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PHARMACOGNOSTIC PROPERTIES OF KHAMIN KHRUEA

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for the Degree of Master of Science in Pharmacy
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Arcangelisia flava (L.) Merr., *Coscinium fenestratum* (Gaertn.) Colebr., *Fibraurea tinctoria* Lour. และ *Combretum latifolium* Blume ซึ่งมีชื่อไทยพ้องกันว่า “ขมิ้นเครือ” สามารถจำแนกลักษณะโดยอาศัยคุณสมบัติต่างๆ ทางเภสัชเวท การตรวจเอกลักษณ์ทางมหาทรรศน์ ทางจุลทรรศน์ และกระสวนขององค์ประกอบทางเคมีบนโครมาโตแกรมฉาบบางของสมุนไพรส่วนลำต้น รวมถึงการหาค่าคงที่ของใบ พบว่ามีความแตกต่างกันอย่างมีนัยสำคัญซึ่งสามารถนำมาใช้ในการพิสูจน์เอกลักษณ์ของสมุนไพรทั้งสี่ชนิดได้ การหาน้ำหนักที่หายไปเมื่อทำให้แห้ง ปริมาณเถ้า และ ปริมาณสิ่งสกัด สามารถนำไปกำหนดมาตรฐานของสมุนไพรแต่ละชนิด นอกจากนี้ได้ศึกษาลักษณะทางเภสัชเวทของ *Coscinium* อีกชนิดไว้ด้วย

ภาควิชา เภสัชเวท **ลายมือชื่อนิสิต** .....

สาขาวิชา เภสัชเวท **ลายมือชื่ออาจารย์ที่ปรึกษา** .....

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Arcangelisia flava (L.) Merr., *Coscinium fenestratum* (Gaertn.) Colebr., *Fibraurea tinctoria* Lour. and *Combretum latifolium* Blume under the same Thai vernacular name “Khamin khruea” were characterized by the detail pharmacognostic properties. Macro-, microscopic characters and thin-layer chromatographic patterns of stem including leaf measurements showed the significant differences among these 4 species which can be used for identification. Loss on drying, ash content and extractive value represent the specifications of each species. Moreover, another species of *Coscinium* has also been studied.

Department of Pharmacognosy **Student’s signature ……..**
Field of study Pharmacognosy **Advisor’s signature …….**
Academic year 2001 **Co-advisor’s signature ……**

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ABBREVIATIONS

°C	=	Degree Celsius
cm	=	Centimeter
conc.	=	Concentration
ED_{50}	=	50% Effective dose
g	=	Gram
IC_{50}	=	50% Inhibition Concentration
kg	=	Kilogram
LD_{50}	=	50% Lethal dose
m	=	Meter
mg	=	Milligram
ml	=	Milliliter
mm	=	Millimeter
mm^2	=	Square millimeter
nm	=	Nanometer
No.	=	Number
S.D.	=	Standard Deviation
sp.	=	Species (singular)
spp.	=	Species (plural)
SPSS	=	Statistical Package for the Social Sciences
TLC	=	Thin-layer chromatography
μg	=	Microgram
μm	=	Micrometer
μM	=	Micromolar
UV	=	Ultraviolet

CHAPTER I

INTRODUCTION

In Thailand, “Khamin khruea” is a folkloric medicine referred to the woody climber with yellow wood and root. Various parts have been used in a wide variety of diseases. Traditionally, leaves are used for emmenagogue; flowers are used to treat dysentery; stem has been used to treat stomachic symptoms, as a tonic, emmenagogue and astringent; root is used to treat orchitis, lymphatic system malfunction and eyes disease (เสงี่ยม พงษ์บุญรอด, 2493; สมาคมเภสัชและอายุรเวชโบราณแห่งประเทศไทย, 2507; สายสนม กิตติขจร, 2526; จีรเดช มโนสร้อย และ อรัญญา มโนสร้อย, 2537).

Under the name “Khamin khruea”, it belongs to several species from 2 families (Menispermaceae and Combretaceae). In the South-eastern of Thailand, “Khamin khruea” is called for *Arcangelisia flava* (L.) Merr. and *Coscinium fenestratum* (Gaertn.) Colebr. from Menispermaceae while in the South part it is a *Fibraurea tinctoria* Lour. from the same family (Forman, 1991). Thai traditional medicine books said that “Khamin khruea” is a *Combretum latifolium* Blume (*C. extensum* Roxb.) from Combretaceae (เสงี่ยม พงษ์บุญรอด, 2493; หน่วยงานศึกษาวิจัยคัมภีร์ใบลาน, 2525), or a *Arcangelisia flava* (L.) Merr. (คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล, 2535; นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร, 2541), or a *Fibraurea tinctoria* Lour. (สายสนม กิตติขจร, 2526; จีรเดช มโนสร้อย และ อรัญญา มโนสร้อย, 2537; นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร, 2541). All species are claimed to use the same therapeutic purposes.

Arcangelisia flava (L.) Merr. is mostly used in South East Asia Region. In Peninsular Malaysia, a decoction of the stem is taken internally for jaundice, worms, indigestion and other intestinal complaints. The smoke from the burning wood is inhaled for troubles of the mucous membrane of the nose and mouth. In the

Philippines, yellow-fruited moonseed is a popular antiseptic: a decoction of the wood is use to clean wounds, ulcers and other skin irritations. Traditional applications include the use of a decoction or infusion of the stem as a stomachic, febrifuge, expectorant, tonic and emmenagogue or abortivum (depending on the quantity administered). In Indonesia, the stems are sold as “kayu seriawan”, meaning “wood against sprue”. The sap which flows abundantly from cut stems is drunk against fever and sprue. In the Philippines, the Moluccas and New Guinea a yellow dye is extracted from the woody stem (De Padua *et al*., 1999).

Coscinium is the Latin transcription of the Greek koskinion (= little sieve), the name was given to the genus by Colebrooke because of the cribriform perforation of the cotyledonary leaves. In India, *C. fenestratum* known as Dharhadi, is used to prepare a yellow dye. It is also widely used as a medicine: aqueous or alcoholic extracts are applied as a bitter tonic; a paste of pounded roots and stems is used to dress bruises and contusions. Darvi, an Ayurvedic drug, used against ulcers and affections of the eye, is derived from the berberine containing plants *C. fenestratum* or *Berberis* species. Dr.Phan-Quoc-Kinh, Faculty of Pharmacy, Hanoi, Vietnam, named “Codan B”, containing a crude alcoholic extract of *C. usitatum* (*C. fenestratum*). In Vietnam, this medicine is prescribed to cure dysentery (Siwon *et al*., 1980). In Cambodia, the wood produces a yellow dye (used together with *Curcuma*). It has been known in Europe as False Calumba, being a substitute for Calumba (*Jateorhiza*). The plant has alleged antiseptic properties and is used in Malaya to dress wounds and ulcers. The species is used as an ingredient for arrow poisons in Malaya (Forman, 1978).

In China, *Fibraurea tinctoria* Lour. is used as a substitute for *Coptis*. In Indo-china, The root and lower part of the stem are employed as a tonic, a deobstruent, a diuretic and a febrifuge. In Malay Peninsula, a decoction of the root is used as a post partum protective medicine; the wood is made into cigars and smoked to make an inhalation for ulcerated nose. In Indonesia, an infusion of the stem is utilized as an eye wash, and internal medicine for bloody excrements; further it is recommended to treat diabetes; a poultice of the leaves is applied to relieve headache (Perry and Metzger, 1980). The decoction of this plant is employed in Malaysia as a tonic after

childbirth, for the treatment of diabetes, dysentery and ulcerated nose (Zakaria *et al*., 1989).

Combretum latifolium Blume (*C. extensum* Roxb.), the astringent fruit is used as a tonic (Perry and Metzger, 1980).

A previous pharmacognostic study of Khamin khruea under the name *Arcangelisia flava* (L.) Merr. has been reported describing the macroscopic and microscopic characters of the root (ถนอมหวัง อมาตยกุล และ ครุณ เพ็ชรพลาย, 2517). The phytochemical study of Khamin khruea which were collected from the different places indicated that Khamin khruea samples from Chanthaburi and Songkhla provinces were not the same species because of their different chemical constituents. The rhizome from Chanthaburi sample contained berberine and palmatine and the stem contained only berberine. While Songkhla sample, the rhizome and the stem contained palmatine and jatrorrhizine. This experiment also compared the berberine content in Khamin khruea between sample from Chanthaburi province and sample from old-styled drugstores in Bangkok. The sample from old-styled drugstores contained lower berberine content than the sample which was collected from Chanthaburi province. However, those Khamin khruea were not informed the scientific name. (เรืองศักดิ์ พันธ์วิศวาส และ คณะ, 2515). Another study is “Quality Control study of *Arcangelisia flava* Stem” by Jewvachdamrongkul *et al.* in 1993. The plant materials included the authentic sample of Khamin khruea (*Arcangelisia flava* (L.) Merr. from Chanthaburi province and crude drug samples which were purchased from different traditional drugstores in Bangkok and Nonthaburi provinces.

This investigation was aimed to characterize the 4 species of Khamin khruea according to this vernacular name and Thai folkloric claimed used. These are *Arcangelisia flava* (L.) Merr., *Coscinium fenestratum* (Gaertn.) Colebr., *Fibraurea tinctoria* Lour. and *Combretum latifolium* Blume (*C. extensum* Roxb.), which will be examined for their macroscopic, microscopic characters of stem and the thin-layer chromatographic patterns of the stem extract including the leaf measurements. The quality controls of crude drugs which are purchased from traditional drugstores throughout Thailand were investigated. The results of this study should provide

valuable information for differentiating in each species of “Khamin khruea” and also aid in referring this Thai vernacular name to the right scientific name.

CHAPTER II

HISTORICAL

1. Botanical aspects

1.1 Menispermaceae

Plants of the family Menispermaceae are dioecious climbers, rarely erect plants. Stipules absent. **Leaves** spiral, often palmatinerved at base; petiole often swollen at base. **Flowers** small, usually 3-merous; sepals, petals and stamens free or united; carpels free, usually 1-6. **Fruits** consisting of usually 1-6 drupes with style-scar sometimes close to base or lateral; endocarp usually bony and often ornamented; sometimes horseshoe-shaped, usually with a condyle, *i.e*. a ventral intrusion into the seed-cavity. **Seed** with endosperm present or absent (Forman, 1991).

According to Forman (1978, 1982 and 1985) the members of Menispermaceae were divided into five tribes, there are *Coscinieae*, *Menispermeae*, *Tiliacoreae*, *Tinosporeae* and *Fibraureae*. But two of these, *Tinosporeae* and *Fibraureae* should probably be combined. The tribes in Asia are characterized by the following combinations of characters.

Coscinieae: Sepals imbricates. Petals 0. Stamens either all or only the inner 3 connate. Carpels 3-6. Drupe with style-scar sublateral towards base on lateral. Endocarp smooth or fibrio-pilose subglobose with condyle obsolete, or subhemispherical with condyle deeply intrusive and 2-chambered. Endosperm present, sometimes ruminate. Seed broadly ellipsoidal or cup-shaped. Embryo with thin foliaceous divaricate cotyledons which are sometimes much folded.

Menispermeae: sepals usually free in 1-few whorls or sometimes connate when in 1 whorl, the innermost whorl sometimes valvate, or sepals spiral. Petals (0-)3-6(-9) sometimes connate. Female flowers with perianth sometimes reduced to 1-2 parts. Stamens free or partly connate or united into a peltate synandrium. Carpels 1-6. Drupe strongly curved with style near base. Endocarp with ± horseshoe-shaped dorsal region usually ornamented with projections or transverse ridges; condyle deeply intrusive, either lamelliform and ± ovate with the seed-cavity curved around its margin or hollow with 1-2 chambers, sometimes perforate. Endosperm usually present, but absent in *Pachygone*. Seed elongate, strongly curved. Embryo elongate and curved with contiguous cotyledons.

Tiliacoreae: sepals imbricate or inner whorl valvate and sometimes connate. Petals rarely absent. Stamens free or connate. Carpels 3-10. Drupe with style-scar near base or lateral. Endocarp smooth, wrinkled, rugose or coarsely reticulate; straight and condyle absent or curved with condyle intrusive and septiform. Endosperm usually absent, but present and ruminate in *Tiliacora*. Seed ellipsoidal, straight. Embryo with thick accumbent cotyledons or elongate and strongly curved with elongate contiguous cotyledons.

Tinosporeae (include ***Fibraureae***): sepals imbricate, rarely connate at the base. Petals 6 or 0. Stamens free or united into a peltate synandrium. Carpels 3(-4). Drupe with style-scar terminal. Endocarp spiny, verrucose, rugose or smooth, condyle a ventral hollow or longitudinal groove or deeply intrusive and clavate endosperm present, sometimes ventrally ruminate. Seed usually straight and ventrally hollowed or grooved, sometimes cup-shaped. Embryo with foliaceous divericate or imbricate cotyledons.

The family contains 73 genera, and about 350 species; which are almost entirely tropical. There are 22 genera, and 51 species in Thailand; of which 9 species are endemic (shown by asterisk) (Forman, 1991). All of them are as follows:

1. *Albertisia*

   Albertisia papuana Becc.

   A. puberula Forman*

2. *Anamirta*

Anamirta cocculus (L.) Wight & Arn.

[Mae nam nong (แม่น้ำนอง) (Northern); thaowan thong (เถาวัลย์ทอง) (South-western); wai din (หวายดิน), Kho khlan (โคคลาน) (Central); thao kha-nom (เถาขะโนม), lumpri (ลุมพรี) (South-eastern)].

3. *Arcangelisia*

Arcangelisia flava (L.) Merr.

[Khamin khruea (ขมิ้นเครือ) (South-eastern); khamin ruesi (ขมิ้นฤาษี), hap (ฮับ) (Peninsular)].

4. *Aspidocarya*

Aspidocarya uvifera Hook.f. & Thoms.

5. *Cissampelos*

Cissampelos hispida Forman*

C. pareira L. var. *hirsuta* (Buch. ex DC.) Forman

[Khong khamao (ขงเขมา) (Northern); khruea ma noi (เครือหมาน้อย) (Eastern); kon pit (ก้นปิด) (South-western); krung khamoa (กรุงเขมา), sifan (สีฟัน) (Peninsular)].

6. *Cocculus*

Cocculus hirsutus (L.) Theob.

C. laurifolius DC.

[Yang nan ton (ย่านน่านต้น) (North-eastern, Central); sakae dong (สะแกดง) (North-eastern); suramarit (สุรามฤต) (Eastern)].

C. orbiculatus (L.) DC.

7. *Coscinium*

Coscinium blumeanum Miers

C. fenestratum (Gaertn.) Colebr.

[Khruea hen (เครือเห็น) (North-eastern); kramin khruea (ขมิ้นเครือ) (South-eastern)].

8. *Cyclea*

Cyclea atjehensis Forman

C. barbata Miers

[Krung badan (กรุงบาดาล) (South-eastern); krung khamao (กรุงเขมา) (Peninsular)].

C. laxiflora Miers

C. polypetala Dunn

C. varians Craib*

9. *Diploclisia*

D. glaucesces (Blume) Diels

[Ma nim dam (มะหนิมดำ), duk khruea (ดูกเครือ) (Northern); khruea sai kai (เครือไส้ไก่) (Shan/Northern); tap tao (ตับเต่า) (Peninsular)].

10. *Fibraurea*

Fibraurea tinctoria Lour.

[Khamin ruesi (ขมิ้นฤาษี), khamin khruea (ขมิ้นเครือ), man miat (มันเมียด) (Peninsular); thaowan thong (เถาวัลย์ทอง) (South-western); kamphaeng chet chan (กำแพงเจ็ดชั้น) (Central)].

11. *Haematocarpus*

Haematocarpus validus (Miers) Bakh.f. ex Forman

12. *Hypserpa*

Hypserpa nitida Miers

[Haen kuem (แฮนกึม) (North-eastern)].

13. *Limacia*

Limacia blumei (Boerl.) Diels

L. oblonga Hook.f. & Thoms.

L. scandens Lour.

14. *Pachygone*

Pachygone dasycarpa Kurz

[Nam phrom (น้ำพรม) (Northern); ya nang chang (หญ้านางช้าง) (Eastern)].

P. odorifera Miers

15. *Parabaena*

Parabaena sagittata Miers

[Phak nang (ผักหนัง) (Shan/Northern)].

16. *Pericampylus*

Pericampylus glaucus (Lamk.) Merr.

[Salit hom kha (สลิดหม่มคา) (Northern); yan tap tao (ย่างตับเต่า) (Peninsular)].

17. *Pycnarrhena*

Pycnarrhena lucida (Teijsm. & Binn.) Miq.

[Ya nang ton (ย่านางต้น) (South-western)].

P. poilanei (Gagnep) Forman

18. *Sinomenium*

Sinomenium acutum (Thunb.) Rehder & Wilson

19. *Stephania*

Stephania brevipes Craib*

[Bua khruea (บัวเครือ) (Northern)].

S. capitata (Blume) Spreng.

S. crebra Forman*

S. elegans Hook.f. & Thoms.

[Se-khi-pho (เส่มิพอ) (Karen/Northern)].

S. glabra (Roxb.) Miers

[Phanang nang (ผะนังนั่ง) (Northern)].

S. glandulifera Miers

S. japonica (Thunb.) Miers

[Kon pit (ก้นปิด), bai kon pit (ใบก้นปิด) (Central); pang pon (ปังปอน) (Northern); tap tao (ตับเต่า), yan pot (ย่านปด) (Peninsular)].

S. oblata Craib

S. papillosa Craib*

S. pierrei Diels

[Bua khruea (บัวเครือ) (North-eastern); bua bok (บัวบก) (South-western, Eastern and Central); kot hua bua (โกฐหัวบัว), sabu lueat (สบู่เลือด) (Central)].

S. reticulata Forman

[Tap tao (ตับเต่า) (Peninsular)].

S. rotunda Lour.

S. suberosa Forman*

[Bua bok (บัวบก) (Central); boraphet phung chang (บอระเพ็ดพุงช้าง) (South-western)].

S. subpetata H.S. Lo

S. tomentella Forman*

S. venosa (Blume) Spreng.

[Plao lueat khruea (เปล้าเลือดเครือ) (Northern); cho koe tho (ชอเกอะทอ) (Karen/Northern); krathom lueat (กระท่อมเลือด) (North-eastern); kling klang dong (กลิ้งกลางดง) (South-western); boraphet yang daeng (บอระเพ็ดยางแดง) (Peninsular)].

20. *Tiliacora*

Tiliacora triandra (Colebr.) Diels

[Choi nang (จ๊อยนาง) (Northern); Thao ya nang (เถาย่านาง) (Eastern, Central); thaowan khieo (เถาวัลย์เขียว) (South-eastern)].

21. *Tinomiscium*

Tinomiscium petiolare Hook.f. & Thoms.

[Pharai hothong (ผ้าร้ายห่อทอง) (Peninsular)].

22. *Tinospora*

Tinospora baenzigeri Forman

[Chung cha ling (จุงจาลิง), Chingcha chali (ชิงช้าชาลี) (General)].

T. crispa (L.) Hook.f. & Thoms.

[Boraphet (บอระเพ็ด) (Central)].

T. siamensis Forman *

T. sinensis (Lour.) Merr.

[Ping kaling (ปิงกะลิง) (Northern); sali thao chali (สลีเถาชาลี) (Central)].

Arcangelisia

Lianes. **Leaves** palmately nerved, at base with small papillose region, on upper surface above insertion of petiole, hollow domatia, sometimes hairy, present in the axils of the nerves and main veins, lamina otherwise glabrous. **Inflorescences** axillary or cauliflorous paniculate with lateral branches spicate or subspicate. **Male flowers** sessile or subsessile. **Sepals** 9-10, glabrous; the outermost, 3-4 minute; larger inner sepals, 3+3. **Petals** 0. **Synandrium** a sessile, globose cluster of 9-12 anthers. **Female flowers**: **Sepals** as in male. **Petals** 0. **Staminodes** present. **Carpels** 3, stigma broad. **Infructescences** with club-shaped, unbranched carpophores. **Drupes** transversely subovoid, or subglobose with style-scar lateral, large; endocarp not sculptured but bearing a layer of radially arranged fibers; condyle inconspicuous or absent. **Seeds** broadly ellipsoidal; endosperm deeply ruminate; cotyledons divergent and much folded (Forman, 1991).

Two species, one is found in Hainan, Indochina, Peninsular Thailand, Malaya to New Guinea, the other is found only in New Guinea (Forman, 1991).

Key to species of *Arcangelisia*

1. Fruits transversely subovoid, 2.2-3.0 cm long, 2.5-3.3 cm broad (long axis) endocarp covered with a dense mat of radially arranged fibers **1. *A. flava***

1. Fruits subglobose, 4.5-5.5 cm diameter; endocarp bearing an interrupted layer of radially arranged fibers, which from a dense dorsal ridge as well as thin lateral transverse plates **2. *A. tympanopoda***

1. *Arcangelisia flava* (L.) Merr.

Synonym: *Menispermum flavum* L., *M. flavescens* (Lam.) DC., *Cocculus flavescens* (Lam.) DC., *Anamirta flavescens* (Lam.) Miq., *A. lemniscata Miers* , *A. luctuosa* Miers, *A. loureiri* Pierre, *Arcangelisia lemniscata* (Miers) Beccari, *A. inchyta* Beccari, *A. loureiri* (Pierre) Diels, *Mirtana loureiri* (Pierre) Pierre, *Tinospora havilandii* Diels

Thai name: Khamin khruea (ขมิ้นเครือ) (South-eastern); khamin ruesi (ขมิ้นฤาษี), hap (ฮับ) (Peninsular)

Other name: Yellow-fruited moonseed (English), Jamu (Indonesia), areuy ki koneng (Sundan), sirawan (Java), daun bulan (Moluccas), mengkunyit (Malaysia), abutra, suma (Philippines)

Distribution: Hainan, Indochina, Malaya to New Guinea.

Ecology: In evergreen forests; at low altitude.

Plant glabrous apart from leaf-domatia; stem with yellow wood and exuding yellow sap when cut, bearing prominent cut-like, petiole-scars. **Leaves** usually ovate, elliptic-ovate or broadly ovate, (10-)12-25 by (5.5)8-19 cm, base usually rounded, truncate or slightly cordate, apex abruptly acuminate, palmately 5-nerved, at the base and with 1-3 pairs of lateral nerves, usually arising from above halfway along the midrib; both surfaces usually drying matt with a rather obscure reticulum, coriaceous, petioles (4-)7-15(-20) cm, swollen at both ends, geniculate at base. **Inflorescences** 10-50 cm, lateral branches, 1-5 cm. **Male flowers** sessile or subsessile subtended by an ovate bracteole, 1 mm long, which is strongly thickened at the base; outer sepals, 3-4,

less than 1 mm long; inner sepals larger, 3+3, elliptic, ovate or narrowly obovate, 1.5-2.5 mm long. **Synandrium** 0.5-1 mm long. **Female flowers**: main **sepals** 6, narrowly oblong with the apex becoming reflexed, 2.5-4 mm long. **Staminodes** minute, scale-like. **Carpels** 3, 1.5 mm long; sigma broad, sessile, papillose. **Infructescences** cauliflorous, usually branched, (5-)7-30(-45) cm, with thickened axis and branches, 3-6 mm diameter, **the fruits** plus carpophores borne on the lateral branches, 1-3 borne together on a club-shaped, unbranched carpophore swollen at the apex, up to 4 cm. **Drupes** yellow, slightly laterally compressed, transversely subovoid, 2.2-3 by 2.5-3.3 cm (long axis), 2-2.5 cm thick, drying finely rugulose, glabrous; endocarp woody (Forman, 1991).

2. *Arcangelisia tympanopoda* (Lauterb. & K. Schum.) Diels

Distribution: New Guinea.

Incompletely known. **Leaves** apparently indistinguishable from those of *A. flava* (L.) Merr. **Male inflorescences and flowers** apparently as in *A. flava* (L.) Merr. **Female flowers** unknown. **Infructescence** cauliflorous, either slender, 2-3 mm diameter, unbranched, 23-40 cm long, bearing a gynophore at the end, or branched with axis up to 5 mm diameter; gynophores thick, claviform, 18-28 mm long. **Drupes** yellow, subglobose, slightly laterally compressed with a faint longitudinal dorsal ridge running all round, 4.5-5.5 cm diameter, surface drying finely granular, glabrous, endocarp woody, surface bearing an interrupted layer of radially arranged fibers, those forming a dense dorsal ridge as well as thin lateral, transverse plates (Forman, 1978).

Coscinium

Lianes with yellow sap. **Leaves** often peltate, palmately nerved, lamina tomentellous, often whitish below. Inflorescences supra-axillary or ramiflorous, composed of a raceme of peduncled, ± globose heads of flowers. **Male flowers**:

Sepals 9, imbricate in 3 whorls, externally sericeous. **Petals** 0. **Stamens** 6, the outer 3 free with 1-locular introrse anthers, the inner 3 with connate filaments and with 2-locular latrorse anthers. **Female flowers**: **Sepals** as in male. **Petals** 0. **Staminodes** 6. **Carpels** 3, densely pilose; style filiform recurved. **Infructescences** with globose carpophore. **Drupes** (only fully known in *C. fenestratum*) subglobose, style-scar sublateral; endocarp covered with anastomosing fibrous ridges; condyle deeply intrusive, thickly clavate. **Seeds** subglobose, hollow, enveloping the condyle; endosperm surrounding the divaricate, folded and divided cotyledons (Forman, 1991).

Two species in Ceylon, India, Thailand, Indochina, Malaya, Sumatra, Java and Borneo (Forman, 1991).

Key to the species of *Coscinium*

1. Lamina ± broadly ovate, less than 1½ times as long as broad, peltate with petiole inserted, 0.8(-2.7) cm from margin or not peltate, upper surface drying fairly smooth. Male flowers in several-flowered heads, about 7 mm diameter **1. *C. fenestratum***

1. Lamina elongate, more than 1½ times as long as broad, peltate with petiole inserted, 1.5-5 cm from margin, upper surface often drying rugose with main nerves markedly impressed. Male flowers in many-flowered heads, about 13 mm diameter. **2. *C. blumeanum***

1. *Coscinium fenestratum* (Gaertn.) Colebr.

Synonym: *Menispermum fenestratum* Gaertn., *Coscinium wallichianum* Miers, *C. blumeanum* auct. non Miers ex Hook.f. & Thoms., *C. maingayi* Pierre, C. usitatum Pierre, *C. blumeanum* Mies var.*epeltatum* Boerl., *C. wightianum* Miers, *C. miosepalum* Diels, *C. peltatum* Merr., *C. fenestratum* var. *macrophyllum* Yamamoto, *C. fenestratum* var. *ovalifolium* Yamamoto, *C. blumeanum* sensu Miq.

Thai name: Khruea hen (เครือเห็น) (North-eastern); kramin khruea (ขมิ้นเครือ) (South-eastern)

Other name: False columba (English), Vang dang (Cambodia), akar kuning, kunyit-kunyit, kunyit-kunyit babi, tol, kupak, kopak (Malaya), akar kuning (Java), abang asuh, binap kokop, upak-upak, perawan, dipang (Borneo)

Distribution: Ceylon, India, Indochina, Malay Peninsular, Sumatra, Java and Borneo.

Ecology: In evergreen forests; at 200 m altitude.

Leaves usually broadly ovate or ovate, rarely subpanduriform with basal, lateral lobes, 11-33 by 8-23 cm, base broadly rounded, truncate or shallowly cordate, rarely broadly obtuse, apex acuminate, upper surface glabrescent, usually drying smooth, midrib and other main nerves sunken; lower surface often whitish tomentellous, palmately 5-7 nerved at base and also usually two pairs of distal lateral nerves thinly coriaceous; petiole 3-16 cm, inserted up to 0.8(-2.7) cm from basal margin of lamina. **Inflorescences**: flowers in several-flowered, globose heads, 6-7 mm diameter, on peduncles 10-30 mm, arranged in a raceme 5-11 cm, inflorescences arising singly or a few together; bracts subulate, 4-5 mm long. **Male flowers** sessile or with pedicels, up to 1 mm. **Sepals** broadly elliptic to obovate; the inner 3-6 spreading, yellow, 1.5-2 mm long; the outermost. Smaller, 1-1.5 mm long, inserted lower. **Stamens** 6, 1 mm long. **Female flowers**: Sepals as in male flowers. **Staminodes** 6, claviform, 1 mm long. **Carpels** 3, curved-ellipsoidal, 2 mm long, densely pilose; style filiform, recurved. **Influctescences** with carpophore globose, tomentellous, 7-8 mm diameter, bearing 1-3 drupes. **Drupes** subglobose, tomentellous, brown to orange or yellowish, 2.8-3 cm diameter; pericarp drying woody, 1 mm thick; endocarp bony, 2.2-2.5 cm diameter, wall 3 mm thick covered with anastomosing fibrous ridges; condyle deeply intrusive, thickly clavate. **Seeds** whitish, subglobose, enveloping the condyle (Forman, 1991).

2. *Coscinium blumenum* Miers

Distribution: Malay Peninsula.

Ecology: In evergreen forests, on limestone rocks or near shore; at low altitudes.

Leaves, ± ovate (in Thailand), 12-35 by 6-20 cm, base broadly rounded or truncate, apex acuminate to rounded, upper surface glabrous, often drying, ± bullate, lower surface whitish tomentellous, palmately 7-11 nerved, at base with 2-3 pairs of distal lateral nerves, thinly coriaceous; petiole 6-20 cm, inserted 1.5-5 cm from basal margin of lamina. **Inflorescences**: flowers in globose, densely and ∞- flowered heads, 10-13 mm diameter on peduncles, 10-25 mm, arranged in a raceme, 12-14 mm; bracts inconspicuous, scale-like, 1-2 mm long. **Male flowers** with pedicels, 1.5-2 mm. The inner **sepals** 3-6, spreading at anthesis, broadly elliptic to spathulate-obovate, 2.5-3 mm long; the outermost 3 elliptic, 1.5-2 mm long, inserted lower. **Stamens** 6, 1 mm long. **Female flowers**: inner **sepals** 6, oblong to oblanceolate, 4-4.5 mm long. Staminodes 6. Carpels 3, as in *C. fenestratum*. **Drupes** tomentose to glabrescent in young state, otherwise unknown (Forman, 1991).

Fibraurea

Woody climbers with yellow wood, entirely glabrous, young stems smoothly and finely striate. **Leaves**, ± elliptic to ovate, base 3(-5)-nerved with the main basal laterals running alongside, the midrib for several (-15) mm, before curving outwards, with 2-4 pairs of distal lateral nerves. **Inflorescences** lax panicles, often ramiflorous. **Male flowers**: main **sepals** 6, with 2-3 minute outer ones. **Petals** 0. **Stamens** 3 or 6, the filaments thick with a prominent collar around the base of the anthers, dehiscence longitudinal to oblique. **Females flowers**: **Sepals** as in male. **Petals** 0. **Staminodes** 6, subulate. **Carpels** 3; stigma cleft-like. **Drupes** radiating from a small knob-like carpophore, drying coarsely wrinkled; endocarp subellipsoidal with ventral narrow longitudinal groove. **Seeds** subellipsoidal, with narrow longitudinal groove; endosperm abundant around the embryo; cotyledons thin, foliaceous (Forman, 1991).

A genus of two species, one with 6 stamens which widespread from the Nicobar, Burma, Peninsular Thailand, Indochina, South China, and from Sumatra and Malaya to Celebes and Philippines. The other with 3 stamens found only in South China, Vietnam and Cambodia (Forman, 1991).

