การศึกษาทางเภสัชเวทของเกสรทั้งห้า

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต สาขาวิชาเภสัชพฤกษศาสตร์ ภาควิชาเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ จุพาลงกรณ์มหาวิทยาลัย

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#### PHARMACOGNOSTIC STUDY OF KAYSORN -TUNG - HAA

Miss Pairin Thongkhoom

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacy Department of Pharmaceutical Botany Faculty of Pharmaceutical Sciences Chulalongkorn University Academic Year 2000 ISBN 974-03-0046-4

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ได้ทำการศึกษาสมุนไพร เพื่อพิสูจน์เอกลักษณ์และจัดทำข้อมูลจำเพาะทางเภสัชเวทของ เกสรทั้งห้า ได้แก่ ดอกพิกุล ดอกบุนนา<mark>ค ดอกสาร</mark>ภี ดอกมะลิ และเกสรบัวหลวง

จัดทำข้อมูลจำเพาะทางเภสัชเวท โดยได้ทำการศึกษาทางสัณฐานวิทยาของสมุนไพร ได้ แก่ รูปร่าง ลักษณะ ขนาด สี กลิ่น รส ศึกษาทางจุลทรรศลักษณะ ได้แก่ ศึกษารูปร่าง ลักษณะของ เซลล์ และโครงสร้างของเนื้อเยื่อ ศึกษาลักษณะของผงสมุนไพร นำส่วนสกัดของเกสรทั้งห้าแต่ละ ชนิดมาศึกษาทางโครมาโตกราฟฟีชนิดผิวบาง โดยวิธี 1 มิติ (one-dimensional) ศึกษาการดูดกลืน แสงของสารประกอบทางเคมีภายใต้แสงเหนือม่วง (ultraviolet) และยังได้ศึกษาเปรียบเทียบกับ สมุนไพรอื่นที่มีลักษณะทางพฤกษศาสตร์ หรือมีชื่อภาษาไทยใกล้เคียงกับเกสรทั้งห้า

ผลการศึกษาสามารถพิสูจน์เอกลักษณ์ และได้ข้อมูลจำเพาะทางเภสัชเวทของเกสรทั้งห้า แต่ละชนิด ซึ่งแสดงผลด้วยภาพ และตารางประกอบคำบรรยาย นอกจากนั้นยังได้ทำการเปรียบ เทียบลักษณะต่างๆ ที่ได้ทำการศึกษาเกสรทั้งห้า เพื่อสามารถนำข้อมูลไปใช้ในการจำแนกชนิดของ เกสรทั้งห้าได้

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#### MAJOR PHARMACEUTICAL BOTANY

KEY WORD : KAYSORN-TUNG-HAA / PHARMACOGNOSTIC STUDY PAIRIN THONGKHOOM : PHARMACOGNOSTIC STUDY OF KAYSORN-TUNG-HAA. THESIS ADVISOR : ASSISTANT PROFESSOR SURAPONG KENGTONG

An investigation of selected group of crude drugs offer identification as well as establishment of pharmacognostic specification of Kaysorn-tung-haa. These are Phi-kun, Bun-naak, Saa-ra-phee, Ma-li and Bua-luang.

Pharmacognostical specification were established by detailed studying of each kind of Kaysorn included morphology as well as other properties of medicinal plants such as shape, size, color, odor and taste, microscopical study concerning cell characters and cell structures, characters of powder drug; chromatographic study of their alcoholic extracts one-dimensional thin-layer chromatography, ultraviolet absorptions of the extract, comparison of pharmacognostic specification of Kaysorntung-haa with similar crude drug and title .

Pharmacognostic studies of each kind of crude drug in Kaysorn-tung-haa revealed specific comparative data of which displayed in the form of tables. The result of this investigation offer a valuable tool for the identification of each crude drug in Kaysorn-tung-haa.

Department.....Pharmaceutical.Botany.....Student's signature..... Field of study...Pharmaceutical.Botany.... Advisor's signature..... Academic year.2000.....

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#### ABBREVIATIONS

°C	degree celsius
cm	centimeter
Fig.	figure
g	gram
hr	hour
L.B.	Liebermann-Burchard detection
μΙ	microliter
μm	micromillimeter
ml	milliliter
min	minute
mm	millimeter
nm	nanometer
TLC	Thin-layer chromatography
UV	ultraviolet
λmax	wavelength at maximum absorption in

nanometer

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### CHAPTER I

Usually a Thai traditional preparation consisted of many herbal materials. It is very rare to find a single drug preparation. In drug preparations, groups of specified number and types of herbs, usually, not being employed separately more over, each ingredient being used with same quantity and therapeutic action, these groups of materials are called "Pi-gud" or "Pi-gud-yaa". (1)

For example, "Pi-gud of 3 drugs" called "Tree-pha-laa " will consisted of 3 kinds of fruits of *Terminalia bellirica* Roxb., *Terminalia chebula* Retz. and *Phyllanthus emblica* Linn. with same quantity (weight). (2)

"Pi-gud of 5 drugs" when applied to 5 kinds of flowers or flower parts called Kaysorn-tung-haa.

Pi-gud Kaysorn is divided in to 3 groups according to the number of component contained, as followed (2) :

- 1. Kaysorn-tung-haa which consisted of 5 kinds of flower part, are listed below:-
  - 1.1 Corolla together with stamen of Phi-kun (Bullet Wood, *Mimusops elengi* Linn.)
  - 1.2 Whole flower of Bun-naak (Iron Wood, *Mesua ferrea* Linn.)
  - 1.3 Whole flower of Saa-ra-phee (Neg, Negkassar, *Mammea siamensis* Kosterm.)
  - 1.4 Stamen of Bua-luang (Lotus, Sacred Lotus, Nelumbo nucifera Gaertn.)
  - 1.5 Corolla together with stamen of Ma-li (Arabian Jasmine, *Jasminum sambac* Ait.)
- Kaysorn-tung-chet which consisted of 7 kinds of flowers or flower parts, are listed below:-
  - 2.1 Corolla together with stamen of Phi-kun (Bullet Wood, *Mimusops elengi* Linn.)
  - 2.2 Whole flower of Bun-naak (Iron Wood, Mesua ferrea Linn.)
  - 2.3 Whole flower of Saa-ra-phee (Neg, Negkassar, *Mammea siamensis* Kosterm.)

- 2.4 Stamen of Bua-luang (Lotus, Sacred Lotus, Nelumbo nucifera Gaertn.)
- 2.5 Corolla together with stamen of Ma-li (Arabian Jasmine, *Jasminum sambac* Ait.)
- 2.6 Whole flower of Cham-pa (Michelia champaca Linn.)
- 2.7 Whole flower of Gra-dung-nga (Ylang Ylang, Cananga odorata Hook f & Th.)
- Kaysorn-tung-kaao which consisted of 9 kinds of flowers or flower parts are listed below:-
  - 3.1 Corolla together with stamen of Phi-kun (Bullet Wood, *Mimusops elengi* Linn.)
  - 3.2 Whole flower of Bun-naak (Iron Wood, Mesua ferrea Linn.)
  - 3.3 Whole flower of Saa-ra-phee (Neg, Negkassar, *Mammea siamensis* Kosterm.)
  - 3.4 Stamen of Bua-luang (Lotus, Sacred Lotus, Nelumbo nucifera Gaertn.)
  - 3.5 Corolla together with stamen of Ma-li (Arabian Jasmine, *Jasminum sambac* Ait.)
  - 3.6 Whole flower of Cham-pa (Michelia champaca Linn.)
  - 3.7 Whole flower of Gra-dung-nga (Ylang Ylang, *Cananga odorata* Hook f.& Th).
  - 3.8 Inflorescence of Lam-jeak (Screw Pine, Pandanus odoratissimus Linn. f.)
  - 3.9 Whole flower of Lam-duan (*Melodolum fruticosum* Lour.)

Some crude drugs in Pi-gud Kaysorn may be imparted of other Pi-gud such as.

- 1. The-wa-tree-su-khon consisted of (4)
  - 1.1 Flower, heartwood and root of Bun-naak (Mesua ferrea Linn.)
  - 1.2 Flower, heartwood and root of Ma-saang (Feronia lucida Swing.)
- 2. Tree-kaysorn-mass consisted of (5)
  - 2.1 Stamen of Bua-luang (Nelumbo nucifera Gaertn.)
  - 2.2 Bark of Fin-ton ( Jatropha multifida Linn.)
  - 2.3 Fruit of Ma-tuum (Aegle marmelos Cor.)
- 3. Cha-tu-tip-khan-thaa consistd of (6)

- 3.1 Flower of Phi-kun ( Mimusops elengi Linn. )
- 3.2 Root of Op-choie (*Cinnamomum* sp.)
- 3.3 Root of Ma-kham-khruea ( Abrus precatorius Linn. )
- 3.3 Rhizome of Khing-khrang (*Globba* sp.)

Many Thai formulations composed of Kaysorn-tung-haa and most of the uses are cardiotonic, antipyretic, antidiarrhea or hematonic.

Kaysorn-tung-haa has been used for a long time. Some particular kinds of crude drugs in Kaysorn-tung-haa sometimes very expensive and difficult to obtain and are contaminated with other similar crude drugs.

Very little is known about the complete identification of Kayaorn-tung-haa. This studies has been performed to prove the identities of Kaysorn-tung-haa. This information would served as referent data for the identification of Kaysorn-tung-haa in the future.

#### CHAPTER II LITERATURE REVIEW

Ph	i-kun	

Botanical Name Mimusops elengi Linn . (7)

Synonym

FamilySapotaceaeCommon nameBullet Wood (18)Thai vernacular namesKun (กุน), Kaeo (แก้ว), Saang-dong (ซางดง), Phi-kun paa(พิกูลป่า) (7)

#### Morphology description (9), (10), (11), (12)

A medium - large glabrous evergreen tree 12 - 15 m high, with a compact leafy head and short erect trunk; bark smooth, scaly. Leaves about 6.3 cm long, 3.2-5 cm wide, elliptic, shortly acuminate, glabrous, base acute or rounded; petioles 1.3-2.5 cm long. Flowers white, fragrant, nearly 2.5 cm across, solitary or in fascicles of 2-6; brown ovoid pedicels 6-20 mm long. Calyx 1 cm long, fulvous-pubescent; segments 8, the 4 outers brown, ovate-lanceolate, acute, the 4 inners pale brown narrower than the outer. Corolla longer than the calyx; tube 1.5 mm long, lobes 8 mm long, about 24, in 2 series, the inner series of 8, the outer of 16 lobes; filament short, glabrous; anther glabrous, slightly twisted, acuminate; staminodes 8, alternate with the stamens, lanceolate, acuminate, densely clothed on the back and margins with white hairs. Ovary appresedly silky- pubescent; style grooved, slightly longer than the corolla. Fruits berry about 2.5 cm long; ovoid, yellow when ripe. Seed solitary, ovoid compressed, brown, shining.

#### Distribution

A native of South East Asia (13)

Chemical constituentes (14), (15), (16)

D-manitol,

 $\beta$ -sitosterol,

 $\beta$ -sitosterol-D-glucoside

#### Traditional uses

Used as cardiotonic, antipyretic and used in sore throat and muscular pain.(17),(18)



Bun-naak

Botanical name	<i>Mesua ferrea</i> Linn. (20)
Synonyms	Mesua specicosa Chois. (20)
	Mesua pedunculata Wight. (20)
	Mesua coromandeliana Wight. (20)
Family	Guttiferae (19)
Common names	Indian Rose Chestnut Tree (21)
	Iron Wood (21)
Thai vernacular names	Bun-n <mark>aak (บุนนาค), Kaa - Ko (ก๊าก่</mark> อ), Kam - Ko (ก้ำก่อ), Pa-Naa-
	Kho (ปะนาคอ), Saa-ra-phee-doi (สารภีดอย) (19)

#### Morphological description(20),(21)

A middle sized, glabrous tree; trunk straight, erect; bark smooth, ash-colored; young branches twiggy, slender. Leaves opposite or partly alternate, whether or not acuminate, with very obscure alternate nerves, lower surface glaucous and prucinose, 4.5-8 cm by 1-2.5 cm young red or white; petioles 4-8 mm long. Flowers fragrant, terminal and in highest leaf-axils solitaly complete flowers; sepals 4, contorted, persistent; concave, green 2 inner ones white-margined, largest; petals 4 contorted; obovate, pure white,3 cm long; stamens very numerous, filaments shortly 1-1.25 cm; superior ovary, ovoid, incompletely 2-celled by 2 basal erect septa; ovules 2 in each cell, basal; style long; stigma peltate-funnel shaped; fruit ellipsoid, acuminate, 3.5 cm long; fleshy exocarp, woody endocarp; pedicels 0.75-1 cm long. Seed 1 - 4, angular, smooth, chestnut - brown.

#### Distribution

A native of Indian to the Malay Peninsula (24)

#### Chemical constituents

mesuanic acid (25) mesuaferrone B (26), (27) mesuol (28)

#### Traditional uses

Used as unguents, embrocation (29), snake poison (30),hematonic, and cardiotonic. (30), (31), (32), (33), (34)



Saa-ra-phee	
Botanical name	Mammea siamensis Kosterm. (35)
Synonym	Ochrocarpus siamensis T. Anders (36)
Family	Guttiferae (37)
Common name	Negkassar (35)
Thai vernacular names	Soi-phee ( สร้อยพี่ ), Saa-ra-phee-nan (สารภี แนน) (37)

#### Morphological description (36), (38)

A medium-large glabrous evergreen tree 10-15 m high, with a compact leafy head, bark scaly, red-brown inner bark, with latex change yellow, red-brown wood. Leaves 14–20 cm long, 4 - 6.5 cm wide, ovate, thick, rounded at the apex, base slender acute, entire, reticulated lateral nerves. Flowers pure white, fragrant, solitary or fascicles, ramicolous, 2 white sepal, synsepalous at base, 6 white corollas, concave. Stamens, numerous, yellow anther, about 0.2 cm long. Fruit spindle, about 2.5 cm long, orange when ripe.

#### Distribution

Thailand, Myanmar, Laos, Cambodia and Vietnam (36), (39)

#### Chemical constituents

6-butyryl-5-hydroxy-4-phenylseselin (40)

6-butyryl-5, 7-dihydroxy-8 (3, 3-dimethylallyl)-4-phenylcoumarin (40)

#### Traditional uses

Used as, cardiotonic, hematonic (41), (42) stomachic, and snuff. (43)

**Bua-luang** 

Botanical nameNelumbo nucifera Gaertn. (44)SynonymNelumbo speciosum Willd. (44)FamilyNelumbonaceae (45)Common namesLotus (44),Egyptian Lotus (46)East Indian Lotus (47)Sacred Lotus (47), (48)Thai vernacular nameBua-uang (บ้วหลวง), Bua (บ้ว), Sat-ta-bong-kot (สัตตบุงการ), Sat-ta-<br/>bong-kot-daeng (สัตตบุงการแดง), Sat-ta-bong-ko-khao (สัตตบุงการ

Morphological description (49), (50)

ขาว) (44)

A large aquatic herb with slender, elongate, branched, creeping stem, sending out roots at the nodes. Leaves borne on subterranean, creeping, long-jointed rhizome; orbicular, glaucous above, purinose, erect, exactly peltate, entire, radiately nerved glabrous, partly flat, floating, with weak petioles, partly shallowly cup-shaped on rigid petioles raised above the water, large leaves 35-60 cm, petiole very long, rough with small distant prickles, milky latex. Flowers axillary solitary; far projecting above the water erect or cernuous, fragrant, 15-25 cm; sepals 2, petals pink, white, inserted at base of receptacle; the outer smallest; the intermediate ones largest; stamens very numerous, filaments narrow; rarely entirely white; appendage of connective readily recurved, white or yellow-white, 4-8mm; anthers yellow; receptacle obconical with flat apex, spongy; receptacle during anthesis yellow, later green, at last black-brown 6-10 cm; ovaries 12-30, sunk in apex of receptacle, free; the short styles and widened stigmas protruding only, ovule 1, hanging from apex of cell; fruit nutlets oval-oblong 1.75-2.5 cm long.

