กรมวิทยาศาสตร์การแพทย์
Department of Medical Sciences

# THAI HERBAL COMPENDIUM ON PHYSICO-CHEMICAL SPECIFICATIONS
## VOLUME II

Department of Medical Sciences, Ministry of Public Health,
Thailand

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# THAI HERBAL COMPENDIUM
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# VOLUME II

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# PREFACE

The use of herbal medicines and preparations for medical or health promoting purposes is growing continuously worldwide. In Thailand, Thai Government has a policy to promote the use of Thai herbs and their products. Recognizing the importance of good quality crude drugs on quality of finished herbal products, the "Thai Herbal Compendium on Physico-chemical Specifications, Volume I" was first published in 2016. The information from this book has been widely used to be a guideline for quality control of plant materials. Since Department of Medical Sciences has concerned the quality specifications of Thai herbal drugs, therefore the information based on systemic scientific research projects of the Medicinal Plant Research Institute is expressed in "Thai Herbal Compendium on Physico-chemical Specifications, Volume II". This volume of the book contains 11 monographs of plant materials and a one monograph of the dry extract. Especially, the physico-chemical specifications of the five roots herbal drug or "Ya Ha Rak" remedy, a famous antipyretic notified in the National List of Essential Drugs, has been first published in this volume. This remedy is composed of the roots of *Capparis micracantha* DC., *Clerodendrum indicum* (L.) Kuntze, *Ficus racemosa* L., *Harrisonia perforata* (Blanco) Merr., and *Tiliacora triandra* Diels. Furthermore, the physico-chemical specifications of *Cyanthillium cinereum* (L.) H. Rob., *Garcinia cowa* Roxb. ex Choisy, *Gymnopetalum chinense* (Lour.) Merr., *Lysiphyllum strychnifolium* (W.G. Craib) A. Schmitz, *Momordica cochinchinensis* (Lour.) Spreng., *Momordica cochinchinensis* (Lour.) Spreng. Dry Extract, and *Musa* × *paradisiaca* L. (ABB group) "kluai namwa" have also been issued. The physico-chemical specifications of each plant materials and preparation were investigated following WHO guideline of quality control method for medicinal plant materials. The related appendices were obtained from "Thai Herbal Pharmacopoeia 2017". The "Thai Herbal Compendium on Physico-chemical Specifications, Volume II" offers valuable information for reference and as a guide pertaining to research and develop for the scientific and industrial communities. It would also be useful as the tool in the quality assurance of plant materials for regulatory authorities to potentiate the good quality of herbal products.

(Sukhum Karnchanaphimai, M.D.)
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# GENERAL NOTICES

# GENERAL NOTICES

The information given in the general notices which is adopted from the Thai Herbal Pharmacopoeia 2017, provides the basic guidelines for the interpretation and applications of the standards, tests and other specifications of the Thai Herbal Compendium on Physico-chemical Specifications.

## Printing Types

In the text, words which refer to reagents, which conform to the requirements specified in the appendices of other parts of the book, and the systematic names of plants are usually printed in italics to distinguish them from the other words in that portion of the text.

## 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 preparations is stored for longer than about 4 weeks at 15° to 25°.

## Test

When the solvent used for a solution is not named, the solvent is Purified Water. In stating the appropriate quantities to be taken for quantitative tests, the use of the word "about" indicates a quantity within 10% of the specified weight or volume. However, the weight or volume taken is accurately determined, and the calculated result is based upon the exact amount taken. The same tolerance applies to specified dimensions.

## Drying to Constant Weight

The specification "dried to constant weight" means that the drying shall be continued until two consecutive weighings do not differ by more than 2.5 mg per g substance taken, the second weighing following an additional hour of drying at the prescribed conditions.

### Ethanol

The term "ethanol" used without other indication means ethanol 95% v/v. Where other strengths are intended, the term "ethanol" is used followed by the statement of the strength.

### Filtration

Where it is directed to "filter", without further quantification, the intent is that the liquid be filtered through suitable filter paper or equivalent device until the filtrate is clear.

### Ignition to Constant Weight

The specification "ignite to constant weight" means that the ignition shall be continued until two consecutive weighings do not differ by more than 0.5 mg per g of substance taken, the second weighing following an additional 15-minute ignition period.

### Percentage Expressions

Percentage concentrations are expressed as follows:

Percent weight in weight (w/w) expresses the number of g of a constituent in 100 g of solution or mixture.

Percent weight in volume (w/v) expresses the number of g of a constituent in 100 ml of solution, and is used regardless of whether water or another liquid is the solvent.

Percent volume in volume (v/v) expresses the number of ml of a constituent in 100 ml of solution.

The term "Percent" used without quantification means, for mixtures of solids and semisolids, percent weight in weight; for solutions or suspensions of solids in liquids, percent weight in volume; for solutions of liquids in liquids, percent volume in volume; and for solutions of gases in liquids, percent weight in volume. For example, a 1% solution is prepared by dissolving 1 g of a solid or semisolid, or 1 ml of a liquid, in sufficient solvent to make 100 ml of solution.

### Reagents

The proper conduct of the analytical procedures described in the Compendium and the reliability of the results depend, in part, upon the quality of the reagents used. The reagents, including the solutions required for the tests of the Compendium, are defined in the Appendices.

## Solutions

Unless otherwise specified in the individual monograph, all solutions called for in tests are prepared with water.

An expression such as "(1 in 10)" means that 1 part by volume of a liquid is to be diluted with, or 1 part by weight of a solid is to be dissolved in, sufficient amount of the diluent or solvent to make the volume of the finished solution 10 parts by volume.

An expression such as "(20:5:2)" means that the respective numbers of parts, by volume, of the designated liquids are to be mixed, unless otherwise indicated.

## Temperatures

Unless otherwise specified, all temperatures in this Compendium are expressed in Celsius degrees, and all measurements are made at 25°. Where "room temperature" is stated, a temperature from 20° to 30° is intended.

## Water-Bath

Where the use of a water-bath is directed without qualification with respect to temperature, a bath of vigorously boiling water is intended.

# MONOGRAPHS

*Capparis micracantha* DC.

# *Capparis micracantha DC.*

**Thai Name** ชิงชี่

**Definition** Dried roots of *Capparis micracantha* DC. (Family Capparaceae).

**Synonyms** *Capparis bariensis* Pierre ex Gagnep.
*Capparis billardieri* DC.
*Capparis callosa* Blume
*Capparis donnaiensis* Pierre ex Gagnep.
*Capparis forsteniana* Miq.
*Capparis hainanensis* Oliv.
*Capparis liangii* Merr. & Chun
*Capparis myrioneura* Hallier f.
*Capparis odorata* Blanco
*Capparis petelotii* Merr.
*Capparis roydsiifolia* Kurz
*Capparis venosa* Merr.

**Part Used** Roots

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Sample solution**: Macerate 1 g of the sample, in *fine powder*, with 10 ml of *methanol* for 30 min and filter (solution 1).

**Chromatographic system**

**Adsorbent**: *Silica gel GF254* precoated HPTLC plate

**Application volume**: 15 µl of *Sample solution*

**Developing solvent system**: *Chloroform, Methanol,* and *Formic acid* (60:30:10)

**Developing distance**: 8 cm

**Spray reagent**: *Ninhydrin TS*

**Analysis**

**Sample**: *Sample Solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Sample* as band (4 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Examine the plate under ultraviolet light (254 and 366 nm). Subsequently, spray the plate with *Spray reagent*, then heat at 105 ° for 5 min and immediately examine the plate in daylight (Table 1); see also Fig. 1.

**Table 1** $hR_f$ Values of Components in Methanolic Extract of the Roots of *Capparis micracantha* DC.

| Band | $hR_f$ Value | Detection | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | *Ninhydrin TS* |
| 1 | 3 – 5 | - | - | brown |
| 2 | 9 – 12 | quenching | - | - |
| 3 | 30 – 34 | - | - | purple brown |
| 4 | 41 – 45 | - | - | purple brown |
| 5 | 95 – 98 | - | green | - |

**B. Colour reaction**

To 2 ml of solution 1, add a few drops of *ninhydrin TS*: a purple color is produced.

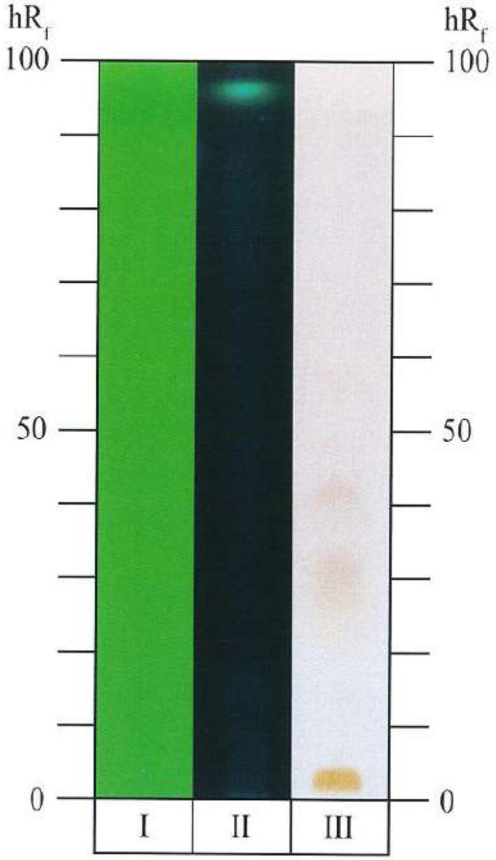


**Fig. 1** Thin-layer Chromatogram of Methanolic Extract of the Roots of *Capparis micracantha* DC.

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

**Loss on drying** Not more than 8.0% w/w after drying at 105° to constant weight (Appendix 3.1).

**Acid-insoluble ash** Not more than 1.0% w/w (Appendix 4.1).

**Total ash** Not more than 4.0% w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 2.0% w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 7.0% w/w (Appendix 4.3).

*Clerodendrum indicum* (L.) Kuntze

# *Clerodendrum indicum* (L.) Kuntze

**Thai Name** เท้ายายม่อม

**Definition** Dried roots of *Clerodendrum indicum* (L.) Kuntze (Family Lamiaceae).

**Synonyms** *Clerodendrum longicolle* G. Mey.
*Clerodendrum mite* (L.) Vatke
*Ovieda mitis* L.
*Siphonanthus angustifolius* Willd.
*Siphonanthus indicus* L.

**Part Used** Roots

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Standard solution**: 0.5 mg/ml of *pectolinarigenin* in *methanol*

**Sample solution**: Reflux 2 g of the sample, in *fine powder*, with 20 ml of *methanol* for 30 min, filter, and concentrate to small volume (2 ml) under reduced pressure.

**Chromatographic system**

**Adsorbent**: *Silica Gel GF254* precoated TLC plate

**Application volume**: each of 5 µl of *Standard solution* and *Sample solution*

**Developing solvent system**: *Hexane, Ethyl acetate* and *Formic acid* (75:25:2)

**Developing distance**: 12 cm

**Spray reagent**: A 1.0% w/v solution of *Aluminium chloride* in *Ethanol*

**Analysis**

**Samples**: *Standard solution* and *Sample solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Samples* as bands (10 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Examine the plate under ultraviolet light (254 and 366 nm). Subsequently, spray the plate with *Spray reagent*, and immediately examine the plate under ultraviolet light at 366 nm (Table 1); see also Fig. 1.

