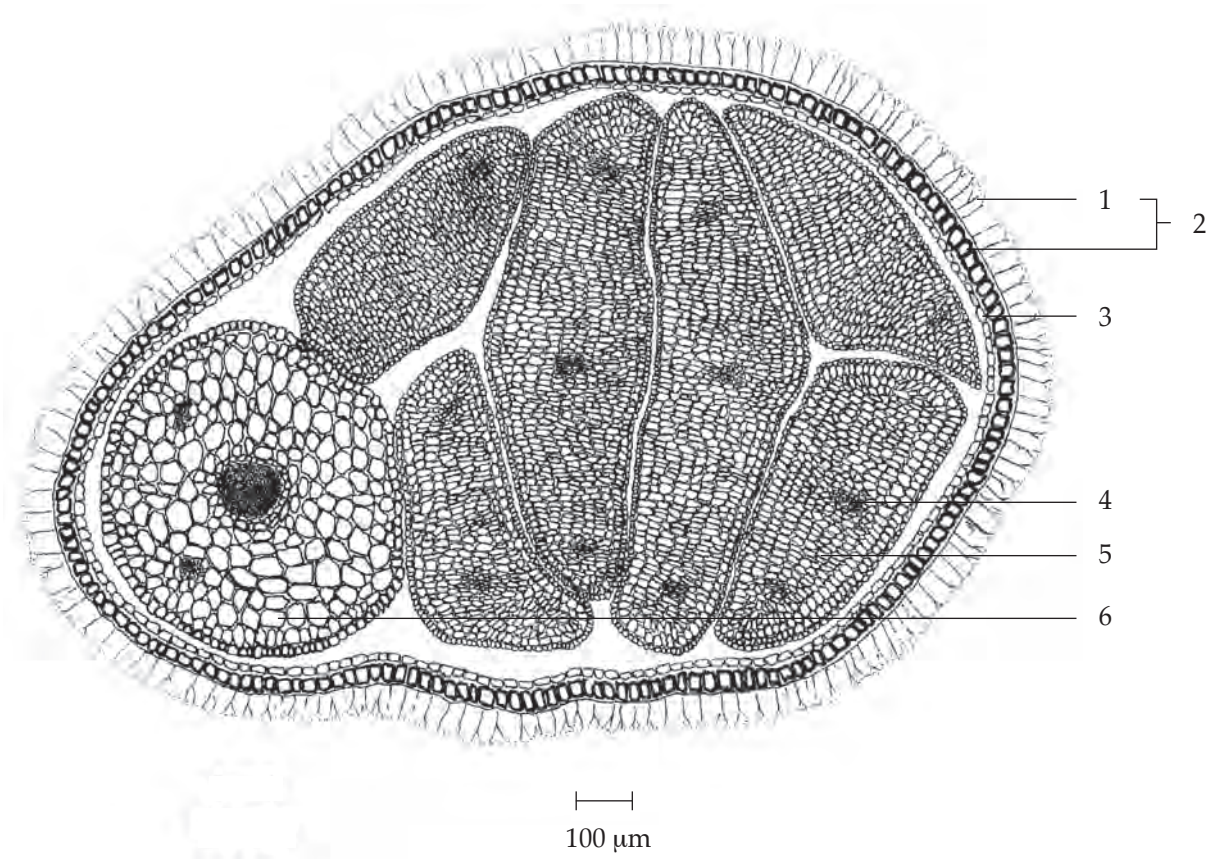


Fig. 2a Transverse Section of the Seed of *Lepidium sativum* L.
1. mucilage layer
2. outer integument
3. inner integument
4. vascular bundle
5. cotyledon
6. part of an embryo

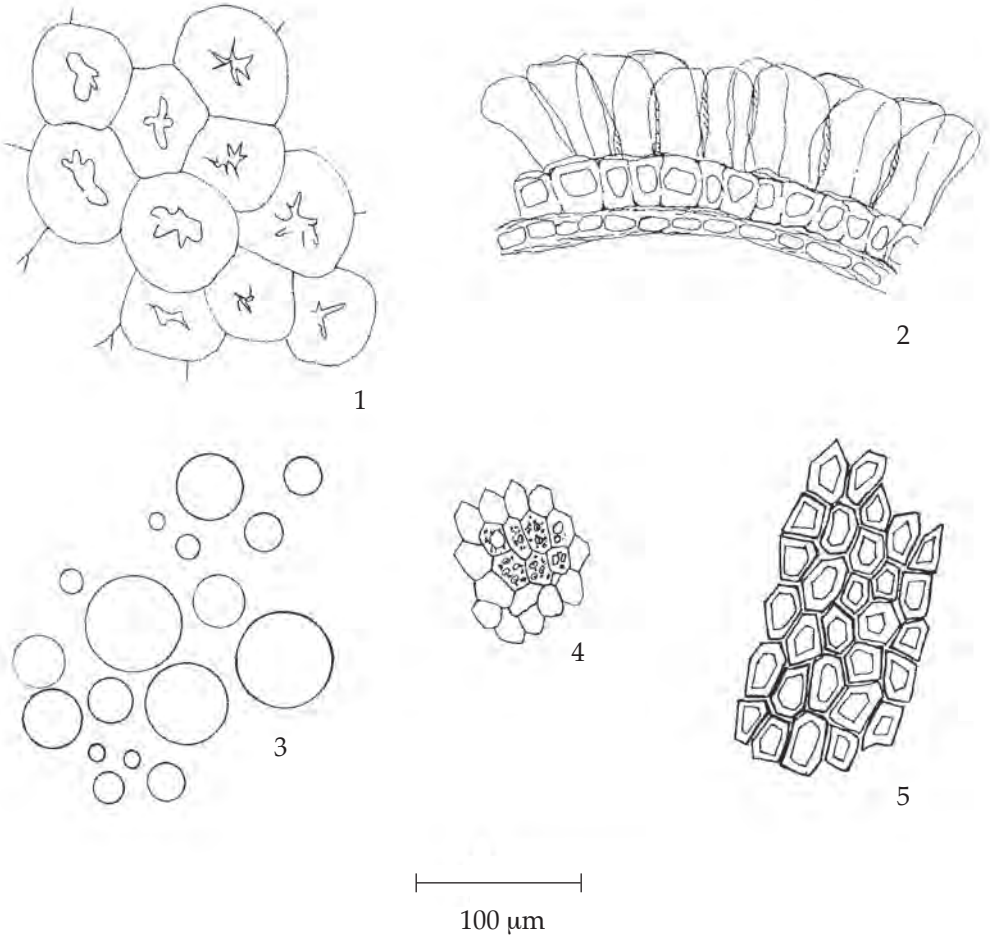


Fig. 2b Powdered Drug of the Seeds of *Lepidium sativum* L.
1. mucilage from outer surface
2. outer integument with protruding mucilage and associated inner integument
3. agglomerated oil globules
4. parenchyma of cotyledon containing oil globules and small aleurone grains
5. outer integument in surface 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 90 volumes of *chloroform* and 10 volumes 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.

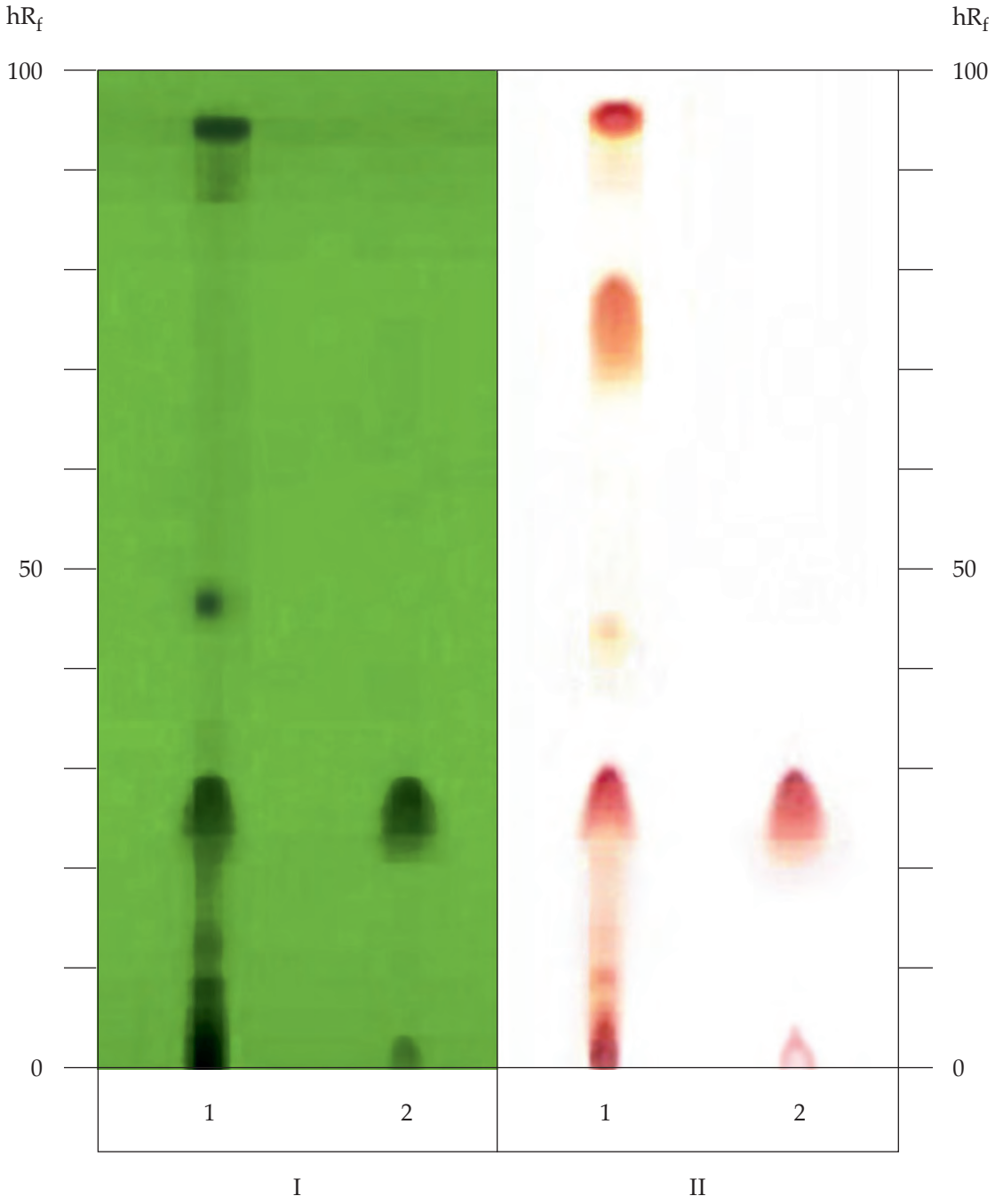


Fig. 3 Thin-Layer Chromatogram of the Extract of the Seeds of *Lepidium sativum* L.
1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with iodine vapour

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. 1) Annual herb 30 to 60 cm tall, branched 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 irregularly lobed, lower leaves small, petiolated, 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, pedicel 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; filament 2.5 to 5.2 mm long, slender; anther 1.5 to 2 mm long; ovary superior, about 5 mm long, smooth, carpels 2 to 4, style and stigma about 7 mm long. Fruit united follicles forming a capsule, ultimately inflated with persistent horn-like styles. Seeds numerous, ovate to lanceolate, trigonal, black.

Description Odourless; taste, bitter, spicy and aromatic.

Macroscopical (Fig. 1) 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 (Figs. 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.

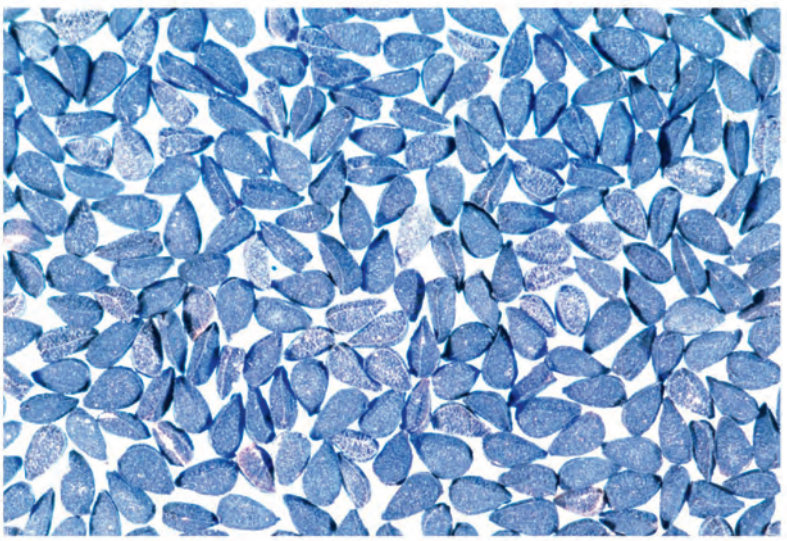
Black Cumin in powder possesses the diagnostic microscopical characters of the unground drug; in surface view, the inner epidermis shows striated wall.

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

1

2

3



1 cm

4

Fig. 1 *Nigella sativa* L.
1. flowering plant 2. flower 3. fruit 4. crude drug

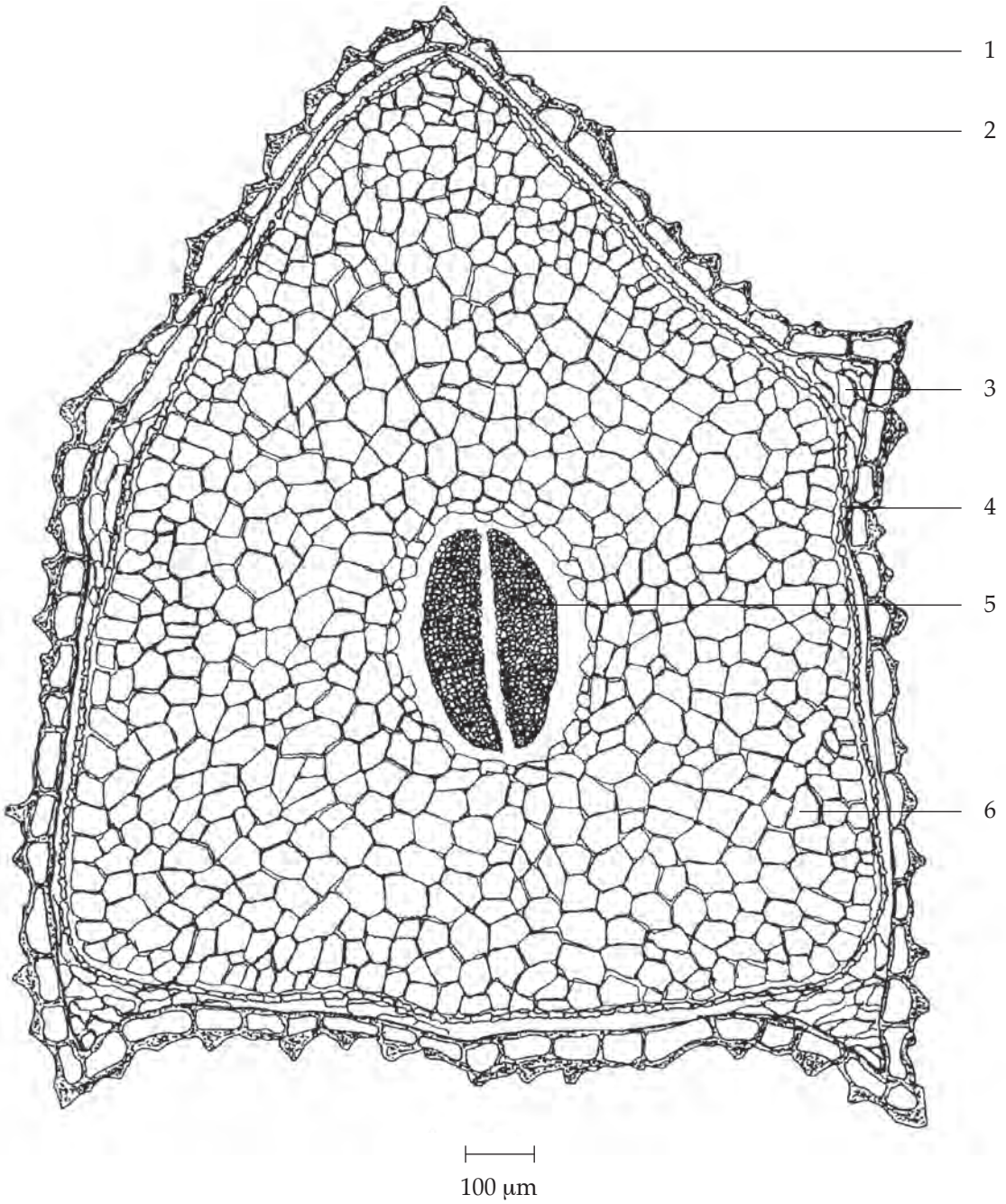


Fig. 2a Transverse Section of the Seed of *Nigella sativa* L.
1. epidermis with irregular papillae
2. papilla
3. layer of collapsed cells
4. inner layer of seed coat
5. cotyledon
6. endosperm

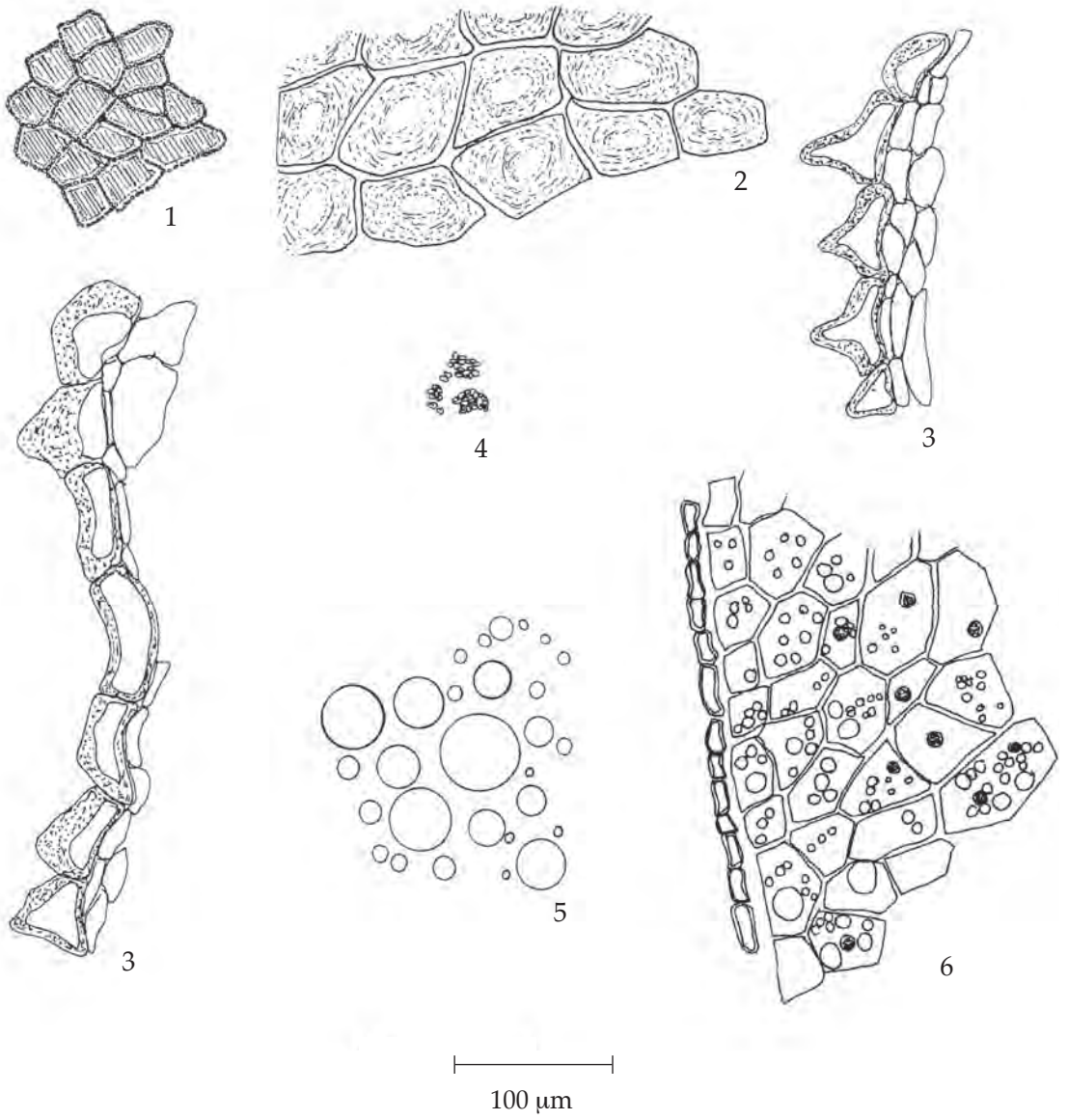


Fig. 2b Powdered Drug of the Seeds of *Nigella sativa* L.

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. Distil at a rate of 2 to 3 mL per minute for 5 hours. Use 2.0 mL of *xylene* in the graduated tube.

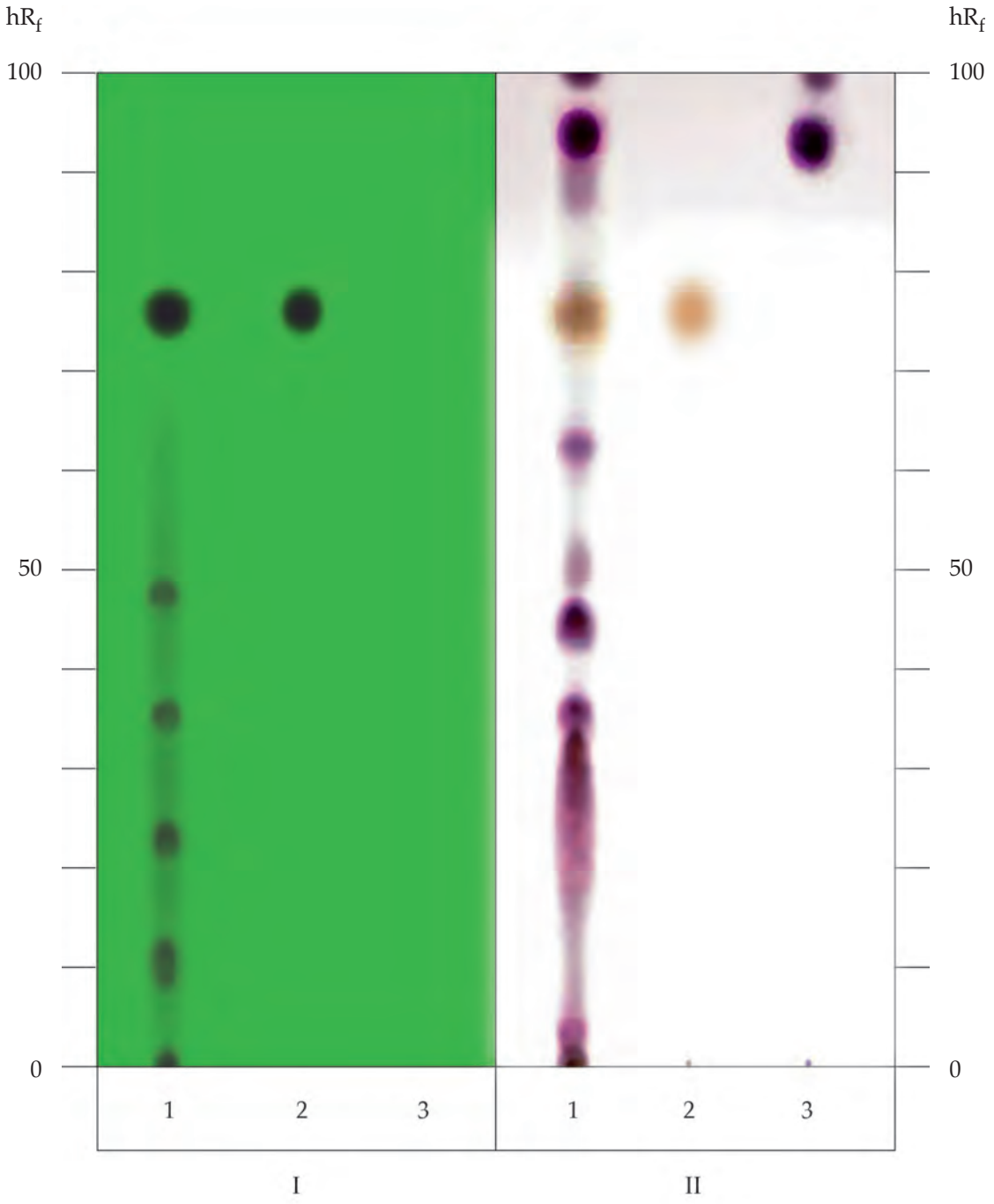


Fig. 3 Thin-Layer Chromatogram of Methanolic Extract of the Seeds of *Nigella sativa* L.

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. 1) Annual herb slender, erect, herbaceous, caulescent, glabrous, 20 to 40 cm tall. 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 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; pedicel 2 to 8 mm long. Flower small; sepal 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 s tyles; carpophore 2-cleft to the base. Fruit mericarp, ovoid-oblong, 4 to 5 mm long, glabrous or setulose

Description Odour, aromatic; taste, spicy.

Macroscopical (Fig. 1) Entire cremocarp with pedicels attached, 4.5 to 7 mm long, 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 parenchyma 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 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.

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

1

2

5 mm

3

4

Fig. 1 *Cuminum cyminum* L.
1. habit 2. young fruit 3. crude drug 4. ground crude drug

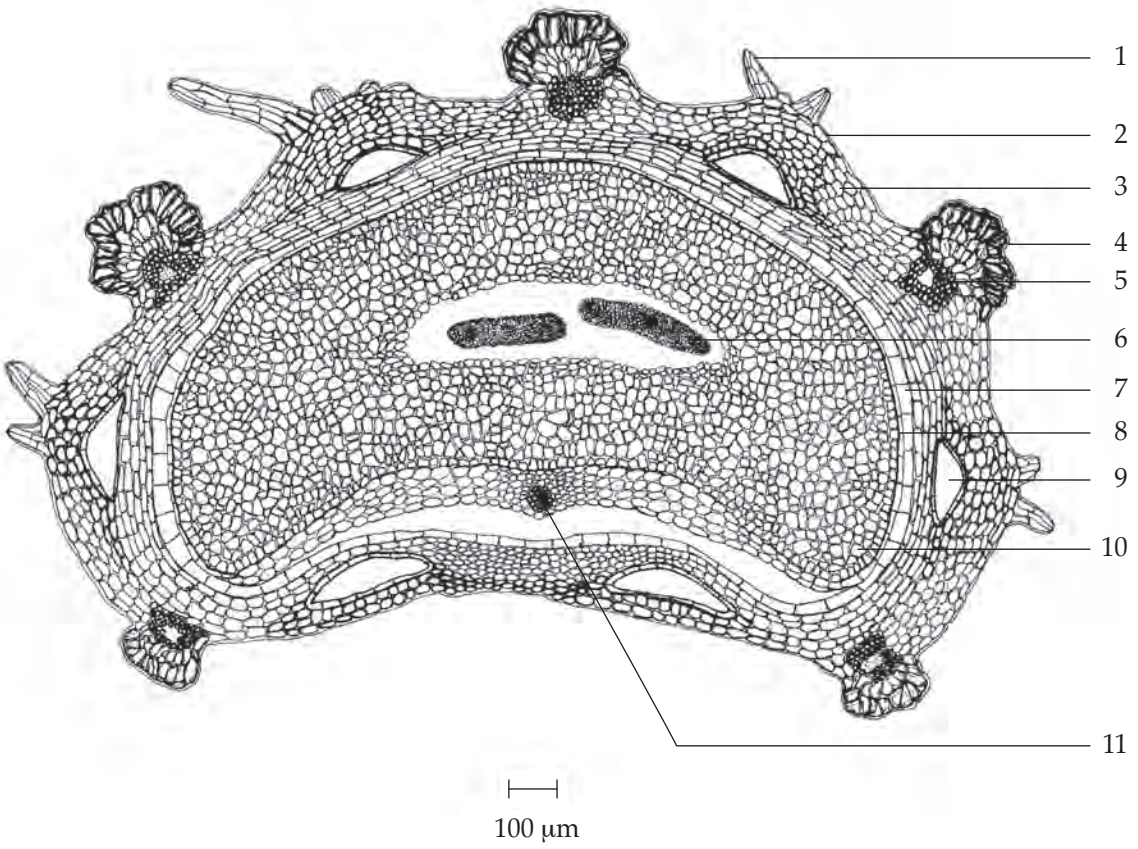


Fig. 2a Transverse Section of the Mericarp of *Cuminum cyminum* L.

1. trichome
2. epicarp
3. parenchyma of mesocarp
4. sclerenchymatous cell
5. vascular bundle
6. cotyledon
7. endocarp
8. spermoderm
9. vitta
10. endosperm
11. raphe

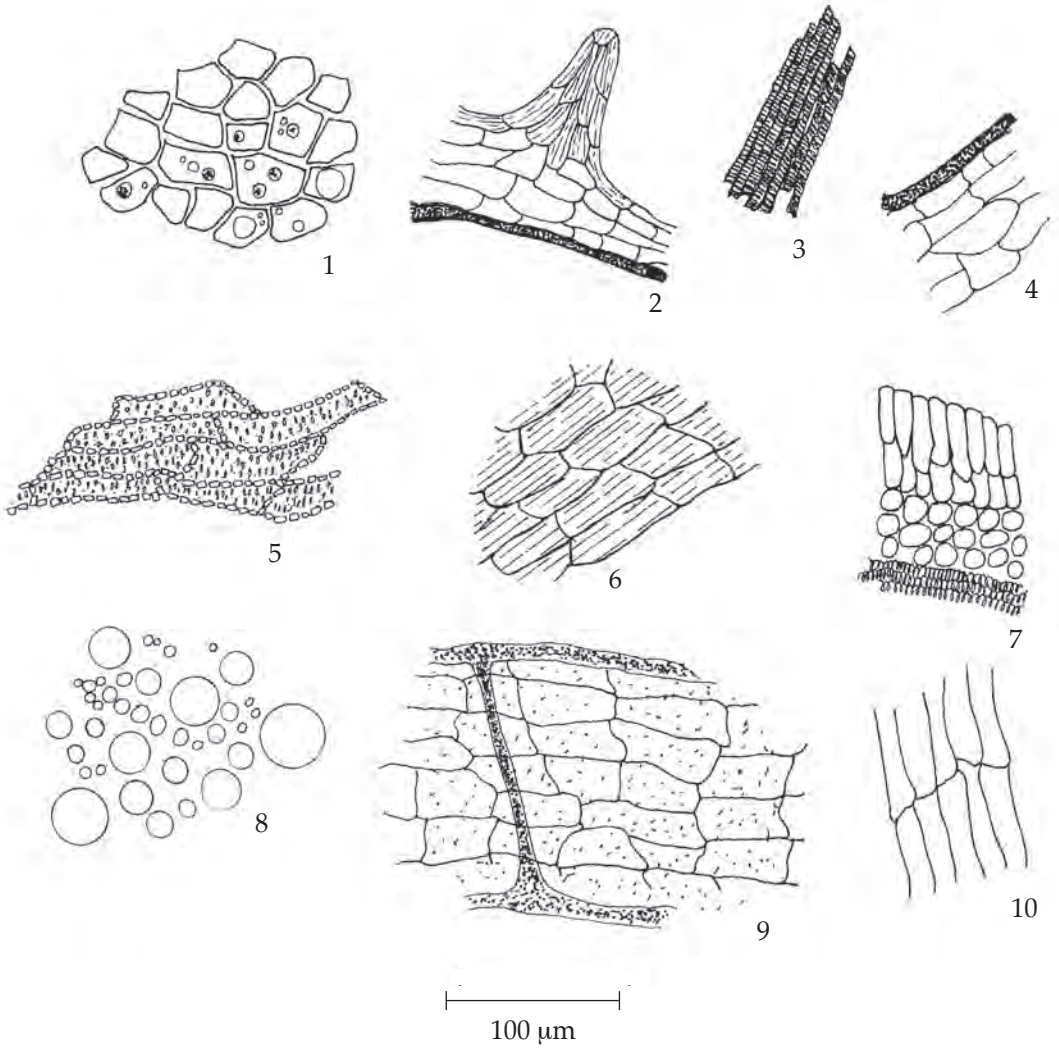


Fig. 2b Powdered Drug of the Fruits of *Cuminum cyminum* L.

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	*Dinitrophenyl-hydrazine 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.

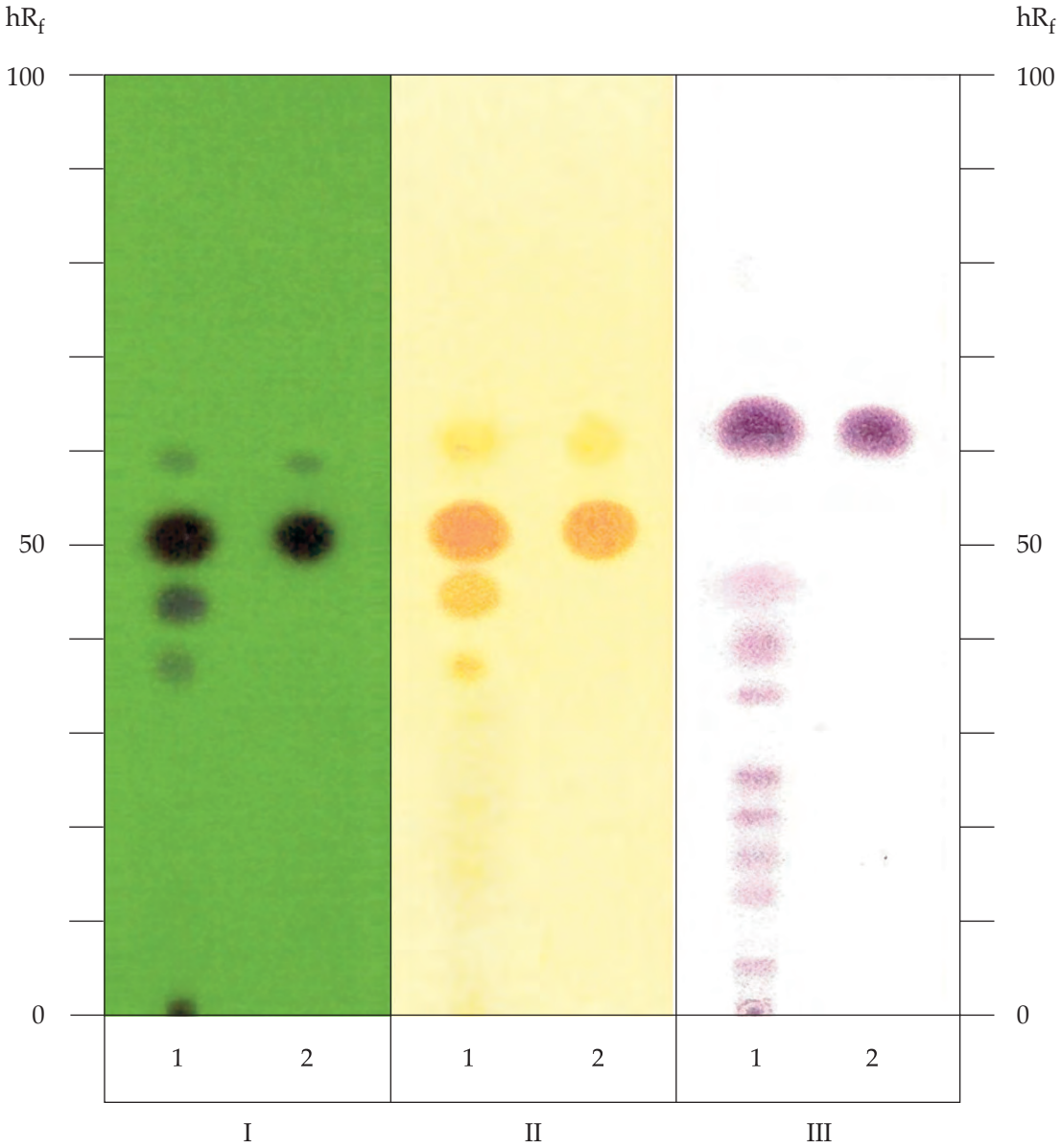


Fig. 3 Thin-Layer Chromatogram of Ethyl Acetate Extract of the Fruits of *Cuminum cyminum* L.

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) Herb annual or perennial, 0.4 to 2 m tall; 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 umbels, terminal and lateral, 5 to 9 cm across; bract and bracteole absent; rays numerous, upward-spreading, unequal; peduncle 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. Fruit cremocarp, oblong, 4 to 6 mm long, terete, glabrous, each mericarp 5-ridged, acute or round-obtuse. Seed 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, 1.1 to 2.5 mm wide, oblong, laterally compressed. Mericarps frequently separate, each being broadly elliptic, more or less curved; dorsal surface convex with 5 prominent, longitudinal ridges, and at the summit a short, conical stylopodium; 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, composed 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 dorsals 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.

1

2

3

4

5 mm

5

Fig. 1a *Foeniculum vulgare* Mill.
1. habit 2. inflorescence 3. umbellet 4. young infructescence 5. crude drug

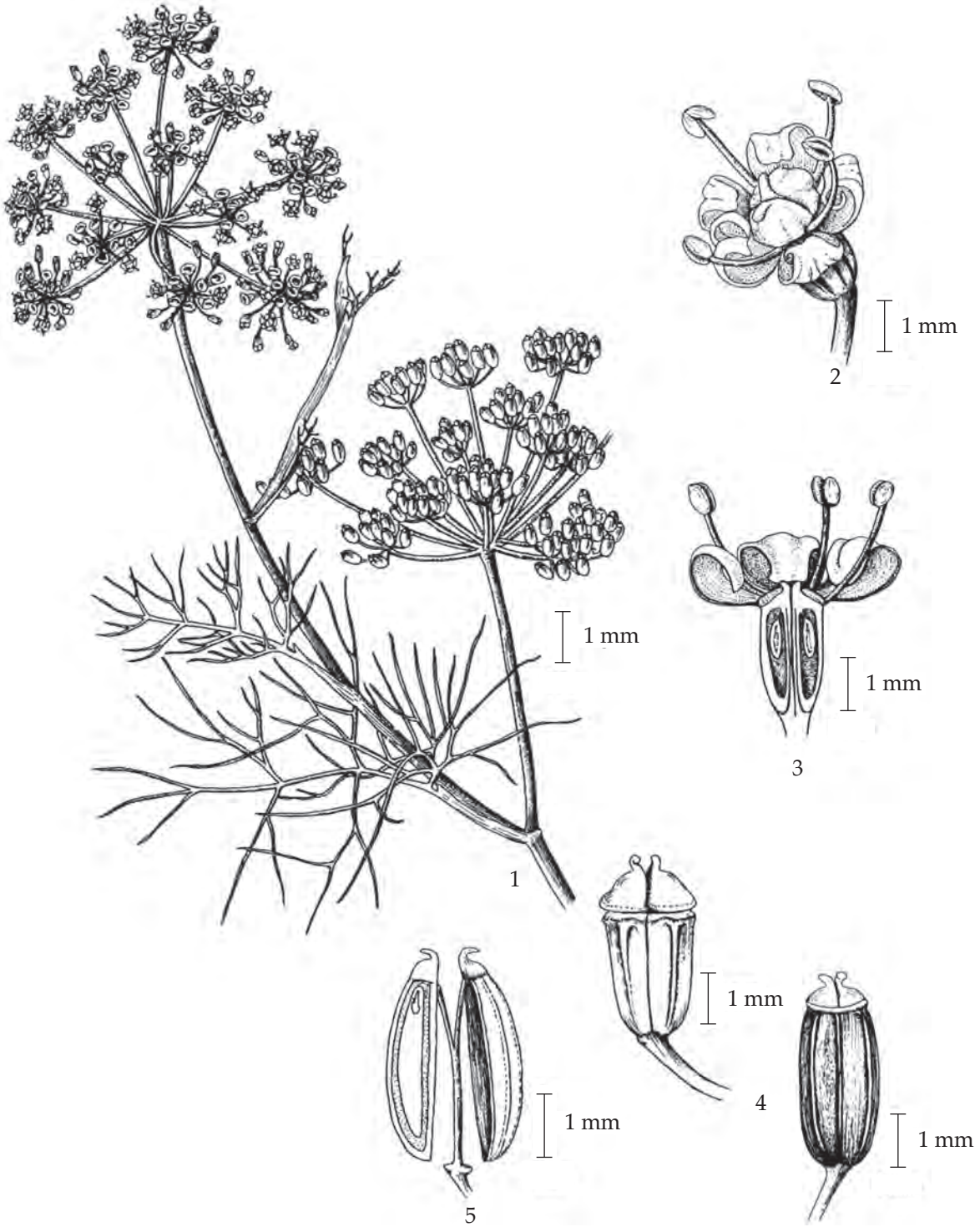


Fig. 1b *Foeniculum vulgare* Mill.
1. part of plant showing inflorescence and infructescence 2. flower
3. flower with longitudinal section of ovary 4. young fruit 5. schizocarp

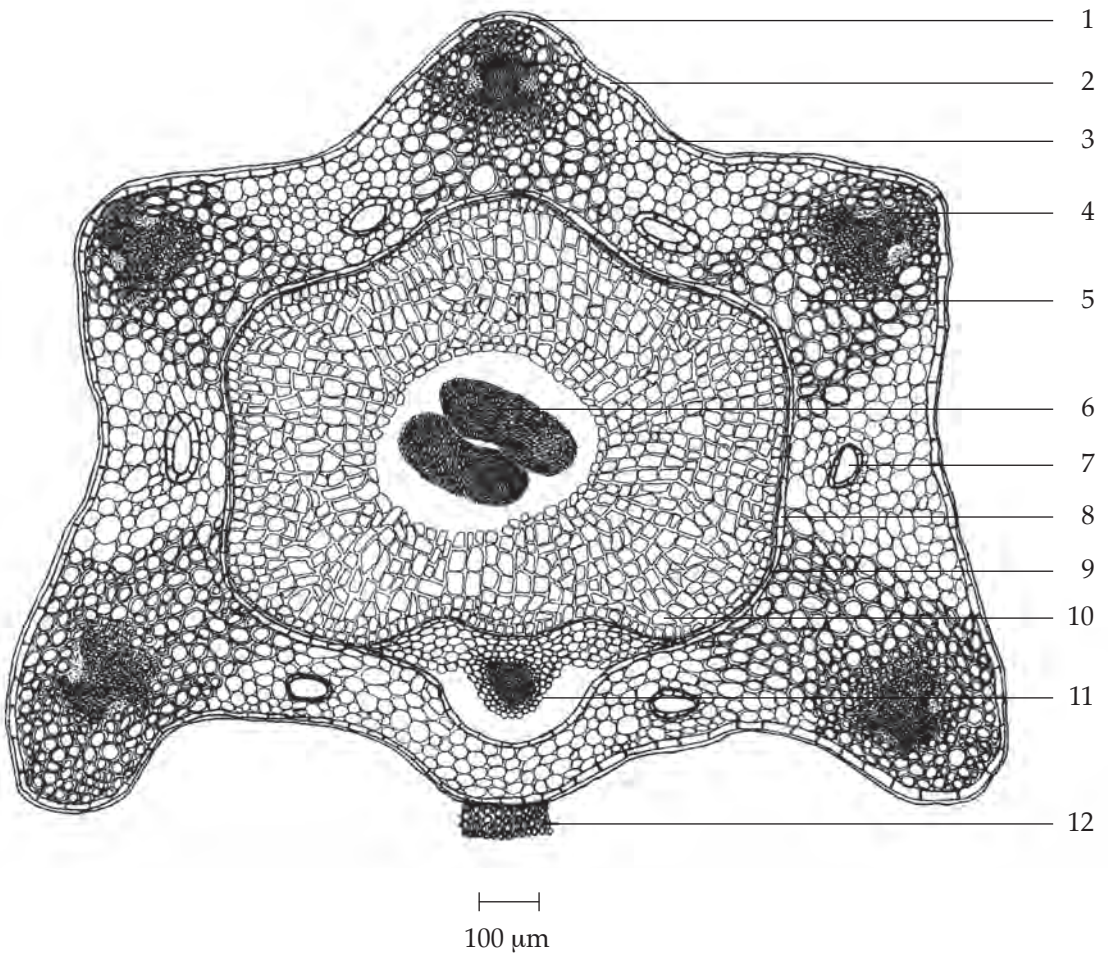


Fig. 2a Transverse Section of the Mericarp of *Foeniculum vulgare* Mill.

1. cuticle layer
2. epicarp
3. parenchyma of mesocarp
4. vascular bundle
5. thick-walled parenchyma
6. cotyledon
7. vitta
8. endocarp
9. spermoderm
10. endosperm
11. raphe
12. carpophore

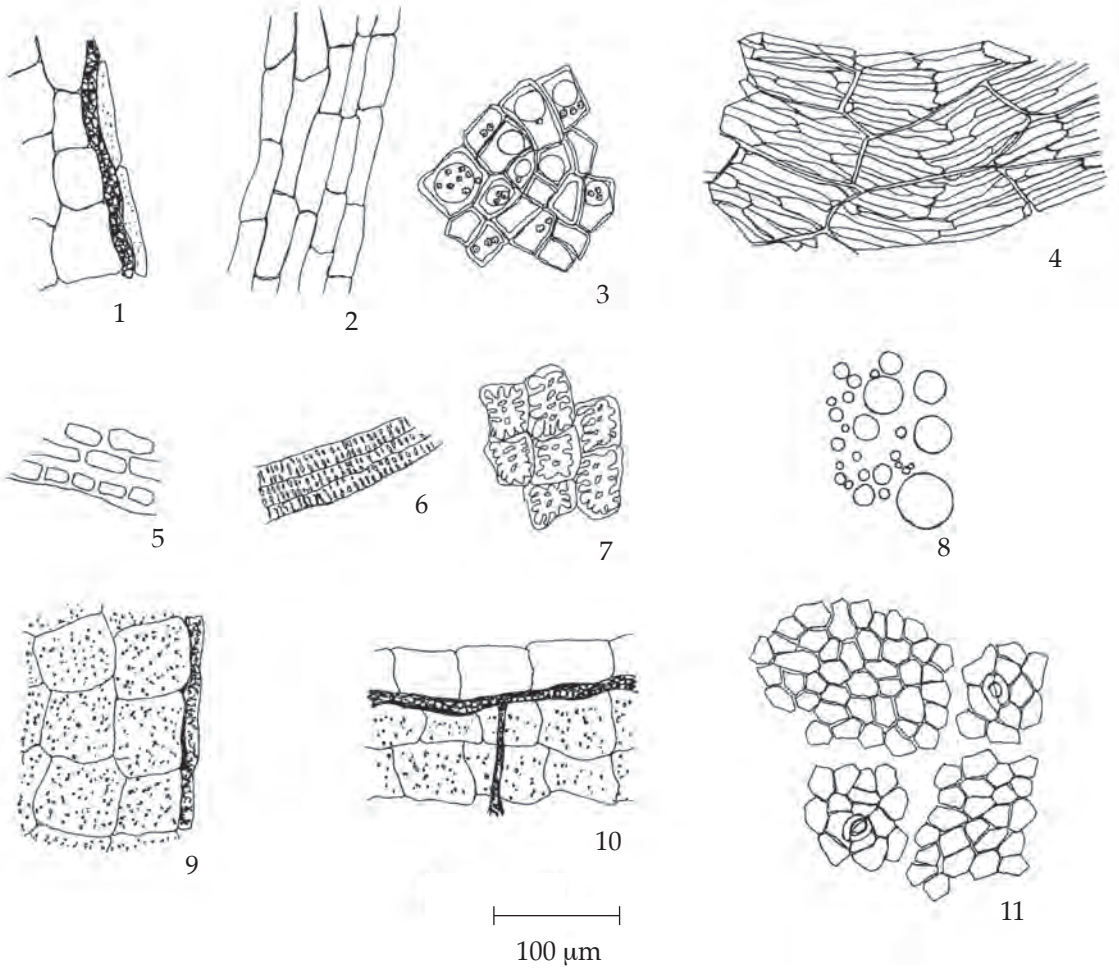


Fig. 2b Powdered Drug of the Fruits of *Foeniculum vulgare* Mill.