#### Bua-luang in thailand:- (51)

There are two kinds of white corolla Bau-laung, they are:-

- Young flower large, oval shape ,slender apex.
   Common name: Hindu Lotus
- Young flower large, plump shape, corolla many.
   Common names: Magnolia lotus, Album Plenum
   Thai vernacular names; Sat-ta-but, Bua-chat-khao, Bua-pom-khao
   This kind is used in Thai traditional medicines. (52)

There are three kinds of rosy color Bua-laung they are:-

- Young flower large, oval shaped, corolla many.
   Common name: East Indian Lotus
   Thai vernacular names: Pa-tum, Pat-ta-ma, Ko-kra-nod
- Young flower large, plump shape, corolla many.
   Common name: Roseum Plenum
   Thai vernacular names: Sat-ta-bong-kot, Bua-lung-pom-daeng, Bua-chatdaeng
- Young flower small, slender or oval shaped.
   Common name: -

Thai vernacular names: Bua-khem-chum-phuu, Bua-pak-king-chom-phuu

#### Distribution

A native of continental Asia (53)

#### Chemical constituents

#### Traditional uses

Used as aphrodisiac, astringent in bleeding piles, anti-inflammatory in mouthsore,(53), antidiarrhea (54) and hematonic (55), (56) Ma-li

Botanical name	Jasminum sambac Ait. (57)
Synonyms	Nyctanthes sambac L. (57)
	Jasminum blancoi Hassk (58)
Family	Oleaceae (57)
Common names	Arabian Jasmine (59)
	Kampopot (60)
	Jasmine (60)
Thai vernacular names	Ma-li-la (มะลิลา), Ma-li (มะลิ), Khao-tak (ข้าวแตก), Ma-li-kae-gai
	(มะลิขี้ไก่), Ma-li-son (มะลิซ้อน) (57)

#### Morphological description (61), (62)

Scandent suberect shrub, branches pubescent. Leaves opposite, membranous, 3-9 cm long , 2-6 cm wide, variable in shape, usually broadly ovate or elliptic, acute, obtuse or acuminate, entire, glabrous or nearly so, base rounded or subcordate, rarely acute; main nerves 4-6 mm long. Flowers white, very fragrant, solitary or usually in 3-flowered terminal cymes; bracts linear up to 6 mm long. Pedicels 6-10 mm long. Calyx 10-13 mm long, hairy; teeth 5 - 9, linear-subulate, 6-10 mm long. Corolla white; double form; tubes narrowly 7-15 mm long, lobes 8 to many, imbricate in bud, oblong-obovate, acute or obtuse, 10-17 mm long and 5 - 8 mm wide. Stamens 2, inserted on corolla tube; yellow, 4 mm long. Stigma oblong, 2 dentate.

#### Distribution

Cultivated throughout India and the tropics of both hemispheres. (61)

#### Chemical constituentes (63), (64)

benzyl 6-O- $\beta$ -D-xylopyranosyl- $\beta$ -D-glucopyranoside( $\beta$ -primeveroside) 2-phenylethyl  $\beta$ -primeveroside 2-phenyl ethyl 6-O- $\alpha$ -L-rhamnopyranosyl- $\beta$ -D-glucopyranoside( $\beta$ -rutinoside) trimeric iridoid glycoside sambbacoside oligomeric iridoid glycosides

molihuaside A-E

#### Traditional uses

Fragrant odored, flowers are added to dried tea to perfume. (65) Used as laxative, antipyretic, lactifuge, antivomiting and antihiccough.(65), (66), (67)



Kra-thingBotanical nameCalophyllum inophyllum Linn. (68)SynonymFamilyGuttiferae (68)Common nameBorneo mahogany (69)Thai vernacular namesKra-thing (กระทิง), Kaa-ka-thing (กากะทิง ), Thing (ทิง ), Nao-wa-<br/>kaan (เนาวกาน), Saa-ra-phee tha-le (สารภีทะเล),<br/>Saa-ra-phee-nane (สารภีแนน) (68)

#### Morphological description(70), (71)

A small or middle-sized glabrous tree, with a crooked trunk; gray bark, smooth Leaves 10-18 cm long, 5-10 cm wide, broadly elliptic, rounded at the apex, often emarginate, with superpand waved margins and very close lateral nerves, giving a striate appearance to the blade, base acute; petioles 9-15 mm long, stout, flat. Flowers 1.9-2.5 cm, racemes 10-15 cm long. Sepals 4, ovate-orbicular, concave, reflexed, ciliolate, the two outer much smaller. Corollas 4, oblong, obtuse, spreading. Stamens numerous, filaments united into 4-6 bundles. Style long, twisted. Stigma large, mushroom-shape. Fruits glabrous, 2.5-3.8 cm, smooth, yellowish; pulp scanty.

#### Distribution

Burma, Andamans, Malay Penisula, Australia (70)

#### Chemical constituent

myricetin-7-glucoside (72)

#### Traditional use

Cardiotonic(73), (74), (75)

#### CHAPTER III

#### MATERIALS AND METHODS

#### Materials

1. Kaysorn-tung-haa, which are flowers of Ma-li, Phi-kun, Bun-naak, Saa-ra-phee and stamens of Bualuang, randomly purchased from 5 traditional drugstroes in Bangkok.

1.1 Thai-hua-chan, Chakkrawad road, Sampanthawong district,Bangkok (purchased date 9/11/98).

1.2 Jae - kan - yoke, Bookkalo road, Bangkok (purchased date

27/11/98).

1.3 Chao-krom-peur, Chakkrawad road, Sampanthawong district, Bangkok (purchased date 9/11/98).

1.4 Vej-ja-pong, Chakkrawad road, Sampanthawong district,

Bangkok(purchased date 9/11/98).

1.5 Tai-un-chan, Chakkrawad road, Sampanthawong district,

Bangkok(purchased date 9/11/98).

2. Saa-ra-phee, Kra-thing, from Chulalongkorn University. (corrected 2/98)

3. Bun-naak from Rayong province.(corrected 6/12/98)

4. Bua-luang (Sat-ta-bong-kot-khao was purchased from Pak-klong-ta-lad (purchased date 10/10/98).

5. TLC Aluminum sheets of precoated silica gel 60  $\mathrm{F_{254}}$  Merck^R 10x10 cm,

0.2 mm thick were employed.

6. Glasswares

erlenmeyer flasks volumetric flasks glass funnels capillary tubes vials etc. 7. Reagents

chloroform, AR

ethyl alcohol, AR

8. Test reagents

Test reagents for microscopy

The following test reagents were prepared according to the methods described by Standard of Asean Herbal Medicine.(see appendix for preparations and uses page 132)

chloral hydrate solution

glycerine water

iodine solution

phloroglucin

hydrochloric acid

Test reagents for TLC

The following test reagents were prepared according to the methods

described by Standard of Asean Herbal Medicine. (see appendix page 132)

vanillin-sulfuric acid

Liebermann-Burchard reagent

Kedde reagent

Dragendorff 's reagent

10. Eguipment for plant identification such as magnifying glass, forceps, razor

blades, needles, etc.

11. Eguipment for photography

12. Electro-freezing microtome

13. Microscope attached with photographic equipment and camera lucida

14. Tank for developing TLC plates

15. UV lamp

16. Ultraviolet spectrophotometer were performed on a Jasco UVIDEC<sub>650</sub> double

beam spectrophotometer.

#### Methods

1. Selection of crude drugs and herbal materials

Five crude drugs in "Kaysorn-tung-haa", Kra-thing, Ma-li-son, and Kaysornthung-haa were purchased from 5 traditional drug stores, following characters were compared:

- morphology of crude drugs
- TLC characters of crude alcoholic extracts
- UV spectra
- 2. Pharmacognostic study

2.1 Morphology of crude drugs.

Flowers were studied as of physical characters including occurrences, color, sizes, shapes, internal appearances as viewed in cross section, odor, and taste. Photographs of crude drugs were taken for the record.

2.2 Histology (76), (77), (78)

Cross or transverse sections of each kind of flowers were prepared on an electro-freezing microtome and the sections were studied under the microscope. Attention should be focused upon the following regions which many show diagnostic characteristics: sepal, corolla, stamens, ovary and pedicel.

Staining test on tissues or cells of the sections using the test reagents for microscopy to study cell contents, cell structure and the compositions of the cells.

Pictures of the sections were taken for the record.

2.3 Powdered drug (79), (80)

The samples of each kind of Kaysorn-tung-haa were ground to

powdered and studied under the microscope to obtain specific characters which can be used to identify the powdered drug. The pictures of powdered drug observed under the microscope were drawn by mean of camera lucida.

3. Chromatographic study

3.1 Preparation of crude alcoholic extract

- Five grams of powder of each kind of Kaysorn-tung-haa were

macerated in15 ml of 95% ethanol for 72 hrs.

- The whole solution was filtered and the filtrate was evaporate until a 5

ml solution was obtianed.

3. 2 One-dimensional TLC to obtain TLC pattern of each kind of flower was carried out using the following conditions:

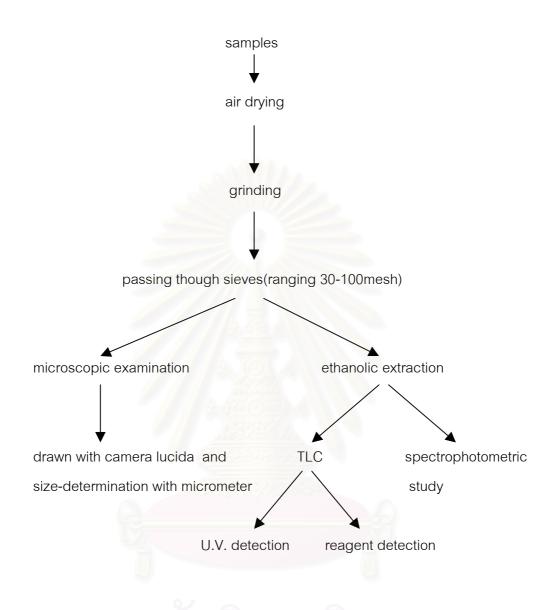
amount	: 20 µl
developing solvent	: chloroform
distance	: 7.5 cm
detection	: 1) UV <sub>254</sub>
	: 2) UV <sub>365</sub>
	: 3) spray with vanillin-sulphuric acid and
	activated by heating at 120°c for 5 min
	: 4) spray with Liebermann-Burchard reagent and
	activated by heating at 100°c for 10 min
	: 5) Kedde reagent

: 6) Dragendorff's reagent

4. Spectrophotometric study

4.1 The crude alcoholic extract of each kind of flower diluted(1:100) with 95% ethanol.

4.2 Ultraviolet spectra of five samples of each kind of flower were taken by using quartz cell with path length 1cm, 95% ethanol was used a reference, scan under185-300 nm.



#### CHAPTER IV RESULTS AND DATA

#### Crude drug randomization

1. Kaysorn-tung-haa were randomly purchased from 5 traditional drug stores.

2. Each ingredient in Kaysorn-tung-haa were randomly purchased from 5 traditional drugstores. Comparisons of the morphology (Fig.1-5) histology (Fig. 0 of each crude drug from various sources revealed that the 5 samples are all difference in characters.

3. Information obtained from the above studies can be used to identify 5 kinds of Kaysorn-tung-haa; Phi-kun, Bun--naak, Saa-ra-phee, Bua-luang and Ma-li.





Fig. 1 Crude drugs of Kaysorn-tung has from Thai-hua-chan

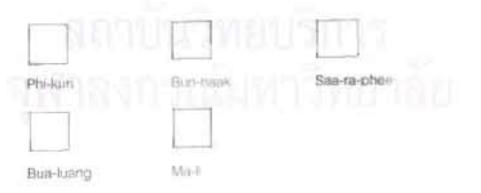


Diagram of photograph

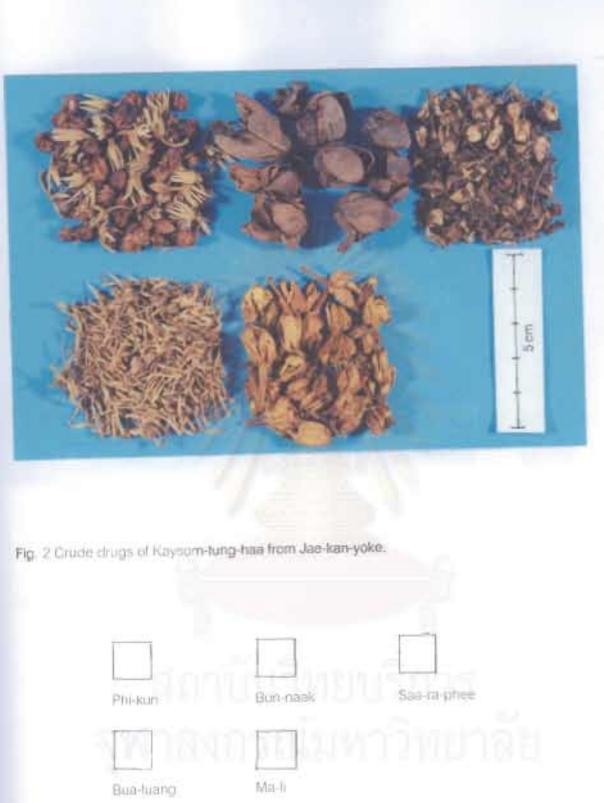


Diagram of photograph.



Fig. 3 Crude drugs of Keysorii-tung-haa from Chao-krom-peur



Diagram of photograph

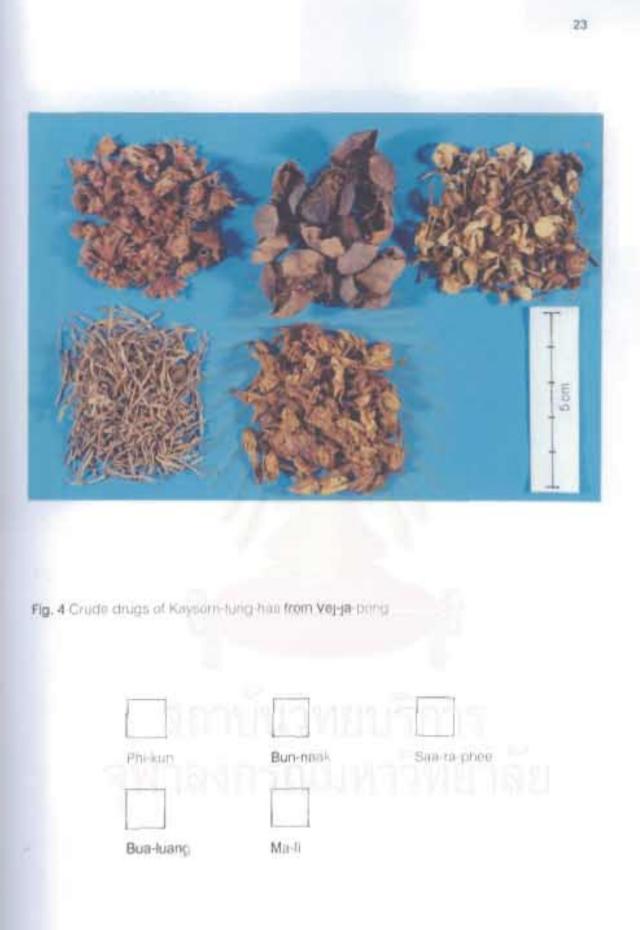


Diagram of photograph



Fig. 5 Crude drugs of Kaysom-tung-heal from Taeon chan

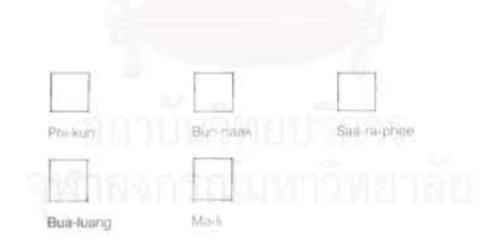


Diagram of photograph:

#### Plant Identification

Identification of plant were carried on the characters of stem, leaves, flowers and fruits of individual ingredient of Kaysorn-thung-haa and orther substitued plants in the "LITERATURE REVIEW" chapter under the "Morphological Description" section.

Each ingredient of Kaysorn-thung-haa can be identified as follows:-

1.	Phi-kun	Mimusops elengi Linn.	Sapotaceae
2.	Bun-naak	Mesua ferrea Linn.	Guttiferae
3.	Saa-ra-phee	Mammea siamensis Kosterm.	Guttiferae
4.	Bua-luang	Nelumbo nucifera Gaertn.	Nelumbonaceae
5.	Ma-li	Jasminum sambac Ait.	Oleaceae

Other substitute plants used for comparisons with Kaysorn-thung-haa can be identified as follow:-

- 1. Kra-thing Calophyllum inophyllum Linn. Guttiferae
- 2. Ma-li-son Jasminum sambac Ait.Oleaceae

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Fig. 6 The Mimusopa elengi Linn.