**Table 1** $hR_f$ Values of Components in Methanolic Extract of the Roots of *Clerodendrum indicum* (L.) Kuntze

| Band | $hR_f$ Value | Detection | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | A 1.0 per cent w/v solution of *Aluminium chloride* in *Ethanol* |
| 1 | 2-3 | - | light blue | - |
| 2 | 4-5 | quenching | blue | light blue |
| 3 | 9-11 | - | light blue | light blue |
| 4 | 22-25 | quenching | dark blue | light blue |
| 5* | 38-40 | quenching | dark blue | light blue |
| 6 | 48-50 | - | - | light blue |
| 7 | 66-70 | - | - | light blue |
| 8 | 72-74 | - | greenish-yellow | light blue |

*pectolinarigenin

**B. Colour reaction**

Macerate 500 mg of the sample, in *fine powder*, with 4 ml of *ethanol* for 10 min and filter. To 2 ml of solution, add 2 or 3 pieces of *magnesium ribbon* and mix with a few drops of *hydrochloric acid*: a yellow-orange colour develops.

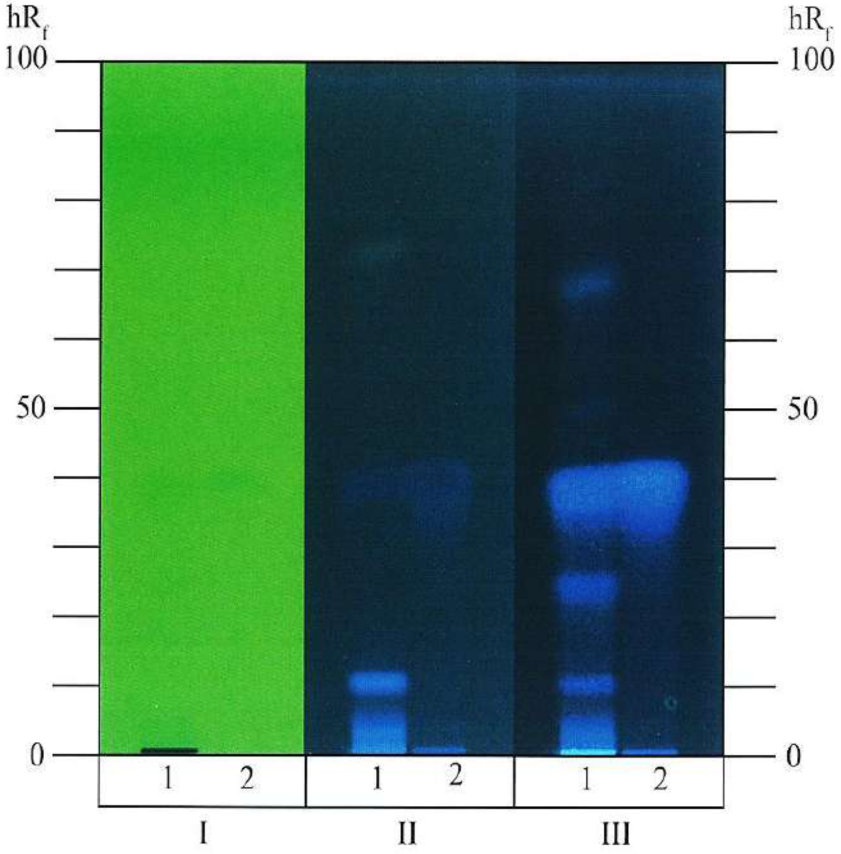


**Fig. 1** Thin-layer Chromatogram of Methanolic Extract of the Roots of *Clerodendrum indicum* (L.) Kuntze

1 = sample solution
2 = standard solution
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection under UV 366 after spraying with a 1.0% w/v solution of *aluminium chloride* in *ethanol*

**Loss on drying** Not more than 8.0% w/w after drying at 105° to constant weight (Appendix 3.1).

**Acid-insoluble ash** Not more than 1.0% w/w (Appendix 4.1).

**Total ash** Not more than 4.0% w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 2.0% w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 5.0% w/w (Appendix 4.3).

*Cyanthillium cinereum* (L.) H.Rob.

# *Cyanthillium cinereum* (L.) H.Rob.

**Thai Name** หญ้าดอกขาว

**Definition** Dried aerial parts of *Cyanthillium cinereum* (L.) H.Rob. (Family Asteraceae).

**Synonyms** *Cacalia cinerea* (L.) Kuntze
*Conyza cinerea* L.
*Cyanopis erigeroides* DC.
*Eupatorium myosotifolium* Jacq.
*Seneciodes cinereum* (L.) Kuntze ex Kuntze
*Serratula cinerea* (L.) Roxb.
*Vernonia cinerea* (L.) Less.
*Vernonia cyanonioides* Walp.
*Vernonia dendigulensis* DC.
*Vernonia diffusa* Decne.
*Vernonia erigeroides* (DC.) DC.
*Vernonia lentii* Volk. & O.Hoffm.
*Vernonia leptophylla* DC.
*Vernonia montana* Hook.f.
*Vernonia parviflora* Reinw.
*Vernonia physalifolia* DC.
*Vernonia rhomboides* Edgew.
*Vernonia villosa* W.F. Wright

**Part Used** Aerial parts

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Standard solution**: 1 mg/ml of *luteoloside* in *absolute ethanol*

**Sample solution**: Reflux 1 g of the sample, in *fine powder*, with 20 ml of *absolute ethanol* for 30 min and filter. Centrifuge and use the supernatant liquid.

**Chromatographic system**

**Adsorbent**: *Silica gel G* precoated TLC plate

**Application volume**: 3 µl of *Standard solution* and 15 µl of *Sample solution*

**Developing solvent system**: *Ethyl acetate, Formic acid, Glacial acetic acid* and *Water* (100:10:10:20)

**Developing distance**: 10 cm

**Spray reagent**: *Natural Products (NP) TS* and *Polyethylene Glycol (PEG) TS*

**Analysis**

**Samples**: *Standard solution* and *Sample solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Samples* as bands (10 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Examine under ultraviolet light (366 nm). Heat the plate at 80° for 10 min and then spray with *natural products (NP) TS* while the plate is still warm. Subsequently spray the plate with *polyethylene glycol (PEG) TS* and observe the colours of the bands under ultraviolet light (366 nm) within 5 to 15 min (Table 1); see also Fig. 1.

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

| Band | $hR_f$ Value | Detection | |
|---|---|---|---|
| | | UV 366 before spraying with *NP/PEG TS* | UV 366 after spraying with *NP/PEG TS* |
| 1 | 45-48 | blue | blue |
| 2* | 61-63 | - | orange |
| 3 | 72-74 | - | green |
| 4 | 82-85 | blue | green |
| 5 | 93-95 | - | green-yellow |
| 6 | 98-99 | red | red |

* luteoloside

### B. Colour Reaction

- Reflux 500 mg of the sample, in *fine powder*, with 20 ml of *absolute ethanol* for 10 min and filter. Evaporate 2 ml of filtrate to dryness. Dissolve the residue in 1 ml of *acetic anhydride* and then slowly add 1 ml of *sulfuric acid* to form two layers: a red-brown ring forms at the zone of contact and the upper layer is green.
- Reflux 500 mg of the sample, in *fine powder*, with 20 ml of *water* for 10 min and filter (solution 1). To 2 ml of solution 1, add a few drops of a 1.0% w/v solution of *iron(III) chloride* and shake well: a green blue colour is produced.
- To 2 ml of solution 1, add 3 or 4 pieces of *magnesium ribbon* and mix with a few drops of *hydrochloric acid*: an orange colour develops.

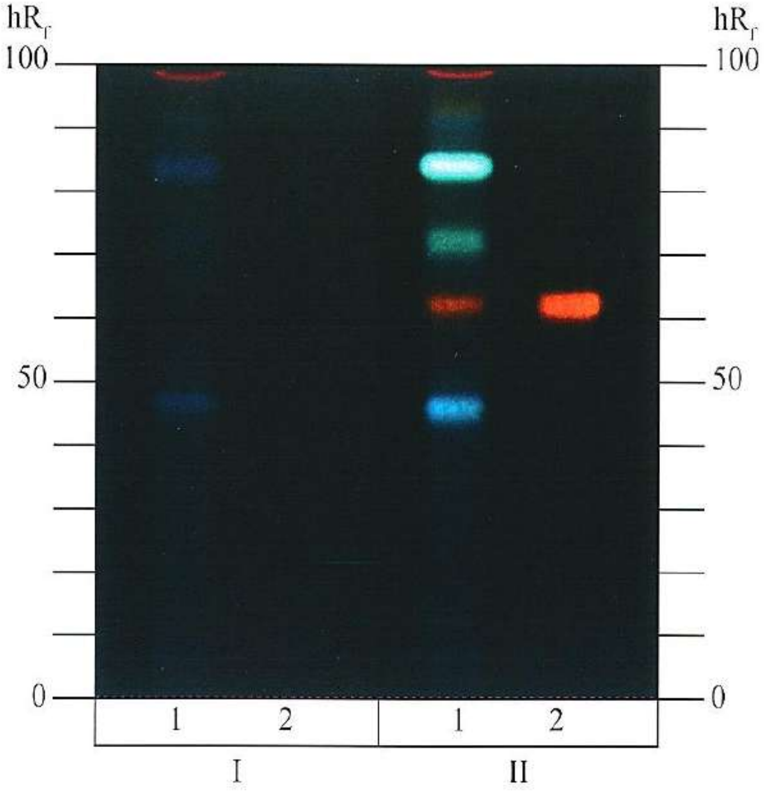


**Fig. 1** Thin-layer Chromatogram of Ethanolic Extract of the Aerial Parts of *Cyanthillium cinereum* (L.) H. Rob.

1 = sample solution
2 = standard solution
I = detection under UV light (366 nm)
II = detection under UV 366 after spraying with *NP/PEG TS*

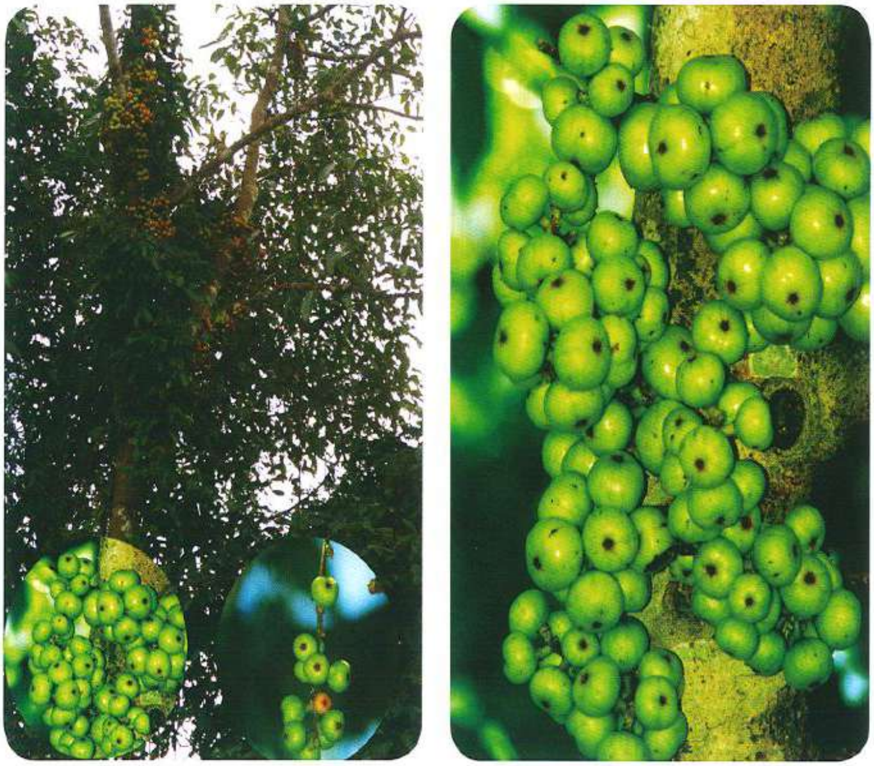
**Loss on drying** Not more than 9.0% w/w after drying at 105° to constant weight (Appendix 3.1).