Key to species of *Fibraurea*

1. Stamens 3. Wall of endocarp very thin firmly crustaceous, less than 0.5 mm thick **1. *F. recisa***

1. Stamens 6. Wall of endocarp much thicker, hard and rigid, 1 mm thick **2. *F. tinctoria***

1. *Fibraurea recisa* Pierre

Synonym: *F. tinctoria* sensu Diels

Other name: Tien sien tan, hoang ten (Chinese), Huang dang, hoang lien nam, day vang giang (Viet Nam)

Distribution: South China, Vietnam and Cambodia.

Ecology: In forests and thickets at 500-1200 m on various soils, including clayey, rocky, schistose granitic and dry sandy.

Woody climber. **Leaves** with petioles 4-10(-13) cm long, drying pale, markedly swollen and geniculate at base, sometimes inserted up to 1.5 mm above the basal margin of lamina; lamina elliptic to subovate oblong-elliptic, base rounded to cuneate, apex acuminate to obtuse, (8-)11-23 x (4-)6-13 cm, upper surface usually drying olivaceous and closely wrinkled, stiffly papyraceous. **Inflorescences** arising from older, leafless stems, lax panicles 11(young)-35 cm long with lateral branches up to 9 cm long. **Male flowers** on pedicels 1.5-4 mm long, main **sepals** broadly elliptic, concave, 2.5 mm long. **Stamens** 3, 2 mm long, filaments thick and broad, widened at the top, anthers broadly rounded at apex with oblique introrse slits. **Female flowers** unknown. **Drupes** yellow on pedicels 1 cm long terminated by a small knob-like carpophore, drying coarsely wrinkled; endocarp 2-2.5 cm long, wall less than 0.5 mm thick, firmly crustaceous (Forman, 1985).

2. *Fibraurea tinctoria* Lour.

Synonym: *Cocculus fibraurea* DC., C. rimosus Blume, *Menispermum tinctorium*

(Lour.) Sprengel, *Fibraurea chloroleuca* Miers, *F. fasciculata* Miers, *F. laxa* Miers, *F. trotteri* Watt ex Diels, *Tinomiscium nicobaricum* Balakrishnan

Thai name: Khamin ruesi (ขมิ้นฤาษี), khamin khruea (ขมิ้นเครือ), man miat (มันเมียด) (Peninsular); thaowan thong (เถาวัลย์ทอง) (South-western); kamphaeng chet chan (กำแพงเจ็ดชั้น) (Central)

Other name: Sekunyit (Malaya), akar stupai, akar kunyit, olor labai (Sumatra), areuy kikoneng (Java)

Distribution: India, Burma to Indo-china, Malay Peninsular and Sumatra to Celebes and Philippines.

Ecology: Locally common in dry evergreen forest, also in bamboo forest and scrub; up to 100 m altitude. Flowering February-May; fruiting April-May.

Large woody climber, up to 40 m, stem containing white latex. **Leaves** elliptic, elliptic-ovate to ovate or oblong-elliptic, (9-)11-21(-28) by (3.5-)5-14 cm, base sometimes subpeltate, usually rounded, apex acuminate, often shortly so, upper surface often drying greyish and smooth with reticulation obscure; thinly coriaceous; petiole (2-)4-13 cm, often drying blackish at least at the swollen base. **Inflorescences** axillary or ramiflorous, 10-38 cm, with lateral branches, up to 12 cm. **Male flowers** sweetly scented, on pedicels, up to 5 mm or sessile, **Main sepals** white or yellow, broadly elliptic, concave, 2.5-4 mm long. **Stamens** 6, 2-2.5 mm long, filament thickly columnar, incurved. **Female flowers**: **sepals** and **petals** as in male. **Staminodes** subulate, 2 mm. **Carpels** ellipsoidal, 1.75 mm long; stigma cleft-like, small. **Infructescences** often ramiflorous, up to 55 cm. **Drupes** yellow to orange on pedicels, 6-15 mm; endocarp 2-2.5 cm long, wall 1 mm thick, hard and rigid (Forman, 1991).

1.2 Combretaceae

Trees, shrubs or lianas, rarely subherbaceous. **Leaves** opposite, verticillate, spiral, or alternate, petioled (rarely sessile), exstipulate, simple, almost always entire. **Flowers** bisexual or unisexual, usually actiomorphic, rarely slighhtly zygomorphic, in axillary or extra-axillary elongated or subcapitate spikes or racemes or in terminal and sometimes axillary panicles. **Calyx-lobes** 4 or 5 (rarely 6-8) or almost absent. sometimes acccrescent. **Petals** 4 or 5 or absent. **Stamens** usually twice as many as the petals, borne inside the upper receptacle usually in two series, exserted or included, anthers dorsifixed, usually versatile (or rarely adnate to the filaments). **Style** usually free. **Ovary** inferior, unilocular, with usually 2 (sometimes 2-6). **Fruit** freshy or dry, usually indehiscent, often variously winged or ridged, 1-seed. Albumen absent (van Steenis, 1954).

The family is distributed throughout the tropics, subtropics; 18 genera with about 450 species distribute in North Australia, Queensland, tropical Asia, Africa, America and Madagascar (van Steenis, 1954).

Combretum spp. are shrubs or woody climbers, rarely small tree; deciduous. **Indumentum** simple hairs or glandular hairs (stalk glands) or scales; most prominent on young parts, leaves, flower and fruit. **Leaves** usually opposite, rarely ternate; hairy or glabrous, often conspicuously scaly; part of petiole sometimes persisting after the leaves are shed forming a thorn, pubescent or glabrous, without glands. **Inflorescence** spikes, racemes or panicles often subtended by bracts. **Flower** bisexual, 4-or 5-merous, sessile or shortly pedicillate, often scaly. **Calyx** cupuliform or infundibuliform, caducuous. **Petals** 4, or usually early caducuous, or absent (in *C. apetalum*). **Stamens** twice the number of calyx-segments. **Ovary** with 2-4 ovules; style free. **Fruit** a drupe with 5 ridges, or a 4-, or 5-winged nut (Weerachai Nanakorn, 1986).

The genus *Combretum* occurs throughout the tropics, except in Australia and Pacific island. It is abundant in tropical Africa with more than 100 species. In Asia about 35 species are represented. Nineteen species of *Combretum* are enumerated for Thailand and 2 are endemic (shown by asterisk) (Weerachai Nanakorn, 1986).

All species of *Combretum* in Thailand are as follows:

1. *Combretum acuminatum* Roxb.

   [Khamin khruea (ขมิ้นเครือ) (Prachin buri, Saraburi)].

2. *C. apetalum* Wall.

   [Dok soi (ดอกสร้อย) (Central, Northern)].

3. *C. chinense* Roxb.

   [Sakae thao (สะแกเถา) (Peninsular)].

4. *C. decandrum* Roxb.

   [Sakae khruea (สะแกเครือ) (Peninsular)].

5. *C. deciduum* Coll. et Hemsl.

   [Haen khruea (แหนเครือ) (Northern)].

6. *C. griffithii* Heurck & Muell.

   [Chaang mang (จ้างมั่ง), chaang mag noi (จ้างมั่งน้อย)(Chiag Mai); haen khruea tua phuu (แหนเครือตัวผู้) (Northern)].

7. *C. latifolium* Blume

   [Khamin khruea (ขมิ้นเครือ) (Prachin buri) เสงี่ยม พงษ์บุญรอด, 2493; ก่องกานดา ชยามฤต, 2528); kae dam (แกดำ) (Nong Khae); thua pae thao (ถั่วแป๊เถา) (Chiang Mai); man daeng (มันแดง), khruea uat chueak (เครืออวดเชือก) (Peninsular); haen lueang (แหนเหลือง) (Kahanaburi) (Weerachai Nanakorn, 1986)].

8. *C. nanum* Buch.

   [Kae dam (แกดำ) (Nong Khai, Laos)].

9. *C. nigrescens* King

   [Sakai (สะไก๋) (Peninsular)].

10. *C. pilosum* Roxb.

    [Khruea khao muak (เครือเขามวก) (Nong Khai); nguang chum (งวงชุ่ม) (Nakhon Phanom); teentang tuamae (ตีนตั่งตัวแม่) (Lampang)].

11. *C. porterianum* (Clarke) Wall. ex Craib

[Nuai sut (หน่วยสุด) (Peninsular)].

12. *C. procursum* Craib*

[Sakae wan (สะแกวัลย์)].

13. *C. punctatum* Blume subsp. *punctatum*

[Sakae khruea (สะแกเครือ), sakae wan (สะแกวัลย์) (Northern)].

C. punctatum Blume subsp. *squamosum* (Roxb. ex G. Don)

[Sakae nuai (สะแกหน่วย) (Peninsular); sakae wan (สะแกวัลย์) (Central)].

14. *C. quadrangulare* Kurz

[Kae (แก) (North-eastern); khon khae (ขอนแข้), chong khae (จองแข้) (Phrae); sang kae (ซังแก)(Khmer-Prachin Buri); phaeng (แพ่ง) (Northern); sakae (สะแก), sakae naa (สะแกนา) (Central)].

15. *C. sundaicum* Miq.

[Sangkae thao (ซังแกเถา) (Peninsular); Akar gambir (อะกาแกเบ้อร์) (Malaysia)].

16. *C. tetralophum* Clarke

[Krot (กรด), thaowan krot (เถาวัลย์กรด) (Central); phum kot (พุ่มกด) (Phitsanulok); sakai nam (สะไก้น้ำ) (Narathiwat); ee-la-ku (อีลากู) (Yawee-Narathiwat)].

17. *C. trifoliatum* Vent.

[Khot sang (คดสัง), yaan tut (ย่านตุด) (Surat Thani); chut (จุด, ชุด) (Peninsular); ben (เบน) (Khon kaen); puei (เปือย) (Nakhon Phanom); yaa yotdam (หญ้ายอดดำ) (Nortern)].

18. *C. winitii* Craib*

[Khruea ma thua nao (เครือมะถั่วเน่า) (Northern)].

19. *C. yunnanense* Exell

[Chaang mang (จ้างมั่ง) (Norhern)].

Combretum latifolium **Blume**

Synonym: *C. extensum* Roxb.

Thai name: Khamin khruea (ขมิ้นเครือ) (Prachin buri) (เสงี่ยม พงษ์บุญรอด, 2493; ก่องกานดา ชยามฤต, 2528); Kae dam (แกดำ) (Nong Khae); thua pae thao (ถั่วแป๊เถา) (Chiang Mai); man daeng (มันแดง), khruea uatcuueak (เครืออวดเชือก) (Peninsular); haen lueang (แหนเหลือง) (Kahanaburi) (Weerachai Nanakorn, 1986).

Distribution: India. Ceylon, Burma, South China (Yunnan Kwangtung), Indochina, Malay Peninsula, New Guinea and Philippines.

Ecology: Common along the edge of the forests, on sandy soil, granite and limestone in open areas near streams and river banks, up to 600 m altitude.

Liana, young parts sparsely pubescent soon glabrous, scaly. **Leaves** coriaceous, broadly elliptic to ovate-elliptic, 8-12(-18.5) by 5-9.5(-13) cm; apex acuminate, base obtuse or slightly cuneate, all parts glabrous; scales, sparse beneath, translucent to pale yellowish, hardly observable; nerves wide spaced, 5-8 pairs; petiole 1.2-2.5 cm, glabrous. **Inflorescence** axillary spikes, usually unbranched or terminal panicles, rachis densely pubescent; spikes 5–10 cm. **Flowers** greenish-white or greenish-yellow, 4-merous, infundibuliform, 10-13 mm, subsessile, outside puberulous with few scales; bracts filiform, very small, early caducuous. **Calyx** funnel-shaped, 6-7 mm long by 3-4 mm in diameter; calyx-segments triangular with acute apex, 3-4 mm long, mature usually recurved; calyx-tube narrowly tubular, 5-6 mm, expanded at the top. **Petals** suborbicular or broadly-oblong, apex emarginate, shorter than the calyx-segments, 1-2 mm. **Stamens** 5-6 mm; disc annular, villous. **Ovary** shortly ellipsoid, 2-2.5 mm; style 8-8.5 mm; ovules 2 or 3. **Fruit** a 4-winged nut, suborbicular to broadly elliptic in outline, 2.5-4 by 2-3.2 cm; puberulous; nut scaly; wings thin, flexible, 1-1.3 cm broad; stipe 2-5 mm long, puberulous (Weerachai Nanakorn, 1986).

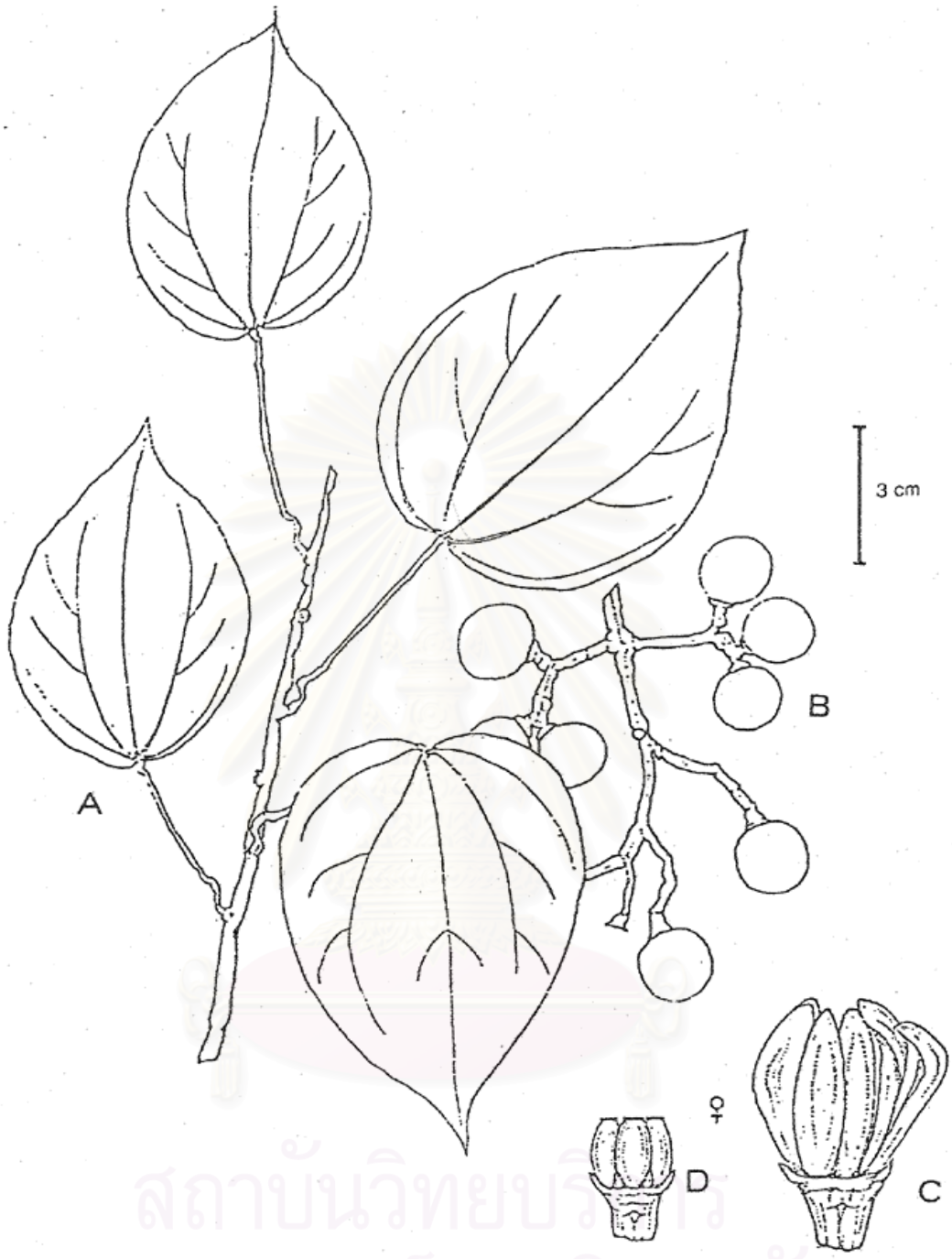


Figure 1. *Arcangelisia flava* (L.) Merr. (ถนอมหวัง อมาตยกุล และ ดรุณ เพ็ชรพลาย, 2517)

A. Leaves B. Fruits

C. Female flower D. Ovaries

(C.-D. Enlarged)

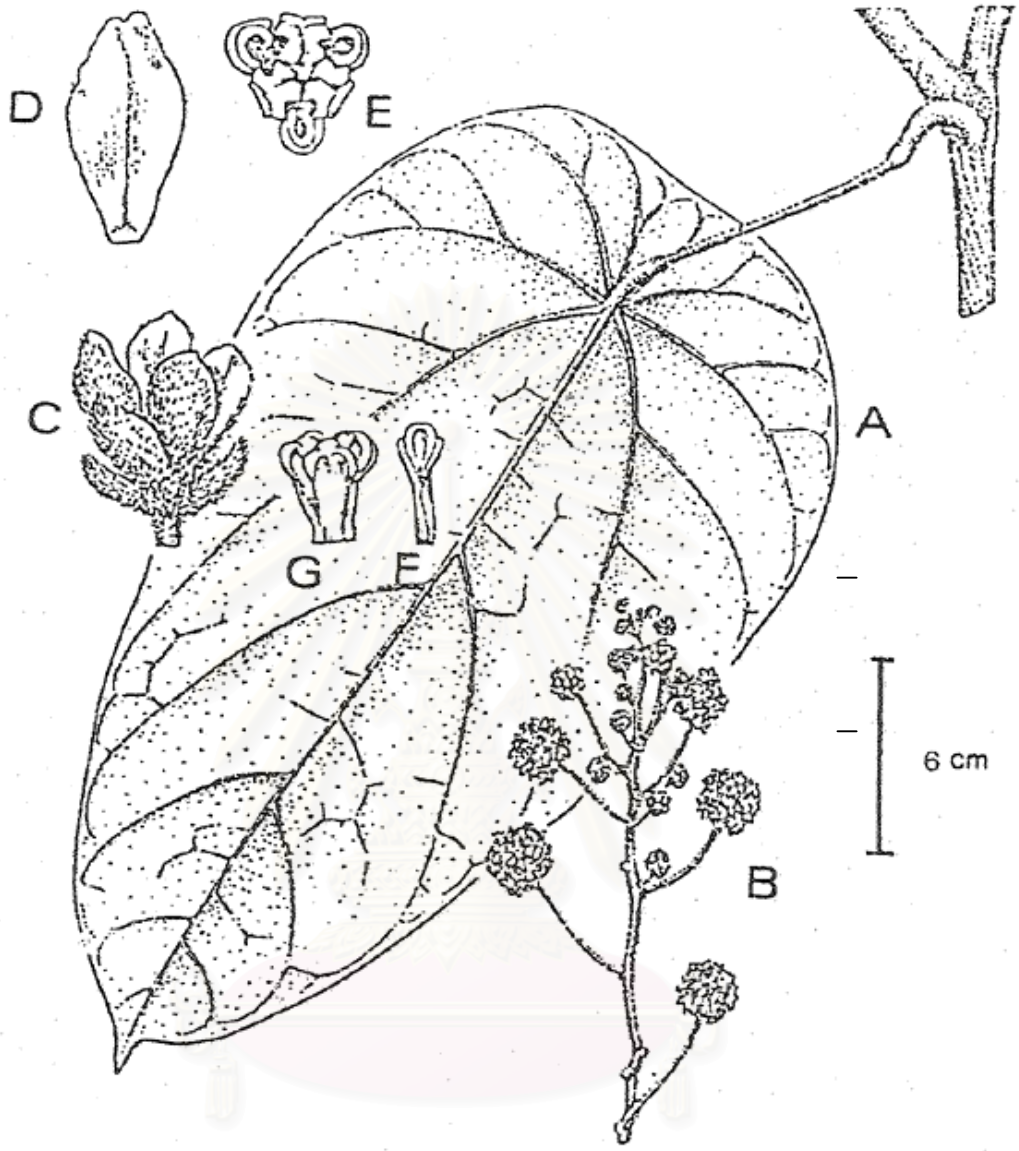


Figure 2. *Coscinium blumeanum* Miers (Forman, 1991)

A. Leaf B. Male inflorescence C. Male flower
D. Sepal E. Anthers (top view) F. Stamen (outer)
G. Stamen (inner) (C.-G. Enlarged)

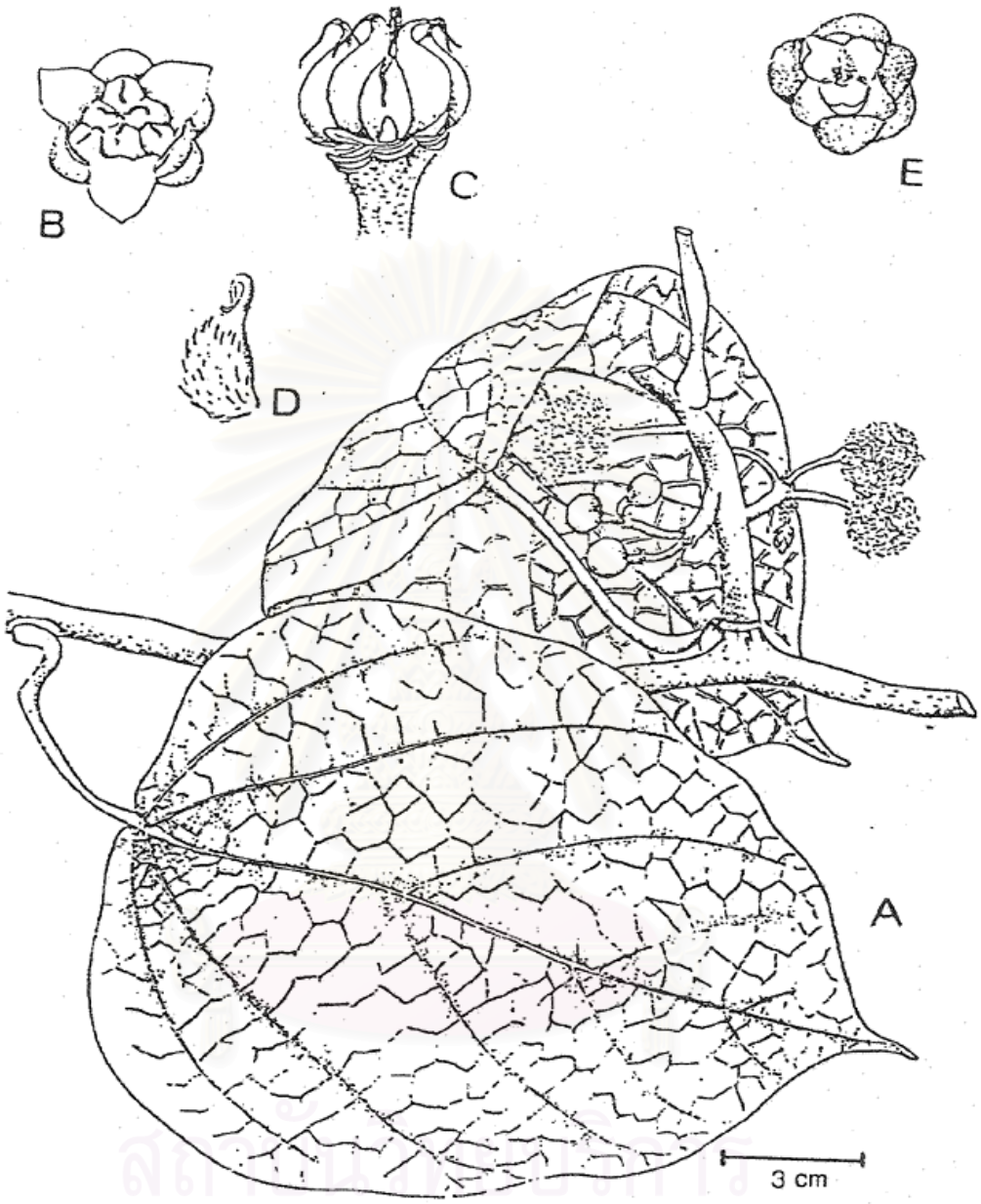


Figure 3. *Coscinium fenestratum* (Gaertn.) Colebr. (Jayaweera, 1982)

A. Branch with leaves and male flower heads B. Female flower

C. Female flower with calyx and corolla removed showing the carpels

D. Carpel E. Stamens (B.-E. Enlarged)

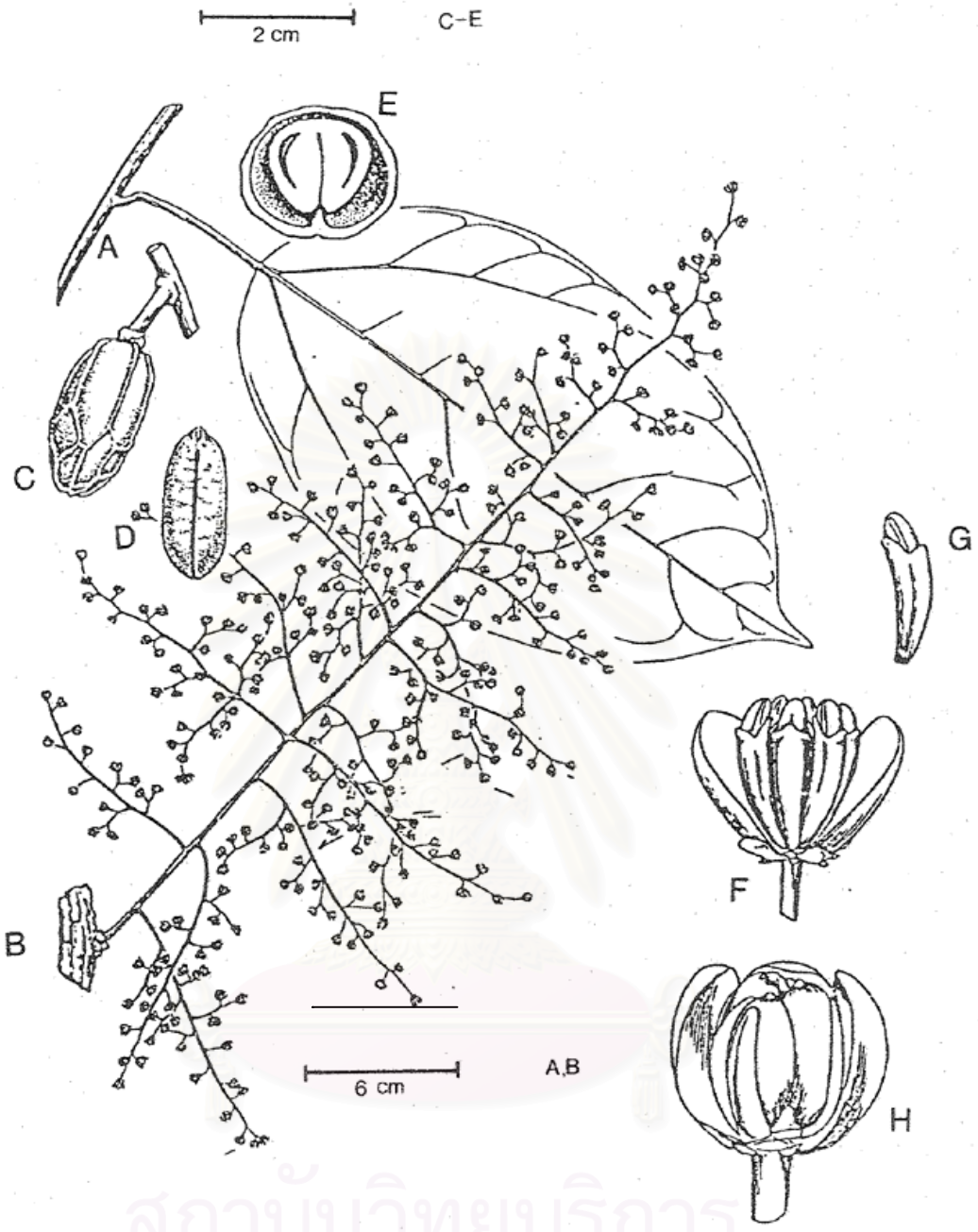


Figure 4. *Fibraurea tinctoria* Lour. (Forman, 1991)

A. Leaf B. Male inflorescence C. Male flower

D. Stamen E. Female flower

F. Drupe G. Endocarp

H. Endocarp (transverse section) (C.-E. Enlarged)

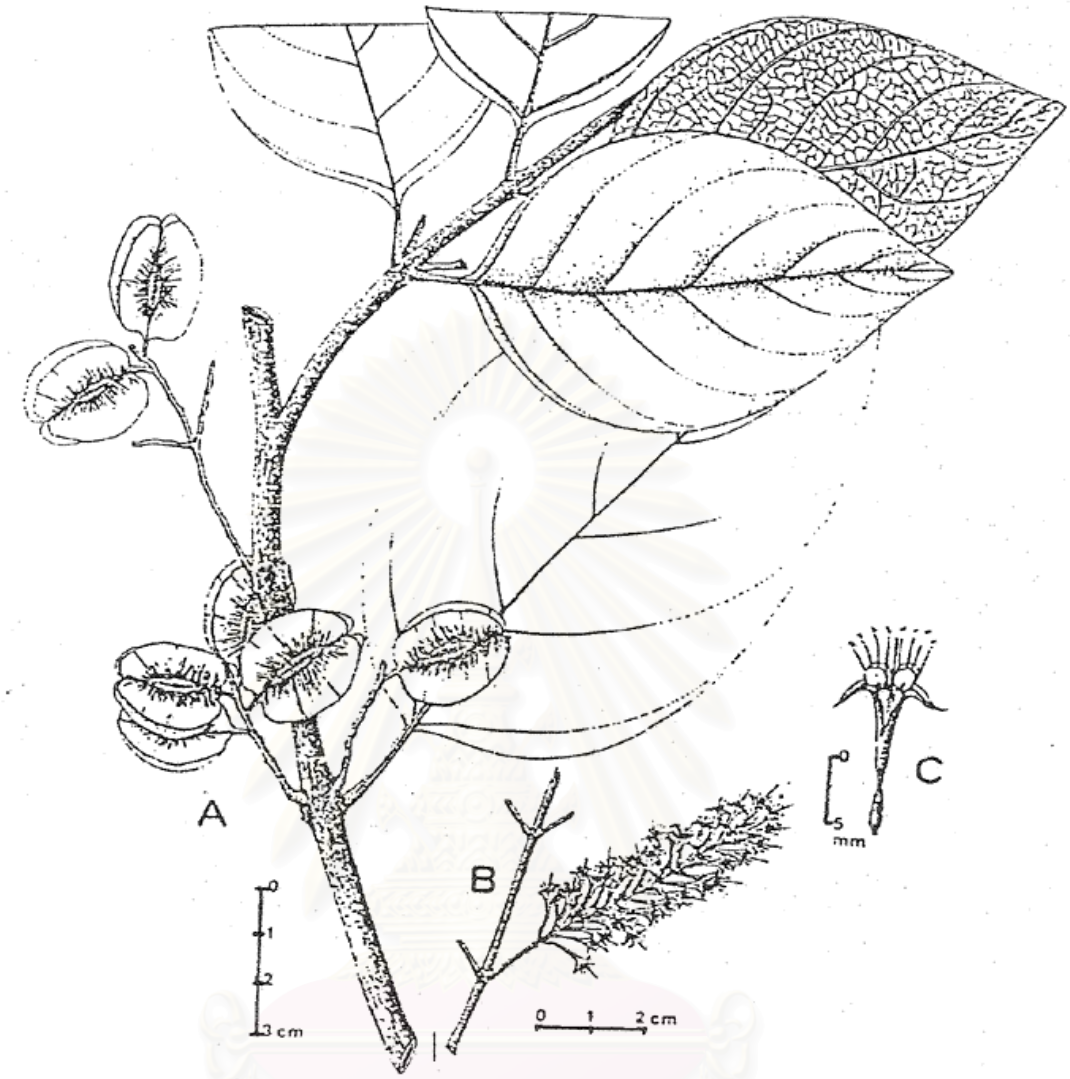


Figure 5. *Combretum latifolium* Blume (Weerachai Nanakorn, 1986)
A. Fruiting twig B. Inflorescence C. Flower

2. Pollen morphology of tribe *Coscinieae* and tribe *Fibraureae*

2.1 Tribe *Coscinieae*

The pollen morphology of 3 genera and 5 species comprising in tribe *Coscinieae* has been studied by Ferguson (1978). The pollen grains in genera of the tribe *Coscinieae* of the Menispermaceae are single, isopolar and tricolporate. The average size is generally small being approximately P (Polar length) 13-23 μm, E (Equatorial width) 12-17 μm. The shape ranges from prolate in *Arcangelisia* (P/E 1.45) to spheroidal in *Anamirta* (P/E 1.1) to oblate-spheroidal in *Coscinium* (P/E 0.9). The ectoapertures are sunken colpi covered with a granular membrane in *Arcangelisia* and *Anamirta*, and rather sunken elliptical pores covered with a finely granular membrane in *Coscinium*. The endoapertures are discrete but clearly evident except in *Coscinium* which has very discrete thinnings adjacent to the pores. The tectum is either partial, reticulate or perforate, smooth. Granules may or may not be present in the lumina.