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Fig. 7 Crude drug appearance of Mimusops elengi Linn

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#### Description of crude drug

Crude drug occur as light brown to reddish brown dried flowers, with a characteristic, aromatic odor, fragrant, composed of circles of 16 outer corolla lobes, 8 inner corolla lobes, fuse basal corolla, each corolla lanceolate, acute, 6-8 mm long, short filaments, glabrous, anther glabrous, acuminate, 8 staminodes, alternate with stamens, lanceolate, acuminate, densely clothed on the back and margins with white hairs, without calyx, ovary and pedicel.

#### Microscopical characters

Sectional and surface view of the corolla exhibit the following microscopic characters.

Upper epidermis composed of a layer of thick-walled polygonal cells with strongly striated cuticle in surface view and in the sectional view showed the rectangular cells covered by thick cuticle.

Mesophyll composed of several layers of thicked-wall circular cells,some containing yellowish pigment. Vascular bundles are located at the central part of this area.

Lower epidermis composed of a layer of cells, similar to those of the upper epidermis but they are more longer in surface view.

Transverse section of the staminode exhibit the following microscopical characters.

Upper epidermis in the sectional view showed the rectangular cells covered by thick cuticle.

Ground tissue composed of several layers of thin-wall circular cells. Vascular bundles are located at the central part of this area.

Lower epidermis composed of a layer of cells , similar to those of the upper epidermis and unequal two-branched unicellular trichome 38-40  $\mu$ m long.

Transverse section of the anther exhibit the following microscipal characters.

The anther composed of four-pollen sacs. The pollen sac wall composed of epidermis or exothecium; that composed of layer of thin-wall rectangular cells.

Endothecium composed of a layer thickening stripe-like fiber located beneath the epidermis.

Tapetum composed of a layer of thin-wall rectangular cells.

Ground tissue composed of several layers of thin-wall polygonal cells. Vascular bundle located at the central part of this area.

#### Powdered drug characters:

The powdered drug is light brown to reddish brown with characteristic aromatic odor, tasteless.

The microscopical characters are:

a. Abundant fragments of the petal in surface view. The upper and lower epidermis is composed of polygonal and elongated cells which rather sinuous in outline; the cuticle is faintly striated. The underlying tissue consists of spiral vessels. The lower epidermis is similar to the upper epidermis except that more elongated cells.

b. The fragments of the exothecium layer of anther composed of rather cuboidal cells, thick-walled and one thin-walled attached to endothecium layer. The endothecium composed of fibrous layer ; rather circular in section view, stripe-like in surface view.

c. Abundant pollen grains, spheriodal or elliptical, with tetra- colpated apertures; the exine smooth and faint granulations , 38-40  $\mu\text{m}$  in diameter.

d. Abundant covering trichomes; two type. Those which occur on the back of basal region stamens; they were unicellular, two branches; short and very long, a single large elongated cell, tapering at both end. The covering trichomes occur on the staminodes, each trichomes has unicellular; forming a T–shaped structure , the walls of this elongated cell, were moderately thick–walled and smooth, tapering at both end.

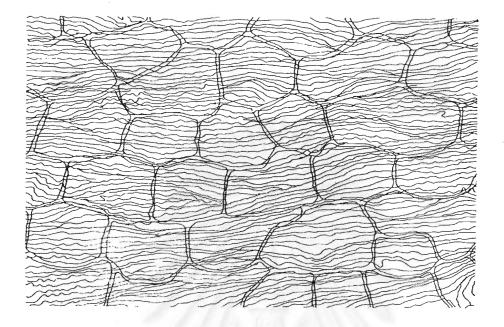


Fig. 8 Upper epidermis of corolla in surface view of *Mimusops elengi* Linn.

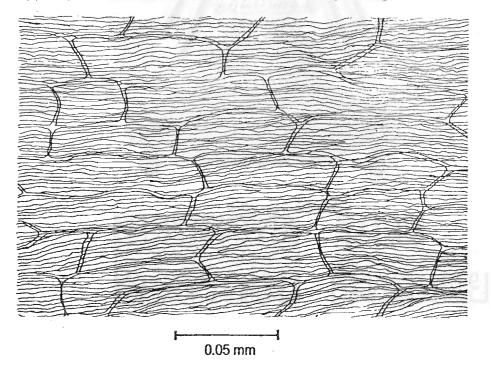


Fig. 9 Lower epidermis of corolla in surface view of Mimusops elengi Linn.

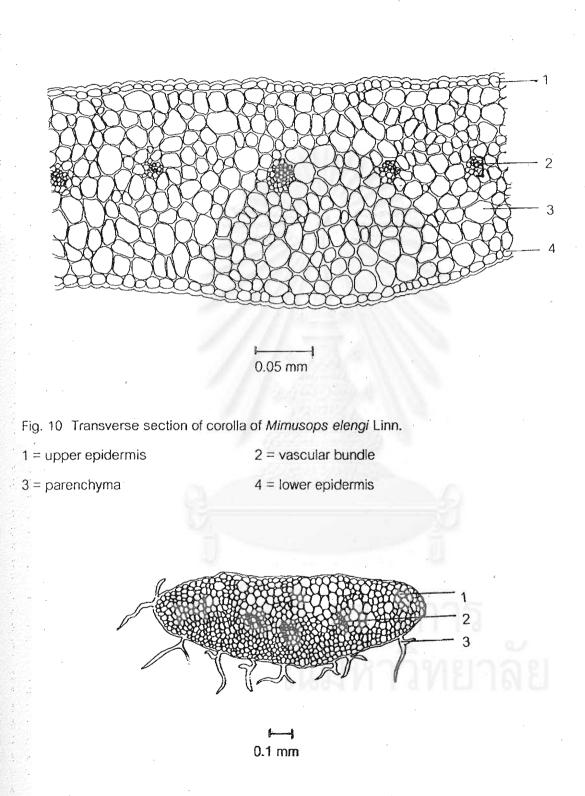


Fig.11 Transverse section of staminode of Mimusops elengi Linn.

1 = groundtissue parenchyma

2 = vascular bundle

3 = trichome

31

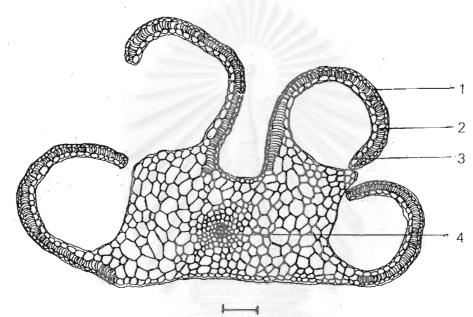




Fig. 12 Transverse section of anther of Mimusops elengi Linn.

1 = exothecium

3 = tapetum

2 = endothecium

4 = vascular bundle

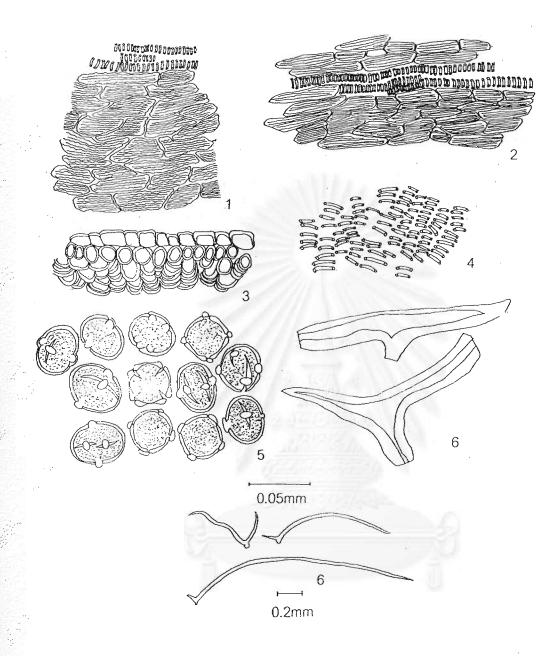


Fig. 13 Microscopical character of powdered drug of Mimusops elengi Linn.

- 1 = upper epidermis of corolla in surface view
- 2 = lower epidermis of corolla in surface view
- 3 = exothecium and endothecium in section view
- 4 = endothecium in surface view
- 5 = pollen grains
- 6 = trichome

Chromatographic characteristics

One dimensional TLC

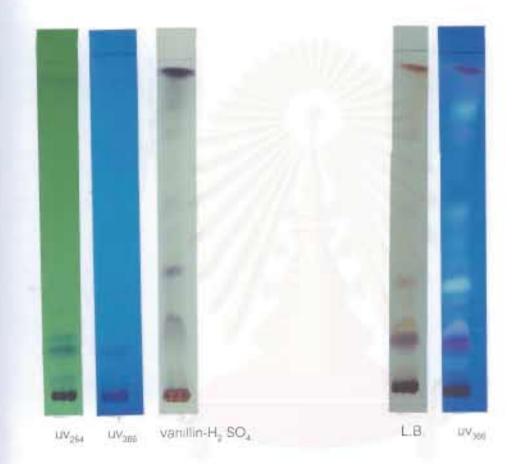


Fig. 14 One-dimensional Thin-layer chromatography patterns of the extract of Mimusops elengi Linn.

Ultraviolet spectroscopic character

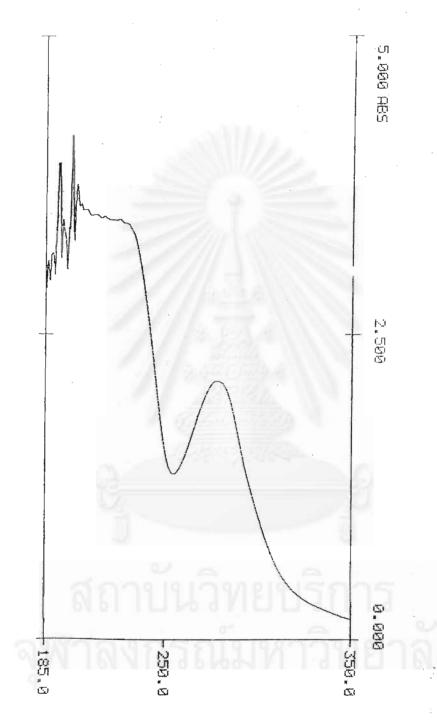


Fig. 15 Ultraviolet spectrum of the extract of Mimusops elengi Linn.

peak	wavelength(nm)	absorption
1	279.2	2.118
2	225.2	3.467
3	216.4	3.492
4	212.8	3.507
5	211.2	3.552
6	206.4	3.552
7	204.0	3.597
8	201.6	3.760
9	198.8	4.169
10	197.6	3.398
11	194.4	3.462
12	192.8	3.937
13	192.0	3.941
14	189.2	3.187
15	186.8	3.117

Table. 1 Ultraviolet absorption of the extract of Mimusops elengi Linn.

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Fig. 16 Crude drug appearance of Mesua ferrea Linn.

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#### Description of crude drug

Crude drug appears as dark brown dried flowers, composed of 2 outer sepals, about 1.5 cm long, about 1.4 cm wide, concave, 2 inner sepals, larger than outer sepals, about 2.2 cm long, about 1.4 cm wide, they are concave and persistent, 4 corollas concave in the closely flower; shattered in the open flower, brown filament, about 0.6 cm long, light brown stamen, about 0.15 cm long, superior ovary, about 1 cm in diameter, 1-2 cm long, peltate-funnel shaped stigma, pedicel 0.5-0.8 cm long.

#### Microscopical characters

Sectional and surface view of the sepal exhibit the following microscopical characters.

Upper epidermis composed of a layer of thick-walled polygonal cells in surface view and in the section showed the rectangular cells covered by thick cuticle and 1 or 2 cellulars trichome, 30-1000  $\mu$ m long.

Mesophyll composed of several layer of thicked-wall circular cells, some containing rosette aggregate of calcium of oxalate, 10-20  $\mu$ m in diameter. Scattered oil glands and vascular bundles located at the central part of this area.

Lower epidermis composed of a layer of cells, similar to those of the upper eopidermis but with 15-40  $\mu$ m long unicellular trichomes.

Sectional and surface view of the corolla exhibit the following microscopical characters.

Upper epidermis composed of a layer of thick-walled polygonal cells.

Mesophyll composed of several layer of thin wall circular cells. Scattered oil grands and vascular bundles are located at the central part of this area.

Lower epidermis composed of a layer of cells, similar to those of upper epidermis but some containing brownish pigment and they are more larger in surface view and in the sectional view showed a layer of cylindical cells of hypodermis layer covered by lower epidermis layer.

Transverse section of the anther exhibit the following microspical characters.

The anther composed of four-pollen sacs. The pollen sac wall composed of epidermis or exothecium; that composed of layer of thick-wall rectangular cells covered with thick cuticle, some containing brownish pigment.

Endothecium composed of a layer of thickening pited-like circular cells

Ground tissue composed of several layer of thin-wall polygonal cells. Vascular bundle are located at the central part of this area.

Transverse section of the pedicel exhibit the following microscopical characters. The epidermis showed the rectangular cells covered by thick cuticle with 1 or 2 cellular trichomes 40-80 µm long, underlying 2 or 3 sclereid layers.

Ground tissue composed of several layers of thicked-wall, scattered oil glands, fibers around the vascular bundles are located at the central part of this area.

Transverse section of the ovary exhibit the following microscopical characters. The epidermis in sectional view shows the rectangular cells covered by thick cuticle. Ground tissue composed of several layers of thicked-wall circular cells. Scattered oil glands and vascular bundles are located at this area. Two chambers of locule at the central.

#### Powdered drug characters

The powdered drug is brown to dark brown, mild odor and slightly sour. The microscopical character of powdered are :

a. The fragments of the sepals in surface view. The cells of the lower epidermis composed of polygonal cells and smooth in outline associated with unicellular trichomes; slender and few incurved, round or tapering at end. Fragments of covering trichomes.

b. Fragments of the corollas composed of elongated rectangular cells, some

39

fragments associated with spiral vessels.

c. Fragments of the exothecium layer of anther composed of rather cuboidal cells; some containing brownish substance. The exothecium layer attached to endothecium layer. Endothecium layer composed of fibrous layer; elongated cells, pitted- like fiber; that attached to tapetum layer; Fragments of fibrous layer are also abundant, composed of rather elliptical cells which in surface view show characteristic, pitted like, thickening and beading of the walls.

d. Pollen grains, scattered, spheroidal or rather triangular outline and smooth exine, with tri-colpated apertures, 39-50  $\mu$ m in diameter.

e. Fragments of spiral and reticulated vessels scattered.



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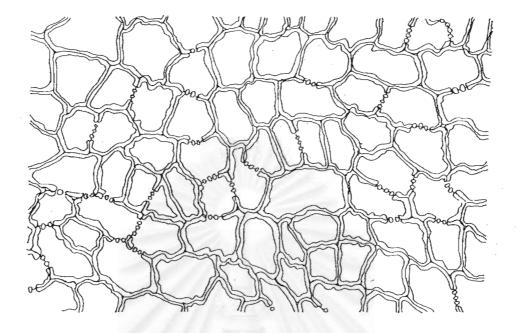
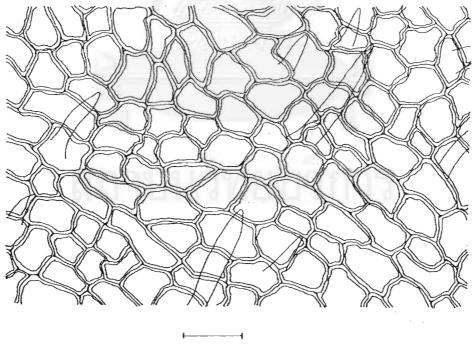
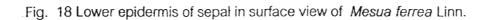


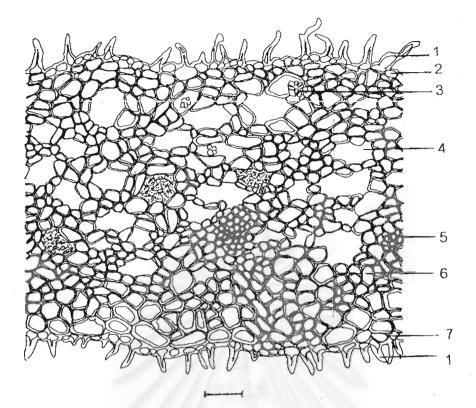
Fig. 17 Upper epidermis of sepal in surface view of Mesua ferrea Linn



0.05mm



41



0.05 mm

Fig. 19 Transverse section of sepal of Mesua ferrea Linn.