**Acid-insoluble ash** Not more than 3.0% w/w (Appendix 4.1).

**Total ash** Not more than 12.0 % w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 6.0%w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 13.0%w/w (Appendix 4.3).



*Ficus racemosa* L.

# *Ficus racemosa* L.

| | |
|---|---|
| **Thai Name** | มะเดื่อชุมพร |
| **Definition** | Dried roots of *Ficus racemosa* L. (Family Moraceae). |
| **Synonyms** | *Covellia glomerata* (Roxb.) Miq.<br>*Ficus acidula* King<br>*Ficus chittagonga* Miq.<br>*Ficus glomerata* Roxb.<br>*Ficus henrici* King<br>*Ficus lanceolata* Buch.-Ham. ex Roxb.<br>*Ficus leucocarpa* (Miq.) Miq.<br>*Ficus lucescens* Blume<br>*Ficus semicostata* F.M.Bailey<br>*Ficus vesca* F.Muell. ex Miq. |
| **Part Used** | Roots |

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Standard solution**: 1 mg/ml of *lupeol* in *methanol*

**Sample solution**: Macerate 1 g of the sample, in *fine powder*, with 10 ml of *methanol* for 20 min, filter, and concentrate to small volume (2 ml) under reduced pressure.

**Chromatographic system**

**Adsorbent**: *Silica Gel GF254* precoated TLC plate

**Application volume**: 2 µl of *Standard solution* and 10 µl of *Sample solution*

**Developing solvent system**: *Hexane* and *Ethyl acetate* (9:1)

**Developing distance**: 12 cm

**Spray reagent**: A 10.0% w/v solution of *Phosphomolybdic acid* in *Ethanol*

**Analysis**

**Samples**: *Standard solution* and *Sample solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Samples* as bands (6 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Examine the plate under ultraviolet light (254 and 366 nm). Subsequently, spray the plate with *Spray reagent*, heat at 105° for 5 min, and immediately examine the plate in daylight (Table 1); see also Fig. 1.

**Table 1** $hR_f$ Values of Components in Methanolic Extract of the Roots of *Ficus racemosa* L.

| Band | $hR_f$ Value | Detection | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | A 10.0 Per cent w/v solution of *Phosphomolybdic Acid* in *Ethanol* |
| 1 | 1-2 | quenching | - | - |
| 2 | 3-4 | - | red | - |
| 3 | 4-5 | quenching | - | - |
| 4 | 7-8 | quenching | blue | - |
| 5 | 9-11 | - | red | blue |
| 6 | 16-18 | - | - | dark blue |
| 7 | 23-25 | - | - | blue |
| 8 | 26-28 | - | light blue | - |
| 9* | 29-32 | - | - | dark blue |
| 10 | 39-41 | - | - | blue |
| 11 | 72-75 | - | - | blue |
| 12 | 80-85 | - | - | dark blue |
| 13 | 94-96 | - | - | dark blue |

*lupeol

**B. Colour reaction**

- Reflux 500 mg of the sample, in *fine powder*, with 10 ml of *chloroform* for 10 min and filter. Evaporate the filtrate to dryness. Dissolve the residue in 1 ml of *acetic anhydride* and then slowly add 1 ml of *sulfuric acid* to form two layers: a red-brown ring forms at the zone of contact and the upper layer is green.
- Macerate 100 mg of the sample, in *fine powder*, with 4 ml of *ethanol* for 5 min and filter. To 2 ml of the filtrate, add 2 or 3 pieces of *magnesium ribbon* and mix with a few drops of *hydrochloric acid*: a pink-orange colour develops.

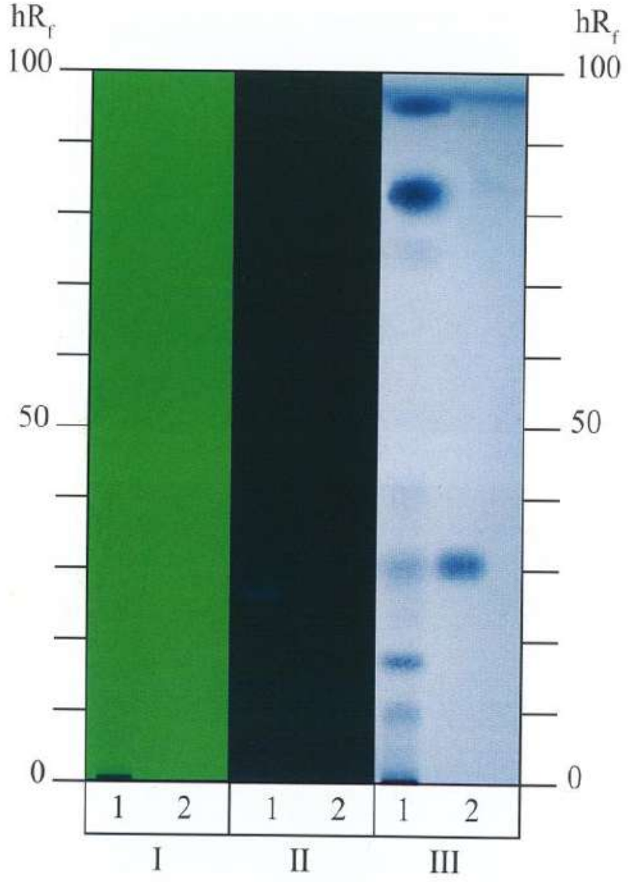


**Fig. 1** Thin-layer Chromatogram of Methanolic Extract of the Roots of *Ficus racemosa* L.

1 = sample solution
2 = standard solution
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection with a 10.0% w/v solution of *phosphomolybdic acid* in *ethanol*

**Loss on drying** Not more than 8.0% w/w after drying at 105° to constant weight (Appendix 3.1).

**Acid-insoluble ash** Not more than 1.0% w/w (Appendix 4.1).

**Total ash** Not more than 6.0% w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 2.0% w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 4.0% w/w (Appendix 4.3).

*Garcinia cowa* Roxb. ex Choisy

# *Garcinia cowa* Roxb. ex Choisy

**Thai Name** ชะมวง

**Definition** Dried leaves of *Garcinia cowa* Roxb. ex Choisy (Family Clusiaceae).

**Synonyms** *Cambogia crassifolia* Blanco
*Garcinia cornea* Roxb. ex Sm.
*Garcinia roxburghii* Wight
*Garcinia wallichii* Choisy
*Oxycarpus gangetica* Buch.-Ham.

**Part Used** Leaves

## Identification

### A. Thin-layer Chromatography Identification Test

**Standard solutions**: 1 mg/ml of *vitexin* in *ethanol* and 1 mg/ml of *orientin* in *ethanol*

**Sample solution**: Reflux 1 g of the sample, in *fine powder*, in 20 ml of *ethanol* for 20 min and immediately filter. Evaporate the filtrate to dryness under reduced pressure at 45° and add 3 ml of *ethanol* to dissolve the residue.

**Chromatographic system**

**Adsorbent**: *Silica gel GF254* precoated TLC plate

**Application volume**: 2 µl of *Standard solutions* and 1 µl of *Sample solution*

**Developing solvent system**: *Ethyl acetate*, *Formic acid*, *Glacial acetic acid* and *Water* (100:11:11:27)

**Developing distance**: 10 cm

**Spray reagent**: *Natural Products (NP) TS* and *Polyethylene Glycol (PEG) TS*

**Analysis**

**Samples**: *Standard solutions* and *Sample solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Samples* as bands (6 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Examine under ultraviolet light (366 nm). Heat the plate at 80° for 10 min and then spray with *natural products (NP) TS* while the plate is still warm. Subsequently spray the plate with *polyethylene glycol (PEG) TS* and observe the colours of the bands under ultraviolet light (366 nm) within 5 to 15 min (Table 1); see also Fig. 1.

**Table 1** $hR_f$ Values of Components in Ethanolic Extract of the Leaves of *Garcinia cowa* Roxb. ex Choisy

| Band | $hR_f$ Value | Detection | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | UV 366 after spraying with *NP/PEG TS* |
| 1 | 34-35 | - | blue | orange |
| 2 | 38-39 | quenching | - | yellow-orange |
| 3 | 44-45 | quenching | - | green-blue |
| 4 | 51-52 | quenching | blue | - |
| 5 | 59-60 | - | - | yellow |
| 6 | 67-68 | quenching | - | orange |
| 7* | 69-70 | quenching | - | yellow |
| 8** | 78-79 | quenching | - | green |
| 9 | 89-90 | quenching | - | yellow |
| 10 | 93-94 | quenching | - | green |

*orientin

**vitexin

**B. Colour reaction**

- Reflux 1 g of the sample, in *fine powder*, with 20 ml of *ethanol* for 20 min and filter (solution 1). Evaporate 2 ml of solution 1 to dryness. Dissolve the residue in 2 ml of *acetic anhydride* and then slowly add 1 ml of *sulfuric acid* to form two layers: a red-brown ring forms at the zone of contact and the upper layer is green.
- Reflux 1 g of the sample, in *fine powder*, with 30 ml of *distilled water* for 20 min and filter (solution 2). To 2 ml of solution 2, add 2 or 3 pieces of *magnesium ribbon* and mix with 1 ml of *hydrochloric acid*: a pink-orange colour develops.
- To 2 ml of solution 1, add 2 or 3 pieces of *magnesium ribbon* and mix with 1 ml of *hydrochloric acid*: a red-pink colour develops.
- To 2 ml of solution 1, add a few drops of a freshly prepared 1% w/v solution of *iron(III) chloride* and shake well: a greenish black colour is produced.
- To 2 ml of solution 2, add a few drops of a freshly prepared 1% w/v solution of *iron(III) chloride* and shake well: a greenish black colour is produced.