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.

Band	hR_f Value	Detection	
		UV 254	*Phosphomolybdic Acid TS*/ 3.3 Per Cent W/V Solution of *Potassium Permanganate* in *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.

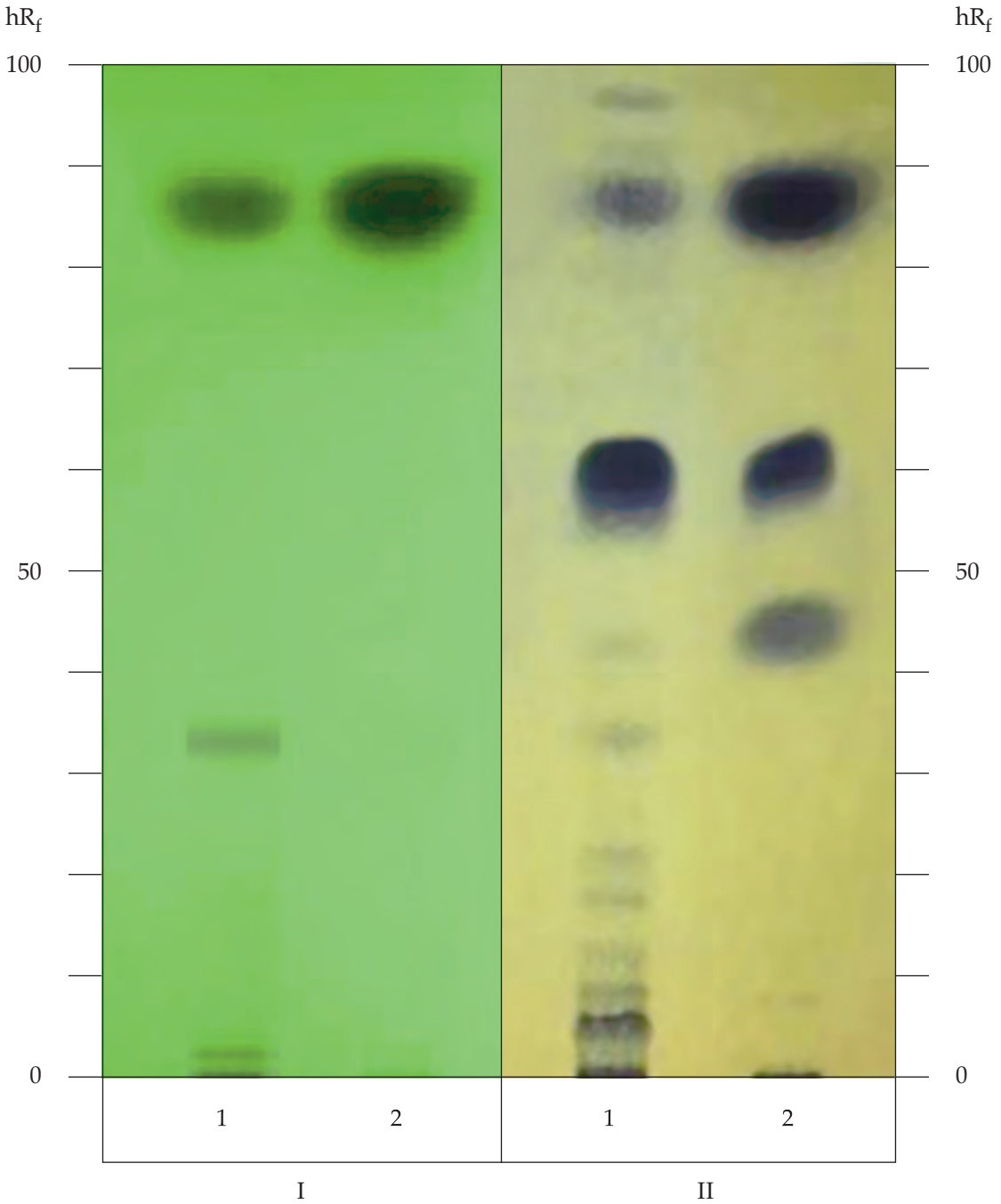


Fig. 3 Thin-Layer Chromatogram of Dichloromethane Extract of the Fruits of *Foeniculum vulgare* Mill.

1 = solution (A)
2 = solution (B)
I = detection under UV light (254 nm)
II = detection with *phosphomolybdic acid TS* and a 3.3 per cent w/v solution of *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 tall. Leaves radical, narrow-linear or filiform, finely acuminate, 7 to 22 cm long, usually 3-nerved, entire or distantly toothed. Scape axillary, longer or shorter than the leaves, glabrous or pubescent; spike ovoid or cylindrical, up to 4 cm long; bract with broad scarious margin, ovate-oblong, obtuse, glabrous. Flower 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, filament, persistent, anther 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, 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.

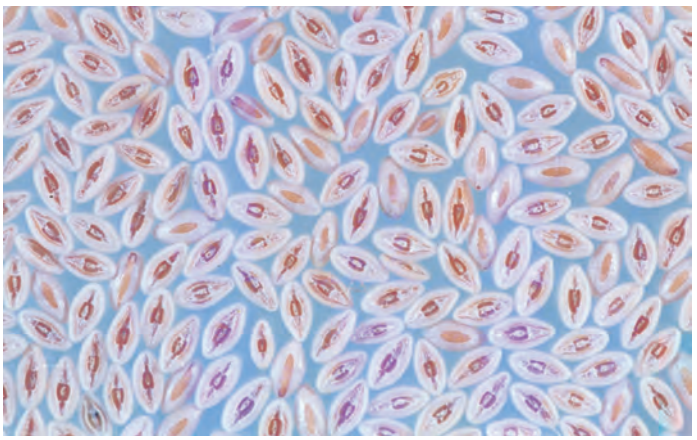
Microscopical (Figs. 2a, 2b, 2c) Transverse section of the seed cut through the cotyledon region shows seed coat, endosperm and cotyledon. The outer layer of seed coat, large, rectangular, mucilaginous epidermal cells; the cells rapidly swelling in water, the outer walls bursting and, liberating numerous 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.

1

2

3



1 cm

4

Fig. 1a *Plantago ovata* Forssk.
1. habit 2. flowering plant 3. inflorescence 4. crude drug

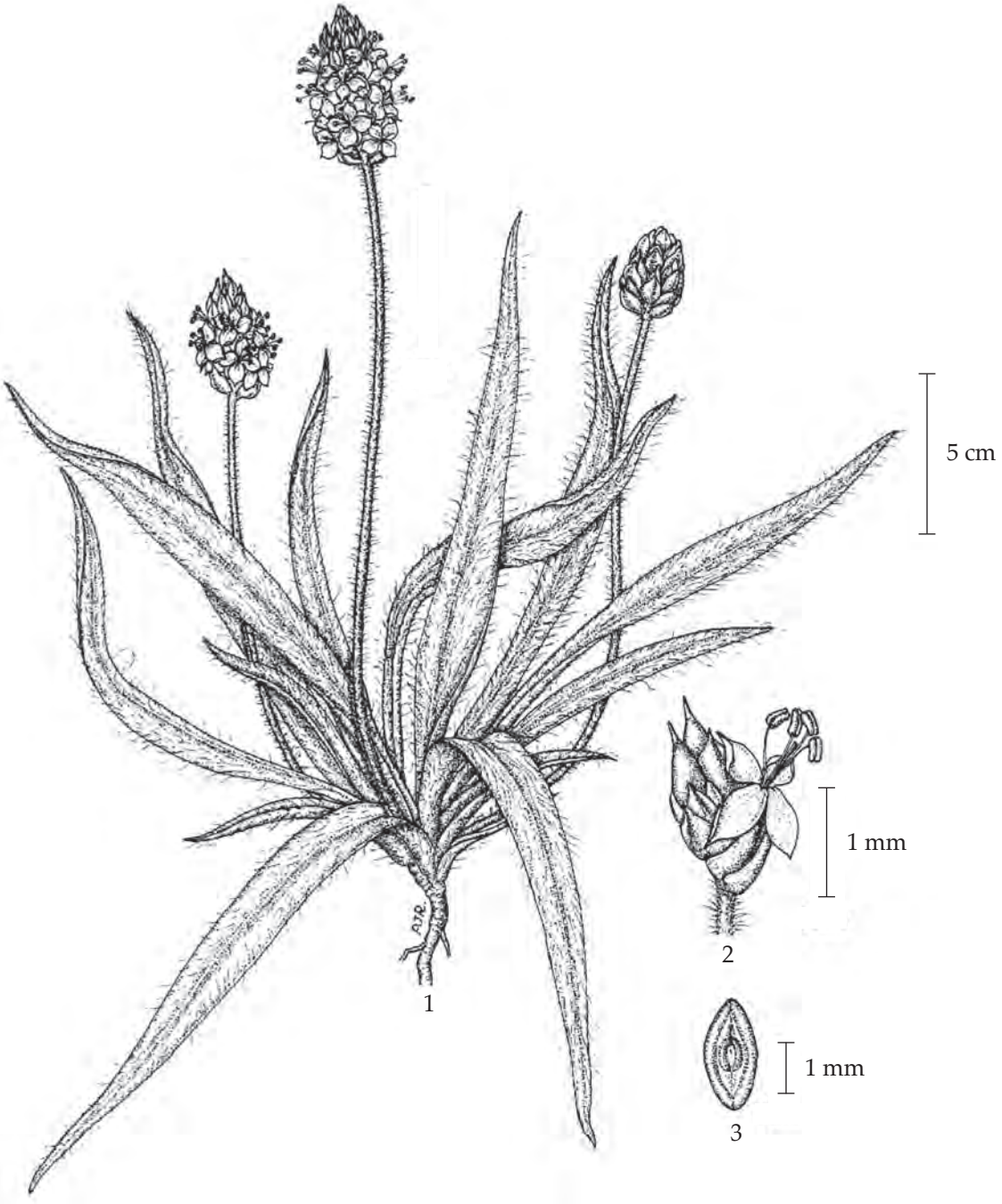


Fig. 1b *Plantago ovata* Forssk.
1. habit 2. inflorescence 3. seed

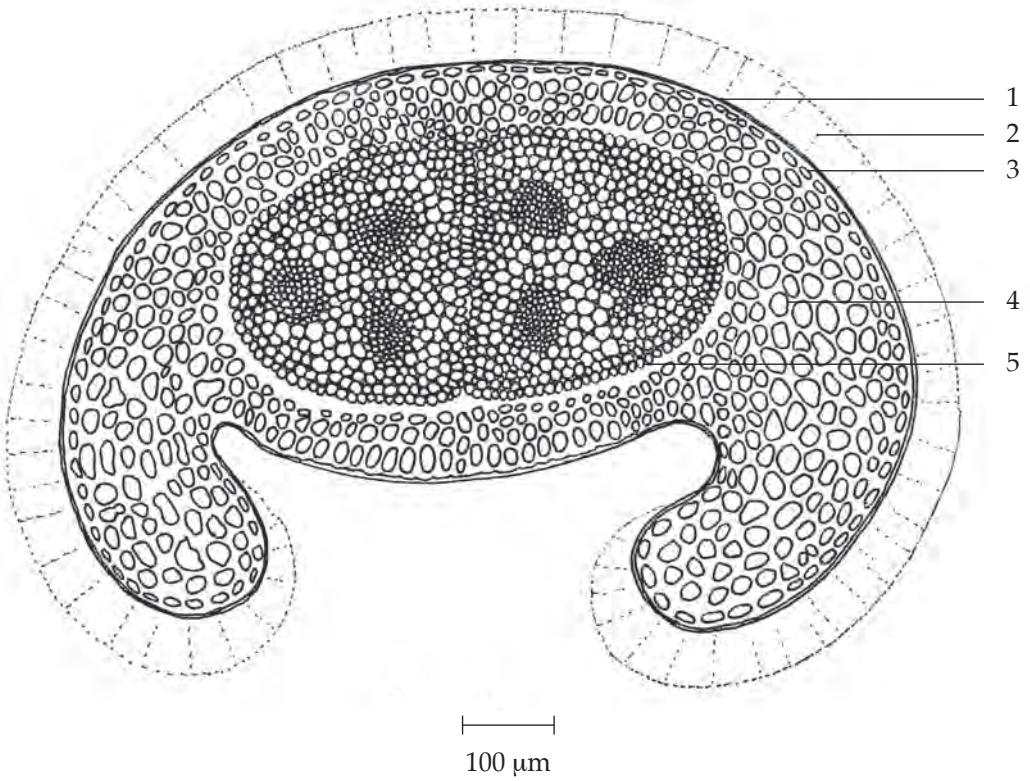
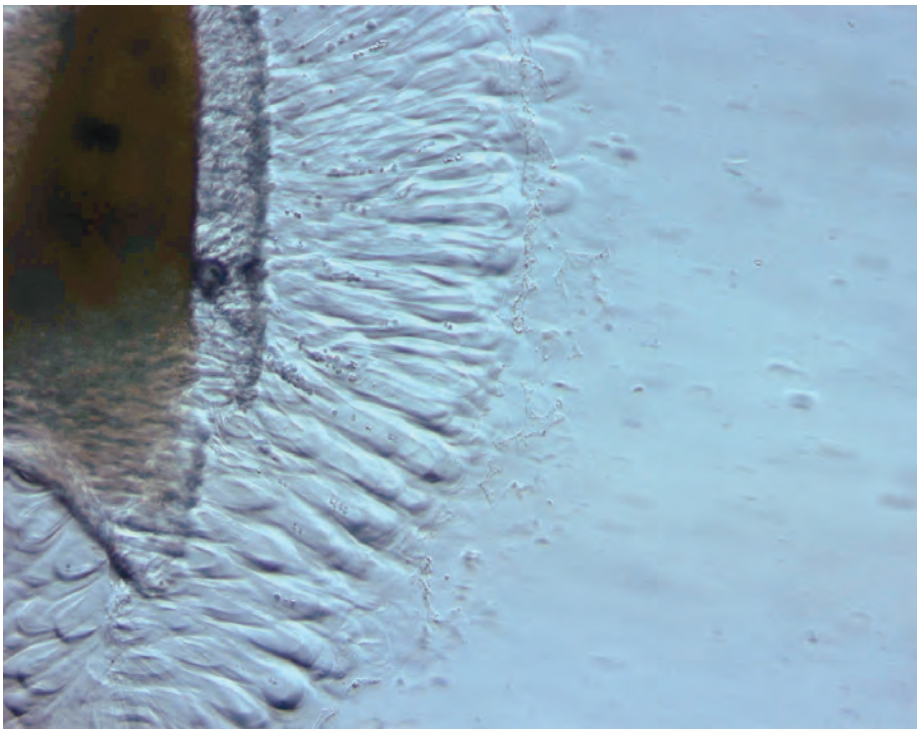


Fig. 2a Transverse Section of the Seed of *Plantago ovata* Forssk., when Mounted in a 50 Per Cent V/V Solution of *Glycerol*

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



200 μm

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*

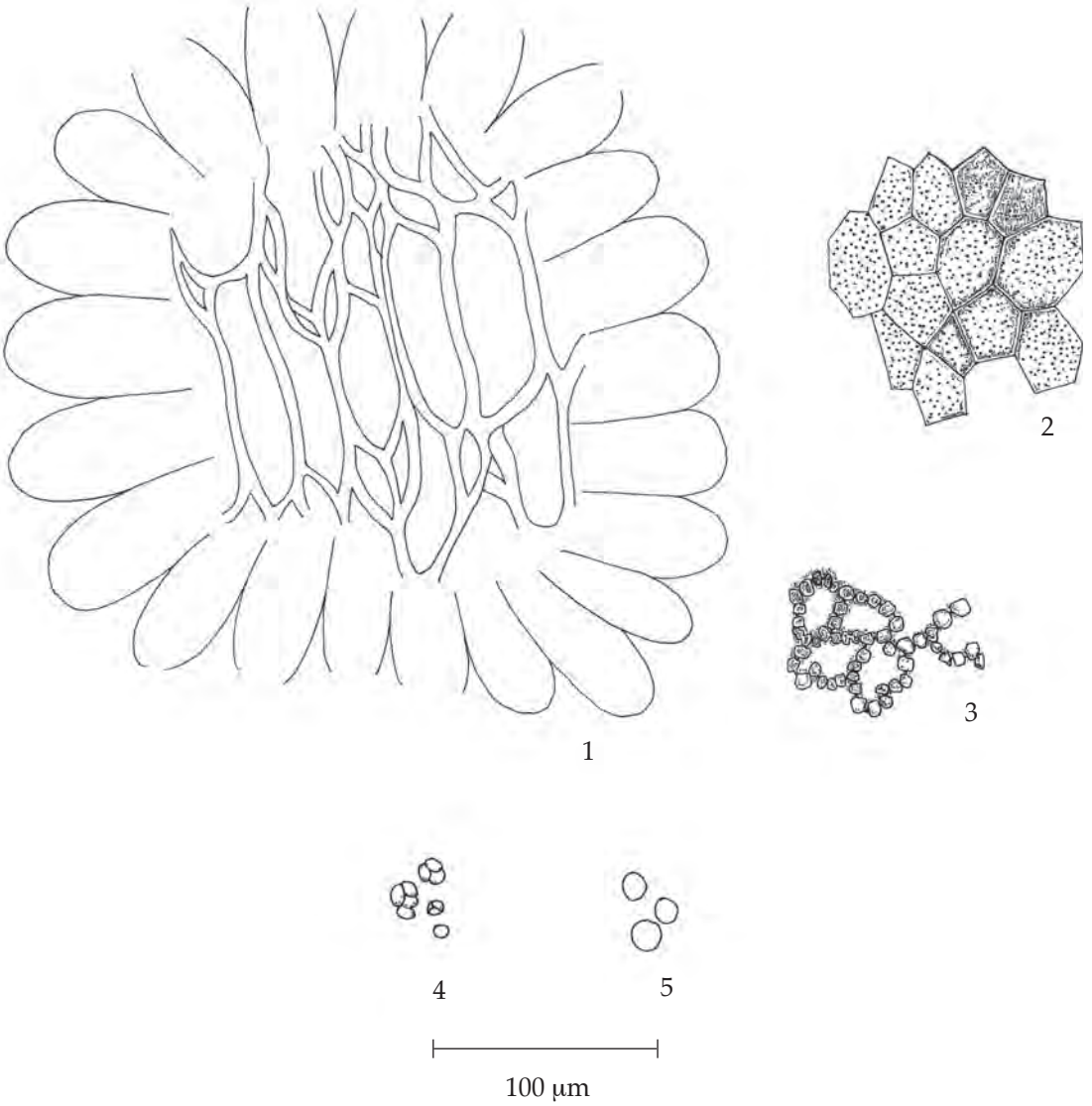


Fig. 2c Powdered Drug of the Seeds of *Plantago ovata* Forssk., when Mounted in *Water*

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
		0.5 Per Cent W/V Solution of *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.

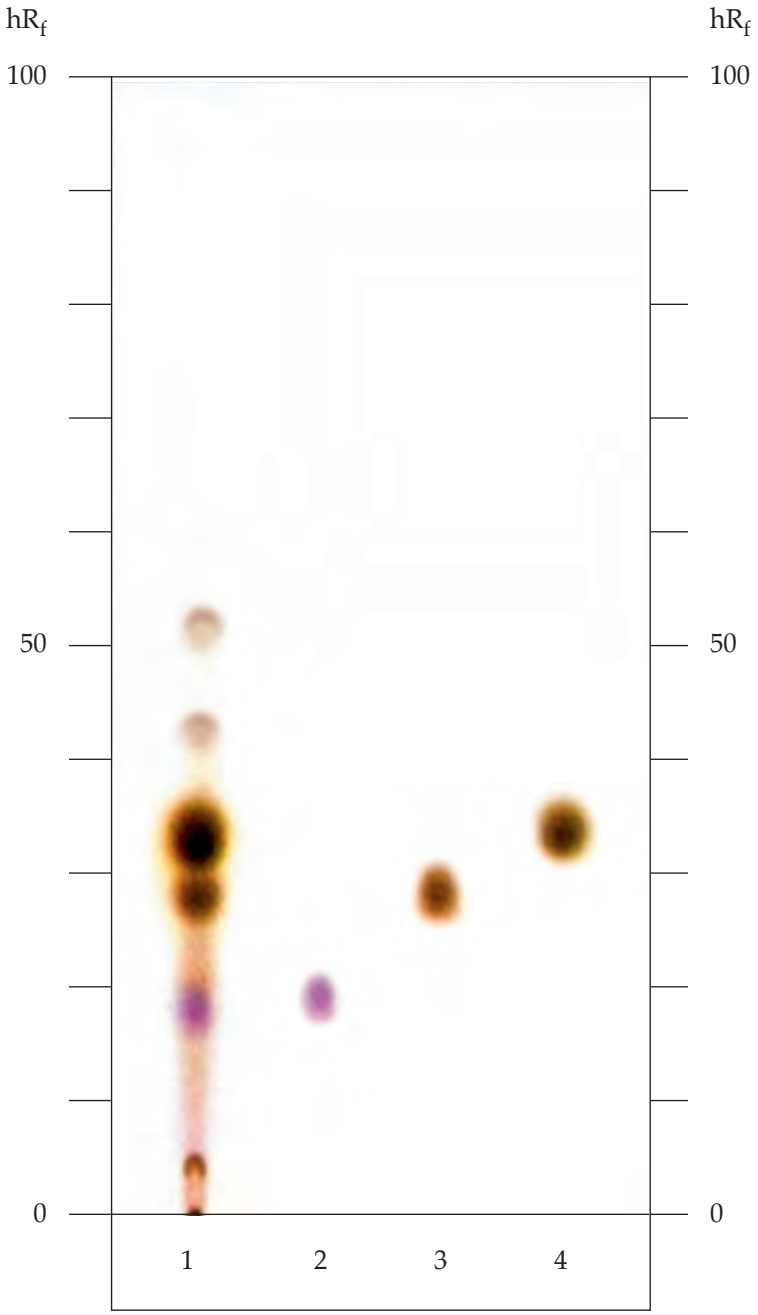


Fig. 3 Thin-Layer Chromatogram of Aqueous Extract of the Seeds of *Plantago ovata* Forssk., Detected with 0.5 Per Cent W/V Solution of *Thymol* in a Mixture of *Sulfuric Acid* and *Ethanol*

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 tall; 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 irregularly toothed, 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 umbel, 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.

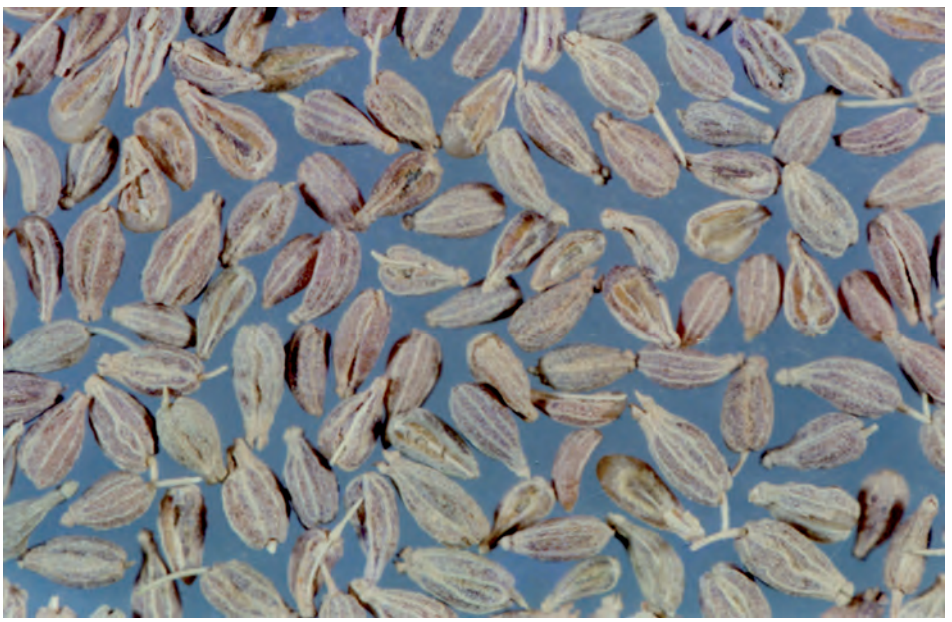
Microscopical (Figs. 2a, 2b) Transverse section of the mericarp through the cotyledon shows epicarp, mesocarp, endocarp, spermoderm, endosperm, and cotyledon. Epicarp, a layer of epidermal cells with numerous papillae and usually unicellular trichomes, covered with striated cuticle, generally 6-ridged. Mesocarp, 3 or 4 layers of elongated parenchyma cells, with a small lignified fibrovascular bundle under the ridge; vittae, elliptic, 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.

1

2

3



4

2 mm

Fig. 1a *Pimpinella anisum* L.
1. habit 2. and 3. inflorescence 4. crude drug

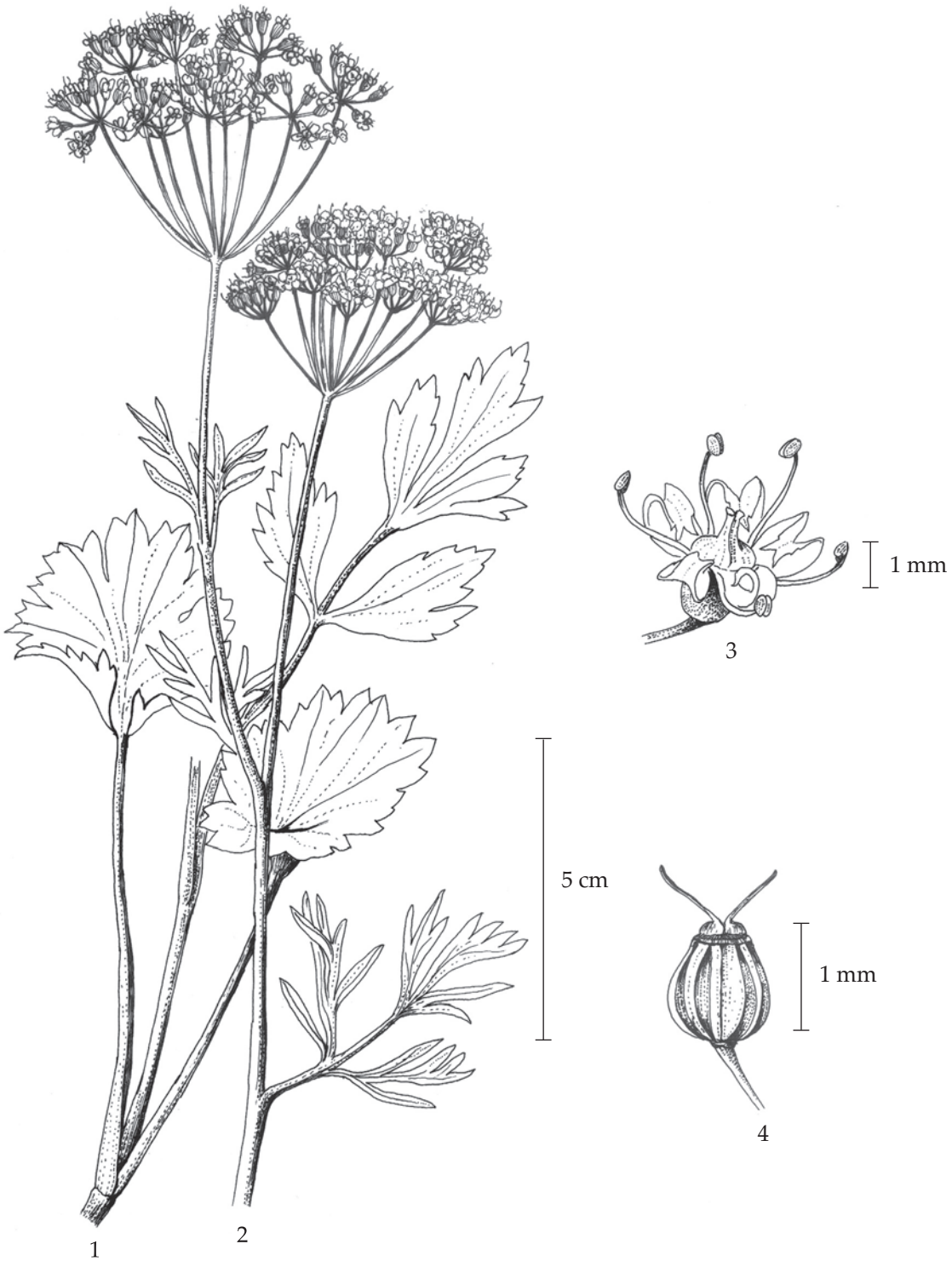


Fig. 1b *Pimpinella anisum* L.
1. leaves on lower stem
2. flowering top with leaves on upper stem and two compound umbels
3. flower 4. fruit (schizocarp)

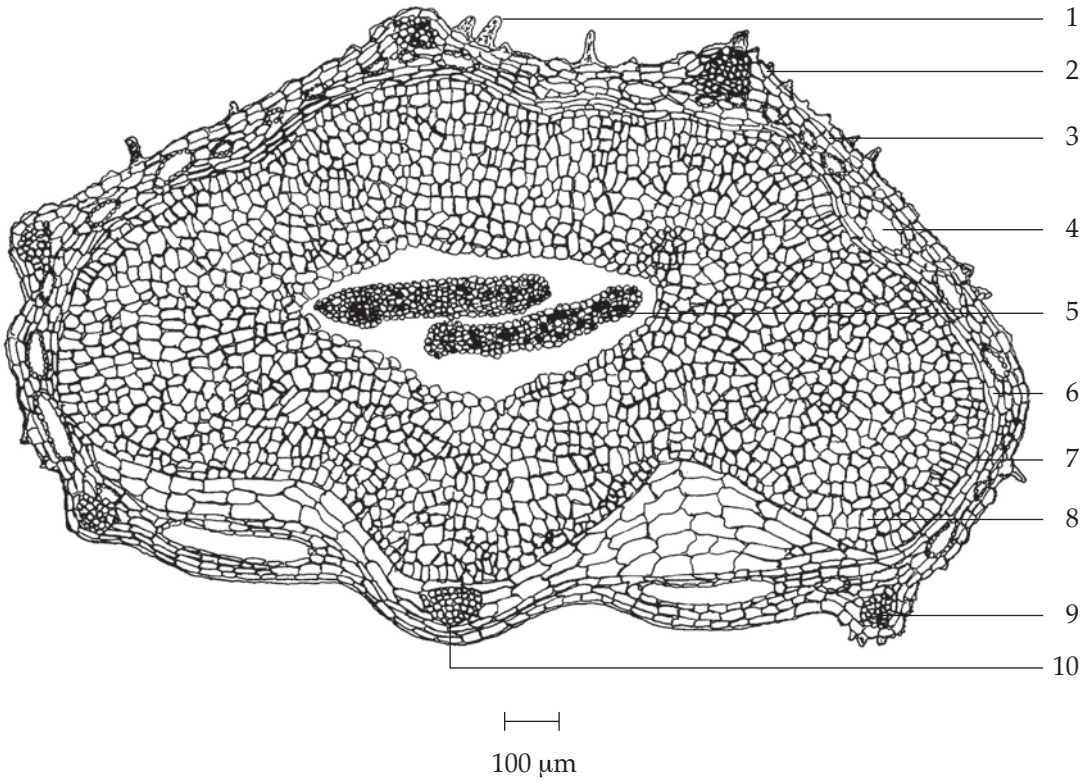


Fig. 2a Transverse Section of the Mericarp of *Pimpinella anisum* L.

1. trichome
2. papilla of the epicarp
3. parenchyma of mesocarp
4. vitta
5. cotyledon
6. endocarp
7. spermoderm
8. endosperm
9. vascular bundle
10. raphe

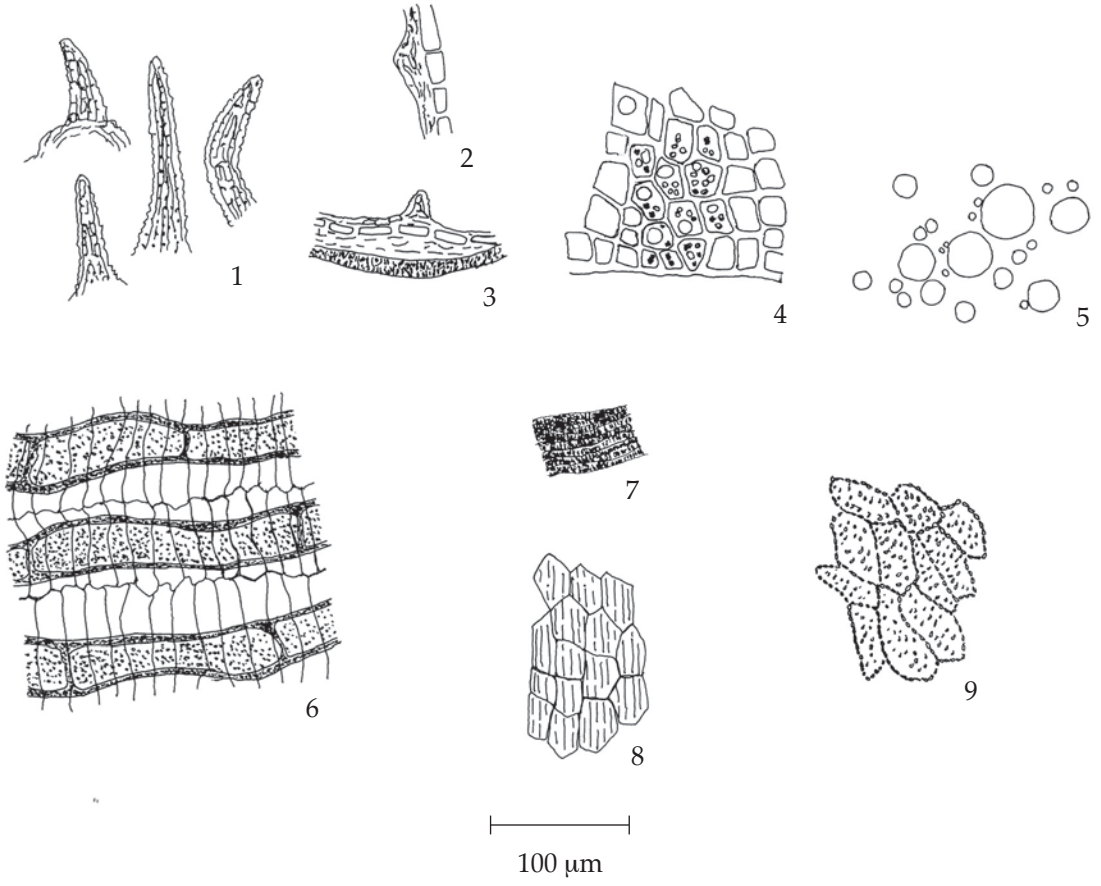


Fig. 2b Powdered Drug of the Fruits of *Pimpinella anisum* L.

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 the specimen 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.

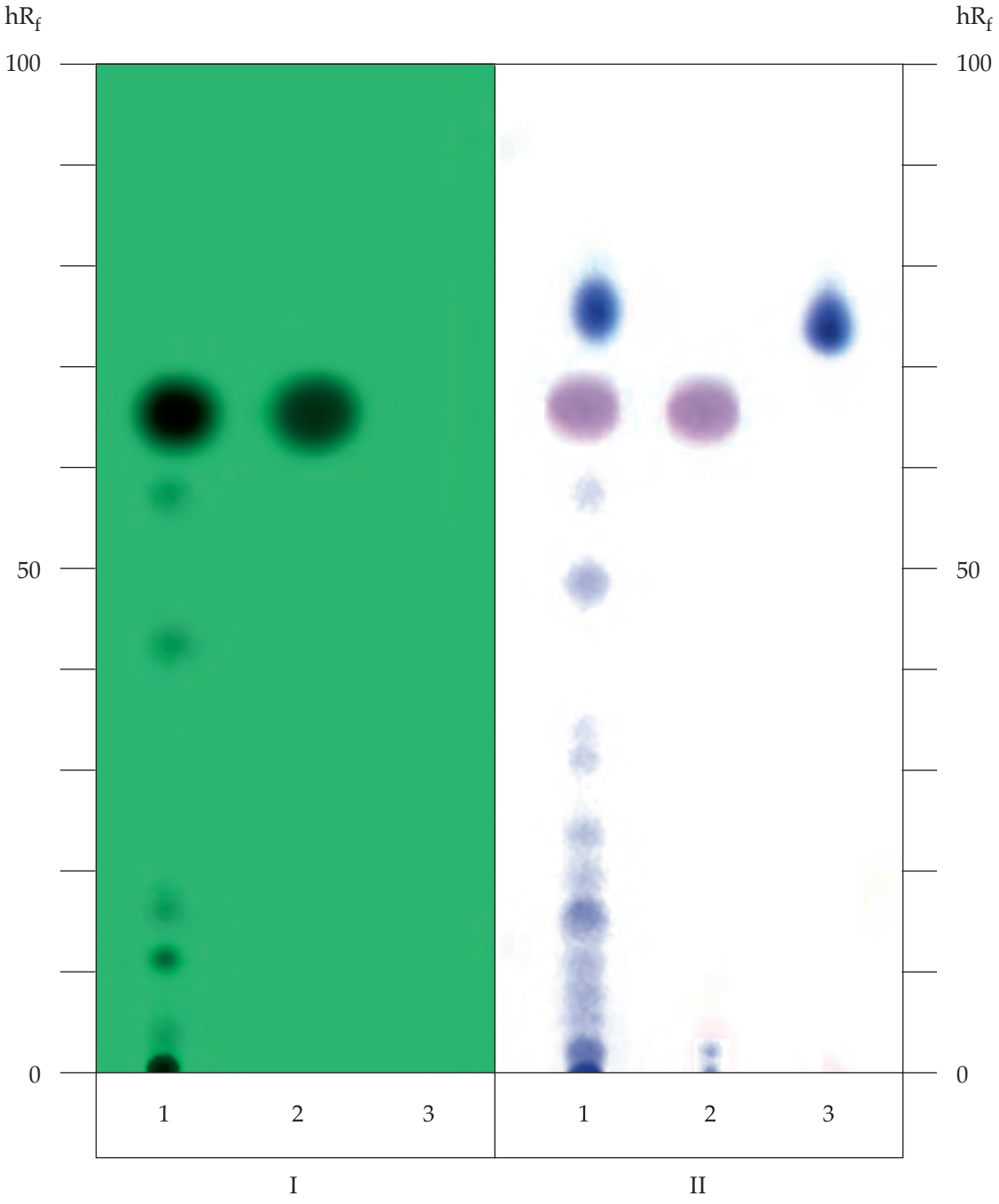


Fig. 3 Thin-Layer Chromatogram of Ethyl Acetate Extract of the Fruits of *Pimpinella anisum* L.

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 tall; 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, elliptic, 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.

1

2

3

2 mm

Fig. 1a *Carum carvi* L.
1. inflorescence 2. infructescence 3. crude drug

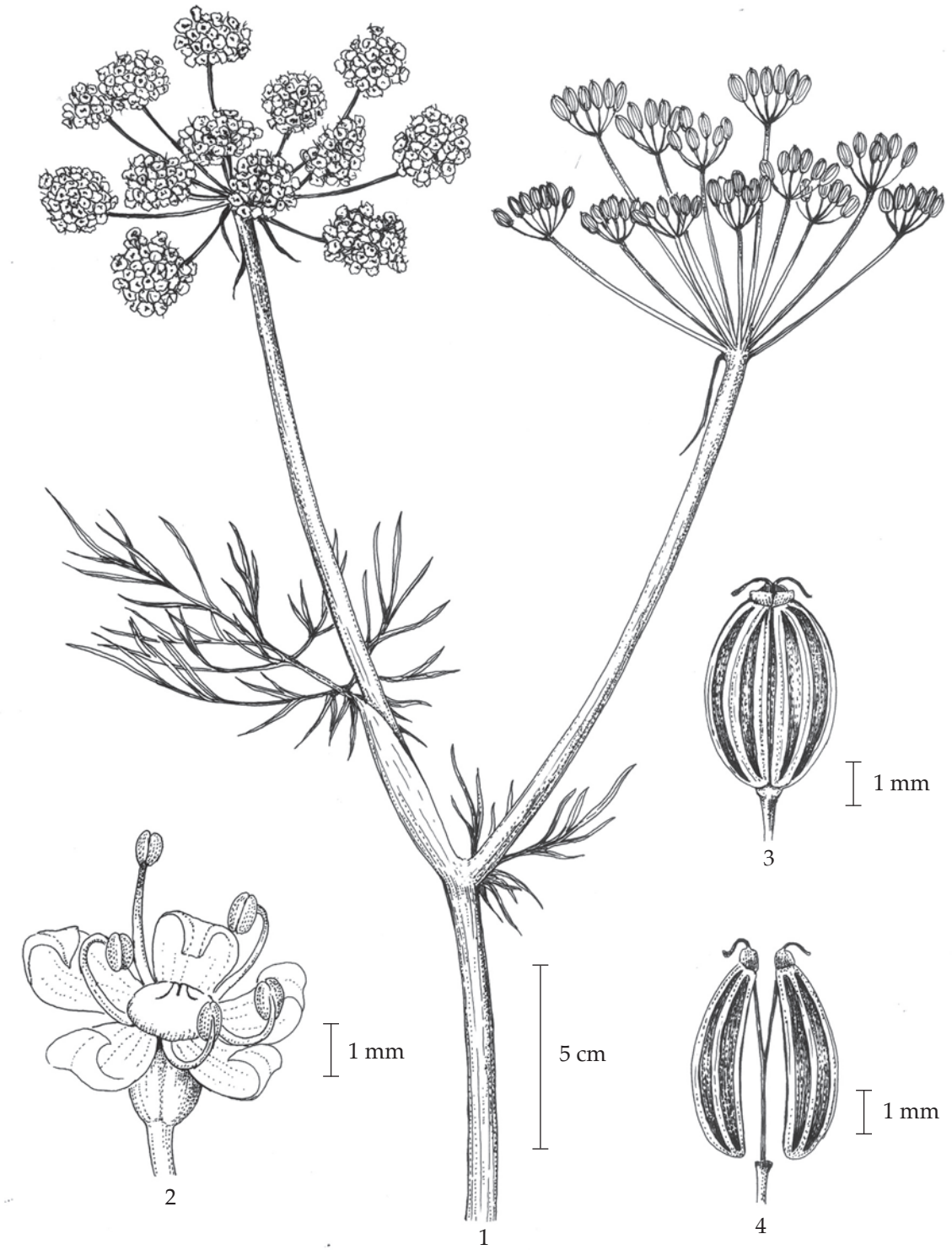


Fig. 1b *Carum carvi* L.
1. flowering top with leaves on upper stem, compound umbels and infructescence
2. flower 3. and 4. fruit (schizocarp)

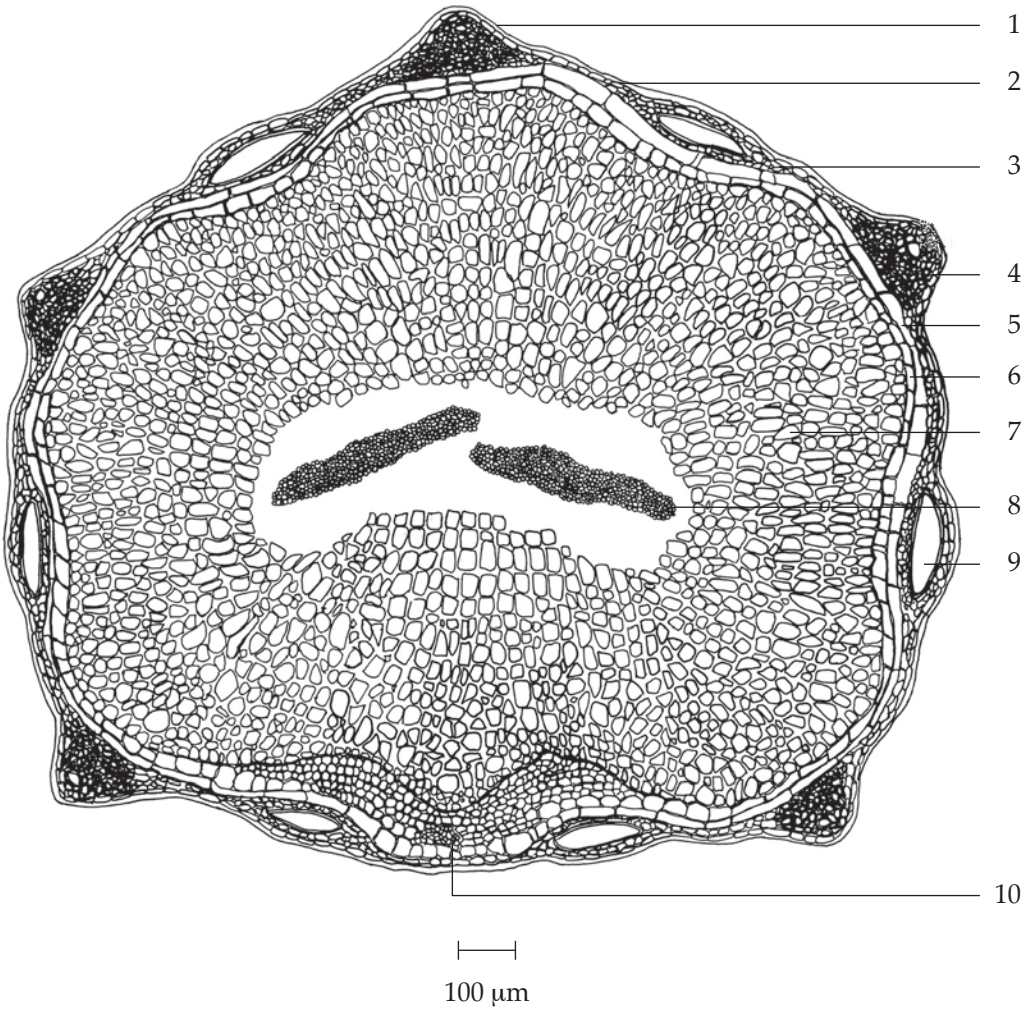


Fig. 2a Transverse Section of the Mericarp of *Carum carvi* L.