1 = trichome

- 2 = upper epidermis
- 3 = rosette aggregate
- 4 = oil gland
- 5 = vascular bundle
- 6 = parenchyma
- 7.= lower epidermis

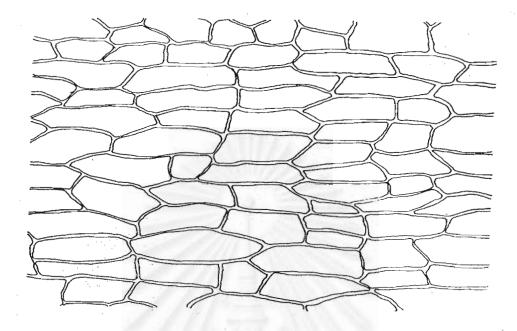


Fig. 20 Upper epidermis of corolla in surface view of Mesua ferrea Linn.

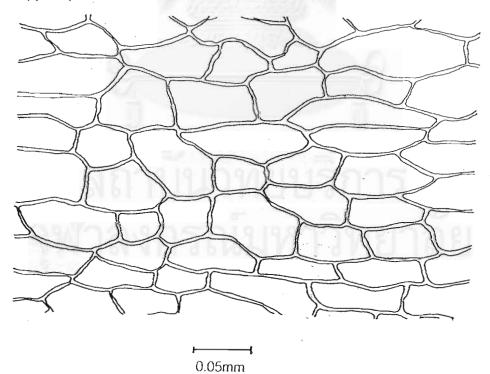
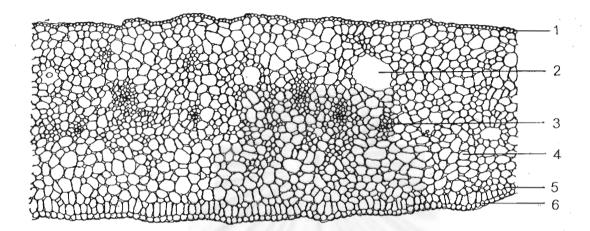


Fig. 21 Lower epidermis of corolla in surface view of Mesua ferrea Linn.

43 .



0.1mm

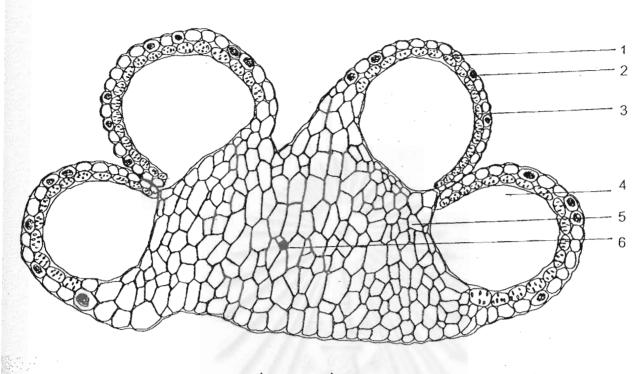
Fig. 22 Transverse section of corolla of Mesua ferrea Linn.

1 = upper epidermis

2 = oil gland

- 3 = vascular bundle
- 4 = parenchyma
- 5 = hypodermis
- 6 = lower epidermis

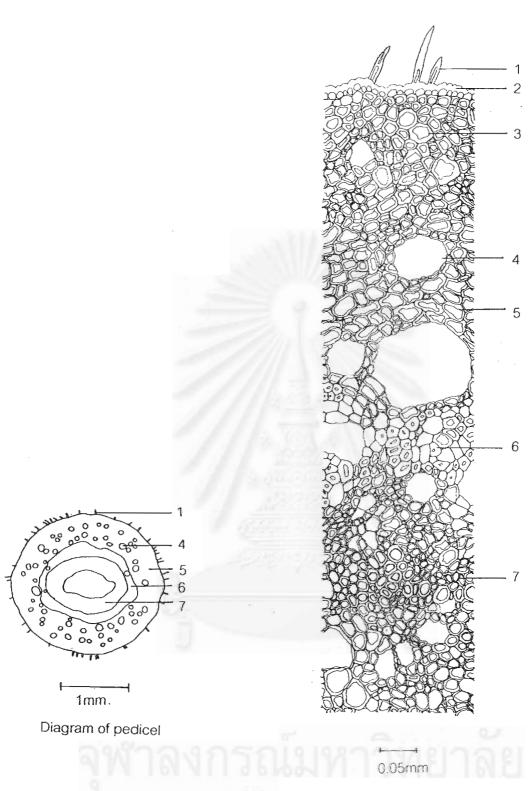
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0.1mm

- Fig. 23 Transverse section of anther of Mesua ferrea Linn.
- 1 = exothecium
- 2 = brownish substance
- 3 = endothecium
- 4 = parenchyma
- 5 = vascular bundle

# จุฬาลงกรณ์มหาวิทยาลัย



2 = cuticle

4 = oil gland

6 = fiber

Fig. 24 Transverse section of pedicel of Mesua ferrea Linn.

1 = trichome

3 = scleried

5 = parenchyma

7 = xylem

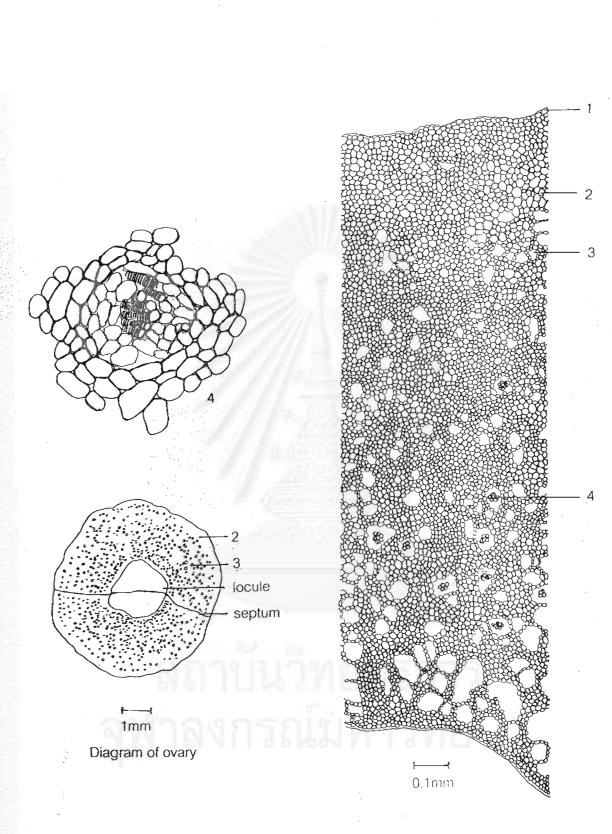


Fig. 25 Transverse section of ovary Mesua ferrea Linn.

1 = epidermis

2 = parenchyma

4 = vascular bundle

3 =oil gland

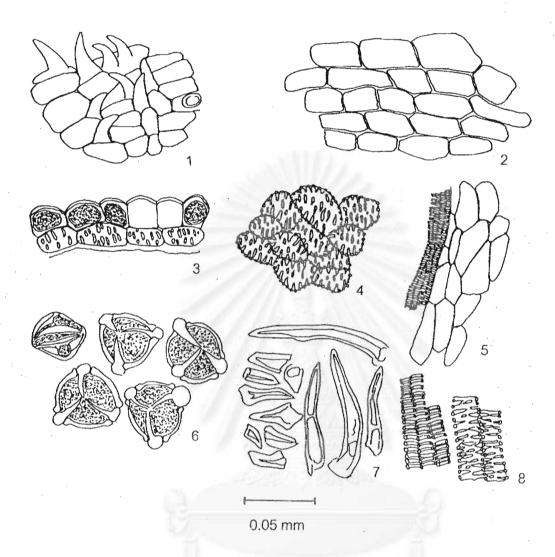


Fig. 26 Microscopical character of powdered drug of Mesua ferrea Linn.

- 1 = outer epidermis of sepal
- 2 = parenchyma of corolla
- 3 = exothecium associated with endothecium
- 4 = surface view of endothecium
- 5 = fragment of parenchyma associated with spiral vessel
- 6 = pollen grain
- 7 = trichomes
- 8 = spiral and reticulated vessel

Chormatographic characteristics

One-dimensional TLC

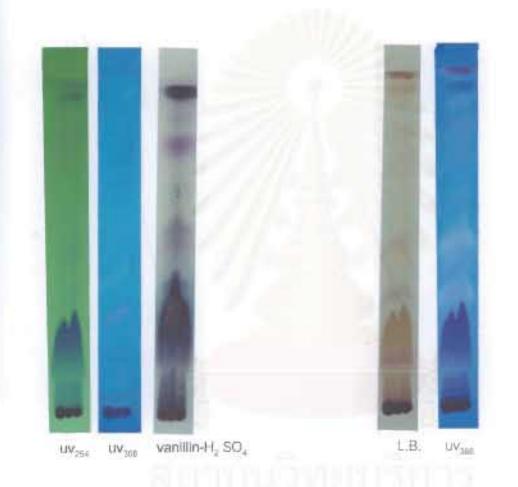


Fig. 27 One-dimensional Thin-layer chromatography patterns of the extract of Mesua ferrea Linn.

### Ultraviolet spectroscopic characters

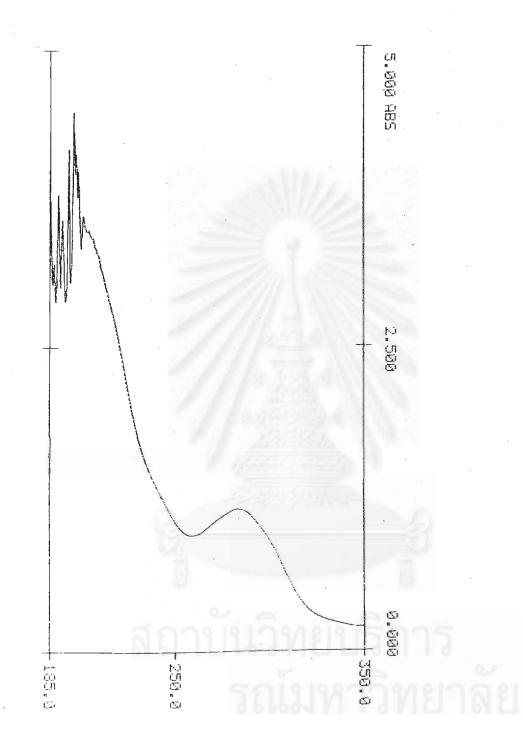


Fig. 28 Ultraviolet spectrum of the extract of Mesua ferrea Linn.

peak	wavelength(nm)	absorption
1	284.4	1.165
2	205.2	3.482
3	202.0	3.604
4	199.6	3.984
5	198.4	4.127
6	197.2	4.476
7	194.8	4.168
8	191.6	3.566
9	199.6	3.785
10	187.6	3.199



#### Saa-ra-phee



Fig. 29 Crude drug appearance of Mammea siamensis Kosterm

สุภาพันวิทยบริการ จุฬาสงกรณ์มีทำวิทยาลัย

#### Description of crude drug

Crude drug occur as light brown flowers composed of 2 sepals, 0.5-0.7 cm long, 0.3-0.5 cm wide, concave, 6 colloras, 0.5-0.8 cm long, easy to fall, numerous stamens, about 0.5 cm long, anther, 0.2cm long, pedicel 0.6-1.5 cm long.

#### Microscopical characters

Sectional and surface view of the sepal exhibit the following microspical characters.

Upper epidermis composed of a layers of thick-walled polygonal cells in surface view and in the sectional view show the rectangular cells covered by thick cuticle.

Mesophyll composed of several layers of rather thicked-walled circular cells some containing cluster crystal of calcium oxalate,  $10-20\mu$ m in diameter. Vascular bundles and oil glands are located at the central part of this area.

Lower epidermis composed of a layer of cells similar to those of upper epidermis together with paracytic stomata.

Sectional and surface view of the corolla exhibit the following microscopical characters

Upper epidermis composed of a layer of thick-walled polygonal cells, underlying spiral vessels in surface view, and in the sectional view show the rectangular cells covered by the thick cuticle.

Mesophyll composed of several layers of thin wall circular cells. Vascular bundles and oil glands are located at the central part of this area.

Lower epidermis composed of a layer of similar to those of upper epidermis but showed rather thick wall, slightly sinuate polygonal cells .

Transverse section of the anther exhibit the following microscopical characters.

The anther composed of four-pollen sacs. The pollen sac wall exhibited following microscopical characters:-

Epidermis or exothecium composed of a layer of thin-walled rectangular cells, some containing brownish pigment.

Endothecium composed of a layer thickening circular cells.

Ground tissue composed of several thin-walled polygonal cells. Vascular bundle are located at the central part of this area.

Transverse section of the pedicel exhibit the following microscopical characters.

Epidermis composed of a layer of thin-walled rectangular cells covered by thin cuticle.

Ground tissue composed of several layers of thin-wall circular cells. Vascular bundle are located at the central part of this area. Several layers at the central part of vascular bundle some containing cluster crystal of calcium oxalate 10-20  $\mu$ m in diameter.

#### Powdered drug characters

The powdered drug is brown, mild odor and astringent.

The microscopical characters of powdered drug are:

a. Abundant epidermis of the sepals in surface view. The lower epidermis is composed of thin-walled rather elongated cells, paracytic stomata.

b. Abundant epidermis of corollas in surface view. The lower epidermis composed of thick–walled, slightly sinuate and polygonal cells.

c. Fragments of anther, exothecium composed rather round, thick-walled; attatched to endothecium layer. The endothecium layer composed of fibrous layer; rather cylindrical in section view and stripe-like in surface view. The endothecium layer associated with ground tissue; small and large polygonal cells.

d. Pollen grains, scattered, spheroidal or rather triangular out line, 35-45  $\mu\text{m}$  in diameter and smooth exine, with tri-colpated apertures with fainted granulations.

e. Epidermis of pedicel composed of polygonal longitudinally cell, paracytic stomata.

f. Scattered cluster crystals of calcium oxalate.

g. Fragments of spiral vessels.



## สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

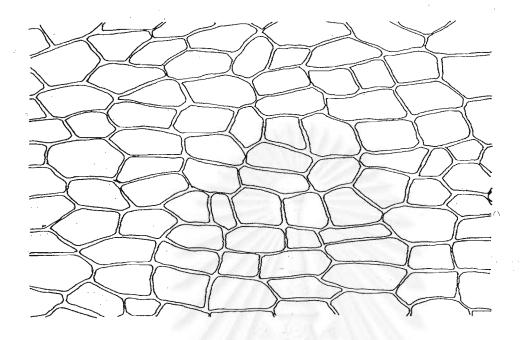
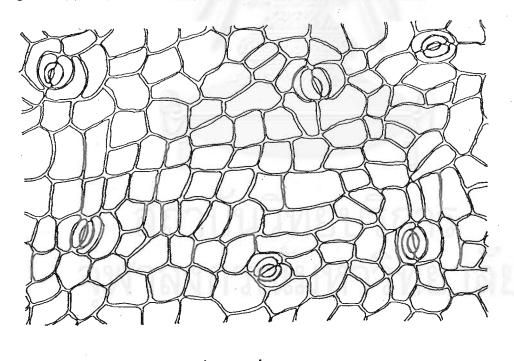
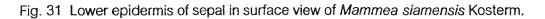


Fig. 30 Upper epidermis of sepal in surface view of Mammea siamensis Kosterm.



0.05mm



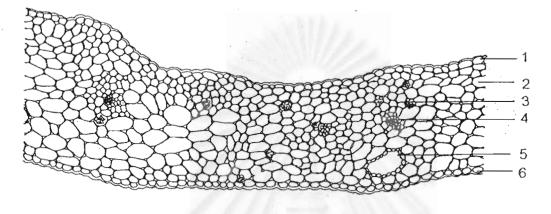




Fig. 32 Transverse section of sepal of Mammea siamensis Kosterm.