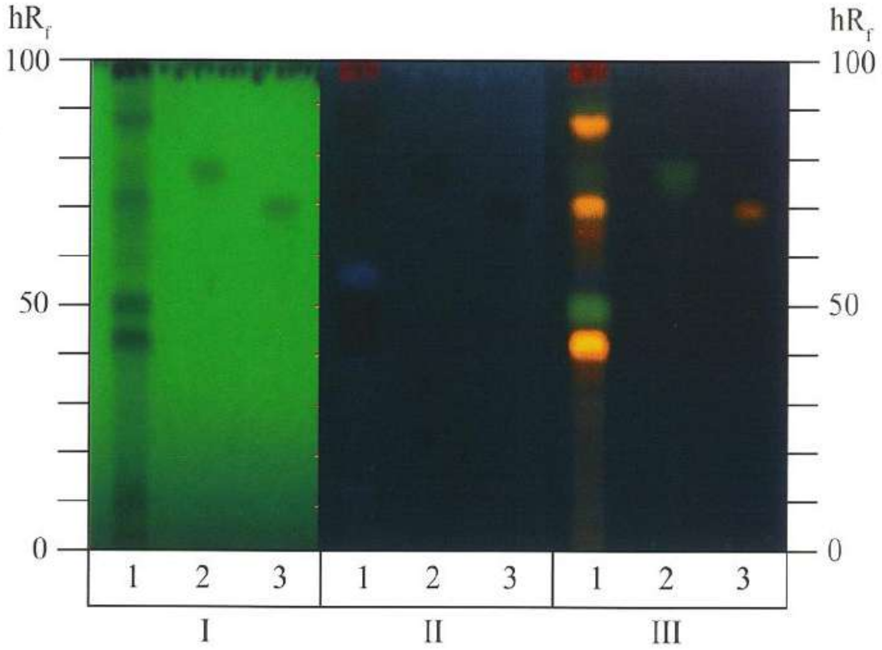


**Fig. 1** Thin-layer Chromatogram of Ethanolic Extract of the Leaves of *Garcinia cowa* Roxb. ex Choisy

1 = sample solution
2 = standard solution of vitexin
3 = standard solution of orientin
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection under UV 366 after spraying with *NP/PEG TS*

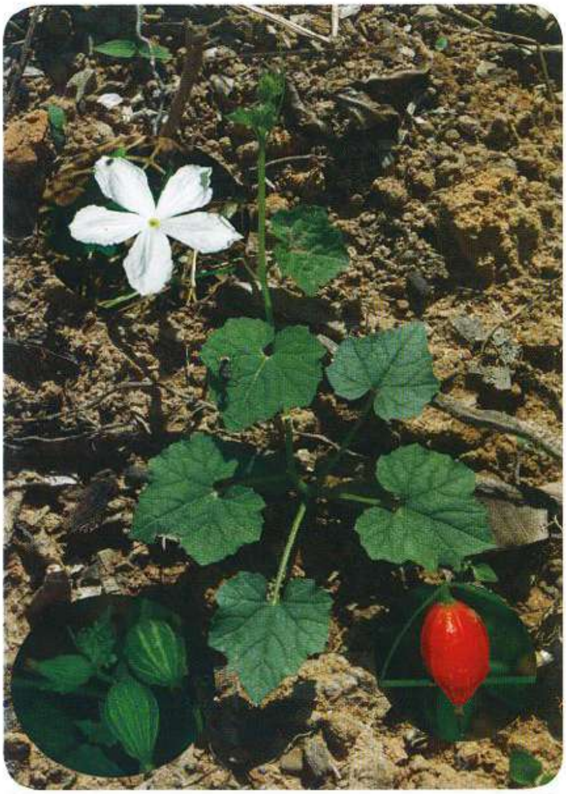
**Loss on drying** Not more than 8.0% w/w after drying at 105° to constant weight (Appendix 3.1).

**Acid insoluble ash** Not more than 0.1 % w/w (Appendix 4.1).

**Total ash** Not more than 7.0% w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 18.0% w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 21.0% w/w (Appendix 4.3).



*Gymnopetalum chinense* (Lour.) Merr.

# *Gymnopetalum chinense* (Lour.) Merr.

**Thai Name** กะดอม

**Definition** Dried young fruits of *Gymnopetalum chinense* (Lour.) Merr. (Family Cucurbitaceae).

**Synonyms** *Bryonia cochinchinensis* Lour.
*Cucumis tubiflorus* Roxb. ex Wight & Arn.
*Euonymus chinensis* Lour.
*Gymnopetalum chinensis* (Lour.) Merr.
*Gymnopetalum cochinchinense* (Lour.) Kurz
*Gymnopetalum cochinchinensis* (Lour.) Kurz
*Gymnopetalum heterophyllum* Kurz
*Gymnopetalum quinquelobatum* Merr.
*Gymnopetalum quinquelobum* Miq.
*Momordica tubiflora* Roxb.
*Tripodanthera cochinchinensis* (Lour.) M. Roem.

**Part Used** Fruits

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Sample solution:** Reflux 500 mg of the sample, in *fine powder*, with 20 ml of *ethanol* for 20 min and filter. Evaporate the filtrate to dryness under reduced pressure and add 1 ml of *ethanol* to dissolve the residue.

**Chromatographic system**

**Adsorbent:** *Siliga gel G* precoated TLC plate
**Application volume:** 2 µl of *Sample solution*
**Developing solvent system:** *Hexane* and *Acetone* (5:2)
**Developing distance:** 8 cm
**Spray reagent:** *Anisaldehyde TS*

**Analysis**

**Sample:** *Sample solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Sample* as band (6 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Examine the plate under ultraviolet light (254 nm). Subsequently, spray the plate with *Spray reagent*, heat at 105° for 5 min, and immediately examine the plate in daylight (Table 1); see also Fig 1.

**Table 1** $hR_f$ Values of Components in Ethanolic Extract of the Fruits of *Gymnopetalum chinense* (Lour.) Merr.

| Band | $hR_f$ Value | Detection | |
|---|---|---|---|
| | | UV 254 | *Anisaldehyde TS* |
| 1 | 4-6 | - | purple |
| 2 | 7-9 | quenching | - |
| 3 | 9-12 | - | purple |
| 4 | 22-24 | - | purple |
| 5 | 28-31 | quenching | purple |
| 6 | 41-44 | quenching | purple |
| 7 | 46-48 | quenching | purple |
| 8 | 60-62 | quenching | - |
| 9 | 63-74 | - | purple |

**B. Colour reaction**

- Reflux 500 mg of the sample, in *fine powder*, with 10 ml of *methanol* on water-bath for 5 min and filter. Evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in 2 ml of *acetic anhydride* and slowly add 2 ml of *sulfuric acid*: a brownish red colour ring develops.
- Reflux 500 mg of the sample, in *fine powder*, with 10 ml of *methanol* on water-bath for 5 min and filter. Evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in 2 ml of *ethanol* and add 1 ml of *ninhydrin TS*, warm in water bath: a purple colour is produced.

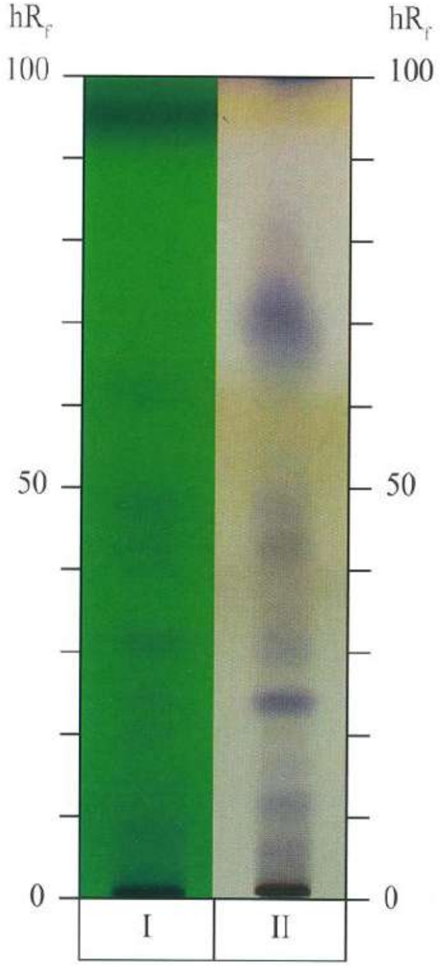


**Fig. 1** Thin-layer Chromatogram of Ethanolic Extract of the Fruits of *Gymnopetalum chinense* (Lour.) Merr.

I = detection under UV light (254 mm)
II = detection with *anisaldehyde TS*

**Loss on drying** Not more than 9.0% w/w after drying at 105° to constant weight (Appendix 3.1).

**Acid-insoluble ash** Not more than 1.0% w/w (Appendix 4.1).

**Total ash** Not more than 11.0% w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 11.0% w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 26.0% w/w (Appendix 4.3).

*Harrisonia perforata* (Blanco) Merr.

# *Harrisonia perforata* (Blanco) Merr.

**Thai Name** คนทา

**Definition** Dried roots of *Harrisonia perforata* (Blanco) Merr. (Family Rutaceae).

**Synonyms** *Anisifolium pubescens* (Wall.) Kuntze
*Feroniella puberula* Yu.Tanaka
*Feroniella pubescens* (Wall. ex Hook.f.) Yu.Tanaka
*Harrisonia citrinaecarpa* Elmer
*Lasiolepis multijuga* Benn.
*Lasiolepis paucijuga* Benn. & R. Br.
*Limonia pubescens* Wall. ex Hook.f.
*Paliurus dubius* Blanco
*Paliurus perforatus* Blanco

**Part Used** Roots

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Sample solution:** Macerate 500 mg of the sample, in *fine powder*, with 4 ml of *methanol* for 15 min and filter.

**Chromatographic system**

**Adsorbent:** *Silica Gel GF254* precoated HPTLC plate

**Application volume:** 10 µl of *Sample solution*

**Developing solvent system:** *Chloroform*, *Methanol* and *Formic acid* (95:5:0.5)

**Developing distance:** 7 cm

**Spray reagent:** *Natural Products (NP) TS* and *Polyethylene Glycol (PEG) TS*

**Analysis**

**Sample:** *Sample solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Sample* as band (6 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Examine under ultraviolet light (254 and 366 nm). Heat the plate at 80° for 10 min and then spray with *natural products* (*NP) TS* while the plate is still warm. Subsequently spray the plate with *polyethylene glycol* (*PEG) TS* and observe the colours of the band under ultraviolet light (366 nm) within 5 to 15 min (Table 1); see also Fig. 1.

**Table 1** $hR_f$ Values of Components in Methanolic Extract of the Roots of *Harrisonia perforata* (Blanco) Merr.

| Band | $hR_f$ Value | Detection | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | *NP/PEG TS* |
| 1 | 6-8 | - | - | blue |
| 2 | 17-21 | quenching | black | black |
| 3 | 21-22 | - | - | pale blue |
| 4 | 36-37 | quenching | | pale yellowish-green |
| 5 | 47-48 | quenching | - | black |
| 6 | 58-60 | - | - | pale blue |
| 7 | 68-72 | - | blue | blue |
| 8 | 73-76 | - | pale blue | blue |
| 9 | 77-81 | quenching | light blue | light blue |
| 10 | 90-94 | quenching | black | yellowish-green |

### B. Colour reaction

Macerate 100 mg of the sample, in *fine powder*, with 4 ml of *ethanol* for 5 min and filter. To 2 ml of solution, add 2 or 3 pieces of *magnesium ribbon* and mix with a few drops of *hydrochloric acid*: a yellow-orange colour develops.

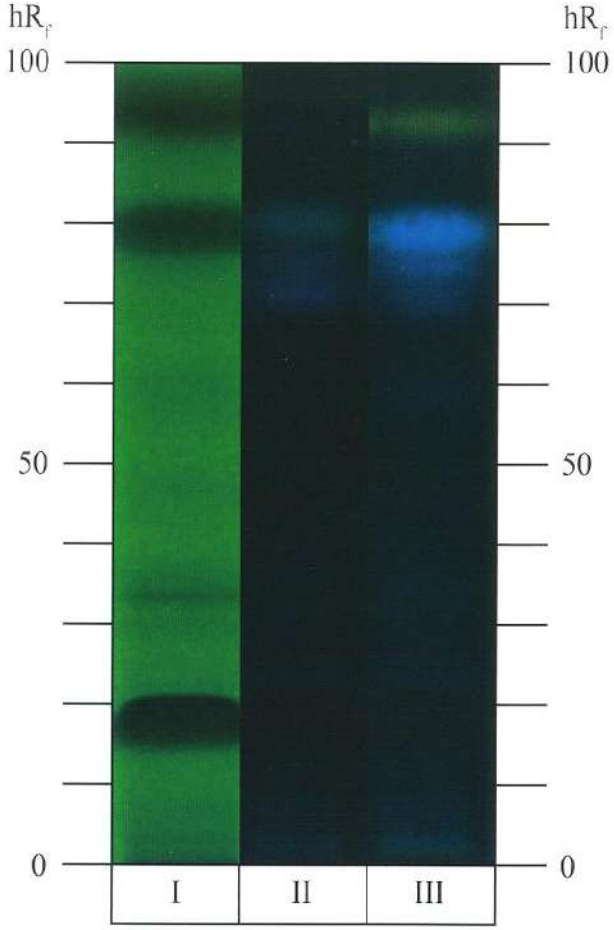


**Fig. 1** Thin-layer Chromatogram of Methanolic Extract of the Roots of *Harrisonia perforata* (Blanco) Merr.

I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
III = detection under UV 366 after spraying with *NP/PEG TS*

**Loss on drying** Not more than 9.0% w/w after drying at 105° to constant weight (Appendix 3.1).

**Acid-insoluble ash** Not more than 1.0% w/w (Appendix 4.1).

**Total ash** Not more than 4.0% w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 3.0% w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 4.0% w/w (Appendix 4.3).