The pollen morphology of the genus *Coscinium* is very distinctive in the tribe and this fact supports the taxonomic results of Forman (1978) who regards this genus as being rather more isolate from *Arcangelisia* and *Anamirta*, the other two genera in the tribe. These two latter genera Forman suggest are more closely related to each other than *Coscinium* and the palynological evidence would support this view.

The pollen morphology of the tribe *Coscineae* appears to show no distinctive palynological characters when considered in relation to that of other tribes in the Menispermaceae. Very similar pollen types to those found in *Arcangelisia* and *Anamirta* in the investigation occur in the tribe *Triclisieae* and also in the tribe *Cocculeae*. The ectoapertures of *Coscinium* are not dissimilar to those found in *Stephania* (Tribe *Cocculeae*).

Pollen types

The pollen can be divided into two very distinct types and two further subtypes as follows:

Type 1 Pollen grains oblate-spheroidal, triporate. Tectum reticulate, lumina with fine granules. Found to occur in the genus *Coscinium*. It is not possible to separate the two species of the genus on the basis of pollen morphology.

Type 2 Pollen grains tricolporate. Tectum perforate or finely reticulate.

Subtype 2a Pollen grains spheroidal P/E 1.1. Found in the monotypic genus *Anamirta*.

Subtype 2b Pollen grains prolate P/E 1.45. Found in the two species of *Arcangelisia*. It has not been found possible to distinguish the species on the basis of pollen morphology.

Pollen description of genus *Arcangelisia*

Pollen grains prolate, P/E 1.45, equatorial outline trilobed, tricolporate. Size: P (18-)20.4(-23) μm, E (12-)14(16) μm. Colpi 13-15 μm long, 2.5 μm wide at the equator, sunken, ends acute or very slightly rounded, membrane rather coarsely granular-papillose. Endoapertures elliptical, meridionally elongated, 3-3.5 μm long, 1.5-2 μm wide. Total wall thickness 1-1.5 μm. Ectexine much thicker than endexine plus foot layer. Foot layer narrow, less than 0.1 μm thick. Endexine (excluding foot layer) 0.2 μm thick. Endexine 1, 0.2 μm thick, much thicker towards the apertures, endexine 2 very narrow and diffuse corresponding to the very finely granular papillose inner surface of acetolysed pollen grain walls seen with the S.E.M.(Scanning Electron Microscopy). Columellae 0.5 μm high. Tectum perforate or sometimes finely reticulate, complete on the margins of the colpi, microperforations 0.1 μm or larger lumina (when finely reticulate in some samples) 0.4-0.6 μm in diameter, generally isodiametric or elliptical. Muri 0.3-0.6 μm wide, 0.3 μm high, cylindrical, smooth.

Pollen description of genus *Coscinium*

Pollen grains oblate-spheroidal or rarely spheroidal, P/E 0.9, equatorial outline circular or slightly elliptical, triporate. Size: P (13-)14.2(-17) μm, E (14-)15.1(-17) μm. Pores elliptical 2.5-3 μm long, 1 μm wide, membrane finely granular.

Endoapertures : there appears to be a slight thinning of the endexine adjacent to the pores in T.E.M. (Transmission Electron Microscopy) section. Total wall thickness 1-1.5 μm. Ektexine much thicker than endexine plus foot layer. Foot layer narrow, less than 0.1 μm thick. Endexine (excluding foot layer) 0.3-0.4 μm thick. Endexine 1, 0.3 μm thicker towards the apertures; endexine 2 very narrow and diffuse corresponding to the finely granular papillose inner surface of acetolysed pollen grain walls seen with the S.E.M.. Columellae 0.4-0.5 μm high. Tectum reticulate, lumina rather variable in size from 1.0-3 μm in diameter (the main variation is between samples and not within samples), irregular in shape (muri often undulate), with fine granules 0.1-0.2 μm high. Muri 0.4 μm wide, 0.3 μm high, cylindrical, smooth.

2.2 Tribe *Fibraureae*

The Pollen morphology of 4 genera and 5 species of tribe *Fibraureae* has been studied by Harley (1985). Pollen grains in this group are single, isopolar and usually tricolpate-operculate or tricolporate. Pollen size is small, ranging from 11-12 μm polar length in *Borismene japurensis* to 19 μm in two of the four collections of *Tinomiscium petiolare*. Pollen shape is subprolate, prolate-spheroidal or, occasionally ± spheroidal. The equatorial outline is ± circular with regularly spaced apertures in *Borimene* and *Burasaia* whilst in *Fibraurea* and *Tinomiscium* it is trilobed and fossaperturate. Colpus length ranges from about 2/3 the total polar length in *Burasaia* to about 3/4 the total polar length in *Fibraurea* and *Tinomiscium*.

The endoapertures in both *Fibraurea* and *Tinomiscium* are distinctly lolongate; in *Borismene* they are apparently small and ± circular. *Burasaia* has no endoaperture but the colpus is covered by a narrow operculum. In the other three genera the colpus membrane supports slightly anastomosing granular ectexinous elements; the coarseness of these granules varies slightly between collections and genera.

Wall stratification is comparable with that seen in other tribes of the family. The foot layer is very thin in *Burasaia madagascariensis* and *Fibraurea recisa*; in

Tinomiscium petiolare, it is much thicker. About 5/6 thick of the mesocolpial wall thickness, in all species examined, consists of ectexine. Towards and across the apertural region the endexine is much thicker, 1/2 - 2/3 of the total wall thickness. There appears to be no notable reduction in the thickness of the endexine in the region subtending the aperture. More or less vertical interruptions in the endexine are apparent on either side of the aperture in transverse section and the endexious material appears less dense than in the areas immediately preceding and under the apertures.

Pollen grains in the *Fibraureae* with a perforate tectum have proportionately more columellae than those with a reticulate tectum. This has also been noted in the Menispermeae. The narrow, darker staining inner layer of the endexine, observed in other members of the family, is also present. Total wall thickness ranges from 0.7-0.9 μm. The tectum of *Tinomiscium* and *Fibraurea* is coarsely perforate, the density of the perforations varies slightly between species and also between collections. A very slight reduction in perforation size is sometimes apparent along the colpus margins.

Pollen types

Type 1 Pollen grains prolate-spheroidal, rarely subprolate, amb trilobed, tricolporate, narrowly lolongate endoapertures, 4-9 μm in length, fossaperturate. Colpus narrow, colpal membrane supporting slightly anastomosing, fine-coarse granular ectexinous elements. Tectum coarsely perforate smooth. Species included : *Tinomiscium petiolare*, *Fibraurea recisa* and *F. tinctoria*.

Type 2 Pollen grains prolate-spheroidal, amb circular, tricolpate-operculate, apertures regularly spaced. Colpus narrow covered by an operculum consisting of + fused ectexinous elements ornamented with spinulae. Tectum reticulate, ornamented with spinulae. Species included : *Burasaia mdagascariensis.*

Type 3 Pollen grains + spheroidal, amb circular, tricolporate, apparently small circular endoapertures, apertures regularly spaced. Colpus narrow ornamented with slightly anastomosing granular elements. Tectum reticulate, muri smooth. Species included : *Borismene japurensis*.

Pollen description of genus *Fibraurea*

Pollen grains prolate-spheroidal, P/E 1.10 (*F. tinctoria*) & 1.12 (*F. recisa*), and trilobed, tricolporate, fossaperturate. Size: P (15-)16.2(-17) μm, E (14-)15.1(-16) μm. Colpi 3/4 the length of the polar axis; the colpus is narrow and the underlying membrane supports slightly anastomosinng granular ectexinous elements. Endoapertures narrowly lolongate (*i.e.* elongated in the direction of the polar axis) 5–8 μm long. Total wall thickness 0.9-1 μm. Sexine thicker than nexine in mesocolpial region. Endexine 0.15-0.2 μm thick, noticeably thicker towards and under the colpus, 0.8-0.9 μm. There is apparently no reduction of endexine beneath the endapertures; there are however, partial interruptions in the endexine on either side of the aperture which suggest that the endexine form an endoapertural “plug”. Foot layer thin, 0.05 μm, continuous. Columellae short, 0.2-0.25 μm. Tectum coarse perforate, 0.3-0.5 μm thick, smooth, perforations 0.1-0.5 μm in diameter.

3. The chemical constituents

The chemical constituents of “Khamin khruea” in the family Menispermaceae have been investigated as summarized in Table 1-4. However, the *Combretum latifolium* Blume (*C. extensum* Roxb.) is the Khamin khruea of which the phytochemical study has not been carried out.

Table 1. The chemical constituents of *Arcangelisia flava* (L.) Merr.

Plant part	Category	Chemical constituent	Reference
Stem	Alkaloid	Berberine, palmatine, columbamine, jatrorrhizine	Santos, 1931
Stem	Alkaloid	Berberine, palmatine, jatrorrhizine	Garcia *et al*., 1970
Root	Alkaloid	Berberine, jatrorrhizine	
Stem and root	Alkaloid	Berberine, jatrorrhizine, pycnarrhine, dehydrocorydalmine, thalifendine, palmatine, hydroxyberberine, limacine, homoaromoline	Verpoorte *et al*., 1982
Stem	Furanoditerpene	6-Hydroxyarcangelisin, 2-dehydroarcangelisinol, tinophyllol, 6-hydroxyfibleucin, fibleucin, fibraurin, 6-hydroxyfibraurin	Kunii *et al*., 1985 and Kawakami *et al*., 1987
Root	Alkaloid	Berberine, palmatine, jatrorrhizine, 8-hydroxyberberine, tetrahydropalmatine	กัลยา ภราไดย และ คณะ, 2532
Stem	Alkane	Heneitetradecan-1-ol, nonadecan-1-ol, octadecane, tetradecan-4-ol	Agusta and Dan Chairul, 1996
	Monoterpene	Limonene	
	Lipid	Linoleic acid	

Table 2. The chemical constituents of *Coscinium fenestratum* (Gaertn.) Colebr.

Plant part	Category	Chemical constituent	Reference
Stem	Alkaloids	Berberine, palmatine, jatrorrhizine	Garcia *et al*, 1970
Root			
Stem and root	Alkaloid	Berberine, berberrubine, jatrorrhizine, N,N-dimethyllindcarpine, palmatine, thalifendine	Siwon *et al*., 1980
Stem	Alkaloid	Berberine, oxyberberine, tetrahydroberberine, 12,13-dihydro-8-oxo-berberine	Malhotra *et al*., 1989
	Steroid	Sitosterol and stigmasterol	
Stem	Alkaloid	Berberine, oxyberberine, oxypalmatine, 8-oxocanadine, 8-oxotetra hydrothalifendine, 8-oxoisocorypalmine, 8-oxothaicanine	Pinho *et al*., 1992
Stem	Alkaloid	Berberine, jatrorrhizine, tetrahydropalmatine, crebanine	Supranee Keawpradub, 1992

Table 3. The chemical constituents of *Coscinium blumeanum* Miers.

Plant part	Category	Chemical constituent	Reference
Not stated	Alkaloid	Berberine	Tamita and Tani, 1941

Table 4. The chemical constituents of *Fibraurea tinctoria* Lour.

Plant part	Category	Chemical constituent	Reference
Tuber	Alkaloid	Palmatine, jatrorrhizine	Tamita and Tani, 1941
Root	Alkaloid	Palmatine	Chu *et al.*, 1962
Bark and stem	Furanoditerpene	Fibraurin, chasmanthin 6-hydroxyfibraurin	Hori *et al*., 1967
Stem and root	Alkaloid	Magnoflorine, pseudocolumbamine, dehydrocorydalmine, palmatrubine, berberine, berberrubine	Siwon *et al*., 1981
Stem	Alkaloid	Palmatine, jatrorrhizine	Boonyaprakarn *et al*., 1983
Stem	Protein	Protein	Pongpan *et al*., 1983
Rhizome	Furanoditerpene	Fibleucin, fibraurin	Itokawa *et al*., 1986
	Furanoditerpene glucoside	Tinophylloloside, fibleucinoside, fibraurinoside	
Entire plant	Alkaloid	Tetrahydropalmatine	Pan *et al*., 1988
Stem	Furanoditerpene	Fibraurin, chasmanthin, palmarin	Zakaria *et al*., 1989
Root	Alkaloid	Berberine chloride, Berberrubine chloride, thalifendine chloride	Dai *et al*., 1993
	Phytoecdysteroid	20-Hydroxyecdysone	

Some alkaloids which are present in the plants under the name “Khamin khruea”:

Berberine ($C_{20}H_{18}O_4N$)

This protoberberine alkaloid was first isolated from *Xanthoxylum clava* Herculis under the name of “Xanthopicrit” and obtained independently from *Berberis vulgaris* L. (Berberidaceae). The base occurs in several plants including those of the Ranunculaceae (*Coptis japonica* Makino, *C. trifolia* (L.) Salisb., *C. occidentalis* Torr. and *Hydrastis canadensis* L. and *Thalictrum foliolosum* DC.), the Berberidaceae (*Berberis buxifolia* Lam., *B. darwinii* Hook, *B. glauca* DC., *B. nervosa* Pursh, *Mahonia aquifolium* Nutt., *M. trifoliata* Fedde, and *Nandinna domestica* Thunb.), the Annonaceae (*Coelocline polycarpa* DC.), the Menispermaceae (*Arcangelisia flava* (L.) Merr., *Coscinium blumeanum* Miers, *C. fenestratum* (Gaertn.) Colebr.), the Papaveraceae (*Argemone mexicana* L., *Chelidonium majus* L., *Corydalis cheilantheifolia* Hemsl., *C. ophiocarpa* Hook.) and the Rutaceae (*Evodia meliifolia* Benth, *Phellodendron amurense* Rupr., *Toddalia aculeata* Pers. and *Zanthoxylum caribaeum* Lam.).

The alkaloid crystallizes (melting point 145°C) from water or aqueous ethanol as the hexahydrate or from chloroform with one mole of solvent as yellow needles. The basic is readily purified via the acetone compound, acetone which forms reddish-yellow tablets. The salts are mostly yellow in color and crystallize well: the hydrochloride dihydrate as small yellow needles; the hydriodide also as yellow needles; the sulphate as slender yellow needles and the nitrate a green-yellow needles. The phosphate sesquihydrate is a bright yellow and also crystalline (Glasby, 1975).

Columbamine ($C_{20}H_{21}O_5N$)

A quaternary alkaloid which has been isolated from the root bark of various species including Ranunculaceae (*Coptis japonica* Mak.), Berberidaceae (*Berberis heteropoda* Schrenk, *B. lambertii*, *B. japonica* Mak., *B. thunbergii* DC. var. *maximowiczii*, *B. vulgaris* L.), and Menispermaceae (*Arcangelisia flava* (L.) Merr., *Jateorhiza palmata* (Lam.) Miers).

The iodide has melting point 223-224°C and is the form in which the alkaloid is normally isolated. When reduced with zinc in acetic-sulphuric acid, the base yields

the tetrahydro derivative, melting point 223°C which furnishes tetrahydropalmatine with CH_2N_2 (Glasby, 1975).

Jatrorrhizine (Jateorhizine) ($C_{20}H_{20}O_4N^+$)

This quaternary protoberberine alkaloid is unknown in the free state and is obtained as the iodide, yellow-red needles of the monohydrate, melting point 208-210°C or the chloride, yellow needles, again of the monohydrate, melting point 206°C. The alkaloid occurs both in the Berberidaceae (*Berberis heteropoda* Schrenk., *B. thunbergii* DC. var. *maximowiczii*, *B. vulgaris* L., and *Mahonia philippinensis* Nutt.) and in the Menispermaceae (*Arcangelisia flava* (L.) Merr., *Coscinium blumenum* Miers., *C. fenestratum* (Gaertn.) Colebr., *Fibraurea chloroleuca* Miers., and *Jateorhiza palmata* Lam.).

A crystalline nitrate, melting point 225°C and a picrate, melting point 217-220°C have also been prepared. With CH_2N_2, the alkaloid yields the O-methyl derivative in the form of the iodide which is identical with palmatine iodide. On reduction, the iodide is converted into *dl*-tetrahydrojatrorrhizine, melting point 217-218 °C, the *d*-form of which is corypalmine (Glasby, 1975).

Magnoflorine ($C_{20}H_{24}O_4N^+$)

A wide variety of plants contain this quaternary alkaloid which is widespread among the genera, *Aquilegia*, *Magnolia* and *Michelia*. It has been isolated and characterized as the iodide, melting point 248-249°C. The optically inactive base also yields an iodide which crystallizes from water, melting point 243°C (Glasby, 1975).

Palmatine ($C_{21}H_{23}O_5N^+$)

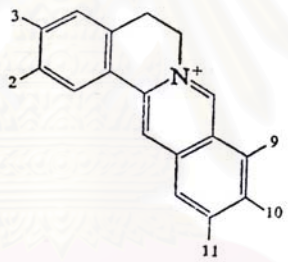
This quaternary alkaloid occurs frequently in the Rhoeadales, being found in Ranunculaceae (*Coptis japonica* Mak.), Berberidaceae (*Berberis heteropoda* Schrenk, *B. vulgaris* L.), Menispermaceae (*Coscinium blumeanum* Miers, *C. fenestratum* (Gaertn.) Colebr., *Fibraurea chloroleuca* Miers, *Jateorhiza palmata* (Lam.) Miers), and Rutaceae (*Phellodendron amurense* Rupr.).

The base is usually obtained as the iodide dihydrate forming orange-yellow needles from water, melting point 241°C. Other salts that have been prepared include the chloride, green yellow needles from water, melting point 205°C; the nitrate,

yellow needles, from water, melting point 239°C; the perchlorate, melting point 262°C; the sulphate, melting point 250°C; the platinichoride, melting point 236°C and the thiocyanate, melting point 210°C. The alkaloid resembles berberine in yielding addition compounds with chloroform and acetone. On catalytic hydrogenation it gives tetrahydropalmatine, while on oxidation with alkaline potassium permanganate it furnishes corydaldine and hemipinic acid (Glasby, 1975).

Thalifendine ($C_{19}H_{16}O_4N^+$)

A quaternary alkaloid presents in *Thalictrum fendleri* Engelm. The base is isolated as the chloride which forms yellow crystals, sintering above 230°C. The ultraviolet spectrum of this salt has absorption maxima at 231, 269 and 348 nm (Glasby, 1975).



	2	3	9	10	11
Berberine	-O-CH_2-O-		OCH_3	OCH_3	H
Palmatine	OCH_3	OCH_3	OCH_3	OCH_3	H
Jatrorrhizine	OCH_3	OH	OCH_3	OCH_3	H
Columbamine	OH	OCH_3	OCH_3	OCH_3	H
Pseudojatrorrhizine	OCH_3	OH	H	OCH_3	OCH_3
Pseudocolumbamine	OH	OCH_3	H	OCH_3	OCH_3
Palmatrubine	OCH_3	OCH_3	OH	OCH_3	H
Berberrubine	-O-CH_2-O-		OH	OCH_3	H
Thalifendine	-O-CH_2-O-		OCH_3	OH	H
Dehydrocorydalmine	OCH_3	OCH_3	OCH_3	OH	H

Figure 6. Structure of some alkaloids in Khamin khruea

4. The biological activities

The biological activities from plant extract of Khamin khruea have been studied including *Arcangelisia flava* (L.) Merr., *Fibraurea tinctoria* Lour. and *Coscinium fenestratum* (Gaertn.) Colebr. as shown in Table 5-7. According to previous phytochemical studies, the main constituents of stem and root are alkaloids that possesses pharmacological effects. The toxicological effects of these constituents have also been studied.

Table 5. The biological activities of *Arcangelisia flava* (L.) Merr. extract

Part used	Extraction	Activity	Reference
Root	Hot water	Menstruation induction effect: human adult (oral)	Quisumbing, 1951
	Ethanol 95%	Hypotensive activity: dog (IV)	Estrada *et al*., 1963
		Skeletal muscle stimulant: dog (intraarterial)	
		Cardiotonic activity: turtle (perfusion)	
	Ethanol 95%	1. Antibacterial activity: agar plate (*Staphylococcus aureus* and *Shigella dysenteriae*, conc. 100.0 mg/disc) 2. Antiyeast activity: (*Candida albicans*, conc. 100.0 mg/disc)	Avirutnant and Pongpan, 1983
	Chloroform	Antimalarial activity: *Plasmodium falciparum* , MIC < 25.0 μg/ml)	Ayudhaya *et al*., 1987
	Methanol	Glucose transport inhibition: cell culture(cells-Ehrlich, conc. 10.0 μg/ml)	Yamasaki, 1996

Table 5. The biological activities of *Arcangelisia flava* (L.) Merr. extract (continued)

Part used	Extraction	Activity	Reference
Stem	Ethanol 95%	Antimycobacterial activity: agar plate (*Mycobacterium smegmatis*, conc. 1.0 mg/disc)	Pongpan *et al.*, 1982
	Chloroform		
	Petroleum ether		
	Petroleum ether	Antimicrobial activity: agar plate (*Bacillus subtilis* and *Candida albicans*, conc. 1.0 mg/disc)	
Bark	Methanol	Glucose transport stimulation: cell culture (CA-Ehrlich-ascites, conc. 1.0 μg/ml)	Murakami *et al*., 1993

Table 6. The biological activity of *Fibraurea tinctoria* Lour. extract

Part used	Extraction	Activity	Reference
Stem	Ethanol 95%	Antimycobacterial activity: agar plate (*Mycobacterium smegmatis*, conc. 1.0 mg/disc)	Pongpan *et al*., 1982

Table 7. The biological activities of *Coscinium fenestratum* (Gaertn.) Colebr. extract

Part used	Extraction	Activity	Reference
Gall	Methanol	Plaque formation suppressant: cell culture (Streptococcus mutans, IC_{50} 140.0 μg/ml)	Namba *et al.*, 1985
	Methanol:water (1:1)	Plaque formation suppressant: cell culture (*Streptococcus mutans*, IC_{50} 240.0 μg/ml)	
	Methanol	Mitogenic activity: cell culture(Lymphocytes, conc. 5 μg/ml)	Namba *et al*., 1989
Stem	Ethanol 95 %	1. Antiyeast activity: agar plate (*Candida albicans*) 2. Antifungal activity: agar plate (*Trichophyton mentagrophytes* and *T. rubrum*)	Ray and Majumdar, 1976
	Water	Antibacterial activity: agar plate (*Clostridium tetani*, conc. 6.25 mg/ml; *C. perfringens, C. novyi, C.botulinum* and *C.sporogenes*, conc. 12.5 mg/ml; *Staphylococcus aureus*, conc. 18.75 mg/ml)	Palasuntheram *et al*., 1982
	Ethanol 50%	Hypotensive activity: dog and rat (IV,dose 5.0 mg/kg); guinea pig (IV,dose 10.0 mg/kg)	Singh *et al*., 1990

Pharmacology and toxicology of some constituents

Berberine is a supplementary drug in the Extra Pharmacopoeia, Martindale (1989) and in Pharmacopoeias of Chinese (1985), India (1985) and Japan (1986) include berberine chloride. Japan Pharmacopoeia also includes berberine tannate. Berberine has been used as a bitter. It possesses antimicrobial activity and has been tried in a number of infections including cutaneous leishmaniasis and cholera. Both the sulphate and the chloride have been used.

Two controlled studies have examined the effect of berberine alone, in combination with tetracycline, and by tetracycline alone on fluid loss by diarrhea in patients with cholera or in non-cholera diarrhea (Lampe, 1992). In the first study, in which berberine chloride was employed in a dose of 100 mg four times daily, berberine did not exhibit a significant vibrostatic effect, exhibited only a slight effect on stool volume, and possibly reduced the vibriostatic effect of tetracycline. Neither tetracycline nor berberine demonstrated any benefit over placebo in patients with noncholera diarrhea of unspecified etiologies (Khin-Maung-U *et al*., 1986). In a later study, different dosage regimens were studied: 400 mg berberine sulphate as a single-bolus dose for enterotoxigenic *Escherichia coli*- induced diarrhea and either 400 mg as a single-bolus dose or 1200 mg berberine sulphate in 400 mg doses every 8 hours for 24 hours for cholera. These doses of berberine produced a significant reduction of mean stool volume during enterotoxigenic *Escherichia coli*-diarrhea regardless of strain (ST or LT). Although berberine produced a modest antisecretory effect in cholera patients, this was overshadowed by the far more powerful and specific activity of tetracycline (Rabbani *et al*., 1987).

Berberine (as the chloride) has been found to be active against a number of gram-positive as well as gram-negative bacteria, such as *Diplococus pneumoniae*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Salmonella typhosa*, *Shigella dysenteriae*, *Staphylococcus aureus*, *S. hemolyticus* and *S. paradysenteria* in different media. It had about the same antibacterial activity as some sulphonamides, berberine also had an effect in broth supplemented with serum, whereas the sulphonamides were antagonized. However, it was found possible for the microorganisms to acquire resistance when left in contact with berberine for a long time (De Padua *et al*., 1999).

Berberine (as the sulphate) has been shown to be bactericidal to *Vibrio cholerae* at a concentration of 35 μg/ml and bacteriostatic to *Staphylococcus aureus* at a concentration of 50 μg/ml. In both these organisms berberine at the concentrations mentioned inhibited RNA and protein synthesis almost immediately after addition. Cell-free preparations made from vibrios pretreated with berberine did not produce choleraic symptoms in infant rabbits, suggesting that the toxin was either inactivated or neutralized. Oral administration of berberine to infant rabbits 18-24 hours before a single fatal intra-intestinal dose of choleragenic toxin prevented toxin-induced diarrhoea and consequently prolonged survival when compared with untreated choleragenic animals. The quaternary ammonium group in berberine seems necessary for its antibacterial activity. Derivatives without the quaternary ammonium group, such as tetrahydroberberine, showed only little antibacterial effect. Berberine (sulphate) in concentrations of 10-25 mg/ml inhibited the growth of the fungi *Alternaria* spp., *Aspergillus flavus*, *A.fumigatus*, *Candida albicans*, *Curvularia* spp., *Drechslera* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus oryzae* and *Scopulariopsis* spp. Oral administration of berberine sulphate at doses of 350-700 mg/kg was effective in treating *Candida albicans* infections of the intestine in mice (De Padua *et al*., 1999).

Berberine (sulphate) administered to rats at doses of 100 mg/kg body weight, 10 days after experimentally induced intestinal amoebiasis was effective in 80 % of the animals. It completely inhibited the growth to trophozoites of *Entamoeba histolytica* at concentrations of 0.5-1 mg/ml *in vitro*, and was active *in vivo* against infections with *E. histolytica* in hamsters and rats. Berberine has also been found to be trypanocidal against *Trypanosoma brucei rhodesiense*. *In vitro* activities with IC_{50} values of 0.4 μg/ml were determined (De Padua *et al*., 1999).

Both berberine sulphate (50 μg/ml) and berberine chloride (25 μg/ml) showed growth inhibition of Ehrlich and lymphoma ascites tumour cells. The presence of berberine in granules inside the cells was detected by its fluorescence. The cytotoxic ED_{50} values in HeLa cell cultures were 3.5-30 μg/ml, and in KB cells a 70% inhibition of protein synthesis was found at a concentration of 1 μg/ml. Berberine chloride inhibited the formation of DNA, RNA, proteins and lipids, as well as the

oxidation of [^{14}C] glucose to $^{14}CO_2$ when incubated with S180 (Swiss mouse ascites sarcoma) cells *in vitro*. Protein and RNA syntheses were most sensitive to berberine. However, berberine failed to inhibit the growth of S180 ascites tumours in mice, which may be explained by the effect of different glucose levels in biological fluids. The binding of the alkaloids to DNA was investigated by means of spectroscopy. Calf thymus DNA produced systematic changes in the absorption spectrum of berberine, which suggests that berberine forms a complex with DNA and binds to the extent of one alkaloid molecule per two base pairs. These binding properties seem to be influenced by the presence of charge and the position and type of substituents in the molecule. From other experiments it was also concluded that berberine is a potent activator for macrophages, to induce inhibition of tumour cells *in vitro* (De Padua *et al*., 1999).

Intravenous infusion of berberine sulphate to rats was found to lower the blood pressure in a dose-dependent manner. A significant hypotensive effect was followed by bradycardia. These effect were also observed in bilaterally vagotomized rats. Berberine chloride at doses of 0.5-5.0 mg/kg administered to rabbits anaesthetized with urethane produced a long lasting, dose-related decrease in blood pressure. The berberine induced hypotension seems attributable to alpha-adrenoceptor blockade, and not to a direct relaxant effect on vascular smooth muscle. Berberine had no direct vasodilatory effects on isolated rabbit pulmonary and cat coronary arteries either, however, the alkaloid reversed vasoconstriction mediated by alpha-adrenergic agents in both preparations (De Padua *et al*., 1999).

Glucocorticoid receptors (GR) have found significantly higher in hepatoma (Hep) than in adjacent liver tissues. GR were expressed not only in G0-G1 phase, but also in S and G2+M phases. Continuous exposure of HepG2 cells to various concentrations (1-50 μM) of berberine resulted in growth inhibition in a dose dependent manner. The viability of berberine-treated HepG2 cells was greater than 90% in all treatment groups. Flowcytometric analysis of berberine-treated HepG2 cells showed that the S phase fraction was significantly reduced. In addition, the secretion of alpha-fetoprotein by HepG2 cells was inhibited by berberine. Finally, the berberine induced cell growth arrest was partially reversible in HepG2 cells (Chi *et al*., 1994).

Berberine chloride demonstrated significant cytotoxic activity for P-388 cells, in addition to three of the human cancer cell lines, namely, breast cancer (ED_{50} 2.7 μM), fibrosarcoma (ED_{50} 2.4 μM) and nasopharyngeal carcinoma (KB) (ED_{50} 8.4 μM). The other protoberberine alkaloid, berberrubine chloride and thalifendine chloride were also found to exhibit cytotoxic activity against P-388 cells, but not against any of the human cancer cell lines utilized (Dai *et al*., 1993).

The protoberberine alkaloids such as columbamine iodide (IC_{50} 58 μg/ml), coptisine picrate (IC_{50} 56 μg/ml), jatrorrhizine chloride (IC_{50} 71 μg/ml) and berberine chloride (IC_{50} 100 μg/ml), demonstrated moderately potent activity in the human immunodeficiency virus type 1 reverse transcriptase (HIV-1RT) system, whereas compounds that lacked the quaternary nitrogen, e.g., tetrahydropalmatine and tetrahydroberberine were inactive (25% inhibition at 200 μg/ml). The unavailability of more diversified analogues of the protoberberines prevented significant structure-activity correlation in this series of alkaloids. Inhibition of human immunodeficiency virus reverse transcriptase is currently considered a useful approach in the prophylaxis and intervention of acquired immunodeficiency syndrome (AIDS) (Tan *et al*., 1991).