- 1 = upper epidermis
- 2 = parenchyma
- 3 = cluster crystal of calcium oxalate
- 4 = vascular bundle
- 5 = oil gland
- 6 =lower epidermis

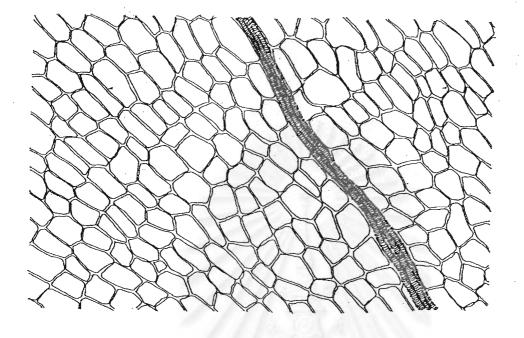
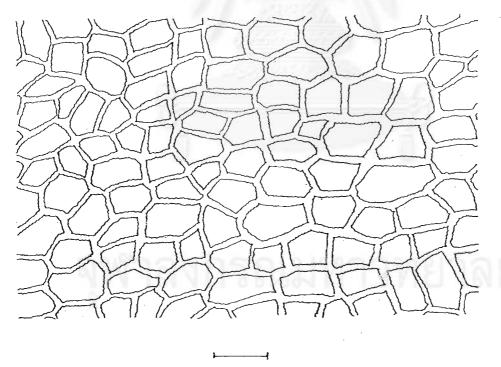


Fig. 33 Upper epidermis of corolla in surface view of Mammea siamensis Kosterm.



0.05mm



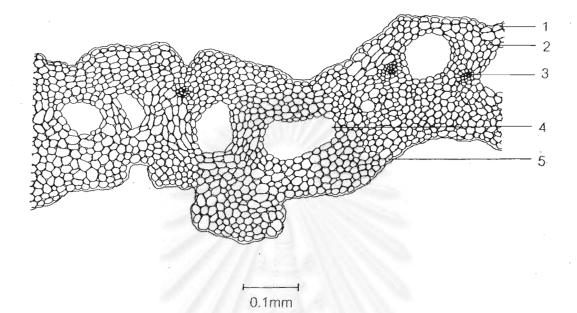
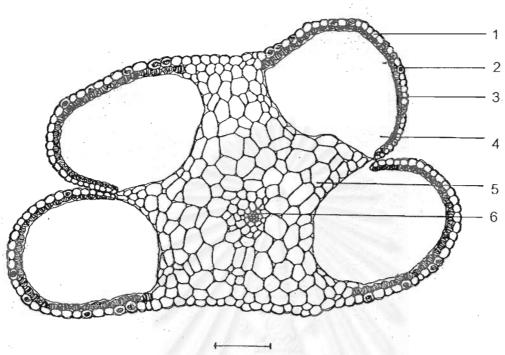


Fig. 35 Transverse section of corolla of Mammea siamensis Kosterm.

- 1 = upper epidermis
- 2 = parenchyma
- 3 = vascular bundle
- 4 = oil gland
- 5 = lower epidermis

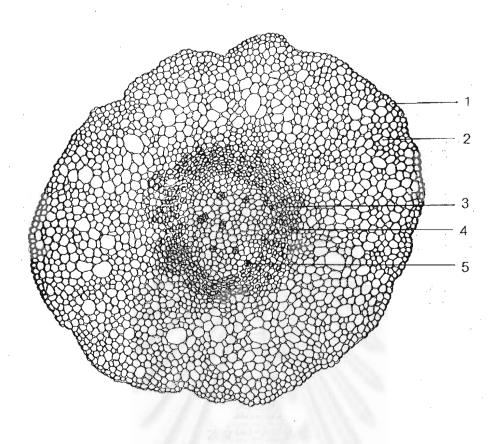
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0.1mm

Fig. 36 Transverse section of anther of Mammea siamensis Kosterm.

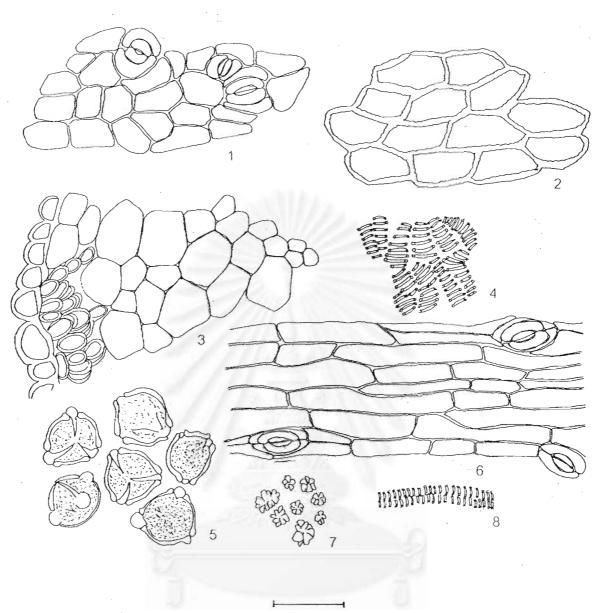
- 1 = exothecium
- 2 = brownish substance
- 3 = endothecium
- 4 = parenchyma
- 6 = vascular bundle



→ → 0.1 mm

Fig. 37 Transverse section of pedicel of Mammea siamensis Kostem.

- 1 = epidermis
- 2 = parenchyma
- 3 = phloem
- 4 = xylem
- 5 = clustal crystal of calcium oxalate



62

0.05mm

Fig. 38 Microscopical character of powdered drug of Mammea siamensis Kosterm.

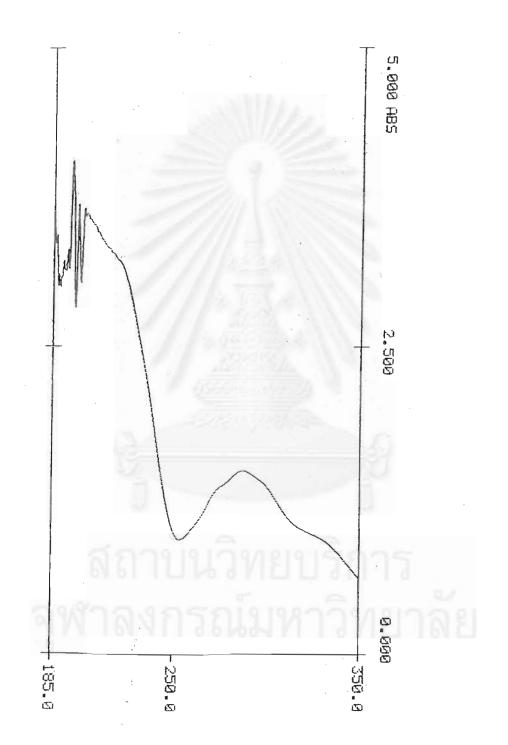
- 1 = fragment of lower epidermis of sepal in surface view
- 2 = fragment of lower epidermis of corolla
- 3 = fragment of anther
- 4= fragment of endothecium
- 5 = pollen grains
- 6 = epidermis of pedicel in surface view
- 7 = clustal crystal of calcium oxalate
- 8 = spiral vessel

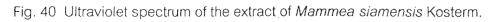
Chromatographic characteristics

One-dimensional TLC



Fig. 39 One-dimensional Thin-layer chromatography patterns of the extract of Mammea siamensis Kosterm.





peak	wavelength(nm)	absorption
1	288.4	1.489
2	209.2	3.465
3	204.8	3.567
4	203.2	3.616
5	201.6	3.662
6	198.4	3.688
7	195.2	4.056
8	193.2	3.311
9	190.8	3.216
10	188.4	3.102
11	187.2	3.434

Table 3 Ultraviolet absorption of extract of Mammea siamensis Kosterm.



สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย **Bua-luang** 



Fig. 41 Nelumbo nucifera Gaertn.



Fig. 42 Crude drug appearance of Neiumbo nuclfera Gaeitn.

สถาบันวิทยบริการ พัฒนาสถายกับทาลิท Crude drug appears as light yellow or brown dried stamen composed of filament, 0.4 cm long, anther 0.5 -1.4 cm long and appendage, few curved, 0.3 - 0.4 cm long.

#### Microscopical characters

Transverse section of the appendage exhibits the following microscopical characters.

Epidermis composed of a papilla layer covered by thick cuticle.

Ground tissue composed of several layers of thin-wall circular cells,some containing brownish pigment.

Transverse section of the anther exhibits the following microscopical characters.

The anther composed of four-pollen sacs. The pollen sac wall composed of

epidermis or exothecium; that composed of papilla layer covered by thick cuticle.

Endothecium composed of a layer of thickening pitted-like fiber.

Tapetum composed of 2 or 3 layers of thin-wall cylindrical cells underlying yellowish layer.

Ground tissue composed of several thin-walled cylindrical cells. Vascular bundle located at the central part of this area.

Transverse sectional of the filament exhibits the following microscopical characters.

Epidermis composed of papilla layer covered by thick cuticle.

Ground tissue composed of several layers of thin-wall circular cells. Vascular bundles and air space scattered are located at this area.

#### Powdered drug characters:

The powdered drug is light brown or yellow, mild cooled odor.

The microscopical characters are:

a. Abundant fragments of papilla layer, anther and appendage of stamens.

b. Abundant fragment of fibrous layers; which are two types, one is longitudinal cells tapering at both ends and beading on the surface of the walls. The other is similar in surface, but thicker than the first type.

c. Abundant pollen grains, spheroidal, striated exine, with tri-colpate apertures awith fainted granulations, 61-78  $\mu$ m in diameter.

d. Fragment of spiral and annular vessels.

e. Brownish substances are scattered, in parenchymatous cells of appendage.

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

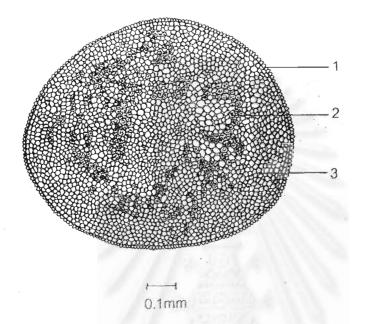


Fig. 43 Transverse section of appendage of Nelumbo nucifera Gaertn.

- 1 = papillae of epidermis
- 2 = brownish substance
- 3 = parenchyma

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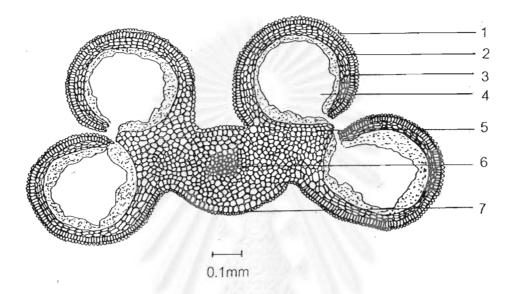
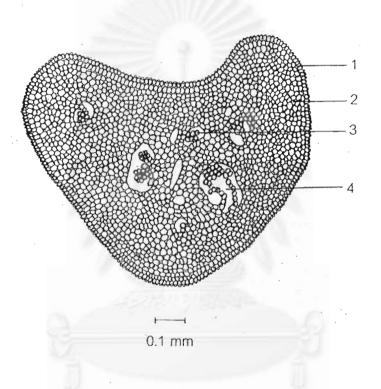


Fig. 44 Transverse section of anther of Nelumbo nucifera Gaertn.

- 1 = exothecium
- 2 = endothecium
- 3 = tapetum
- 4 = pollen sac
- 5 = parenchyma
- 6 = vascular bundle
- 7 = papillae epidermis

ถาบันวิทยบริการ ลงกรณ์มหาวิทยาลัย



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Fig. 45 Transverse section of filament of Nelumbo nucifera Gaertn.

- 1 = papillae epidermis
- 2 = parenchyma
- 3 = vascular bundle
- 4 = interceliular space

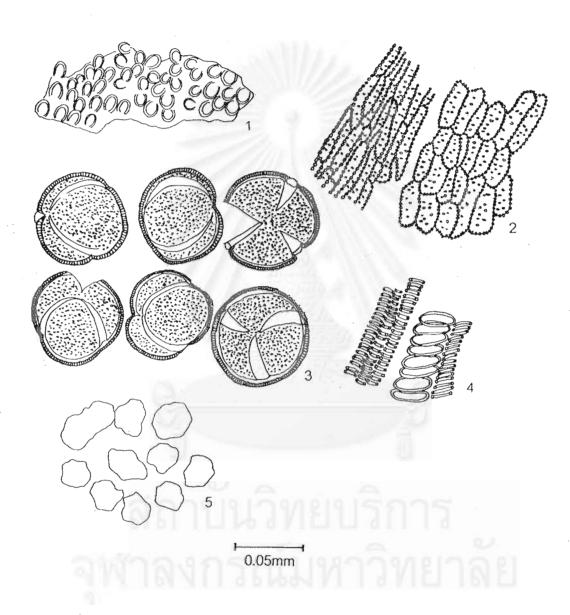
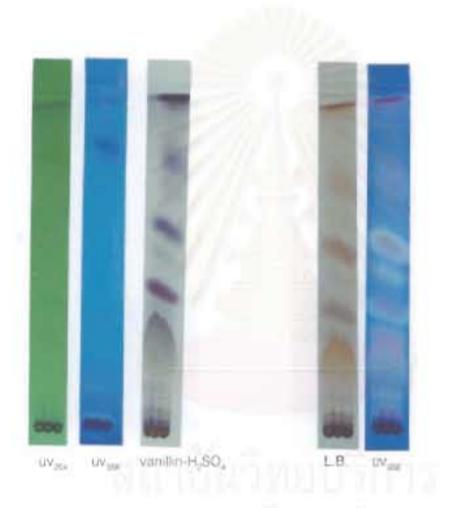


Fig. 46 Microscopical character of powdered drug of Nelumbo nucifera Gaertn.

- 1 = surface view of papillae layer
- 2 = pited- like fiber in surface view of endothecium
- 3 = pollen grains
- 4 = spiral and anular vessel
- 5 = brownish-substance

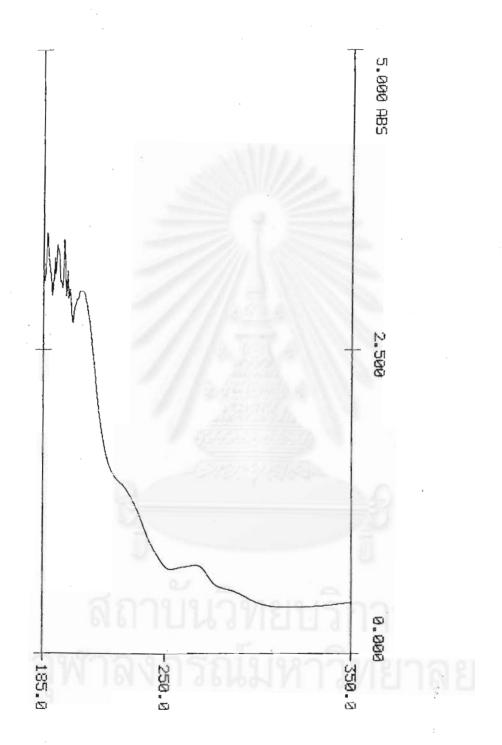
Chromatographic characteristics

One-dimensional TLC





#### Ultraviolet spectroscopic characters



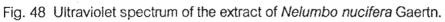


Table. 4 Ultraviolet of extract of Nelumbo nucifera Gaertn.

peak	wavelength(nm)	absorption
1	267.6	0.726
2	204.4	2.991
3	199.2	3.004
4	198.0	3.158
5	196.0	3.410
6	194.8	3.070
7	192.4	3.371
8	191.2	3.263
9	187.2	3.468







Fig. 49 Jasminum sambac Ait.

## สถาบันวิทยบริการ จฬาลงกรณ์มหาวิทยาลัย



Fig. 50 Crude drug appearance of Jasminum sambac Art

## สถาบันวิทยบริเการ ขัดเป็นที่การการการค

#### Description of crude drug

Crude drug appears as light brown dried flowers, mild odor, composed of 7-10 corolla lobes, oblong-obivate, acute or obtuse, 0.9-1.7 cm long, stamen inserted on corolla tube, about 0.3 cm long and 2 anthers

#### Microscopical characters

Transverse section and surface view of the corolla lobe and corolla tube exhibit the following microscopical characters.

Upper epidermis composed of a layer of thick-walled polygonal cells with strongly striated cuticle in surface view, and in the sectional view shows the rectangular cells covered by thick cuticle.

Mesophyll composed of several layers of thin wall circular cells in corolla lobe and thicked-wall circular cells in corolla tube some containing small starch grains. Vascular bundles are located at the central part of this area.

Lower epidermis composed of layer of cells, similar to those of the upper epidermis but some containing greenish-yellow pigment and not striated cuticle in surface view.