*Lysiphyllum strychnifolium* (W. G. Craib) A. Schmitz

# *Lysiphyllum strychnifolium* (W. G. Craib) A. Schmitz

**Thai Name** ย่านางแดง

**Definition** Dried leaves of *Lysiphyllum strychnifolium* (W.G. Craib) A. Schmitz (Family Fabaceae).

**Synonym** *Buahinia strychnifolia* Craib

**Part Used** Leaves

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Sample solution:** Reflux 500 mg of the sample, in *fine powder*, with 20 ml of *ethanol* for 20 min and filter. Evaporate the filtrate to dryness under reduced pressure and add 1 ml of *ethanol* to dissolve the residue.

**Chromatographic system**

**Adsorbent:** *Silica gel G* precoated TLC plate

**Application volume:** 2 µl

**Developing solvent system:** *Ethyl acetate*, *Formic acid*, *Glacial acetic acid*, and *Water* (25:2:2:3)

**Developing distance:** 12 cm

**Spray reagent:** *Natural Products (NP) TS* and *Polyethylene Glycol (PEG) TS*

**Analysis**

**Sample:** *Sample solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Sample* as band (6 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Examine under ultraviolet light (366 nm). Heat the plate at 80° for 10 min and then spray with *natural products (NP) TS* while the plate is still warm. Subsequently spray the plate with *polyethylene glycol (PEG) TS* and observe the colours of the band under ultraviolet light (366 nm) within 5 to 15 min (Table 1); see also Fig. 1.

**Table 1** $hR_f$ Values of Compotents in Ethanolic Extract of the Leaves of *Lysiphyllum strychnifolium* (W.G. Craib) A. Schmitz

| Band | $hR_f$ Value | Detection | |
|---|---|---|---|
| | | UV 366 | UV 366 after spraying with *NP/PEG TS* |
| 1 | 5-7 | blue | - |
| 2 | 8-11 | blue | - |
| 3 | 36-39 | green | - |
| 4 | 45-48 | - | - |
| 5 | 58-62 | blue | - |
| 6 | 60-64 | - | orange |
| 7 | 66-72 | - | green |
| 8 | 77-82 | blue | orange |
| 9 | 95-98 | blue | - |

**B. Colour reaction**

- Reflux 500 mg of the sample, in *fine powder*, with 10 ml of *methanol* on water-bath for 5 min and filter (solution1). Evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in 2 ml of *acetic anhydride* and slowly add 2 ml of *sulfuric acid*: a brownish red colour ring develops.
- Reflux 500 mg of the sample, in *fine powder*, with 10 ml of *methanol* on water-bath for 5 min and filter. To 2 ml of solution 1, add 2 or 3 pieces of *magnesium ribbon* and mix with a few drops of *hydrochloric acid*: a pink-red colour develops.

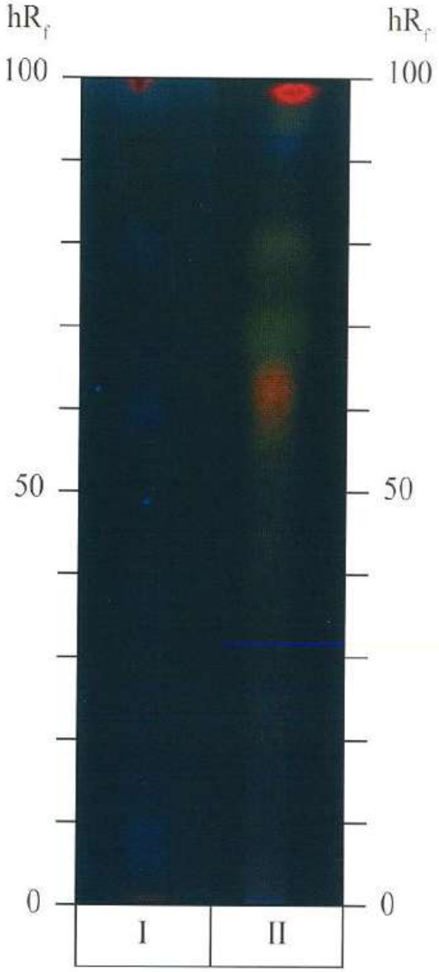


**Fig. 1** Thin-layer Chromatogram of Ethanolic Extract of the Leaves of *Lysiphyllum strychnifolium* (W. G. Craib) A. Schmitz

I = detection under UV light (366mm)

II = detection under UV 366 after spraying with *NP/PEG TS*

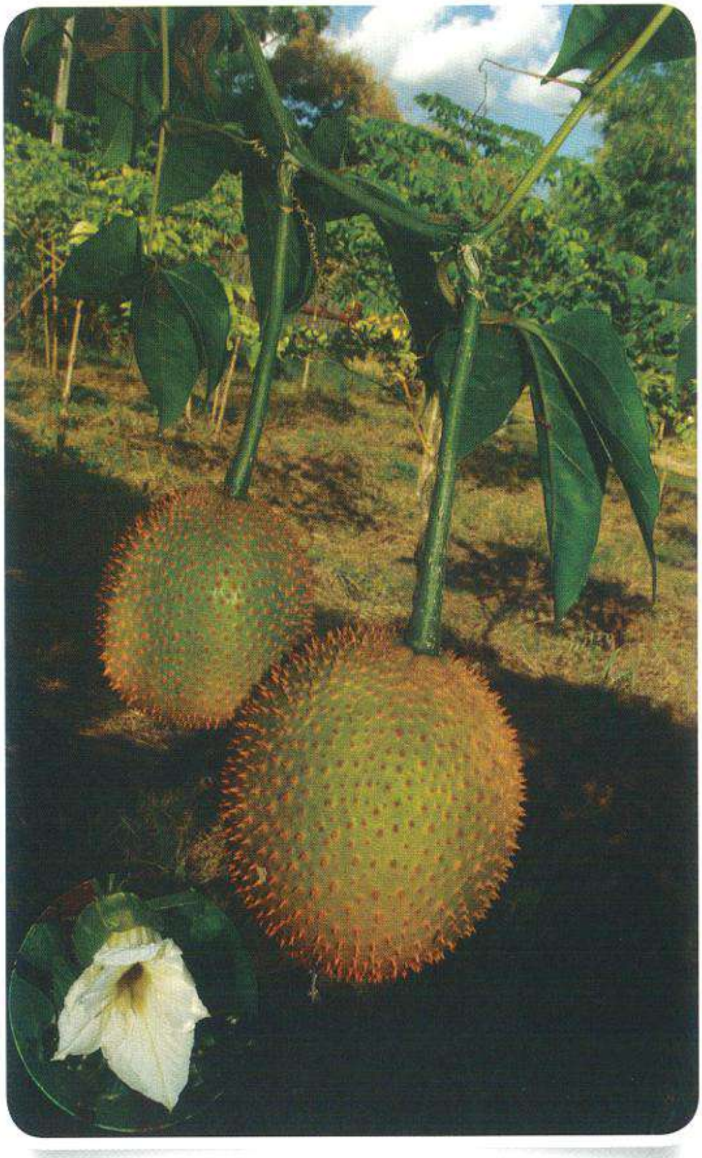
**Loss on drying** Not more than 9.0% w/w after drying at 105° to constant weight (Appendix 3.1).

**Acid-insoluble ash** Not more than 1.0% w/w (Appendix 4.1).

**Total ash** Not more than 7.0% w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 23.0% w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 16.0% w/w (Appendix 4.3).



*Momordica cochinchinensis* (Lour.) Spreng.

# *Momordica cochinchinensis* (Lour.) Spreng.

**Thai Name** ฟักข้าว

**Definition** Dried leaves of *Momordica cochinchinensis* (Lour.) Spreng. (Family Cucurbitaceae).

**Synonyms** *Momordica macrophylla* Gage
*Momordica meloniflora* Hand.-Mazz.
*Momordica mixta* Roxb.
*Muricia cochinchinensis* Lour.
*Zucca commersoniana* Ser.

**Part Used** Leaves

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Standard solutions:** 1 mg/ml of L-*glutamic acid* in *methanol*, 1 mg/ml of L-*tryptophan* in *methanol* and 1 mg/ml of 4-*aminobutyric acid* in *methanol*.

**Sample solution:** Sonicate 400 mg of the sample, in *fine powder*, in 4 ml of *methanol* for 30 min and filter.

**Chromatographic system**

**Adsorbent:** *Silica gel GF254* precoated TLC plate

**Application volume:** 15 µl of *Sample solution*, each of 1 µl of *Standard solutions*

**Developing solvent system:** *n-Butanol*, 1-*Propanol*, *Glacial acetic acid* and *Water* (30:10:11:10)

**Developing distance:** 8 cm

**Spray reagent:** *Ninhydrin TS*

**Analysis**

**Samples:** *Sample solution* and *Standard solutions*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Samples* as bands (5 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Examine the plate under ultraviolet light (254 and 366 nm). Subsequently, spray the plate with *Spray reagent*, heat at 110° for 10 min and examine the plate in daylight (Table 1); see also Fig 1.

**Table 1** $hR_f$ Values of Components in Methanolic Extract of the Leaves of *Momordica cochinchinensis* (Lour.) Spreng.

| Band | $hR_f$ Value | Detection | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | *Ninhydrin TS* |
| 1 | 24-28 | - | - | red-brown |
| 2* | 35-40 | - | - | pink-purple |
| 3** | 45-51 | - | - | red-brown |
| 4 | 53-56 | - | blue | pink-purple |
| 5 | 60-63 | - | blue | - |
| 6*** | 66-71 | quenching | blue | red-brown |
| 7 | 74-79 | quenching | - | - |
| 8 | 81-89 | - | blue | - |
| 9 | 95-99 | quenching | red | green |

*L-glutamic acid

**4-aminobutyric acid

***L-tryptophan

**B. Colour reaction**

- Reflux 500 mg of the sample, in *fine powder*, with 10 ml of *water* for 15 min, and filter. To 2 ml of the filtrate, add a few drops of *potassium cupri-tartrate TS*: an orange-red colour of precipitate is produced.
- Reflux 500 mg of the sample, in *fine powder*, with 10 ml of *methanol* for 15 min, filter, and evaporate to dryness. Dissolve the residue with 2 ml of *acetic anhydride*, slowly add a few drops of *sulfuric acid*: a reddish-brown ring forms at the zone of contact and the upper layer is green.
- Reflux 1 g of the sample, in *fine powder*, with 20 ml of *water* for 15 min, and filter. To 2 ml of the filtrate, add a few drops of *ninhydrin TS*: a violet colour is produced.
- Reflux 1 g of the sample, in *fine powder*, with 25 ml of *water* for 15 min and filter. To 2 ml of the filtrate, add a few drops of a 1.0% w/v solution of *iron(III) chloride*: a greenish blue colour is produced.
- To 500 mg of the sample, in *fine powder*, in a stoppered test-tube, add 10 ml of *water*. Shake for 30 sec: the persisting foam for over 15 min is produced.