Both berberine and palmatine inhibited specific cholinesterase in rabbit spleen and pseudocholinesterase in normal horse serum. Both compounds were less effective inhibitory agents than neostigmine, but palmatine exhibited lower toxicity than berberine. Tetrahydropalmatine and tetrahydroberberine had no anticholinesterase effect, suggesting that the quaternary ammonium group is crucial for the effect of isoquinoline alkaloids on this enzyme (Berezhinskaya *et al*, 1968).

Palmatine inhibits the effect of adrenaline on the blood pressure of rabbits. *dl*-Tetrahydropalmatine inhibits the effect of 5-hydroxytryptamine (5-HT) on the isolated rat organ *in vitro* but could not release 5-HT from the tissue. Both alkaloids have ACTH-like and bactericidal actions, and palmatine had an anticholinesterase effect. Most likely palmatine, *dl*-tetrahydropalmatine and ergot alkaloids have analogous pharmacology mechanism (Mu-Ch'un Ch'en and Chen-Yu Ch'i, 1965).

Berberine, as well as columbamine, stimulates biliary secretion in patients with toxic hepatitis and berberine triples the secretion of bile in 1.5 hours (Velluda *et al*., 1958). Tetrahydroberberine possess central depressant effects which was equipotent to tetrahydropalmatine and tetrahydrocoptisine, but more potent than tetrahydrojatrorrhizine. The acute toxicity of tetrahydroberberine was less than that of chlorpromazine and chlordiazepoxide (Yamahara, 1976). Tetrahydropalmatine has been reported to exhibit strong analgesic, sedative and hypnotic effects. It is a weaker analgesic than morphine, but it possesses stronger hypnotic properties. It stimulates rabbit intestine and guinea-pig uterus (Kettenes and Salemink, 1981). Tetrahydrocolumbamine antagonises acetylcholine (Kitabatake *et al*., 1964).

Topoisomerase II-mediated DNA cleavage assays showed that berberrubine poisons the enzyme by stabilizing topoisomerase II-DNA cleavable complexes. Berberrubine induces DNA cleavage in a site-specific and concentration-dependent manner. Comparison of the cleavage pattern of berberrubine with that of etoposide (prototypical topoisomerase II poison) revealed that they share many common sites of cleavage (Kim *et al*., 1998).

Magnoflorine act as neuromuscular blocking agents and significantly lower blood pressure in various animals due to their ganglionic blockade and half as active as that of hexamethonium (ganglion-blocker). The acute LD_{50} of magnoflorine by intravenous injection in mice is 0.02 g/kg, oral medication with a 10-fold dose daily for 4 weeks neither elicited any toxic symptoms or retarded the growth (Chang *et al*.,1964). It exhibited curarelike gangionic blocking effects (Shimamoto *et al.*, 1958). Substitution of acetyl groups for the 2 hydroxyl groups of corytuberine or magnoflorine increase their toxicity and the LD_{50} of O,O-diacetylmagnoflorine is 6.6 times greater than that magnoflorine (Fakhrutdinov, 1971).

Of the bisbenzyisoquinoline alkaloids investigated, (+)-homoaromaline showed inhibition of the histamine production by RBL-2H3 cells and both (+)-homoaromaline and (-)-limacine were capable of inhibiting *in vitro*, the growth of cultured *Plasmodium falciparum* strains and tumour cell lines. However, their ‘selectivity index’ (activity against mammalian cells/activity against cultured *P. falciparum* strains) typically ranges from 2-100; a selectivity index of >1000 appears

to indicate that a component merits further investigation as an anti-malarial (De Padua *et al*., 1999).

Ecdysteroids were initially demonstrated as insectmoulting hormones, and phytoecdysteroids of plant origin were first discovered in 1966 by Nakanishi *et al*. during a screen for anticancer compounds. Subsequently, multiple physiological and ecological functions have been established for phytoecdysteroids, such as inducing metamorphosis in insects and stimulating protein synthesis in mouse liver. This range of activities has sustained interest in these compounds. Phytoecdysteroids have been found in 111 plant families, and it has been suggested that they play a protective role. These compounds appear to be particularly common in the Pteridophyta but also occur in many families of the Gymnospermae and Angiospermae, such as Amaranthaceae, Caryophyllaceae, Compositae, Moraceae, Menispermaceae, Osmundaceae, Podocarpaceae, Polypodiaceae, Ranunculaceae and Taxaceae. Phytoecdysteroids had been found to occur in only two species in the Menispermaceae, namely, *Abuta velutina* Gleason and *Diploclisia glaucescens* (Blume) Diels. The present isolation of 20-hydroxyecdysone from the roots of *F. chloroleuca* suggests that phytoecdysteroids may exist in other species in the genus *Fibraurea* (Dai *et al*., 1993).

Some metabolic and toxicological data on berberine are available from experiments on rats. The blood level of orally administered [^{3}H] berberine chloride plateaued after 4-24 hours, and maximal levels in the liver and muscles were achieved at 12 hours. Urinary berberine excretion reached a maximum at 12-24 hours. Excretion in the urine and faeces at 48 hours amounted to respectively 2.7% and 86% of the administered dose. Faecal elimination as the main excretion route indicates that berberine is not readily absorbed by the gastro-intestinal tract. The biological half-life of berberine chloride was 5.2 hours after intraperitoneal administration and 5.4 hours after oral administration. Perfusion experiments (in dogs and rabbits) indicated oxidation of berberine chloride in the liver. The LD_{50} value of berberine sulphate was more than 1 g/kg after oral administration in the rat and about 90 mg/kg after intraperitoneal administration. Histopathological examinations revealed no changes in tissues and organs, even in cases when berberine sulphate had been given for 6 weeks at daily doses of 500 mg/kg (De Padua *et al*., 1999).

An oral dose of 2.75 g berberine to dogs (weight unspecified) produced severe gastrointestinal irritation, profuse, watery diarrhea, salivation, muscular tremors, and paralysis. Respiration was not affected. After death the intestines were found to be contracted, generally empty or containing mucous and watery fluid, and inflamed. Oral doses of 100 mg/kg berberine sulphate were reported to be well tolerated by rats, with the comment that 'no marked CNS effects were seen'. An oral dose of 25 mg/kg the sulphate to cats induced depression in about 1 hour lasting for 6 to 8 hours; 50 mg/kg caused salivation and sporadic emesis but with recovery within 24 hours. A dose of 100 mg/kg induced persistant emesis for 6 to 8 hours and the death of all animals 8 to 10 days later. The chronic oral administration of 25 mg/kg berberine sulphate for 10 days to cats was not associated with any gross or microscopic changes. Doses of 50 or 100 mg/kg induced serous hemorrhagic inflammation of the small and large intestine. The intraperitoneal administration of 5, 20, or 40 mg/kg berberine choride to cats produced sedation at all doses beginning within 3 to 5 minutes and persisting for less than 2 hours. A single cat exhibited a transient rage reaction. The intraperitoneal administration of 5mg/kg berberine chloride markedly reduced amphetamine-induced motor hyperactivity in mice. Lethal intravenous doses of berberine sulphate in the dog or cat cause central respiratory failure prior to cardiac arrest. These doses are associated also with pulmonary edema and hemorrhage (Lampe, 1992).

Acute toxicity studies on ethanol extract of *Arcangelisia flava* Merr. (contain berberine hydrochloride and traces of other isoquinoline alkaloids) in mice was reported by Utaipatana and Chumsri (1987). Physical signs were observed shortly after alkaloids administration. Hypoactivity and respiratory depression were detected in mice after oral administration at dose level up 1.667 g/kg. The LD_{50} of isoquinoline alkaloids of *Arcangelisia flava* (L.) Merr. which were 1.1085 and 1.1150 g/kg in female and male mice respectively.

No human pharmacokinetic data for berberine is available, although a quantitative observation has been made that, unlike animals, a considerable quantity of berberine is excreted unchanged in the urine of man. Berberine has been reported to be well tolerated in therapeutic doses of 0.5 g; serious intoxications in man being unknown. It should be noted carefully, however, that no systematic studies have been

conducted during which contemporary laboratory methods have been employed to assess organ function during either the acute or chronic administration of berberine salts or of berberine-containing plant extracts. Many of these could not be verified, nor is it clear if some of the stated adverse effects were found only in animal studies utilizing parenteral rather than oral administration. A short general review of the safety of herbal medication lists berberine as possibly hepatotoxic, but also without reference. No side effect related to cardiovascular activity have been noted with oral preparations containing berberine or its salts in animal studies or for the treatment of diarrhea in man. The safety of berberine or extracts of berberine-containig plants has not been established in respect to fertility, pregnancy, lactation and to potential mutagenic or carcinogenic effects (Lampe, 1992).

CHAPTER III

PHARMACOGNOSTIC STUDY

Pharmacognostic study is used to characterized and identified crude drugs. It consists of the macroscopic, microscopic characterization and phytochemical screening. In the macroscopic method, organoleptic sensation is used to determine the size, shape, color, odor and taste of the crude drugs. The microscopic method revealed plant histology. The thin-layer chromatographic technique is used to differentiate extracts of different biological origins. The phytochemical screening is employed to identify important chemical constituents in the crude extract. Methods for quality control of crude drugs are described in Pharmacopoeia.

Apparatus for microscopic measurements

Microscopic measurements can be carried out using a stage micrometer in conjunction with an eyepiece micrometer and drawing attachment.

1. Micrometers

Two scales are required, known, respectively, as a stage micrometer and an eyepiece micrometer. The stage micrometer is a glass slide 7.6 x 2.5 cm (3 x 1 inch) with a scale engraved on it. The scale is usually 1 mm long and is divided into 0.1 and 0.01 parts of a millimetre. The eyepiece micrometer may be a linear scale and the scale 0-10 or it may be ruled in squares. The value of one eyepiece division is determined for every optical combination to be used, a note being made in each case of the objective eyepiece and length of raw-tube.

To do this, unscrew the upper lens of the eyepiece, place the eyepiece micrometer on the ridge inside, and replace the lens. Put the stage micrometer on the stage and focus it in the ordinary way. The two micrometer scales now appear as in Figure 7, when the objective (x 4) is in use. It will be seen that when the 7 line of the stage micrometer coincides with the 0 of the eyepiece, the 10 of the stage coincides

with 7.7 of the eyepiece. As the distance between 7 and 10 on the stage scale is 0.3 mm, 77 of the small eyepiece divisions equal to 0.3 mm or 300 μm; therefore, 1 eyepiece division of this eyepiece micrometer which is used with the objective (x 4) of this microscope equals to 300/77 or 3.9 μm.

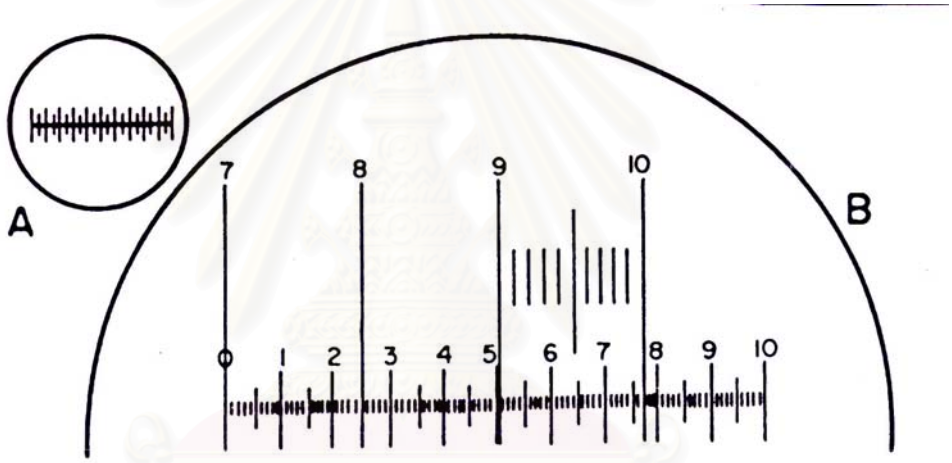


Figure 7. A. Eyepiece micrometer
B. Eyepiece micrometer superimposed on portion of stage micrometer scale.
(Trease and Evans, 1996)

2. Drawing attachment (Olympus model BH2-DA)

The model BH2-DA is used to visually superimpose the image of a specimen over the surface image of a drawing paper placed beside the microscope so that the specimen image can be traced on the paper. Different from the ordinary microprojection system where an image is projected on a screen, the model BH2-DA permits drawing in a bright room without any more light intensity than required by ordinary microscopy. Start drawing by place drawing paper steadily in position, occasionally touching up the light intensity of the microscope light source so that it can be well balanced with the brightness of the drawing surface. If the drawing surface is dark, increase the brightness of the room by additional use of fluorescent lamp to illuminate the drawing surface, which makes drawing easier.

Figure 8. Microscope with Drawing attachment apparatus

3. Photomicrographic equipment (Olympus model PM-10AD)

This equipment is uniquely qualified to be used with the microscope (Olympus model BHT) for routine and advanced photomicrography.

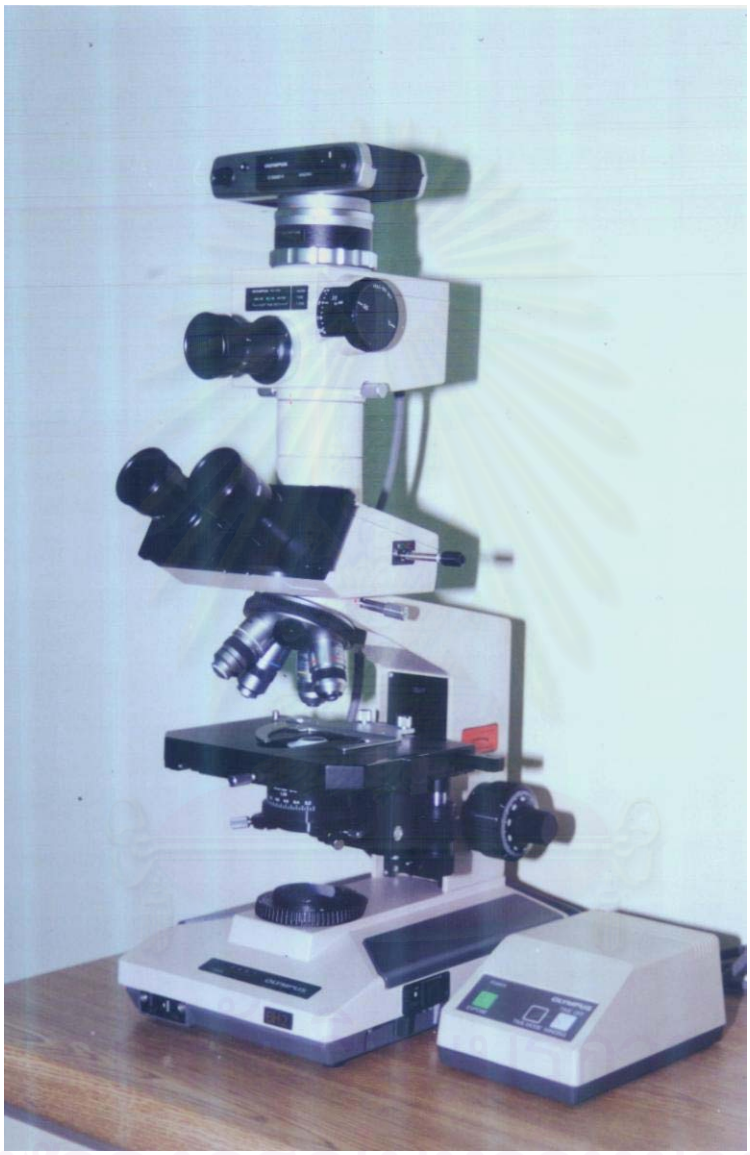


Figure 9. Microscope with Photomicrographic equipment

Leaf measurements

The leaf measurements are used to distinguish closely related species that are not easily characterized by general microscopy.

1. Stomatal number
2. Stomatal index
3. Palisade ratio
4. Vein-islet number
5. Vein-terminal number

1. Stomatal number

Stomata are another type of epidermal structure possessing great diagnostic value. A stoma consists of two similar cells, the guard cells, placed with their long axis parallel and having a small cellular space, the porous between them. By variations of the turgidity of the guard cells, the size of the porous is altered. In surface view the guard cells often appear crescent shaped, their concave faces being adjacent to one another. During the formation of a stoma, the cells cut off from the mother cell often acquire a shape and size differing from those of the other epidermal cells and are therefore termed the subsidiary cells (Wallis, 1960). The two guard cells and the porous counted as 1 cell stoma. The average number of stomata per square millimetre of epidermis is termed the stomatal number. In recording results the range as well as the average value should be recorded or each surface of the leaf and the ratio of values for the two surfaces (Trease an Evans, 1996). The actual number of stomata per square millimetre is variable for the same plant, this being especially noticeable if records are made for different years (Wallis, 1960).

2. Stomatal index

The significance of the number of stomata per unit area of leaf was investigated by Timmerman in 1927. Salisbury showed that a high correlation coefficient exists between the number of stomata and the number of epidermal cell per unit area of leaf surface of a given species (Youngken, 1948).

$$\text{Stomatal index} = \frac{S}{S+E} \times 100$$

The stomatal index is the percentage which the number of stomata form of the total number of epidermal cells, each stoma being counted as one cell. Thus, if **S** represent the number of stomata per unit area and **E** the number of epidermal cells in the same unit area. The figure so obtained is fairly constant for any species and can be used as a specific character (Wallis, 1960).

3. Palisade ratio

The average number of palisade cells beneath each upper epidermal cell is termed the palisade ratio. Quite fine powders can be used for the determination(Trease and Evans, 1996). The palisade cells of the mesophyll bear a definite relation to the epidermal cells, a fact which becomes evident from a study of the palisade ratio. This ratio has been shown to be sufficiently constant to serve as a diagnostic character of species belonging to the same genus (Wallis, 1960). The term ‘palisade ratio’ was introduced by two British pharmacognosists, T.E. Wallis and T. Dewar, in 1933. It represents a figure obtained by counting the total number of palisade cells beneath four epidermal cells and dividing the number by four (Youngken, 1948).

4. Vein-islet number

The term ‘vein-islet’ is used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. The number of vein-islets per mm^2 calculated from four contiguous square millimetres in the central part of the lamina, midway between the midrib and the margin, is termed the vein-islet number (Trease and Evans, 1996). The shape of the vein-islets is frequently characteristic and will often enable one to sort out a mixture of leaves which have been broken into small fragments. It has been shown that the number of vein-islets per unit area of leaf surface is constant for any given species of plant and can be used as a character for the identification of species (Wallis, 1960).

5. Vein-terminal number

Hall and Melville (1951) determined veinlet termination number, which they define as the number of veinlet terminations per mm^2 of leaf surface. A vein termination is the ultimate free termination of a veinlet or branch of a veinlet (Trease and Evans, 1996).

Phytochemical screening

The important of plant-derived medicinals in modern medicine is often underestimated. A knowledge of the biological activities and/or chemical constituents of plants is desirable, not only for the discovery of new therapeutic agents but because such information may be of value in disclosing new sources of such economic materials. A knowledge of the chemical constituents of plants would further be valuable to those interested in the expanding area of chemotaxonomy (biochemical systematics), to those interested in biosynthesis, and to those interested in deciphering the actual value of folkloric remedies.

The method for use in phytochemical screening should be (a) simple, (b) rapid, (c) designed for a minimum of equipment, (d) reasonably selective for the class of compounds under study, (e) quantitative in so far as having a knowledge of the lower limit of detection is concerned, and if possible, (f) should give additional information as to the presence or absence of specific members of the group being evaluated (Farnsworth, 1966).

Quality Controls

One possible problem in devising standards for crude drugs concerns the requirement for an assay of the active constituents when the latter may not have been precisely ascertained. Furthermore, one of the tenets of the herbal medicine is that the maximum effectiveness of the drug derives from the whole drug or its crude extract rather than from isolated components. In cases where an assay is lacking it is

therefore of paramount importance that the crude drug is properly authenticated, its general quality verified and all formulations of it prepared in accordance with good manufacturing practice. Although official standards are necessary to control the quality of drugs their use doses raise certain problems. Of necessity, to accommodate the considerable variation that occurs between different batches of a natural product it is necessary to set relatively low standards which allow the use of commercial materials available in any season. There are a number of standards, numerical in nature, which can be applied to the evaluation of crude drugs either in the whole or the powdered condition (Trease and Evans, 1996). For this investigation, these are following:

Loss on drying

This is employed in the EP, BP, USP and TP. Although the loss in weight, in the samples so tested, principally is due to water, small amounts of other volatile materials will also contribute to the weight loss. For materials which contain little volatile material, drying (105°C) to constant weigh will be employed. The moisture balance combines both the drying process and weight recording; it is suitable where large numbers of samples are handled and where a continuous record of loss in weight with time is required (Trease and Evans, 1996).

Ash content

The determination of ash is useful to detecting low-grade products, exhausted drugs and excess of sandy or earthy matter; it is more especially applicable to powdered drugs. Different types of ash figures are used such as total ash, acid-insoluble ash and water soluble ash. A total ash figure is useful to exclude drugs which have been coated with chalk, lime or calcium sulphate to improve their appearance. The acid-insoluble ash, i.e., the ash insoluble in dilute hydrochloric acid, is often of more value than the total ash. The majority of drugs contain calcium oxalate, sometimes in large amounts and the amount is often very variable. Since, however, the calcium oxide or carbonate, yielded by the incinerated oxalate, is soluble in hydrochloric acid, one can remove all the variable constituent of the ash by means

of dilute hydrochloric acid and weigh the residue, which is known as the acid-insoluble ash (Wallis, 1960).

Extractive value

The determination of water-soluble or ethanol-soluble extractive is used as a means of evaluating drugs the constituents of which are not readily estimated by other means. In certain cases extraction of the drug is by maceration, in others by a continuous extraction process. For the latter the Soxhlet extractor is particularly useful and has been in use for many years, not only for the determination of extractives but also for small-scale isolations (Trease and Evans, 1996).

CHAPTER IV

EXPERIMENTAL

Scopes of investigation

1. Leaf measurements from each species: stomatal number, stomatal index, palisade ratio, vein-islet number and vein-terminal number.
2. Study of the macroscopic and microscopic characters of stem from authentic samples.
3. Illustration of thin-layer chromatographic patterns of each authentic species and crude drugs which were purchased from traditional drugstores throughout Thailand.
4. Phytochemical screening of alkaloid and tannin groups.
5. Quality controls of crude drugs which were purchased from traditional drugstores throughout Thailand according to the Pharmacopoeia: loss on drying, total ash, acid-insoluble ash and extractive value.

Apparatus

1. Beaker : 150, 250 ml
2. Chromatographic chamber
3. Crucible
4. Day-light lamp
5. Desiccator
6. Drawing attachment (Olympus, model BH-2DA)
7. Filter paper, harden ashless (Whatman) diameter 7 cm
8. Filter paper (Whatman) diameter 7 and 11 cm
9. Erlenmeyer flask: 150, 250 ml
10. Glass filter
11. Hot Air Oven
12. Hot plate
13. Micro-pipettes
14. Microscope (Olympus, model CH)

15. Microscope with Photomicrography (Olympus, model PM-10AD)
16. Muffle Furnace (Gallenkamp size 2)
17. pH paper (Whatman)
18. Stirring rod
19. Test tubes
20. TLC plates : TLC aluminium sheets silica gel 60 F_{254} pre-coated 20x20 cm, layer thickness 0.2 mm (E. Merck., Germany)
21. Ultraviolet lamp
22. Water-bath

Chemical substances and solvents

1. Acetic acid, glacial GR. grade (E. Merck., Germany)
2. Anisaldehyde (E. Merck., Germany)
3. Berberine sulphate
4. Bismuth oxynitrate
5. Bromine water
6. n-Butanol
7. Chloroform
8. Choral hydrate B.P.
9. Cyclohexane
10. Diethylamine (E. Merck., Germany)
11. Ethanol
12. Gelatin
13. Glycerin U.S.P.
14. Hydrochloric acid (E. Merck., Germany)
15. Iodine
16. Iron (III) chloride
17. Mercury (II) chloride
18. Methanol
19. Phloroglucinol
20. Potassium iodide
21. Sulphuric acid 95-97% (E. Merck., Germany)
22. Water, distilled

Part 1. Leaf measurements

1.1 Materials: The fresh mature authentic leaves, collected date as shown in parenthesis.

* *Arcangelisia flava* (L.) Merr. (5 samples):
  - The Siri Ruchachat Medicinal Plant Garden, Mahidol University Salaya Campus, Nakhon Pathom province (July, 2000).
  - Klaeng district, Rayong province (November, 2000).
  - The Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok province (July, 2000).
  - Institute of Thai Traditional Medicine, Nonthaburi province (July, 2000).
  - Medicinal Plant Garden, Department of Medical Science, Ministry of Public Health, Chanthaburi province (September, 2000).

* *Coscinium fenestratum* (Gaertn.) Colebr. (1 sample):
  - Beung karn district, Nong Khai province (August, 2000).

* *Fibraurea tinctoria* Lour. (3 samples):
  - Faculty of Pharmacy, Prince of Songkla University, Songkhla province (January, 2001).
  - Sa ba yoi district, Songkhla province (February, 2001).
  - Sa ba yoi district, Songkhla province (February, 2001).

* *Combretum latifolium* Blume (2 samples):
  - Sa nam chai khet district, Chachoengsao province (September, 2000).
  - Sakaeraj district, Nakhon Ratchasima province (January, 2001).

The leaves of the two families were identified by comparing with those described in the Flora of Thailand for Menispermaceae by Forman (1991) and Thai Forest Bulletin for Combretaceae by Weerachai Nanakorn (1986) and also comparing with the authentic herbarium specimens preserved at the Forest herbarium, Royal

forest department, Bangkok and at herbarium of the Botany and Weed Division, Department of Agriculture, Ministry of Agriculture and Co-operation, Bangkok.

The leaves and stems of *Coscinium fenestratum* (Gaertn.) Colebr. and *Fibraurea tinctoria* Lour. were obtained as authentic samples from Associate Professor Rapepol Bavovada, Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University and Assistant Professor Niwat Keawpradub, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy, Prince of Songkla University respectively.

The leaves of the other species of *Coscinium* (2 samples) were taken from Si kao district, Trang province (October, 2000) and Sa ba yoi district, Songkhla province (February, 2001) had been studied together with those authentic samples.

1.2 Procedure

1.2.1 Preparation of leaves.

The fresh mature leaves wear cleaned and cut into small pieces (1x1 cm), in the central part of lamina, midway between the midrib and the margin. Fragments of leaves are bleached by gently warming in chloral hydrate solution (4 g/ml in distilled water). This solution was frequently shaken and changed for rapid removing of chlorophyll from the leaf fragments. When the leaf fragments were cleared, they were rinsed off in distilled water at least 2 times and finally kept in glycerin to maintain the structure and moisture of the cells.

1.2.2 Method for leaf determination

(a). Stomatal number and stomatal index

Prior to the determination, the drawing attachment has already set for the microscope and the stage micrometer was used for measuring the diameter of the drawing magnification on the paper surface. The area of this circle was calculated in square millimeter and this area was specific for each magnification of lens. If the lens and the magnification wear changed, the area must be calculated again. The ordinary epidermis and the stomata wear traced and counted. The trichome or its cicatrix was

also counted as 1 ordinary epidermal cell. Counted incomplete cell of epidermal and stoma on the bordered of circle as 1 cell from only one side of half circle. Concerning the covering trichomes that were difficult to focused and traced on the circled area of paper. Thus, the stomatal number and the stomatal index were determined using glandular trichomes instead of stomata and defined in terms of glandular number and glandular index respectively.

$$\text{Stomatal number} = \frac{S}{1\ mm^2}$$

$$\text{Stomatal index} = \frac{S \times 100}{S+E}$$

S = the total numbers of stomata (Glandular trichomes were use instead of stomata in case of *Coscinium.*)

E = the total numbers of epidermal cells

(b). Palisade ratio

Examined and determined the palisade ratio by counting the number of palisade cells under 4 contiguous epidermal cells and divided by 4. Counted incomplete palisade cell underlying the two epidermal cells as 1 cell. Caution must be observed not to count any incomplete palisade cell underlying outside of the 2 epidermal cells.

(c). Vein-islet number and veinlet termination number

A drawing attachment apparatus was set up and by means of a stage micrometer the paper was divided into square of 4 mm^2. The stage micrometer was then replaced by the cleared preparation and the vein were traced in a square 2 mm x 2 mm on the paper. When counting, it is convenient to number each vein-islet on the tracing. Counted the number of the vein-islets that complete enclosed by veins and count the incomplete vein-islets which were cut by two adjacent sides. The total number of vein-islets divided by 4 mm^2 is termed ‘vein-islet number’. Counted the

free termination of veinlet and divided the total vein termination by 4 mm^2 and defined as 'veinlet termination number'.

1.2.3 Evaluated all of data collected using SPSS program.

Part 2 The macroscopic and microscopic characterizations of stem.

2.1 Materials: Fresh stem authentic samples, collected date as shown in parenthesis.

* *Arcangelisia flava* (L.) Merr. (3 samples):
  - The Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok province (October, 2000).
  - Medicinal Plant Garden, Department of Medical Science, Ministry of Public Health, Chanthaburi province (September, 2000).
  - Klaeng district, Rayong province (November, 2000).

* *Coscinium fenestratum* (Gaertn.) Colebr. (1 sample):
  - Beung karn district, Nong Khai province (August, 2000).

* *Fibraurea tinctoria* Lour.(1 samples):
  - Natawee district, Songkhla province (January, 2001).

* *Combretum latifolium* Blume (1 sample):
  - Sa nam chai khet district, Chachoengsao province (September, 2000).
  - Sakaeraj district, Nakhon Ratchasima province (January, 2001).

Authentic samples of stem were identified alike their leaves.

The stem of the other species of *Coscinium* (2 samples) from Si kao district, Trang province (October, 2000) and Sa ba yoi district, Songkhla province (January, 2001) had been studied together with those authentic samples.

2.2 Procedure

The fresh authentic stems were chopped into small pieces and dried in a hot air oven at 50°C, then ground and passed through a sieve with mesh number 60. Kept in a well-closed container.

2.2.1 The macroscopic method: determine the size, shape, color, odor and taste of the authentic and crude drugs.

2.2.2 The microscopic method: plant histology

The drawings were made using microscope and drawing attachment. In preparing the drawings the objective has been to emphasize the most diagnostic characters by which each powder may be identified, particularly within the morphological group to which it belongs. The cells and cell inclusion were taken photographs by Photomicrographic equipment which is attached to microscope. Most of particles were drawn from preparations of the powdered which had been first mounted in water. Subsequently sections should be cleared by means of chloral hydrate and some stained as follows.

Chloral hydrate solution BP

A valuable and widely used clearing agent. This dissolves starch, proteins, chlorophyll, resins and volatile oils, and causes shrunken cells to expand. Chloral hydrate may be used, not only for sections but also for whole leaves, flower, pollen grain etc. It does not dissolve calcium oxalate and is therefore a good reagent for detection of these crystals.

Iodine solution

This give a blue color with starch and hemicelluloses.

Phloroglucinol solution and Hydrochloric acid

A solution of Phloroglucinol with hydrochloric acid as a test for lignin. Mounted the section in the solution of Phloroglucinol and allowed to stand for about 2 minutes; removed any alcohol which has not evaporated with a piece of filter paper; added concentrated hydrochloric acid covered and examined. All lignified walls stain pink or red.