Transverse section of the anther exhibit the following microscopical characters.

The anther composed of four-pollen sacs. The pollen sac wall composed of epidermis or exothecium; that composed of layer of thick-walled rectangular cells covered by thick cuticle, some containing greenish-yellow pigment.

Endothecium composed of a layer thickening stripe-like fiber. Tapetum composed of 2 or 3 layers of thin-wall circular cells.

Ground tissue composed of several thin-walled circular cells containing small starch grains. Vascular bundles are located at the central part of this area.

#### Powdered drug characters

The powdered drug is light brown or yellowish, mild odor and bitter.

The microscopical characters of powdered drug are:

a. The very abundant fragments of corollas in surface view. The upper epidermis is composed of parenchymatous cells ; the cuticle is faintly striated curvature. The lower epidermis is composed of parenchymatous cells containing greenish–yellow substance.

b. Fragments of anthers in which the exothecium composed of rather ellipticalcells, moderately rough and thickened walls; containing green-yellow substance. The exothecium attached to endothecium layer. The endothecium layer composed of rather elliptical of fibrous layers in section views. The fragments of exothecium layer composed of thin-walled cells, rather elliptical cell; round at both end, few faintly striated and containing greenish-yellow substance in surface views.

c. Pollen grains, few, spheroidal and elliptical pollen grains, tri or tetra-colpa apertures, tapering end of striate exine with granulations, 40-50  $\mu$ m in diameter.

c. Small starch grains, hilum at the center of starch grains.

e. Scattered of spiral and annular vessels.

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

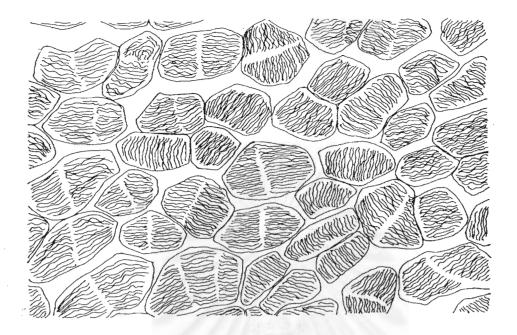
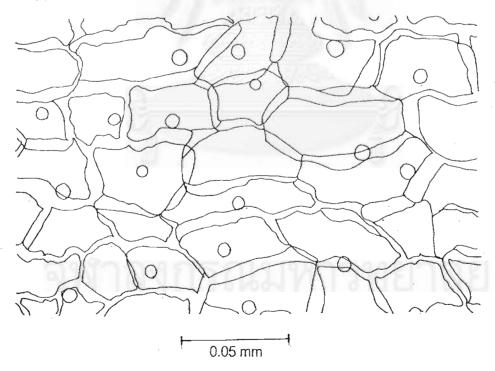
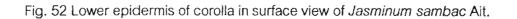


Fig. 51 Upper epidermis of corolla in surface view of Jasminum sambac Ait.





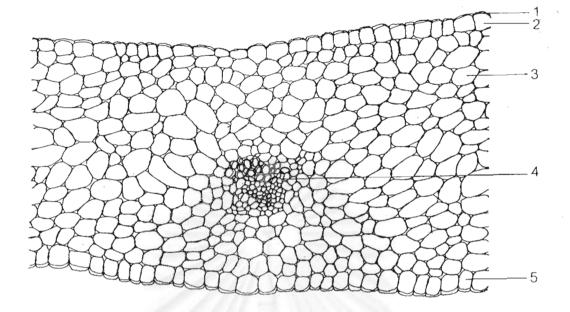


Fig. 53 Transverse section of corolla lobe of Jasminum sambac Ait.

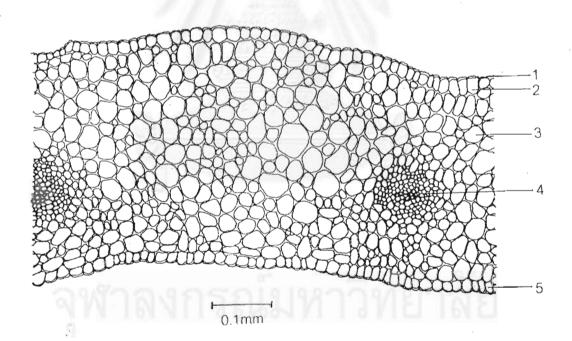


Fig. 54 Transverse section of corolla tube of Jasminum sambac Ait.

- 1 = cuticle 2 = upper epidermis
- 3 = parenchyma 4 = vascular bundle
- 5 =lower epidermis

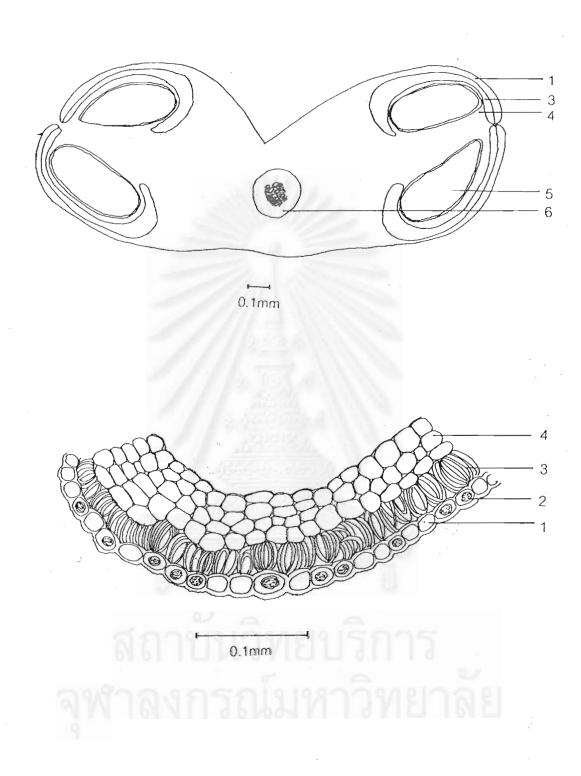


Fig. 55 Transverse section of anther of Jasminum sambac Ait.

- 1 = exothecium
- 2 = greenish-yellow substance
- 3 = endothecium 4 = tapetum
- 5 = pollensac 6 = vascular bundle

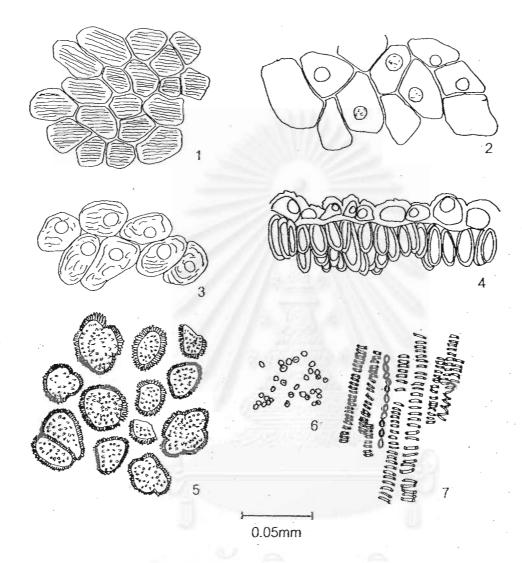


Fig. 56 Microscopical character of powdered drug of Jasminum sambac Ait.

- 1 = upper epidermis of corolla lobe in surface view
- 2 = lower epidermis of corolla lobe in surface view containing greenish-yellow substance
- 3 = exothecium in surface view containing greenish-yellow substance
- 4 = exothecium and endothecium in section view
- 5 = pollen grains
- 6 = starch grain
- 7 = annular and spiral vessel

Chromatographic characteristic

One-dimensional TLC

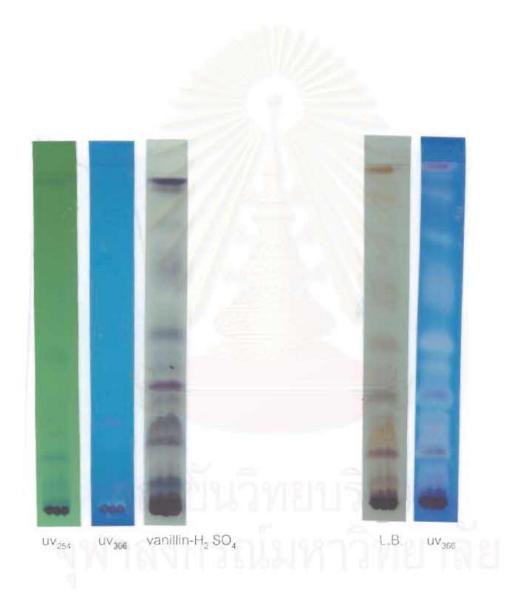
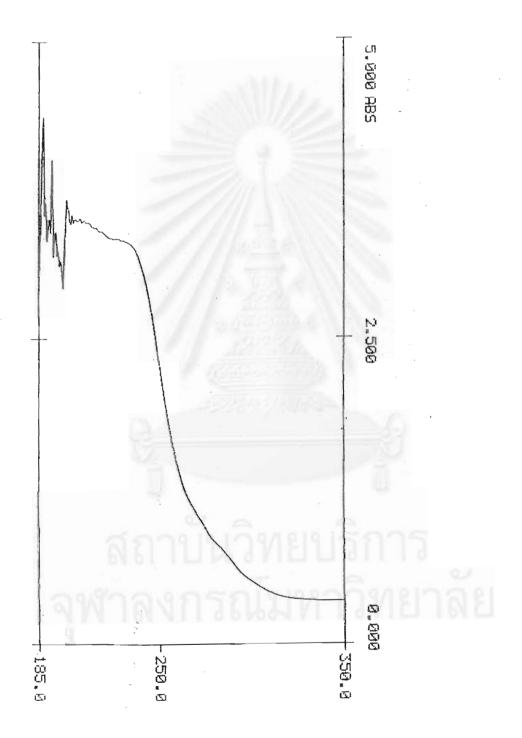
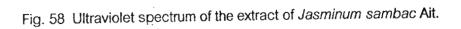


Fig. 57 One-dimensional Thin-layer chromatography patterns of the extract of Jasminum sambac Ait.

### Ultraviolet spectroscopic characters.





peak	wavelength(nm)	absorption
1	209.2	3.495
2	207.2	3.509
3	204.4	3.505
4	202.8	3.538
5	200.0	3.665
6	196.8	3.145
7	195.6	3.241
8	194.4	3.394
9	192.2	4.005
10	190.8	3.503
11	188.8	3.647
12	187.6	4.363

Table 5 Ultraviolet absorption of the extract of Jasminum sambac Ait.



Ma-li-son



# Fig. 59 Ma-Loon (Jasminum sambac Alt.)



Fig. 60 Crude drug appearance of Ma-li-son (Jasminum sambac Ait.)



#### Description of crude drug

Crude drug appears as pale brown dried flower, composed of many corolla lobe, obovate, acute or obtuse, corolla tube 0.3-0.6 cm long, stamen insert on corolla tube, about 0.3 cm long.

#### Microscopical characters

Sectional and surface view of the corolla lobe and corolla tube, exhibit the following microscopical characters.

Upper epidermis composed of a layer of thick-walled polygonal cells with strongly striated cuticle underlying containing pale yellowish pigmen in surface view and in the sectional view showed the rectangular cells covered by thick cuticle. The upper epidermis cells are longer than those of corolla tube.

Mesophyll composed of several layers of thin-wall circular cells. Vascular bundles are located at the central part of this area.

Lower epidermis composed of a layers large thick-walled polygonal cells and in sectional view showed the rectangular cells covered by thick cuticle. The lower epidermis cells in corolla lobe are more longer than thos of corolla tube.

Sectional of the anther exhibit the following microscopical characters.

The anther composed of four pollen sacs. The pollen sac wall composed of epidermis or exothecium; that composed of layer of thick-walled rectangular cells covered by thick cuticle, some containing greenish-yellow pigment.

Endothecium composed of a layer thickening strips-like fiber associated with yellowish layer

Ground tissue composed of several thin-walled circular cells. Vascular bundle located at the central part of this area.

#### Powdered drug characters

The powdered drug is pale brownish color, mild odor, slightly bitter.

The microscopical characters of powdered drug are:

a. Abundant fragments of corollas in surface view. The upper epidermis composed of parenchymatous cells; the cuticle in faintly striated, containing greenish– yellow substance. The lower epidermis composed of small and large parenchymatous cells.

b. Fragments of anther of. The exothecium composed of rather elliptical cells, moderately thickened and smooth walls; that containing greenish – yellow substance. The endothecium layer composed of rather elliptical of fibrous layers in section views and striped–like fibrous layer in surface views.

c. Few pollen grains, round, some like two pollens are adnate longitudinal , bicolpated apertures, strigted exine, 50-70  $\mu$ m in diameter.

d. Fragments of rather round and elliptical cells containing small starch grains and scatterd small starch grains.

e. Fragments of spiral vessel.

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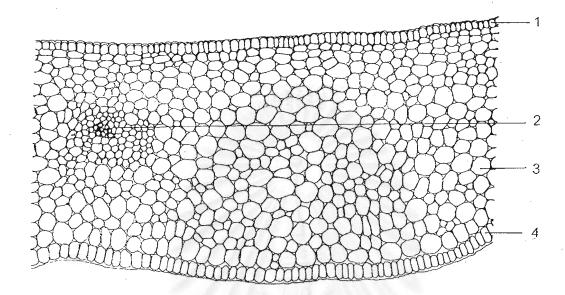


Fig. 61 Transverse section of corolla lobe of Ma-li-son (Jasminum sambac Ait

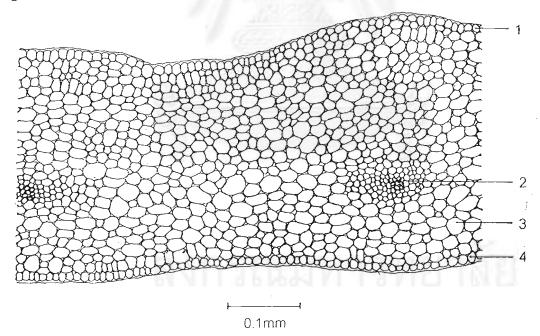


Fig. 62 Transverse section of corolla tube of Ma-li-son (Jasminum sambac Ait.)

1 = upper epidermis

- 2 = vascular bundle
- 3 = parenchyma
- 4 = lower epidermis

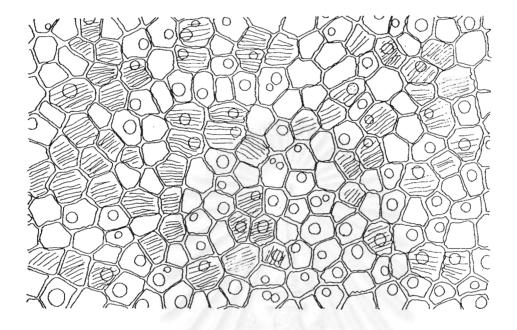
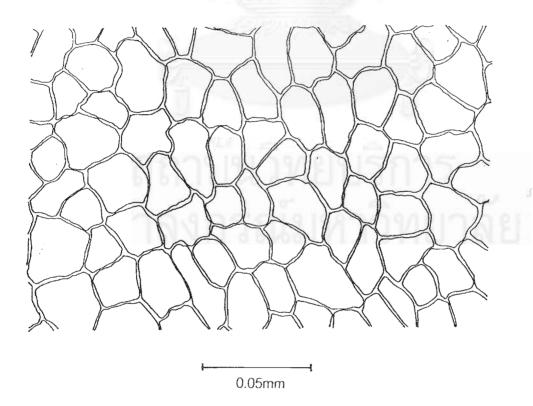
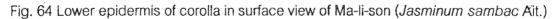


Fig. 63 Upper epidermis of corolla in surface view of Ma-li-son (Jasminum sambac Ait.)





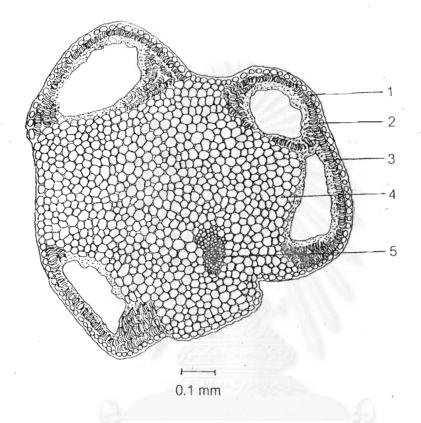


Fig. 65 Transverse section of anther Ma-li-son (Jasminum sambac Ait.)