1. cuticle layer
2. epicarp
3. mesocarp
4. vascular bundle
5. endocarp
6. spermoderm
7. endosperm
8. cotyledon
9. vitta
10. raphe

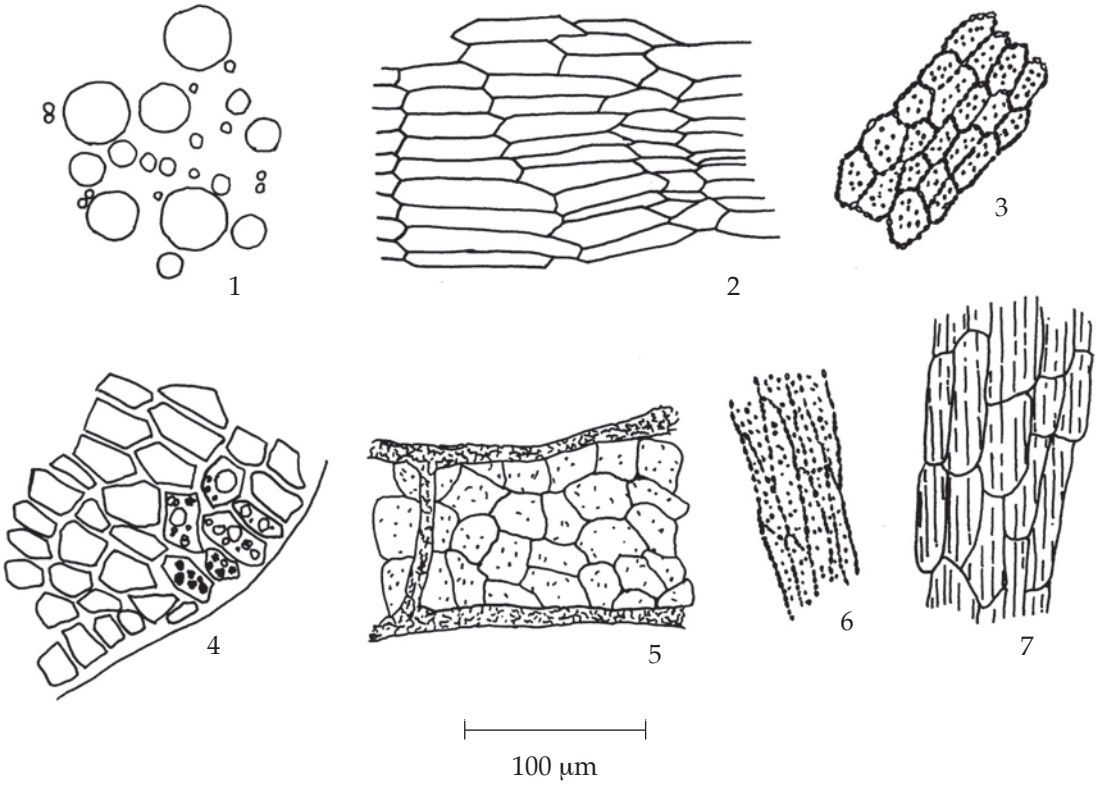


Fig. 2b Powdered Drug of the Fruits of *Carum carvi* L.

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 the specimen 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). 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 *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.

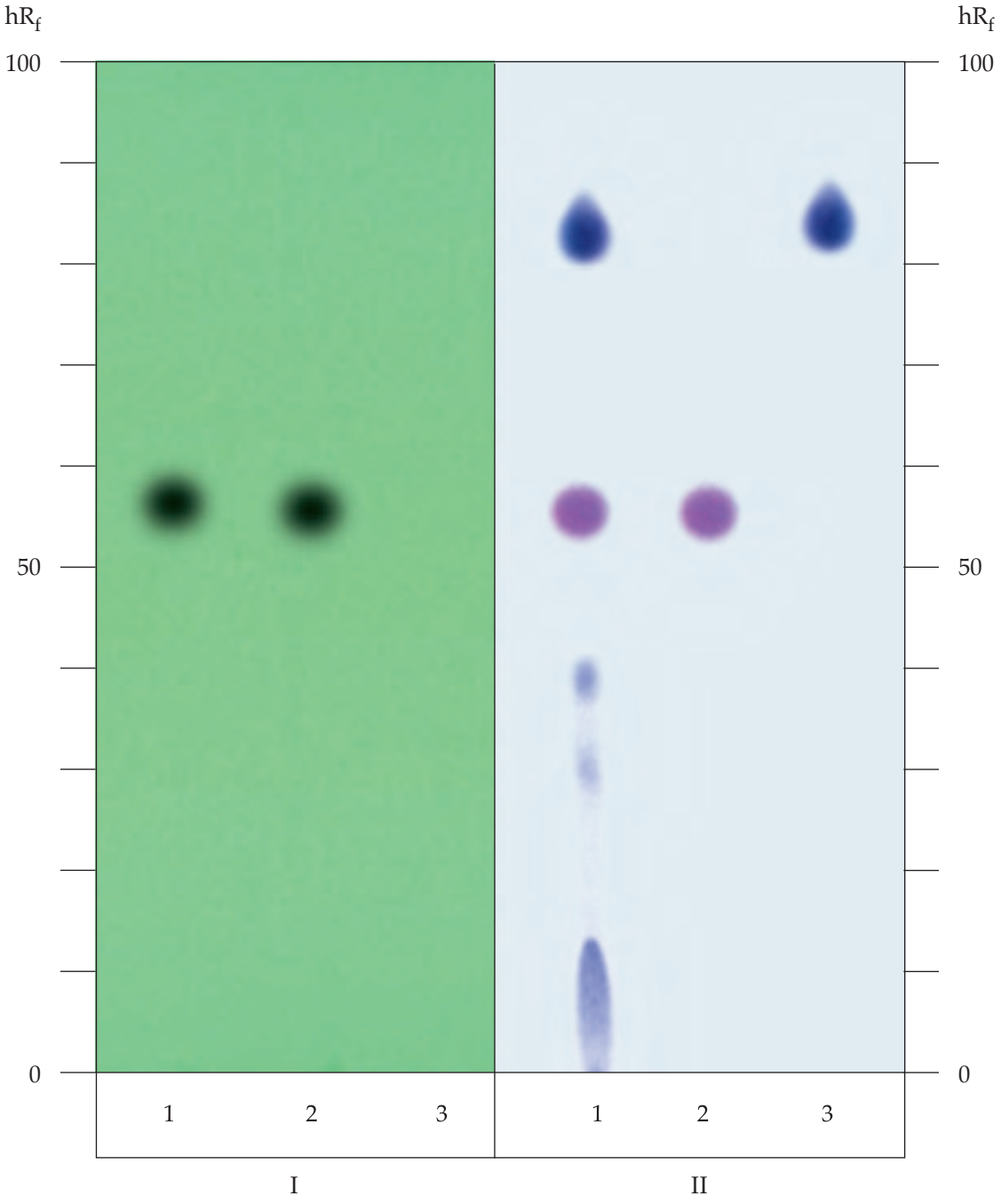


Fig. 3 Thin-Layer Chromatogram of Ethyl Acetate Extract of the Fruits of *Carum carvi* L.

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 TAKKATAEN)

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 tall; terete, striate, with subfusiform or fusiform taproot; main stem, hollow, glaucous, cylindrical, dichotomously branched with 5 to 8 branches. Leaves bi- or tri-pinnately compound with linear-filiform ultimate segments, 4 to 20 mm long; petiole 1 to 3.5 cm long; petiole sheathing, 5 to 6 cm long; upper cauline leaves greatly reduced. Inflorescence loose compound umbels; peduncle 7 to 20 cm long; rays 10 to 45, 3 to 15 cm in spread, bearing 10 to 45 umbellules; pedicel 6 to 16 mm long. Flower small, about 2 mm in diameter; sepal 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. Fruit ovoid, flattened dorsally, consisting of 2 dry, 1-seeded, indehiscent mericarps, up to about 5 mm long, 2 to 3 mm wide and about 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 parenchyma cells; each of the ridge, a lignified fibrovascular bundle, surrounded by reticulate and porous, thickened, lignified parenchyma 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

5 mm

Fig. 1a *Anethum graveolens* L.
1. habit 2. inflorescence 3. infructescence 4. crude drug

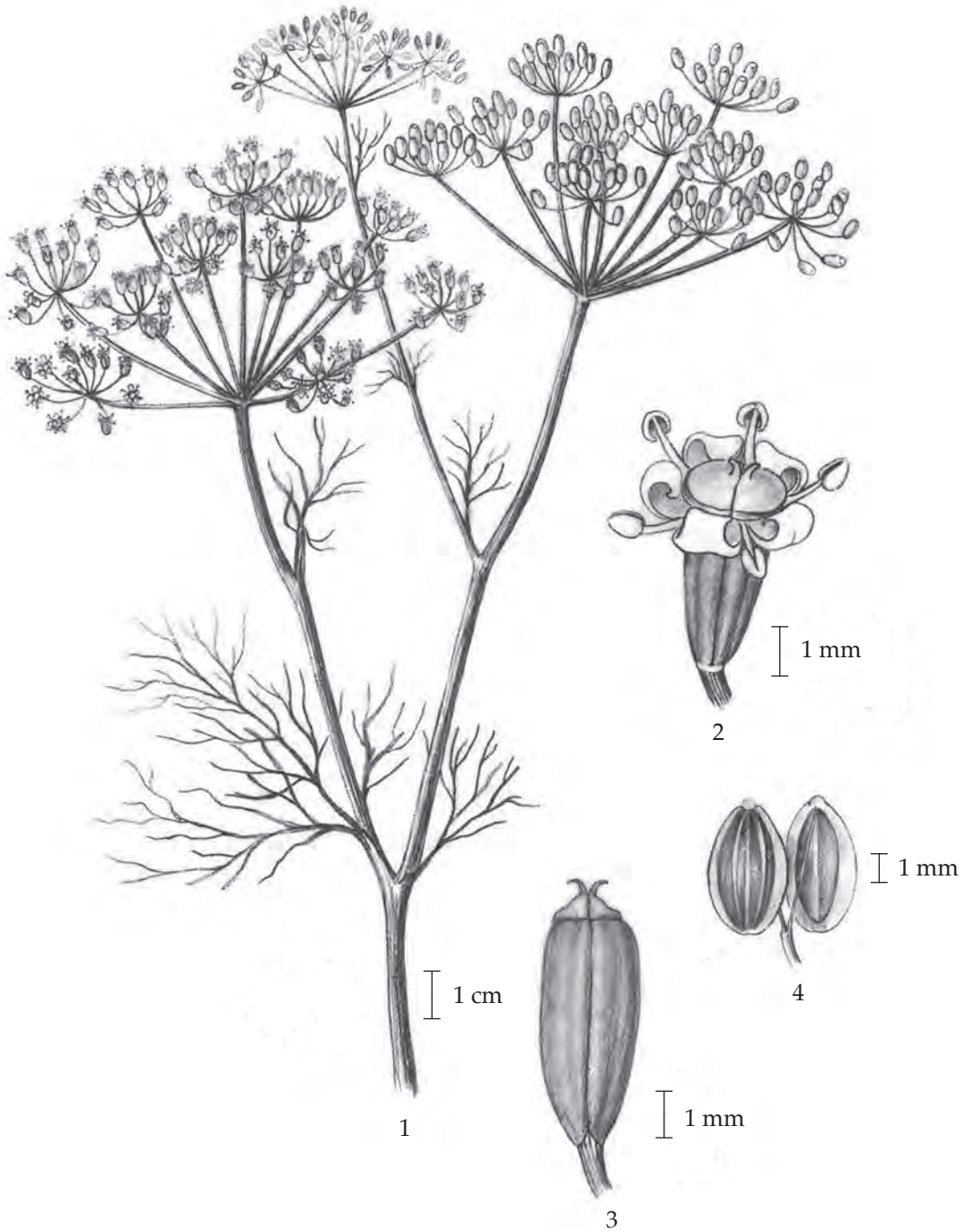


Fig. 1b *Anethum graveolens* L.
1. part of plant showing inflorescences and infructescence
2. flower 3. young fruit 4. schizocarp

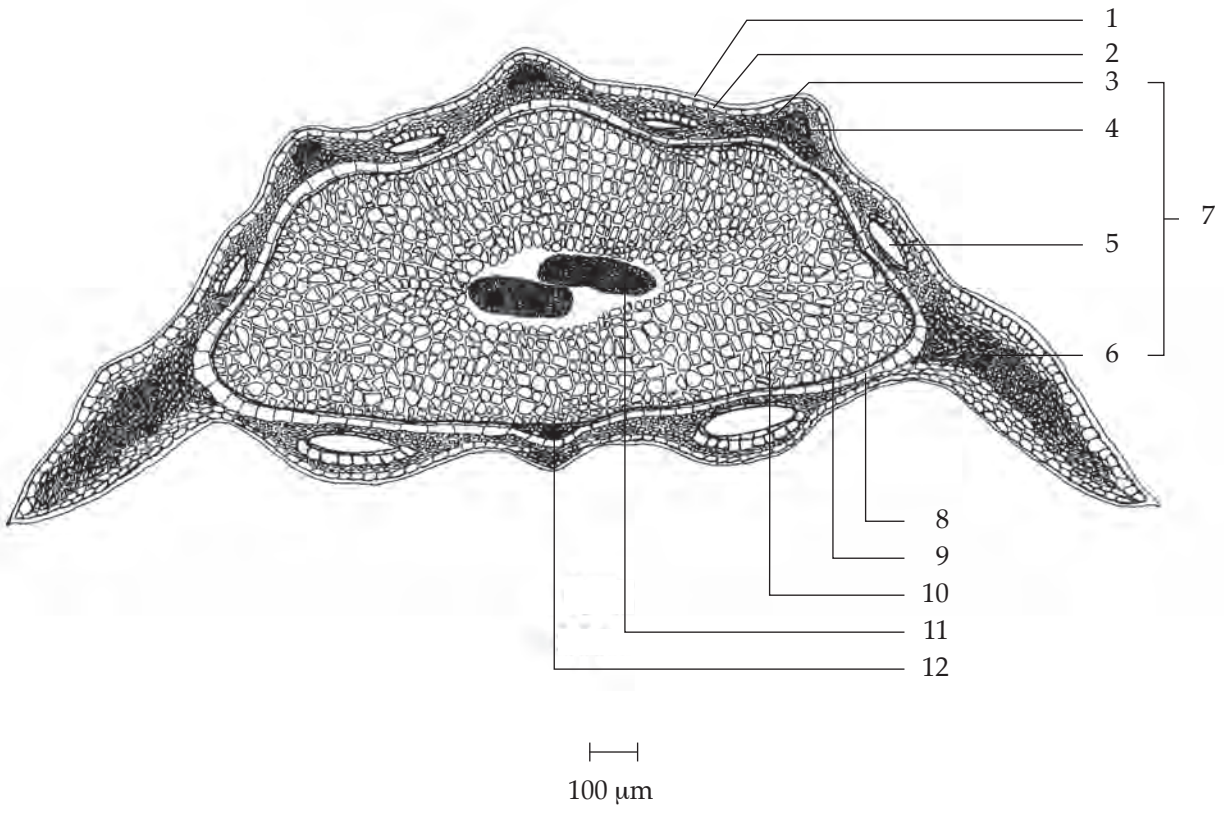


Fig. 2a Transverse Section of the Mericarp of *Anethum graveolens* L.

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

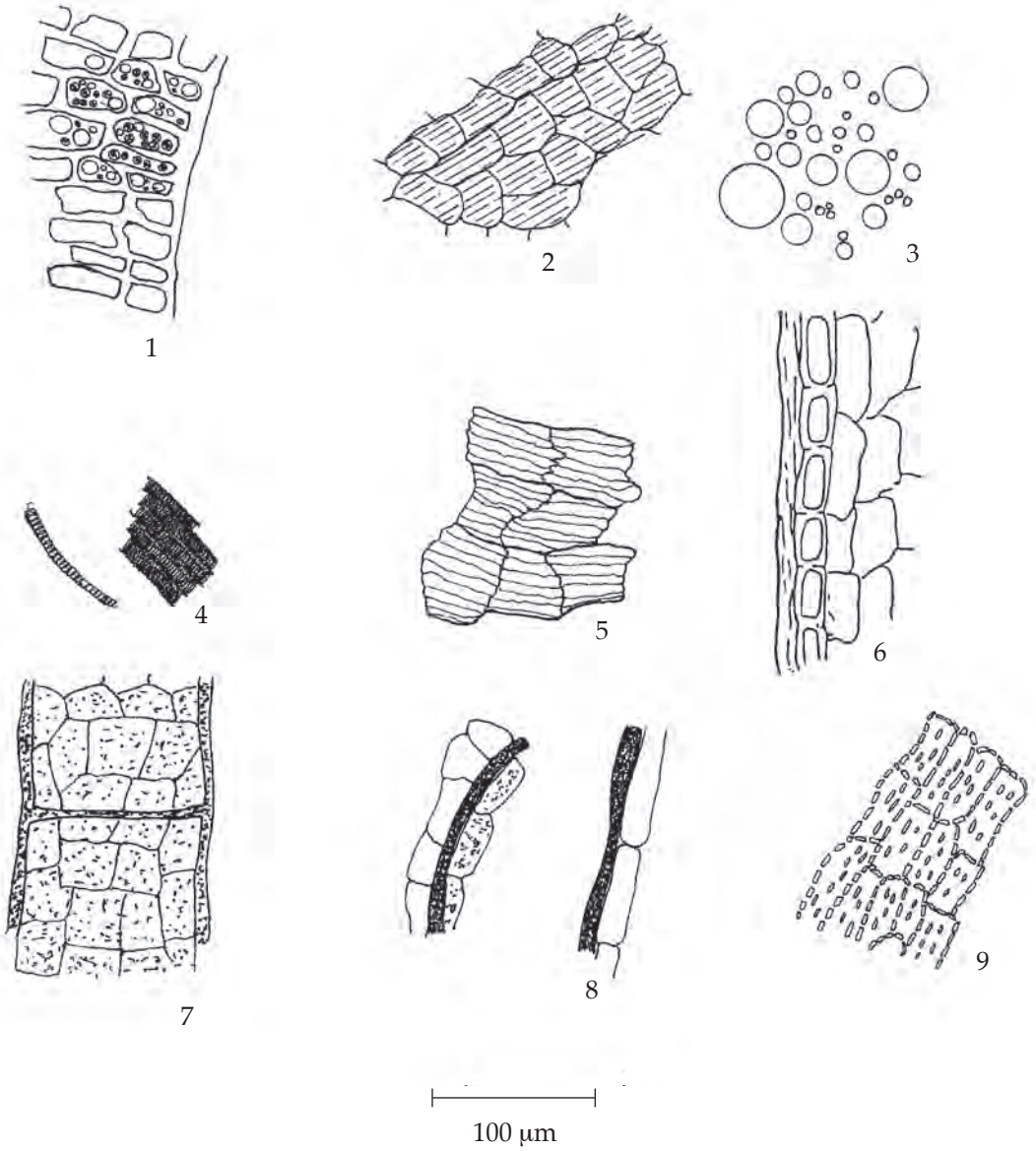


Fig. 2b Powdered Drug of the Fruits of *Anethum graveolens* L.

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. Several 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 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.

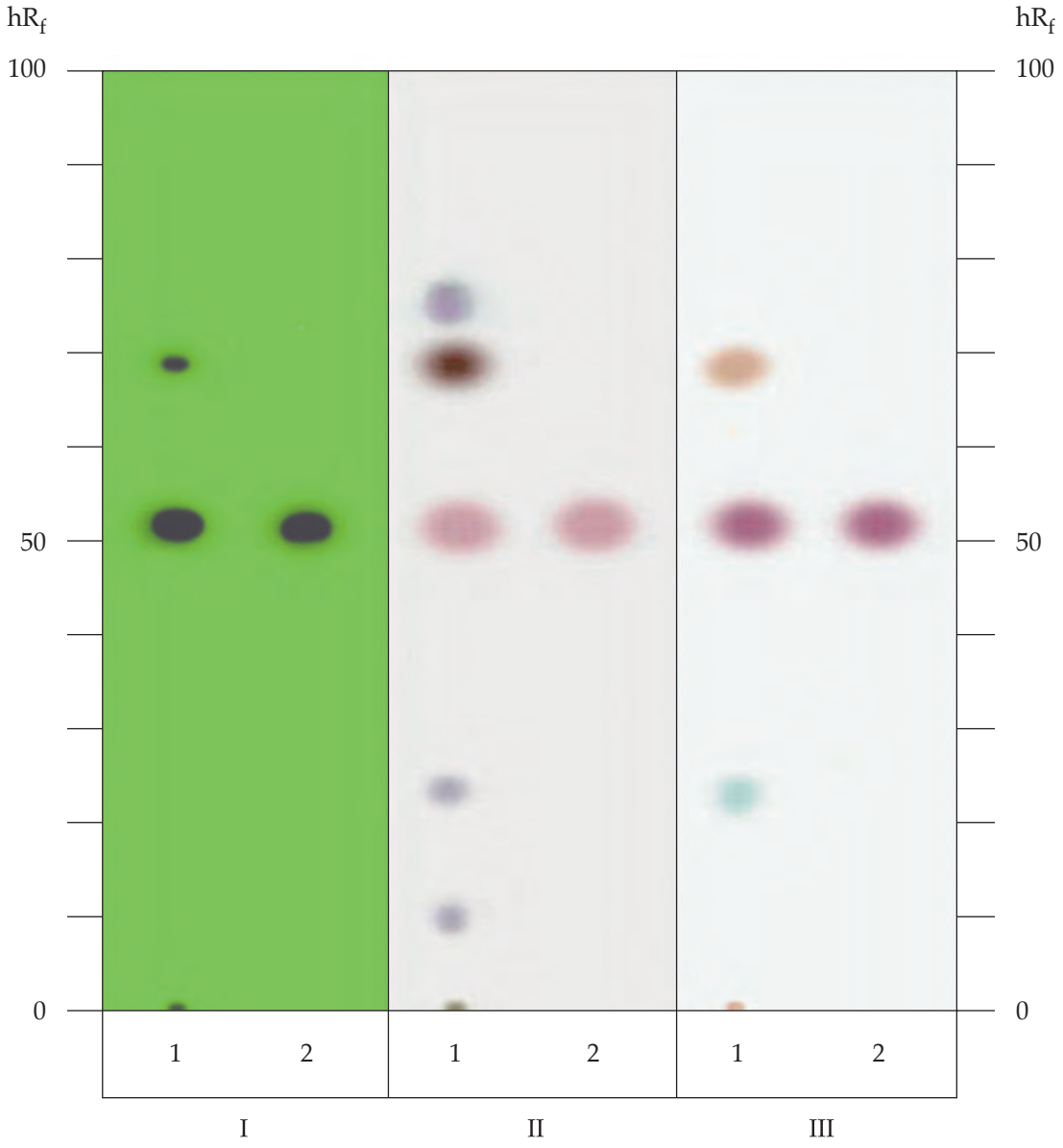


Fig. 3 Thin-Layer Chromatogram of Ethanolic Extract of the Fruits of *Anethum graveolens* L.

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 tall; taproot 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; pedicel 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; stamens 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.

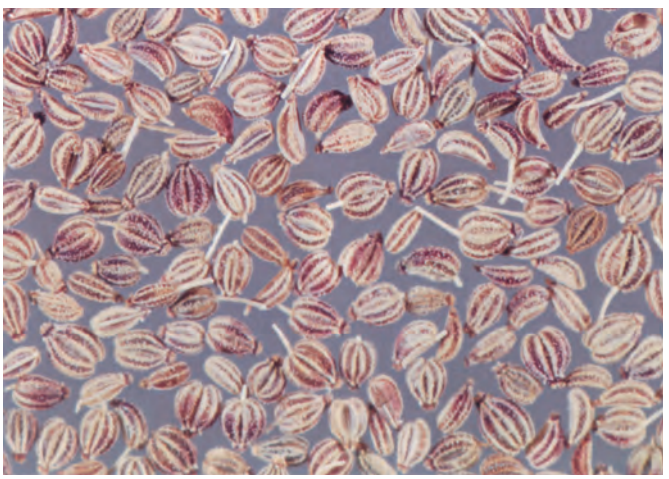
Microscopical (Figs. 2a, 2b) Transverse section of the mericarp through the cotyledon shows an epicarp layer, epidermal cells covered with striated cuticle layer, numerous thick-walled papillae of small and large sizes. Large papilla, surrounded by about 6 small papillae. Mesocarp, several layers of elongated parenchyma 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. Cotyledon, parenchyma cells with aleurone grains and oil globules.

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

1

2

3



2 mm

4

Fig. 1a *Trachyspermum ammi* (L.) Sprague
1. habit 2. inflorescences 3. closed-up of flowers 4. crude drug

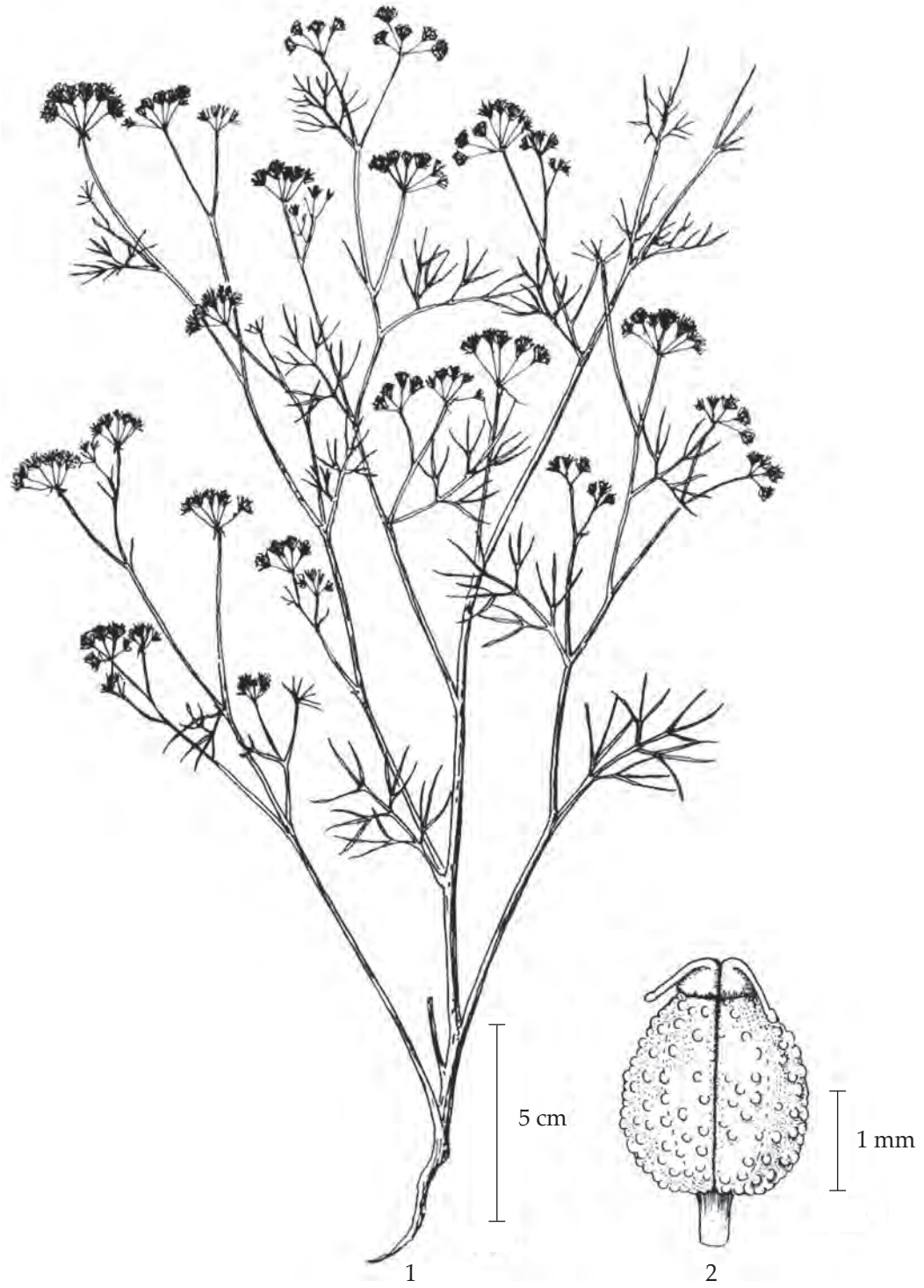


Fig. 1b *Trachyspermum ammi* (L.) Sprague
1. part of plant showing inflorescences and infructescences 2. fruit

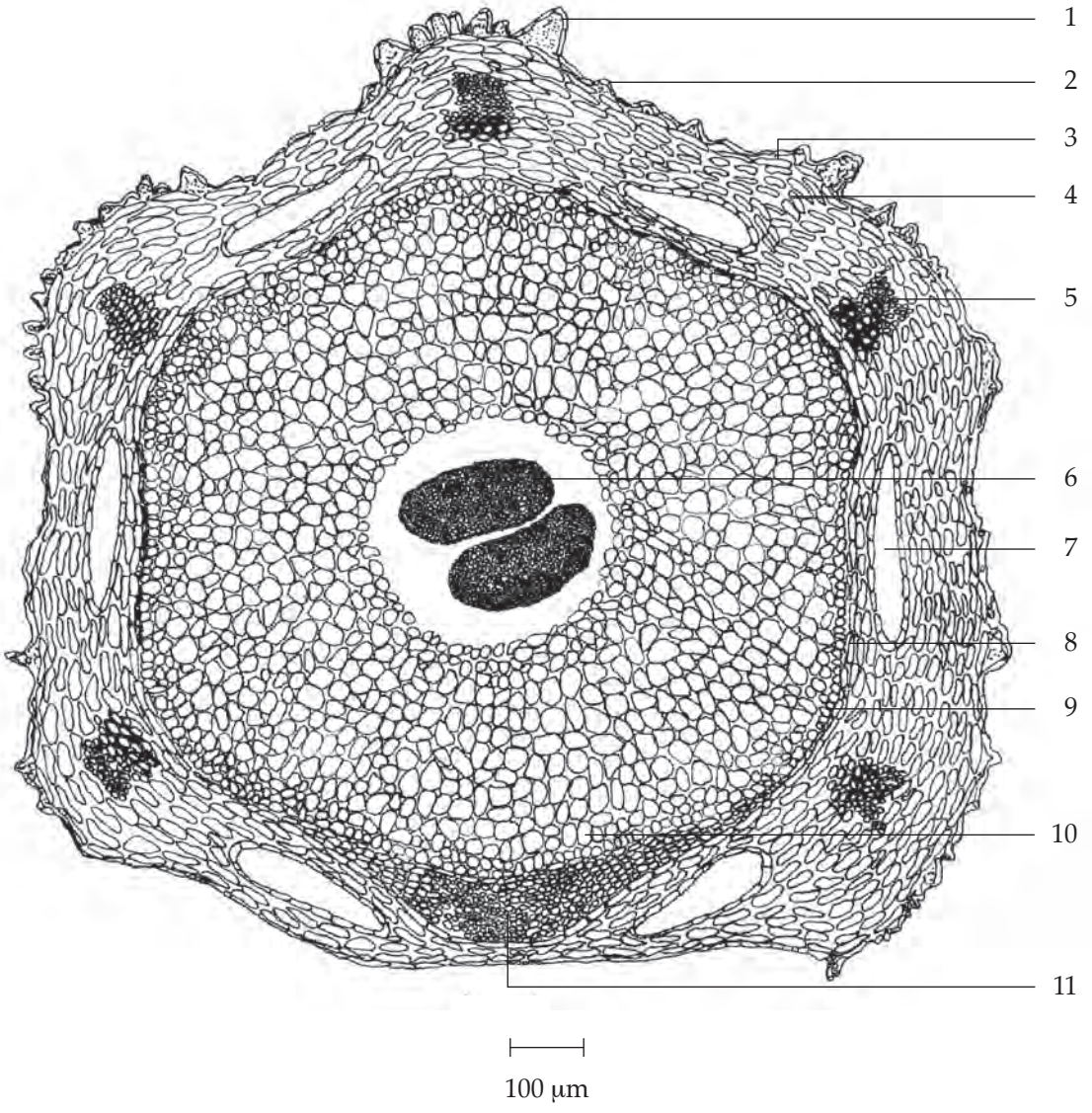


Fig. 2a Transverse Section of the Mericarp of *Trachyspermum ammi* (L.) Sprague

1. large papilla
2. small papilla
3. epicarp
4. parenchyma of mesocarp
5. vascular bundle
6. cotyledon
7. vitta
8. endocarp
9. seed coat
10. endosperm
11. raphe

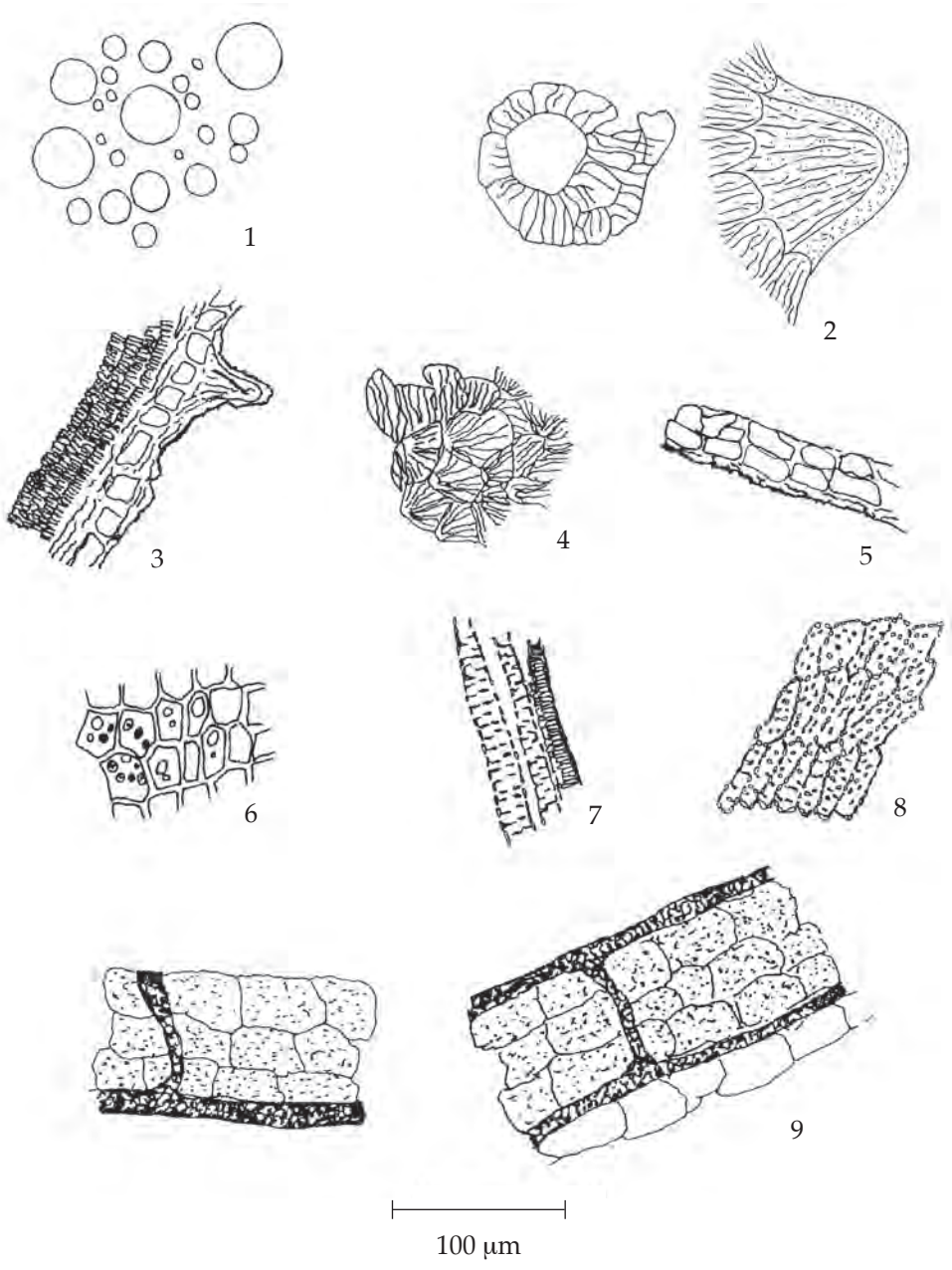


Fig. 2b Powdered Drug of the Fruits of *Trachyspermum ammi* (L.) Sprague

1. oil globules
2. large papilla surrounded by small papillae
3. epicarp in sectional view with 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). Several other 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.

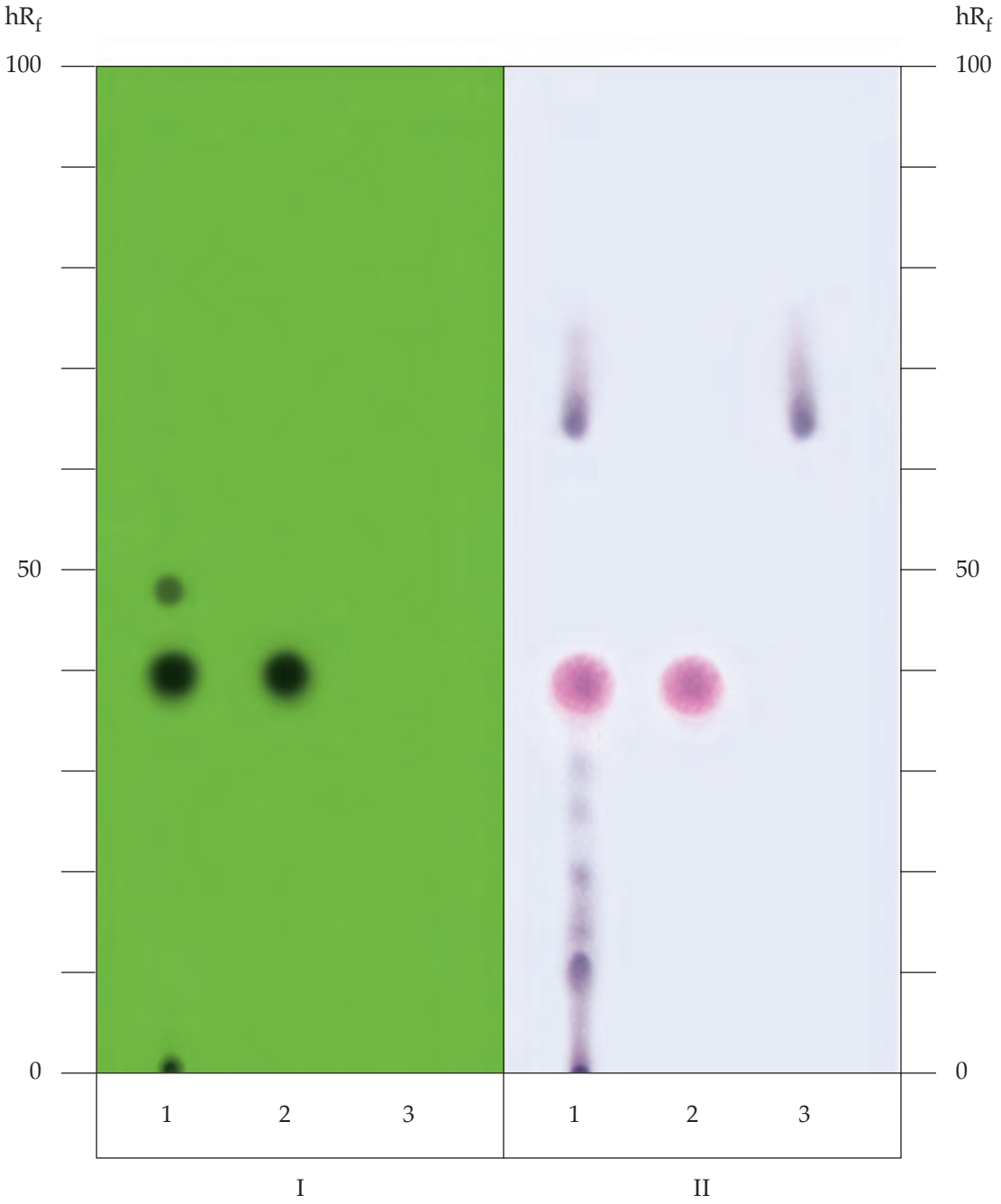


Fig. 3 Thin-Layer Chromatogram of Ethanolic Extract of the Fruits of *Trachyspermum ammi* (L.) Sprague

1 = solution (A)
2 = solution (B)
3 = solution (C)
I = detection under UV light (254 nm)
II = detection with *vanillin-sulfuric acid TS1*

ทิ้งถ่อน (THING THON)

ถ่อน (THON)
Albiziae Procerae Cortex
Forest Siris Bark

Synonyms Rain Siris Bark, White Siris Bark

Category Carminative, stomachic.

Forest Siris Bark is the dried stem bark of *Albizia procera* (Roxb.) Benth. [*Acacia procera* (Roxb.) Willd, *Mimosa procera* Roxb.] (Family Leguminosae-Mimosoideae), Herbarium Specimen Number: DMSC 5251, Crude Drug Number: DMSc 1177.

Constituents Forest Siris Bark contains phenolics (e.g., (+)-catechin, protocatechuic acid). It also contains flavonoids (e.g., quercetin, rutin), triterpenoids, saponins, tannins, etc.

Description of the plant (Fig. 1) Tree up to 15 m tall; bark pale greyish green, yellowish brown to brown, rough; inner bark pink; branchlet terete, glabrous. Leaves bipinnate; rachis 10 to 45 cm long, with narrowly elliptic gland 0.6 to 1 cm long at base; pinnae 2 to 5 pairs, 12 to 20 cm long; leaflets (4–)5 to 11(–16) pairs, opposite; petiolule very short; blade ovate to subrhomboid, 3 to 4.5 cm long, 1.2 to 2.2 cm wide, apex obtuse, rounded, emarginate with a tiny mucro, base asymmetrical, truncate or cuneate, chartaceous, faintly puberulous on both surfaces. Inflorescence panicle, 15 to 30 peduncled heads, axillary or terminal, up to 30 cm long; peduncle 1.5 to 2.5 cm long. Flower sessile, uniform; calyx 2 to 3 mm long, tubular to narrowly funnel-shaped, glabrous, lobes 5-toothed, 0.7 to 1.2 mm long; corolla whitish, funnel-shaped, 6 to 6.5 mm long, tube glabrous, lobes 5, elliptic, 2 to 2.5 mm long, acute and puberulous at apex; stamens numerous; lower part of filament united into staminal tube, longer than corolla tube; ovary superior, subsessile, glabrous. Fruit dehiscent pod, flat, 10 to 20 cm long, 1.5 to 2.5 cm wide, shortly stalked, reddish brown to brown, glabrous, with distinct marks over the seed. Seeds 6 to 12, elliptic to obovoid, about 7.5 mm long, about 4.5 mm wide, flat.

Description Odour, mild; taste, slightly bitter.

Macroscopical (Fig. 1) Bark, irregularly shaped; outer part rough, yellowish brown to brown; middle part, pinkish; inner part smooth, yellowish to straw-coloured.

Microscopical (Figs. 2a, 2b) Transverse section of the stem bark shows outer and inner barks. Outer bark: layers of yellow thin-walled rectangular cork cells; groups of lignified sclereids, some containing tannin mass and resinous substance; and parenchyma, some containing tannin mass and resinous substance. Inner bark: phloem parenchyma, some containing starch grains, prismatic crystals, oil droplets, resinous substance, and tannin mass; sieve tube; phloem rays, some containing starch grains; and phloem fibres.

Forest Siris Bark in powder possesses the diagnostic microscopical characters of the unground drug. Prismatic sheath and various shapes and sizes of sclereids, some containing tannin mass and resinous substance, are frequently observed.