Hydrochloric acid is a powerful clearing agent and it must be remembered that it will dissolve many cell contents, including calcium oxalate. To prevent damage to the microscope either by liquid contact or by vapors, preparations mounted in concentrated hydrochloric acid should be free of excess acid and must be removed from the microscope stage as soon as possible.

Part 3 Thin-layer Chromatographic patterns of stem extract

3.1 Materials:

3.1.1 Authentic drug samples as part 2

3.1.2 Crude drugs which were purchased from traditional drugstores throughout Thailand (Table 8).

Table 8. Khamin khruea which were purchased from traditional drugstores

No. of sample	Called name	Place	Purchased date
1	Khamin khruea, Ham	Trang province	October, 2000
2	Ham	Songkhla province	February, 2001
3	Ham	Ubon Ratchathani province	August, 2000
4	Khamin khruea	Bangkok province	August, 2000
5	Ham	Ubon Ratchathani province	May, 2000
6	Khamin khruea	Prachin Buri province	May, 2000
7	Khamin khruea, Ham	Kanchanaburi province	July, 2000
8	Ham	Nong Khai province	August, 2000
9	Khamin khruea	Nakhon Pathom province	September, 2000
10	Khamin khruea, Ham	Bangkok province	September, 2000
11	Khamin khruea, Ham	Bangkok province	September, 2000
12	Khamin khruea, Ham	Bangkok province	September, 2000
13	Khamin khruea	Uttaradit province	September, 2000
14	Khamin khruea, Ham	Chiang Mai province	September, 2000
15	Khamin khruea, Ham	Chiang Mai province	September, 2000
16	Khamin khruea, Ham	Nan province	September, 2000
17	Khamin khruea, Ham	Nakhon Ratchasima province	October, 2000
18	Khamin khruea, Ham	Nakhon Ratchasima province	October, 2000
19	Khamin khruea ,Ham	Lop Buri province	October, 2000
20	Khamin khruea, Ham	Chiang Rai province	November, 2000
21	Khamin khruea, Ham	Suphan Buri province	November, 2000
22	Khamin khruea, Ham	Suphan Buri province	November, 2000
23	Khamin khruea, Ham	Phuket province	November, 2000
24	Khamin khruea, Ham	Phuket province	November, 2000
25	Khamin khruea	Chumphon province	December, 2000
26	Khamin khruea	Chumphon province	December, 2000
27	Khamin khruea	Songkhla province	February, 2001
28	Khamin khruea	Songkhla province	February, 2001
29	Khamin khruea	Ubon Ratchathani province	August, 2000
30	Khamin khruea	Bangkok province	September, 2000
31	Khamin khruea	Bangkok province	September, 2000
32	Khamin khruea	Ubon Ratchathani province	January, 2001

3.2 Procedure

3.2.1 The fresh authentic stems were chopped into small pieces and dried in a hot air oven at 50°C, then ground and passed through a sieve with mesh number 3. Kept in a well-closed container. The purchased sample also did as described above.

3.2.2 Prepared 0.02% berberine sulphate in alcohol as control.

3.2.3 One-dimensional chromatography.

1. Five grams of dried stem powdered drug were macerated in 10 ml of methanol for 24 hours, then filtered through filter paper (Whatman) and kept in well-closed container prior to spotted on TLC plate.

2. Selected suitable solvent system that can provide the pattern for separation and identification.

System 1 : Butanol : acetic acid : water (7:1:2)

System 2 : Methanol : diethylamine : water (8:1:1)

and Cyclohexane : diethylamine (9:1)

3. Spotted the extract amount 4 μl per each sample by micropipetted on TLC plate, allowed to dried by air.

4. Developed the chromatogram after solvent system saturating in chamber. In system 2, developed the chromatogram after first solvent system saturating in chamber and removed the plate from the tank, allowed to dried by air. Re-developed the same plate in the same direction in the second solvent system until ascended 5 cm then removed the plate and allowed to dried by air. The developing distance was 5 cm at room temperature (30-35°C).

5. Detection of the chromatogram.

- Visible in daylight
- Fluorescence under UV 254 and 365 nm
- Detection with Dragendorff's reagent
- Detection with Anisaldehyde-sulphuric acid reagent

3.2.2 Two-dimensional chromatography.

1. Crude extract of authentic samples were prepared as one-dimensional chromatography.

2. Solvent systems
   - The first dimension was Butanol: acetic acid : water (7:1:2)
   - The second dimension was Methanol : diethylamine : water (8:1:1).

3. Spotted the extract amount 30 µl per each sample by micropipetted on TLC plate at the left side angle of plate, allowed to dried by air.

4. Developed the chromatogram after first solvent system saturating in chamber. The developing distance is 15 cm, removed the plate from the tank and allowed to dried by air. Re-developed the same plate in the second direction commenced in the perpendicular direction with the second solvent system until ascended 15 cm, removed the plate and allowed to dried by air.

5. Detection of the chromatogram
   - Fluorescence under UV 254 and 365 nm

6. Record R_f value.

Recording for two-dimensional chromatography

Rf grid was used as an aid in recording the values as shown in Figure 10. The Rf values were coded as follows (Bubpachart, 1981, quoting Vichiara, 1964).

00 (Zero,Zero)	Corresponds to Rf	.000 to .075
08	”	.075 to .125
10	”	.125 to .175
18	”	.175 to .225
20	”	.225 to .275
28	”	.275 to .325
30	”	.325 to .375
38	”	.375 to .425
40	”	.425 to .475
48	”	.475 to .525
50	”	.525 to .575
58	”	.575 to .625
60	”	.625 to .675
68	”	.675 to .725

70	Corresponds to R_f	.725 to .775
78	”	.775 to .825
80	”	.825 to .875
88	”	.875 to .925
90	”	.925 to 1.000

The colors were arranged in a continuous disc system according to the solar spectrum colors. It was often difficult to indicate color with precision (e.g. shades of pink and pale violet), but moderate errors would not invalidate the present system. Number 1 to 7 were used, the first digit for the basic color itself and the second digit “0” (zero) to “5” for light or pale shade to darker such as

10	pink	13	purplish-red	15	red
20	pale orange	21	pinkish-orange		
		24	yellowish-orange	25	orange
30	brown			35	dark brown
				38	yellowish-brown
40	light yellow			45	yellow
50	green	53	brownish-green	55	dark green
60	blue			65	dark blue
70	heliotrope	73	bluish-violet	75	violet
80	gray			85	black
90	quenching			95	strong quenching (under UV)
00	nil				

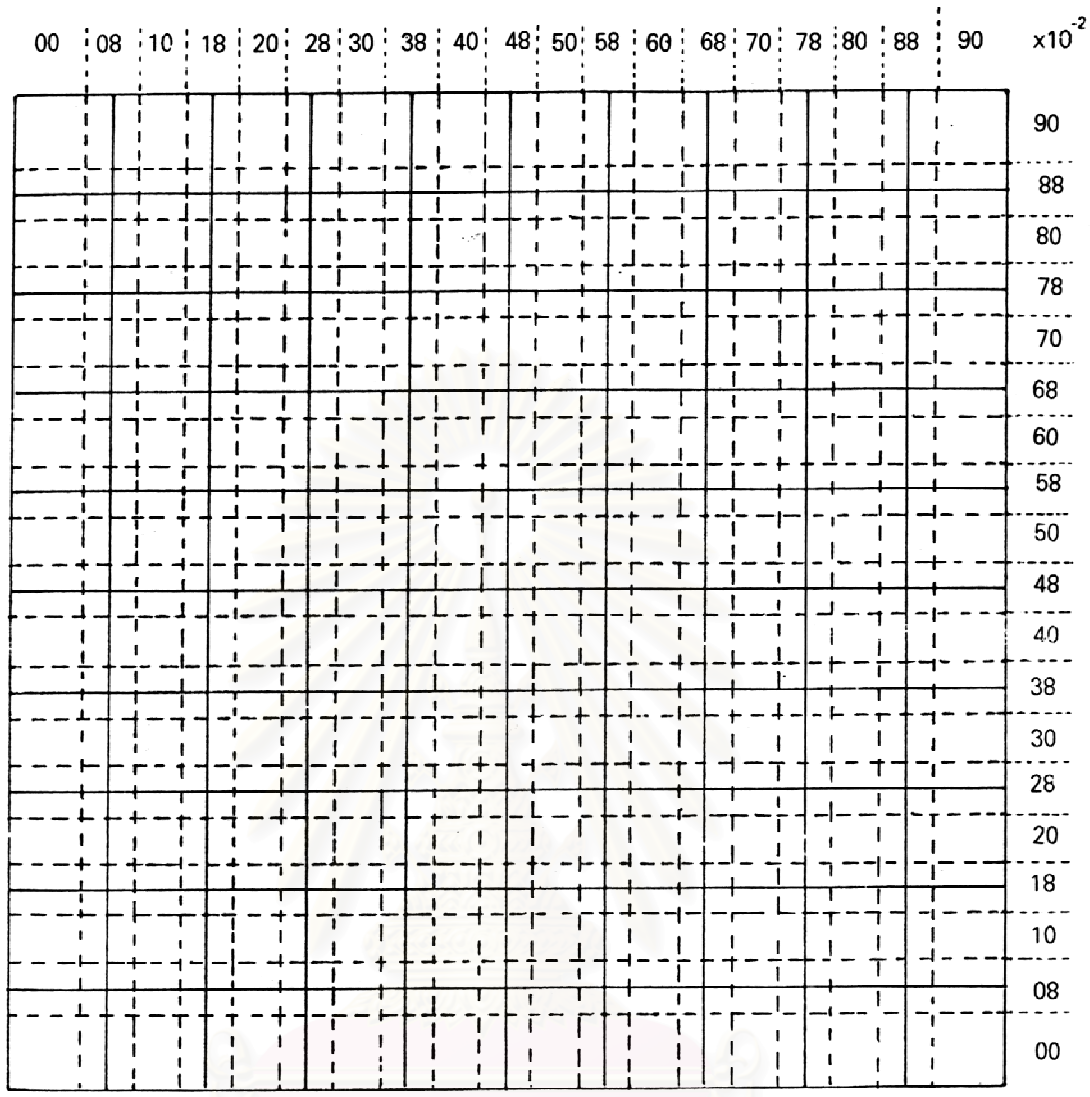


Figure 10. R_f grid (1:1)

Part 4 Phytochemical screening of alkaloid and tannin groups

4.1 Materials: as 3.1

4.2 Procedure

4.2.1 Alkaloid test

1. Twenty grams of dried coarse powdered drug was macerated in 50 ml of 95% ethanol for 24 hours, filtered and then evaporated to dryness on water-bath.

2. Dissolved the crude extract in 10 ml of dilute sulphuric acid (2%), filtered and separated the filtrate into 2 portions:

portion 1 added a few drops of Mayer's reagent, an alkaloid-positive reaction must be observed white precipitation.

portion 2 added a few drops of Dragendorff's reagent, an alkaloid-positive reaction must be observed orange precipitation.

3. The observed precipitation must not dissolve in alcohol.

4.2.2 Tannin test

The five grams of dried coarse powdered drug was boiled in 30 ml of distilled water, filtered and separated the filtrate into 4 portions:

portion 1 As control

portion 2 added a few drops of gelatin solution, positive reaction with tannin must be observed white precipitation.

portion 3 added a few drops of ferric chloride solution, positive test with tannin must be observed blue or green color and precipitation.

- positive test with pyrogallol resulted in blue color and precipitation.
- positive test with catechol resulted in green color and precipitation.

portion 4 added a few drops of bromine water,

- If positive test with pyrogallol, observed no change.
- If positive test with catechol resulted in light precipitation.

Part 5 Quality controls

5.1 Materials: as 3.1

5.2 Procedure

5.2.1 Loss on drying

As directed in the monograph (Thai Pharmacopoeia, 1987), conducted the determination on 2 to 5 grams of crude drugs, previously mixed and accurately weighed. Tared a glass-stoppered, shallow weighing bottle that has been dried until constant weight or 30 minutes under the same condition to be employed in the determination. Put the test specimen in the bottle, replaced the cover, and accurately weighed the bottle and the contents. Dried the test specimen at the temperature 105°C to constant weight. Upon opening the chamber, closed the bottle promptly and allowed it to come to room temperature in the desiccator before weighing. Calculated the percentage of loss on drying with reference to the air-dried substance.

5.2.2 Total ash and acid-insoluble ash (Thai Pharmacopoeia, 1987)

Total ash

Placed 2 to 4 g of the ground sample, accurately weighed in a suitable tared crucible (usually of platinum or silica), previously ignited, cooled and weighed. Incinerated the sample by gradually increasing the temperature, not exceeding 450°C, until free from carbon; cooled and weighed. If a carbon-free ash cannot be obtained in this way, cooled the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrated. Dried on a water-bath and then on a hot plate and incinerate to constant weight. Calculated the percentage of total ash with reference to the air-dried substance.

Acid-insoluble ash

Boiled the total ash for 5 minutes with 25 ml of diluted hydrochloric acid, collected the insoluble matter on an ashless filter paper, washed with hot water until the filtrate was neutral, and ignited at about 500°C. Calculated the percentage of acid-insoluble ash with reference to the air-dried substance.

5.2.3 Extractive value (British Pharmacopoeia, 1993)

Ethanol-soluble extractive

Macerated 5 g of the air-dried drug, coarsely powdered, with 100 ml of ethanol of the specified strength in a closed flask or 24 hours, shaking frequently during the first 6 hours and then allowing to stand for 18 hours. Filtered rapidly, taking precautions against loss of ethanol, evaporated 20 ml of the filtrate to dryness in a tared, flat-bottomed, shallow dish and dried at 105°C to constant weight. Calculated the percentage of ethanol-soluble extractive with reference to the air-dried drug.

Water-soluble extractive

Proceeded as directed for ethanol-soluble extractive, but using chloroform water in place of ethanol.

5.3 Each crude drug sampling was tripled for loss on drying and ash content, but was seconded for extractive value.

CHAPTER V

RESULTS AND DISCUSSION

Part 1 The results of leaf measurement

The leaf measurements data are shown in Table 9-34.

Table 9-13. Stomatal number and stomatal index of *Arcangelisia flava* (L.) Merr.

Table 14. Glandular number and glandular index of *Coscinium fenestratum* (Gaertn.) Colebr.

Table 15-16. Glandular number and glandular index of *Coscinium* sp.

Table 17-19. Stomatal number and stomatal index of *Fibraurea tinctoria* Lour.

Table 20-21. Stomatal number and stomatal index of *Combretum latifolium* Blume

Table 22-26. Vein-islet number, veinlet termination number and palisade ratio of *Arcangelisia flava* (L.) Merr.

Table 27. Vein-islet number, veinlet termination number and palisade ratio of *Coscinium fenestratum* (Gaertn.) Colebr.

Table 28-29. Vein-islet number, veinlet termination number and palisade ratio of *Coscinium* sp.

Table 30-32. Vein-islet number, veinlet termination number and palisade ratio of *Fibraurea tictoria* Lour.

Table 33-34. Vein-islet number, veinlet termination number and palisade ratio of *Combretum latifolium* Blume

The outline drawing of each species of leaf measurements including stomata, glandular trichomes, vein structure and four epidermal cells with underlying palisade cells are shown in Figure 11-14 and the microscopic illustration of their leaves are shown in Figure15-19.

Table 9. Stomatal number and stomatal index of *Arcangelisia flava* (L.) Merr. (1)

Area determination = 0.1134 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
31	342	273.37	8.31
36	319	317.46	10.14
35	326	308.64	9.69
31	327	273.37	8.66
27	327	238.10	7.63
32	376	282.19	7.84
29	336	255.73	7.95
33	353	291.01	8.55
31	327	273.37	8.66
32	334	282.19	8.74
35	346	308.64	9.19
30	297	264.55	9.17
27	299	238.10	8.28
38	393	335.10	8.82
33	377	291.01	8.05
35	390	308.64	8.24
37	388	326.28	8.71
35	405	308.64	7.95
38	378	335.10	9.13
32	327	282.19	8.91
32	330	282.19	8.84
33	371	291.01	8.17
34	380	299.82	8.21
30	280	264.55	9.67
39	350	343.92	10.03
28	290	246.91	8.81
44	384	388.01	10.28
32	330	282.19	8.84
33	371	291.01	8.17
34	380	299.82	8.21
	mean	292.77	8.73
	S.D.	32.62	0.70

Table 10. Stomatal number and stomatal index of *Arcangelisia flava* (L.) Merr. (2)

Area determination = 0.1134 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
25	274	220.46	8.36
22	276	194.00	7.38
22	275	194.00	7.41
28	295	246.91	8.67
25	296	220.46	7.79
22	286	194.00	7.14
24	300	211.64	7.41
22	269	194.00	7.56
23	290	202.82	7.4
22	285	194.00	7.17
25	293	220.46	7.86
24	287	211.64	7.72
24	300	211.64	7.41
27	300	238.10	8.26
25	282	220.46	8.14
26	304	229.28	7.88
22	279	194.00	7.31
23	286	202.82	7.44
22	281	194.00	7.26
25	317	220.46	7.31
20	253	176.37	7.33
23	290	202.82	7.35
25	300	220.46	7.69
24	285	211.64	7.77
29	333	255.73	8.01
25	295	220.46	7.81
26	310	229.28	7.74
27	332	238.10	7.52
29	320	255.73	8.31
24	310	211.64	7.19
	mean	214.58	7.65
	S.D.	19.56	0.40

Table 11. Stomatal number and stomatal index of *Arcangelisia flava* (L.) Merr. (3)

Area determination = 0.1134 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
30	278	264.55	9.74
34	308	299.82	9.94
32	304	282.19	9.52
25	280	220.46	8.2
30	312	264.55	8.77
30	326	264.55	8.43
27	299	238.10	8.28
26	295	229.28	8.1
29	313	255.73	8.48
30	322	264.55	8.52
36	339	317.46	9.50
29	280	255.73	9.39
26	283	229.28	8.41
27	285	238.10	8.65
30	282	264.55	9.62
31	287	273.37	9.75
27	298	238.10	8.31
28	256	246.91	9.86
30	300	264.55	9.09
29	298	255.73	8.87
32	330	282.19	8.84
29	307	255.73	8.63
27	310	238.10	8.01
30	319	264.55	8.6
32	322	282.19	9.04
26	311	229.28	7.72
28	298	246.91	8.59
32	284	282.19	10.13
27	300	238.10	8.26
30	330	264.55	8.33
	mean	258.38	8.85
	S.D.	22.23	0.65

Table 12. Stomatal number and stomatal index of *Arcangelisia flava* (L.) Merr. (4)
Area determination = 0.1134 mm^2

Number of Stomata	Number of epidermal cells	Stomatal number	Stomatal index
27	289	238.10	8.54
28	292	246.91	8.75
35	329	308.64	9.62
30	296	264.55	9.2
35	324	308.64	9.75
31	326	273.37	8.68
28	282	246.91	9.03
33	316	291.10	9.46
33	301	291.01	8.99
35	320	308.64	9.86
32	322	282.19	9.04
32	321	282.19	9.07
33	328	291.01	9.14
34	331	299.82	9.32
33	340	291.01	8.85
30	290	264.55	9.38
35	315	308.64	10
29	300	255.73	8.81
32	330	282.19	8.84
31	320	273.37	8.83
31	307	273.37	9.17
34	327	299.82	9.42
39	328	343.92	10.63
28	297	246.91	8.62
32	319	282.19	9.12
31	307	273.37	9.17
30	285	264.55	9.52
38	308	335.10	10.98
32	325	282.19	8.96
33	337	291.01	8.92
	mean	283.36	9.26
	S.D.	24.91	0.56

Table 13. Stomatal number and stomatal index of *Arcangelisia flava* (L.) Merr. (5)

Area determination = 0.1134 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
31	338	273.37	8.40
32	346	282.19	8.47
27	335	238.10	7.46
33	358	291.01	8.44
29	348	255.73	7.69
36	380	317.46	8.65
28	324	246.91	7.95
34	348	299.82	8.90
31	351	273.37	8.12
27	327	238.10	7.63
28	336	246.91	7.69
33	371	291.01	8.17
32	366	282.19	8.04
31	340	273.37	8.36
30	349	264.55	7.92
28	332	246.91	7.78
29	340	255.73	7.86
27	330	238.10	7.56
30	335	264.55	8.22
33	345	291.01	8.73
31	345	273.37	8.24
30	333	264.55	8.26
29	343	255.73	7.80
26	310	229.28	7.74
34	353	299.82	8.79
30	339	264.55	8.13
28	380	246.91	6.86
29	345	255.73	7.75
34	340	299.82	8.95
32	360	282.19	8.16
	mean	268.08	8.09
	S.D.	22.28	0.47

Table 14. Glandular number and glandular index of *Coscinium fenestratum* (Gaertn.) Colebr.

Area determination = 0.1134 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular number	Glandular index
6	245	52.91	2.39
9	244	79.37	3.56
9	253	79.37	3.44
8	241	70.55	3.21
7	238	61.73	2.86
8	226	70.55	3.42
7	230	61.73	2.95
7	226	61.73	3.00
7	256	61.73	2.66
9	252	79.37	3.45
8	227	70.55	3.40
6	221	52.91	2.64
7	226	61.73	3.00
6	214	52.91	2.73
8	242	70.55	3.20
6	221	52.91	2.64
7	226	61.73	3.00
6	214	52.91	2.72
8	242	70.55	3.20
8	232	70.55	3.33
8	254	70.55	3.05
9	253	79.37	3.44
7	215	61.73	3.15
7	231	61.73	2.94
6	239	52.91	2.45
8	240	70.55	3.23
7	238	61.73	2.86
9	259	79.37	3.36
8	235	70.55	3.29
8	245	70.55	3.16
	mean	65.85	3.06
	S.D.	8.89	0.32

Table 15. Glandular number and glandular index of *Coscinium* sp. (1)

Area determination = 0.1134 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular number	Glandular index
3	135	26.46	2.17
3	133	26.46	2.21
2	125	17.64	1.57
4	126	35.27	3.08
3	129	26.46	2.27
2	123	17.64	1.60
2	112	17.64	1.75
3	130	26.46	2.26
3	125	26.46	2.34
4	141	35.27	2.76
4	132	35.27	2.94
3	129	26.46	2.27
4	128	35.27	3.03
3	129	26.46	2.27
3	129	26.46	2.27
4	135	35.27	2.88
2	120	17.64	1.64
3	132	26.46	2.22
3	133	26.46	2.21
4	130	35.27	2.99
3	129	26.46	2.27
2	122	17.64	1.61
3	133	26.46	2.21
3	132	26.46	2.22
4	140	35.27	2.78
2	120	17.64	1.64
3	128	26.46	2.29
4	135	35.27	2.88
3	130	26.46	2.26
3	139	26.46	2.11
	mean	27.05	2.30
	S.D.	6.10	0.45

Table 16. Glandular number and glandular index of *Coscinium* sp. (2)

Area determination = 0.1134 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular number	Glandular index
2	191	17.64	1.04
3	194	26.46	1.52
2	196	17.64	1.01
3	202	26.46	1.46
3	185	26.46	1.60
3	185	26.46	1.60
2	182	17.64	1.09
3	181	26.46	1.63
2	195	17.64	1.02
3	189	26.46	1.56
2	176	17.64	1.12
3	177	26.46	1.67
3	187	26.46	1.58
4	189	35.27	2.07
2	182	17.64	1.09
2	172	17.64	1.15
3	173	26.46	1.70
3	179	26.46	1.65
3	173	26.46	1.70
4	190	35.27	2.06
3	179	26.46	1.65
2	170	17.64	1.16
3	173	26.46	1.70
3	182	26.46	1.62
4	190	35.27	2.06
2	180	17.64	1.10
3	178	26.46	1.66
4	195	35.27	2.01
3	180	26.46	1.64
3	189	26.46	1.56
	mean	25.00	1.52
	S.D.	5.71	0.33

Table 17. Stomatal number and stomatal index of *Fibraurea tinctoria* Lour. (1)

Area determination = 0.1134 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
27	277	238.10	8.88
24	262	211.64	8.39
23	263	202.82	8.04
21	261	185.19	7.45
21	253	185.19	7.66
22	283	194.00	7.21
20	258	176.37	7.19
29	306	255.73	8.66
31	297	273.37	9.45
23	240	202.82	8.75
21	255	185.19	7.61
23	278	202.82	7.64
22	259	194.00	7.83
22	262	194.00	7.75
23	270	202.82	7.85
22	253	194.00	8.00
28	262	246.91	9.66
27	258	238.10	9.47
25	267	220.46	8.56
22	251	194.00	8.06
23	250	202.82	8.42
25	261	220.46	8.74
26	260	229.28	9.09
24	271	211.64	8.14
22	265	194.00	7.67
23	268	202.82	7.90
26	250	229.28	9.42
24	280	211.64	7.89
25	291	220.46	7.91
30	275	264.55	9.84
	mean	212.82	8.30
	S.D.	24.70	0.74

Table 18. Stomatal number and stomatal index of *Fibraurea tinctoria* Lour. (2)

Area determination = 0.1134 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
34	302	299.82	10.12
25	262	220.46	8.71
30	296	264.55	9.20
26	287	229.28	8.31
28	283	246.91	9.00
28	293	246.91	8.72
30	274	264.55	9.87
29	269	255.73	9.73
33	289	291.01	10.25
26	290	229.28	8.23
29	267	255.73	9.80
34	285	299.82	10.66
33	293	291.01	10.12
31	295	273.37	9.51
33	307	291.01	9.71
30	327	264.55	8.40
31	288	273.37	9.72
25	268	220.46	8.53
29	283	255.73	9.30
31	256	273.37	10.80
25	267	220.46	8.56
26	289	226.28	8.25
30	318	264.55	8.62
23	258	202.82	8.19
28	298	246.91	8.59
30	302	264.55	9.04
36	318	317.46	10.17
32	298	282.19	9.69
30	272	264.55	9.93
27	284	238.10	8.68
	mean	259.26	9.28
	S.D.	27.75	0.77

Table 19. Stomatal number and stomatal index of *Fibraurea tinctoria* Lour. (3)

Area determination = 0.1134 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
41	355	361.55	10.35
37	327	326.28	10.16
35	341	308.64	9.31
42	355	370.37	10.58
42	357	370.37	10.53
43	376	379.19	10.26
44	383	388.00	10.30
43	373	379.19	10.34
33	323	291.01	9.27
45	366	396.83	10.95
38	377	335.10	9.16
45	375	396.83	10.71
31	324	273.37	8.73
37	318	326.28	10.42
36	328	317.46	9.89
43	364	379.19	10.57
34	307	299.82	9.97
44	342	388.01	11.39
38	335	335.10	10.19
33	298	291.01	9.97
32	354	282.19	8.29
40	319	352.73	11.14
33	362	291.01	8.35
41	309	361.55	11.71
31	338	273.37	8.40
42	346	370.37	10.82
37	395	326.28	8.56
44	355	388.01	11.03
39	379	343.92	9.33
45	379	396.83	10.61
	mean	343.33	10.04
	S.D.	40.64	0.93

Table 20. Stomatal number and stomatal index of *Combretum latifolium* Blume (1)

Area determination = 0.1134 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
36	248	317.46	12.68
55	334	485.01	14.14
39	266	343.92	12.79
40	289	352.73	12.16
36	265	317.46	11.96
47	326	414.46	12.60
50	305	440.92	14.08
40	274	352.73	12.74
37	270	326.28	12.05
46	300	405.64	13.29
54	330	476.19	14.06
45	283	396.83	13.72
37	253	326.28	12.76
33	235	291.01	12.31
36	249	317.46	12.63
41	295	361.55	12.20
37	269	326.28	12.09
40	276	352.73	12.66
42	306	370.37	12.07
36	256	317.46	12.33
33	231	291.01	12.50
45	261	396.83	14.71
36	229	317.46	13.58
38	250	335.10	13.19
46	306	405.64	13.07
44	315	388.01	12.26
36	251	317.46	12.54
35	210	308.64	14.29
36	228	317.46	13.64
43	301	379.19	12.50
	mean	358.32	12.92
	S.D.	51.18	0.77

Table 21. Stomatal number and stomatal index of *Combretum latifolium* Blume (2)

Area determination = 0.1134 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
45	277	396.83	13.98
47	267	414.46	14.97
48	249	423.28	16.16
39	233	343.92	14.34
41	243	361.55	14.44
40	224	352.73	15.15
38	231	335.10	14.13
50	266	440.92	15.82
41	250	361.55	14.09
51	257	449.74	16.56
52	269	458.55	16.20
52	275	458.55	15.90
39	246	343.92	13.68
38	240	335.10	13.67
39	250	343.92	13.49
50	250	440.92	16.66
44	260	388.01	14.47
42	238	370.37	15.00
46	259	405.64	15.08
48	251	423.28	16.05
40	238	352.73	14.39
43	238	379.19	14.29
49	257	432.10	16.01
45	284	396.83	13.68
48	240	423.28	16.67
45	267	396.83	14.42
41	258	341.55	13.71
45	260	396.83	14.75
44	257	388.01	14.62
47	284	414.46	14.20
	mean	392.34	14.89
	S.D.	39.11	1.00

Table 22. Vein-islet number, veinlet termination number and palisade ratio of *Arcangelisia flava* (L.) Merr. (1)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm²	**Vein-islet number**	**Count in 4 mm²**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
9	2.25	36	9.00	18	4.50
7	1.75	27	6.75	22	5.50
10	2.50	30	7.50	24	6.00
7	1.75	27	6.75	20	5.00
7	1.75	22	5.50	22	5.50
9	2.25	23	5.75	27	6.75
7	1.75	30	7.50	20	5.00
11	2.75	22	5.50	26	6.50
9	2.25	35	8.75	22	5.50
7	1.75	34	8.50	23	5.75
9	2.25	36	9.00	24	6.00
7	1.75	33	8.25	18	4.50
9	2.25	33	8.25	18	4.50
8	2.00	26	6.50	25	6.25
6	1.50	28	7.00	25	6.25
8	2.00	32	8.00	23	5.75
9	2.25	24	6.00	24	6.00
12	3.00	29	7.25	23	5.75
9	2.25	26	6.50	22	5.50
11	2.75	25	6.25	25	6.25
10	2.50	31	7.75	21	5.25
8	2.00	28	7.00	23	5.75
8	2.00	25	6.25	26	6.50
8	2.00	27	6.75	24	6.00
10	2.50	29	7.25	25	6.25
9	2.25	28	7.00	23	5.75
8	2.00	30	7.50	22	5.50
9	2.25	34	8.50	24	6.00
9	2.25	33	8.25	19	4.75
7	1.75	28	7.00	22	5.50
mean	2.14	**mean**	7.26	**mean**	5.67
S.D.	0.35	**S.D.**	1.01	**S.D.**	0.61

Table 23. Vein-islet number, veinlet termination number and palisade ratio of *Arcangelisia flava* (L.) Merr. (2)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm²	**Vein-islet number**	**Count in 4 mm²**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
7	1.75	21	5.25	23	5.75
7	1.75	16	4.00	25	6.25
8	2.00	20	5.00	23	5.75
8	2.00	18	4.50	17	4.25
8	2.00	18	4.50	24	6.00
9	2.25	18	4.50	22	5.50
7	1.75	22	5.50	17	4.25
6	1.50	20	5.00	21	5.25
8	2.00	22	5.50	25	6.25
10	2.50	13	3.25	18	4.50
7	1.75	16	4.00	22	5.50
11	2.75	22	5.50	20	5.00
9	2.25	30	7.50	21	5.25
8	2.00	19	4.75	23	5.75
7	1.75	19	4.75	21	5.25
9	2.25	19	4.75	20	5.00
8	2.00	22	5.50	18	4.50
7	1.75	23	5.75	22	5.50
8	2.00	24	6.00	19	4.75
6	1.50	21	5.25	23	5.75
9	2.25	18	4.50	19	4.75
10	2.50	13	3.25	25	6.25
9	2.25	18	4.50	20	5.00
7	1.75	20	5.00	22	5.50
6	1.50	23	5.75	25	6.25
9	2.25	23	5.75	23	5.75
7	1.75	20	5.00	24	6.00
8	2.00	25	6.25	24	6.00
7	1.75	21	5.25	25	6.25
9	2.25	25	6.25	27	6.75
mean	1.99	**mean**	5.08	**mean**	5.48
S.D.	0.31	**S.D.**	0.88	**S.D.**	0.66