- 1 = exothecium
- 2 = endothecium
- 3 = tapetum
- 4 = parenchyma
- 5 = vascular bundle

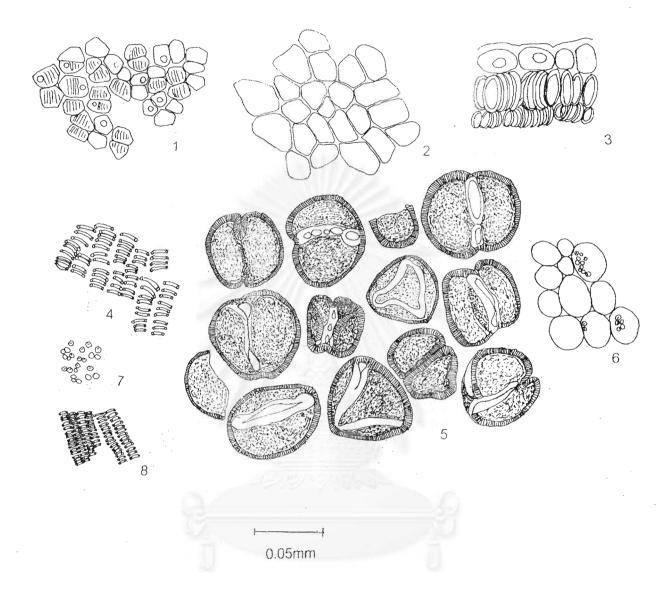


Fig. 66 Microscopical character of powdered drug of Ma-li-son (Jasminum sambac Ait.)

- 1 = upper epidermis of corolla containing yellowish substance
- 2 = lower epidermis of corolla
- 3 = exothecium containing yellowish substance associated with fibrous layer of anther
  - (endothecium)
- 4 = fibrous fiber
- 5 = pollen grains
- 6 = parenchyma containing starch grains
- 7 = starch grain
- 8 = spiral vessel

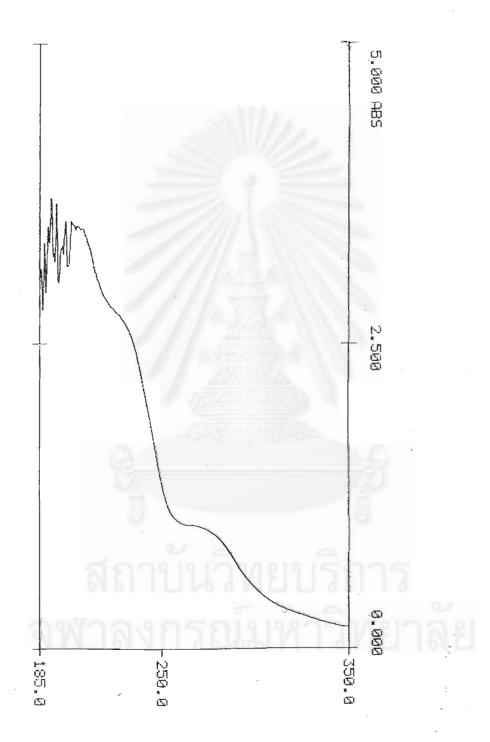
Chromatographic characteristics.

One-dimensional TLC



Fig. 67 One-dimensional Thin-layer chromatography patterns of the extract of Ma-li-son (Jasminum sambac Ait.)

Ultraviolet spectroscopic characters.





peak	wavelenght(nm)	absorption
1	208.0	3.455
2	204.8	3.478
3	202.2	3.512
4	199.2	3.511
5	197.6	3.309
6	194.4	3.666
7	193.2	3.249
8	191.6	3.709
9	190.0	3.471
10	188.0	3.333
11	186.4	3.118

Table 6 Ultraviolet absorption of the extract of Ma-li-son (Jasminum sambac Ait.)



Kra-thing



Fig. 69 Calaphyllum inophyllum Linn.





Fig. 70 Crude drug appearance of Calophyllum inophyllum Linn.



#### Description of crude drug

Crude drug occur as light brown or brown dried flowers, composed of 2 outer sepals, ovate-orbicular, concave, 0.5-0.6 cm long, 0.4-0.5 cm wide, 2 inner sepals, 0.8-0.9 cm long, 0.4-0.5 cm wide.

#### Microscopical characters

Sectional and surface view of the sepal exhibit the following microscopical characters.

Upper epidermis composed of a layer of thick-walled polygonal cells underyling cluster crystal of calcium oxalate 6-9  $\mu$ m in diameter in surface view and in the sectional view shows the rectangular cells covered by thick cuticle.

Mesophyll composed of several layers of thin-wall circular cells, some containing cluster crystal of calcium oxalate, air space. Vascular bundle located at the central part of this area.

Lower epidermis composed of a layer of thick-walled polygonal cells associated paracytic stomata, underlying cluster crystal of calcium oxalate in surface view and in the sectional shows the rectangular cells covered by thick cuticle.

Sectional and surface view of the corolla exhibit the following microscopical characters.

Upper epidermis composed of a layer of thick-walled polygonal cells in surface view and in the sectional view show the rectangular cells covered by thick cuticle.

Mesophyll composed of several layers of rather thicked-wall circular cells, some containing cluster crystal of calcium oxalate 20-30  $\mu$ m in diameter. Vascular bundles and oil glands located at the central part of this area.

Lower epidermis of corolla composed of a layer of cells, similar to those of the upper epidermis.

Transverse section of the anther exhibit the following microscopical characters.

The anther composed of four pollen sacs. The pollen sac wall composed of epidermis or exothecium; that composed of layer of thick-walled rectangular cells covered by thick cuticle some containing yellowish-brown pigment.

Endothecium composed of a layer thickening pitted-like cells.

Ground tissue composed of several thin-walled polygonal cells. Vascular bundle located at the central part of this area.

Transverse section of the pedicel exhibit the following microscopical characters. Epidermis composed of rectangular cells covered by thick cuticle.

Ground tissue composed of several layers of thin-wall circular cells, scattered oil gland in this area. Vascular bundle located at the central part of this area and air space located at the central part of vascular bundle.

Transverse section of the ovary exhibit the following microscopical characters. The ovary composed of outer ovary wall, inner ovary wall with one locule.

The outer ovary wall composed of epidermis; that composed of rectangular cells covered by thick cuticle. Ground tissue composed of several layers of thin-wall circular cells, scattered oil glands and vascular bundles.

The inner ovary wall composed of layer of thin-walled circular cells, one locule located at the central part of this area.

Inner layer composed of one layer of square cells. Ground tissue composed of many layers of circular cells; smaller than ground tissue of outer part with one locule at the central part of this area.

#### Powdered drug characters

The powdered drug is light brown, mild odor

The microscopical characters of powdered drug are:

a. Fragments of the sepals in surface view. The upper epidermis composed of polygonal cells with slightly thickened walls. The lower epidermis composed of polygonal cells with slightly thickened walls, paracytic stomata , underlying the epidermis contains cluster crystals of calcium oxalate.

b. Fragments of the corollas in surface view. The lower epidermis composed of polygonal cells with slightly thickened walls, underlying the epidermis contains cluster crystals of calcium oxalate.

c. The abundant fragments of fibrous layer of the anther composed of rather elliptical and polygonal cell, pitted-like cell in surface view.

d. Pollen grains scattered, spheroidal or rather triangular outline and smooth exine, with tri-tetra colpated apertures and also has faint granulations.

e. Scattered cluster crystals of calcium oxalate.

f. Scattered spiral vessel.

g. Fragments of pedicel in surface view ; that composed of longitudinal elongated cells, paracytic stomata, underlying epidermis contains cluster crystals of calcium oxalate.

h. Fragments of covering trichomes, occur at the margin of sepal. Multicellular ;two or three cells in each row, round or tapering at end.

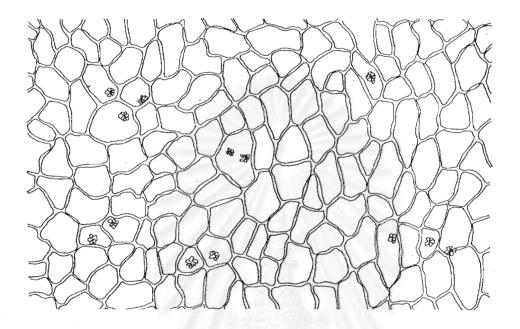


Fig. 71 Upper epidermis of sepal in surface view of Calophyllum inophyllum Linn.

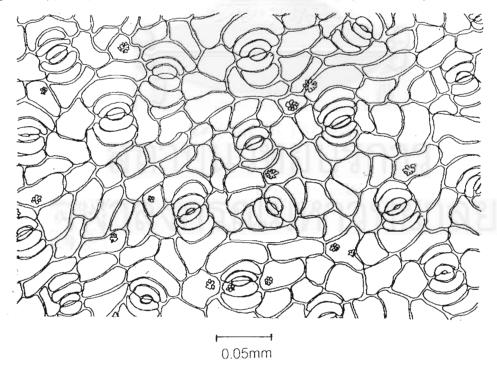
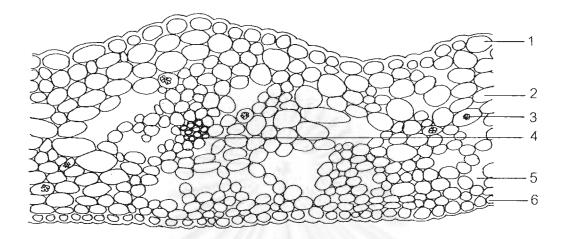


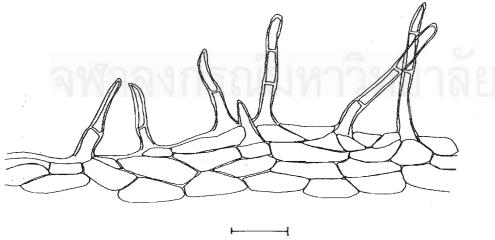
Fig. 72 Lower epidermis of sepal in surface view of Calophyllum inophyllum Linn.



0.05mm

Fig. 73 Transverse section of sepal of Calophyllum inophyllum Linn.

- 1 = upper epidermis
- 2 = parenchyma
- 3 = rosette aggregate of calcium oxalate
- 4 = vascular bundle
- 5 = intercellular space
- 6 = lower epidermis



0.05mm

Fig. 74 Multicellular trichome at margin of sepal of Calophyllum inophyllum Linn.

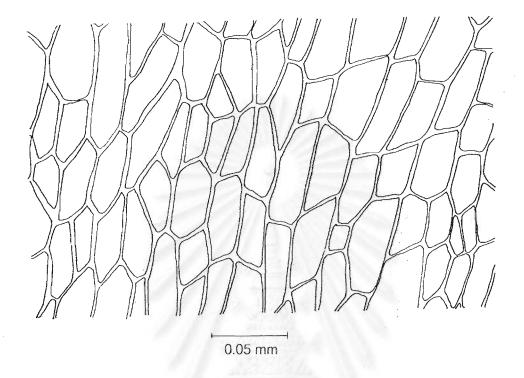
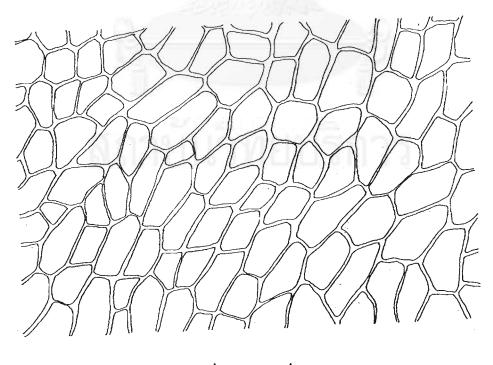
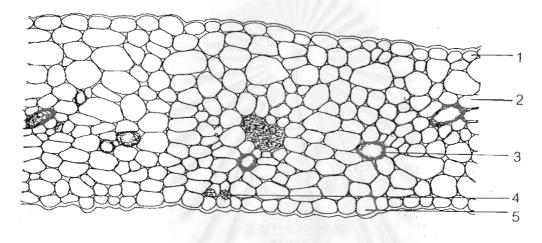


Fig. 75 Upper epidermis of corolla in surface view of Calophyllum inophyllum Linn.



0.05mm

Fig. 76 Lower epidermis of corolla in surface view of Calophyllum inophyllum Linn.



0.1mm

Fig. 77 Transverse section of corolla of Calophyllun inophyllum Linn.

- 1 = upper epidermis
- 2 = ground tissue parenchyma
- 3 =oil gland
- 4 = cluster crystal of calcium oxalate
- 5 =lower epidermis

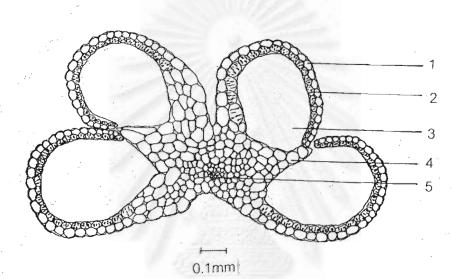


Fig. 78 Transverse section of anther of Calophyllum inophyllum Linn.

- 1 = exothecium
- 2 = endothecium
- 3 = pollen sac
- 4 = parenchyma
- 5 = vascular bundle

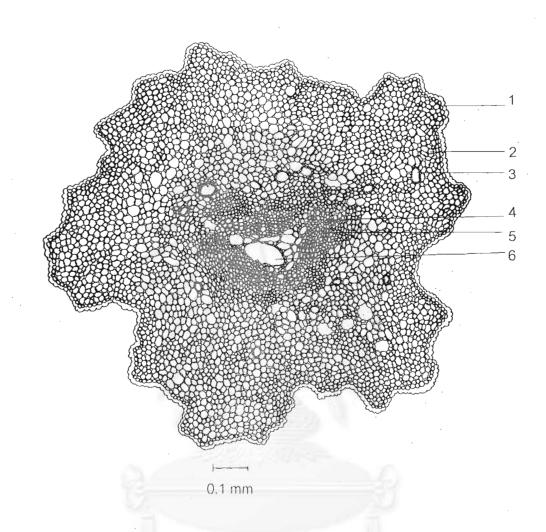


Fig.79 Transverse section of pedicel of Calophyllum inophyllum Linn.

- 1 = cuticle
- 2 = parenchyma
- 3 = oil gland
- 4 = phloem
- 5 = xylem
- 6 = central lumen

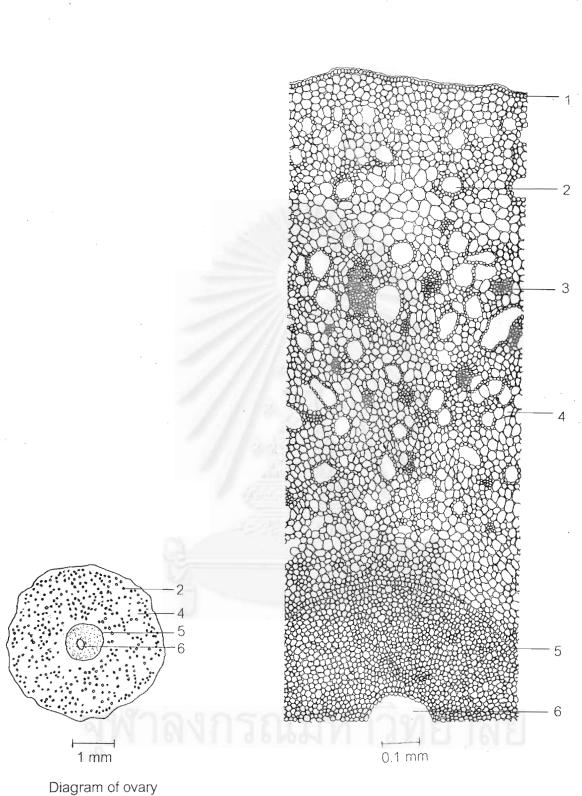


Fig. 80 Transverse section of ovary of Calophyllum inophyllum Linn.