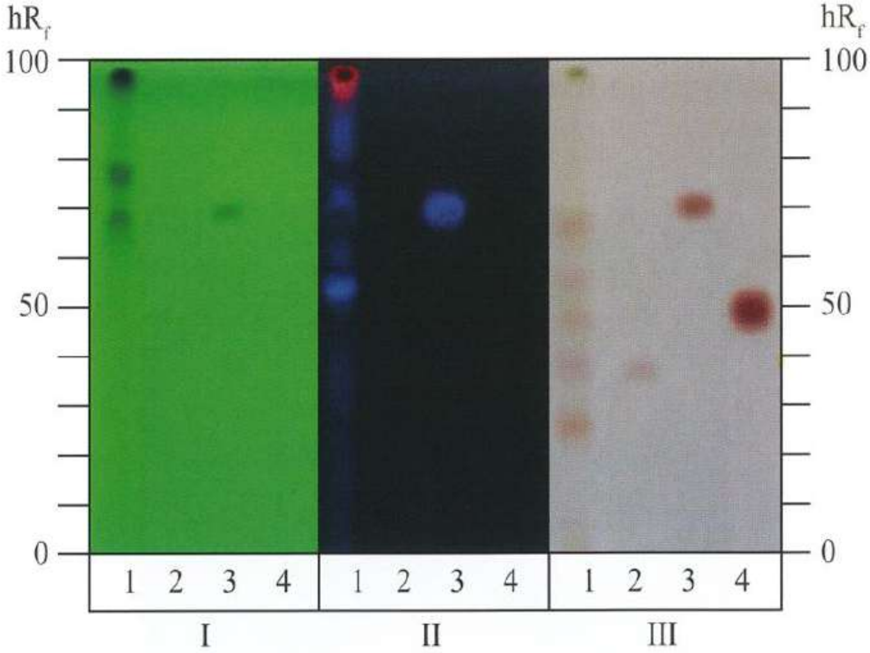


**Fig. 1** Thin-layer chromatogram of Methanolic Extract of the Leaves of *Momordica cochinchinensis* (Lour.) Spreng

1 = sample solution
2 = standard solution of *L*-glutamic acid
3 = standard solution of *L*-tryptophan
4 = standard solution of *4*-aminobutyric acid
I = detection under UV light (254 nm)
II = detection under UV light (366 nm)
II = detection under daylight after spraying with *ninhydrin TS*

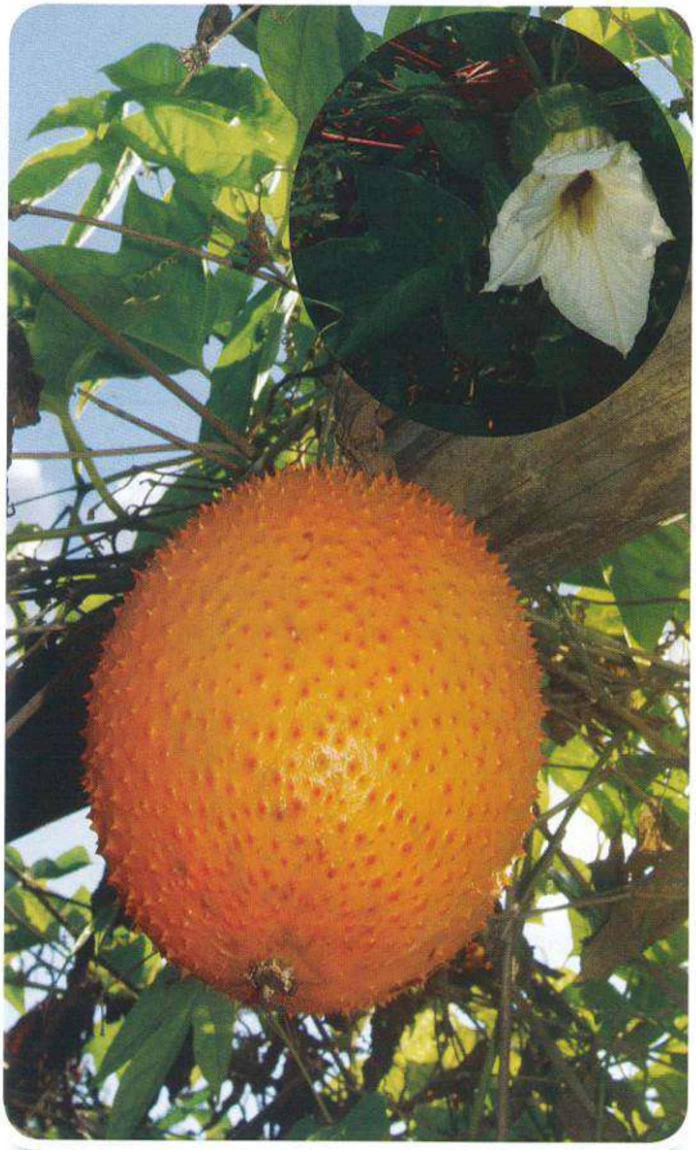
**Loss on drying** Not more than 9.0 % w/w after drying at 105 °C to constant weight (Appendix 3.1).

**Acid insoluble ash** Not more than 2.0 % w/w (Appendix 4.1).

**Total ash** Not more than 17.0 % w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 9.0 % w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 28.0 % w/w (Appendix 4.3).



*Momordica cochinchinensis* (Lour.) Spreng.

# *Momordica cochinchinensis* (Lour.) Spreng. Dry extract

**Thai Name** สารสกัดแห้งฟักข้าว

**Definition** The *Momordica cochinchinensis* (Lour.) Spreng. Dry Extract is prepared from the arils of *Momordica cochinchinensis* (Lour.) Spreng. (Family Cucurbitaceae) by compression through filter bag, concentration under reduced pressure, and using a freeze-dried apparatus until dryness.

**Synonyms** *Momordica macrophylla* Gage
*Momordica meloniflora* Hand.-Mazz.
*Momordica mixta* Roxb.
*Muricia cochinchinensis* Lour.
*Zucca commersoniana* Ser.

**Part Used** Arils

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Standard solution:** 1 mg/ml of *β-carotene* in *hexane*

**Sample solution:** Sonicate 200 mg of the sample, in *fine powder*, in 3 ml of *hexane* for 30 min and filter.

**Chromatographic system**

**Adsorbent:** *Silica gel G* precoated TLC plate

**Application volume:** 10 µl of *Sample solution*, 1 µl of *Standard solution*

**Developing solvent system:** *Hexane* and *Dichloromethane* (5:1)

**Developing distance:** 8 cm

**Spray reagent:** *Anisaldehyde TS*

**Analysis**

**Samples:** *Sample solution* and *Standard solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Samples* as bands (5 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Subsequently, spray the plate with *Spray reagent*, heat at 105 ° for 5 min and examine the plate in daylight (Table 1); see also Fig 1.

**Table 1** $hR_f$ Values of Components in Hexane Extract of the *Momordica cochinchinensis* (Lour.) Spreng. Dry Extract

| Band | $hR_f$ Value | Detection | |
|---|---|---|---|
| | | Daylight | *Anisaldehyde TS* |
| 1 | 10-11 | orange-yellow | violet |
| 2 | 13-15 | - | violet |
| 3 | 29-33 | orange-yellow | violet |
| 4 | 41-45 | orange-yellow | violet |
| 5* | 56-60 | orange-yellow | violet |

*β-carotene

B. Colour reaction

- Moisture 1 g of the sample, in *fine powder*, with 0.5 ml of *concentrated ammonia*, 5 ml of *chloroform*, then continuously shake for 10 min, filter, and evaporate to dryness. Dissolve the residue with 1 ml *ethanol*. To 2 ml of the filtrate, add a few drops of *Dragendorff TS*: an orange colour of precipitate is produced.
- Reflux 500 mg of the sample, in *fine powder*, with 10 ml of *water* for 15 min, and filter. To 2 ml of the filtrate, add a few drops of *potassium cupri-tartrate TS*: an orange-red colour of precipitate is produced.
- Reflux 500 mg of the sample, in *fine powder*, with 10 ml of *methanol* for 15 min, filter, and evaporate to dryness. Dissolve the residue with 2 ml of *acetic anhydride*, slowly add a few drops of *sulfuric acid*: a reddish-brown ring forms at the zone of contact and the upper layer is green.
- Reflux 1 g of the sample with 20 ml of *water* for 15 min, and filter. To 2 ml of the filtrate, add a few drops of *ninhydrin TS*: a violet colour is produced.

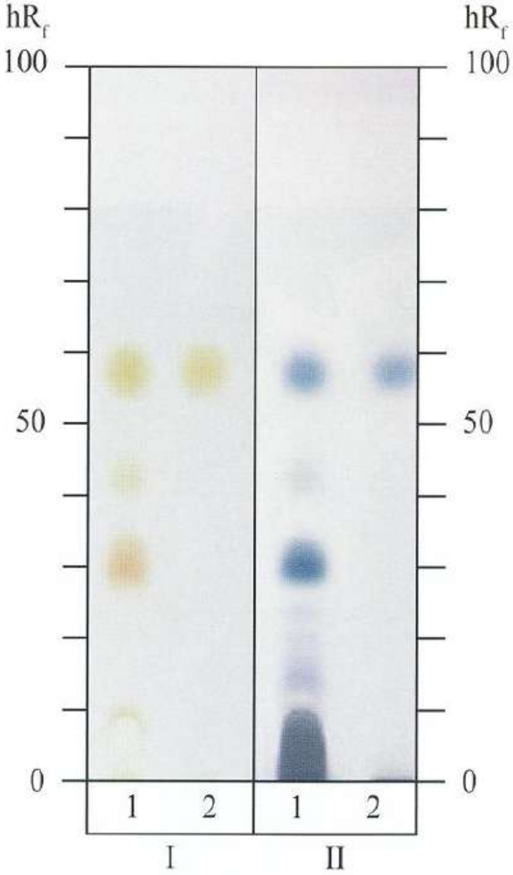


**Fig. 1** Thin-layer Chromatogram of Hexane Extract of the *Momordica cochinchinensis* (Lour.) Spreng. Dry Extract

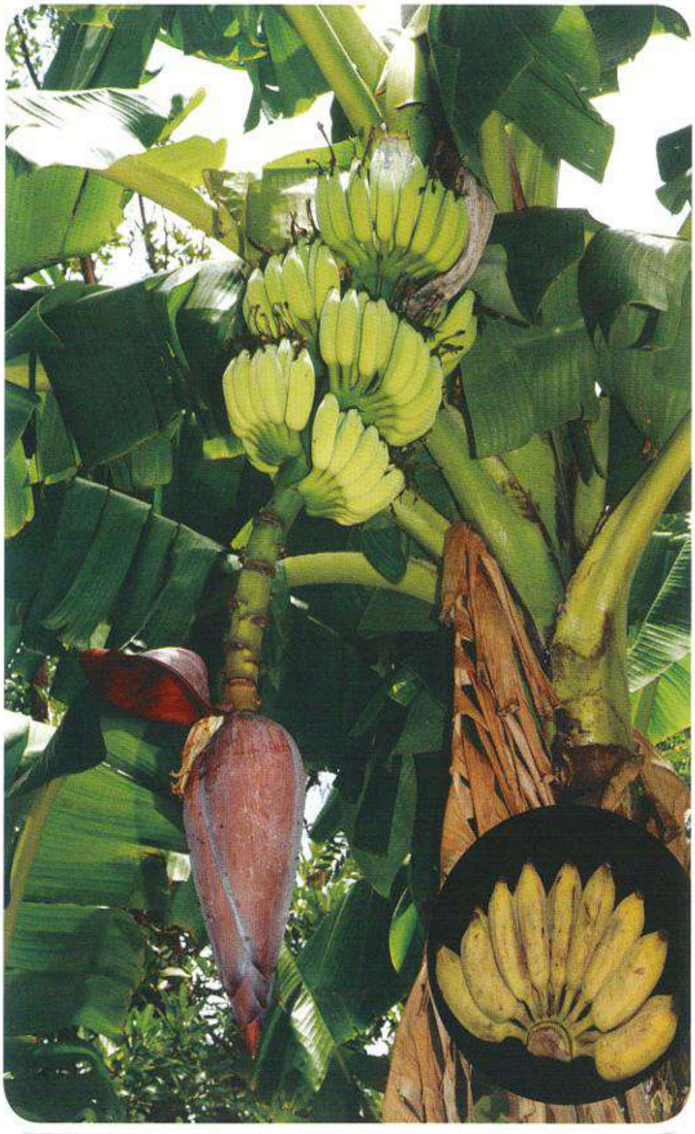
1 = sample solution
2 = standard solution
I = detection in daylight
II = detection in daylight after spraying with *anisaldehyde TS*

**Loss on drying** Not more than 7.0 % w/w after drying at 105°C to constant weight, using 1.0 g of the sample (Appendix 3.1).