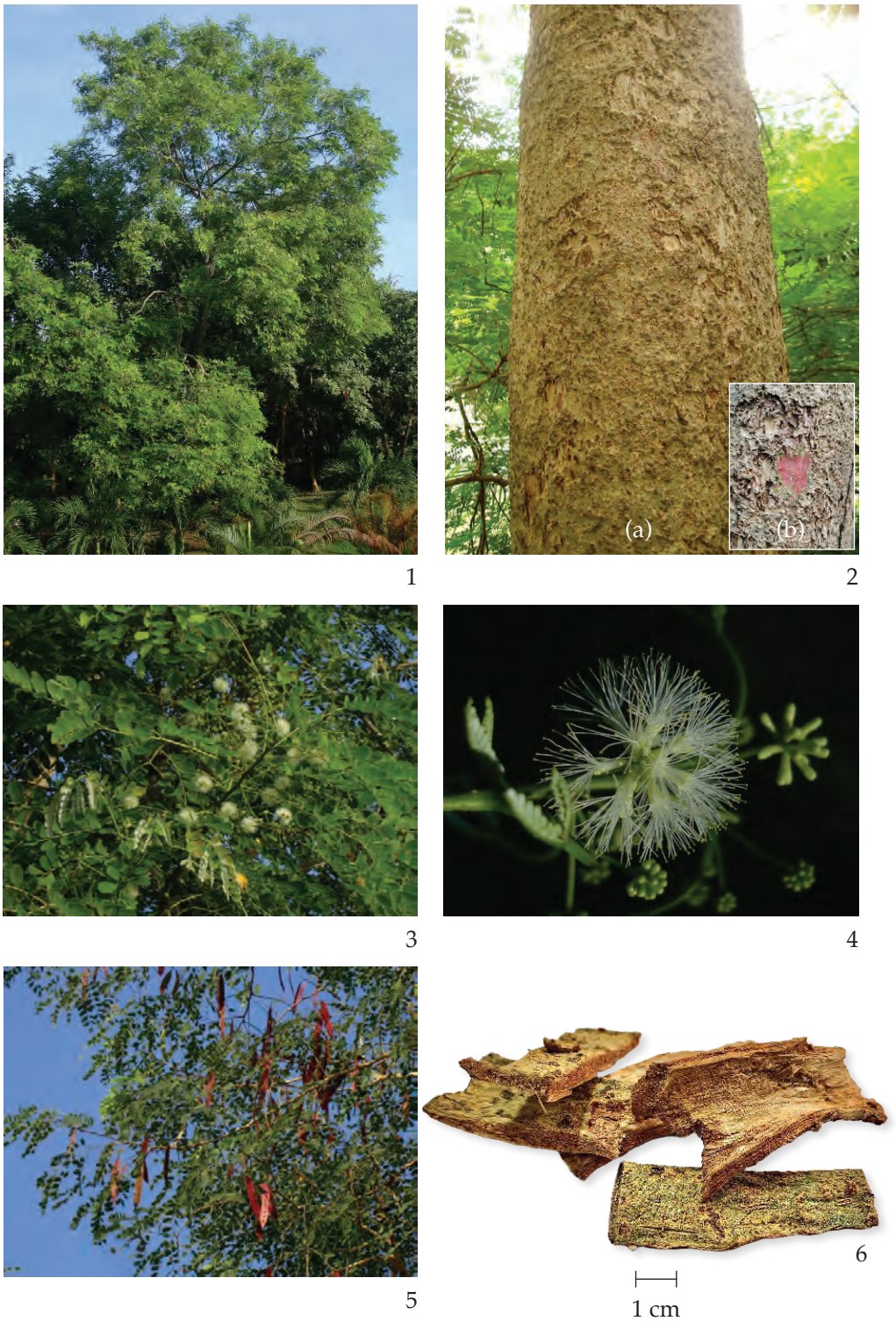


Fig. 1 *Albizia procera* (Roxb.) Benth.
1. habit 2. stem bark (a), showing pink inner bark (b) 3. flowering branch
4. flowering heads 5. fruiting branches 6. crude drug

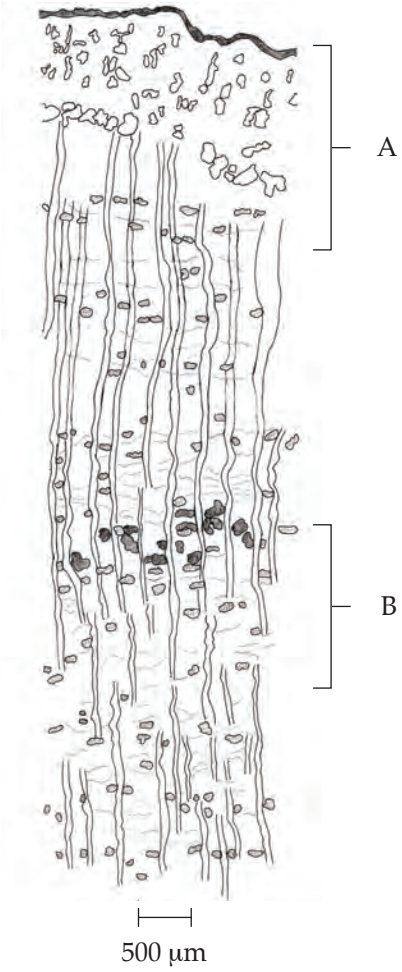


Diagram of the Stem Bark

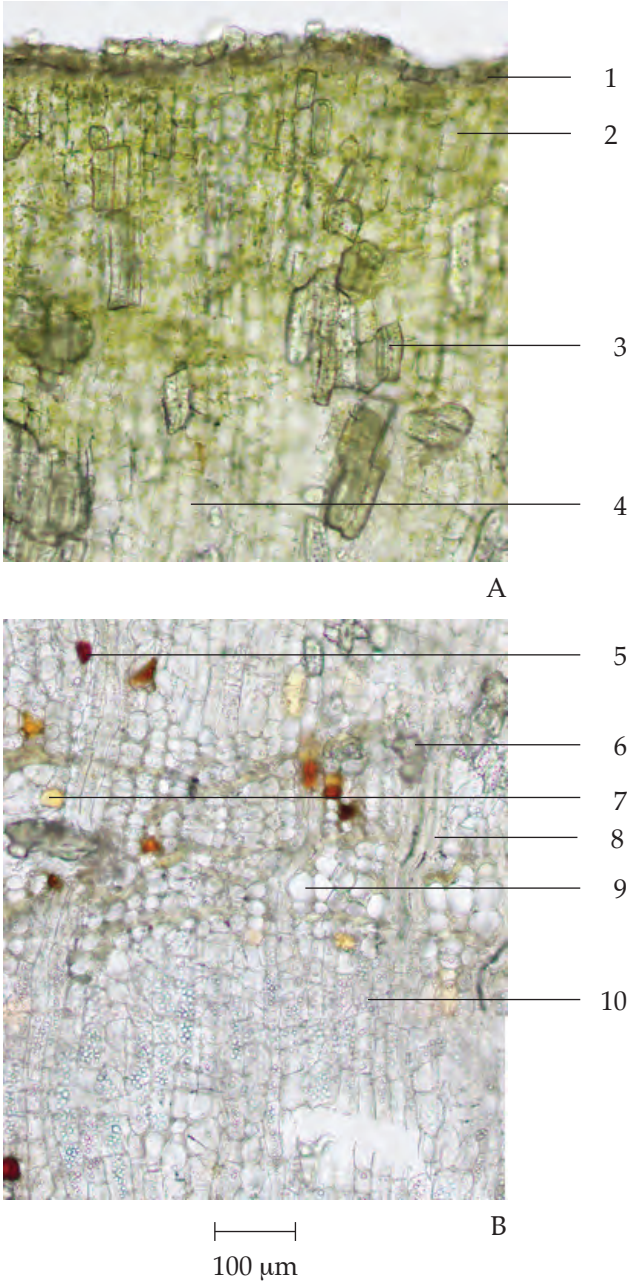


Fig. 2a Photomicrographs of Transverse Section of the Stem Bark of *Albizia procera* (Roxb.) Benth.

A. Outer Bark and Part of Inner Bark
B. Part of Inner Bark

1. cork
2. parenchyma
3. sclereid
4. phloem ray
5. resinous substance
6. prismatic crystal
7. parenchyma containing tannin mass
8. phloem parenchyma
9. sieve tube
10. parenchyma containing starch grains

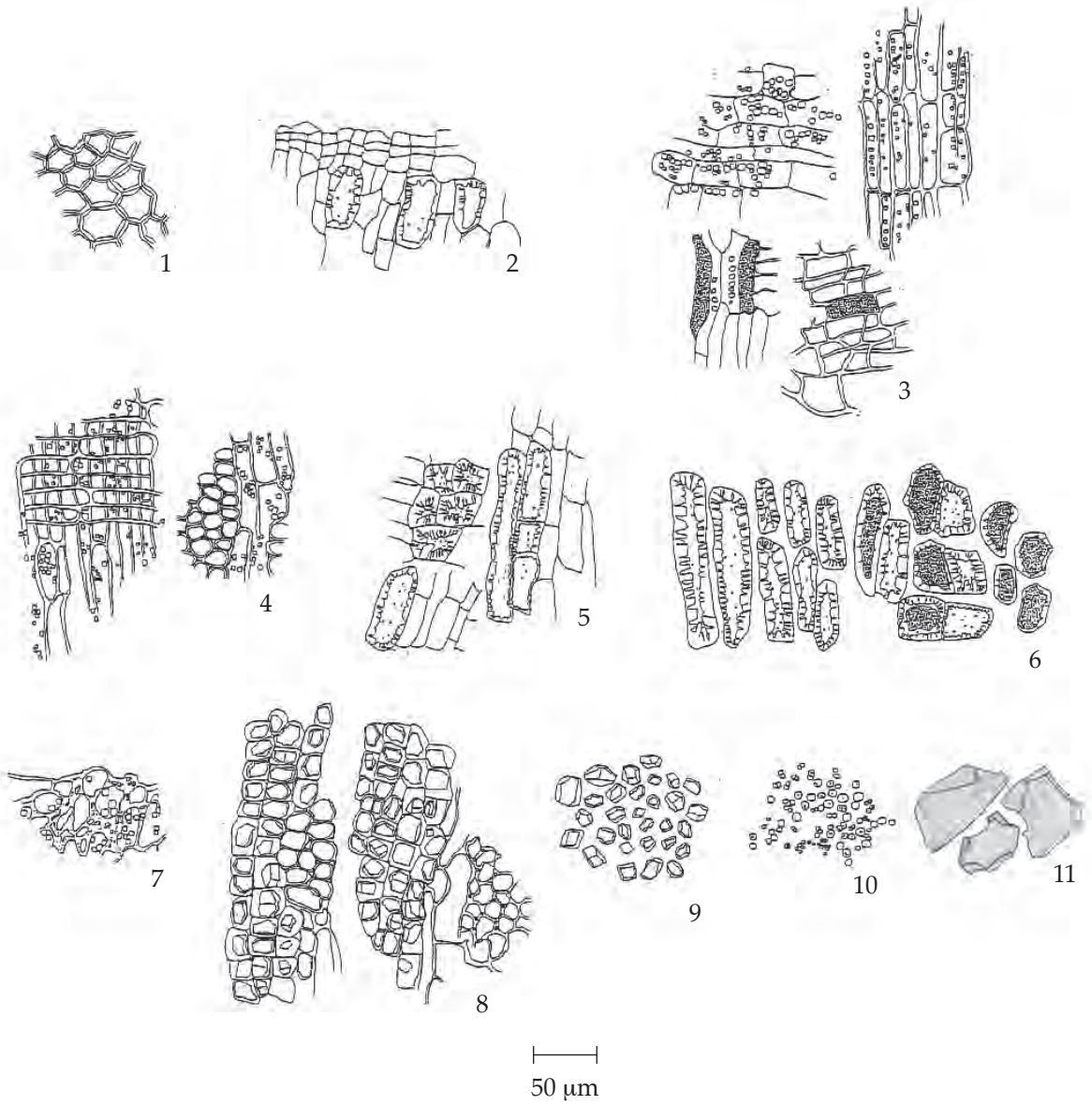


Fig. 2b Powdered Drug of the Stem Barks of *Albizia procera* (Roxb.) Benth.

1. cork in surface view
2. cork, sclereids and parenchyma in sectional view
3. parenchyma, some containing starch grains and tannin mass
4. phloem parenchyma and phloem rays, some containing starch grains
5. sclereids associated with parenchyma
6. sclereids, some containing tannin mass
7. phloem parenchyma, some containing starch grains, and collapsed cells in sectional view
8. prismatic sheaths
9. prismatic crystals
10. simple and compound starch grains
11. resinous substance

Packaging and storage Forest Siris Bark 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 *fine powder*, with 10 mL of *methanol* for 15 minutes and filter. To 2 mL of the filtrate, add 1 or 2 pieces of *magnesium ribbon*, mix with 4 or 5 drops of *hydrochloric acid* and warm on a water-bath for 5 minutes: a red colour develops.

B. Heat 500 mg of the sample, in *fine powder*, with 10 mL of *water* on a water-bath for 15 minutes and filter. Shake 5 mL of the filtrate in a screw-capped tube for 15 seconds: a persisting foam is produced for over 30 minutes.

C. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1), using *silica gel F254* as the coating substance and a mixture of 50 volumes of *toluene*, 50 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 as bands of 8 mm, 5 μL of solution (A) and 1 μL of solution (B). Prepare solution (A) by refluxing 500 mg of the sample, in *fine powder*, with 10 mL of a mixture of equal volumes of *ethyl acetate* and *methanol* for 10 minutes and filtering. Evaporate the filtrate to dryness and dissolve the residue in 2 mL of *methanol*. For solution (B), dissolve 1 mg of (+)-*catechin hydrate* in 1 mL of *methanol*. After removal of the plate, allow it to dry in air and examine the plate under ultraviolet light (254 nm), marking the quenching bands. The chromatogram obtained from solution (A) shows a quenching band (hR_f value 30 to 40) corresponding to the (+)-catechin hydrate band from solution (B); other quenching bands are also observed. Subsequently spray the plate with *anisaldehyde TS* and heat at 105° for 3 minutes; the band due to (+)-catechin hydrate is yellowish brown. Several other bands of different colours are observed. (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in a Mixture of Ethyl Acetate and Methanolic Extract of the Stem Barks of *Albizia procera* (Roxb.) Benth.

Band	hR_f Value	Detection	
		UV 254	*Anisaldehyde TS*
1	5–7	quenching	brown
2	12–18	–	violet
3*	30–40	quenching	yellowish brown
4	45–55	–	violet
5	78–85	–	pale purple
6	85–95	–	purple

*(+)-catechin hydrate

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).

Total ash Not more than 11.0 per cent w/w (Appendix 7.7).

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

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

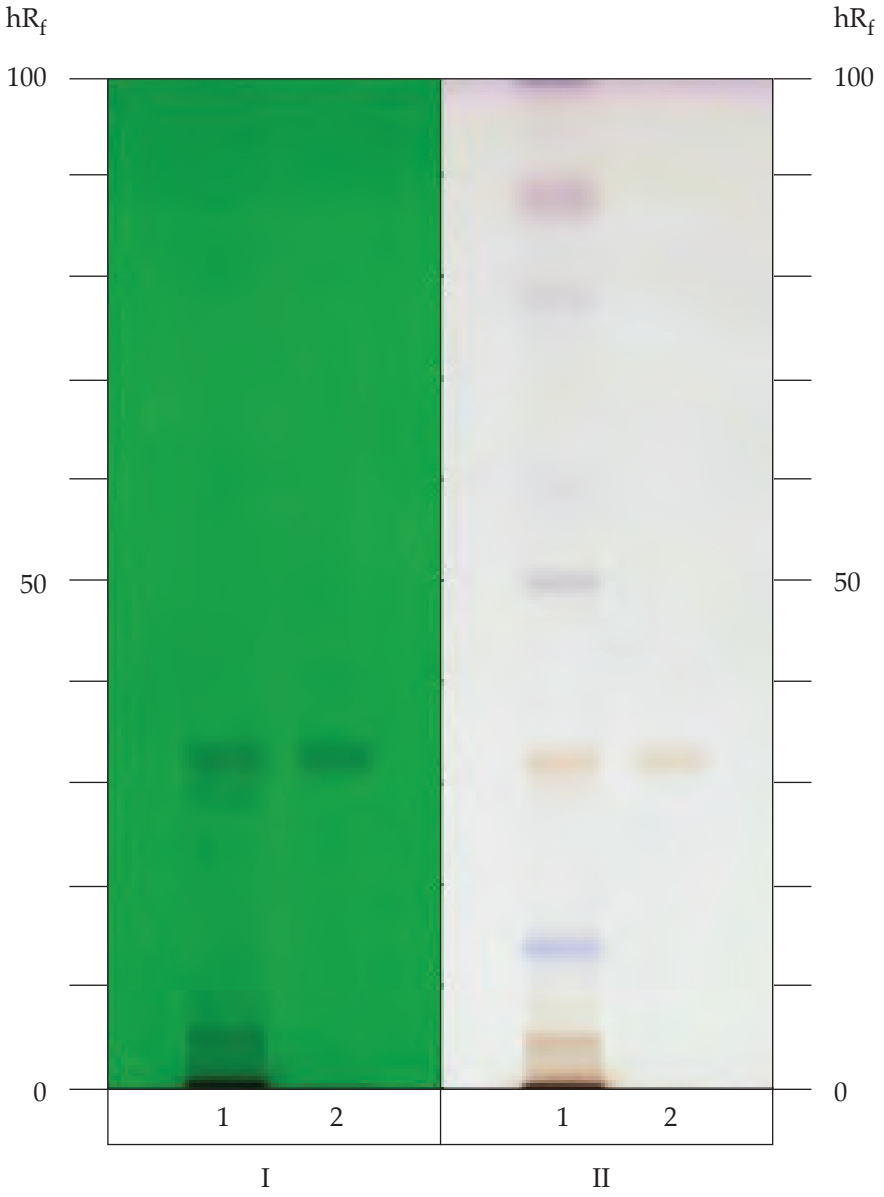


Fig. 3 Thin-Layer Chromatogram of a Mixture of Ethyl Acetate and Methanolic of the Stem Barks of *Albizia procera* (Roxb.) Benth.

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

ว่านน้ำ (WANNAM)

Acori Calami Rhizoma
Sweet Flag Root

Synonyms Acorus Root, Calamus Root

Category Carminative.

Sweet Flag Root is the dried rhizome of *Acorus calamus* L. (Family Acoraceae), Herbarium Specimen Number: DMSC 890.

Constituents Sweet 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 sesquiterpenoids (e.g., acoragermacrone, acolamone and iso-acolamone), 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. Fruit baccate.

Description Odour, sweet and aromatic; taste, bitter and pungent.

Macroscopical (Fig. 1a) The rhizome is longitudinally splitted into subcylindrical pieces, 5 to 20 cm long, 0.5 to 1.5 cm in diameter. The unpeeled drug is covered in a thin brown cork and is much shrunken and deeply longitudinally wrinkled. 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, surrounded 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.

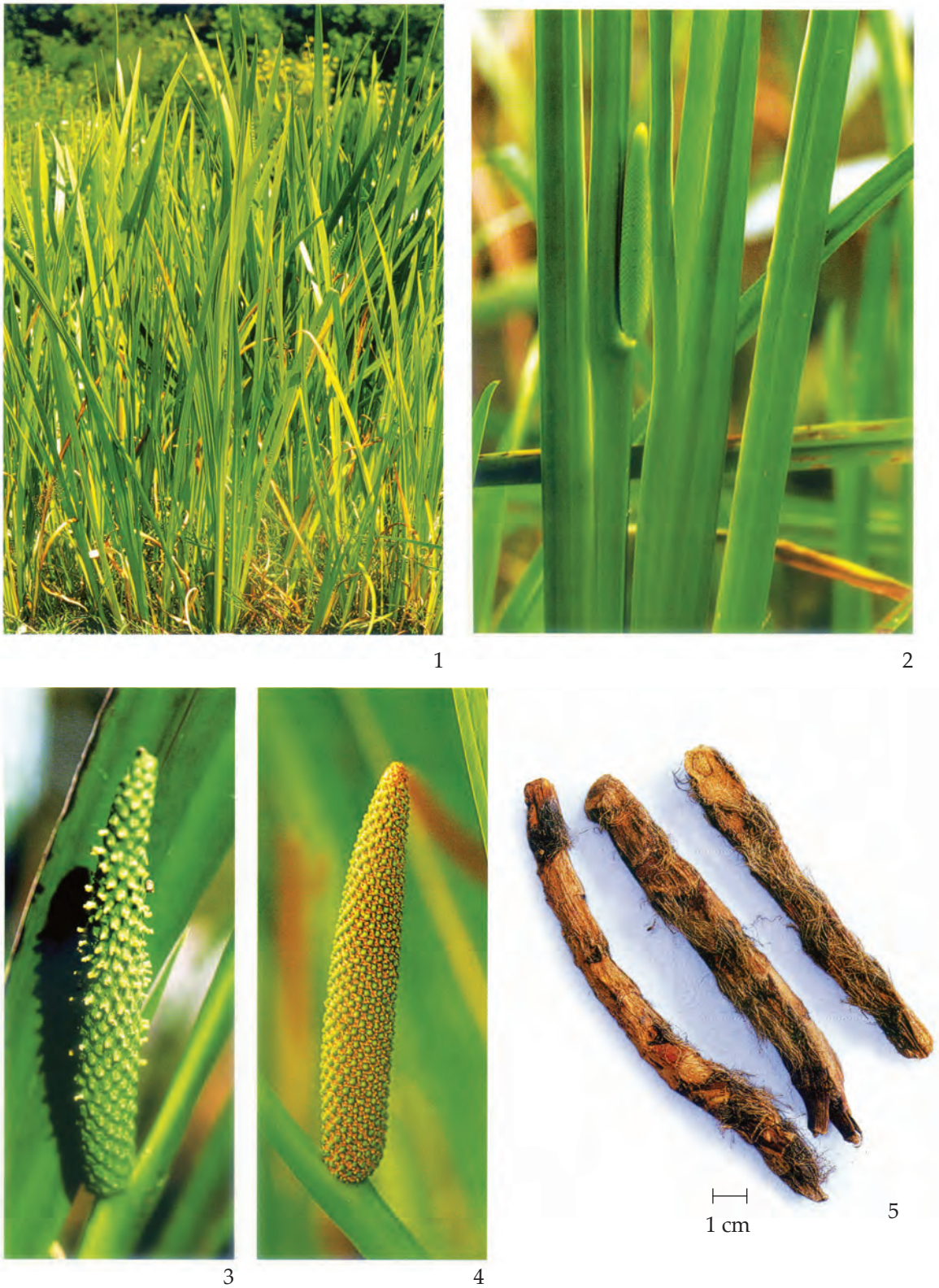


Fig. 1a *Acorus calamus* L.
1. habit 2. juvenile spadix 3. flowering spadix 4. fruiting spadix 5. crude drug

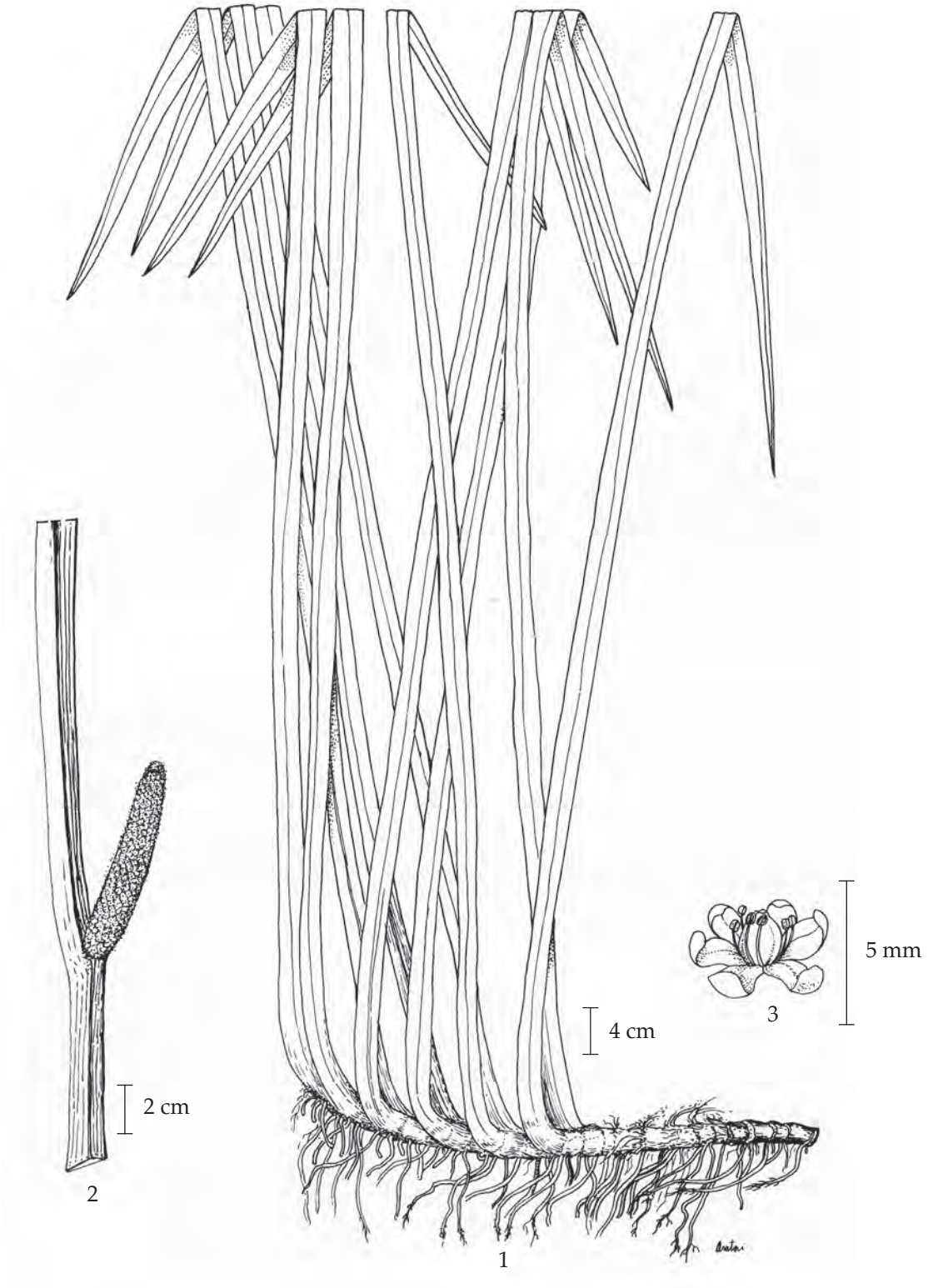
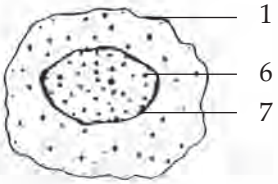
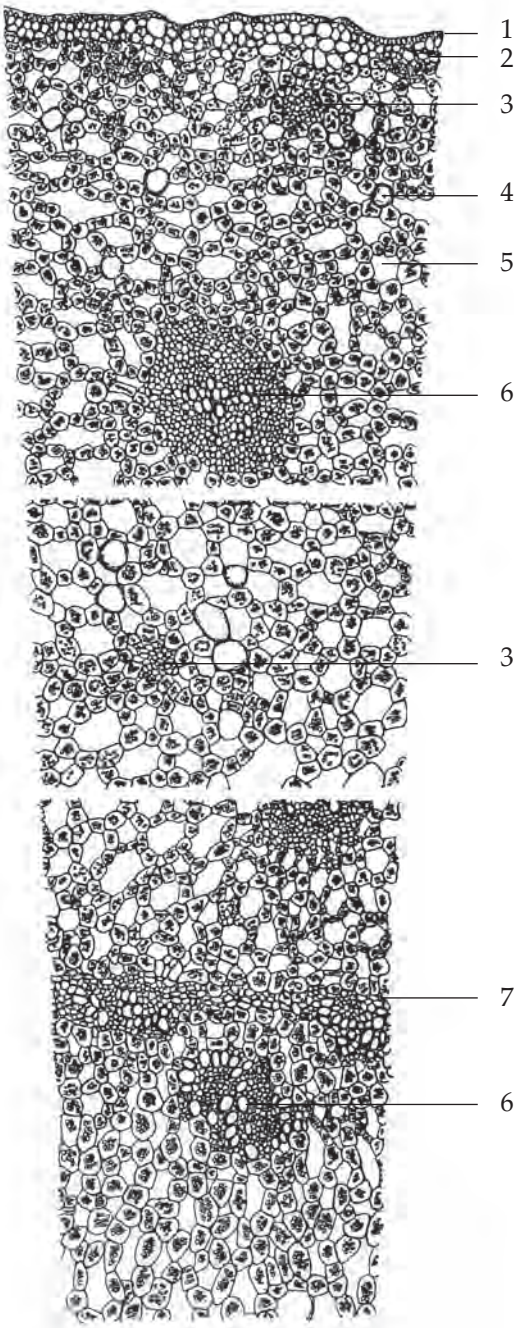


Fig. 1b *Acorus calamus* L.
1. habit 2. flowering spadix 3. flower



5 mm

Diagram of the Rhizome



50 μm

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

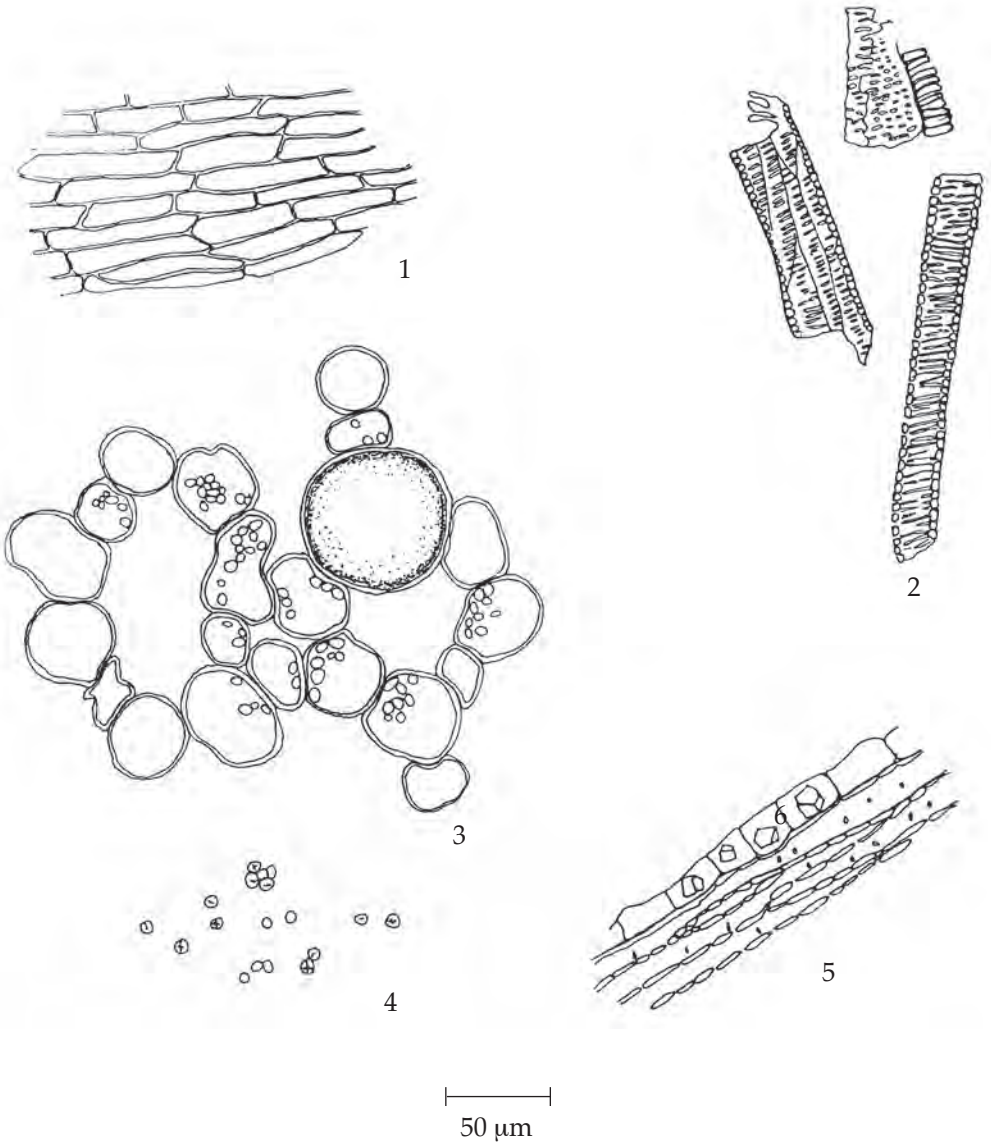


Fig. 2b Powdered Drug of the Rhizomes of *Acorus calamus* L.

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. Several Other 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.

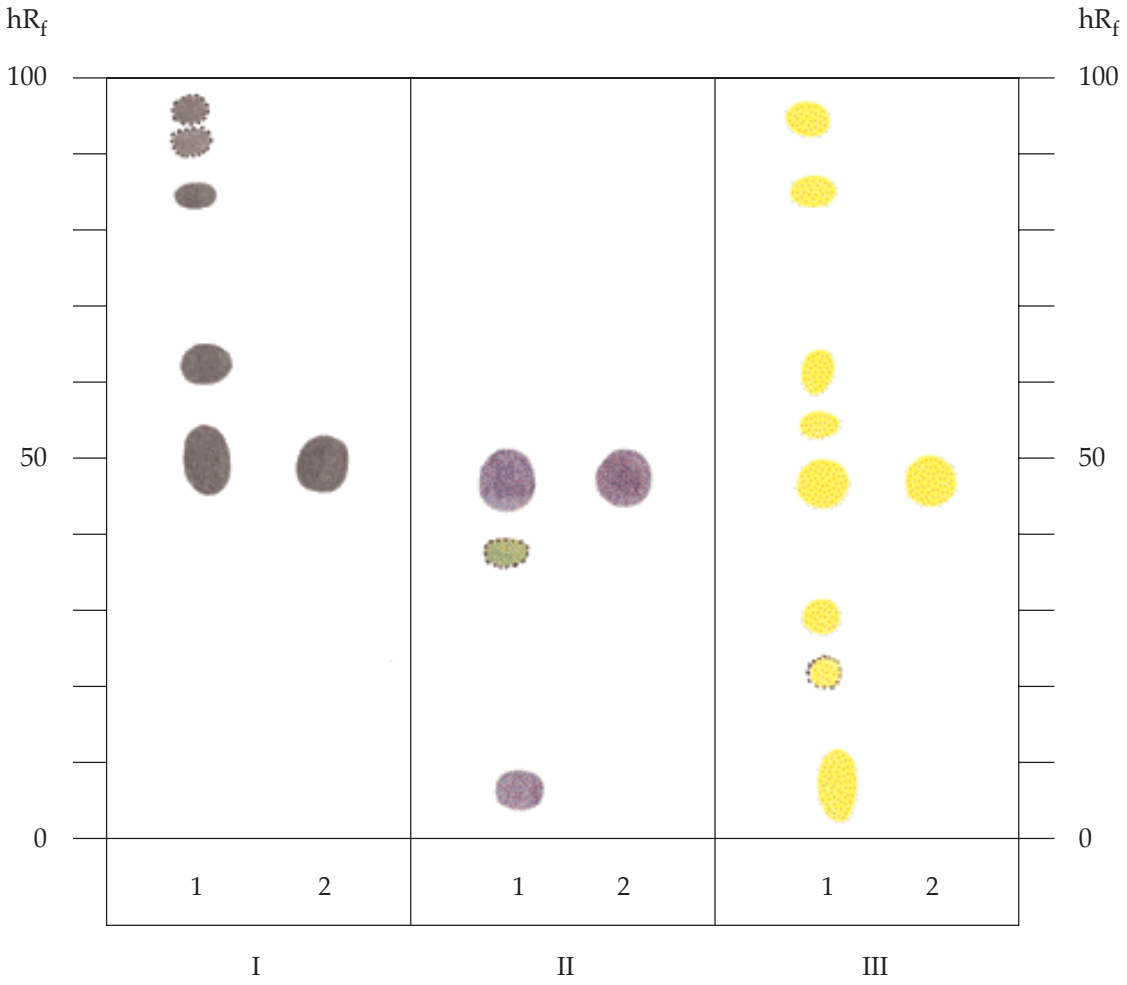


Fig. 3 Thin-Layer Chromatogram of Ethanolic Extract of the Rhizomes of *Acorus calamus* L.

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

หญ้าดอกขาว (YA DOK KHAO)

หมอน้อย (MO NOI), หญ้าละออง (YA LA-ONG)
Cyanthillii Cinerei Herba
Little Ironweed Herb

Category Smoking cessation aid**.**

Little Ironweed Herb is the dried aerial part of *Cyanthillium cinereum* (L.) H. Rob. [*Conyza cinerea* L., *Vernonia cinerea* (L.) Less.] (Family Compositae), Herbarium Specimen Number: DMSC 5258, Crude Drug Number: DMSc 1171.

Constituents Little Ironweed Herb contains triterpenoids (e.g., lupeol), flavonoids and sesquiterpene lactones (e.g., hirsutinolides, vercinolides). It also contains nicotine, nitrates, nitrites, potassium salts, sterols, etc.

Description of the plant (Fig. 1) Herb, annual to perennial, up to 1 m tall; stem erect, conspicuously ribbed, pubescent. Leaves simple, spirally arranged, lanceolate or ovate to broadly ovate, 3.5 to 6.5(−10) cm long, 1.5 to 3 cm wide, upper leaves gradually smaller, apex acute to acuminate or obtuse, base cuneate or attenuate, margin serrate or undulate-serrulate, upper surface puberulous, lower surface greyish pubescent to tomentose, especially on nerves, glandular, lateral veins 5 to 7 pairs; petiole 1 to 2.5 cm long, often winged. Inflorescence terminal and axillary, broadly paniculate; capitulum campanulate, 5 to 8 mm long; peduncle 0.3 to 1 cm long; involucres campanulate, 4 to 5 mm long, 2.5 to 3 mm wide; phyllaris green with purple apex, 3- to 4-seriate, imbricate, linear to oblong or lanceolate, 1.5 to 2 mm long, apex acute to acuminate, outer surface sericeous glandular. Florets 25 to 30; corolla pink to purple, rarely white, funnel-form, 4.5 to 6 mm long, puberulous, glandular, basal tube 3 to 3.5 mm long, lobes 5, 1 to 2 mm long; anther short, apical appendage acute, base obtuse; style purple, about 3 mm long, 2-branched, branches about 0.5 mm long, inner surface with stigmatic papillae, outer surface with sweeping hairs. Fruit achene, clavate, 1.5 to 2 mm long, about 0.7 mm wide, densely hairs and glandular dotted; pappus bristles, whitish, 2-seriate, 3 to 5 mm long, persistent.

Description Odour, characteristic; taste, mild.

Macroscopical (Fig. 1) A mixture of broken, crisp, cylindrical, ribbed stems, flowering heads, whitish fruiting heads with bristled pappi, and broken dark-green lanceolate to broadly ovate leaves.

Microscopical (Figs. 2a, 2b, 2c, 2d) Transverse sections of the leaf through the midrib and lamina show upper epidermis, mesophyll, vascular bundle, and lower epidermis. Upper epidermis: a layer of rectangular cells; stomata; trichomes, multicellular uniseriate and T-shaped. Mesophyll: palisade cells, a layer of columnar cells, some containing oil droplets and/or rosette aggregate crystals; spongy cells, irregularly shaped, thin-walled, some containing oil droplets; collenchyma, angular and lamellar, in the upper and lower parts of midrib. Vascular bundle: xylem in the upper part and phloem in the upper and lower parts. Lower epidermis: small rectangular cells; stomata; trichomes, T-shaped, multicellular uniseriate and glandular.

In surface view, upper epidermis of the lamina shows slightly wavy-walled cells, anomocytic stomata, T-shaped and multicellular uniseriate trichomes; lower epidermis illustrates wavy-walled cells, anomocytic stomata, T-shaped, multicellular uniseriate and glandular trichomes.

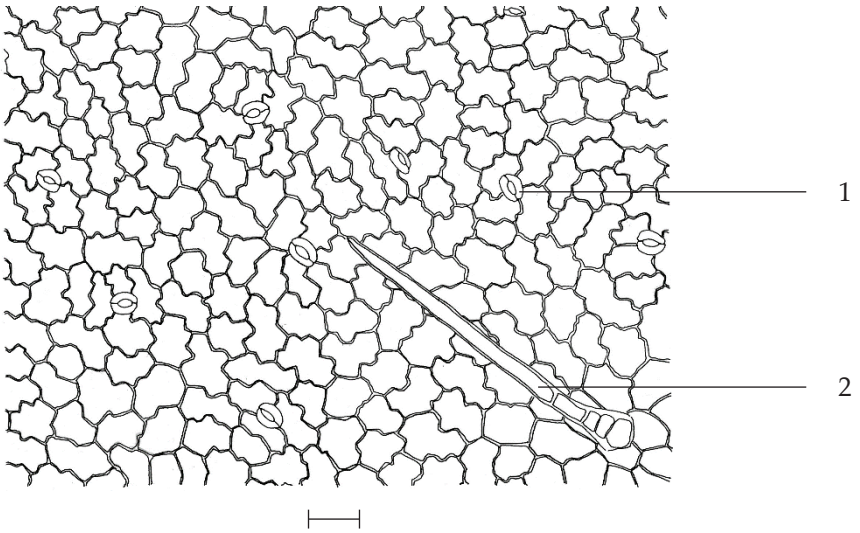
1

2

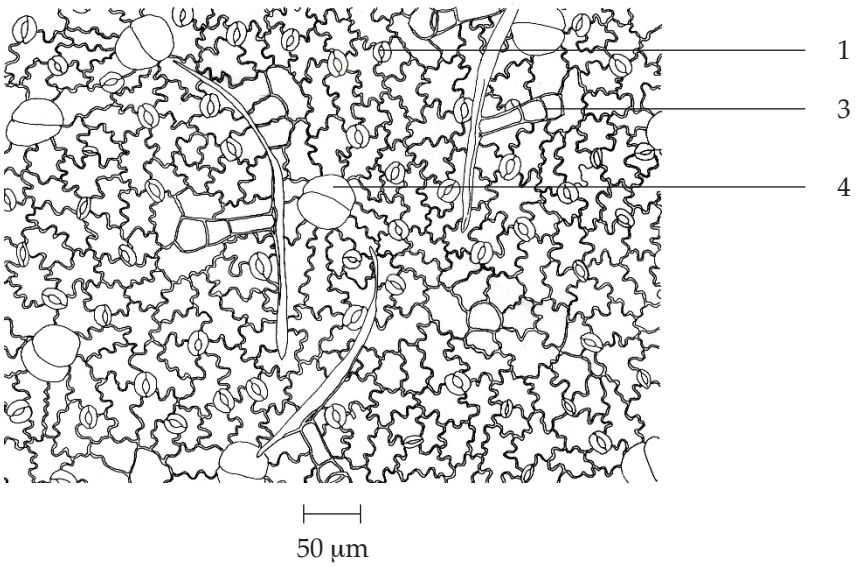
3

4

Fig. 1 *Cyanthillium cinereum* (L.) H. Rob.
1. habit 2. inflorescences 3. capitulum 4. infructescences 5. crude drug



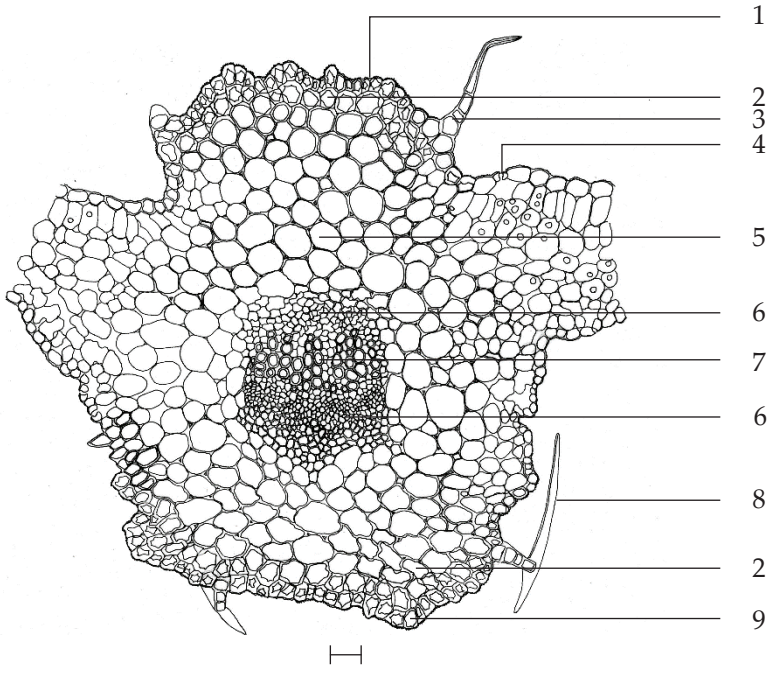
Upper Epidermis of the Lamina



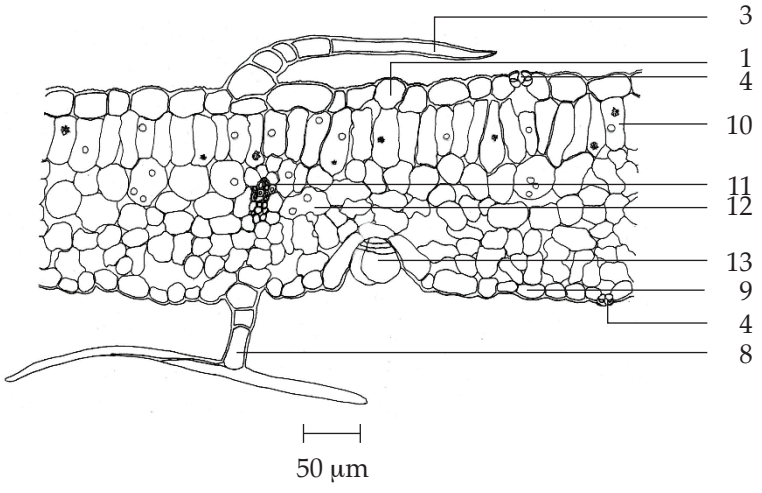
Lower Epidermis of the Lamina

Fig. 2a Epidermises of the Leaf of *Cyanthillium cinereum* (L.) H. Rob.

1. anomocytic stoma
2. multicellular uniseriate trichome
3. T-shaped trichome
4. glandular trichome



Transverse Section of the Midrib



Transverse Section of the Lamina

Fig. 2b Transverse Sections of the Leaf of *Cyanthillium cinereum* (L.) H. Rob.

1. upper epidermis
2. collenchyma
3. multicellular uniseriate trichome
4. stoma
5. parenchyma
6. phloem
7. vessel
8. T-shaped trichome
9. lower epidermis
10. palisade cell containing oil droplet and rossette aggregate
11. vascular tissue
12. spongy cell containing oil droplets
13. glandular trichome

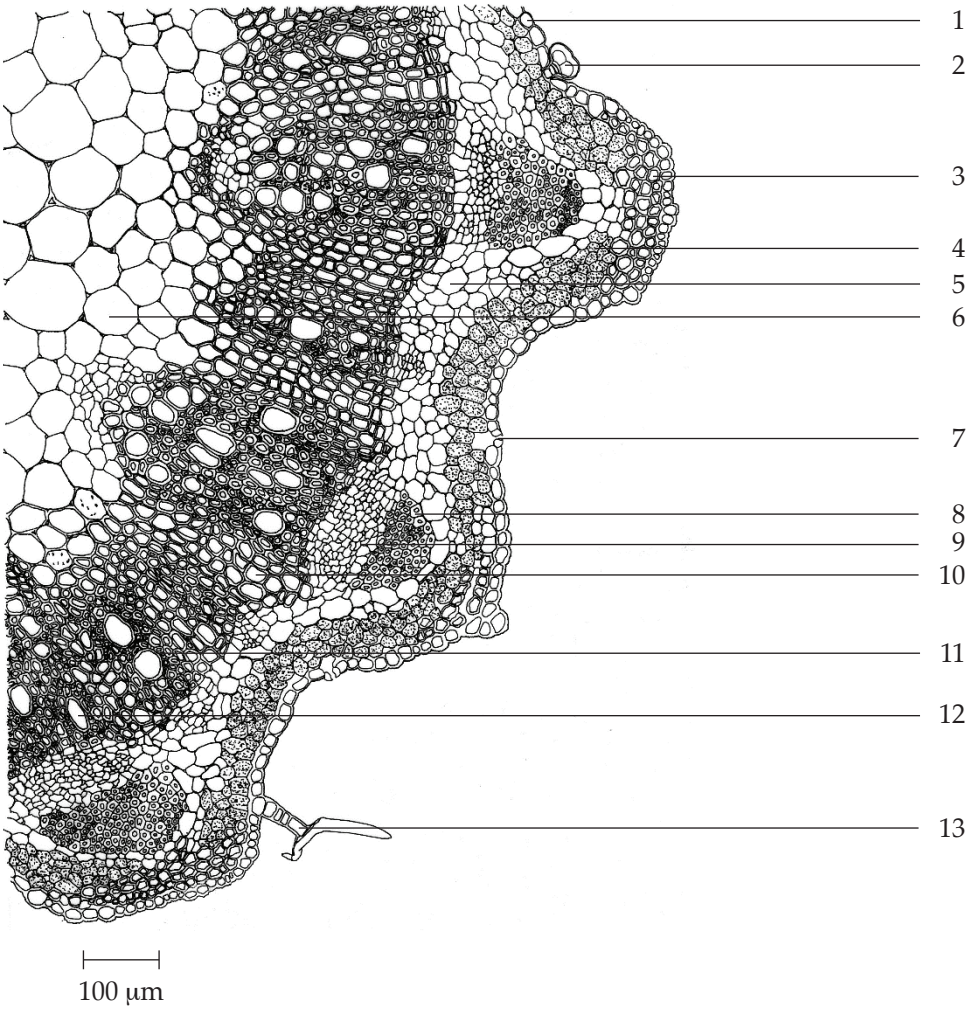


Fig. 2c Transverse Section of the Stem of *Cyanthillium cinereum* (L.) H. Rob.