Table 24. Vein-islet number, veinlet termination number and palisade ratio of *Arcangelisia flava* (L.) Merr. (3)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm^2	**Vein-islet number**	**Count in 4 mm^2**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
13	3.25	22	5.50	22	5.50
7	1.75	24	6.00	25	6.25
9	2.25	26	6.50	25	6.25
9	2.25	29	7.25	26	6.50
13	3.25	31	7.75	22	5.50
9	2.25	31	7.75	20	5.00
10	2.50	25	6.25	27	6.75
13	3.25	35	8.75	20	5.00
13	3.25	29	7.25	23	5.75
10	2.50	20	5.00	21	5.25
11	2.75	31	7.75	23	5.75
10	2.50	29	7.25	18	4.50
9	2.25	31	7.75	28	7.00
9	2.25	31	7.75	22	5.50
11	2.75	29	7.25	18	4.50
15	3.75	24	6.00	24	6.00
15	3.75	27	6.75	21	5.25
12	3.00	34	8.50	24	6.00
9	2.25	35	8.75	22	5.50
9	2.25	31	7.75	25	6.25
9	2.25	29	7.25	26	6.50
8	2.00	29	7.25	22	5.50
9	2.25	33	8.25	24	6.00
9	2.25	35	8.75	24	6.00
7	1.75	26	6.50	23	5.75
8	2.00	34	8.50	26	6.50
9	2.25	32	8.00	28	7.00
10	2.50	21	5.25	28	7.00
15	3.75	25	6.25	27	6.75
12	3.00	28	7.00	25	6.25
mean	2.60	**mean**	7.22	**mean**	5.91
S.D.	0.57	**S.D.**	1.04	**S.D.**	0.69

Table 25. Vein-islet number, veinlet termination number and palisade ratio of *Arcangelisia flava* (L.) Merr. (4)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm²	**Vein-islet number**	**Count in 4 mm²**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
9	2.25	32	8.00	19	4.75
9	2.25	25	6.25	22	5.50
7	1.75	27	6.75	19	4.75
6	1.50	29	7.25	21	5.25
6	1.50	29	7.25	17	4.25
9	2.25	34	8.50	19	4.75
12	3.00	32	8.00	19	4.75
7	1.75	26	6.50	22	5.50
9	2.25	35	8.75	18	4.50
10	2.50	29	7.25	24	6.00
7	1.75	32	8.00	23	5.75
10	2.50	31	7.75	17	4.25
13	3.25	34	8.50	20	5.00
10	2.50	38	9.50	18	4.50
10	2.50	32	8.00	22	5.50
10	2.50	32	8.00	25	6.25
12	3.00	33	8.25	21	5.25
10	2.50	34	8.50	18	4.50
10	2.50	32	8.00	24	6.00
12	3.00	29	7.25	24	6.00
13	3.25	30	7.50	23	5.75
14	3.50	29	7.25	19	4.75
12	3.00	32	8.00	18	4.50
10	2.50	34	8.50	22	5.50
12	3.00	28	7.00	18	4.50
7	1.75	34	8.50	18	4.50
9	2.25	30	7.50	18	4.50
10	2.50	33	8.25	17	4.25
10	2.50	34	8.50	25	6.25
11	2.75	29	7.25	22	5.50
mean	2.47	**mean**	7.82	**mean**	5.10
S.D.	0.52	**S.D.**	0.72	**S.D.**	0.65

Table 26. Vein-islet number, veinlet termination number and palisade ratio of *Arcangelisia flava* (L.) Merr. (5)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm²	**Vein-islet number**	**Count in 4 mm²**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
8	2.00	29	7.25	20	5.00
8	2.00	28	7.00	22	5.50
8	2.00	23	5.75	17	4.25
8	2.00	34	8.50	24	6.00
8	2.00	27	6.75	24	6.00
7	1.75	22	5.50	25	6.25
7	1.75	28	7.00	22	5.50
8	2.00	29	7.25	28	7.00
9	2.25	25	6.25	21	5.25
7	1.75	19	4.75	19	4.75
7	1.75	18	4.50	19	4.75
7	1.75	17	4.25	25	6.25
9	2.25	18	4.50	27	6.75
9	2.25	20	5.00	22	5.50
10	2.50	22	5.50	25	6.25
8	2.00	22	5.50	25	6.25
6	1.50	22	5.50	20	5.00
8	2.00	25	6.25	23	5.75
8	2.00	32	8.00	20	5.00
10	2.50	22	5.50	19	4.75
7	1.75	28	7.00	26	6.50
8	2.00	35	8.75	24	6.00
6	1.50	31	7.75	22	5.50
6	1.50	27	6.75	22	5.50
8	2.00	26	6.50	23	5.75
7	1.75	21	5.25	23	5.75
11	2.75	28	7.00	26	6.50
9	2.25	27	6.75	27	6.75
8	2.00	25	6.25	28	7.00
7	1.75	28	7.00	25	6.25
mean	1.98	**mean**	6.32	**mean**	5.78
S.D.	0.30	**S.D.**	1.18	**S.D.**	0.72

Table 27. Vein-islet number, veinlet termination number and palisade ratio of *Coscinium fenestratum* (Gaertn.) Colebr.

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm^2	**Vein-islet number**	**Count in 4 mm^2**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
103	25.75	76	19.00	18	4.50
97	24.25	73	18.25	19	4.75
109	27.25	79	19.75	20	5.00
106	26.50	75	18.75	18	4.50
96	24.00	65	16.25	18	4.50
113	28.25	59	14.75	20	5.00
126	31.50	66	16.50	18	4.50
123	30.75	65	16.25	22	5.50
134	33.50	60	15.00	18	4.50
96	24.00	62	15.50	22	5.50
103	25.75	61	15.25	19	4.75
103	25.75	66	16.50	27	6.75
96	24.00	63	15.75	27	6.75
134	33.50	60	15.00	18	4.50
133	33.25	63	15.75	23	5.75
137	34.25	70	17.50	18	4.50
138	34.50	71	17.75	18	4.50
138	34.50	76	19.00	18	4.50
123	30.75	67	16.75	18	4.50
144	36.00	75	18.75	19	4.75
132	33.00	66	16.50	17	4.25
123	30.75	68	17.00	24	6.00
113	28.25	62	15.50	15	3.75
113	28.25	70	17.50	21	5.25
98	24.50	66	16.50	18	4.50
121	30.25	60	15.00	25	6.25
113	28.25	70	17.50	23	5.75
120	30.00	60	15.00	24	6.00
135	33.75	62	15.50	20	5.00
125	31.25	70	17.50	19	4.75
mean	29.54	**mean**	16.72	**mean**	5.03
S.D.	3.76	**S.D.**	1.42	**S.D.**	0.75

Table 28. Vein-islet number, veinlet termination number and palisade ratio of *Coscinium* sp. (1)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm²	**Vein-islet number**	**Count in 4 mm²**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
54	13.50	46	11.50	36	9.00
50	12.50	39	9.75	43	10.75
56	14.00	34	8.50	44	11.00
42	10.50	35	8.75	44	11.00
46	11.50	35	8.75	39	9.75
50	12.50	38	9.50	33	8.25
48	12.00	42	10.50	42	10.50
59	14.75	36	9.00	46	11.50
56	14.00	35	8.75	55	13.75
52	13.00	34	8.50	49	12.25
58	14.50	40	10.00	46	11.50
42	10.50	34	8.50	52	13.00
57	11.75	37	9.25	53	13.25
49	12.25	35	8.75	54	13.50
50	12.50	42	10.50	52	13.00
53	13.25	34	8.50	40	10.00
41	10.25	40	10.00	48	12.00
45	11.25	53	13.25	44	11.00
47	11.75	42	10.50	39	9.75
41	10.25	44	11.00	47	11.75
53	13.25	37	9.25	52	13.00
55	13.75	38	9.50	40	10.00
43	10.75	38	9.50	40	10.00
54	13.50	40	10.00	50	12.50
49	12.25	37	9.25	51	12.75
52	13.00	45	11.25	58	14.50
45	11.25	35	8.75	48	12.00
55	13.75	47	11.75	45	11.25
54	13.50	39	9.75	42	10.50
45	11.25	33	8.25	50	12.50
mean	12.43	**mean**	9.70	**mean**	11.52
S.D.	1.31	**S.D.**	1.17	**S.D.**	1.50

Table 29. Vein-islet number, veinlet termination number and palisade ratio of *Coscinium* sp. (2)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm^2	**Vein-islet number**	**Count in 4 mm^2**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
53	13.25	60	15.00	40	10.00
56	14.00	51	12.75	43	10.75
59	12.25	55	13.75	50	12.50
55	13.75	45	11.25	52	13.00
66	16.50	60	15.00	42	10.50
60	15.00	50	12.50	50	12.50
52	13.00	60	15.00	43	10.75
61	15.25	46	11.50	44	11.00
57	14.25	59	14.75	45	11.25
67	16.75	50	12.50	45	11.25
67	16.75	56	14.00	43	10.75
60	15.00	60	15.00	46	11.50
59	14.75	47	11.75	41	10.25
65	16.25	61	15.25	52	13.00
49	12.25	62	15.50	44	11.00
55	13.75	58	14.50	50	12.50
58	14.50	61	15.25	53	13.25
54	13.50	59	14.75	44	11.00
68	15.00	50	12.50	48	12.00
51	12.75	52	13.00	48	12.00
57	14.25	53	13.25	52	13.00
57	14.25	56	14.00	43	10.75
53	13.25	58	14.50	53	13.25
54	13.50	46	11.50	47	11.75
49	12.25	54	13.50	41	10.25
52	13.00	49	12.25	45	11.25
55	13.75	58	14.50	55	13.75
55	13.75	61	15.25	40	10.00
54	13.50	53	13.25	41	10.25
55	13.75	54	13.50	45	11.25
mean	14.13	**mean**	13.70	**mean**	11.54
S.D.	1.27	**S.D.**	1.30	**S.D.**	1.10

Table 30. Vein-islet number, veinlet termination number and palisade ratio of *Fibraurea tinctoria* Lour. (1)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm²	**Vein-islet number**	**Count in 4 mm²**	**Veinlet termination Number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
7	1.75	16	4.00	18	4.50
6	1.50	12	3.00	17	4.25
5	1.25	16	4.00	17	4.25
6	1.50	17	4.25	15	3.75
9	2.25	16	4.00	19	4.75
7	1.75	11	2.75	17	4.25
9	2.25	16	4.00	21	5.25
6	1.50	19	4.75	16	4.00
8	2.00	19	4.75	16	4.00
6	1.50	13	3.25	16	4.00
9	2.25	18	4.50	21	5.25
6	1.50	12	3.00	18	4.50
8	2.00	16	4.00	16	4.00
8	2.00	18	4.50	18	4.50
9	2.25	18	4.50	20	5.00
9	2.25	16	4.00	21	5.25
8	2.00	15	3.75	14	3.50
9	2.25	17	4.25	17	4.25
6	1.50	13	3.25	16	4.00
6	1.50	12	3.00	19	4.75
6	1.50	16	4.00	18	4.50
10	2.50	13	3.25	14	3.50
8	2.00	11	2.75	17	4.25
9	2.25	11	2.75	17	4.25
5	1.25	18	4.50	17	4.25
6	1.50	12	3.00	14	3.50
8	2.00	12	3.00	14	3.50
7	1.75	15	3.75	18	4.50
8	2.00	17	4.25	20	5.00
8	2.00	18	4.50	20	5.00
mean	1.85	**mean**	3.78	**mean**	4.34
S.D.	0.35	**S.D.**	0.66	**S.D.**	0.53

Table 31. Vein-islet number, veinlet termination number and palisade ratio of *Fibraurea tinctoria* Lour. (2)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm^2	**Vein-islet number**	**Count in 4 mm^2**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
7	1.75	17	4.25	20	5.00
10	2.50	16	4.00	18	4.50
6	1.50	17	4.25	21	5.25
5	1.25	18	4.50	16	4.00
9	2.25	14	3.50	18	4.50
8	2.00	16	4.00	20	5.00
8	2.00	13	3.25	16	4.00
10	2.50	15	3.75	17	4.25
8	2.00	21	3.25	18	4.50
8	2.00	14	3.50	17	4.25
8	2.00	14	3.50	18	4.50
9	2.25	17	4.25	19	4.75
6	1.50	18	4.50	15	3.75
8	2.00	19	4.75	18	4.50
6	1.50	17	4.25	18	4.50
6	1.50	16	4.00	16	4.00
8	2.00	18	4.50	14	3.50
9	2.25	20	5.00	14	3.50
5	1.25	18	4.50	14	3.50
10	2.50	19	4.75	16	4.00
9	2.25	16	4.00	14	3.50
10	2.50	15	3.75	18	4.50
9	2.25	16	4.00	17	4.25
9	2.25	15	3.75	16	4.00
8	2.00	22	5.50	17	4.25
10	2.50	16	4.00	16	4.00
8	2.00	20	5.00	14	3.50
6	1.50	18	4.50	18	4.50
7	1.75	16	4.00	20	5.00
8	2.00	18	4.50	18	4.50
mean	1.98	**mean**	4.24	**mean**	4.26
S.D.	0.37	**S.D.**	0.54	**S.D.**	0.49

Table 32. Vein-islet number, veinlet termination number and palisade ratio of *Fibraurea tinctoria* Lour. (3)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm^2	**Vein-islet number**	**Count in 4 mm^2**	**Veinlet termination Number**	**No. beneath 4 epidermal cells**	**Palisade Ratio**
8	2.00	13	3.25	16	4.00
7	1.75	17	4.25	18	4.50
6	1.50	22	5.50	19	4.75
7	1.75	14	3.50	19	4.75
9	2.25	18	4.50	18	4.50
9	2.25	17	4.25	19	4.75
5	1.25	12	3.00	16	4.00
7	1.75	14	3.50	19	4.75
7	1.75	12	3.00	15	3.75
8	2.00	12	3.00	18	4.50
8	2.00	15	3.75	15	3.75
6	1.50	16	4.00	18	4.50
5	1.25	18	4.50	17	4.25
7	1.75	11	2.75	18	4.50
6	1.50	15	3.75	18	4.50
5	1.25	17	4.25	16	4.00
6	1.50	17	4.25	16	4.00
7	1.75	10	2.50	17	4.25
8	2.00	16	4.00	19	4.75
6	1.50	14	3.50	19	4.75
5	1.25	12	3.00	17	4.25
7	1.75	12	3.00	16	4.00
8	2.00	16	4.00	17	4.25
5	1.25	13	3.25	16	4.00
5	1.25	17	4.25	18	4.50
8	2.00	13	3.25	19	4.75
9	2.25	15	3.75	18	4.50
7	1.75	14	3.50	16	4.00
7	1.75	12	3.00	17	4.25
8	2.00	15	3.75	18	4.50
mean	1.72	**mean**	3.66	**mean**	4.35
S.D.	0.32	**S.D.**	0.65	**S.D.**	0.32

Table 33. Vein-islet number, veinlet termination number and palisade ratio of *Combretum latifolium* Blume (1)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm²	**Vein-islet number**	**Count in 4 mm²**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
14	3.50	7	1.75	59	14.75
19	4.75	4	1.00	57	14.25
10	2.50	5	1.25	52	13.00
20	5.00	9	2.25	63	15.75
12	3.00	6	1.50	64	16.00
23	5.75	6	1.50	46	11.50
11	2.75	7	1.75	63	15.75
16	4.00	6	1.50	55	13.75
12	3.00	6	1.50	65	16.25
15	3.75	6	1.50	51	12.75
15	3.75	7	1.75	58	14.50
13	3.25	6	1.50	56	14.00
14	3.50	4	1.00	69	17.25
18	4.50	11	2.75	65	16.25
19	4.75	12	3.00	61	15.25
21	5.25	10	2.50	46	11.50
14	3.50	8	2.00	60	15.00
16	4.00	8	2.00	66	16.50
20	5.00	10	2.50	59	14.75
20	5.00	10	2.50	62	15.50
18	4.50	11	2.75	60	15.00
14	3.50	11	2.75	64	16.00
14	3.50	9	2.25	70	17.50
14	3.50	6	1.50	62	15.50
13	3.25	7	1.75	68	17.00
12	3.00	9	2.25	61	15.25
14	3.50	7	1.75	61	15.25
14	3.50	7	1.75	64	16.00
11	2.75	9	2.25	56	14.00
14	3.50	10	2.50	57	14.25
mean	3.83	**mean**	1.97	**mean**	15.00
S.D.	0.83	**S.D.**	0.53	**S.D.**	1.49

Table 34. Vein-islet number, veinlet termination number and palisade ratio of *Combretum latifolium* Blume (2)

Vein-islet		**Veinlet termination**		**Palisade cell**	
Count in 4 mm²	**Vein-islet number**	**Count in 4 mm²**	**Veinlet termination number**	**Number beneath 4 epidermal cells**	**Palisade Ratio**
13	3.25	10	2.50	51	12.75
15	3.75	9	2.25	51	12.75
18	4.50	4	1.00	59	14.75
13	3.25	11	2.75	51	12.75
15	3.75	6	1.50	63	15.75
16	4.00	12	3.00	51	12.75
15	3.75	9	2.25	69	17.25
15	3.75	6	1.50	67	16.75
16	4.00	8	2.00	67	16.75
15	3.75	9	2.25	51	12.75
14	3.50	4	1.00	61	15.25
16	4.00	4	1.00	69	17.25
12	3.00	7	1.75	63	15.75
14	3.50	8	2.00	69	17.25
14	3.50	12	3.00	70	17.50
14	3.50	12	3.00	61	15.25
18	4.50	9	2.25	53	13.25
13	3.25	9	2.25	60	15.00
15	3.75	5	1.25	63	15.75
11	2.75	6	1.50	71	17.75
14	3.50	11	2.75	69	17.25
21	5.25	10	2.50	58	14.50
17	4.25	12	3.00	58	14.50
14	3.50	6	1.50	62	15.50
15	3.75	10	2.50	65	16.25
13	3.25	10	2.50	57	14.25
17	4.25	9	2.25	55	13.75
12	3.00	8	2.00	64	16.00
14	3.50	9	2.25	60	15.00
15	3.75	7	1.75	59	14.75
mean	3.70	**mean**	2.10	**mean**	15.22
S.D.	0.51	**S.D.**	0.61	**S.D.**	1.60

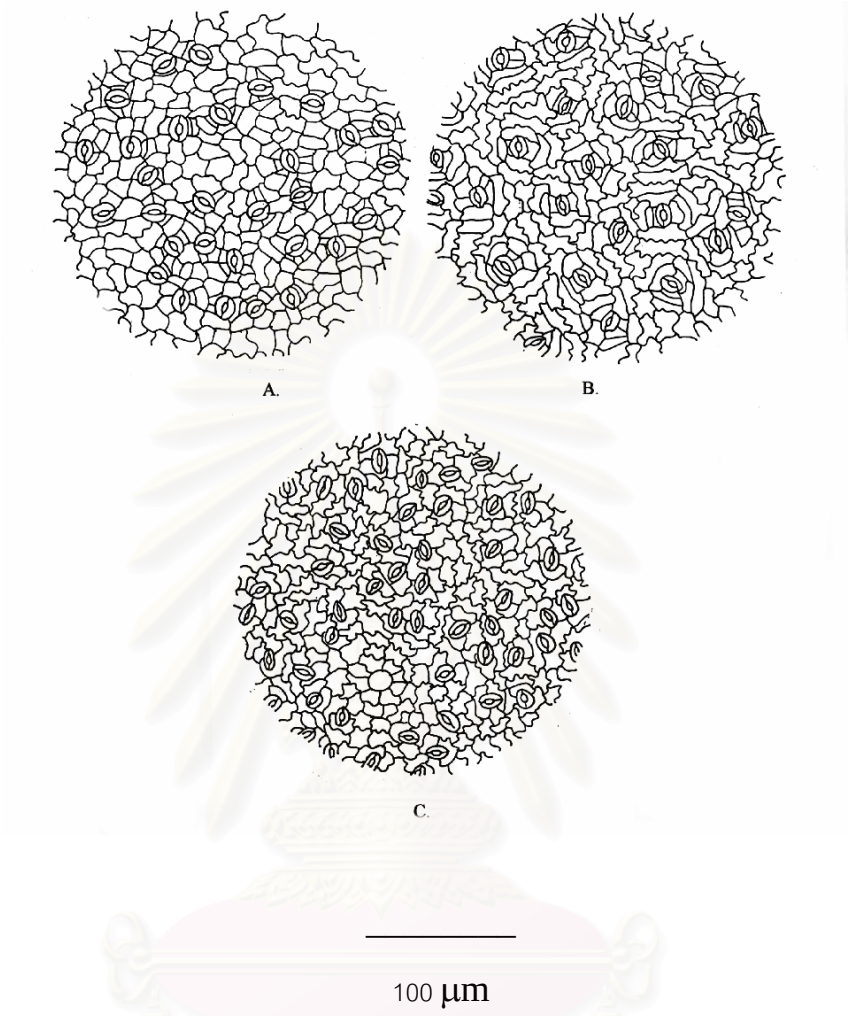


Figure 11. Lower epidermis of the leaves in surface view

A. *Arcangelisia flava* (L.) Merr.

B. *Fibraurea tinctoria* Lour.

C. *Combretum latifolium* Blume

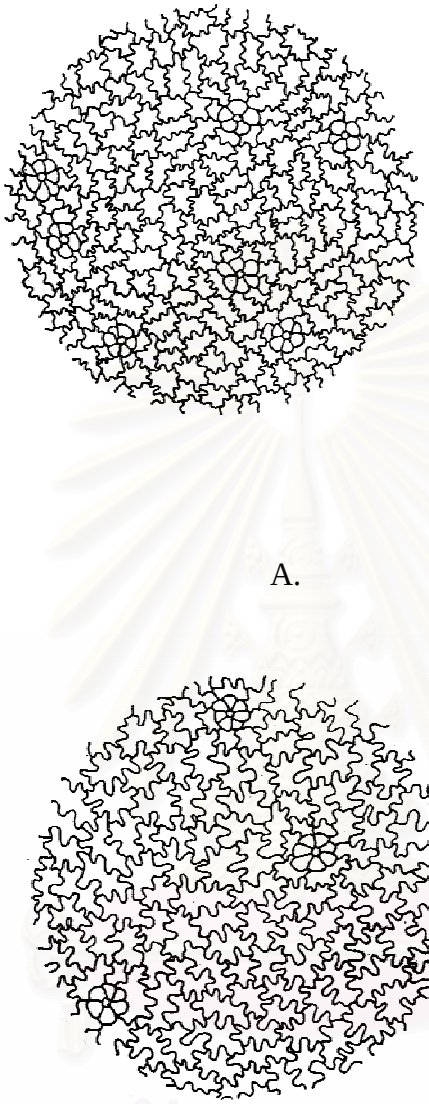


Figure 12. Upper epidermis of the leaves in surface view

A. *Coscinium fenestratum* (Gaertn.) Colebr.

B. *Coscinium* sp.

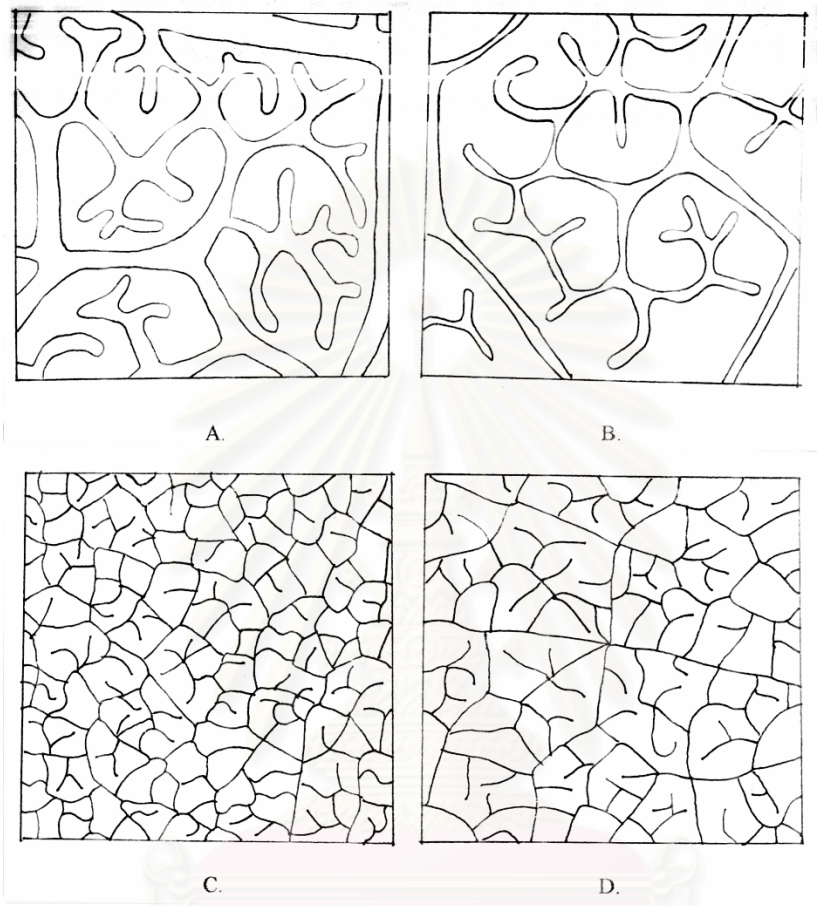


Figure 13. Vein-islet and veinlet termination of leaf in surface view

A. *Arcangelisia flava* (L.) Merr.

B. *Fibraurea tinctoria* Lour.

C. *Coscinium fenestratum* (Gaertn.) Colebr.

D. *Coscinium* sp.

E.

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1 mm

Figure 13. Vein-islet and veinlet termination of leaf in surface view (continued)

E. *Combretum latifolium* Blume

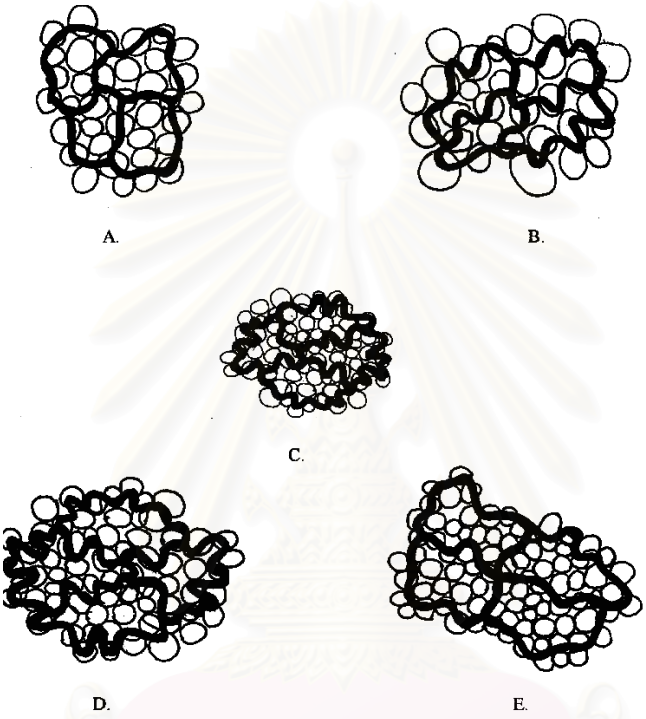

Figure 14. Four upper contiguous epidermal cells with underlying palisade cells in surface view (x 600)

A. *Arcangelisia flava* (L.) Merr.

B. *Fibraurea tinctoria* Lour.

C. *Coscinium fenestratum* (Gaertn.) Colebr.

D. *Coscinium* sp.

E. *Combretum latifolium* Blume

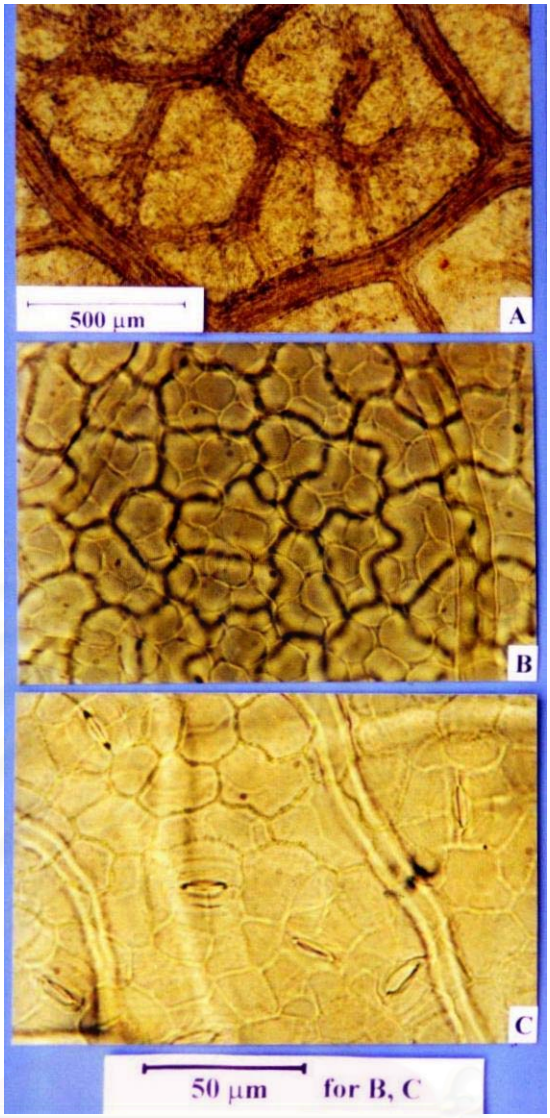

Figure 15. Microscopic illustration of *Arcangelisia flava* (L.) Merr. leaf

A. Vein-islet and veinlet termination

B. Upper epidermis with underlying palisade cells

C. Lower epidermis with stomata

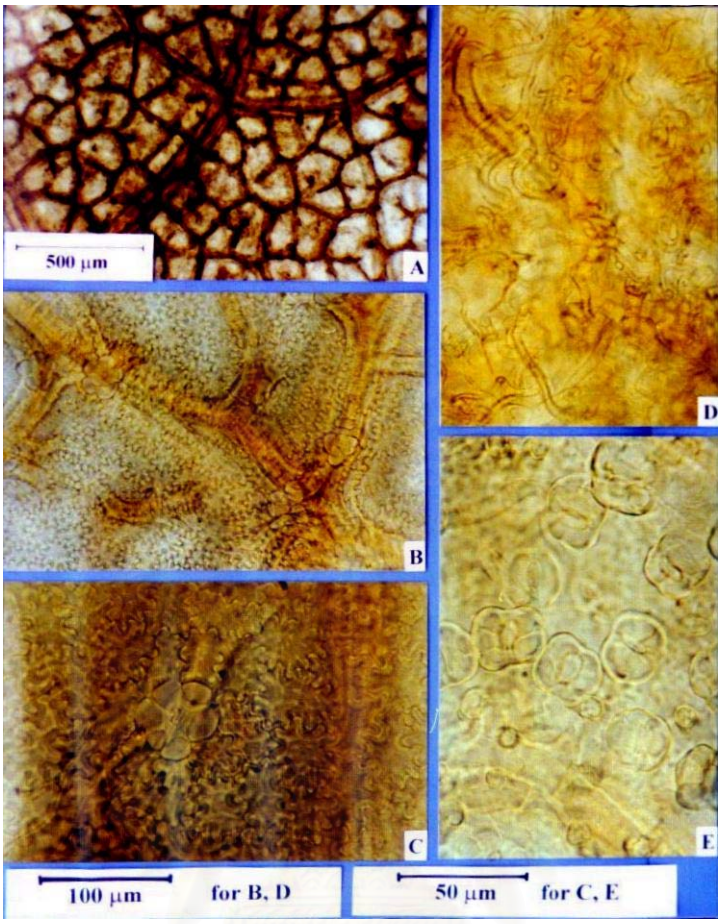

Figure 16. Microscopic illustration of *Coscinium fenestratum* (Gaertn.) Colebr. leaf