**Total ash** Not more than 7.0 % w/w, using 1.0 g of the sample (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 44.0 % w/w, using 1.0 g of the sample (Appendix 4.3).

**Water-soluble extractive** Not less than 54.0 % w/w, using 1.0 g of the sample (Appendix 4.3).



*Musa* × *paradisiaca* L. (ABB group) "kluai namwa"

# *Musa × paradisiaca* L. (ABB group) "kluai namwa"

**Thai Name** กล้วยน้ำว้า

**Definition** Unripe pulp of *Musa × paradisiaca* L. (ABB group) "kluai namwa" (Family Musaceae).

**Part Used** Unripe pulp

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Standard solution:** 1% w/v *gallic acid* in *methanol*

**Sample solution:** Macerate 5 g of the sample, in *fine powder*, with 50 ml of *ethanol* for 3 hours and filter. Evaporate the filtrate to dryness and add 2 ml of *absolute ethanol* to dissolve the residue.

**Chromatographic system**

**Adsorbent:** *Silica gel G* precoated TLC plate

**Application volume:** 1 ml of *Standard solution* and 4 ml of *Sample solution*

**Developing solvent system:** *Ethyl acetate*, *Toluene*, *Methanol* and *Formic acid* (30:30:8:2)

**Developing distance:** 10 cm

**Spray reagent:** A 20.0% w/v solution of *Phosphomolybdic acid* in *Ethanol*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Samples* as bands (8 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Spray the plate with *Spray reagent*, heat at 105-110 ° for 5 to 10 min, and examine the plate in daylight (Table 1); see also Fig. 1.

**Table 1** $hR_f$ Values of Components in Ethanolic Extract of the Unripe Pulp of *Musa* × *paradisiaca* L. (ABB group) "kluai namwa"

| Band | $hR_f$ Value | Detection |
|---|---|---|
| | | A 20.0 per cent w/v solution of *Phosphomolybdic Acid* in *Ethanol* |
| 1 | 2-5 | blue |
| 2* | 42-47 | blue |
| 3 | 60-63 | blue |
| 4 | 85-89 | blue |

* gallic acid

**B. Colour Reaction**

- Reflux 500 mg of the sample, in *fine powder*, with 20 ml of *water* for 10 min and filter. To 2 ml of filtrate, add 3 or 4 pieces of *magnesium ribbon* and mix with a few drops of *hydrochloric acid*: a pale pink colour develops.
- Add 10 ml of *water* to 500 mg of the sample, in *fine powder*, boil for 2 min and filter. To 3 ml of the filtrate, add 1 drop of *potassium cupri-tartrate TS* and warm: a brick-red precipitate is produced.
- Reflux 1 g of the sample, in *fine powder*, with 20 ml of *water* for 10 min and filter. To 2 ml of solution add 1 ml of *ethanol* and shake well: a white gelatinous mass is produced.

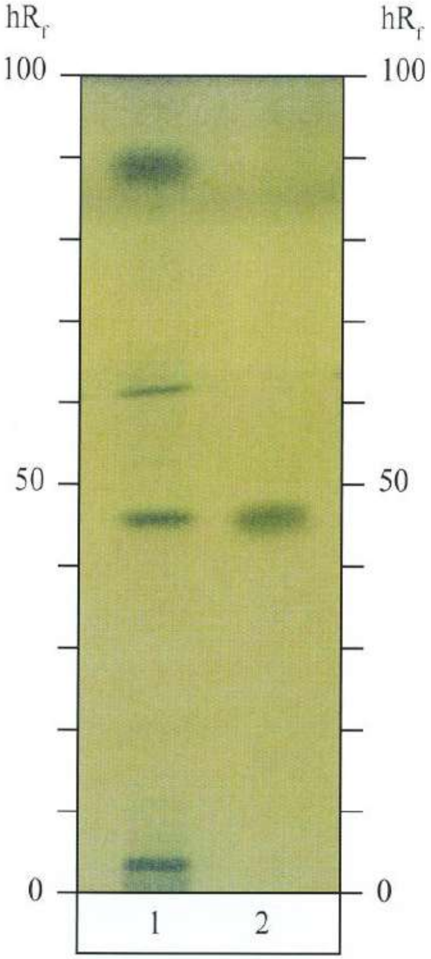


**Fig. 1** Thin-layer Chromatogram of Ethanolic Extract of the Unripe Pulp of *Musa* × *paradisiaca* L. (ABB group) "kluai namwa".

1 = sample solution
2 = standard solution

**Loss on drying** Not more than 10.0% w/w after drying at 105° to constant weight (Appendix 3.1).

**Acid-insoluble ash** Not more than 0.5% w/w (Appendix 4.1).

**Total ash** Not more than 3.0% w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 2.0% w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 6.0% w/w (Appendix 4.3).

*Tiliacora triandra* Diels

# *Tiliacora triandra* Diels

**Thai Name** ย่านาง

**Definition** Dried roots of *Tiliacora triandra* Diels (Family Menispermaceae).

**Part Used** Roots

**Identification**

**A. Thin-layer Chromatography Identification Test**

**Sample solution:** Macerate 1 g of the sample, in *fine powder*, with 10 ml of *methanol* for 15 min and filter. Evaporate the filtrate to dryness and add 2 ml of *methanol* to dissolve the residue.

**Chromatographic system**

**Adsorbent:** *Silica gel GF254* precoated TLC plate

**Application volume:** 5 µl of *Sample solution*

**Developing solvent system:** *Chloroform* and *Ethanol* (70:30)

**Developing distance:** 8 cm

**Spray reagent:** *Dragendorff TS, Modified*

**Analysis**

**Sample:** *Sample Solution*

Carry out the test as described in the "Thin-layer Chromatography" (Appendix 2.1). Apply the *Sample* as band (4 mm) to the plate and dry in air. Develop the chromatogram in a saturated chamber. Remove the plate from the chamber and dry in air. Examine the plate under ultraviolet light (254 and 366 nm). Subsequently, spray the plate with *Spray reagent*, then heat at 105° for 5 min and immediately examine the plate in daylight (Table 1); see also Fig. 1.

**Table 1** $hR_f$ Values of Components in Methanolic Extract of the Roots of *Tiliacora triandra* Diels

| Band | $hR_f$ Value | Detection | | |
|---|---|---|---|---|
| | | UV 254 | UV 366 | *Dragendorff TS, Modified* |
| 1 | 5 – 6 | quenching | - | orange |
| 2 | 12 – 15 | quenching | blue | - |
| 3 | 34 – 38 | quenching | - | orange |
| 4 | 48 – 50 | quenching | - | orange |
| 5 | 53 – 56 | quenching | green | orange |
| 6 | 76 – 78 | - | red | - |
| 7 | 84 – 86 | - | light blue | - |

**B. Colour reaction**

Macerate 200 mg of the sample, in *fine powder*, with 4 ml of *chloroform* for 5 min and filter. To 2 ml of the filtrate, add a few drops of *Dragendorff TS, Modified*: an orange precipitated is produced.

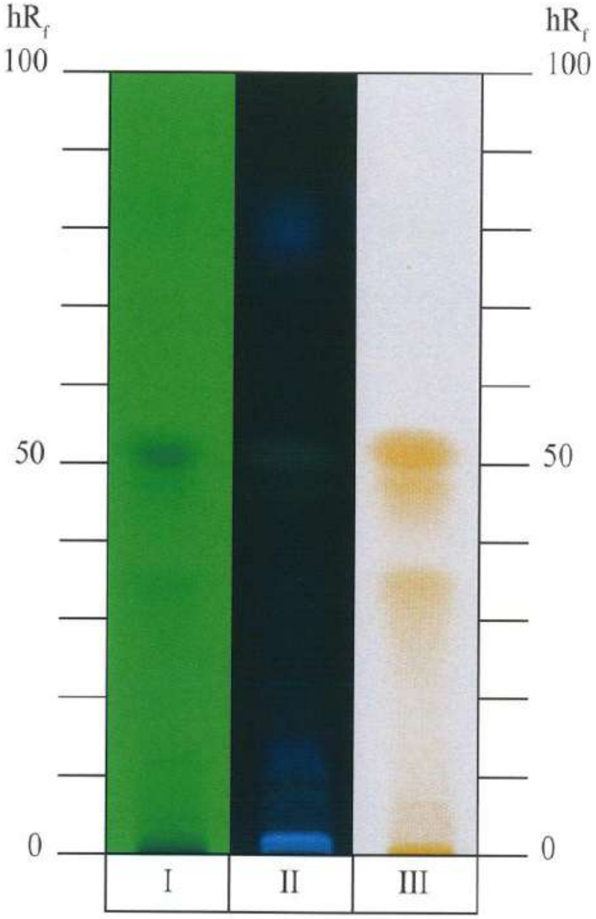


**Fig. 1** Thin-layer Chromatogram of Methanolic Extract of the Roots of *Tiliacora triandra* Diels

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

**Loss on drying** Not more than 8.0% w/w after drying at 105° to constant weight (Appendix 3.1).

**Acid-insoluble ash** Not more than 1.0% w/w (Appendix 4.1).

**Total ash** Not more than 6.0% w/w (Appendix 4.2).

**Ethanol-soluble extractive** Not less than 4.0% w/w (Appendix 4.3).

**Water-soluble extractive** Not less than 7.0% w/w (Appendix 4.3).

# APPENDICES

Reference:

Department of Medical Sciences, Ministry of Public Health. Thai Herbal Pharmacopoeia 2017. 1st edition. Bangkok: The Agricultural Co-operative Federation of Thailand, Ltd.; 2017.

# Content of the Appendices

**Appendix 1 General Information**

**Appendix 2 Chromatography**

**Appendix 3 Physical Tests**

**Appendix 4 Crude Drugs**

# Appendix 1 General Information

The specifications given below are strictly for the use of the materials as reagents. The inclusion of 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.

**Acetic Acid** To glacial acetic acid, add sufficient water to produce solution containing 33.0% w/w of $C_2H_4O_2$.

**Acetic acid, Glacial** $C_2H_4O_2 = 60.05$

Use analytical reagent grade of commerce containing not less than 99.0% w/w of $C_2H_4O_2$.

DESCRIPTION Clear, colourless liquid; odour, pungent.

**Acetic anhydride** $C_4H_6O_3 = 102.09$

Use analytical reagent grade of commerce containing not less than 97.0% w/v of $C_4H_6O_3$.