1. epidermis
2. glandular trichome
3. collenchyma
4. chlorenchyma
5. cortical parenchyma
6. pith parenchyma
7. stoma
8. phloem fibre
9. phloem
10. xylem ray
11. xylem fibre
12. vessel
13. T-shaped trichome

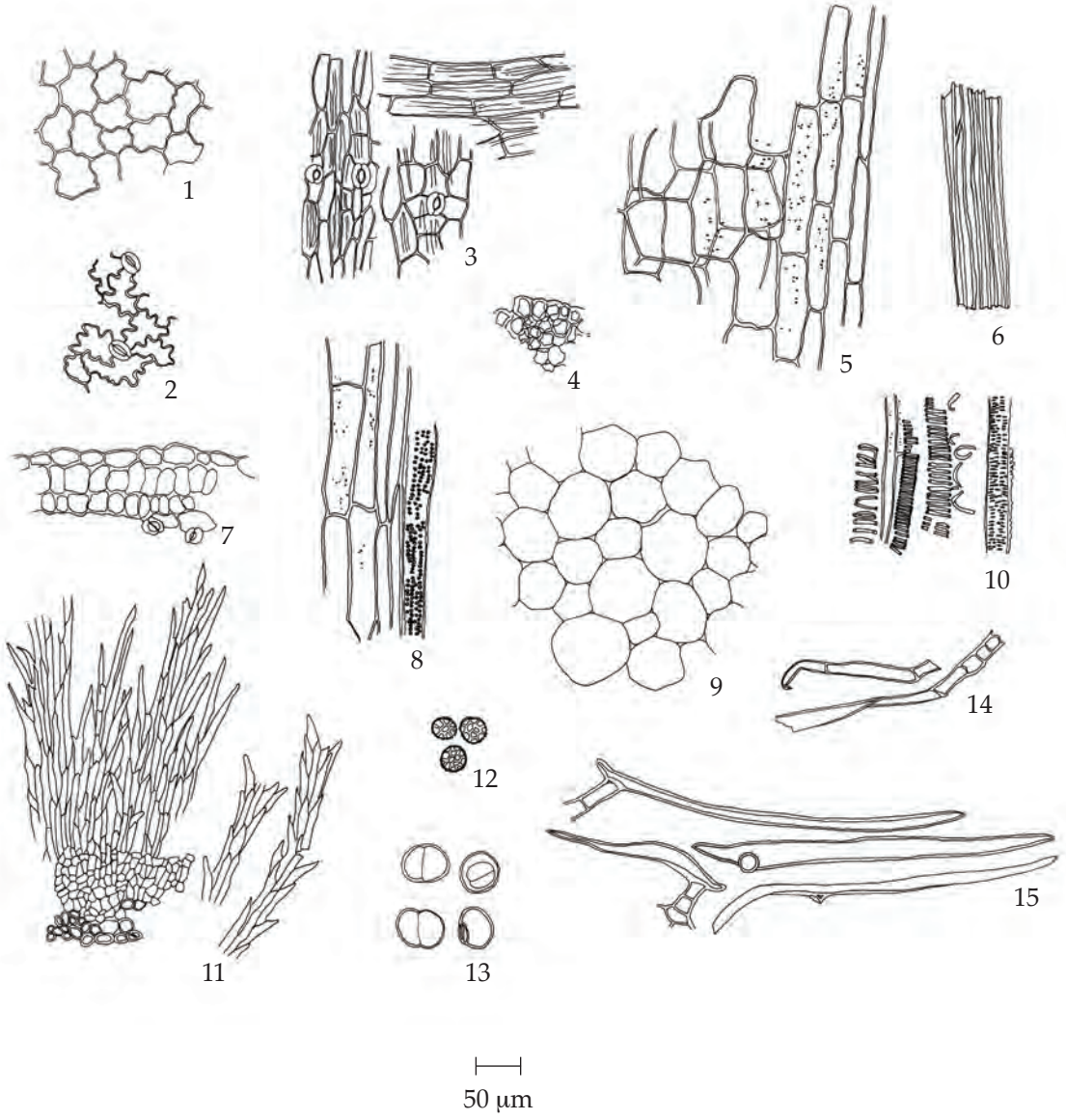


Fig. 2d Powdered Drug of the Aerial Parts of *Cyanthillium cinereum* (L.) H. Rob.

1. upper epidermis
2. lower epidermis with anomocytic stomata
3. epidermis of stem in surface view
4. group of collenchyma cells
5. parenchyma in longitudinal view
6. group of fibres
7. lamina in sectional view
8. parenchyma associated with bordered-pitted vessel and fibres in longitudinal view
9. parenchyma in sectional view
10. spiral vessels associated with fibres, and bordered-pitted vessel
11. pappi
12. pollen grains
13. glandular trichomes
14. multicellular uniseriate trichomes
15. T-shaped trichomes

Transverse section of the stem shows epidermis, cortex, vascular tissue, and pith. Epidermis: a layer of round cells; trichomes, T-shaped, multicellular uniseriate and glandular. Cortex: layers of lamellar collenchyma cells, round parenchyma and groups of fibres in the mature stem. Vascular tissue: phloem, group of fibres and parenchyma; xylem, rays, fibres and vessels. Pith: numerous large round parenchyma.

Little Ironweed Herb in powder possesses the diagnostic microscopical characters of the unground drug. T-shaped trichomes, pappi and anomocytic stomata are frequently observed and characteristic.

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

Warning It should be used with caution in patients with impaired cardiac and/or renal function.

Identification

A. Boil 1 g of the sample in *coarse powder*, with 20 mL of *water* for 1 to 2 minutes and filter (solution 1). To 1 mL of solution 1, add 1 or 2 pieces of *magnesium ribbon* and a few drops of *hydrochloric acid*: a pinkish red colour is produced.

B. To 1 mL of solution 1, add a few drops of *iron(III) chloride TS* and shake well: a greenish brown colour is produced.

C. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1), using *silica gel F254* as the coating substance and a mixture of 57 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 of solution (A) and 2 μL of solution (B). Prepare solution (A) by refluxing 1 g of the sample, in *fine powder*, with 20 mL of *ethanol* for 30 minutes and filtering. Evaporate the filtrate to dryness under reduced pressure at 30° and dissolve the residue in 2 mL of *ethanol*. For solution (B) dissolve 3 mg of *lupeol* in 1 mL of *methanol*. After removal of the plate, allow it to dry in air and 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 28 to 30) corresponding to the lupeol spot from solution (B). Other pink spots are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Aerial Parts of *Cyanthillium cinereum* (L.) H. Rob.

Spot	hR_f Value	Detection
		Anisaldehyde TS
1*	28–30	purple
2	41–42	pink
3	54–55	pink
4	61–62	pink

*lupeol

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 3.0 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 7.0 per cent w/w (Appendix 7.12).

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

Dose 2 g, prepared as an infusion by soaking in 120 to 200 mL of boiling water, three or four times a day after meals.

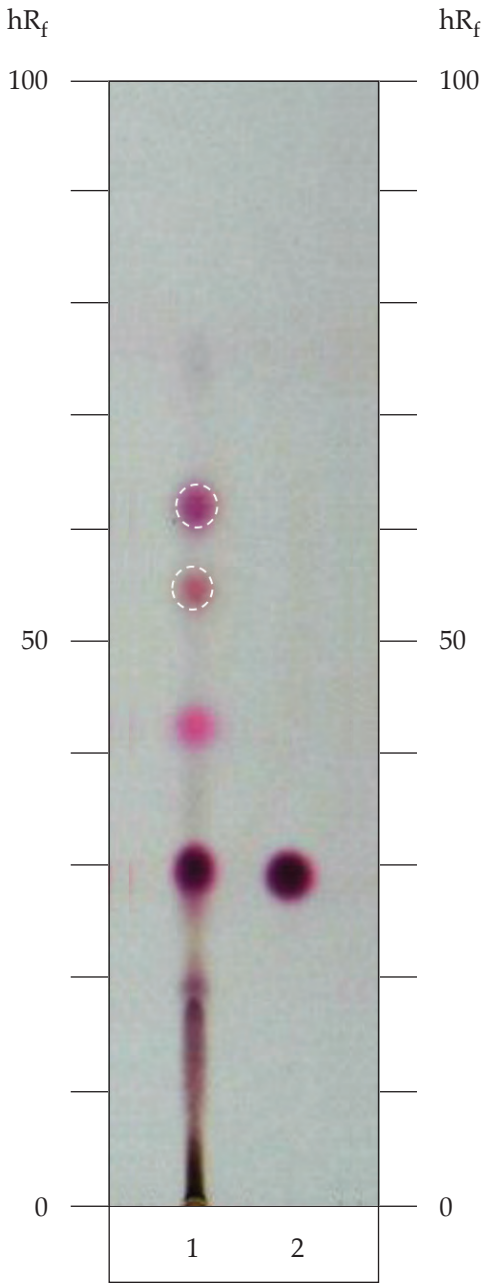


Fig. 3 Thin-Layer Chromatogram of Ethanolic Extract of the Aerial Parts of *Cyanthillium cinereum* (L.) H. Rob., Detected with *Anisaldehyde TS*

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

ย่านาง (YA NANG)

เถาย่านาง (THAO YA NANG), เถาวัลย์เขียว (THAOWAN KHIAO), ย่านนาง (YAN NANG)
Tiliacorae Triandrae Radix
Tiliacora Triandra Root

Category Antipyretic.

Tiliacora Triandra Root is the dried root of *Tiliacora triandra* (Colebr.) Diels (*Coccolus triandrus* Colebr., *Limacia triandra* (Colebr.) Hook. f.) (Family Menispermaceae), Herbarium Specimen Number: DMSC 5210, Crude Drug Number: DMSc 1071.

Constituents Tiliacora Triandra Root contains bisbenzylisoquinoline alkaloids (e.g., 2′-nortiliacorinine, tiliacorine, tiliacorinine, yanangcorinine).

Description of the plant (Figs. 1a, 1b) Climber or straggling shrub; stem usually slender, glabrous or pubescent, striated. Leaves simple, alternate, ovate, elliptic or lanceolate, 5 to 11(−17) cm long, 2 to 6(−10) cm wide, apex obtuse, acute or acuminate, base attenuate, obtuse or subcordate, margin entire, glabrous, papyraceous, pinnately nerved, often with steeply ascending basal nerves, seemingly subpalmately nerved, midrib of lower surface rugulose near base; petiole 0.5 to 2 cm long, rugulose. Inflorescence paniculate, 1- to few-flowered, axillary or cauliflorous, 2 to 8(−17) cm long; peduncle about 5 mm long. Male flower yellowish, about 2 mm in diameter; sepals 6, 2-seriate, 3 outer ones smaller, 3 inner ones broadly elliptic, about 2 mm long, subglabrous; petals 3 or 6, minute glabrous; stamens 3, clavate, about 2 mm long. Female flower reddish; sepal as in male flower; petals 6, oblong-elliptic, about 1 mm long; carpels 8 or 9, less than 1 mm long, borne on short gynophore, glabrous, stigma sessile; staminode absent. Fruit drupe, obovoid, 0.7 to 1 cm long, 6 to 7 mm wide, subcompressed, glabrous, green when young becoming yel lowish or reddish when ripe; stalk 3 to 4 mm long; endocarp transversely and irregularly ridged. Seed 1, horseshoe-shaped, curved, rugulose.

Description Odourless; taste, bitter.

Macroscopical (Fig. 1a) Transverse slices of roots; bark thin, brown; sectional surface of wood yellowish to light brown, with several successive and distinct concentric and radiate zones.

Microscopical (Figs. 2a, 2b, 2c) Transverse section of the root shows periderm, cortex and vascular tissue. Periderm, several layers of thin-walled rectangular cork cells. Cortex, polygonal shape of thin-walled parenchyma cells, some containing starch grains or prismatic crystals, and several layers of thick-walled sclereids in the inner zone. Vascular tissue, primary and secondary vascular tissues; primary vascular tissue, metaphloem and metaxylem; secondary vascular tissue, anomalous type, radially arranged, some containing secondary phloem, secondary xylem and broad zone of ray cells, some of which contain starch grains.

Radial and tangential longitudinal sections of the woody part of the root illustrate medullary ray cells with starch grains, bordered-pitted vessels and bundles of fibres.

Tiliacora Triandra Root in powder possesses the diagnostic microscopical characters of the unground drug. Various shapes of sclereids, some containing prismatic crystals, simple and compound starch grains, scattered prismatic crystals and thin-walled corks are frequently observed.

Fig. 1a *Tiliacora triandra* (Colebr.) Diels
1. habit 2. female inflorescence 3. male inflorescences
4. unripe infructescences 5. ripe infructescence 6. seeds 7. crude drug

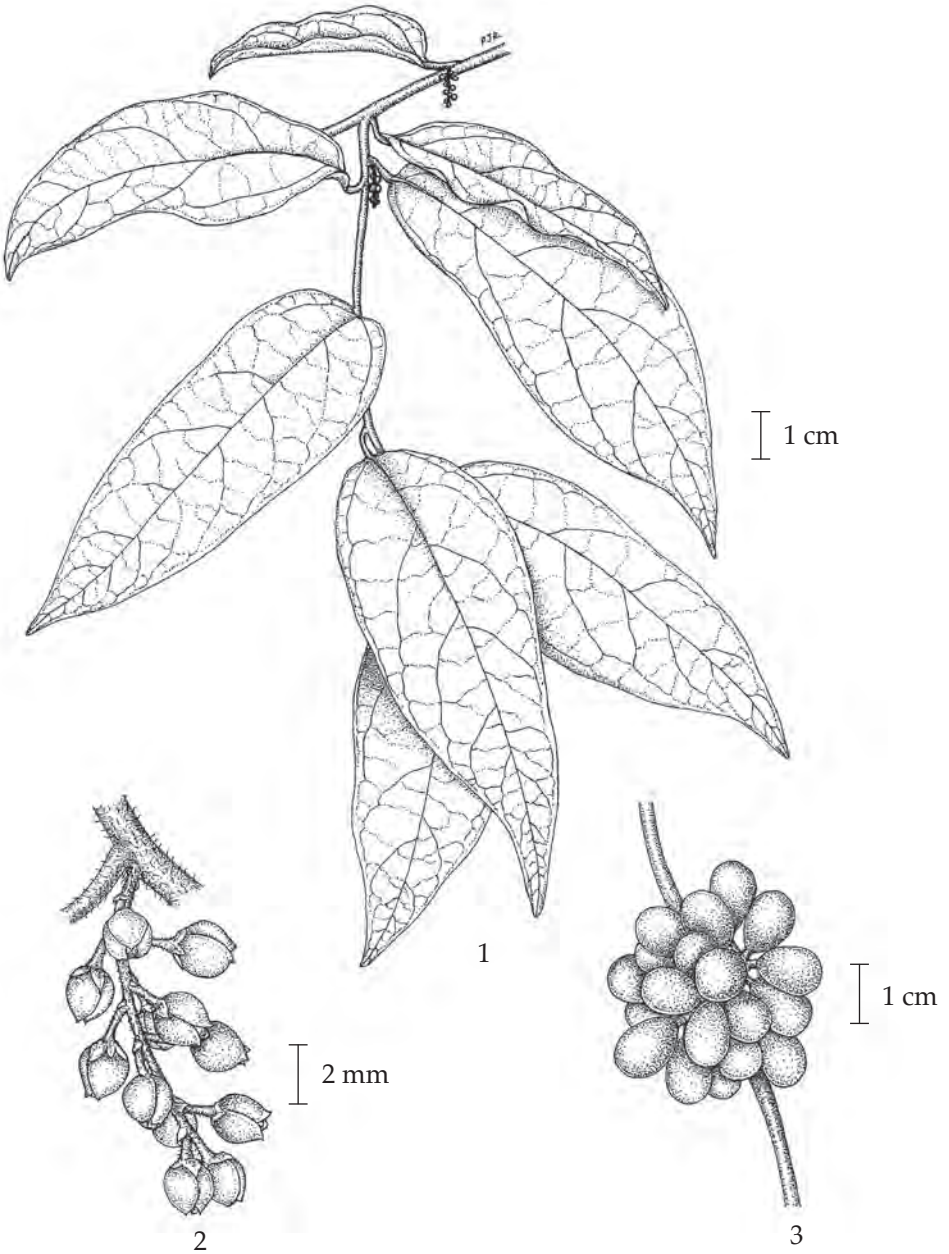


Fig. 1b *Tiliacora triandra* (Colebr.) Diels
1. twig showing leaves and inflorescences 2. inflorescence
3. part of infructescence

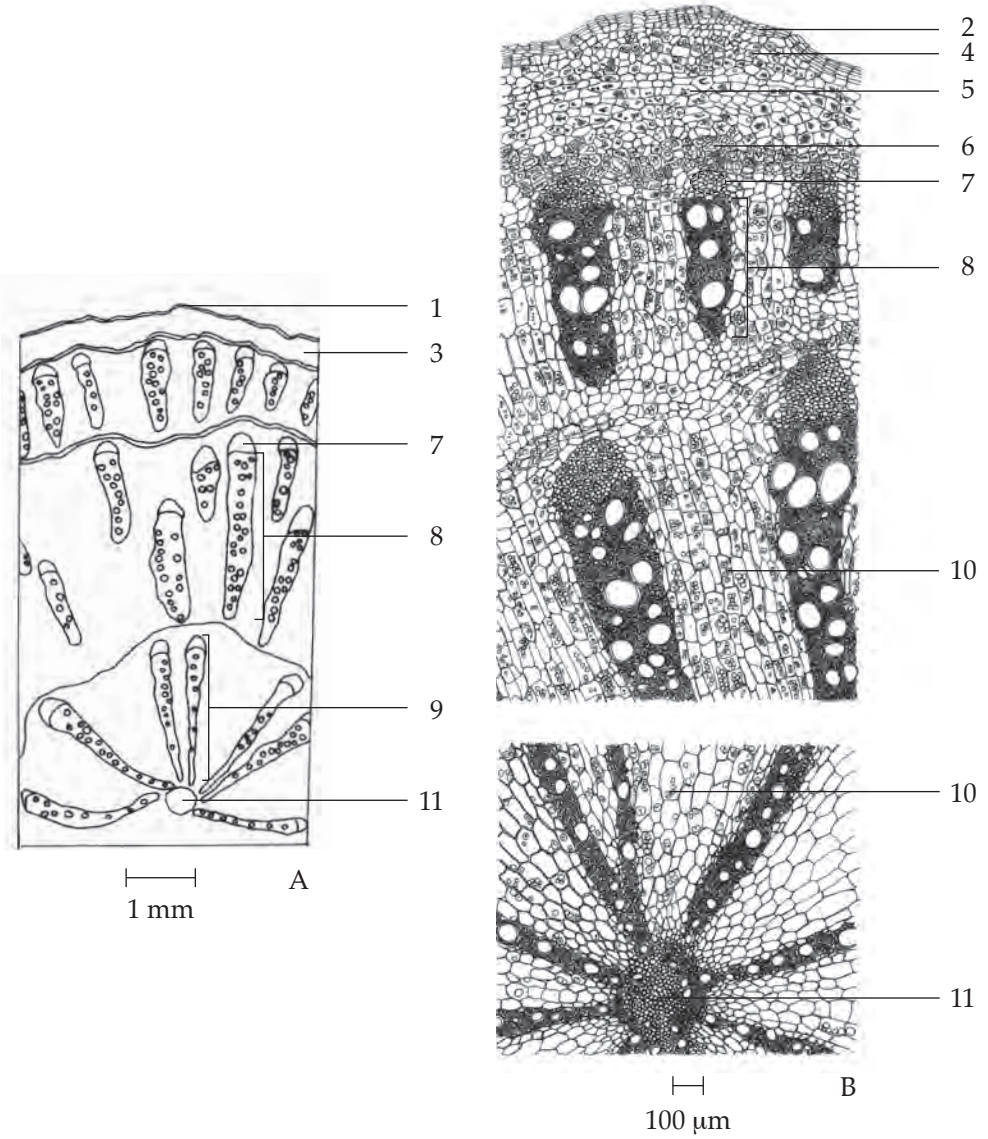


Fig. 2a Transverse Section of the Root of *Tiliacora triandra* (Colebr.) Diels

A. Diagram

B. Part of Transverse Section

1. periderm
2. cork
3. cortex
4. parenchyma with prismatic crystal
5. parenchyma with starch grains
6. sclereid
7. secondary phloem
8. secondary xylem
9. secondary vascular tissue
10. medullary ray with starch grains
11. primary vascular tissue

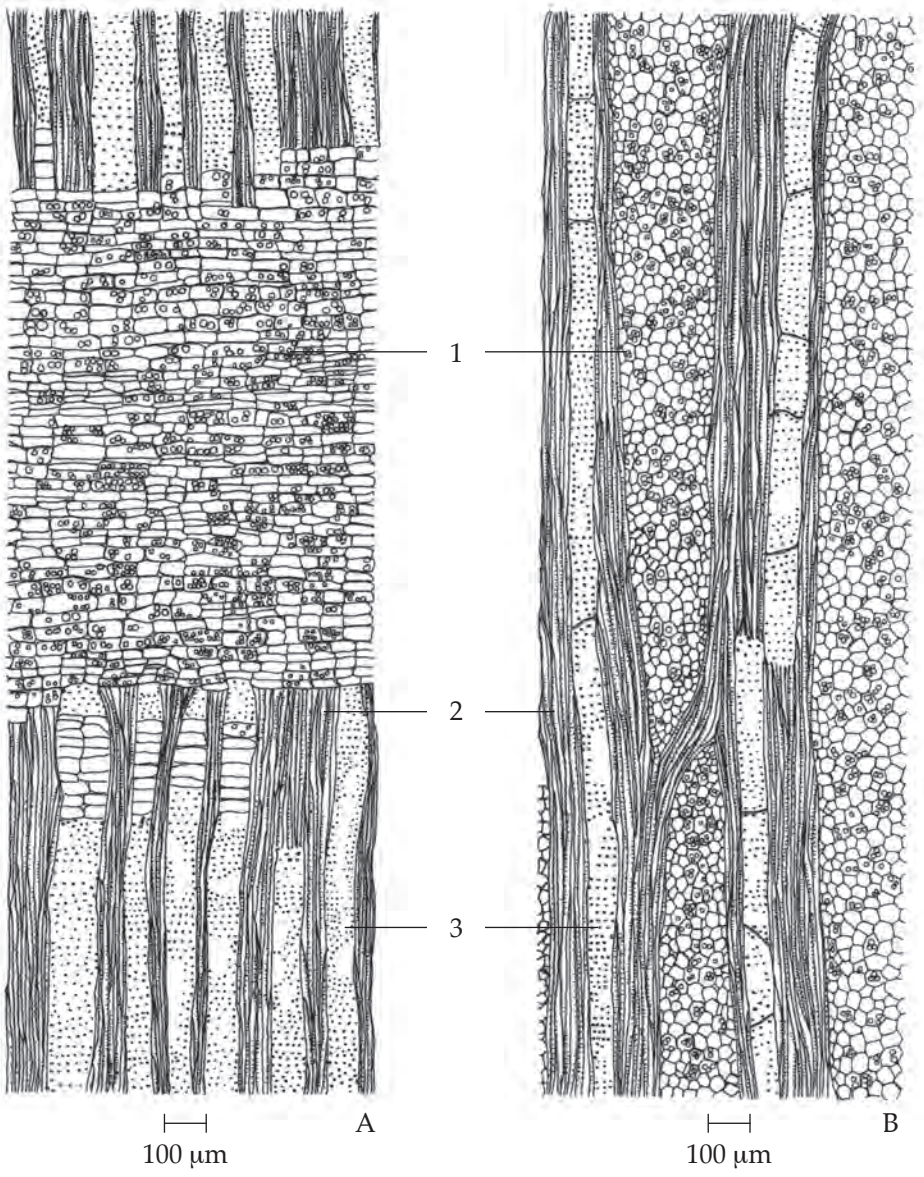


Fig. 2b Radial and Tangential Longitudinal Sections of the Woody Part of the Root of *Tiliacora triandra* (Colebr.) Diels

A. Radial Longitudinal Section

B. Tangential Longitudinal Section

1. medullary ray with starch grains
2. fibre
3. vessel

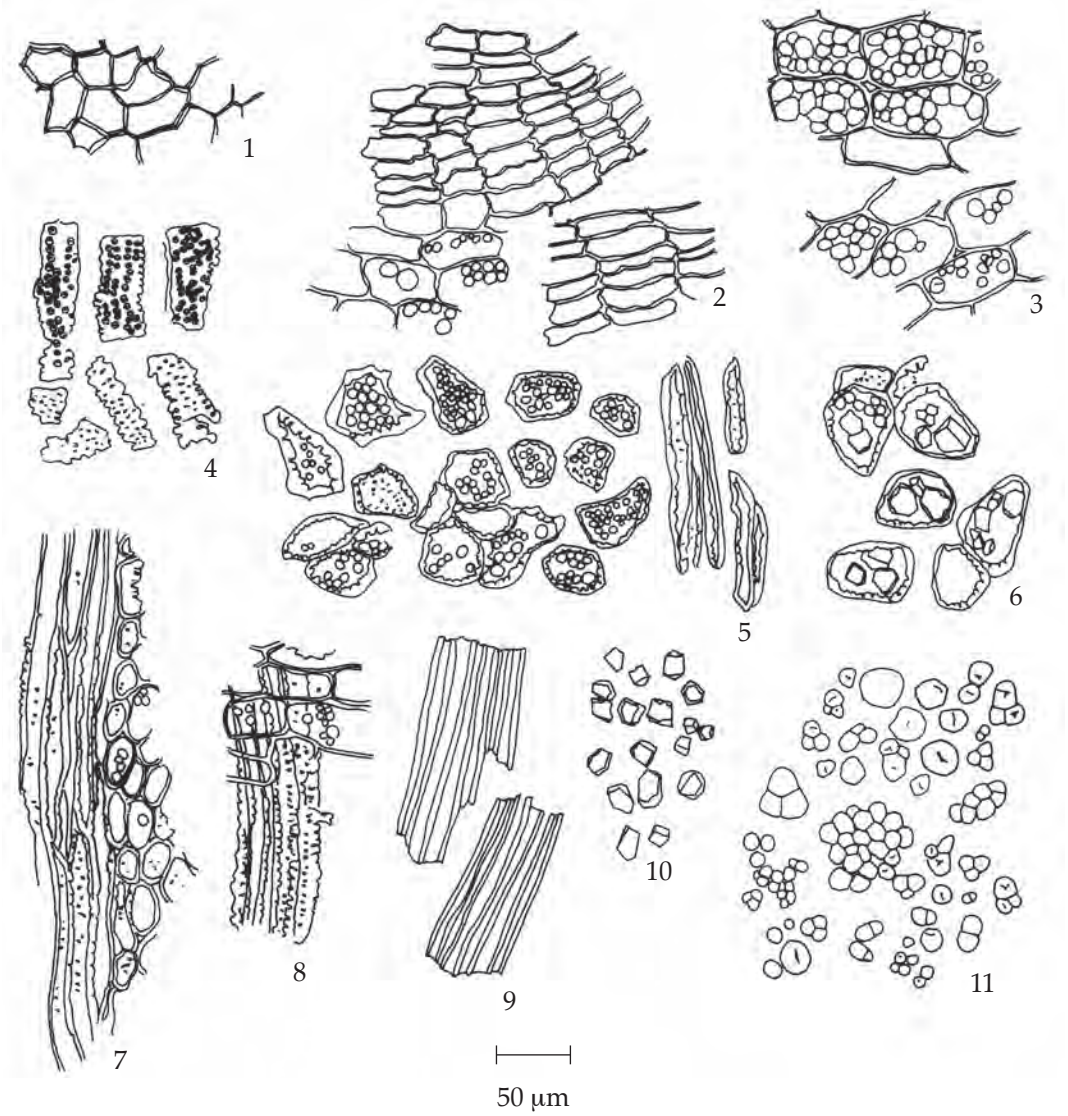


Fig. 2c Powdered Drug of the Roots of *Tiliacora triandra* (Colebr.) Diels

1. cork in surface view
2. cork and parenchyma in sectional view, some containing starch grains
3. parenchyma, some containing starch grains
4. bordered-pitted vessels
5. sclereids, some containing starch grains
6. sclereids, some containing prismatic crystals and/or starch grains
7. fibres associated with medullary ray in tangential longitudinal view
8. fibres associated with medullary ray in radial longitudinal view
9. fragments of fibres
10. prismatic crystals
11. simple and compound starch grains

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

Additional information

1. In Thai herbal markets, the adulteration of tiliacora triandra root with stems, branches, etc., may be found.
2. Risk-benefit should be considered when consuming since safety data in humans are not available at the present time.

Identification

A. Macerate 200 mg of the sample, in *fine powder*, with 4 mL of *chloroform* for 5 minutes and filter. To 2 mL of the filtrate, add a few drops of *modified Dragendorff TS2* and shake well: 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 70 volumes of *chloroform* and 30 volumes of *ethanol* as the mobile phase and allowing the solvent front to ascend 8 cm above the line of application. Apply to the plate as a band of 8 mm, 5 μL of the test solution prepared by macerating 1 g 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 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 bands. 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 *modified Dragendorff TS2* and immediately examine the plate; three orange bands are observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Methanolic Extract of the Roots of *Tiliacora triandra* (Colebr.) Diels

Band	hR_f Value	Detection		
		UV 254	UV 366	*Modified Dragendorff TS2*
1	8–9	–	light blue	–
2	13–14	–	light blue	–
3	41–45	quenching	–	orange
4	55–58	quenching	light green	orange
5	60–65	strong quenching	green	orange
6	89–92	strong quenching	blue	–
7	93–96	quenching	light blue	–

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).

Total ash Not more than 8.0 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 6.0 per cent w/w (Appendix 7.12).

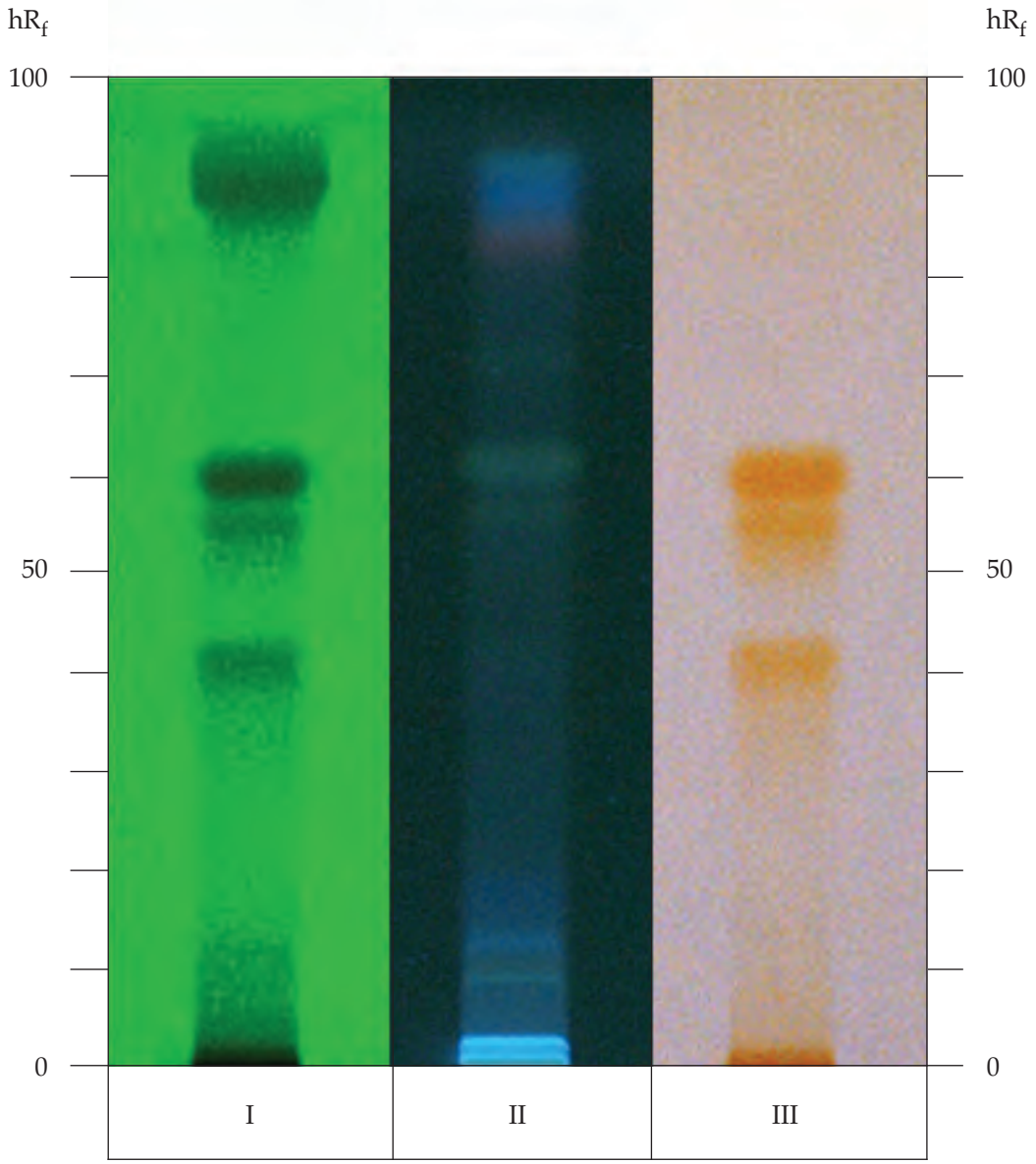


Fig. 3 Thin-Layer Chromatogram of Methanolic Extract of the Roots of *Tiliacora triandra* (Colebr.) Diels

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with *modified Dragendorff TS2*

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 tall, usually branched 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 elongated 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, anther 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.

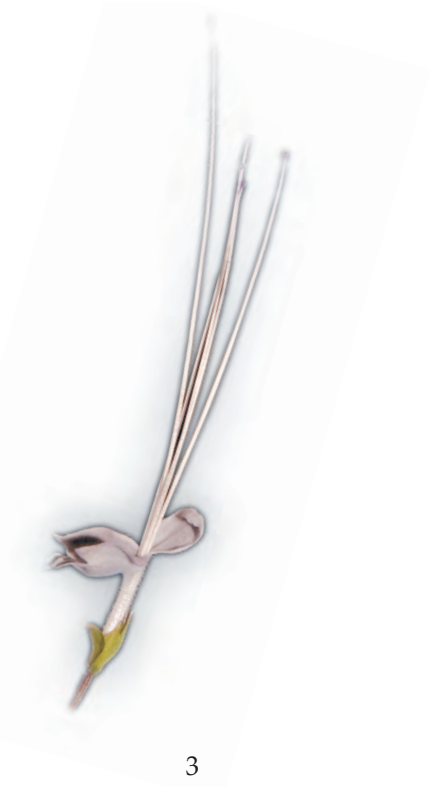
Macroscopical (Figs. 1a) A mixture of entire and broken, crisp, mainly dark green ovate or ovate-lanceolate leaves and quadrangular stems with a deep longitudinal groove down two opposite sides.

Microscopical (Figs. 2a, 2b, 2c, 2d) Transverse section of the leaf through the midrib and surface view of the lamina shows upper epidermis, a single layer of rectangular cells covered with thick cuticle, wavy-walled in surface view; diacytic and anomocytic stomata; covering trichome, unicellular or 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 parenchyma cells associated with fibre group. Vascular bundle, phloem and xylem; vessel, spiral, scalariform, reticulate and pitted. Pith, thin-walled and polygonal large parenchyma cells.

1

2



3

Fig. 1a *Orthosiphon aristatus* (Blume) Miq.
1. and 2. inflorescences 3. flower 4. crude drug

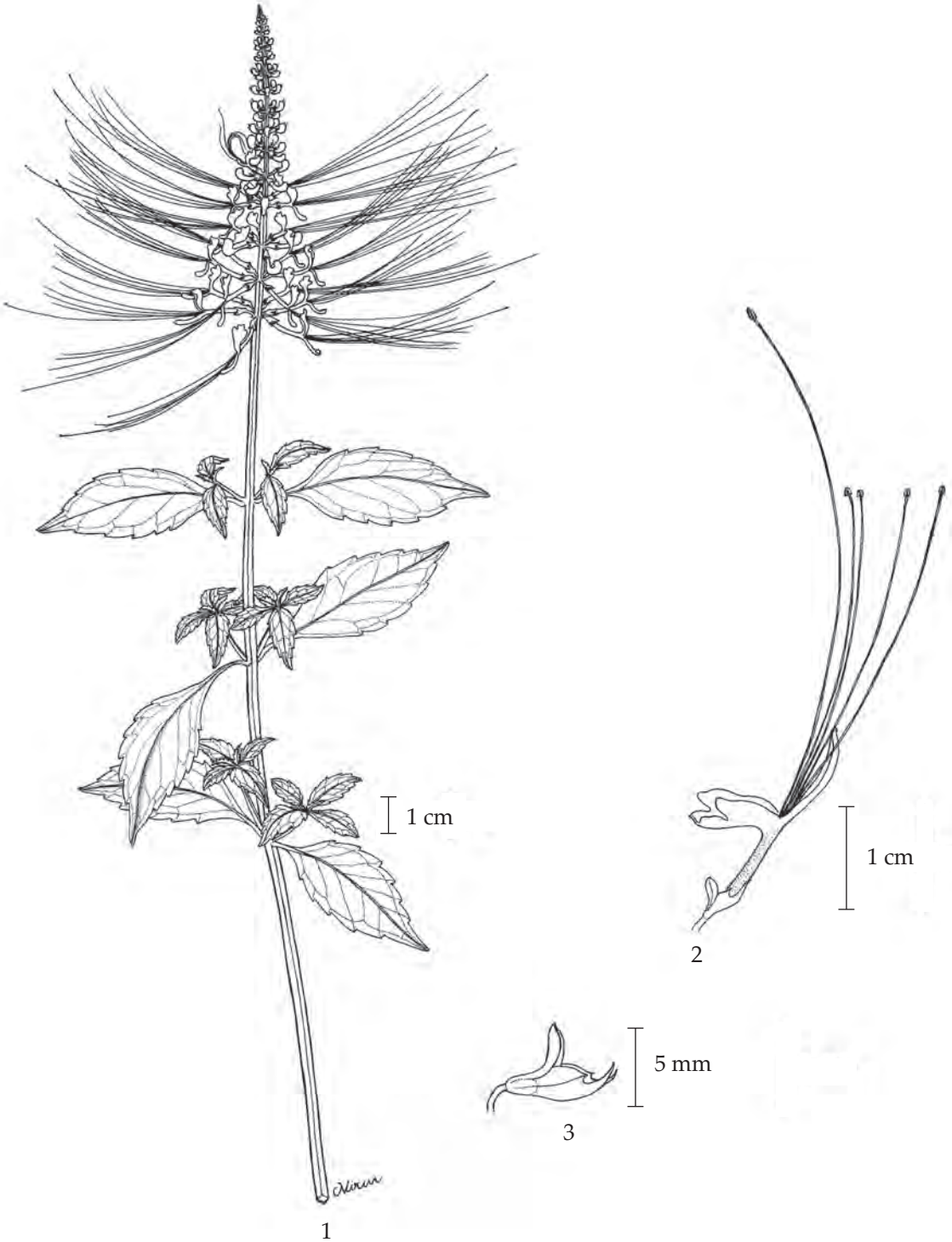


Fig. 1b *Orthosiphon aristatus* (Blume) Miq.
1. flowering top 2. flower 3. fruit with persistent calyx

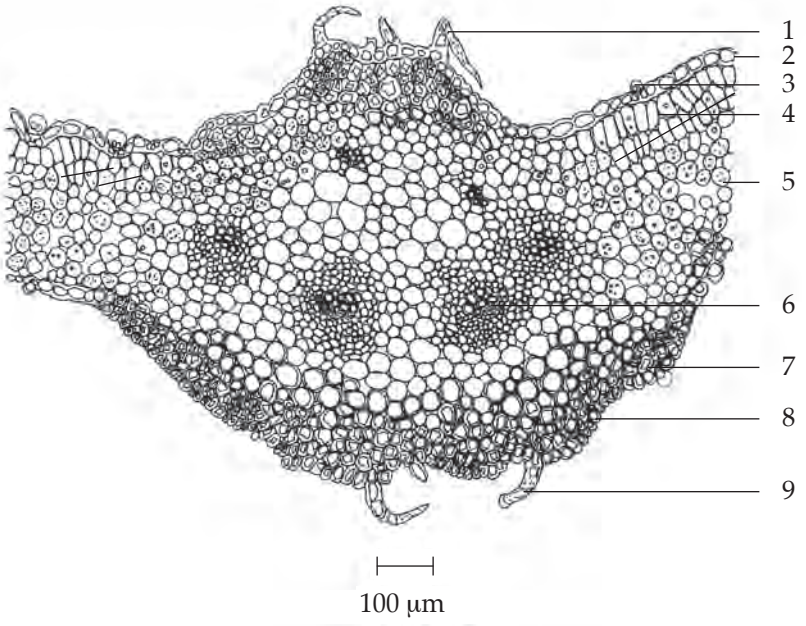


Fig. 2a Transverse Section of the Leaf through the Midrib of *Orthosiphon aristatus* (Blume) Miq.

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

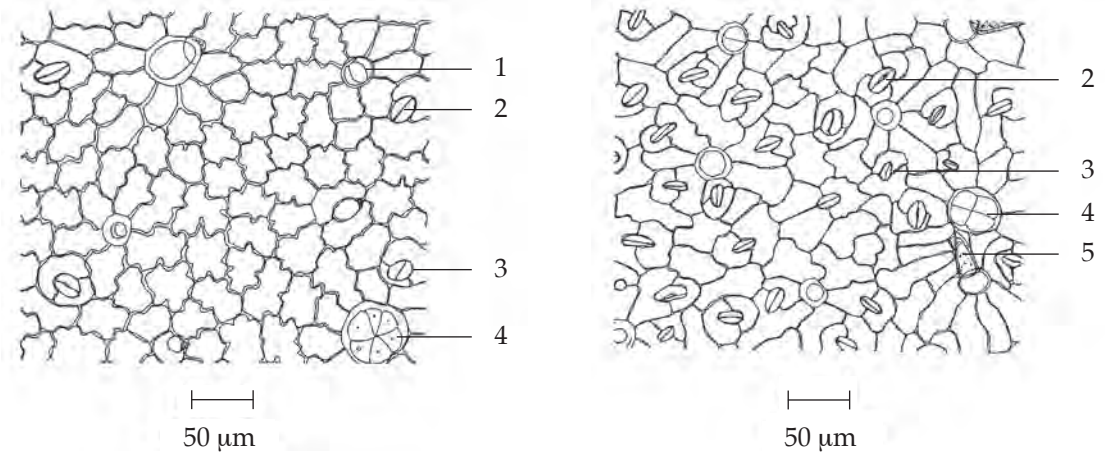


Fig. 2b Epidermises of the Leaf of *Orthosiphon aristatus* (Blume) Miq. in Surface View

1. cicatrice
2. anomocytic stomata
3. diacytic stomata
4. glandular trichome with radiated head
5. covering trichome

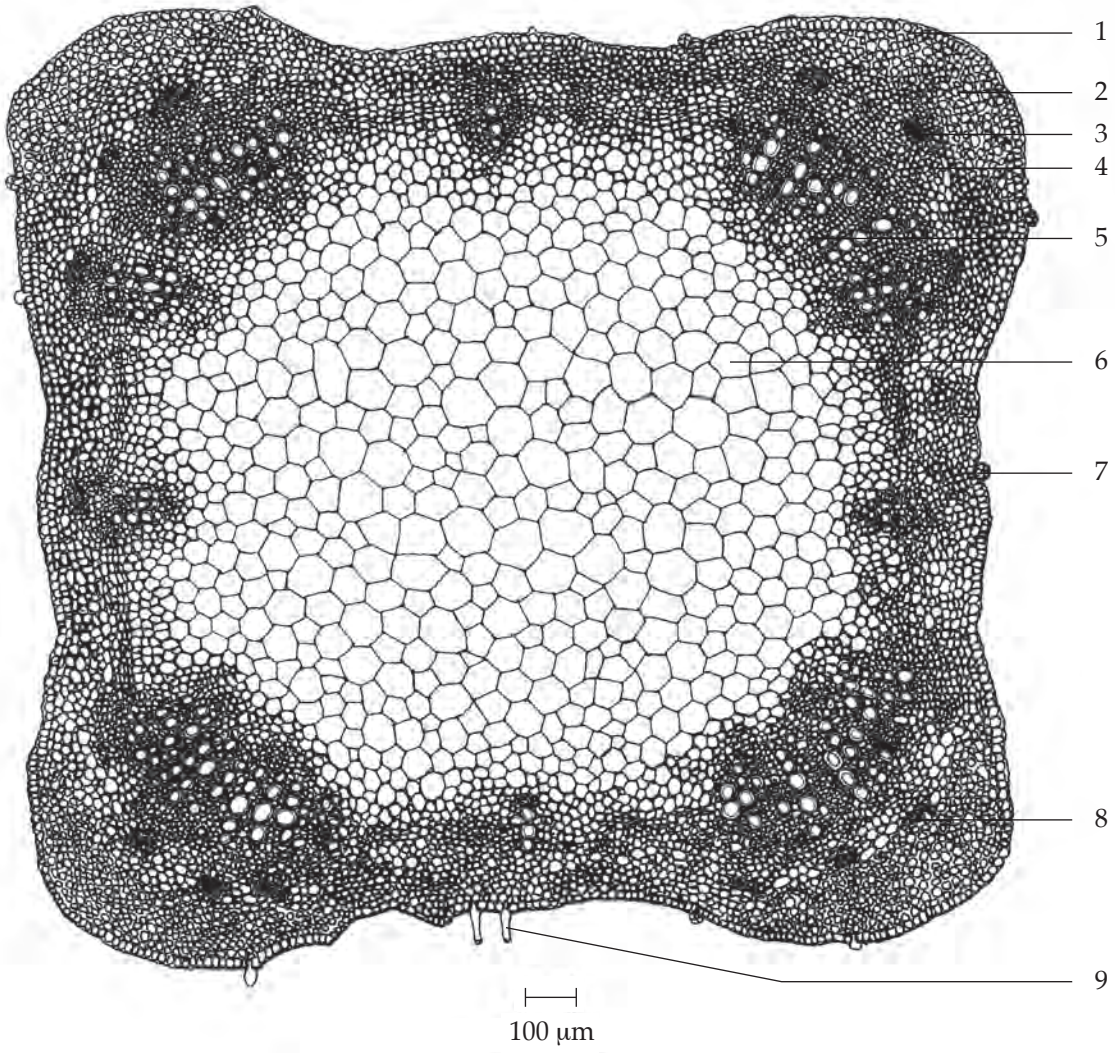


Fig. 2c Transverse Section of the Stem of *Orthosiphon aristatus* (Blume) Miq.