A. Vein-islet and veinlet termination
B. Upper epidermis with glandular trichomes
C. Upper epidermis with glandular trichome
D. Lower epidermis with trichomes
E. Lower epidermis with stomata

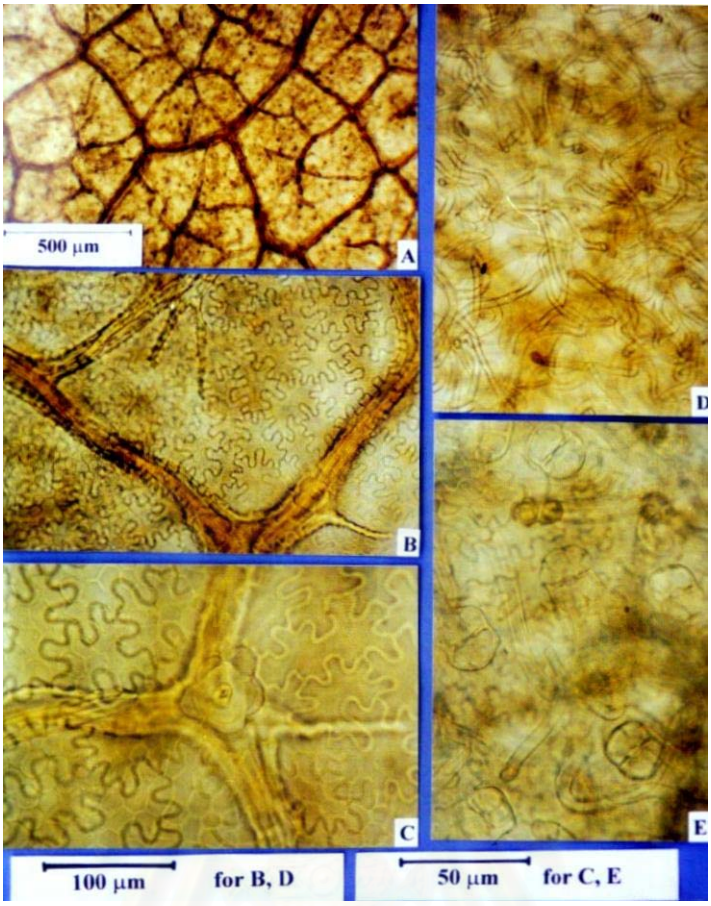

Figure 17. Microscopic illustration of *Coscinium* sp. leaf

A. Vein-islet and veinlet termination (x 40)

B. Upper epidermis with glandular trichomes (x 200)

C. Upper epidermis with glandular trichome (x 400)

D. Lower epidermis with trichomes (x 200)

E. Lower epidermis with stomata (x 400)

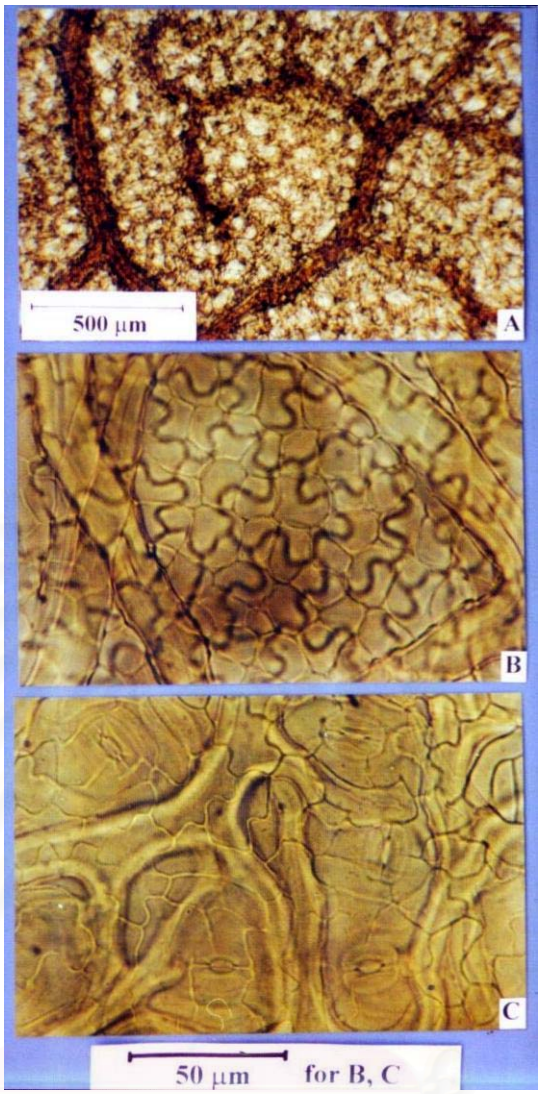

Figure 18. Microscopic illustration of *Fibraurea tinctoria* Lour. leaf

A. Vein-islet and veinlet termination

B. Upper epidermis with underlying palisade cells

C. Lower epidermis with stomata

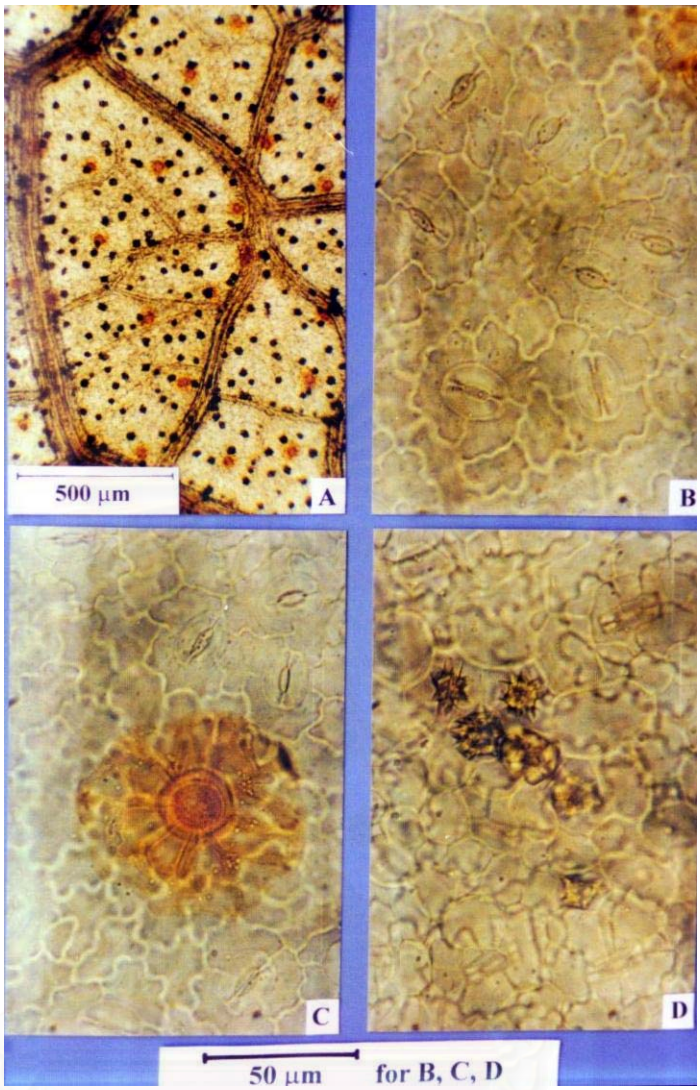

Figure 19. Microscopic illustration of *Combretum latifolium* Blume leaf

A. Vein-islet and veinlet termination

B, C. Lower epidermis with stomata and gland

D. Rosette aggregate crystals of calcium oxalate

Part 2 The results of the macroscopic and microscopic characterizations of stem

1. Authentic samples

Stem of authentic samples in each species is shown in Figure 20 and the microscopic characters of these samples were shown as follows (Figure 21-30).

2. The macroscopic character of "Khamin khruea" purchased from the traditional drugstores throughout Thailand (according to Table 8) are shown in Table 35 and Figure 31.

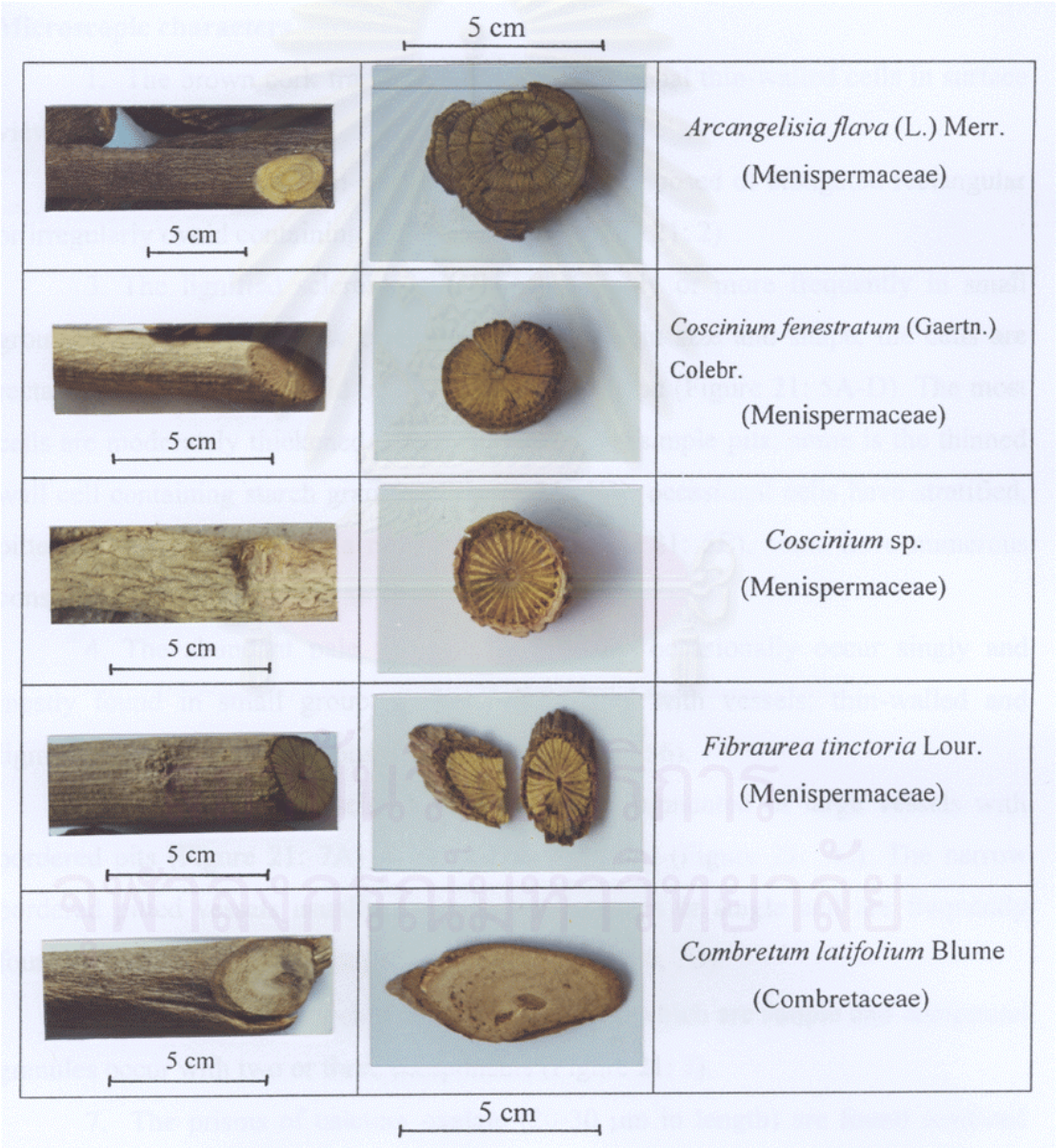

Figure 20. Stem of authentic samples

***Arcangelisia flava* (L.) Merr.**

Macroscopic characters

The main stem is subcylindrical with occasional nodes, more or less tortuous and up to 5 cm in diameter. Externally, greenish-dark brown to blackish brown, where occasionally longitudinal fissures or irregularly wrinkled. The fresh stem exhibit bright yellow wood with exuding yellow sap and fade down to yellowish brown when dried. The transversely cut surface show prominent annual rings and distinctly radiate concentric zone of projecting medullary rays with in each ring about 20-30, 65-80, 100-120, 130-140 respectively from the inner ring. The bark up to 4 mm in thickness. The powder is greenish-yellow in color with odorless and bitter taste.

Microscopic characters

1. The brown cork fragment which are polygonal thin-walled cells in surface view (Figure 21: 1).

2. The abundant thin-walled parenchyma composed of elongated rectangular or irregularly ovoid containing starch granules (Figure 21: 2).

3. The lignified sclereids, which occur singly or more frequently in small group. Individual cells show considerable variation in size and shape, the cells are rectangular, irregularly ovoid or small polygonal shape (Figure 21: 5A-D). The most cells are moderately thickened walls with numerous simple pits; some is the thinned wall cell containing starch granules (Figure 21: 5D); occasional cells have stratified, pitted, thickened wall with a narrow lumen (Figure 21: 5E), some have numerous conspicuous pits (Figure 21: 5F).

4. The abundant pale yellow fibers, which occasionally occur singly and mostly found in small group or found associated with vessels; thin-walled and lignified cells with conspicuous simple pits (Figure 21: 6).

5. The lignified vessels, frequently found fragmented of large vessels with bordered pits (Figure 21: 7A) and rarely found spiral (Figure 21: 7C). The narrow bordered pitted vessels usually occur in small groups or single and are frequently found associated with the groups of fibers (Figure 21: 6, 7B).

6. The abundant starch granules (10-30 μm), which are simple and compound granules occur with two or three components (Figure 21: 3).

7. The prisms of calcium oxalate (20-30 μm in length) are found scattered (Figure 21: 4).

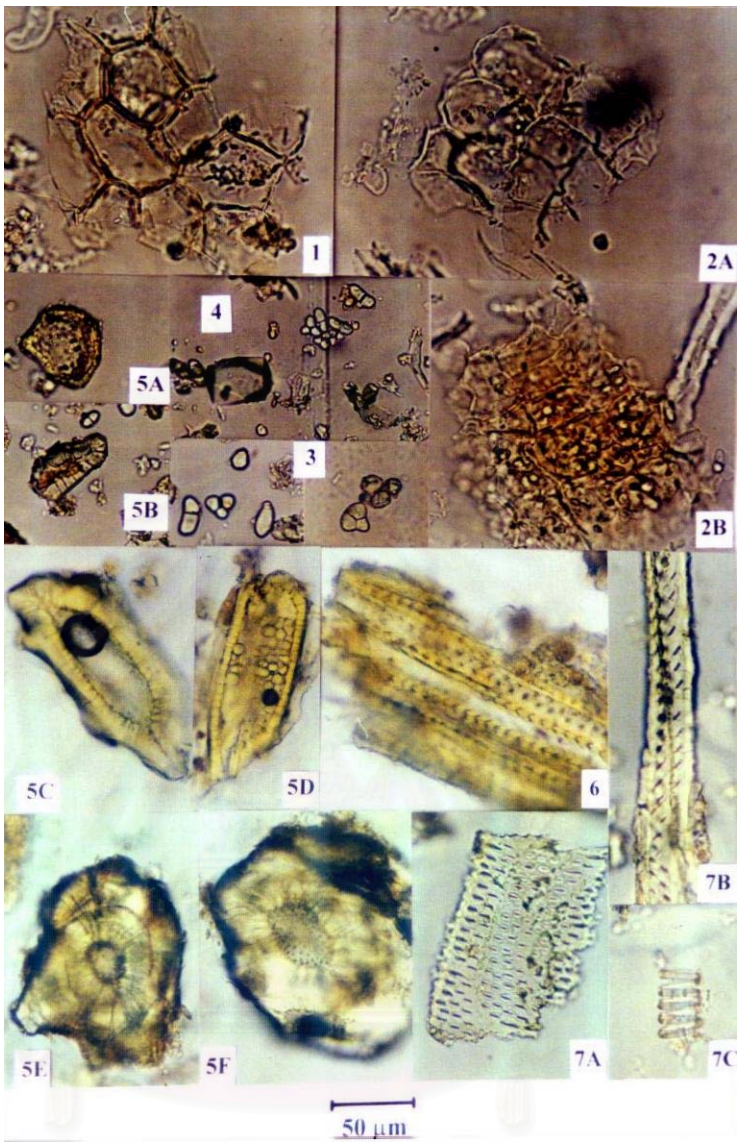

Figure 21. Microscopic characters of *Arcangelisia flava* (L.) Merr. stem

1. Cork
2. Parenchyma
3. Starch granules
4. Prisms of calcium oxalate
5. Sclereids
6. Group of fibers
7. Vessels

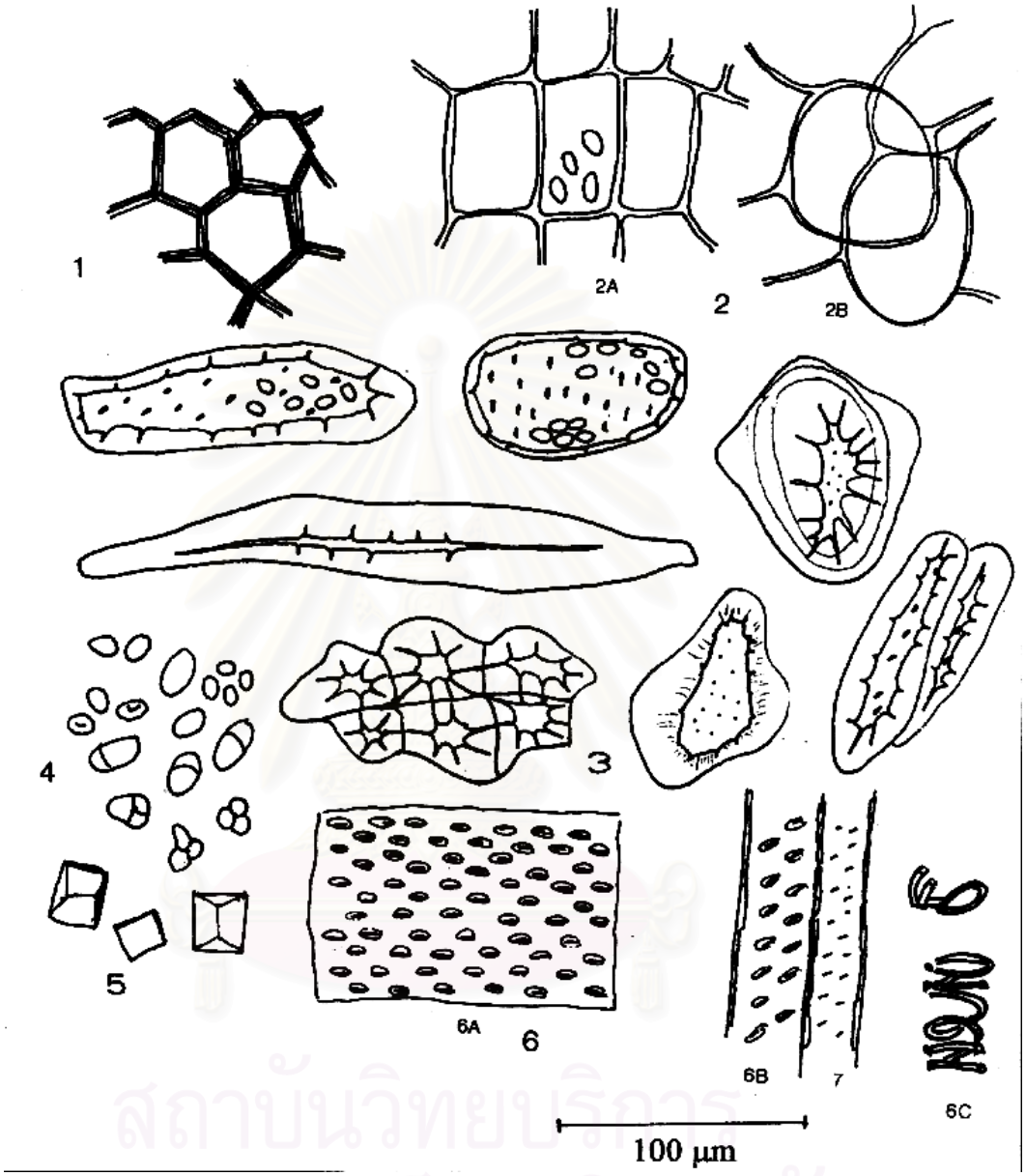

Figure 22. Microscopic characters of *Arcangelisia flava* (L.) Merr. stem

1. Cork
2. Parenchyma
3. Sclereids
4. Starch granules
5. Prisms of calcium oxalate
6. Vessels
7. Fibers

***Coscinium fenestratum* (Gaertn.) Colebr.**

Macroscopic characters

The stem is subcylindrical up to 5 cm in diameter, with occasional enlarge nodes up to 7 cm in diameter. Externally, moderate brown to blackish brown where nearly smooth, irregularly wrinkled and fissures. The transversely cut surface exhibit yellowish brown wood with about 30 to 40 radius lines structure distinctly between the medullary rays and the more lighter xylem. The bark is about 2 to 4 mm in thickness. It occurs in commerce mostly in cylindrical pieces about 2 to 5 cm in length and 1 to 4 cm in diameter, occasionally in oblique slices 2 to 5 mm in thickness, up to 12 cm in length and 2 to 5 cm in width. The powder is greenish-yellow, odorless and bitter taste.

Microscopic characters

1. The occasional fragments of brownish cork in surface view composed of polygonal cells (Figure 23: 1A) and several layers of rectangular thin-walled cells in sectional view (Figure 23: 1B).

2. The parenchyma is thin-walled and lignified; they are mostly quadrilateral cells (Figure 23: 2A-B).

3. The abundant lignified sclereids occur singly or in small groups, they show considerable variation in size and shape; The very large sclereids usually more or less ovoid to rectangular, very thin-walled with few pits (Figure 23: 4A) and fairly found asymmetrical thick walled sclereids containing prisms of calcium oxalate (Figure 23: 4B). The walls of cells show great variation; mostly are moderately to heavily thickened with narrow lumen; striation are sometimes visible (Figure 23: 4C-E). The shape varies from more or less isodiametric to elongated tapered ends (Figure 23: 4D-F).

4. The lignified fibers usually found in small groups with numerous pits (Figure 23: 5).

5. The vessels, frequently found fragmented of large simple and bordered pitted vessels (Figure 23: 3A-B), and also found singly or in small group vessels with bordered pits (Figure 23: 3C). Few vessels also occur with reticulate or spiral thickening.

6. The starch granules are simple and occasionally found compound having two or three components, 5-25 μm in diameter, not abundant (Figure 23: 6).

7. The prisms of calcium oxalate are scattered and occasionally found up to 20 μm (Figure 23: 7).

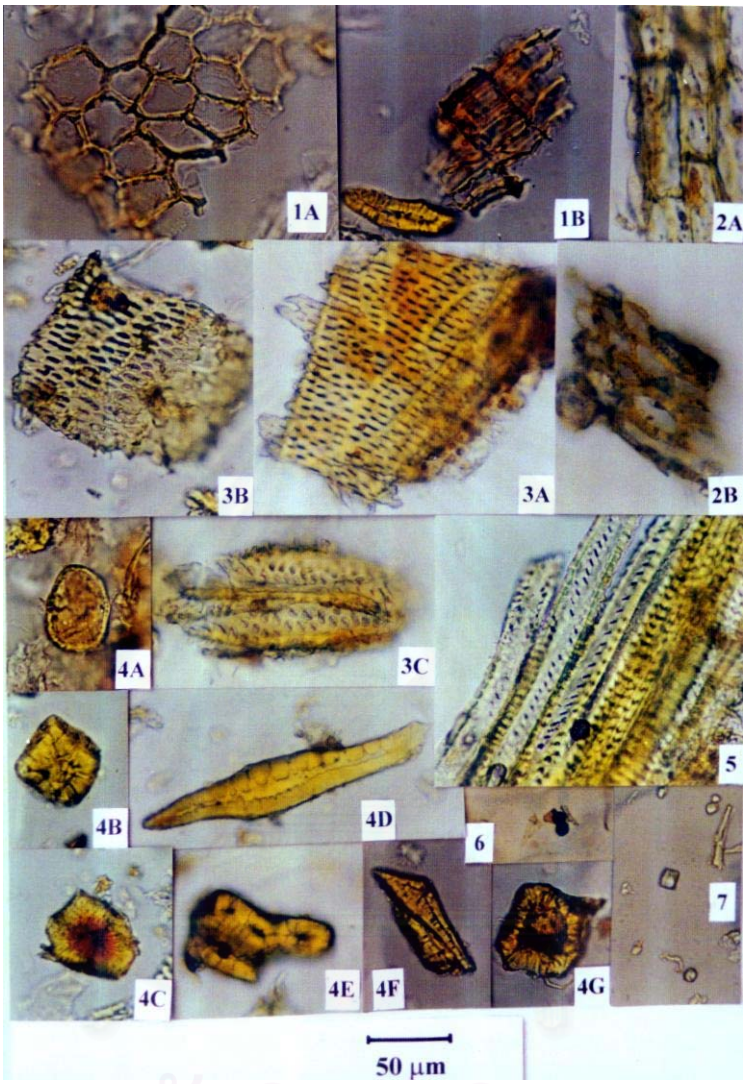

Figure 23. Microscopic characters of *Coscinium fenestratum* (Gaertn.) Colebr. stem
1A. Cork (surface view) 1B. Cork (sectional view) and a sclereid
2. Parenchyma 3. Vessels 4. Sclereids
5. Fibers 6. Starch granules
7. Starch granules and a prism of calcium oxalate

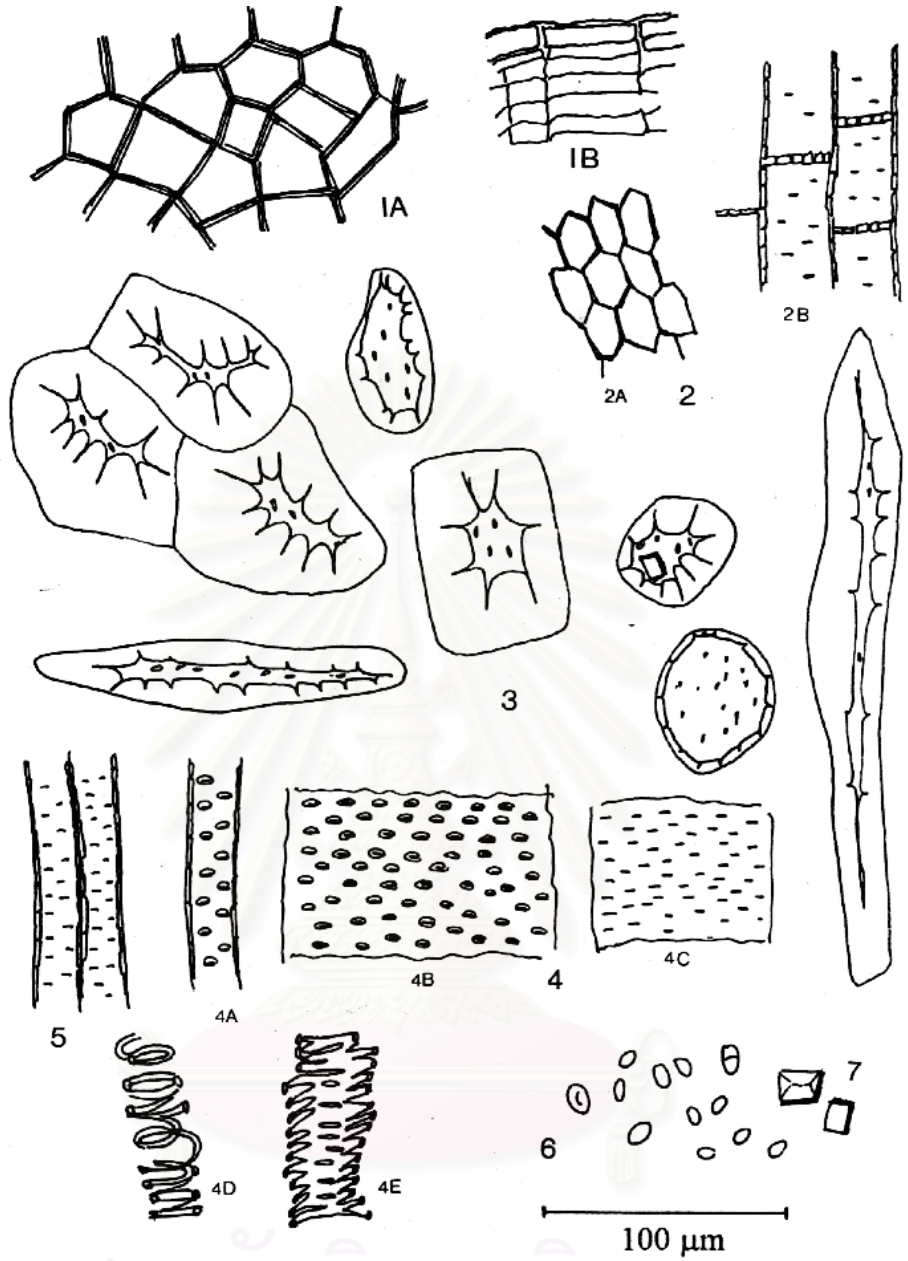

Figure 24. Microscopic characters of *Coscinium fenestratum* (Gaertn.) Colebr. stem

1A. Cork (surface view) 1B. Cork (sectional view)

2. Parenchyma 3. Sclereids

4. Vessels 5. Fibers

6. Starch granules 7. Prisms of calcium oxalate

***Coscinium* sp.**

Macroscopic characters

The stem is subcylindrical up to 4 cm in diameter, with occasional nodes. Externally, blackish brown or creamy white where nearly smooth or irregularly wrinkled to fissures. The transversely cut surface exhibit yellowish brown wood with about 20 to 35 radius lines structure distinctly between the medullary rays and the more lighter xylem. The bark is about 2 to 4 mm in thickness. The powder is greenish-yellow, odorless and bitter taste.

Microscopic characters

1. The numerous fragments of brown cork, polygonal in surface view and composed of several layers of rectangular thin-walled cells in sectional view (Figure 25: 1A-B).

2. The parenchyma, the irregular ovoid with thin-walled (Figure 25: 3A). The yellow quadrilateral thick-walled, lignified cells with simple pits containing starch granules which are markedly found singly or in small group (Figure 25: 3B-C, 8).

3. The abundant lignified sclereids, considerable variation in size and shape; which are usually found in small group. The thickened cells are frequently found containing a number of prisms of calcium oxalate (Figure 25: 5A, 5C); some are thickened and irregularly rectangular with numerous pits (Figure 25: 5B, 5E), striations are visible (Figure 25: 5D).

4. The lignified fibers occur in small group usually associated with the vessels; thin and lignified walls (Figure 25: 4), occasionally found large elongated thickened wall with few pits (Figure 25: 9).

5. The abundant vessels, which usually are found fragmented; they are large, simple or bordered pitted walls; occasionally have both simple and bordered pits (Figure 25: 2).

6. The fairly abundant starch granules, mostly simple and occasionally found compound having two or three components, 5-25 μm in diameter (Figure 25: 6).