DESCRIPTION Colourless liquid.

**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.

**Aluminium chloride** $AlCl_3 = 133.34$

Use analytical reagent grade of commerce.

DESCRIPTION White to light yellow powder.

**4-Aminobutyric acid** $C_4H_9NO_2$ = 204.23

Use analytical reagent grade of commerce containing not less than 99% of $C_4H_9NO_2$.

DESCRIPTION White powder.

Store in tightly closed containers, protected from light.

**Ammonia Solution, Strong** $NH_3$ = 17.03

Contains not less than 25.0% w/w and not more than 30.0% 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.

Store protected from air, at a temperature not exceeding 20°.

**Anisaldehyde** (4-Methoxybenzaldehyde) $C_8H_8O_2$ = 136.15

Use general reagent grade of commerce.

DESCRIPTION Colourless to pale yellow, oily liquid; odour, aromatic.

SOLUBILITY Slightly soluble in water; miscible with ethanol and with ether.

**Bismuth subnitrate** (Bismuth Oxynitrate) $Bi_5O(OH)_9(NO_3)_4$ = 1461.99

DESCRIPTION White, microcrystalline powder.

SOLUBILITY Insoluble in water and ethanol; readily soluble in dilute nitric acid and in dilute hydrochloric acid.

**n-Butanol** (n-Butyl Alcohol; 1-Butanol) $C_4H_{10}O$ = 74.12

Use analytical reagent grade of commerce.

DESCRIPTION Clear, colourless liquid.

SOLUBILITY Soluble in 11 parts of water, at 15.5°.

**β-Carotene** $C_{40}H_{56}$ = 536.9

Use analytical reagent grade of commerce containing not less than 94% of $C_{40}H_{56}$.

DESCRIPTION Red powder.

Store in tightly closed containers, protected from light.

**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 gas.

Use analytical reagent grade of commerce containing 0.4 to 1.0% 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.

Store protected from light.

**Copper(II) Sulfate**

Use analytical reagent grade of commerce.

**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.

**Diphenylboric Acid-Aminoethyl Ester** (Diphenylboric Acid-2-Aminoethyl Ester; Diphenylboric Acid-Ethanolamine Reagent) $C_{14}H_{16}BNO = 225.10$

Use a suitable grade.

**Ethanol** Use Ethanol (95%).

**Ethanol, Absolute** $C_2H_6O = 46.07$

Use analytical reagent grade of commerce.

DESCRIPTION Colourless, clear, mobile and volatile liquid; odour, characteristic and spirituous. Flammable, burning with a blue, smokeless flame; Hygroscopic.

**Ethanol (95%)** (Ethyl Alcohol)

**A mixture of ethanol and water. Contains not less than 92.3% w/w and not more than 93.8% w/w, corresponding to not less than 94.9% v/v and not more than 96.0% 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.

**Ethyl Acetate** $C_4H_8O_2 = 88.11$

Use analytical reagent grade of commerce.

DESCRIPTION Colourless liquid; odour, fruity-like.

**Formic Acid** $CH_2O_2 = 46.03$

Use analytical reagent grade of commerce containing about 90% w/w of $CH_2O_2$ and about 23.6 M in strength.

DESCRIPTION Colourless, corrosive liquid; odour, pungent.

**Gallic Acid** (3,4,5-Trihydroxybenzoic Acid) $C_7H_6O_5.H_2O$ = 188.14

DESCRIPTION White or almost white crystals or powder.

SOLUBILITY Sparingly soluble in cold water; very soluble in boiling water and in ethanol.

**L-Glutamic acid** $C_5H_9NO_4$ = 147.10

Use analytical reagent grade of commerce containing not less than 99% of $C_5H_9NO_4$.

DESCRIPTION White powder.

Store in tightly closed containers, protected from light.

**Hexane** $C_6H_{14}$ = 86.18

The hexane fraction from petroleum.

DESCRIPTION Colourless, mobile, highly flammable liquid.

**n-Hexane** $C_6H_{14}$ = 86.18

Use analytical reagent grade of commerce usually containing not less than 99% of the pure isomer, n-$C_6H_{14}$.

DESCRIPTION Colourless, flammable liquid.

**Hydrochloric Acid** HCl = 36.46

Use analytical reagent grade of commerce.

When no molarity is indicated, use analytical reagent grade of commerce with relative density of about 1.18, containing not less than 35% w/w and not more than 38% w/w of HCl and about 11.5 M in strength.

DESCRIPTION Clear, colourless, fuming liquid; odour, pungent.

SOLUBILITY Miscible with water.

Solution of molarity *x*M should be prepared by diluting 85×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°.

**Iron(III) Chloride** (Ferric chloride) $FeCl_3.6H_2O$ = 270.30

Use analytical grade of commerce.

DESCRIPTION Yellowish orange or brownish, crystalline masses; deliquescent.

Store in well-closed containers.

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

Use analytical reagent grade of commerce containing not less than 99% of $C_{30}H_{50}O$.

**Luteoloside** $C_{21}H_{20}O_{11}$ = 448.38

Use analytical reagent grade of commerce containing not less than 98% of $C_{21}H_{20}O_{11}$.

**Magnesium Ribbon**

Use a suitable grade.

**Methanol** (Methyl Alcohol) $CH_4O$ = 32.04

Use analytical reagent grade of commerce.

DESCRIPTION Colourless liquid.

SOLUBILITY Miscible with water, forming a clear colourless liquid.

**Ninhydrin** $C_9H_6O_4$ = 178.14

DESCRIPTION Pale yellow prisms from water or alcohol.

SOLUBILITY Freely soluble in water.

**Orientin** $C_{21}H_{20}O_{11}$ = 448.38

Use analytical reagent grade of commerce containing not less than 97% of $C_{21}H_{20}O_{11}$.

Store at 4° in a dry place.

**Pectolinarigenin** $C_{17}H_{14}O_6$ = 314.29

Use analytical reagent grade of commerce containing not less than 98% of $C_{17}H_{14}O_6$.

**Phosphomolybdic Acid** $H_3PO_4.12MoO_3.24H_2O$ = 2257.62

Use analytical reagent grade of commerce

DESCRIPTION Fine, orange-yellow crystals.

SOLUBILITY Very soluble in water

**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.

**Potassium Iodide** KI = 166.00

Use analytical reagent grade of commerce.

DESCRIPTION White crystalline powder.

**Potassium Sodium Tartrate** $KNaC_4H_4O_6.4H_2O$ = 282.22

Use analytical reagent grade of commerce.

**1-Propanol** (n-Propyl Alcohol) $C_3H_8O$ = 60.10

DESCRIPTION Colourless liquid; odour, characteristic.

SOLUBILITY Miscible with water and with ethanol.

**Sodium Hydroxide** NaOH = 40.00

Use analytical reagent grade of commerce.

**Sulfuric Acid** $H_2SO_4$ = 98.07

When no molarity is indicated, use analytical reagent grade of commerce containing about 96% w/w of sulfuric acid and about 18 M in strength.

DESCRIPTION Colourless, oily, corrosive liquid.

When solutions of molarity $x$M are required, they should be prepared by carefully adding 54×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.

**Toluene** (Methylbenzene) $C_7H_8$ = 92.14

Use analytical reagent grade of commerce.

DESCRIPTION Clear, colourless liquid; odour, characteristic. Flammable.

SOLUBILITY Miscible with water and with ethanol.

**L-Tryptophan** $C_{11}H_{12}N_2O_2$ = 204.23

Use analytical reagent grade of commerce containing not less than 99% of $C_{11}H_{12}N_2O_2$.

DESCRIPTION White powder.

Store in tightly closed containers, protected from light.

**Vitexin** $C_{21}H_{20}O_{10}$ = 432.38

DESCRIPTION Yellow powder

Use analytical reagent grade of commerce containing not less than 98% of $C_{21}H_{20}O_{10}$.

Store in +4° in a dry place.

**Water** $H_2O$ = 18.02

Use Purified Water of the Official Pharmacopoeia.

## 1.2 Test Solutions

**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.

**Dragendorff TS** 0.85 g of basic bismuth nitrate is dissolved in 40 ml of water and 10 ml of glacial acetic acid, followed by addition of 8 g of potassium iodide dissolved in 20 ml of water.

**Dragendorff TS, Modified**

Solution A: Dissolve 0.85 g of bismuth subnitrate 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.

**Iron(III) Chloride TS** Dissolve 9 g of iron(III) chloride in water to make 100 ml.

**Ninhydrin TS** (Triketohydrindene Hydrate TS) Dissolve 0.2 g of ninhydrin in 10 ml of water.

**Ninhydrin TS, Ethanolic** Dissolve 1 g of ninhydrin in 50 ml of ethanol and 10 ml of glacial acetic acid.

**Natural Products (NP) TS** Dissolve 1 g of diphenylboric acid-2-aminoethyl ester in sufficient methanol to make 100 ml.

**Polyethylene Glycol (PEG) TS** Dissolve 5 g of polyethylene glycol 4000 in sufficient ethanol to make 100 ml.

**Potassium Cupri-tartate 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 product 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 solution No.1 and No.2 immediately before use.

## 1.3 Materials for Chromatograph

### Thin-layer Chromatography

The coating substances described below are used to prepare thin-layer chromatoplates in accordance with the procedure described in Appendix 2.1. Commercial precoated chromatoplates may be used for Pharmacopoeial tests provided they comply with the test for chromatographic separation described for the corresponding coating substance.

**Silica Gel G** A fine, white, homogeneous powder of an average particle size between 10 and 40 μm containing about 13% w/w of calcium sulfate hemihydrate.

**Silica Gel GF254** A fine, white, homogeneous powder of an average particle size between 10 and 40 μm containing about 13% w/w of calcium sulfate hemihydrate and about 1.5% w/w of a fluorescent indicator having a maximum intensity at 254 nm.

## 1.4 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% 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% 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% 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 describes 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 tailing 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 sifted 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 on 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%.

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[1] US National Institute of Standards and Technology

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 from during the sifting. Weight accurately the amount remaining on the sieve and in the receiving pan.

The fineness of a powdered drug or chemical may be determined also by screening through the sieves in mechanical sieve shaker, which reproduces the circular and tapping motion given to testing sieves in hand sifting but with a uniform mechanical action, following the directions provided by the manufacturer of the shaker.

| Number of Sieve* | Nominal Mesh Aperture Size | Perferred Average Wire Diameter | Percentage Sieving Area | US Sieve No.** |
|---|---|---|---|---|
| µm | mm | mm | | |
| **4000** | **4.00** | **1.40** | | |
| 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 size".

** The list of United States standard sieves is included for information purposes.

# Appendix 2 Chromatography

## 2.1 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.

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.

**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.

**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 need, 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 bands 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 edge 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 hr. 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.

**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.

# Appendix 3 Physical Tests

## 3.1 Loss on Drying

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 min 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 hr 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.

# Appendix 4 Crude Drugs

## 4.1 Acid-insoluble Ash

Use Method I unless otherwise indicated in the monograph.

### Method I

Boil the total ash for 5 min 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 min; 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 desiccators 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.

## 4.2 Total Ash

Use Method I unless otherwise directed in the compendium monograph.

### Method I

**For vegetable drugs** Incinerate 2 to 3 g to 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 min, allow to cool in a desiccators 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 hr 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 filter paper. Combine the filtrate with the ash, carefully evaporate to dryness and ignite to constant weight.

## 4.3 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 hr, shaking frequently during the first 6 hr and then allowing to stand for 18 hr. 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 hr. Attach a reflux condenser to the flask. Boil gently for 1 hr, 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 hr, cool in a desiccator for 30 min, 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.

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NOTE

NOTE

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