1. epidermis
2. collenchyma
3. parenchyma
4. phloem
5. xylem
6. thin-walled parenchyma
7. glandular trichome
8. fibre
9. covering trichome

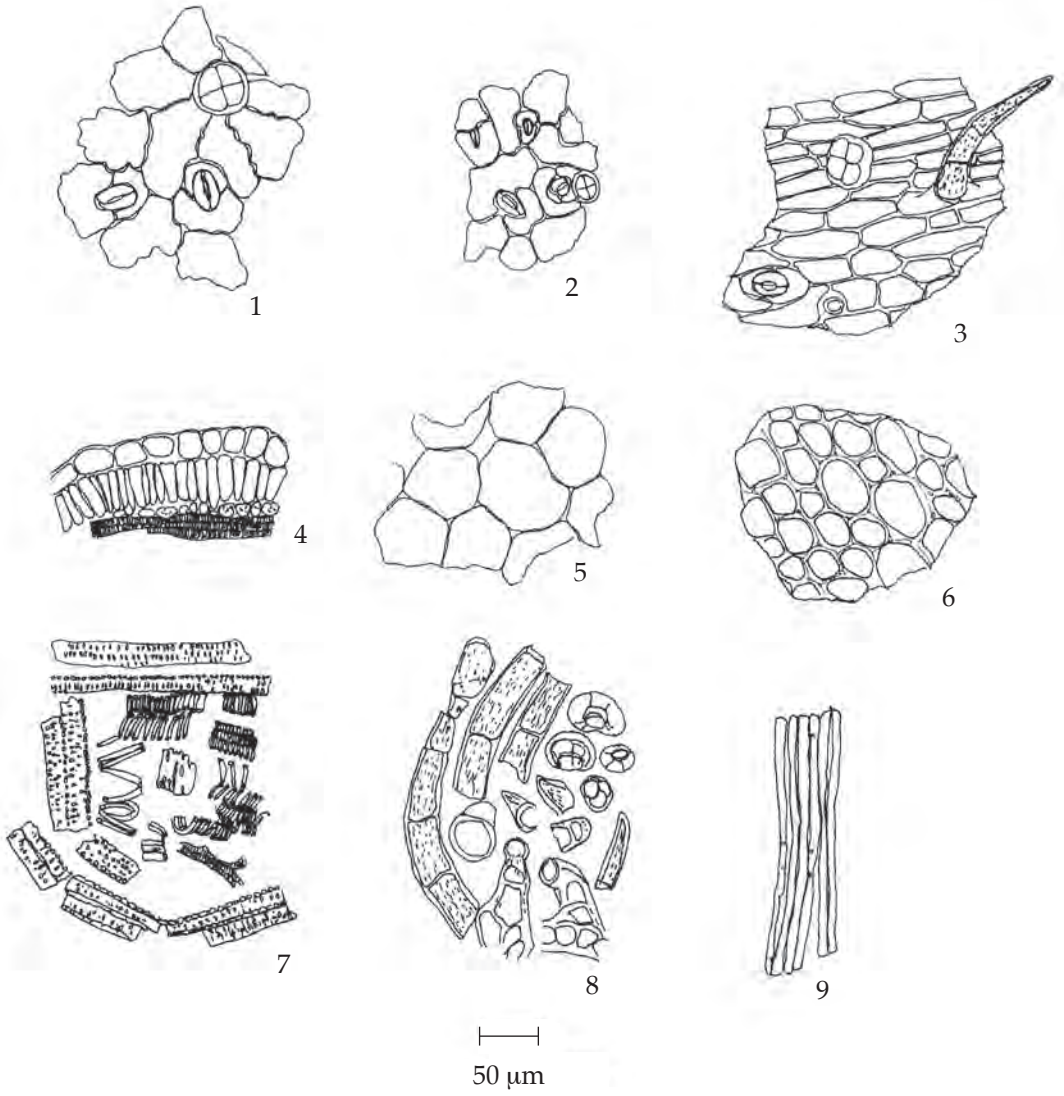


Fig. 2d Powdered Drug of the Leaves and Stem Tips of *Orthosiphon aristatus* (Blume) Miq.

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 quenching spots. The chromatogram obtained from solution (A) shows three quenching spots (hR_f values 30 to 37, 44 to 48 and 52 to 60) corresponding to rosmarinic acid, sinensetin and caffeic acid spots from solutions (B), (C) and (D), respectively. Examine the same plate under ultraviolet light (366 nm), three blue fluorescent spots corresponding to rosmarinic acid, sinensetin, 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 spots due to rosmarinic acid, sinensetin and caffeic acid are blue fluorescent. Another spot of lower hR_f value appears also as a 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	blue	blue
3**	44–48	weak quenching	blue	blue
4***	52–60	quenching	blue	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.

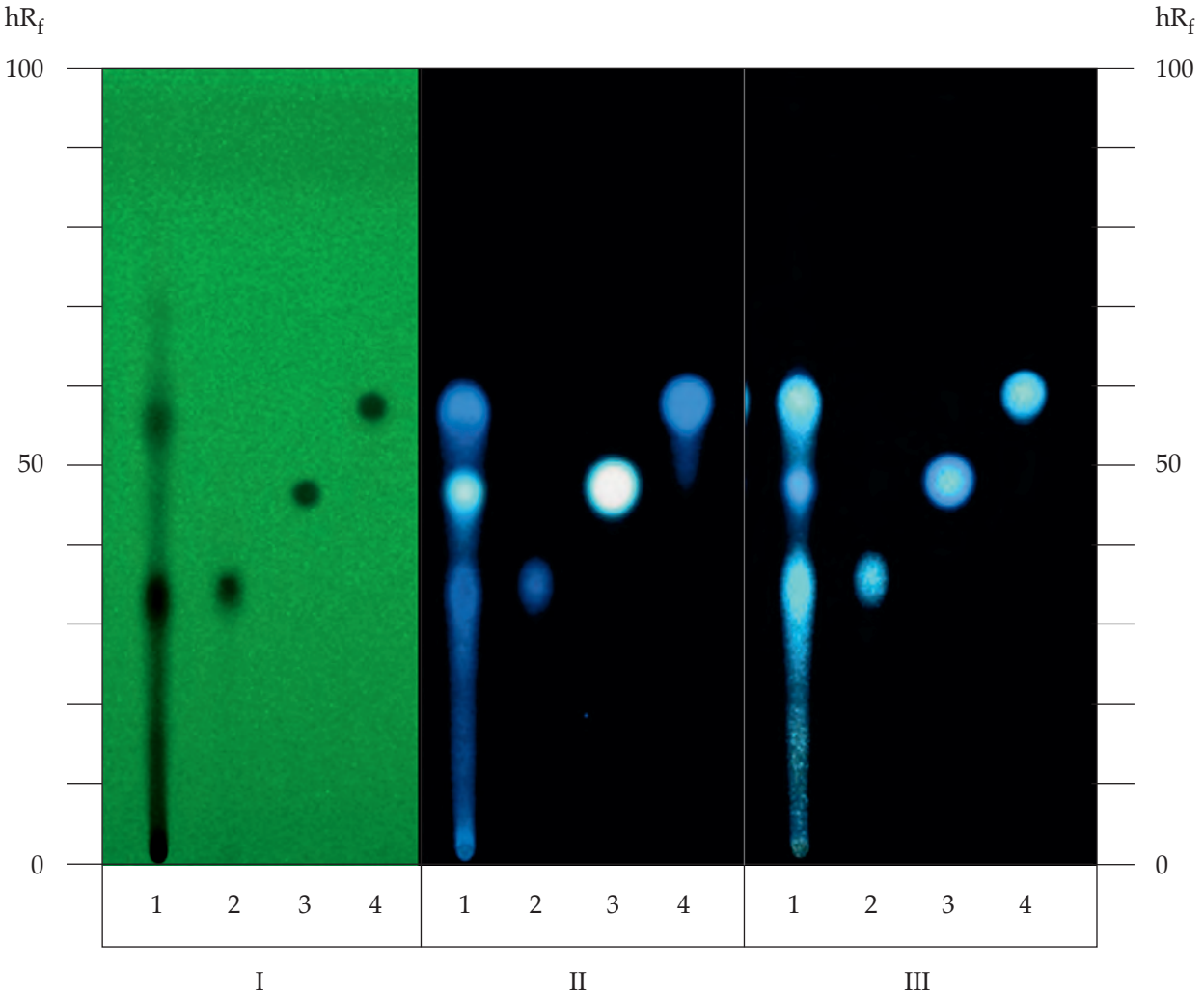


Fig. 3 Thin-Layer Chromatogram of the Extract of the Leaves and Stem Tips of *Orthosiphon aristatus* (Blume) Miq.

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*

หญ้าปักกิ่ง (YA PAK KING)

หญ้าเทวดา (YA THEWADA)
Murdanniae Loriformidis Herba
Murdannia Loriformis Herb

Synonyms Angel Grass Herb, Beijing Grass Herb, Peking Grass Herb

Category Tonic.

Murdannia Loriformis Herb is the dried aerial part of *Murdannia loriformis* (Hassk.) R. S. Rao & Kammathy [*Aneilema angustifolium* N. E. Br., *A. loriforme* Hassk., *A. terminale* Wight] (Family Commelinaceae), Herbarium Specimen Number: BKF 133708, Crude Drug Number: DMSc 1174.

Constituents Murdannia Loriformis Herb contains a glycosphingolipid which is *β*-*O*-D-glucopyranosyl-2-(2′-hydroxy-*Z*-6′-enecosamide)sphingosine. It also contains glycosidic compounds which are *β*-sitosteryl-*β*-D-glucoside and digalactosyl diglycerides and inorganic salts including sodium, potassium and magnesium.

Description of the plant (Figs. 1a, 1b) Perennial rosette herb, diffuse or ascending, 15 to 50(–100) cm long, glabrous or puberulous, rarely densely hirsute. Leaves simple, spirally arranged, blade linear, basal leaves 10 to 20 cm long, 1 to 1.5 cm wide, cauline leaves shorter, apex acute, base clasping stem into sheath, margin proximally ciliate, glabrous, rarely densely hirsutulous, parallel-veined; leaf sheath ciliate only on one side of sheath mouth. Inflorescence cincinnus, terminal, sometimes axillary, solitary or 2 or 3 forming a panicle, subcapitate of several densely arranged flowers; peduncle 3 to 6 cm long; bract caducous; bracteole suborbicular, 4 to 8 mm long, 4 to 6 mm wide, translucent, greenish. Flower with pedicel about 3 mm long, erect, slightly curved, up to 4 mm long when in fruit; sepals 3, translucent green, ovate-elliptic, 3 to 5 mm long, 2 to 3 mm wide; petals 3, whitish, purplish to bluish, obovate-orbicular, 3.5 to 6 mm long, 3 to 5 mm wide; fertile stamens 2, filament pubescent; staminodes 4, 1 antisepalous and 3 antipetalous; ovary superior, 3-loculed. Fruit capsule, ovoid-globose, 3 to 4 mm long, trigonous. Seeds 2 per locule, brown, radiate striate, finely white reticulate.

Description Odour, faint and characteristic; taste, irritating.

Macroscopical (Fig. 1a) Aerial part, greenish grey; leaves, varied in size and shape, linear, longitudinally wrinkled and twisted, glabrous.

Microscopical (Figs. 2a, 2b, 2c, 2d) Transverse section of the leaf through the midrib shows upper epidermis, mesophyll, vascular bundles, and lower epidermis. Upper epidermis, a layer of rectangular cells, with a few multicellular uniserate trichomes and covering reticulate cuticle striation. Mesophyll: 3 to 6 layers of large polygonal parenchyma cells in the upper part, some containing yellowish brown pigment; 2 to 8 layers of small round chlorenchyma; 1 to 3 layers of small polygonal parenchyma cells in the lower part. Vascular bundle, phloem tissue and xylem tissue, surrounded by 1 to 3 layers of bundle sheath. Lower epidermis, rectangular cells, multicellular uniseriate trichomes, hexacytic stomata, and covering reticulate cuticle striation. Collenchyma, 2 or 3 layers, found beneath lower epidermis in the midrib.

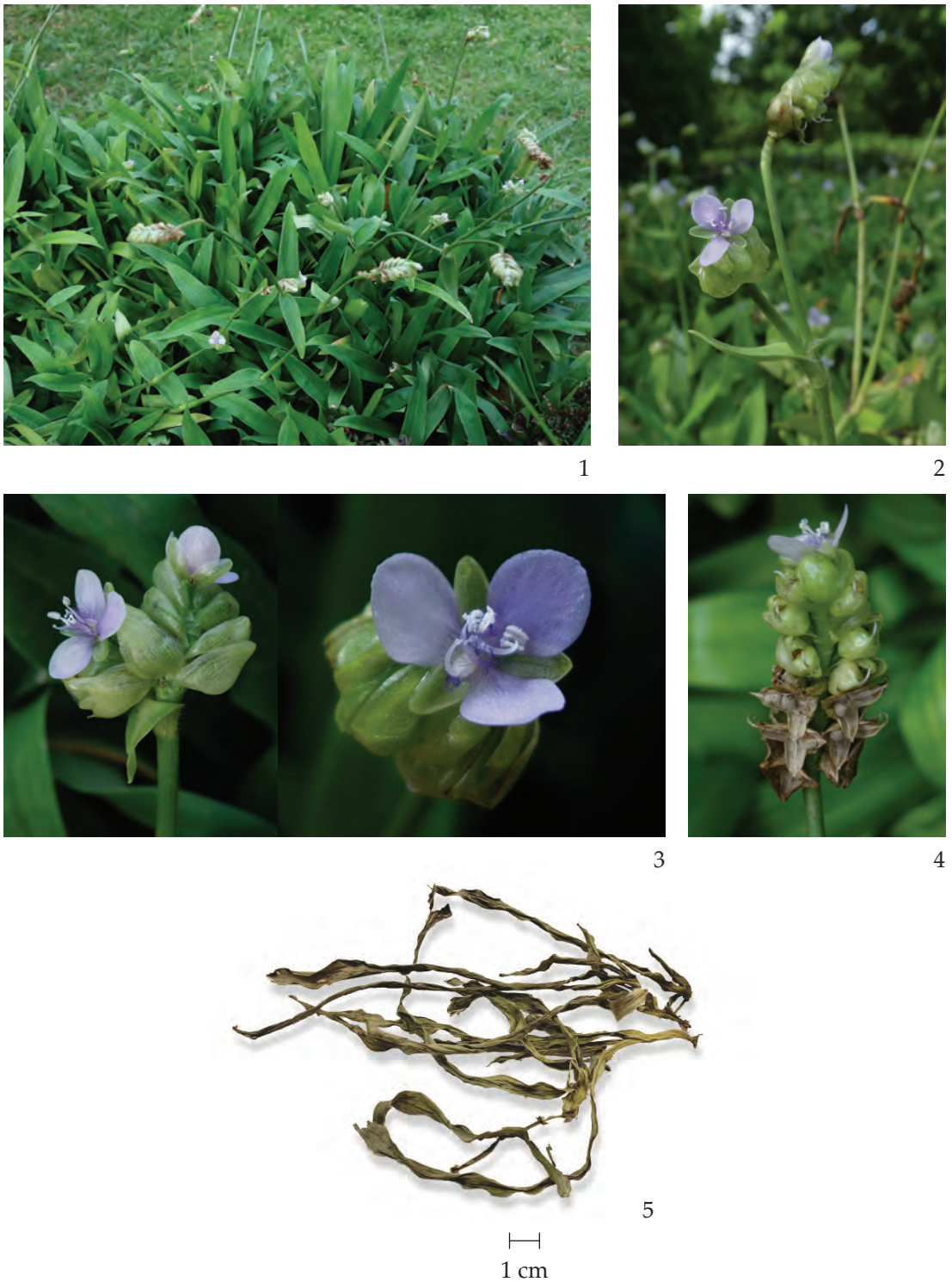


Fig. 1a *Murdannia loriformis* (Hassk.) R. S. Rao & Kammathy
1. habit 2. and 3. inflorescences 4. infructescence bearing fruits and flower 5. crude drug

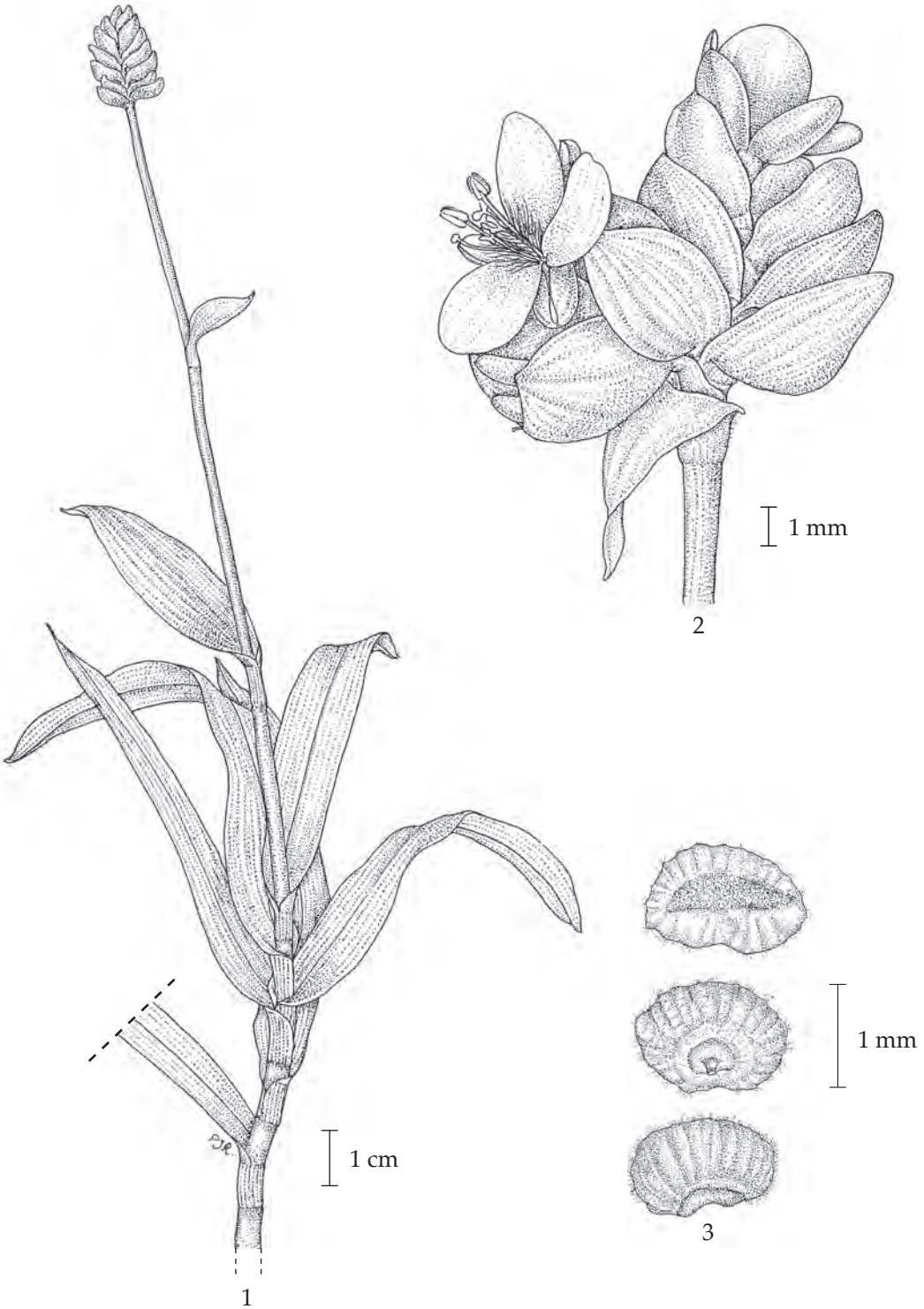
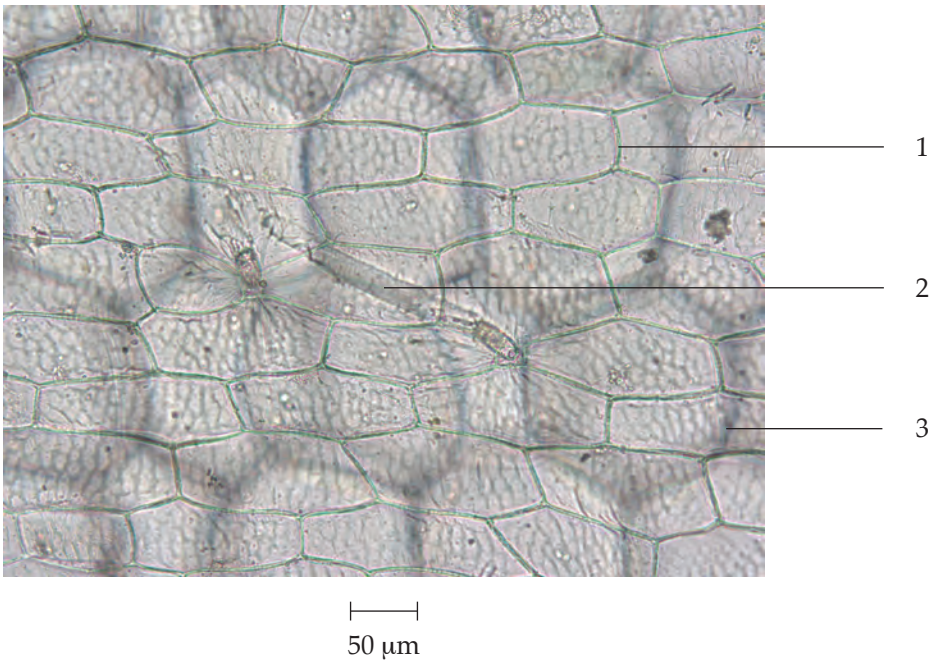
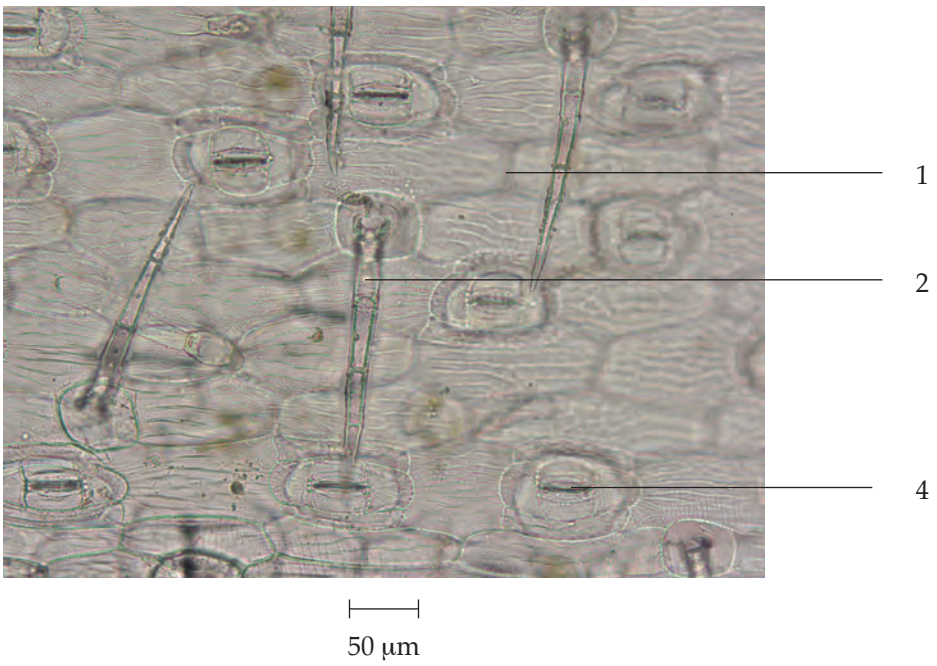


Fig. 1b *Murdannia loriformis* (Hassk.) R. S. Rao & Kammathy
1. flowering branch 2. inflorescence 3. seeds



Upper Epidermis of the Lamina



Lower Epidermis of the Lamina

Fig. 2a Photomicrographs of Epidermises of the Leaf of *Murdannia loriformis* (Hassk.) R. S. Rao & Kammathy

1. epidermis
2. multicellular uniseriate trichrome
3. parenchyma
4. hexacytic stoma

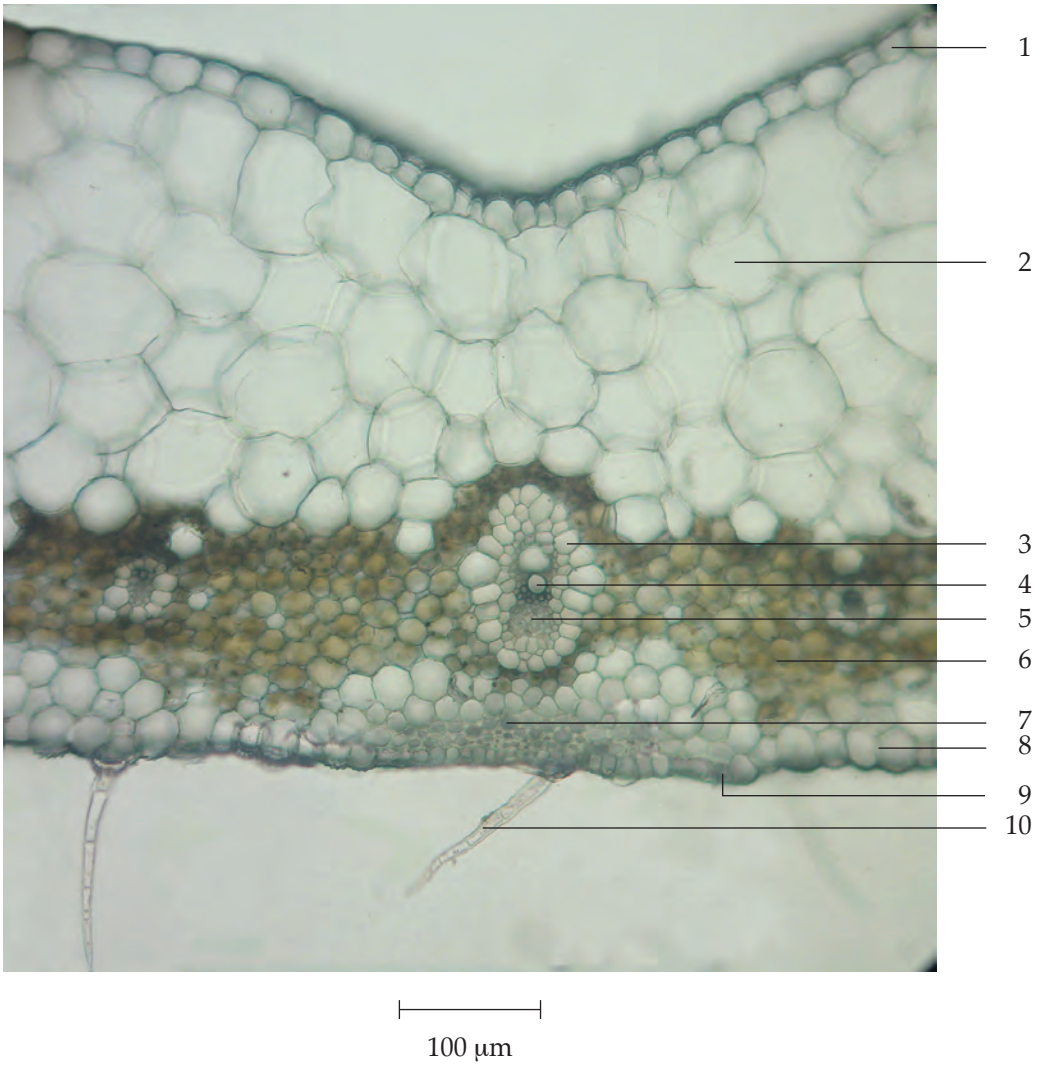


Fig. 2b Photomicrograph of Transverse Section of the Midrib of the Leaf of *Murdannia loriformis* (Hassk.) R. S. Rao & Kammathy

1. upper epidermis
2. parenchyma
3. bundle sheath
4. xylem
5. phloem
6. chlorenchyma
7. collenchyma
8. lower epidermis
9. stoma
10. multicellular uniseriate trichrome

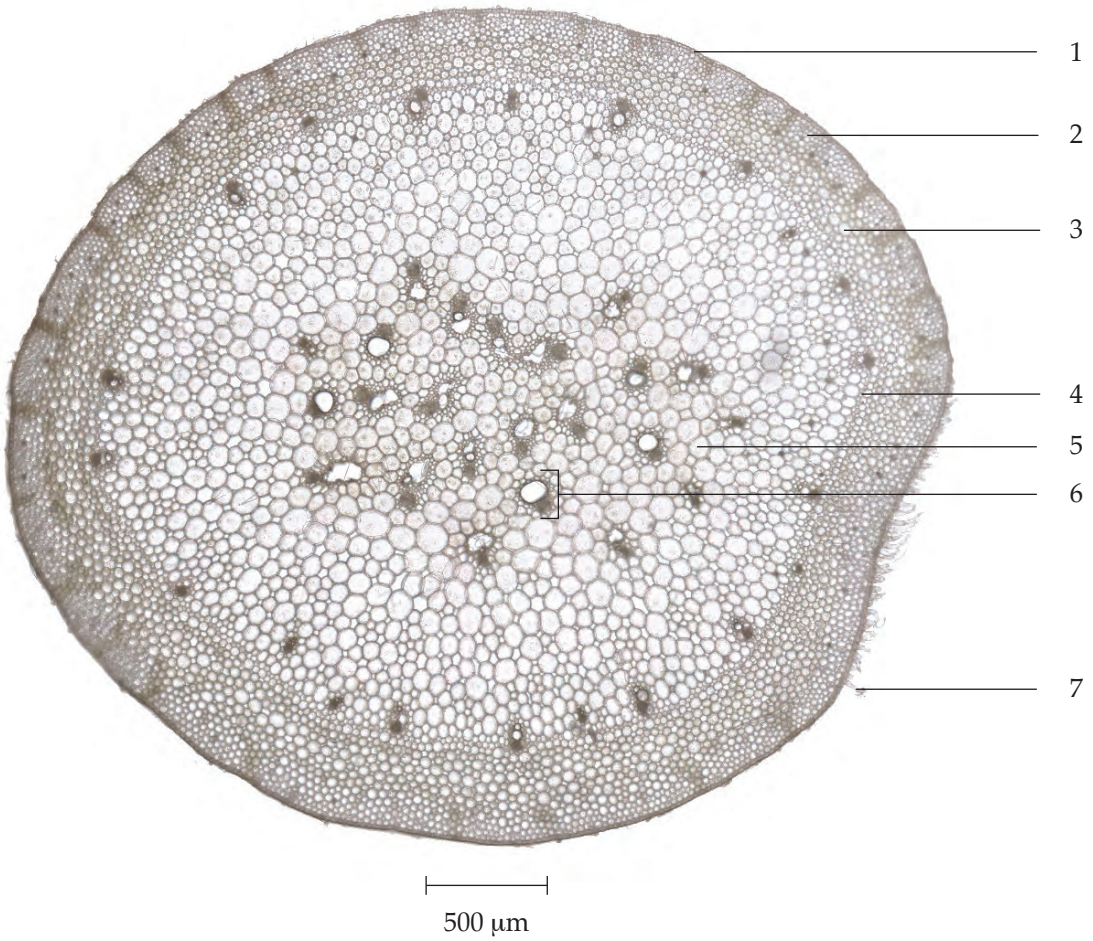


Fig. 2c Photomicrograph of Transverse Section of the Stem of *Murdannia loriformis* (Hassk.) R. S. Rao & Kammathy

1. epidermis
2. collenchyma
3. chlorenchyma
4. pseudoendodermis
5. parenchyma
6. vascular bundle
7. multicellular uniseriate trichome

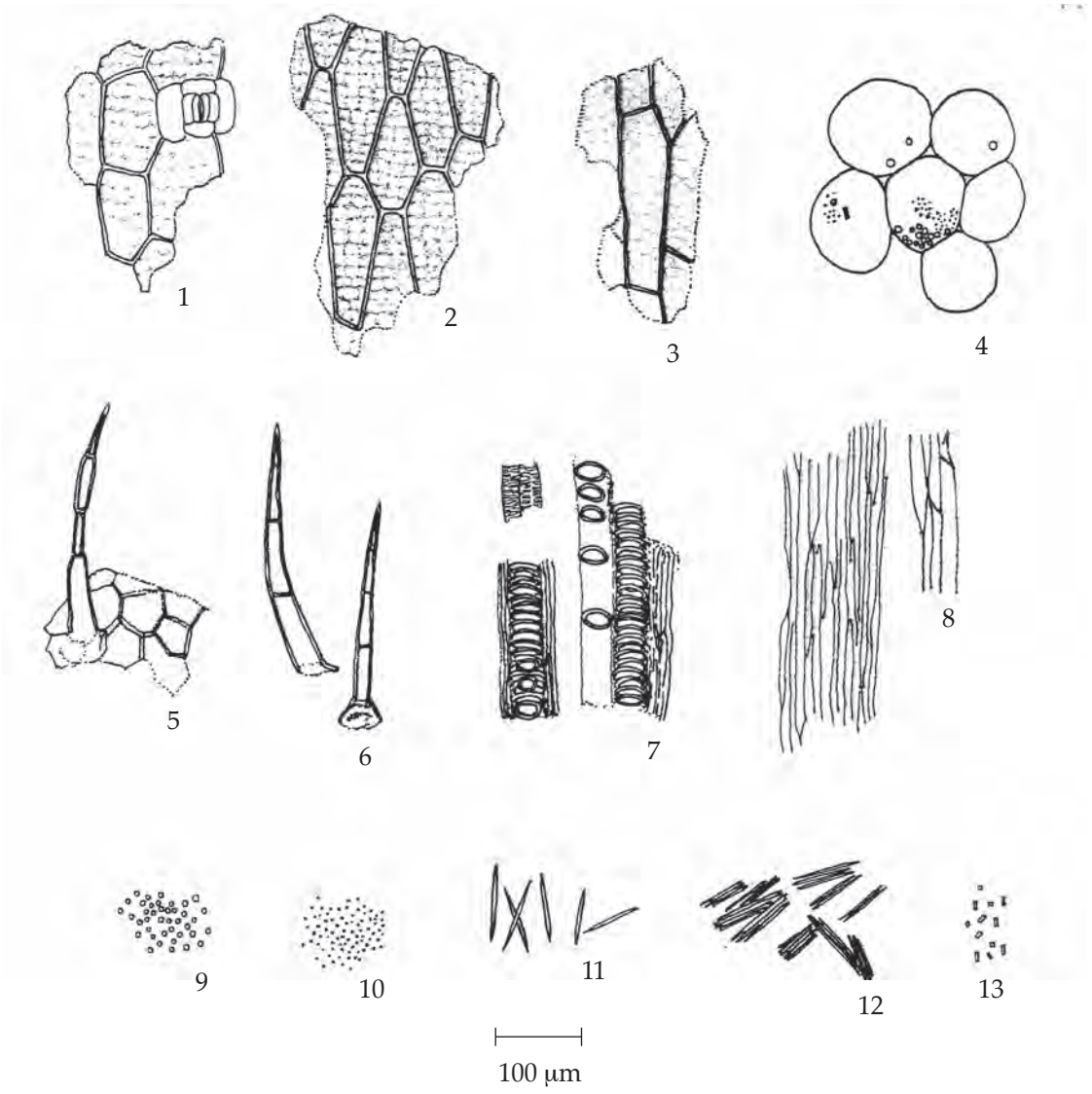


Fig. 2d Powdered Drug of the Aerial Parts of *Murdannia loriformis* (Hassk.) R. S. Rao & Kammathy

1. lower epidermis with hexacytic stoma and reticulate cuticle striation in surface view
2. upper epidermis with reticulate cuticle striation in surface view
3. epidermis of stem in surface view
4. parenchyma with microcrystals and starch grains
5. epidermis with multicellular uniseriate trichome in surface view
6. multicellular uniseriate trichomes
7. annular, spiral and reticulate vessels with fibre bundle
8. group of fibres
9. starch grains
10. microcrystals
11. acicular crystals
12. raphides
13. rod-shaped crystals

Transverse section of the stem shows epidermis, cortex and vascular bundles. Epidermis, rectangular cells, multicellular uniseriate trichomes and covering cuticle layer. Cortex, collenchyma, chlorenchyma, polygonal parenchyma cells, some containing starch grains, yellowish brown pigment, microcrystals, raphide, acicular and rod-shaped crystals, and a layer of pseudoendodermis. Vascular bundles, scattering in the cortex.

Murdannia Loriformis Herb in powder possesses the diagnostic microscopical characters of the unground drug. Epidermal layers with reticulate cuticle striation and multicellular uniseriate trichome (mostly 4 cells) are characteristic. Large parenchyma with microcrystals, and acicular, rod-shaped or raphide crystals, can also be seen.

Additional information Ethnomedically, the ground fresh aerial parts of murdannia loriformis herb are squeezed with sufficient amount of drinking water and allowed to settle. The clear supernatant is orally taken.

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

Identification

A. To 1 g of the sample, in powder, add 10 mL of *ethanol*, warm on a water-bath for 15 minutes and filter. Evaporate the filtrate to dryness in a porcelain dish, dissolve the residue in 5 drops of *acetic anhydride* and slowly add a few drops of *sulfuric acid* along the inner side of the dish: a blue-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 85 volumes of *chloroform* and 15 volumes of *methanol* as the mobile phase. Apply separately to the plate, 30 μL of solution (A) as a band of 8 mm and 3 μL of solution (B) as a spot. Prepare solution (A) by refluxing 1 g of the sample, in powder, with 20 mL of *ethanol* on a water-bath for 15 minutes, filtering and evaporating the filtrate to dryness. Dissolve the residue in 1 mL of *methanol*. For solution (B), dissolve 1 mg of β-*sitosteryl*-β-*D*-*glucoside* 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. Subsequently examine the plate under ultraviolet light (366 nm); several fluorescent bands of different colours are observed (Table 1); see also Fig. 3. 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 purple band (hR_f value 28 to 29) corresponding to the spot of *β*-sitosteryl-*β*-D-glucoside from solution (B). Several other purple bands are also observed (Table 1); see also Fig. 3.

Table 1 hR_f Values of Components in Ethanolic Extract of the Aerial Parts of *Murdannia loriformis* (Hassk.) R. S. Rao & Kammathy

Band	hR_f Values	Detection		
		UV 254	UV 366	10 Per Cent V/V Solution of *Sulfuric Acid* in *Ethanol*
1	6–7	–	–	pale purple
2	10–11	–	–	pale purple
3	16–18	weak quenching	–	–
4*	24–25	–	–	pale purple
5	26–27	–	violet	–
6**	28–29	–	–	purple
7	37–40	quenching	red	–
8	40–42	–	–	pale purple
9	42–44	quenching	–	–
10	45–47	–	red	–
11	52–54	–	–	pale purple
12	56–59	weak quenching	red	–
13	67–69	–	pink	purple

*β-O-D-glucopyranosyl-2-(2′-hydroxy-Z-6′-enecosamide)sphingosine (from experiments)
**β-sitosteryl-β-D-glucoside (available as a standard substance)

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 14.0 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 19.0 per cent w/w (Appendix 7.12).

Swelling index Not less than 7.0, when determined by the following method.

Place about 1 g of Murdannia Loriformis Herb, in *coarse powder*, accurately weighed, in a 25-mL glass-stoppered cylinder graduated over a height of 125±5 mm in 0.5-mL divisions. 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 in the layer of the Herb and any particles of the Herb floating at the surface of the liquid by rotating the cylinder about a vertical axis. Measure the volume occupied by the Herb, including any adhering mucilage. Carry out three tests at the same time. The swelling index is determined by the mean of the three tests.

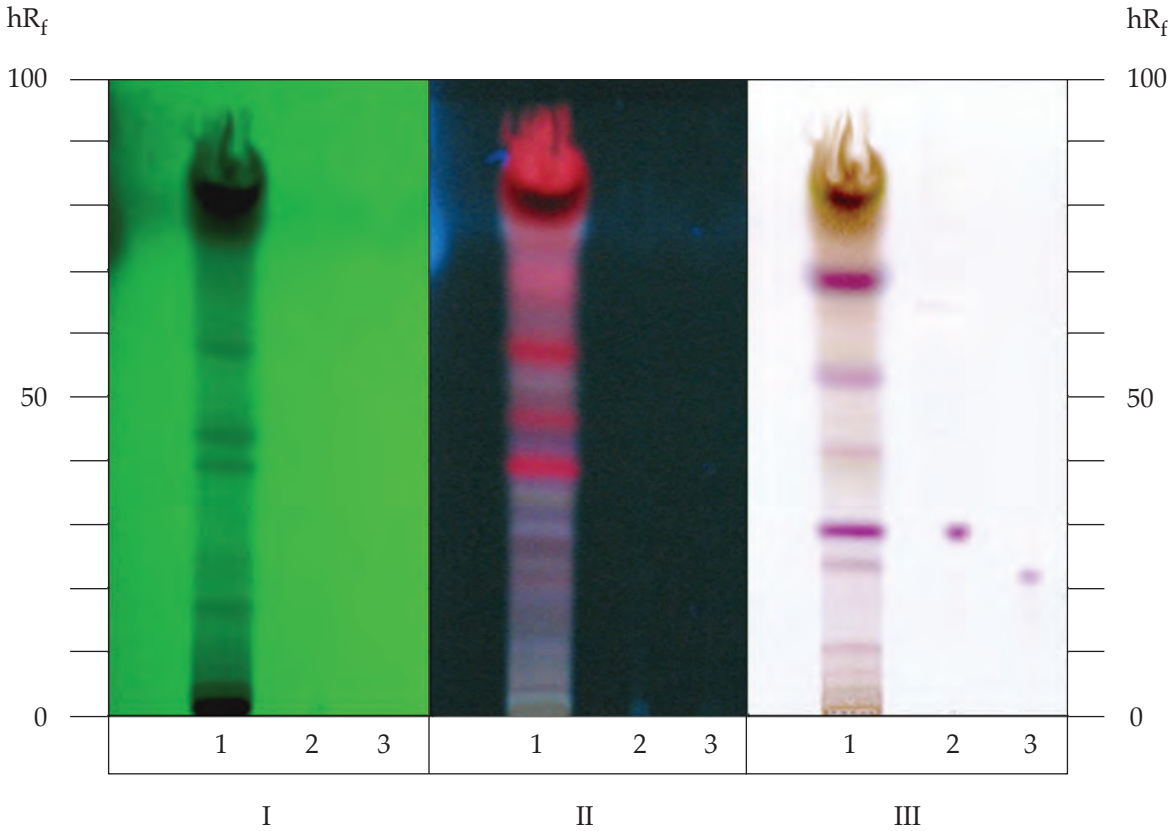


Fig. 3 Thin-Layer Chromatogram of Ethanolic Extract of the Aerial Parts of *Murdannia loriformis* (Hassk.) R. S. Rao & Kammathy

1 = solution (A)
2 = solution (B)
3 = a 0.1 per cent w/v solution of *β*-O-D-glucopyranosyl-2-(2′-hydroxy-Z-6′-enecosamide)sphingosine in *methanol* (3 μL)
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with a 10 per cent v/v solution of *sulfuric acid* in *ethanol*

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 $C_2H_4O_2 = 60.05$

Use an analytical reagent grade of commerce containing not less than 99.5 per cent w/w and not more than 100.5 per cent w/w of $C_2H_4O_2$.

DESCRIPTION Clear, colourless liquid; odour, pungent and characteristic. Boils at about 118°.

Acetic Anhydride $C_4H_6O_3 = 102.09$

Use an 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 $C_3H_6O = 58.08$

DESCRIPTION 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 reweighed. 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°.

Ammonium Dihydrogenphosphate (Ammonium Phosphate, Monobasic) $(NH_4)\ H_2PO_4$ = 115.03
Use analytical reagent grade of commerce.

pH About 4.2, in a 2.3 per cent w/v solution (Appendix 4.11).

Andrographolide $C_{20}H_{30}O_5$ = 350.46

Use an 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 a 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 an analytical reagent grade of commerce containing not less than 98.0 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.

Astragalin (Kaempferol 3-*β*-D-glucopyranoside) $C_{21}H_{20}O_{11}$ = 448.38

Use analytical reagent grade of commerce containing not less than 97.0 per cent.

DESCRIPTION White to light yellow powder or crystals.

SOLUBILITY Sparingly soluble in *ethanol* and *methanol*.

MELTING RANGE 218° to 220° (Appendix 4.3).

Store in tightly closed containers at a temperature between 2° and 8°.

Asiatic Acid $C_{30}H_{48}O_5$ = 488.70

Use an analytical reagent grade of commerce containing not less than 95.0 per cent of $C_{30}H_{48}O_5$.

Asiaticoside $C_{48}H_{78}O_{19}$ = 959.12

Use an analytical reagent grade of commerce containing not less than 97.0 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 an analytical reagent grade of commerce containing not less than 95.0 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 a 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).

(+)-Catechin Hydrate $C_{15}H_{14}O_6 \cdot xH_2O$= 290.27

MELTING TEMPERATURE 175° to 177° (Appendix 4.3).

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 an 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 an analytical reagent grade of commerce containing not less than 95.0 per cent of $C_{10}H_{16}O$.

Citronellal $C_{10}H_{18}O$ = 154.25

Use an analytical reagent grade of commerce containing not less than 95.0 per cent w/w of $C_{10}H_{18}O$.

SOLUBILITY Very slightly soluble in *water*, soluble in *ethanol*.

REFRACTIVE INDEX About 1.446, at 20° (Appendix 4.7).

SPECIFIC GRAVITY 0.848 to 0.856 (Appendix 4.9).

Store in tightly closed containers, in a cool and dry place, protected from light.

Citronellol $C_{10}H_{20}O$ = 156.27

Use an analytical reagent grade of commerce containing not less than 95.0 per cent w/w of $C_{10}H_{20}O$.

DESCRIPTION Clear, colourless liquid.

SOLUBILITY Practically insoluble in *water*, miscible with *ethanol*.

BOILING RANGE 220° to 222° (Appendix 4.5).

REFRACTIVE INDEX 1.456, at 20° (Appendix 4.7).

SPECIFIC GRAVITY About 0.857 (Appendix 4.9).

Store in tightly closed containers, protected from light.

Citronellyl Acetate $C_{12}H_{22}O_2$ = 198.30

Use an analytical reagent grade of commerce containing not less than 95.0 per cent w/w of $C_{12}H_{22}O_2$.

BOILING POINT 229° (Appendix 4.5).

REFRACTIVE INDEX 1.443, at 20° (Appendix 4.7).

SPECIFIC GRAVITY About 0.89 (Appendix 4.9).

Store in tightly closed containers, protected from light.

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 a 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, colourless 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 $C_{14}H_{11}N$.

Diethyl ether $C_4H_{10}O = 74.12$

Use an analytical reagent grade of commerce.

DESCRIPTION Colourless liquid; odour, characteristic.

BOILING POINT 34.6° (Appendix 4.5).

WEIGHT PER MILLILITRE 0.71 g (Appendix 4.9).

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 (Minutes)	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 an 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 an 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 an 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).

Eurycomanone $C_{20}H_{24}O_9 = 408.40$

Use an analytical reagent grade of commerce containing not less than 95.0 per cent w/w of $C_{20}H_{24}O_9$.