1 = epidermis 2 =oil gland

3 = vascular bundle 4 = parenchyma

5 = square cell

6 = locule

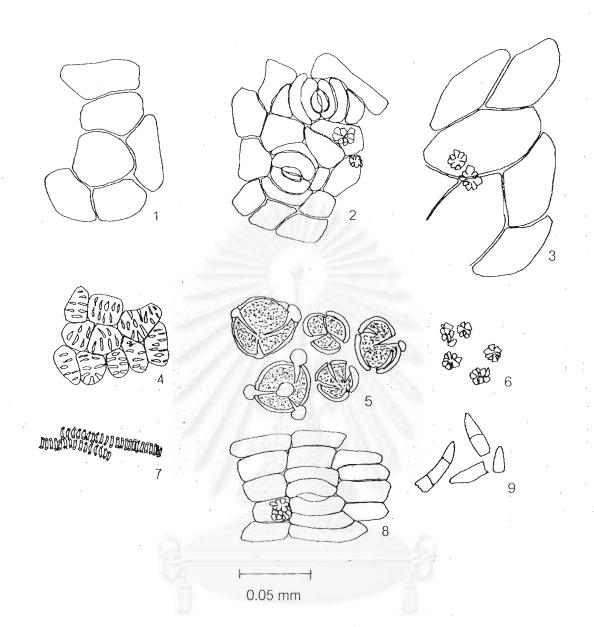


Fig. 81 Microscopical character of powdered drug of Calophyllum inophyllum Linn.

1 = upper epidermis of sepal

2 = lower epidermis of sepal

- 3 = epidermis of corolla
- 4 = endothecium in surface view
- 5 = pollen grains
- 6 = rosette aggregate of calcium oxalate
- 7 = spiral vessels
- 8 = epidermis of pedicel in surface view
- 9 = trichomes

Chromatographic characteristic

One-dimensional TLC

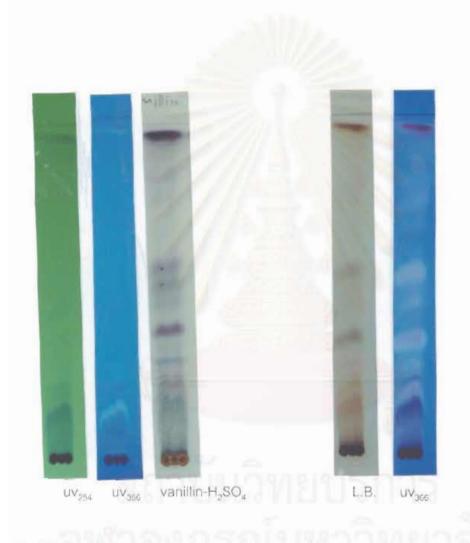
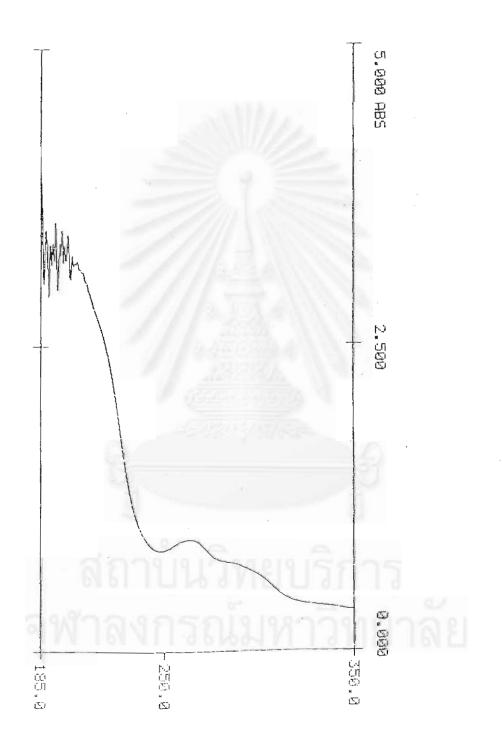


Fig.82 One-dimensional Thin-layer chromatography patterns of the extract of Calophyllum inophyllum Linn.





peak	wavelenght(nm)	absorption
1	265.2	0.904
2	206.0	3.140
3	204.8	3.207
4	203.2	3.195
5	202.0	3.253
6	199.6	3.422
7	197.6	3.346
8	196.4	3.460
9	195.2	3.358
10	193.2	3.528
11	191.6	3.354
12	190.4	3.345
13	188.4	3.471
14	186.0	3.865

Table.7 Ultraviolet absorption of extract of Calophyllum inophyllum Linn.

### CHAPTER V CONCLUSION AND DISCUSSION

Microscopical characters of the powdered drug of Ma-li and Ma-li-son are nearly similar of greenish mass, starch grains, ground tissue parenchyma cells of anther.

Table 8 Comparison of the microscopical characters of the powdered drugs of Ma-li-laand Ma-li- son.

Character	Ma-li	Ma-li-son	
upper epidermis of corolla	striated curvature	Attenuated striated	
/	232232	curvature	
lower epidermis of corolla	not striated	not striated	
tapetum	thin-walled	Yellowish layer	
	parenchymatous cells		
pollen grains	tri-tetra colpate apertures,	bi-colpate apertures	
	40-50 <b>µ</b> m	50-70 <b>μ</b> m	

Microscopical characters of the powdered drug of Saa-ra-phee and Kra-thing are nearly similar of lower epidermis of sepal, pollen grains, spiral vessel, rosette aggregate of calcium oxalate, epidermis of pedicels.

 Table 9 Comparison of the microscopical characters of powdered drug of Saa-ra-phee

 and Kra-thing.

powdered drug	Saa-ra-phee	Kra-thing
sepal	without trichome	trichome 2-3 septum,
		46-129 <b>μ</b> m
lower epidermis of corolla	thick- wall	thin wall
endothecium	stripe-like cells	pited-like fiber
pollen grians	tri-colpate aperture,	tri or tetra-colpate aperture,
	spheroidal, 35-45 $\mu$ m	spheroidal, 30-45 $\mu$ m

Microcopical characters of pollen grains of Kaysorn-tung-haa are nearly similar, as shown in table 10.

Table 10 Comparison of the characters of pollen grains of Kaysorn-tung-haa

character of	Ma-li	Phi-kun	Bun-naak	Saa-ra-phee	Bua-luang
size	40-50 <b>µ</b> m	38-40 <b>µ</b> m	39-50 <b>µ</b> m	35-45 <b>µ</b> m	61-78 <b>µ</b> m
shape	Spheroidal	elliptical	spheroidal	spheroidal	spheroidal
outer wall	Striated	smooth	smooth	smooth	smooth
colpate	tri or tetra	tetra-colpate	tri-colpate	tri-colpate	tri-colpate
aperture	colpate	apertures	apertures	apertures	apertures
	apertures				

Microscopiral characters of the anther of Kaysorn-tung-haa are nearly similar and difference show in the table 11

Table 11 Comparison of the microscopical characters of anther of Kaysorn-tung-haa.

character	Ma-li	Phi-kun	Bun-naak	Saa-ra-phee	Bua-luang
exothecium	- circular,	- circular,	- ci <mark>rcular,</mark>	- circular	- with
	elliptical	ellipticall	elliptical	cell	papillae
	cells	cells	cells		appendage
	- contianing		- contaning	- containing	
	greenish-		brownish	brownish	
	yellow		substance	substance	
	substance				
endothecium	- stripe-like	- stripe-like	- pitted-like	- stripe-like	- pitted-like
	cells	cells	circular or	cells	elliptical or
			elliptical		orbicular
			cells		thick wall
tapetum	- many layer	-	-	<u>.</u>	- many layer
	of thin wall	2.6			of thin wall
	parenchyma	านวง	1ยบว่	การ	parenchyma
	cells	e e e e e e e e e e e e e e e e e e e		9	cells and
2	1.1911	1319	มหาว	<b>VIE</b> 16	yellowish
9					layer

Microscopical characters of the powdered drug of Kaysorn-tung-haa are nearly similar and difference showed in the table 12

Table 12 Comparison of microscopical characters of powered drug of Kaysorn-tung-haa

character	Ma-li	Phi-kun	Bun-naak	Saa-ra-phee	Bua-luang
lower	-		1 or 2	- paracytic	-
epidermis of			unicellular,	stomata	
sepal			trichomes	- rosette	
upper	- striated	striated	not striated	not striate	-
epidermis of	- yellowish				
corolla	substance				
lower	- not	striated	not striated	not striated	-
epidermis of	striated,		ALAK		
corolla	yellowish	(Janethe	Selfield B		
	substance	135319V	2/ Allan		
vessel	annular,	spiral	spiral	spiral	annular,
	spiral				spiral
crystal	_ 🥹	-	-	cluster crystal	-

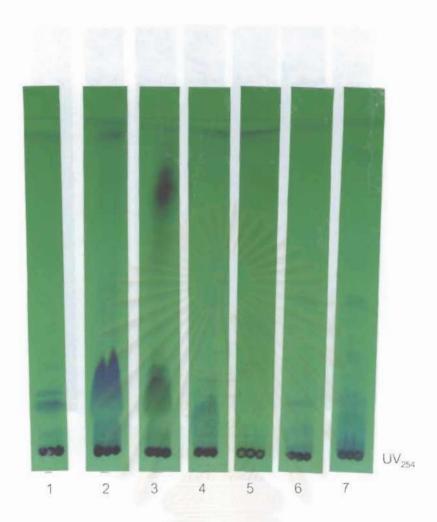


Fig 84 One dimensional TLCpatterns of the extracts of Kaysorn-tung-haa, Ma-li-son and Kra-thing.flowers dection UV. 254..



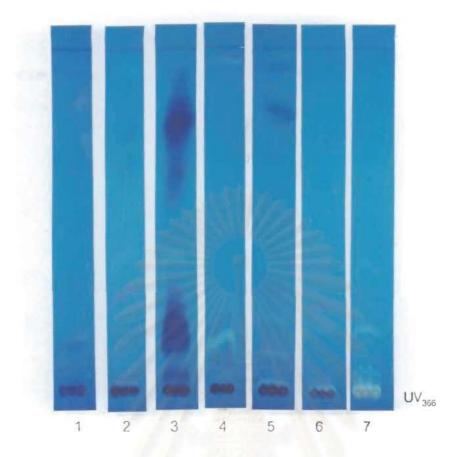


Fig 85 One dimensional TLC patterns of the extracts of Kaysorn-tung-haa, Ma-li-son and Kra-thing.flowers dection UV. 366.



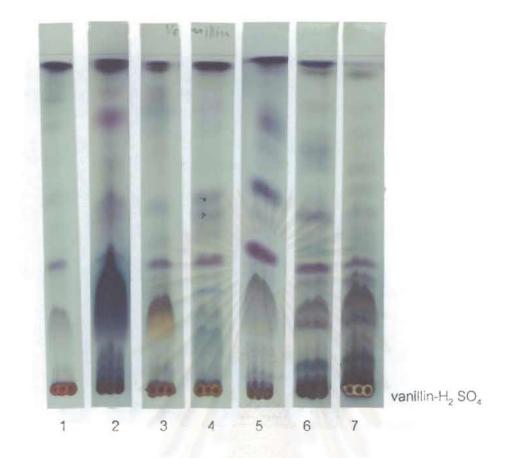


Fig 86 One dimensional TLC characteristics of the extracts of Kaysorn-tung-haa, Ma-lison and Kra-thing.flowers dection Vanillin-H<sub>2</sub> SO<sub>4</sub>

1=Phi-kun 2=Bun-naak 3=Saa-ra-phee 4=Kra-thing 5=Bua-luang

6=Ma-li 7=Ma-li-son

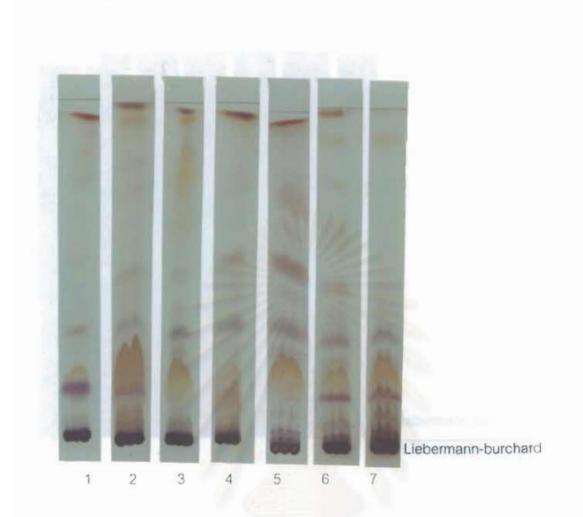


Fig 87 One dimensional TLC patterns of the extracts of Kaysorn-tung-haa, Ma-li-son and Kra-thing.flowers dection Liebermann-burchard.



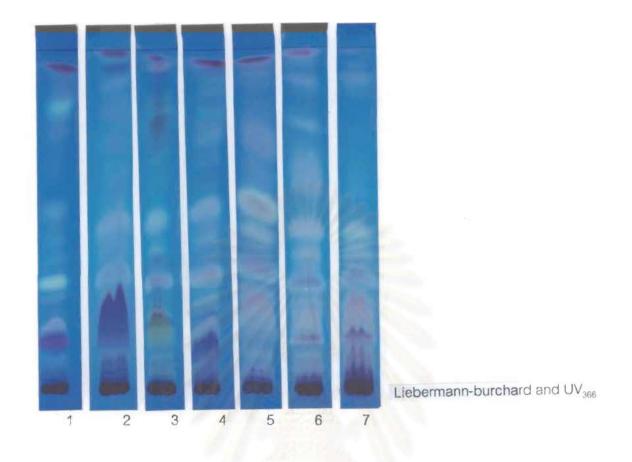
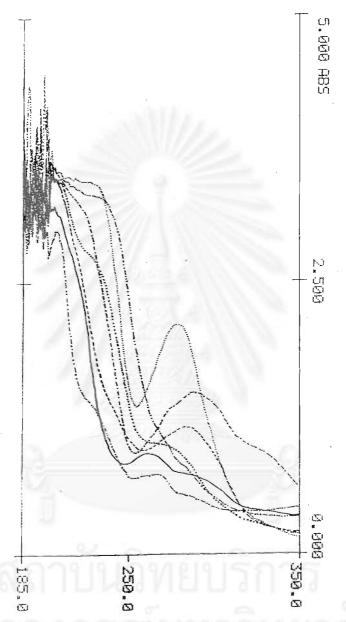
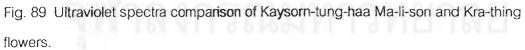


Fig 88 One dimensional TLC patterns of the extracts of Kaysorn-tung-flower. Ma-li-son and Kra-thing.flowers dection Liebermann-burchard and UV<sub>366</sub>

1=Phi-kun	2=Bun-naak	3=Saa-ra-phee	4=Kra-thing	5=Bua-luang	
6=Ma-li	7=Ma-li-son			· · · ·	
			มหาวิ		





	Phi-kun		Bun-naak
· _ · - · - · - ·	Saa-ra-phee	<b>_</b>	Bua-luang
	Ma-li	•••••••••••••	Ma-li-son
	Kra thing		

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สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX

#### Test reagent for microscopy

#### Chloral hydrate

A solution prepared by dissolving 25 gm in 10 ml of water is an excellent clearing agent. It dissolves starch, resin, protein, chlorophyll and causes more or less expansion of shrunken cells.

#### Glycerin, dilute

This is prepared by adding equal parts of pure glycerin and distilled water. It is use as a mounting medium.

#### Hydrochloric acid

The concentrated acid is used with an equal volume of phloroglucin solution in determining lignification. Dilute solutions are employed for neutralizing alkaline solutions, etc.

#### lodine water

Add as much iodine to distilled water as it will dissolve. Stains starch pale blue to purplish blue.

#### Phloroglucin solution

Dissolve 1 gm of phloroglucin in 50 ml of 95 percent alcohol. This solution gradually darkens and loses strength with age. It is unfit for use after 3 or 4 months. The lignified walls of elements first treated with several drops or two of concentrated hydrochloric acid are colored red.

#### Test reagent for TLC

Lieberman-Burchard reagent(Acetic anhydric-sulfuride acid reagent) Spray solution : Mix cautiously, while cooling, 5 ml of acetic anhydride with 5 ml of sulfuric acid. The mixture is added slowly,while cooling, to 50 ml of absolute ethanol. Pepare freshly before use.

Treatment : Heat the sprayed chromatogram at 110<sup>°</sup> for 10 min, examine in daylight and ultra-violet light (366 nm).

Vanillin-sulfuric acid

Spray solution : Dissolve 3 g of vanillin in 100 ml of absolute ethanol, and 0.5 ml of sulfuric acid is added to the solution.

Treatment : Heat the sprayed chromatogram at 120<sup>°</sup> until the spots At in maximum color intensity.



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