DESCRIPTION White powder.

Store in tightly closed containers, protected from light and air, at a temperature not exceeding 20°.

Fenchone $C_{10}H_{16}O = 152.23$

Use an 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 an analytical reagent grade of commerce containing not less than 95.0 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 an 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 an 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 an 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 Monohydrate) $C_7H_6O_5{\cdot}H_2O$ = 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*.

Gelatin

Use a general reagent grade of commerce.

DESCRIPTION Colourless or slightly yellow, transparent, brittle, tasteless sheets, flakes, or powder; odourless.

SOLUBILITY Soluble in hot *water*, *acetic acid* and *glycerol*; insoluble in organic solvents.

Geraniol $C_{10}H_{18}O$ = 154.25

Use an analytical reagent grade of commerce containing not less than 97.0 per cent w/w of $C_{10}H_{18}O$.

DESCRIPTION Oily liquid, slight odour of rose.

SOLUBILITY Practically insoluble in *water*, miscible with *ethanol*.

Store in tightly closed containers, protected from light.

Geranyl Acetate $C_{12}H_{20}O_2$ = 196.27

Use an analytical reagent grade of commerce containing not less than 97.0 per cent w/w of $C_{12}H_{20}O_2$.

DESCRIPTION Colourless or slightly yellow liquid, slight odour of rose and lavender.

Store in tightly closed containers, in a cool and dry place.

6-Gingerol $C_{17}H_{26}O_4$ = 294.39

Use an analytical reagent grade of commerce containing not less than 98.0 per cent w/w of $C_{17}H_{26}O_4$.

DESCRIPTION Yellowish white or yellow liquid or solid.

Gypenoside III (Ginsenoside Rb1) $C_{54}H_{92}O_{23}\cdot 3H_2O$ = 1163.34

Use an analytical reagent grade of commerce containing not less than 95.0 per cent w/w of $C_{54}H_{92}O_{23}\cdot 3H_2O$.

DESCRIPTION A colourless solid.

SOLUBILITY Soluble in *water*, in anhydrous *ethanol* and in *methanol*.

MELTING POINT About 199° (Appendix 4.3).

SPECIFIC ROTATION About + 11.3 determined in a 1 per cent w/v solution in methanol (Appendix 4.8).

Store in tightly closed containers, in a cool and dry place.

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 an 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 an 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.

Hydrochloric Acid, Heavy Metal-Free
Use a suitable reagent grade of commerce. Complies with the requirements prescribed for *hydrochloric acid* with the following maximum contents of heavy metals in ppm: Arsenic 0.005, Cadmium 0.003, Copper 0.003, Iron 0.05, Mercury 0.005, Nickel 0.004, Lead 0.001, and Zinc 0.005.

Hydrochloric Acid, Dilute, Heavy Metal-Free
Use a suitable reagent grade of commerce. Complies with the requirements prescribed for *hydrochloric acid* with the following maximum contents of heavy metals in ppm: Arsenic 0.005, Cadmium 0.003, Copper 0.003, Iron 0.05, Mercury 0.005, Nickel 0.004, Lead 0.001, and Zinc 0.005.

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 an analytical reagent grade of commerce containing not less than 98.0 per cent w/w of $C_{16}H_{14}O_4$.

DESCRIPTION Off-white or light brown powder.

Iron(III) Chloride (Ferric Chloride) $FeCl_3 \cdot 6H_2O$ = 270.30

Use an 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 a general reagent grade of commerce.

DESCRIPTION Bluish green crystals or pale, crystalline powder; odourless. Efflorescent 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 a 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 an 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.

Limonene $C_{10}H_{16}$ = 136.23

Use an analytical reagent grade of commerce containing not less than 99.0 per cent w/w of $C_{10}H_{16}$.

DESCRIPTION Colourless liquid.

SOLUBILITY Practically insoluble in *water*, soluble in *ethanol*.

BOILING RANGE 175° to 177° (Appendix 4.5).

REFRACTIVE INDEX 1.471 to 1.474, at 20° (Appendix 4.7).

SPECIFIC ROTATION About + 124 (Appendix 4.8).

SPECIFIC GRAVITY About 0.84 (Appendix 4.9).

Store in tightly closed containers, in a cool and dry place, protected from light.

Lupeol $C_{30}H_{50}O$ = 426.72

Use a general reagent grade of commerce.

Madecassoside $C_{48}H_{78}O_{20}$ = 975.12

Use an analytical reagent grade of commerce containing not less than 95.0 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 Nitrate (Magnesium Nitrate Hexahydrate) $Mg(NO_3)_2{\cdot}6H_2O$ = 256.41

Use analytical reagent grade of commerce.

DESCRIPTION Colourless, clear crystals, deliquescent.

SOLUBILITY Very soluble in *water*, freely soluble in *ethanol*.

Store in tightly closed containers, in a cool and dry place.

Magnesium Ribbon

Use a suitable grade.

Methanol (Methyl Alcohol) CH_4O = 32.04

Use an 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) $C_5H_{12}O$ = 88.15

Use a 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, Dilute, Heavy Metal-Free

Use a suitable reagent grade of commerce. Complies with the requirements prescribed for *hydrochloric acid* with the following maximum contents of heavy metals in ppm: Arsenic 0.005, Cadmium 0.005, Copper 0.001, Iron 0.02, Mercury 0.002, Nickel 0.005, Lead 0.001, and Zinc 0.01.

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.

Nitric Acid, Heavy Metal-Free

Use a suitable reagent grade of commerce. Complies with the requirements prescribed for *hydrochloric acid* with the following maximum contents of heavy metals in ppm: Arsenic 0.005, Cadmium 0.005, Copper 0.001, Iron 0.02, Mercury 0.002, Nickel 0.005, Lead 0.001, and Zinc 0.01.

Nitrogen N_2 = 28.01

Use a laboratory cylinder grade of commerce, 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 a 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*.

Pectolinarigenin $C_{17}H_{14}O_6$ = 314.30

Use analytical reagent grade of commerce containing not less than 98.0 per cent.

DESCRIPTION Yellow powder.

SOLUBILITY Sparingly soluble in *ethanol* and *methanol*.

Store in tightly closed containers at a temperature not exceeding 25°, protected from light.

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 an 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 .

Piceatannol $C_{14}H_{12}O_4$ = 244.24

Use analytical reagent grade of commerce containing not less than 98.0 per cent.

DESCRIPTION Yellow-tan to brown-pink powder.

SOLUBILITY Soluble in *ethanol*.

Store in tightly closed containers at a temperature between 2° and 8°.

Piperine $C_{17}H_{19}NO_3$ = 285.34

Use an analytical reagent grade of commerce containing not less than 95.0 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 an 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 an analytical reagent grade of commerce.

DESCRIPTION White crystalline powder.

Potassium Permanganate $KMnO_4$ = 158.03

Use an 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 an 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 an 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 an analytical reagent grade of commerce containing not less than 95.0 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 an analytical reagent grade of commerce containing not less than 95.0 per cent of $C_{18}H_{16}O_8$.

Ruthenium Red (Ammoniated Ruthenium Oxychloride) $C_{l6}H_{42}N_{14}O_2Ru_3$ = 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 an analytical reagent grade of commerce containing not less than 95.0 per cent of $C_{27}H_{30}O_{16}$.

6-Shogaol $C_{17}H_{24}O_3$ = 276.37

Use an analytical reagent grade of commerce containing not less than 98.0 per cent w/w of $C_{17}H_{24}O_3$.

DESCRIPTION Pale yellow liquid.

Sinapic Acid (Sinapinic Acid) $C_{11}H_{12}O_5$ = 224.21

Use a general reagent grade of commerce.

MELTING TEMPERATURE About 202° (Appendix 4.3).

Sinensetin $C_{20}H_{20}O_7$ = 372.37

Use a general reagent grade of commerce.

***β*-sitosterol *β*-D-glucoside** $C_{35}H_{60}O_6$ = 576.85

Use analytical reagent grade of commerce containing not less than 75.0 per cent.

DESCRIPTION White to off-white powder or crystals.

Store in tightly closed containers at a temperature not exceeding 25°.

Sodium Acetate $C_2H_3NaO_2{\cdot}3H_2O$ = 136.08

Use an analytical reagent grade of commerce.

Sodium Borohydride (Sodium Tetrahydroborate) $NaBH_4$ = 37.83

Use analytical reagent grade of commerce.

DESCRIPTION Colourless, hygroscopic crystals.

SOLUBILITY Freely soluble in *water*, soluble in *anhydrous ethanol*, decomposing at higher temperature or in the presence of acids or certain metal salts forming borax and hydrogen.

Store in tightly closed containers, in a cool and dry place, protected from light.

Sodium Dodecyl Sulfate (Sodium Lauryl Sulfate) $C_{12}H_{25}NaO_4S$ = 288.38

Use a 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 an analytical reagent grade of commerce.

Sodium Hydroxide NaOH = 40.00

Use an 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 an 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 *x*M are required, they should be prepared by carefully adding 54*x* 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).

Tetrahydrofuran (Tetramethylene Oxide) C_4H_8O = 72.11

Use an analytical reagent grade of commerce.

DESCRIPTION Clear, colourless, flammable liquid.

BOILING TEMPERATURE About 66° (Appendix 4.5).

WEIGHT PER MILLILITRE About 0.89 g (Appendix 4.9).

Do not distil until it complies with the following test.

PEROXIDES Place 8 mL of *starch-iodide TS* in a groundglass-stoppered cylinder with a capacity of 12 mL and about 1.5 cm in diameter and add sufficient of the substance being examined to fill the cylinder completely, shake vigorously and allow to stand for 30 minutes protected from light. No colour is produced.

Tetrahydrofuran used in spectrophotometry complies with the following additional requirement.

TRANSMITTANCE Not less than 20 per cent at 255 nm, 80 per cent at 270 nm and 98 per cent at 310 nm, determined using *water* in the reference cell.

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 an analytical reagent grade of commerce containing not less than 95.0 per cent of $C_{10}H_{12}O_2$.

Tin(II) Chloride (Stannous Chloride, Tin Chloride Dihydrate) $SnC_{12}{\cdot}2H_2O$ = 225.65

Use analytical reagent grade of commerce.

DESCRIPTION Colourless crystals.

SOLUBILITY Soluble in *dilute hydrochloric acid*.

Toluene (Methylbenzene) C_7H_8 = 92.14

Use an 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 a 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.

Ursolic acid $C_{30}H_{48}O_3$ = 456.71

Use an analytical reagent grade of commerce containing not less than 98.0 per cent w/w of $C_{30}H_{48}O_3$.

DESCRIPTION White powder.

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, Carbon Dioxide-free
Water that has been boiled vigorously for a few minutes and protected from the atmosphere during cooling and storage.

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 stand 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 an 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. (**Note** Use a suitable grade available such as Celite 545.)

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. (**Note** Use a suitable grade available such as Celite 545-AW.)

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. (**Note** Use suitable grades available such as Anachrome Q, Gas-Chrom Q and Varaport 30.)

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, nitriol, vinyl, or fluoroalkyl groups, or mixtures of these), and microporous 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.

Kieselguhr G A fine, greyish white powder, the grey colour becoming more pronounced on triturating with water. The average particle size is between 10 and 40 μm. It consists of natural kieselguhr which has been extracted with hydrochloric acid and calcined, and to which about 15 per cent w/w of calcium sulfate hemihydrate has been added. It complies with the following requirements.

CONTENT OF CALCIUM SULFATE Carry out the test described under Silica gel G.

SEPARATING POWER Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1), using a mixture of 65 volumes of *ethyl acetate*, 23 volumes of *2-propanol* and 12 volumes of *water* as the mobile phase. Prepare the chromatoplates using a slurry of the sample in 0.02 M *sodium acetate*. Apply to the plate, 5 μL of a solution in *pyridin*e containing 0.01 per cent w/v of each of *lactose, sucrose, fructose*, *D-glucose*, and *D-galactose*. After removal of the plate, dry it at 105° to 110°, and allow to cool. Spray the plate with about 10 mL of *anisaldehyde TS* and heat at 105° to 110° for 5 to 10 minutes; the chromatogram shows five, well-defined, well-separated spots with no tailing.

ALKALINITY pH of a suspension prepared by shaking 1 g with 10 mL of *carbon dioxide-free water* for 5 minutes, 7.0 to 8.0 (Appendix 4.11).

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 $CaSO_4 \cdot ½H_2O$.

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 *indophenols 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 indophenols 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 (Appendix 4.11).

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, but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, ten portions from 1 to 10 μL of a 1 mg per mL solution of *benzoic acid* in the same solvent mixture. After removal of the plate, 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.

Silica gel H A fine, white, homogenous powder of an average particle size between 10 and 40 μm. It is free from calcium sulfate hemihydrate and complies with the tests for Separating Power and Alkalinity stated under Silica gel G.

Silica gel HF254 A fine, white homogenous powder of an average particle size between 10 and 40 μm containing about 1.5 per cent w/w of a fluorescent indicator having a maximum intensity at about 254 nm. It is free from calcium sulfate hemihydrates and complies with the tests for Alkalinity and Separating Power stated under Silica gel G, and with the test for Fluorescence stated under Silica gel GF254.

Silica gel F254 A fine, white, homogeneous powder of an average particle size of about 15 μm containing a suitable binding agent and about 1.5 per cent w/w of a fluorescent indicator having a maximum intensity at 254 nm. It complies with the following requirement.

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, but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, ten portions from 1 to 10 μL of a 1 mg per mL solution of *benzoic acid* in the mobile phase. After removal of the plate, 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.

Silica gel, Octadecylsilyl A very finely divided silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups.

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.

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.

[1]US National Institute of Standards and Technology.

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.

Heavy metals See under Arsenic and Heavy Metals in the General Notices of the Thai Herbal Pharmacopoeia.

Microbial limit The products consisting of herbal drugs comply with the "Limits for Microbial Contamination" (Appendix 10.5).

Packaging and storage Herbal drugs shall 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 liquid (liquid extracts), semi-solid (soft extracts and oleoresins) or solid (dry extracts) preparations, obtained from herbal drugs. The constituents of interest are completely or partially separated from other components with the aid of water, ethanol or other suitable solvents.

For this Pharmacopoeia, the solvents preferred are ethanol, water or ethanol-water mixtures. In case of other solvents being used, the test for residual solvents shall be demonstrated by the acceptable validated methods. This extraction process involves the removal of the desired constituents from the herbal matter with suitable menstrua, the evaporation of all or nearly all of the solvent, and the adjustment of the residual fluids, masses, or powders to the prescribed standards. Extracts may be subjected to processes that increase the content of characterized constituents, decrease the content of unwanted constituents, or both. Extracts with no added excipients or inert substances and no processing beyond the extraction are called native or genuine extracts.

Production

Herbal drugs, solvents and other materials used for the preparation of extracts are of suitable quality and where applicable comply with the requirements of any relevant monograph in the Pharmacopoeia. Where justified, herbal drugs used for the production of extracts may exceed the limits for heavy metals specified in the "Herbal Drugs" (Appendix 1.16H) provided that the resulting extract satisfies the requirements for heavy metals (Appendix 5.2).

Different batches of the herbal drug which are compliant with the relevant monograph, or in the absence of an individual monograph with other suitable specifications, may be combined prior to extraction to achieve a certain range of content for one or more constituents in the herbal drug to be extracted. The herbal drug may also undergo a preliminary treatment, for example, grinding, inactivation of enzymes or defatting. In addition, unwanted constituents (e.g., toxic constituents) or unwanted matter (e.g., insoluble matter) may be removed at a suitable stage in the production process.

Where solvents are recovered from the production process, such recovered or recycled solvents may be used, provided that the recovery procedures are controlled and monitored to ensure that solvents meet appropriate standards before re-use or admixture with other approved materials. Water used for preparation of extracts is of suitable quality such as Purified Water.

Where applicable, extraction liquors are concentrated to the intended consistency using suitable methods, usually under reduced pressure and at a temperature at which deterioration of the constituents is reduced to a minimum. Essential oils that have been separated during processing may be restored to the extracts at an appropriate stage in the production process. Suitable excipients or inert substances may be added at various stages of the production process for technological reasons (for example, as part of the drying process or to improve the homogeneity or consistency of an extract). For some extracts, suitable inert excipients may also be added to adjust one or more constituents to a defined

content. Suitable inert substances may be added as carriers or diluents to improve physical characteristics. Suitable stabilizers, antioxidants, antimicrobials, and other preservatives may be added to preserve the integrity, where justified and authorized.

Extraction with a given solvent leads to a typical content of selected constituents in the extracted dry matter; during production of extracts, purification procedures may be applied that increase the content of these selected constituents with respect to the expected values; such extracts are called refined extract.

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.

Pesticide residues Unless otherwise stated in the monograph, the extracts comply with the "Pesticide Residues" (Appendix 7.22H). Where justified, this test may be totally or partially exempt when the complete history (nature and quantity of the pesticides used, date of each treatment, during cultivation and after harvest) of the treatment of the batch is known and can be checked precisely and according to good agricultural and collection practice (GACP).

Labelling The label on the container states (1) the herbal or animal matter used; (2) whether the extract is liquid, soft, oleoresin, or dry; (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 "refined"; (6) the name and amount of any excipient or inert substance used; (7) where applicable, that the content of constituents (markers) have been 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 using ethanol of a suitable concentration and/or water together with, where necessary, other substances to extract the herbal drug, or by dissolving a soft or dry extract of the herbal drug (which has been produced using the same extraction solvent as would be used to prepare the liquid extract by direct extraction) in either ethanol of the required concentration or water.

Liquid extracts produced from soft or dry extracts do not contain any excipients other than those that would be present in the liquid extract prepared by direct extraction. However, exceptions may be justified in certain cases such as when the soft extract used to produce the liquid extract contains stabilizers, antioxidants or antimicrobial preservatives that have been added to ensure its stability.

Liquid extracts are adjusted, if necessary, so that they satisfy the requirements for content of solvent. Liquid extracts may be filtered, if necessary. A slight sediment may form on standing.

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.

2-Propanol 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 *self-indicating silica gel* or other suitable desiccants and weigh. Calculate the result as a percentage or in grams per litre.

Packaging and storage Liquid extracts shall 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 105° for 3 hours. Allow to cool in a desiccator over *self-indicating silica gel* or other suitable desiccants 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 shall be kept in tightly closed containers, protected from light.

Labelling In addition to the requirements listed above, the label on the container states the concentration of any added antimicrobial preservative.

Oleoresins

Oleoresins are semi-solid preparations composed of a resin in solution in an essential and/or fatty oil and are obtained by evaporation of the solvent(s) used for their production.

The following requirements apply to oleoresins produced by extraction and not to natural oleoresins.

Water The oleoresin complies with the limits prescribed in the monograph.

Packaging and storage Oleoresins shall be kept in tightly closed containers, protected from light.

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 105° for 3 hours. Allow to cool in a desiccator over *self-indicating silica gel* or other suitable desiccants 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.

Water Where a test for loss on drying is not applicable, the dry extract complies with the limits prescribed in the monograph.

Packaging and storage Dry extracts shall be kept in tightly 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 monograph, 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 30º 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.

CREAMS Creams are homogeneous, semi-solid preparations consisting of opaque emulsion systems. They are multiphase preparations composed of a lipophilic phase and an aqueous phase.

Hydrophobic creams Hydrophobic creams have the lipophilic phase as the continuous phase. They contain water-in-oil emulsifying agents such as wool fat, sorbitan esters and monoglycerides.

Hydrophilic creams Hydrophilic creams have the aqueous phase as the continuous phase. They contain oil-in-water emulsifying agents such as sodium or triethanolamine soaps, sulfated fatty alcohols and polysorbates, combined, if necessary, with water-in-oil emulsifying agents.

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 ±3 nm for the range 400 to 600 nm.

241.15 nm (Ho)	404.66 nm (Hg)
253.70 nm (Hg)	435.83 nm (Hg)
287.15 nm (Ho)	486.00 nm (Dβ)
302.25 nm (Hg)	486.10 nm (Hβ)
13.16 nm (Hg)	536.30 nm (Ho)
334.15 nm (Hg)	546.07 nm (Hg)
361.50 nm (Ho)	576.96 nm (Hg)
365.48 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

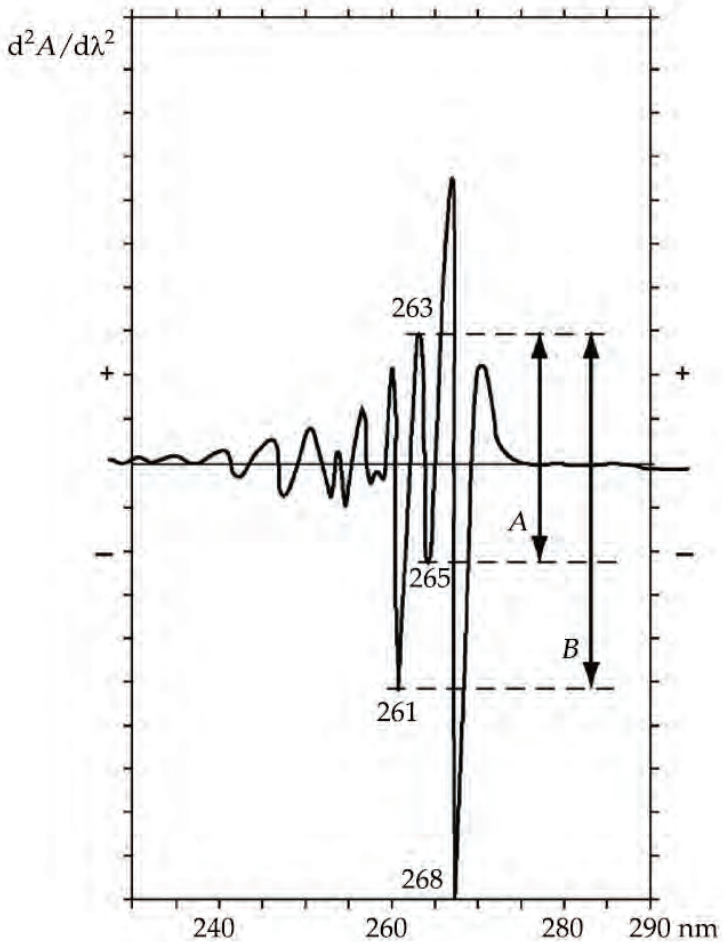
A spectrophotometer complying with the requirements prescribed above and equipped with an analogue resistance-capacitance differentiation module or a digital differentiator or another means of producing second derivative spectra should be used in accordance with the manufacturer's instructions. Some 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 extrema 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.



2.3 ATOMIC SPECTROPHOTOMETRY: EMISSION AND ABSORPTION

These techniques are used to determine the concentration of certain metallic ions by measuring the intensity of emission or absorption of light at a particular wavelength by the atomic vapour of the element generated from the substance, for example, by introducing a solution of the substance into a flame.

FOR ATOMIC EMISSION SPECTROMETRY

Atomic emission is a process that occurs when electromagnetic radiation is emitted by excited atoms or ions. In atomic emission spectrometry the sample is subjected to temperatures high enough to cause not only dissociation into atoms, but also to cause significant amounts of collisional excitation and ionization of the sample atoms to take place. Once the atoms and ions are in the excited states, they can decay to lower states through thermal or radiative (emission) energy transitions and electromagnetic radiation is emitted. An emission spectrum of an element contains several more lines than the corresponding absorption spectrum. Atomic emission spectrometry is a technique for determining the concentration of an element in a sample by measuring the intensity of one of the emission lines of the atomic vapour of the element generated from the sample. The determination is carried out at the wavelength corresponding to this emission line.

Apparatus

The apparatus essentially consists of the following:

(a) a sample introduction and nebulization system;
(b) a flame to generate the atoms to be determined;
(c) a monochromator;
(d) a detector;
(e) a data-acquisition unit.

Oxygen, air and a combustible gas such as hydrogen, acetylene, propane or butane may be used in flames. The atomization source is critical, since it must provide sufficient energy to excite and atomize the atoms. The atomic spectra emitted from flames have the advantage of being simpler than those emitted from other sources, the main limitation being that the flames are not powerful enough to cause emission for many elements allowing their determination. Acidified water is the solvent of choice for preparing test and standard preparations, although organic solvents may also be used if precautions are taken to ensure that the solvent does not interfere with the stability of the flame.

Interferences

Spectral interference is reduced or eliminated by choosing an appropriate emission line for measurement or by adjusting the slit for spectral band-width. Physical interference is corrected by diluting the sample preparation, by matching the matrix or by using the method of standard additions. Chemical interference is reduced by using chemical modifiers or ionization buffers.

Memory effect

The memory effect caused by deposit of analyte in the apparatus may be limited by thoroughly rinsing between runs, diluting the solutions to be measured if possible and thus reducing their salt content, and by aspirating the solutions through as swiftly as possible.

Method

(**Note** Evaluate and select the type of material of construction, pretreatment, and cleaning of analytical labware used in the Atomic Absorption Spectrophotometric analyses. The material must be inert and, depending on the specific application, resistant to caustics, acids, and/or organic solvents, i.e., plastic.)

Operate an atomic emission spectrometer in accordance with the manufacturer's instructions at the prescribed wavelength. Optimize the experimental conditions (flame temperature, burner adjustment, use of an ionic buffer, concentration of solutions) for the specific element to be analyzed and in respect of the sample matrix. Introduce a blank solution into the atomic generator and adjust the instrument reading to zero or to its blank value. Introduce the most concentrated standard preparation and adjust the sensitivity to obtain a suitable reading. It is preferable to use concentrations which fall within the linear part of the calibration curve. If this is not possible, the calibration plots may also be curved and are then to be applied with appropriate calibration software.

Determinations are made by comparison with standard preparations with known concentrations of the element to be determined either by the method of direct calibration (Method I) or the method of standard additions (Method II).

METHOD I: METHOD OF DIRECT CALIBRATION

For routine measurements three standard preparations of the element to be determined and a blank are prepared and examined. Prepare the solution of the substance to be examined (test preparation) as prescribed in the monograph. Prepare not fewer than three standard preparations of the element to be determined, the concentrations of which span the expected value in the test preparation. For assay purposes, optimal calibration levels are between 0.7 and 1.3 times the expected content of the element to be determined or the limit prescribed in the monograph. For purity determination, calibration levels are between the limit of detection and 1.2 times the limit specified for the element to be determined. Any reagents used in the preparation of the test preparation are added to the standard preparations and to the blank solution at the same concentration. Introduce each of the solutions into the instrument using the same number of replicates for each solution, to obtain a steady reading.

Calculation Prepare a calibration curve from the mean of the readings obtained with the standard preparation by plotting the means as a function of concentration. Determine the concentration of the element in the test preparation from the curve obtained.

METHOD II: METHOD OF STANDARD ADDITIONS

Add to at least three similar volumetric flasks equal volumes of the solution of the substance to be examined (test preparation) prepared as prescribed. Add to all but one of the flasks progressively larger volumes of a standard preparation containing a known concentration of the element to be determined to produce a series of solutions containing steadily increasing concentrations of that element known to give responses in the linear part of the curve, if at all possible. Dilute the contents of each flask to volume with solvent. Introduce each of the solutions into the instrument using the same number of replicates for each solution, to obtain a steady reading.

Calculation Calculate the linear equation of the graph using a least-squares fit, and derive from it the concentration of the element to be determined in the test preparation.

Validation of the method

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

LINEARITY

Prepare and analyze not fewer than four standard preparations over the calibration range and a blank solution. Perform not fewer than five replicates. The calibration curve is calculated by least-square regression from all measured data. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

— the correlation coefficient is at least 0.99,

— the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and highest calibration level. When the ratio of the estimated standard deviation of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

ACCURACY

Verify the accuracy preferably by using a certified standard material. Where this is not possible, perform a test for recovery.

RECOVERY

For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. For other determinations, for example for trace element determination, the test is not valid if recovery is outside of the range 80 per cent to 120 per cent at the theoretical value. Recovery may be determined on a suitable standard preparation (matrix solution) which is spiked with a known quantity of analyte (middle concentration of the calibration range).

REPEATABILITY

The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

LIMIT OF QUANTIFICATION

The limit of quantification can be estimated by calculating the standard deviation of not less than six replicate measurements of a blank solution, divided by the slope of a standard curve, and multiplying by 10. If validating a procedure using the method of standard additions, the slope of standards applied to a solution of the substance being examined (test preparation) is used. Other suitable approaches can be used.

FOR ATOMIC ABSORPTION SPECTROMETRY

Atomic absorption is a process that occurs when a ground state-atom absorbs electromagnetic radiation of a specific wavelength and is elevated to an excited state. The atoms in the ground state absorb energy at their resonant frequency and the electromagnetic radiation is attenuated due to resonance absorption. The energy absorption is virtually a direct function of the number of atoms present.

This appendix provides general information and defines the procedures used in element determinations by atomic absorption spectrometry, either atomization by flame, by electrothermal vaporization in a graphite furnace, by hydride generation or by cold vapour technique for mercury.

Atomic absorption spectrometry is a technique for determining the concentration of an element in a sample by measuring the absorption of electromagnetic radiation by the atomic vapour of the element generated from the sample. The determination is carried out at the wavelength of one of the absorption (resonance) lines of the element concerned. The amount of radiation absorbed is, according to the Beer's law, proportional to the element concentration.

Apparatus

The apparatus consists essentially of the following:

(a) a source of radiation;
(b) a sample introduction device;
(c) a sample atomizer;
(d) a monochromator or polychromator;
(e) a detector;
(f) a data-acquisition unit.

The apparatus is usually equipped with a background correction system. Hollow-cathode lamps and electrodeless discharge lamps (EDL) are used as radiation source. The emission of such lamps consists of a spectrum showing very narrow lines with half-width of about 0.002 nm of the element being determined.

There are three types of sample atomizers:

FLAME TECHNIQUE

A flame atomizer is composed of a nebulization system with a pneumatic aerosol production accessory, a gas-flow regulation and a burner. Although other flame types have been documented, the most commonly used flame is an air–acetylene flame. Because the temperature of the air-acetylene flame is not sufficient to destroy oxides that might form or are present, a nitrous oxide-acetylene flame often is used, depending on the analyte and nature of the sample. The air-acetylene flame burns within a temperature range of 2125° to 2400°, but the nitrous oxide-acetylene flame burns within a temperature range of 2650° to 2800°. The configuration of the burner is adapted to the gases used and the gas flow is adjustable. Samples are nebulized, acidified water being the solvent of choice for preparing test and standard preparations. Organic solvents may also be used if precautions are taken to ensure that the solvent does not interfere with the stability of the flame.

ELECTROTHERMAL ATOMIZATION TECHNIQUE

An electrothermal atomizer is generally composed of a graphite tube furnace and an electric power source. Electrothermal atomization in a graphite tube furnace atomizes the entire sample and retains the atomic vapour in the light path for an extended period. This improves the detection limit. Samples, liquid as well as solid, are introduced directly into the graphite tube furnace, which is heated in a programmed series of steps to dry the sample and remove major matrix components by pyrolysis and to then atomize all of the analyte. The furnace is cleaned using a final temperature higher than the atomization temperature. The flow of an inert gas during the pyrolysis step in the graphite tube furnace allows a better performance of the subsequent atomization process.

COLD VAPOUR AND HYDRIDE GENERATION TECHNIQUE

The atomic vapour may also be generated outside the spectrometer. This is notably the case for the cold-vapour method for mercury or for certain hydride-forming elements such as arsenic, antimony, bismuth, selenium and tin. For mercury, atoms are generated by chemical reduction with stannous chloride or sodium borohydride and the atomic vapour is swept by a stream of an inert gas into a cold quartz cell mounted in the optical path of the instrument. Hydrides thus generated are swept by an inert gas into a heated cell in which they are dissociated into atoms. Both cold vapour and hydride generation techniques are very sensitive and have detection limits in the part per billion (ppb) or part per trillion (ppt) range.

Interferences

Chemical, physical, ionization, and spectral interferences are encountered in atomic absorption measurements. Chemical interference is compensated by addition of matrix modifiers, of releasing agents or by using high temperature produced by a nitrous oxide-acetylene flame; the use of specific ionization buffers (for example, lanthanum and caesium) compensates for ionization interference; by dilution of the sample, through the method of standard additions or by matrix matching, physical interference due to high salt content or viscosity is eliminated. Spectral interference results from the overlapping of resonance lines and can be avoided by using a different resonance line. The use of Zeeman background correction also compensates for spectral interference and interferences from molecular absorption, especially when using the electrothermal atomization technique. The use of multi-element hollow-cathode lamps may also cause spectral interference. Specific or non-specific absorption is measured in a spectral range defined by the bandwidth selected by the monochromator (0.2 to 2 nm).

Background correction

Scatter and background in the flame or the electrothermal atomization technique increase the measured absorbance values. Background absorption covers a large range of wavelengths, whereas atomic absorption takes place in a very narrow wavelength range of about 0.005 to 0.02 nm. Background absorption can in principle be corrected by using a blank solution of exactly the same composition as the sample, but without the specific element to be determined, although this method is frequently impracticable. With the electrothermal atomization technique the pyrolysis temperature is to be optimized to eliminate the matrix decomposition products causing background absorption. Background correction can also be made by using two different light sources, the hollow-cathode lamp that measures the total absorption (element and background) and a deuterium lamp with a continuum emission from which the background absorption is measured. Background is corrected by subtracting the deuterium lamp signal from the hollow-cathode lamp signal. This method is limited in the spectral range on account of the spectra emitted by a deuterium lamp from 190 to 400 nm. Background can also be measured by taking readings at a non-absorbing line near the resonance line and then subtracting the results from the measurement at the resonance line. Another method for the correction of background absorption is the Zeeman effect (based on the Zeeman splitting of the absorption line in a magnetic field). This is particularly useful when the background absorption shows fine structure. It permits an efficient background correction in the range of 185 to 900 nm.

Choice of the operating conditions

After selecting the suitable wavelength and slit width for the specific element, the need for the following has to be ascertained:

— correction for non-specific background absorption,

— chemical modifiers or ionization buffers to be added to the sample as well as to blank and standard solutions,

— dilution of the sample to minimize, for example, physical interferences,

— details of the temperature programme, preheating, drying, pyrolysis, post-atomization with ramp and hold times,

— inert gas flow,

— matrix modifiers for electrothermal atomization (furnace),

— chemical reducing reagents for measurements of mercury or other hydride-forming elements along with cold vapour cell or heating cell temperature,

— specification of furnace design.

Method

(**Note** Evaluate and select the type of material of construction, pretreatment, and cleaning of analytical labware used in the Atomic Absorption Spectrophotometric analyses. The material must be inert and, depending on the specific application, resistant to caustics, acids, and/or organic solvents, i.e., plastic.)

The preparation of the sample may require a dissolution, a digestion (mostly microwave-assisted), an ignition step or a combination thereof in order to clear up the sample matrix and/or to remove carbon-containing material. If operating in an open system, the ignition temperature should not exceed 600°, due to the volatility of some metals, unless otherwise stated in the monograph.

Operate an atomic absorption spectrometer in accordance with the manufacturer's instructions at the prescribed wavelength. Introduce a blank solution into the atomic generator and adjust the instrument reading so that it indicates maximum transmission. The blank value may be determined by using solvent to zero the apparatus. Introduce the most concentrated standard preparation and adjust the sensitivity to obtain a maximum absorbance reading. Rinse in order to avoid contamination and memory effects. After completing the analysis, rinse with *water* or acidified water.

If a solid sampling technique is applied, full details of the procedure are provided in the monograph.

Ensure that the concentrations to be determined fall preferably within the linear part of the calibration curve. If this is not possible, the calibration plots may also be curved and are then to be applied with appropriate calibration software.

Determinations are made by comparison with standard preparations with known concentrations of the element to be determined either by the method of direct calibration (Method I) or the method of standard additions (Method II).

METHOD I: DIRECT CALIBRATION

For routine measurements three standard preparations and a blank solution are prepared and examined.

Prepare the solution of the substance to be examined (test preparation) as prescribed in the monograph. Prepare not fewer than three standard preparations of the element to be determined, the concentrations of which span the expected value in the test preparation. For assay purposes, optimal calibration levels are between 0.7 and 1.3 times the expected content of the element to be determined or the limit prescribed in the monograph. For purity determination, calibration levels are the limit of detection and 1.2 times the limit specified for the element to be determined. Any reagents used in the preparation of the test preparation are added to the standard and blank solutions at the same concentration.

Introduce each of the solutions into the instrument using the same number of replicates for each of the solutions to obtain a steady reading.

Calculation Prepare a calibration curve from the mean of the readings obtained with the standard preparations by plotting the means as a function of concentration. Determine the concentration of the element in the test preparation from the curve obtained.

METHOD II: STANDARD ADDITIONS

Add to at least three similar volumetric flasks equal volumes of the solution of the substance to be examined (test preparation) prepared as prescribed. Add to all but one of the flasks progressively larger volumes of a standard preparation containing a known concentration of the element to be determined to produce a series of solutions containing steadily increasing concentrations of that element known to give responses in the linear part of the curve, if possible. Dilute the contents of each flask to volume with solvent.

Introduce each of the solutions into the instrument, using the same number of replicates for each of the solutions, to obtain a steady reading.

Calculation Calculate the linear equation of the graph using a least-squares fit and derive from it the concentration of the element to be determined in the test preparation.

Validation of the method

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

LINEARITY

Prepare and analyze not fewer than four standard preparations over the calibration range and a blank solution. Perform not fewer than five replicates.

The calibration curve is calculated by least-square regression from all measured data. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

— the correlation coefficient is at least 0.99,

— the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and highest calibration level.

When the ratio of the estimated standard deviation of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

ACCURACY

Verify the accuracy preferably by using a certified reference material. Where this is not possible, perform a test for recovery.

RECOVERY

For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. For other determinations, for example, for trace element determination the test is not valid if recovery is outside of the range 80 per cent to 120 per cent at the theoretical value. Recovery may be determined on a suitable standard preparation (matrix solution) which is spiked with a known quantity of analyte (middle concentration of the calibration range).

REPEATABILITY

The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

LIMIT OF QUANTIFICATION

The limit of quantification can be estimated by calculating the standard deviation of not less than six replicate measurements of a blank solution, divided by the slope of a standard curve, and multiplying by 10. If validating a procedure using the method of standard additions, the slope of standards applied to a solution of the substance being examined (test preparation) is used. Other suitable approaches can be used.

2.10 PLASMA SPECTROCHEMISTRY

INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETRY

Inductively coupled plasma-atomic emission spectrometry (ICP-AES); also referred to as inductively coupled plasma-optical emission spectroscopy (ICP-OES), is one of the plasma-based instrumental methods. The inductively coupled plasma (ICP) is a high-temperature excitation source that desolvates, vapourizes, and atomizes aerosol samples and ionizes the resulting atoms. The excited analyte ions and atoms can then subsequently be detected by observing their emission lines, a method termed inductively coupled plasma-atomic emission spectroscopy, or the excited or ground state ions can be determined by a technique known as inductively coupled plasma-mass spectrometry (ICP-MS). The ICP can use either an atomic emission (optical emission) or a mass spectral detection system. In ICP-AES, analyte detection is achived at an emission wavelength of the analyte in question. In ICP-MS, analytes are detected directly at their atomic masses because these masses must be charged to be detected in ICP-MS, the method relies on the ability of the plasma source to both atomize and ionize sample constituents. ICP-AES and ICP-MS may be used for either a single- or multi-element analysis and used for either sequential or simultaneous analyses with good sensitivity over an extended linear range.

ICP formation

The components that make up the ICP excitation source include the argon gas supply, torch, radio frequency (RF) induction coil, impedance-matching unit, and RF generator. Argon gas is almost universally used in the ICP. The plasma torch consists of three concentric tubes designated as the inner, the intermediate, and the outer tube. The intermediate and outer tubes are almost universally made of quartz. The inner tube can be made of quartz or alumina if the analysis is conducted with solutions containing hydrofluoric acid. The nebulizer gas flow carries the aerosol of the sample solution into and through the inner tube of the torch and into the plasma. The intermediate tube carries the intermediate (sometimes referred to as the auxiliary) gas. The intermediate gas flow helps to lift the plasma off the inner and intermediate tubes to prevent their melting and the deposition of carbon and salts on the inner tube. The outer tube carries the outer (sometimes referred to as the plasma or coolant) gas, which is used to form and sustain the toroidal plasma. The toroidal plasma is referred to as the induction region through which the sample is introduced into the centre of the plasma.

An RF induction coil, also called the load coil, surrounds the torch and produces an oscillating magnetic field, which in turn sets up an oscillating current in the ions and electrons produced from the argon. The impedance-matching unit serves to efficiently couple the RF energy from the generator to the load coil. Within the load coil of the RF generator, the energy transfer between the coil and the argon creates a self-sustaining plasma. Collisions of the ions and electrons liberated from the argon ionize and excite the analyte atoms in the high-temperature plasma. The plasma operates at temperatures of 6000 K to 10,000 K (about 5727° to 9727°), so most covalent bonds and analyte-to-analyte interactions are eliminated.

Apparatus

The apparatus consists essentially of the following elements:

(a) a sample-introduction system consisting of a peristaltic pump delivering the solution at constant flow rate into a nebulizer;

(b) an RF generator;

(c) a plasma torch;

(d) transfer optics focussing the image of the plasma at the entrance slit of the spectrometer; radial viewing is better for difficult matrices (alkalis, organics), whereas axial viewing gives more intensity and better detection limits in simple matrices;

(e) wavelength dispersive devices consisting of diffraction gratings, prisms, filters or interferometers;

(f) detectors converting radiant energy into electrical energy;

(g) a data-acquisition unit.

Interference

Interference is anything that causes the signal from an analyte in a sample to be different from the signal for the same concentration of that analyte in a calibration solution. The well-known chemical interference that is encountered in flame atomic absorption spectrometry is usually weak in ICP-AES. In rare cases where interference occurs, it may be necessary to increase the RF power or to reduce the inner support-gas flow to eliminate it. The interference in ICP-AES can be of spectral origin or even the result of high concentrations of certain elements or matrix compounds. Physical interference (due to differences in viscosity and surface tension of the sample and calibration standards) can be minimized by dilution of the sample, matrix matching, use of internal standards, or through application of the method of standard additions. Another type of interference occasionally encountered in ICP-AES is the so-called "easily ionized elements (EIEs) effect". The EIEs are those elements that are ionized much more easily, for example alkaline metals and alkaline earths. In samples that contain high concentrations of EIEs (more than 0.1 per cent), suppression or enhancement of emission signals is likely to occur.

SPECTRAL INTERFERENCE This interference may be due to other lines or shifts in background intensity. These lines may correspond to argon (observed above 300 nm), OH bands due to the decomposition of water (at about 300 nm), NO bands due to the interaction of the plasma with the ambient air (between 200 nm and 300 nm), and other elements in the sample, especially those present at high concentrations. The interference falls into four different categories: simple background shift, sloping background shift, direct spectral overlap, and complex background shift.

ABSORPTION INTERFERENCE This interference arises when part of the emission from an analyte is absorbed before it reaches the detector. This effect is observed particularly when the concentration of a strongly emitting element is so high that the atoms or ions of that element that are in the lower energy state of transition absorb significant amounts of the radiation emitted by the relevant excited species. This effect, known as self-absorption, determines the upper end of the linear working range for a given emission line.

MULTICOMPONENT SPECTRAL FITTING Multiple emission-line determinations are commonly used to overcome problems with spectral interferences. A better, more accurate method for performing spectral interference corrections is to use the information obtained with advanced detector systems through multicomponent spectral fitting. This quantifies not only the interference, but also the background contribution from the matrix, thereby creating a correction formula. Multicomponent spectral fitting utilizes a multiple linear-squares model based on the analysis of pure analyte, the matrix and the blank, creating an interference-corrected mathematical model. This permits the determination of the analyte emission in a complex matrix with improved detection limits and accuracy.

Procedure

SAMPLE PREPARATION AND SAMPLE INTRODUCTION The basic goal for the sample preparation is to ensure that the analyte concentration falls within the working range of the instrument through dilution or preconcentration, and that the sample-containing solution can be nebulized in a reproducible manner. Several sample-introduction systems tolerate high acid concentrations, but the use of sulfuric and phosphoric acids can contribute to background emission observed in the ICP spectra. Therefore, nitric and hydrochloric acids are preferable. The availability of hydrofluoric acid-resistant (for example, perfluoroalkoxy polymer) sample-introduction systems and torches also allows the use of hydrofluoric acid. In selecting a sample-introduction method, the requirements for sensitivity, stability, speed, sample size, corrosion resistance and resistance to clogging have to be considered. The use of a cross-flow nebulizer combined with a spray chamber and torch is suitable for most requirements.
The peristaltic pumps used for ICP-AES usually deliver the standard and sample solutions at a rate of 1 mL per minute or less. In the case of organic solvents being used, the introduction of oxygen must be considered to avoid organic layers.

CHOICE OF OPERATING CONDITIONS The standard operating conditions prescribed by the manufacturer are to be followed. Usually, different sets of operating conditions are used for aqueous solutions and for organic solvents. Suitable operating parameters are to be properly chosen:

(1) wavelength selection;
(2) support-gas flow rates (outer, intermediate and inner tubes of the torch);
(3) RF power;
(4) viewing position (radial or axial);
(5) pump speed;
(6) conditions for the detector (gain/voltage for photomultiplier tube detectors, others for array detectors);
(7) integration time (time set to measure the emission intensity at each wavelength).

Control of instrument performance

SYSTEM SUITABILITY The following tests may be carried out with a multi-element control solution to ensure the adequate performance of the ICP-AES system:

(1) energy transfer (generator, torch, plasma); measurement of the ratio Mg II (280.270 nm)/Mg I (285.213 nm) may be used;
(2) sample transfer, by checking nebulizer efficiency and stability;
(3) resolution (optical system), by measuring peak widths at half height, for example, As (189.042 nm), Mn (257.610 nm), Cu (324.754 nm) or Ba (455.403 nm);
(4) analytical performance, by calculating detection limits of selected elements over the wavelength range.

Validation of the method

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

LINEARITY Prepare and analyze not fewer than four standard solutions over the calibration range plus a blank. Perform not fewer than five replicates. The calibration curve is calculated by least-square regression from all measured data of the calibration test. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

(1) the correlation coefficient is at least 0.99;

(2) the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and for the highest calibration level.

When the ratio of the estimated standard deviations of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

ACCURACY Verify the accuracy preferably by using a certified reference material. Where this is not possible, perform a test for recovery.

RECOVERY For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. The test is not valid if recovery, for example, for trace-element determination, is outside of the range 80 per cent to 120 per cent of the theoretical value. Recovery may be determined on a suitable standard solution (matrix solution) spiked with a known quantity of analyte (concentration range that is relevant to the samples to be determined).

REPEATABILITY The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

LIMIT OF QUANTIFICATION The limit of quantification is estimated by calculating the standard deviation of not less than ten replicate measurements of a blank solution and multiplying by 10. When validating a procedure using the method of standard additions, the slope of standards applied to a solution of the test material is used. Other suitable approaches can be used.

INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

Inductively coupled plasma-mass spectrometry (ICP-MS) is a plasma-based instrumental method that uses mass spectral detection system. When using the ICP-MS, analytes are detected directly at their atomic masses. Because these masses must be charged to be detected in ICP-MS, the method relies on the ability of the plasma source to both atomize and ionize sample constituents. The basic principles of ICP formation are described in ICP Formation under Inductively Coupled Plasma-Atomic Emission Spectrometry.

The sample-introduction system and data-handling techniques of an ICP-AES system are also used in ICP-MS.

Apparatus

The apparatus consists essentially of the following elements:

(a) a sample-introduction system, consisting of a peristaltic pump delivering the solution at constant flow rate into a nebulizer;

(b) an RF generator;

(c) a plasma torch;

(d) an interface region including cones to transport ions to the ion optics;

(e) a mass spectrometer;

(f) a detector;

(g) a data-acquisition unit.

Interference

Mass interference is the major problem, for example by isobaric species that significantly overlap the mass signal of the ions of interest, especially in the central part of the mass range (for example, 40 to 80 a.m.u.). The combination of atomic ions leads to polyatomic or molecular interferences (i.e., $^{40}Ar^{16}O$ with ^{56}Fe or $^{40}Ar^{40}Ar$ with ^{80}Se). Matrix interference may also occur with some analytes. Some samples have an impact on droplet formation or on the ionization temperature in the plasma. These phenomena may lead to the suppression of analyte signals. Physical interference is to be circumvented by using the method of internal standardization or by standard addition. The element used as internal standard depends on the element to be measured: ^{59}Co and ^{115}In, for example, can be used as internal standards.

The prime characteristic of an ICP-MS instrument is its resolution, i.e. the efficiency of separation of two close masses. Quadrupole instruments are, from this point of view, inferior to magnetic-sector spectrometers.

Procedure

SAMPLE PREPARATIONS AND SAMPLE INTRODUCTION The sample preparation usually involves a step of digestion of the matrix by a suitable method, for example in a microwave oven. Furthermore, it is important to ensure that the analyte concentration falls within the working range of the instrument through dilution or preconcentration, and that the sample-containing solution can be nebulized in a reproducible manner.

Several sample-introduction systems tolerate high acid concentrations, but the use of sulfuric and phosphoric acids can contribute to background emission. Therefore, nitric and hydrochloric acids are preferable. The availability of hydrofluoric acid-resistant (for example, perfluoroalkoxy polymer) sample-introduction systems and torches also allows the use of hydrofluoric acid. In selecting a sample-introduction method, the requirements for sensitivity, stability, speed, sample size, corrosion resistance and resistance to clogging have to be considered. The use of a cross-flow nebulizer combined with a spray chamber and torch is suitable for most requirements. The peristaltic pumps usually deliver the standard and sample solutions at a rate of 20 to 1000 μL per minute.

In the case of organic solvents being used, the introduction of oxygen must be considered to avoid organic layers.

CHOICE OF OPERATING CONDITIONS The standard operating conditions prescribed by the manufacturer are to be followed. Usually, different sets of operating conditions are used for aqueous solutions and for organic solvents. Suitable operating parameters are to be properly chosen:

(1) selection of cones (material of sampler and skimmer);
(2) support-gas flow rates (outer, intermediate and inner tubes of the torch);
(3) RF power;
(4) pump speed;
(5) selection of one or more isotopes of the element to be measured (mass).

Isotope selection

Isotope selection is made using several criteria. The most abundant isotope for a given element is selected to obtain maximum sensitivity. Furthermore, an isotope with the least interference from other species in the sample matrix and from the support gas should be selected. Information about isobaric interferences and interferences from polyatomic ions of various types, for example, hydrides, oxides, chlorides, etc., is usually available in the software of ICP-MS instrument manufacturers.

Control of instrument performance

SYSTEM SUITABILITY

(1) Tuning of the instrument allows to monitor and adjust the measurement before running samples. ICP-MS mass accuracy is checked with a tuning solution containing several isotopes covering the whole range of masses, for example, ^{9}Be, ^{59}Co, ^{89}Y, ^{115}In, ^{140}Ce, and ^{209}Bi.

(2) Sensitivity and short- and long-term stability are recorded. The instrument parameters (plasma condition, ion lenses and quadrupole parameter) are to be optimized to obtain the highest possible number of counts.

(3) Tuning for resolution and mass axis is to be done with a solution of Li, Y and Tl to ensure an acceptable response over a wide range of masses.

(4) Evaluation of the efficiency of the plasma to decompose oxides has to be performed in order to minimize these interferences. The ratio Ce/CeO and/or Ba/BaO is a good indicator, and a level less than about 3 per cent is required.

(5) Reduction of the formation of double-charged ions is made with Ba and Ce. The ratio of the signal for double-charged ions to the assigned element should be less than 2 per cent.

(6) Long-term stability is checked by running a standard first and at the end of the sample sequence, controlling whether salt deposits on the cones have reduced the signal throughout the run.

Validation of the method

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

LINEARITY Prepare and analyze not fewer than four standard preparations over the calibration range plus a blank. Perform not fewer than five replicates.

The calibration curve is calculated by least-square regression from all measured data of the calibration test. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

(1) the correlation coefficient is at least 0.99;

(2) the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and for the highest calibration level.

When the ratio of the estimated standard deviations of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed.

If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

ACCURACY Verify the accuracy preferably by using a certified reference material. Where this is not possible, perform a test for recovery.

RECOVERY For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. The test is not valid if recovery, for example for trace-element determination, is outside the range 80 per cent to 120 per cent of the theoretical value. Recovery may be determined on a suitable standard solution (matrix solution) spiked with a known quantity of analyte (concentration range that is relevant to the samples to be determined).

REPEATABILITY The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

LIMIT OF QUANTIFICATION The limit of quantification is estimated by calculating the standard deviation of not less than ten replicate measurements of a blank solution and multiplying by 10. When validating a procedure using the method of standard additions, the slope of standards applied to a solution of the test material is used. Other suitable approaches can be used.

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 LIQUID CHROMATOGRAPHY

The term Liquid chromatography (LC), in this Pharmacopoeia, is referred to as HPLC, both high-pressure and high-performance. It is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations are achieved by partition (mass distribution), adsorption, ion-exchange, or stereochemical-interaction processes, depending upon the type of stationary phase used. Unless otherwise specified, all information below is valid for LC as well as for LC using reduced particle-size columns (e.g., sub-2 μm). The latter requires instrumentation characterized by the capability to apply higher pressures (typically up to 100 MPa, i.e., about 15,000 psi), lower extra-column band broadening, improved gradient mixing and a higher sampling rate in the detection system.

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 acquisition system.

PUMPING SYSTEMS LC pumping systems deliver metered amounts of mobile phase from the solvent reservoirs to the column through high-pressure tubing and fittings. Microprocessor-controlled pumping systems are capable of accurately delivering a mobile phase of either constant (isocratic elution) or varying composition (gradient elution), according to a defined programme. In the case of gradient elution, pumping systems which deliver solvent(s) from several reservoirs are available and solvent mixing can be achieved on either the low- or high-pressure side of the pump(s).

INJECTORS After dissolution in mobile phase or other suitable solution, compounds to be chromatographed are injected into the mobile phase. Fixed-loop and variable volume devices operated manually or by an auto-sampler are used.

STATIONARY PHASES For most pharmaceutical analyses, separation is achieved by partition of compounds in the test solution between the mobile phase and the stationary phase. A system consisting of a polar stationary phase and a nonpolar mobile phase is described as normal-phase chromatography, while the opposite arrangement, a polar mobile phase and a nonpolar stationary phase, is called reversed-phase chromatography. There are many types of stationary phase materials employed in LC, including:

— silica, alumina or porous graphite, used in normal-phase chromatography, where the separation is based on differences in adsorption and/or mass distribution (partition chromatography),

— resins or polymers with acid or basic groups, used in ion-exchange chromatography, where separation is based on competition between the ions to be separated and those in the mobile phase,

— porous silica or polymers, used in size-exclusion chromatography, where separation is based on differences between the volumes of the molecules, corresponding to steric exclusion,

— a variety of chemically modified supports prepared from polymers, silica or porous graphite, used in normal-phase (adsorption chromatography) and reversed-phase LC, where the separation is based principally on partition of the molecules between the mobile phase and the stationary phase,

— special chemically modified stationary phases, e.g., cellulose or amylose derivatives, proteins or peptides, cyclodextrins, etc., for the separation of enantiomers (chromatography on chiral stationary phases).

Most separations are based upon partition mechanisms utilizing chemically modified silica as the stationary phase and polar solvents as the mobile phase. The surface of the support, e.g., the silanol groups of silica, is reacted with various silane reagents to produce covalently bound silyl derivatives covering a varying number of active sites on the surface of the support. The nature of the bonded phase is an important parameter for determining the separation properties of the chromatographic system.

Commonly used bonded phases are shown below:

octyl (C_8)	=	$Si\text{-}[CH_2]_7\text{-}CH_3$
octadecyl (C_{18})	=	$Si\text{-}[CH_2]_{17}\text{-}CH_3$
phenyl (C_6H_5)	=	$Si\text{-}[CH_2]_n\text{-}C_6H_5$
cyanopropyl (CN)	=	$Si\text{-}[CH_2]_3\text{-}CN$
aminopropyl (NH_2)	=	$Si\text{-}[CH_2]_3\text{-}NH_2$
diol	=	$Si\text{-}[CH_2]_3\text{-}O\text{-}CH(OH)\text{-}CH_2\text{-}OH$

Unless otherwise stated by the manufacturer, silica-based reversed-phase columns are considered to be stable in mobile phases having an apparent pH in the range of 2.0 to 8.0. Columns containing porous graphite or particles of polymeric materials such as styrene-divinylbenzene copolymer are stable over a wider pH range. Analysis using normal-phase chromatography with unmodified silica, porous graphite or polar chemically modified silica, e.g., cyanopropyl or diol, as the stationary phase with a nonpolar mobile phase is applicable in certain cases. For analytical separations, the particle size of the most commonly used stationary phases varies between 2 μm and 10 μm. The particles may be spherical or irregular, of varying porosity and specific surface area. These properties contribute to the chromatographic behaviour of a particular stationary phase. In the case of reversed phases, the nature of the stationary phase, the extent of bonding, e.g., expressed as the carbon loading, and whether the stationary phase is end-capped (i.e., part of the residual silanol groups being silylated) are additional determining factors. Tailing of peaks, particularly of basic substances, can occur when residual silanol groups are present. In addition to porous particles, superficially porous or monolithic materials may be used.

COLUMNS Columns, made of stainless steel, lined stainless steel, and polymers of varying length and internal diameter are used for analytical chromatography. Columns with internal diameters of less than 2 mm are often referred to as microbore columns. The temperature of the mobile phase and the column must be kept constant during an analysis. Most separations are performed at room temperature, but some require a different temperature for optimal performance.

In LC procedures, a guard column may be used with the following requirements, unless otherwise indicated in the individual monograph: (a) the length of the guard column must be not more than 15 per cent of the length of the analytical column, (b) the internal diameter must be the same or smaller than that of the analytical column, and (c) the packing material should be the same as the analytical column and contain the same bonded phase. In any case, all system suitability requirements specified in the official procedure must be met with the guard column installed.

MOBILE PHASES

For normal-phase chromatography, less polar organic solvents are generally employed. The residual water content of the solvents used in the mobile phase is to be strictly controlled to obtain reproducible results. In reversed-phase LC, aqueous phases, with or without organic solvents, are employed.

Components of the mobile phase are usually filtered to remove particles greater than 1.45 µm (or 0.2 µm when the stationary phase is made of sub-2 µm particles and when special detectors, e.g., light scattering detectors, are used). Solvents are normally degassed before pumping by sparging with helium, sonication and/or using on-line membrane/vacuum modules to avoid the creation of gas bubbles in the detector cell. Solvents for the preparation of the mobile phase are normally free of stabilizers and, if an ultraviolet detector is employed, are transparent at the wavelength of detection. Solvents and other components employed are to be of appropriate quality. Adjustment of the pH, if necessary, is effected using only the aqueous component of the mobile phase and not the mixture. If buffer solutions or saline solutions are used, adequate rinsing of the system is carried out with a 5 per cent v/v mixture of the organic part of the mobile phase in water to prevent crystallization of salts after completion of the analysis. Mobile phases may contain other components, e.g., a counter-ion for ion-pair chromatography or a chiral selector for chromatography using an achiral stationary phase.

DETECTORS

Ultraviolet/visible (UV/Vis) spectrophotometers, including diode array detectors, are the most commonly employed detectors. Fluorescence spectrophotometers, differential refractometers (RI), electrochemical detectors (ECD), light scattering detectors, charged aerosol detectors (CAD), mass spectrometers (MS), radioactivity detectors or other special detectors may be used.

Procedure

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 in this Appendix.

In general, the steps involved in the liquid chromatographic technique are as follows:

1. Equilibrate the column and detector with the mobile phase at the specified flow rate until a stable baseline is achieved.
2. Prepare the sample and the standard solutions. The solutions must be free from solid particles.
3. Inject the standard/sample solution through the injector, or use an autosampler.
4. Perform the isocratic/gradient programme.
5. Record the chromatogram.
6. Analyze as specified in the monograph.

In quantitative work, particularly where the use of an internal standard is not specified in the monograph, the use of a fixed-volume loop injector is recommended. In certain exceptional cases the use of peak heights alone is prescribed in the monograph; where this is the case peak heights should be used irrespective of the symmetry factor. The column is usually made of stainless steel and its dimensions are stated in the monograph as length × internal diameter. The nominal diameter of the particles of the stationary phase is stated in parentheses following the name of stationary phase prescribed in the monograph. In most cases reference is made to a particular commercial brand that has been found to be suitable for the purpose, but such statements do not imply that a different but equivalent commercial brand may not be used. The separation should be carried out at a constant room temperature unless otherwise specified in the monograph. When using mobile phases of high pH with a silica-based column, it is advisable to use a pre-column before the analytical column.

Unless otherwise specified in the monograph, the detector consists of a photometric detector fitted with a low-volume flow cell (about 10 μL is suitable); the wavelength setting is specified in the monograph.

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, V_T. 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, V_O (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 retention 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

Figure 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.

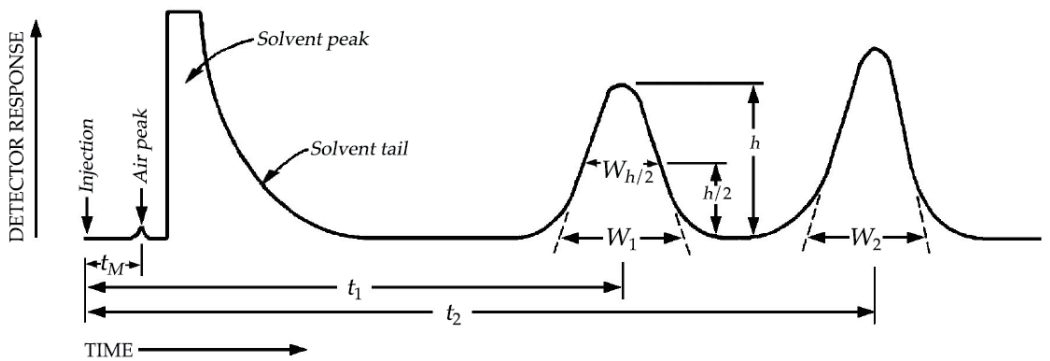


Fig. 1 Chromatographic Separation of Two Substances

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.

The elution time of a compound can be described by the retention 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.

The retention factor (also known as the capacity factor, *k′*) is defined as:

$$k = \frac{\text{amount of substance in stationary phase}}{\text{amount of substance in mobile phase}}$$

or

$$k = \frac{\text{time spent by substance in stationary phase}}{\text{time spent by substance in mobile phase}} = \frac{t}{t_M} - 1,$$

where t is the retention time measured from time of injection to time of elution of peak maximum and t_M is the hold-up time–the time required for elution of a non-retained component, shown in Figure 1 as an air or non-retained solvent peak, with the baseline scale in minutes.

The volume of mobile phase required for elution of a component is defined as retention volume, V. It may be calculated from the retention time and the flow rate, F, in mL/min:

$$V = t \times F$$

The volume of mobile phase required for elution of a non-retained component is defined as the hold-up volume, V_M. It may be calculated from the hold-up time, t_M, and the flow rate, F, in mL/min:

$$V_{\mathrm{M}} = t_{\mathrm{M}} \times F$$

Comparisons are normally made in terms of relative retention times, R_r, unless otherwise specified in the monograph:

$$R_r = \frac{t_2}{t_1},$$

where t_2 and t_1 are the 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 component, 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:

$$R = \frac{2(t_2 - t_1)}{W_2 + W_1},$$

where 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 number of theoretical plates, N, by the equation:

$$N = 5.54(t/W_{2,h/2})^2$$

and to determine the resolution, R, by the equation:

$$R = \frac{2(t_2 - t_1)}{1.70(W_{1,h/2} + W_{2,h/2})},$$

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. Peak areas are generally used but may be less accurate if peak interference occurs. 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.

The peak-to-valley ratio, p/v, may be employed as a system suitability criterion in a test for related substances when baseline separation between two peaks is not achieved. Figure 2 represents a partial separation of two substances, where H_p is the height above the extrapolated baseline of the minor peak and H_v is the height above the extrapolated baseline at the lowest point of the curve separating the minor and major peaks:

$$p/v = H_p/H_v$$

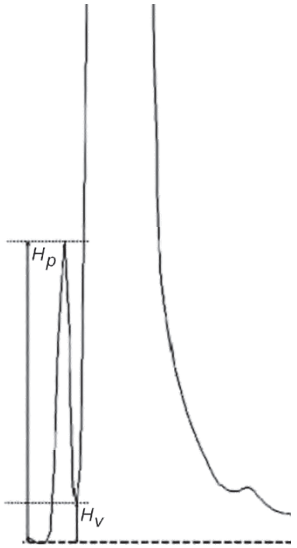


Fig. 2 Peak-to-Valley Ratio Determination

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.

Factors that may affect the chromatographic behaviour include:

— the composition, ionic strength, temperature, and apparent pH of the mobile phase;

— flow rate, column dimensions, column temperature, and pressure;

— stationary phase characteristics including type of chromatographic support (particle-based or monolithic), particle or macropore size, porosity, and specific surface area;

— reversed phase and other surface modification of the stationary phases, the extent of chemical modification (as expressed by end-capping, carbon loading, etc.).

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 signal-to-noise (S/N) ratio is determined by the equation:

$$\text{S/N ratio} = 2H/h,$$

where H is the height of the peak measured from the peak apex to a baseline extrapolated over a distance ≥5 times the peak width at its half-height, and h is the difference between the largest and smallest noise values observed over a distance ≥5 times the width at the half-height of the peak and, if possible, situated equally around the peak of interest (Fig. 3).

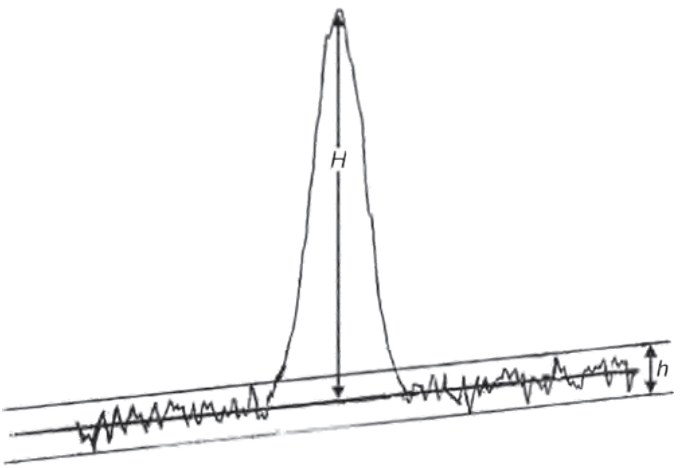


Fig. 3 Noise and Chromatographic Peak, Components of the S/N Ratio

The resolution, R, is a function of the number of theoretical plates, N (also referred to as column efficiency) and may be 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.

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.

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. 4):

$$T = \frac{W_{0.05}}{2f},$$

where T is the symmetry factor (or tailing factor), $W_{0.05}$ is the width of the peak at 5 per cent height and f is the 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. In some cases, values less than unity may be observed. As peak asymmetry increases, integration and hence precision become less reliable.

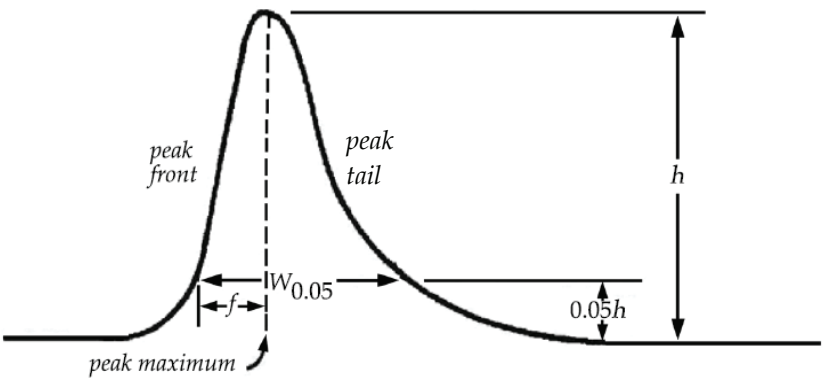


Fig. 4 Asymmetrical Chromatographic Peak

These system suitability 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 solution 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 requirements 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. Adjustment of conditions with gradient elutions is more critical than with isocratic elutions, since it may lead to shifts in peaks to a different step of the gradient, thus leading to the incorrect assignment of peaks, and to the masking of peaks or a shift such that elution occurs beyond the prescribed elution time. Changes other than those indicated require revalidation of the method. The chromatographic conditions described have been validated during the elaboration of the monograph.

The system suitability tests are included to verify that the separation required for satisfactory performance of the test or assay is achieved. 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 reversed-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.

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 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.

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 pH of the value or range specified, or ±1.0 pH 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

For isocratic separations, changes in column length, column internal diameter and particle size are allowed as follows:

— column length: ±70 per cent,
— column internal diameter: ±25 per cent,
— particle size: maximal reduction of 50 per cent, no increase permitted.

For gradient separations, only changes in column length and column internal diameter, are allowed as follows:

— column length: ±70 per cent,
— column internal diameter: ±25 per cent,
— particle size: no adjustment permitted.

FLOW RATE

For isocratic separations, the flow rate can be adjusted by ±50 per cent. When column dimensions are changed, the flow rate is determined by the equation:

$$F_2 = F_1 \frac{L_2 d_2^2}{L_1 d_1^2}$$

where F_1 is the flow rate specified in the monograph, in mL per minute; F_2 is adjusted flow rate, in ml per minute; L_1 is the length of the column specified in the monograph, in mm; L_2 is the length of the column used, in mm; d_2 is the internal diameter of the column specified in the monograph, in mm; d_2 is the internal diameter of the column used, in mm.

For gradient separations, the adjustment of the flow rate is allowed when changing the column dimensions. The flow rate is determined by the equation above.

COLUMN TEMPERATURE The column temperature can be adjusted as follows, unless otherwise specified:

— isocratic separations: ±10°,
— gradient separations: ±5°.

INJECTION VOLUME The injection volume can be reduced as far as is consistent with accepted precision and detection limits. No increase in the injection volume is permitted.

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.

Quantification

Peaks due to solvents and reagents or arising from the mobile phase or the sample matrix are disregarded during quantification.

DETECTOR SENSITIVITY The detector sensitivity is the signal output per unit concentration or unit mass of a substance in the mobile phase entering the detector. The relative detector response factor, commonly referred to as response factor, expresses the sensitivity of a detector for a given substance relative to a standard substance. The correction factor is the reciprocal of the response factor.

EXTERNAL STANDARD METHOD The concentration of the component(s) quantified is determined by comparing the response(s) obtained with the sample solution to the response(s) obtained with a standard solution.

INTERNAL STANDARD METHOD Equal amounts of the internal standard are introduced into the sample solution and a standard solution. The internal standard is chosen so that it does not react with the test material and does not contain impurities with the same retention time as that of the analytes, and is stable and resolved from the component(s) quantified (analytes). The concentrations of the analytes are determined by comparing the ratios of their peak areas or peak heights and the internal standard in the sample solution with the ratios of their peak areas or peak heights and the internal standard in the standard solution.

NORMALIZATION PROCEDURE The per cent content of a component of the test substance is calculated by determining the area of the corresponding peak as a percentage of the total area of all the peaks, excluding those due to solvents or reagents or arising from the mobile phase or the sample matrix and those at or below the limit at which they can be disregarded.

CALIBRATION PROCEDURE The relationship between the measured or evaluated signal y and the quantity (e.g., concentration or mass) of substance x is determined, and the calibration function is calculated. The analytical results are calculated from the measured signal or evaluated signal of the analyte and its position on the calibration curve.

In tests for impurities for both the external standard method, when a dilution of the sample solution is used for comparison, and the normalization procedure, any correction factors indicated in the monograph are applied (e.g., when the relative response factor is outside the range of 0.8 to 1.2).

When the impurity test prescribes the total of impurities or there is a quantitative determination of an impurity, choice of an appropriate threshold setting and appropriate conditions for the integration of the peak areas is important. In such tests the limit at or below which a peak is disregarded is generally 0.05 per cent. Thus, the threshold setting of the data collection system corresponds to at least half of this limit. Integrate the peak area of any impurity that is not completely separated from the principal peak, preferably by valley-to-valley extrapolation (tangential skim).

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 –10° to +360°, 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.

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 may be 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 may be 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.

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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.

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 0º 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 10º 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 100º 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.

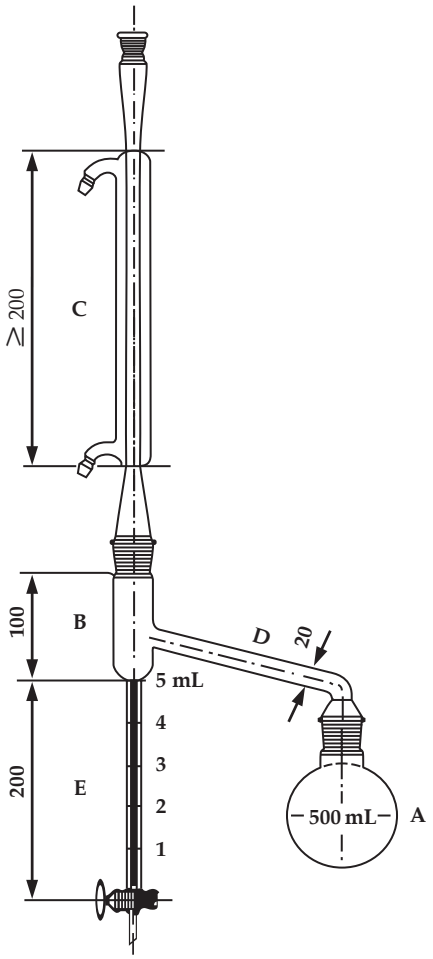
Where the sample is an insoluble solid, the water may be extracted using a suitable anhydrous solvent from which an appropriate quantity, accurately weighed, may be injected into the anolyte solution. Alternatively an evaporation technique may be used in which water is released and evaporated by heating 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, dis til 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

5.2 LIMIT TESTS

HEAVY METALS IN HERBAL DRUGS AND HERBAL DRUG PREPARATIONS

Caution When using closed high-pressure digestion vessels and microwave laboratory equipment, be familiar with the safety and operating instructions given by the manufacturer.

Apparatus

The apparatus typically consists of the following:

(a) a digestion flasks, polytetrafluoroethylene, perfluoroalkoxy polymer, quartz or glass flasks with a volume of 20 to 150 mL, fitted with a tightly closed closure, a valve to adjust the pressure inside the container and a polytetrafluoroethylene tube to allow release of gas;

(b) a programmable microwave oven (e.g., with a magnetron frequency of 2450 MHz, with a selectable output from 0 to 1500±70 W in 1 per cent increments);

(c) an atomic spectrometer, an inductively coupled plasma-atomic emission spectrometer, or an inductively coupled plasma-mass spectrometer.

Method

Carry out the test as described in the "Atomic Spectrometry: Emission and Absorption" (AAS) (Appendix 2.3), "Inductively Coupled Plasma-Atomic Emission Spectrometry" (ICP-AES) (Appendix 2.10), or "Inductively Coupled Plasma-Mass Spectrometry" (ICP-MS) (Appendix 2.10).

Deviations from the experimental parameters of the sample preparation procedure and the method description below are acceptable provided that the validation requirements are met and the system suitability test is fulfilled on the day of the analysis.

SAMPLE PREPARATION

(**Note** Clean all the glassware and laboratory equipment with a 1 per cent w/v solution of *nitric acid* before use. All liquid samples should be weighed.)

Use either of the following methods.

Method I Wet Digestion

FOR ARSENIC, CADMIUM AND LEAD

Test solution Transfer about 250 mg of the herbal drug, coarsely powdered and accurately weighed, to a 100-mL glass beaker (in case of liquid samples, use 5 g). Add 2.5 mL of a 50 per cent v/v solution of *heavy metal-free nitric acid*, mix well, cover, and heat on a water-bath for 15 minutes. Allow to cool and add 5 mL of *heavy metal-free nitric acid*. Reheat on a water-bath for 60 minutes or until completely digested. Transfer the solution to a 50-mL volumetric flask and dilute to volume with water. Filter or centrifuge at 3421 × *g* (3000 rpm) for 10 minutes, if necessary. Prepare simultaneously the blank solution by carrying out the digestion in the same manner as for the test solution.

FOR MERCURY

Proceed as directed under the determination for arsenic, cadmium and lead except using a 100-mL volumetric flask in place of a 100-mL beaker and heat in a water-bath. The temperature of a water-bath is 60°.

Method II Closed Vessel Digestion or Microwave Digestion

TEST SOLUTION In a digestion flask, place the prescribed quantity of the substance to be examined (about 500 mg of the herbal drug, in *No. 1400 powder*). Add 4 mL of *heavy metal-free hydrochloric acid* and 6 mL of *heavy metal-free nitric acid*. Close the flask tightly. Place the digestion flasks in the microwave oven. Carry out the digestion in 3 steps according to the following programme, used for 7 flasks each containing the test solution: 80 per cent power for 15 minutes, 100 per cent power for 5 minutes, 80 per cent power for 20 minutes.

At the end of the cycle, allow the flasks to cool in air or water. After cooling, open each digestion flask and introduce the clear, colourless solution obtained into a 50-mL volumetric flask. Rinse each digestion flask with two 15-mL portions of *heavy metal-free dilute nitric acid*, collect the rinsings in the volumetric flask and dilute to 50.0 mL with *water*. Modifiers (e.g., in the case of AAS with electrothermal atomization, 1.0 mL of a 1 per cent w/v solution of *magnesium nitrate* and 1.0 mL of a 10 per cent w/v solution of *ammonium dihydrogen phosphate*) and stabilizing agents may be used, if necessary.

BLANK SOLUTION Mix 4 mL of *heavy metal-free hydrochloric acid* and 6 mL of *heavy metal-free nitric acid* in a digestion flask. Carry out the digestion in the same manner as for the test solution.

Determination of Arsenic, Cadmium and Lead Using AAS with Electrothermal Atomization

Carry out the test as described by the "Atomic Spectrometry: Emission and Absorption" (Appendix 2.3) and measure the content of arsenic, cadmium and lead by the "Method of Direct Calibration" (Method I, Appendix 2.3) or by the "Method of Standard Additions" (Method II, Appendix 2.3), using standard solutions of each heavy metal and the instrumental parameters recommended in Table 1.

The absorbance value of the blank solution is subtracted from the value obtained with the test solution.

Table 1 Instrumental Parameters for AAS with Electrothermal Atomization

		As	Cd	Pb
Wavelength	nm	193.7	228.8	283.5
Slit width	nm	0.5	0.5	0.5
Lamp current	mA	10	6	5
Ignition temperature	°C	1400	800	800
Atomization temperature	°C	2600	1800	2200
Gas flow rate	L/min	3	3	3

Determination of Arsenic, Cadmium and Lead Using AAS with Cold-Vapour or Hydride Atomization

Carry out the test as described by the "Atomic Spectrometry: Emission and Absorption" (Appendix 2.3) and measure the content of arsenic and mercury by the "Method of Direct Calibration" (Method I, Appendix 2.3) or by the "Method of Standard Additions" (Method II, Appendix 2.3), using standard solutions of arsenic or mercury and an automated continuous-flow hydride vapour generation system.

The absorbance value of the blank solution is subtracted from the value obtained with the test solution.

ARSENIC

Sample solution To 19.0 mL of the test solution or of the blank solution as prescribed above, add 1 mL of a 20 per cent w/v solution of *potassium iodide*. Allow the test solution to stand at room temperature for about 50 minutes or at 70° for about 4 minutes.

Acid reagent Use *Heavy metal-free hydrochloric acid*.

Reducing reagent Prepare a 0.6 per cent w/v solution of *sodium tetrahydroborate* in a 0.5 per cent w/v solution of *sodium hydroxide*.

The recommended instrumental parameters in Table 2 may be used.

MERCURY

Sample solution Use *Test solution* or *Blank solution*, as prescribed above.

Acid reagent Prepare a 51.5 per cent w/v solution of *heavy metal-free hydrochloric acid*.

Reducing reagent Prepare a 1.0 per cent w/v solution of *stannous chloride* in *heavy metal-free dilute hydrochloric acid*.

The recommended instrumental parameters in Table 2 may be used.

Table 2 Instrumental Parameters for AAS with Cold-Vapour or Hydride Atomization

		As	Hg
Wavelength	nm	193.7	253.7
Slit width	nm	0.2	0.5
Lamp current	mA	10	4
Acid reagent flow rate	mL/min	1.0	1.0
Reducing reagent flow rate	mL/min	1.0	1.0
Sample solution flow rate	mL/min	7.0	7.0
Absorption cell		Quartz (heated)	Quartz (unheated)
Nitrogen flow rate	L/min	0.1	0.1

Determination of Arsenic, Cadmium, Mercury, and Lead Using ICP-AES

Carry out the test as described by the "ICP-AES" (Appendix 2.10) and measure the content of arsenic, cadmium, mercury, and lead by the "Method of Direct Calibration" (Method I, Appendix 2.3), using standard solutions of each heavy metal or a mixture of all measured metals, and the instrumental parameters recommended in Table 3.

The emission value of the blank solution is subtracted from the value obtained with the test solution.

Table 3 Instrumental Parameters for ICP-AES

		As	Cd	Hg	Pb
Wavelength	nm	193.696/ 197.197/ 189.042	214.438/ 226.502/ 228.802	184.950/ 253.652/ 435.835	220.351/ 283.306/ 168.215
Ar, Monitorline	nm	430.010	430.010	430.010	430.010
Plasma energy	W	1200	1200	1200	1200
Peak algorithm with background correction		yes	yes	yes	yes

Determination of Arsenic, Cadmium, Mercury, and Lead Using ICP-MS

Carry out the test as described by the "ICP-MS" (Appendix 2.10) and measure the content of arsenic, cadmium, mercury, and lead by the "Method of Direct Calibration" (Method I, Appendix 2.3) using standard solutions of each heavy metal and the analytical isotopes and additional masses recommended in Table 4.

The signal intensity of the blank solution is subtracted from the value obtained with the test solution.

System Suitability

A system suitability test must be carried out on the day of the analysis to ensure that the sample preparation and measurement system are appropriate.

Acceptance criterion for preparation of sample solution A clear solution is obtained.

Acceptance criterion for measurement system The measured concentration of a standard solution of the metal at a concentration within the range of the used calibration curve does not differ from the actual concentration by more than 20 per cent.

Table 4 Recommended Analytical Isotopes and Additional Masses for ICP-MS

Isotope	Element of Interest
75	Arsenic
106, 108, 111, 114	Cadmium
202	Mercury
206, 207, 208	Lead

Validation Requirements

The analytical procedures used must be validated in accordance with the relevant general methods as described in the "Atomic Spectrometry: Emission and Absorption" (Appendix 2.3), "ICP-AES" (Appendix 2.10) and "ICP-MS" (Appendix 2.10). Additionally, the following criteria must be fulfilled.

Specificity

Specificity is the ability to ensure that the analytical procedures for sample preparation and measurement allow a reliable determination of the metal(s) in the presence of components (e.g., carrier gas, impurities, matrix) that may be expected to be present.

Acceptance criteria The procedure must be able to assess unequivocally each heavy metal to be determined with this procedure in the presence of components that may be expected to be present, including other heavy metals, matrix components and other sources of interference; specificity is demonstrated by complying with the accuracy requirement for the metal(s) to be determined.

Range

The calibration range of each metal is within the linear range of the method; test solutions containing residues at a level outside the calibration range may be diluted to concentrations within the calibration range.

Acceptance criterion Range is demonstrated by complying with the recovery requirement.

Accuracy

Verify the accuracy using a certified reference material or by performing a test for recovery.

RECOVERY Recovery may be determined on a sample of the substance to be examined, spiked with a known quantity of a reference standard of the metal (three concentration levels in the range of 50 to 150 per cent of the intended specification limit, even if the original concentration of the reference standard is at the specified value), in triplicate.

Acceptance criterion Spike recovery is within 70 per cent and 150 per cent for the mean of three replicates at each concentration.

Repeatability

TEST SAMPLES Either six independent samples of the substance to be examined spiked with a suitable reference standard at the specified concentration level, or three concentration levels prepared in triplicate.

Acceptance criterion The relative standard deviation is in both cases not greater than the value indicated in Table 5.

Intermediate Precision

The effect of random events (intra-laboratory variations) on the analytical precision of the method must be established. Acceptable experiments for establishing intermediate precision include performing the repeatability analysis on different days, or with different instrumentation, or with different analysts. Only one of the three experiments is required to demonstrate intermediate precision.

Acceptance criterion The relative standard deviation is not greater than the value indicated in Table 5.

Limit of Quantification

Determine the lowest concentration meeting the acceptance criterion. Use the results from the accuracy study.

Acceptance criterion The limit of quantification is below the specification limit.

Table 5

Concentration Range of the Metal (mg/kg)	Repeatability (RSD) (Per Cent)	Intermediate Precision (RSD) (Per Cent)
0.01–1	20	32
> 1	10	16

Limit of Detection (only Applicable to Limit Tests)

Determine the lowest concentration giving a signal clearly distinct from that obtained with a blank solution.

Acceptance criterion The limit of detection is not more than 0.1 times the concentration of the specification limit.

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 800°±25° 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 600°±50° 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 600°±50° 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 cons titutes 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.

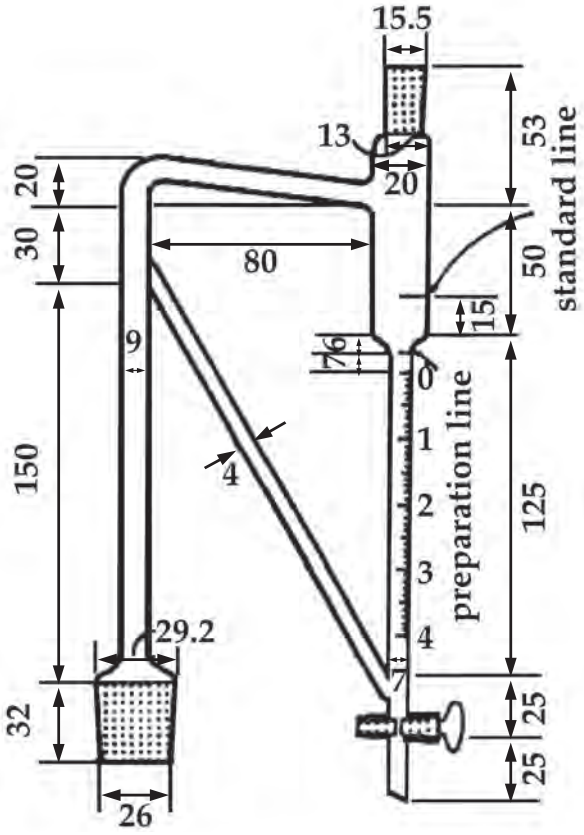
2. *Foreign elements*: matter not coming from the source plant and of either vegetable or mineral origin.

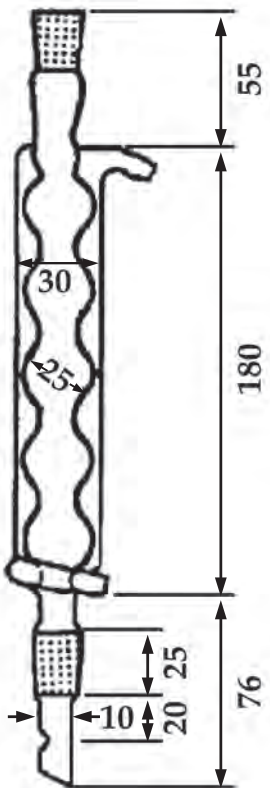
Method

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

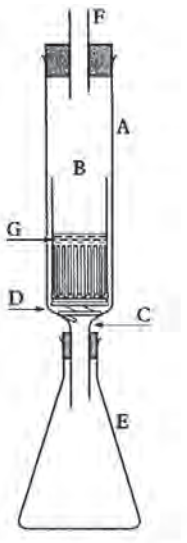
Where the process of maceration or percolation is specified in a monograph, carry out the following procedures with any modification indicated in the monograph.

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

Herbal drugs 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 herbal drug preparations of plant origin are taken over long periods of time; limits for residues should therefore be 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 herbal drugs. 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 herbal drug being examined at least complies with the limits indicated in Table 1. (The calculation is based on 60 kg of body weight.) 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 herbal drug 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.

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 fresh samples should be stored in the refrigerator, but typically no longer than 5 days. Dried samples may be stored at room temperature, but if storage time is expected to exceed two weeks, they should be sub-sampled and stored in the freezer (–10°).

Table 1

Substance	Limit (mg/kg)
Acephate	0.1
Alachlor	0.05
Aldrin and dieldrin (sum of)	0.05
Azinphos-ethyl	0.1
Azinphos-methyl	1.0
Bromide, inorganic (calculated as bromide ion)	125.0
Bromophos-ethyl	0.05
Bromophos-methyl	0.05
Bromopropylate	3.0
Chlordane (sum of *cis*-, *trans*-, and oxychlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyriphos-ethyl	0.2
Chlorpyriphos-methyl	0.1
Chlorthal-dimethyl	0.01
Cyfluthrin and isomers (sum of)	0.1
λ-Cyhalothrin	1.0
Cypermethrin and isomers (sum of)	1.0
DDT (sum of *o*,*p*′-DDE, *p*,*p*′-DDE, *o*,*p*′-DDT, *p*,*p*′-DDT, *o*,*p*′-TDE, and *p*,*p*′-TDE)	1.0
Deltamethrin	0.5
Diazinon	0.5
Dichlofluanid	0.1
Dichlorvos	1.0
Dicofol	0.5
Dimethoate and omethoate (sum of)	0.1
Dithiocarbamates (expressed as CS_2)	2.0
Endosulfan (sum of isomers and endosulfan sulfate)	3.0
Endrin	0.05
Ethion	2.0
Etrimphos	0.05
Fenchlorophos (sum of fenchlorophos and fenchlorophos-oxon)	0.1
Fenitrothion	0.5
Fenpropathrin	0.03
Fensulfothion (sum of fensulfothion, fensulfothion-oxon, fensulfothion-oxon sulfone, and fensulfothion sulfone)	0.05
Fenthion (sum of fenthion, fenthion-oxon, fenthion-oxon sulfone, fenthion-oxon sulfoxide, fenthion sulfone, and fenthion-sulfoxide)	0.05

Table 1 (Continued)

Substance	Limit (mg/kg)
Fenvalerate	1.5
Flucythrinate	0.05
τ-Fluvalinate	0.05
Fonophos	0.05
Heptachlor (sum of heptachlor, *cis*-heptachlorepoxide, and *trans*-heptachlorepoxide)	0.05
Hexachlorbenzene	0.1
Hexachlorocyclohexane (sum of isomers α-, β-, δ-, and ε-)	0.3
Lindan (γ-hexachlorocyclohexane)	0.6
Malathion and malaoxon (sum of)	1.0
Mecarbam	0.05
Methacriphos	0.05
Methamidophos	0.05
Methidathion	0.2
Methoxychlor	0.05
Mirex	0.01
Monocrotophos	0.1
Parathion-ethyl and paraoxon-ethyl (sum of)	0.5
Parathion-methyl and paraoxon-methyl (sum of)	0.2
Pendimethalin	0.1
Pentachloranisole	0.01
Permethrin and isomers (sum of)	1.0
Phosalone	0.1
Phosmet	0.05
Piperonyl butoxide	3.0
Pirimiphos-ethyl	0.05
Pirimiphos-methyl (sum of pirimiphos-methyl and *N*-desethyl-pirimiphos-methyl)	4.0
Procymidone	0.1
Profenophos	0.1
Prothiophos	0.05
Pyrethrum (sum of cinerin I, cinerin II, jasmolin I, jasmolin II, pyrethrin I, and pyrethrin II)	3.0
Quinalphos	0.05
Quintozene (sum of quintozene, pentachloraniline, and methyl pentachlorphenyl sulfide)	1.0
S-421	0.02

Table 1 (Continued)

Substance	Limit (mg/kg)
Tecnazene	0.05
Tetradifon	0.3
Vinclozolin	0.4

Qualitative and quantitative analysis of pesticide residues

Use either of the following analytical procedures:

— Analytical procedures issued by National Authorities or International Standard Organizations, or those published in internationally recognized manuals or publications;

— Analytical procedures with appropriate and reliable performance characteristics validated by a collaborative study or single-laboratory study according to the internationally recognized protocols. The validated analytical procedures shall be assessed in compliance with the latest version of ISO/IEC 17025.

The analytical procedures stated above shall be capable of providing the reliable results of the specified maximum pesticide residue level.

In addition, they shall satisfy the following criteria:

— The chosen method, especially with respect to its purification steps, is suitable for the combination of pesticide residue and substance to be examined, and is not susceptible to interference from co-extractives;

— The limit of quantification for each pesticide matrix combination to be analyzed is not more than the corresponding tolerance limit; the method is shown to recover between 70 and 120 per cent of each pesticide with a repeatability not less than 20 per cent relative standard deviation. (**Note** The lower recoveries may be acceptable in certain cases.)

— The concentration of the test and standard solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

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 applicable	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 applicable	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*
Test for *Salmonella*	Rappaport-Vassiliadis broth Tetrathionate bile brilliant green broth	Growth promoting Inhibitory	*Salmonella enterica* subsp. *enterica* serovar Typhimurium or *Salmonella enterica* subsp. *enterica* serovar Abony *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 Inhibitory	*S. aureus* *E. coli*

Table 4 (Continued)

Test	Medium	Property	Test Strains
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 colonies 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 tests (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.

***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.

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[1]Biggy agar, CHROMagar Candida, or Candida isolation agar is recommended.

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
Yeas t 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-sterile 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.
	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.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

Table 1 (Continued)

Category	Types	Requirements*
	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.
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.
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.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

**The examples are LUKPRAKHOP (herbal compress), YAPHOK (herbal poultice).

Table 2 (Continued)

Category	Types	Requirements*
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).

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