7. The prisms of calcium oxalate, which are found scattered as well as in sclereids, 20-30 μm in length (Figure 25: 7).

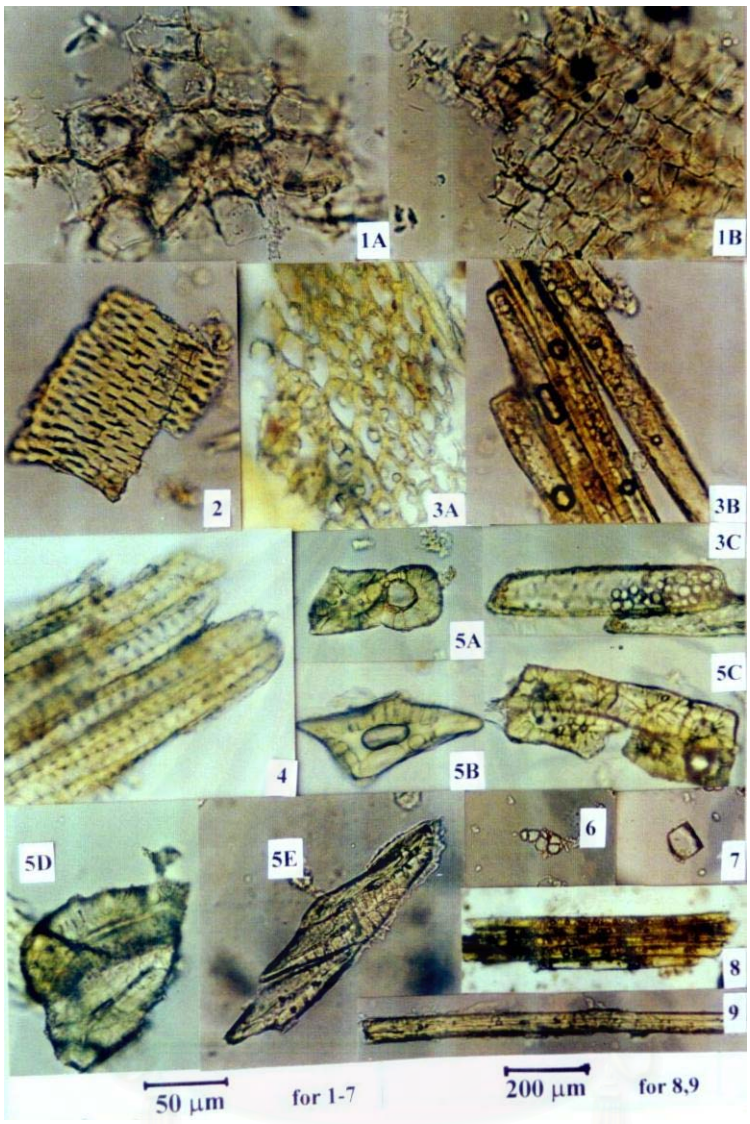

Figure 25. Microscopic characters of *Coscinium* sp. stem

1A. Cork (surface view) 1B. Cork (sectional view)

2. Vessel 3, 8. Parenchyma

4, 9. Fibers 5. Sclereids

6. Starch granules 7. A prism of calcium oxalate

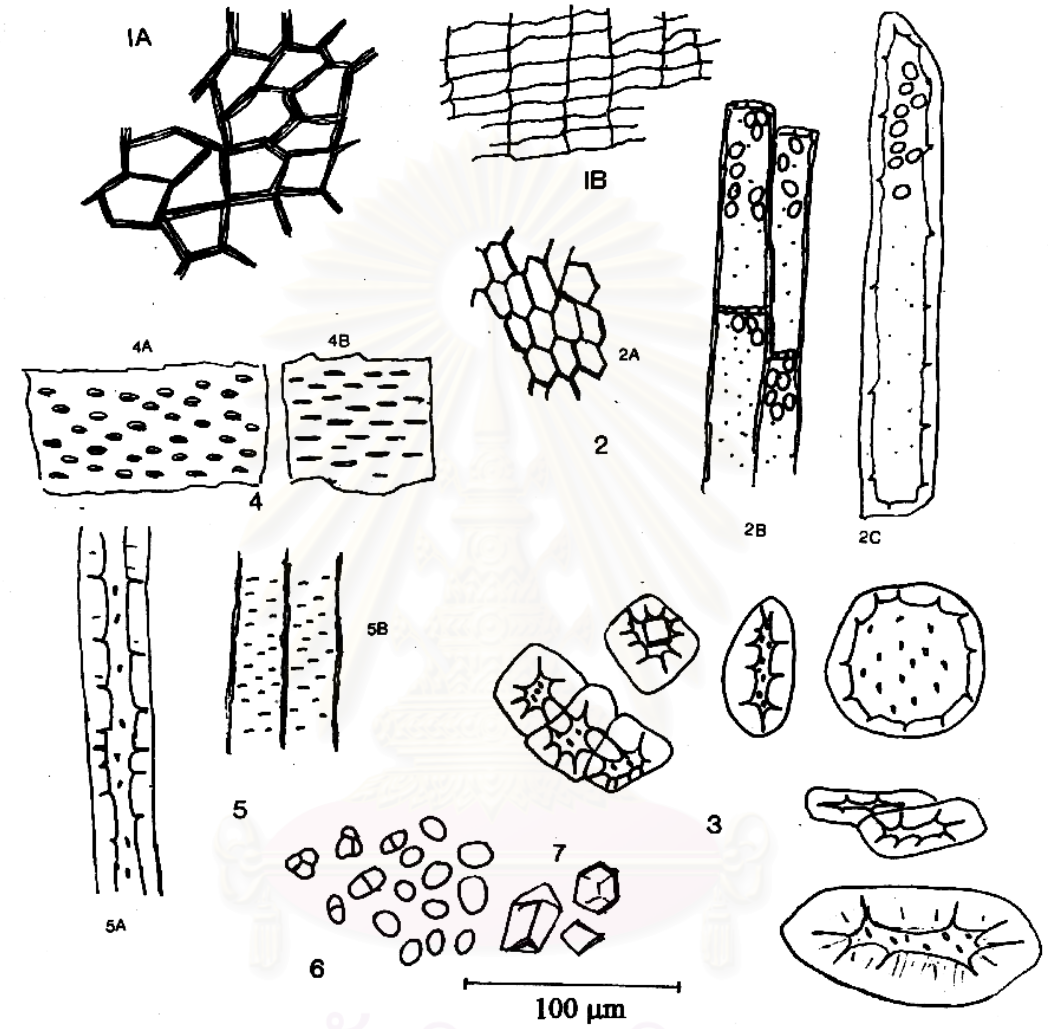

Figure 26. Microscopic characters of *Coscinium* sp. stem

1A. Cork (surface view)	1B. Cork (sectional view)
2. Parenchyma	3. Sclereids
4. Vessels	5. Fibers
6. Starch granules	7. Prisms of calcium oxalate

***Fibraurea tinctoria* Lour.**

Macroscopic characters

The stem is cylindrical up to 4 cm in diameter. Externally, greenish brown to blackish brown where twisted, longitudinal furrows and slightly fissures. The transversely cut surface exhibits yellow wood with about 30 to 60 radius lines structure distinctly between the medullary rays and the more lighter xylem. The bark is about 2 to 3 mm in thickness. It occurs in commerce mostly in cylindrical pieces about 2 to 5 cm in length and 0.5 to 2.5 cm in diameter. The powder is greenish-yellow, odorless and bitter taste.

Microscopic characters

1. The fragments of thin-walled cork, they are brown and polygonal in surface view (Figure 27: 1).

2. The abundant thin-walled parenchyma containing starch granules, irregularly ovoid or rectangular (Figure 27: 2).

3. The fairly abundant sclereids which were found singly or small group show considerable variation in size, shape and the degree of thickness; the irregularly rectangular or ovoid have thick-walled with numerous pits (Figure 27: 6A). The thin-walled sclereids usually found containing prisms of calcium oxalate (Figure 27: 6B, E) and some of them has very thin wall (Figure 27: 6F). The thickened wall with small lumen rectangular sclereids are mostly found connected in small group (Figure 27: 6C) and occasionally found singly large sclereid (Figure 27: 7A). Some sclereid are elongated forming fibrous sclereids (Figure 27: 9).

4. The fibers, which occur singly or in small group. Individual fibers are large with pointed ends, narrow lumen, thickened, lignified walls and few pits (Figure 27: 8). The small group of fibers are frequently found associate with vessels, thin-walled, lignified and few pits.

5. The vessels, which usually occur in fragments, fairly large, lignified, bordered and simple pits (Figure 27: 3A-B). The small vessels usually found associated with fibers (Figure 27: 3D). Spiral vessel is rarely found (Figure 27: 3C).

6. The abundant starch granules, they are simple ovoid 10 to 20 μm in diameter (Figure 27: 4) and the compound granules with two components are rarely found.

7. The prisms of calcium oxalate are not abundant about 10 to 20 μm in length (Figure 27: 5), occasionally found in sclereids.

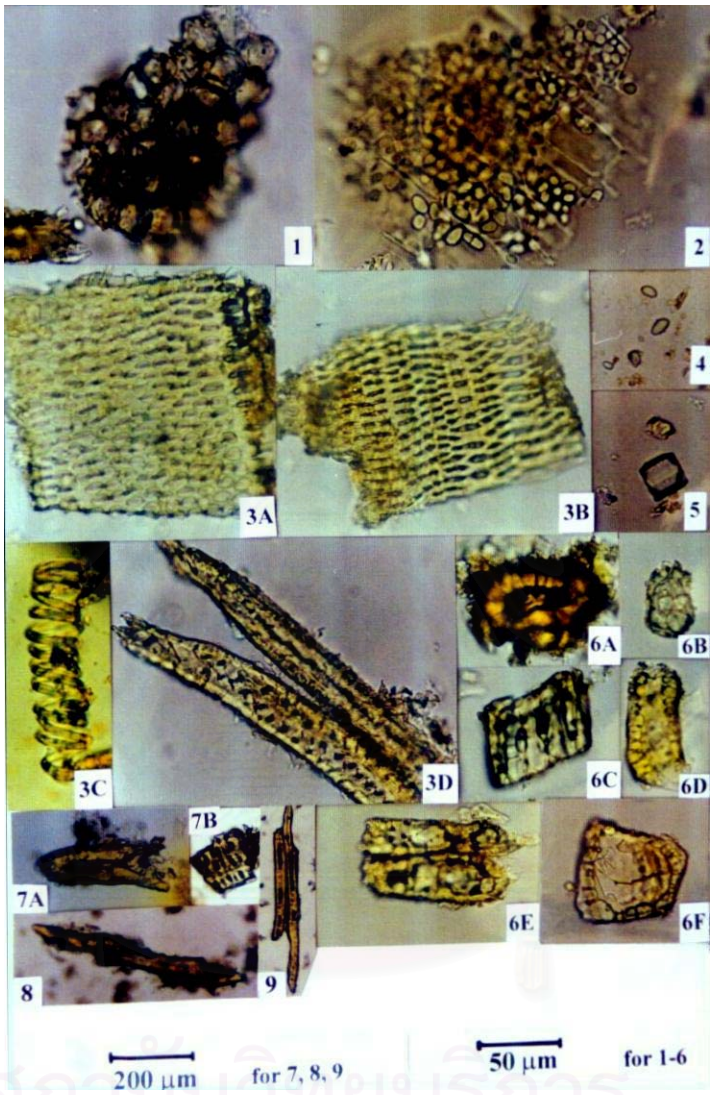

Figure 27. Microscopic characters of *Fibraurea tinctoria* Lour. stem

1. Cork
2. Parenchyma cells containing starch granules
3. Vessels
4. Starch granules
5. A prism of calcium oxalate
6, 7. Sclereids
8. Fiber
9. Fibrous sclereids

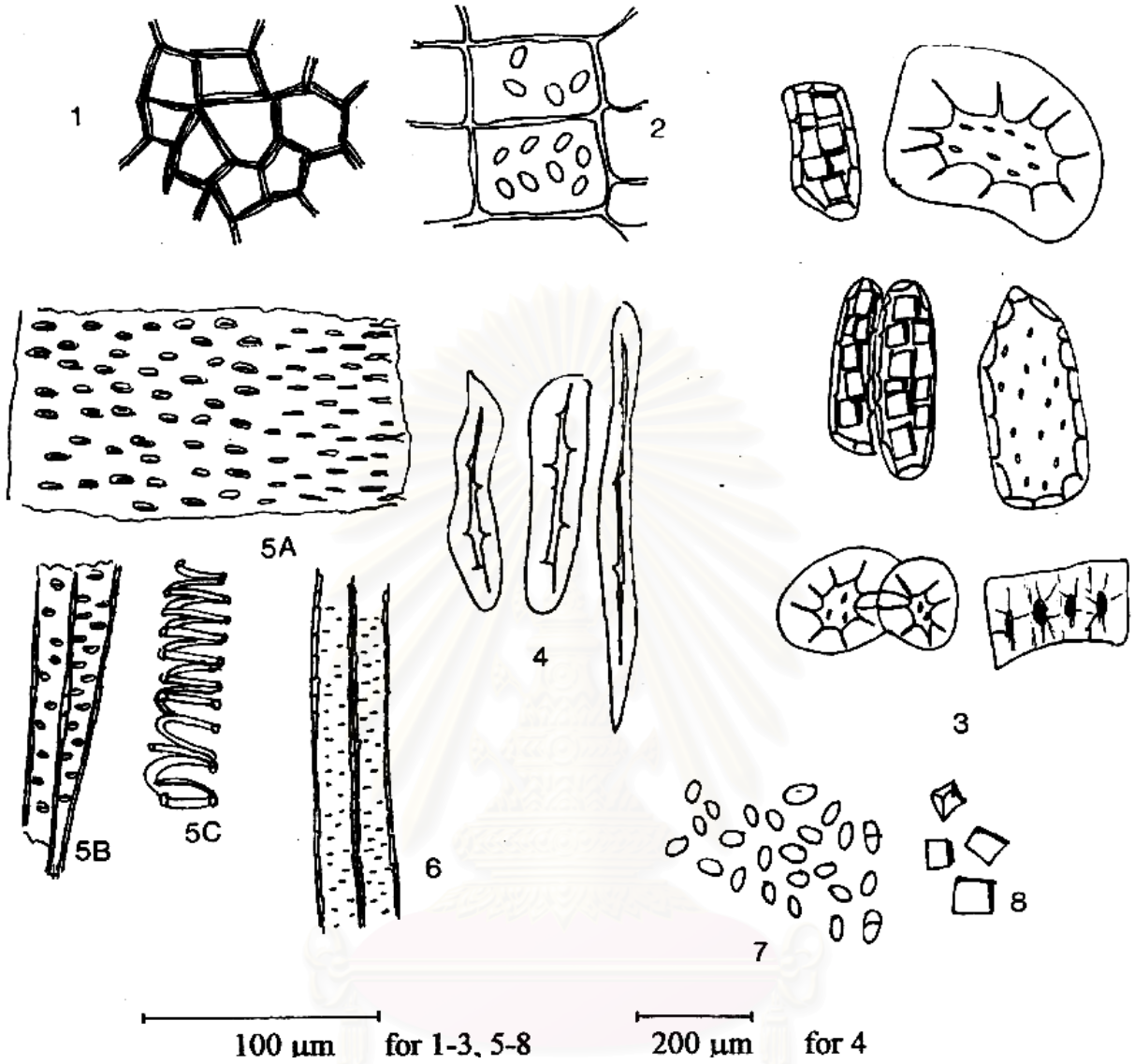

Figure 28. Microscopic characters of *Fibraurea tinctoria* Lour. stem

1. Cork	2. Parenchyma cells containing starch granules
3. Sclereids	4. Fibrous sclereids
5. Vessels	6. Fibers
7. Starch granules	8. Prisms of calcium oxalate

***Combretum latifolium* Blume**

Macroscopic characters

The stem is cylindrical up to 4 cm in diameter. Externally, brown to blackish brown where slightly fissures. The transversely cut surface exhibits creamy whitish yellow to pale brown wood and dark brown pith up to 0.5 cm in diameter. The bark is up to 2 mm in thickness. It occurs in commerce mostly in oblique slices up to 3 mm in thickness, up to 5 cm in length and 2 to 3 cm in width, occasionally in small chopped pieces about 2-5 cm in length or longitudinal quartered segments 6 to 8 cm in length. The powder is pale brown, faint odor and tasteless.

Microscopic characters

1. The cork fragments are brown and polygonal in surface view (Figure 29: 1).

2. The large bordered pitted vessel frequently found fragmented (Figure 29: 2A). The narrowly bordered pitted vessel is occasionally found (Figure 29: 2B).

3. The abundant parenchyma of the medullary rays, the medullary rays are usually found uniseriate and composed of round cells as seen in tangential longitudinal view (Figure 29: 3A, 10) or occasionally found bi-seriate. The medullary rays in radial longitudinal views, the cells are rectangular, thin-walled associated with bordered pitted vessels (Figure 29: 3B) or fibers or parenchyma (Figure 29: 3C). The other is a reserved parenchyma containing starch granules, longitudinally elongated tapered end and thin-walled (Figure 29: 5, 13). Another is lignified, thin-walled cells usually contain a prism of calcium oxalate, arranged in vertical files (Figure 29: 4, 12).

4. The abundant starch granules; they are simple 20 to 30 μm in length and up to 10 μm in width, mostly ellipsoidal and the central point hilum occasionally appears as a line. Individual starch granule occurs scattered (Figure 29: 6) or usually found in the parenchyma (Figure 29: 5).

5. The abundant prisms calcium oxalate 10 to 25 μm in length, which are found scattered (Figure 29: 7) as well as in the parenchyma cells (Figure 29: 4).

6. The lignified fibers usually occur in a small group and occasionally occur singly (Figure 29: 8).

7. The sclereids usually occur in a small group of elongated cells; variable in degree of thickness (thin and thickened walls). Both types have numerous pits, lignified but the thin-walled has large lumen (Figure 29: 9).

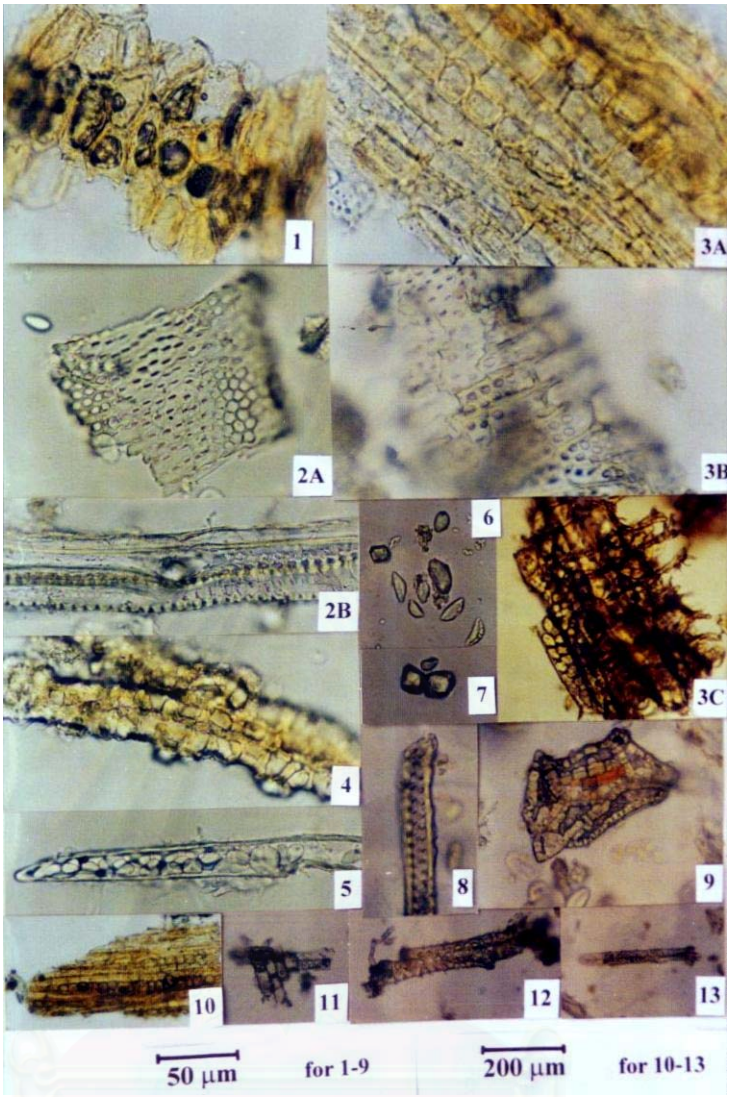

Figure 29. Microscopic characters of *Combretum latifolium* Blume stem

1. Cork
2. Vessels
3, 10, 11. Medullary rays
4, 12. Parenchyma containing prisms of calcium oxalate
5, 13. Parenchyma containing starch granules
6. Starch granules
7. Prisms of calcium oxalate
8. Fiber
9. Sclereids

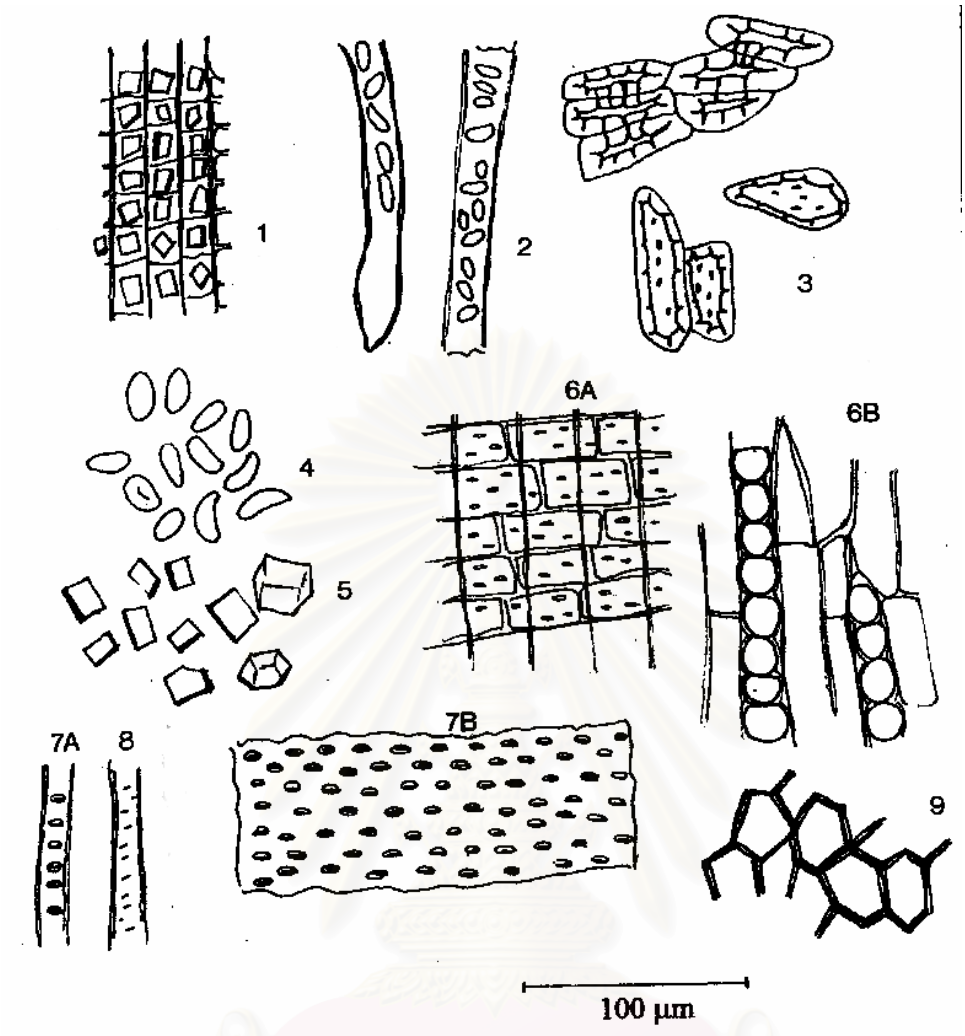

Figure 30. Microscopic characters of *Combretum latifolium* Blume stem

1. Parenchyma containing prisms of calcium oxalate
2. Parenchyma containing starch granules
3. Sclereids 4. Starch granules
5. Prisms of calcium oxalate
6A. Medullary rays (radial view) 6B. Medullary rays (tangential view)
7. Vessels 8. Fiber 9. Cork

Table 35. Macroscopic characters of purchased Khamin khruea

sample	Macroscopic character
1	Yellow stem with white rough fissured bark, 2.5-3 cm in diameter.
2	Yellow stem with dark brown fissured bark, 2.5-3 cm in diameter.
3	Yellow stem with smooth bark, chopped in various sizes, 1.5-4 cm in diameter.
4	Yellow stem, oblique slice 2-4 mm thickness, 2-5 cm in width and up to 10 cm in length .
5	Yellow stem with fissured bark, large with occassional node, mostly 3-4 cm in diameter.
6	Yellow stem with smooth bark, chopped in various sizes and shapes, 1-3 cm in diameter.
7	Yellow stem with smooth bark, chopped in various sizes, 1-3 cm in diameter.
8	Bright yellow stem, oblique slice to 2-5 mm thickness, 2-5 cm in width and up to 12cm in length.
9	Pale yellow stem with fissured bark, chopped in various sizes 2-3 cm in diameter.
10	Yellow stem with smooth bark, chopped in various sizes and shapes, 1-2.5 cm in diameter.
11	Yellow stem with smooth bark, chopped in various cylindrical sizes, 0.7-3 cm in diameter.
12	Yellow stem with smooth bark, chopped in various sizes 1-2.5 cm in diameter.
13	Yellow stem with fissured bark, chopped in various sizes and shape 1.5-2.5 cm in diameter.
14	Pale yellow stem with smooth bark, chopped in various sizes 1-3 cm in diameter.
15	Fine yellow powdered.
16	Yellow stem with fissured bark, chopped in various sizes and shapes, 1-2 cm in diameter.
17	Yellow stem with smooth bark, chopped in various sizes and shapes, 1-2 cm in diameter.
18	Pale yellow stem with smooth bark, chopped in various sizes and shapes, 1-2 cm in diameter.
19	Yellow stem with fissured bark, chopped in various sizes and shapes, 1-2.5 cm in diameter.
20	Bright yellow stem, oblique slice up to 5 mm in thickness, about 3cm in width and up to 12 cm in length.
21	Yellow stem with smooth bark, chopped in various sizes and shapes, 1-2.5 cm in diameter.
22	Yellow stem with smooth bark, chopped in various sizes and shapes, 1-3 cm in diameter.
23	Yellow stem with smooth bark, chopped in cylindrical 5-8 cm long, 1-2.5 cm in diameter.
24	Yellow stem with smooth bark, chopped in various sizes, 4-8 cm long and 1-2.5 cm in diameter.
25	Yellow stem with longitudinal furrow bark, chopped in various sizes, 0.5-2 cm in diameter.
26	Yellow stem with longitudinal furrow bark, chopped in various sizes, 1-2.5 cm in diameter.
27	Yellow stem with longitudinal furrow bark, 2-3 cm in diameter.
28	Yellow stem with longitudinal furrow bark, 2-3 cm in diameter.
29	Brown stem, chopped in various sizes and shapes, up to 3 cm in length..
30	Brown stem, chopped in longitudinal quartered segment, 6-8 cm in length.
31	Brown stem, oblique slice up to 3 mm thickness, 1-3cm in width and up to 6 cm in length.
32	Brown stem, oblique slice up to 3 mm thickness, 2-3cm in width and up to 5 cm in length.

Figure 31. Khamin khruea samples which were purchased from traditional drugstores

Part 3 The results of Thin-layer chromatographic pattern of stem extract

The results of one-dimensional TLC of crude extract are shown as follows:
System 1 n-Butanol:acetic acid: water (7:1:2), Figure 32.
System 2 Methanol:diethylamine: water (8:1:1) and Cyclohexane:diethylamine (9:1), Figure 33.

Labelled on TLC plate,

B = 0.02% Berberine sulphate in alcohol

A = Authentic sample of *Arcangelisia flava* (L.) Merr.

C = Authentic sample of *Coscinium fenestratum* (Gaertn.) Colebr.

F = Authentic sample of *Fibraurea tinctoria* Lour.

CL = Authentic sample of *Combretum latifolium* Blume

The results of two-dimensional TLC of crude extract of authentic sample of each species are shown as follows:

Arcangelisia flava (L.) Merr., Figure 34 and Table 36.

Coscinium fenestratum (Gaertn.) Colebr., Figure 35 and Table 37.

Fibraurea tinctoria Lour., Figure 36 and Table 38.

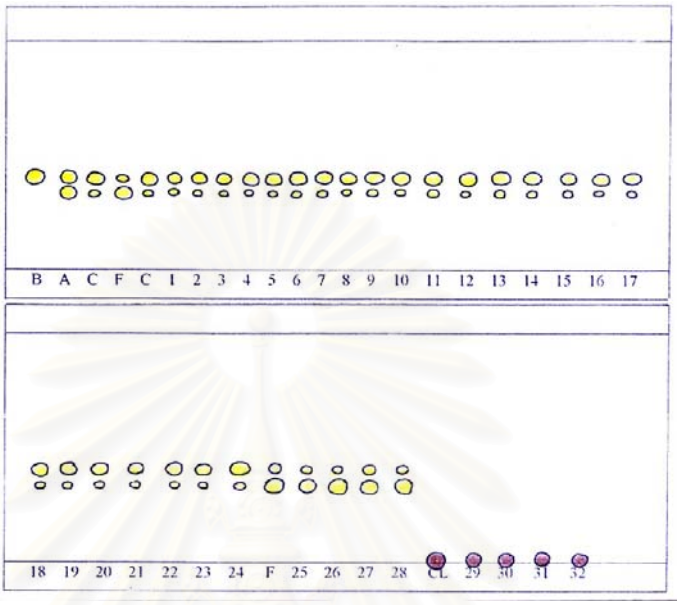

Figure 32. One-dimensional TLC of Khamin khruea stem extract
(system : n-Butanol : acetic acid : water (7:1:2))
A. Visible in daylight.

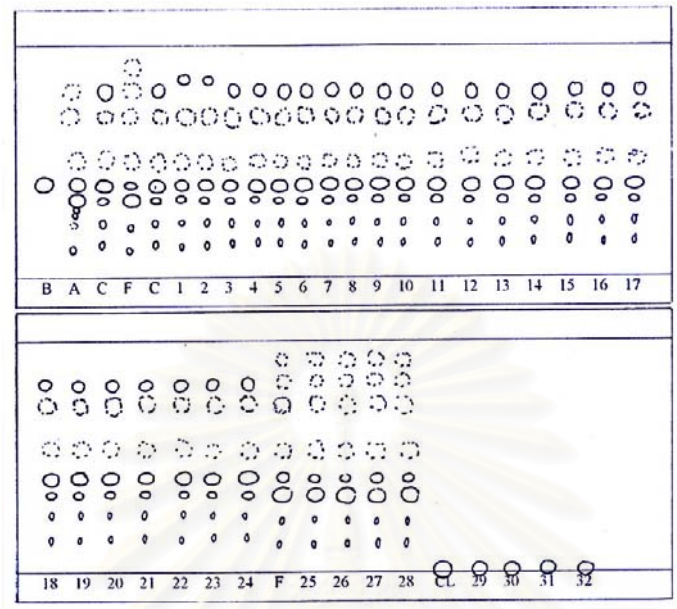

Figure 32. One-dimensional TLC of Khamin khruea stem extract
(system : n-Butanol : acetic acid : water (7:1:2)) (continued)

B. Fluorescence under UV

- - - = Fluorescence under UV 254 nm

…… = Fluorescence under UV 365 nm

____ = Fluorescence under UV both 254 and 365 nm

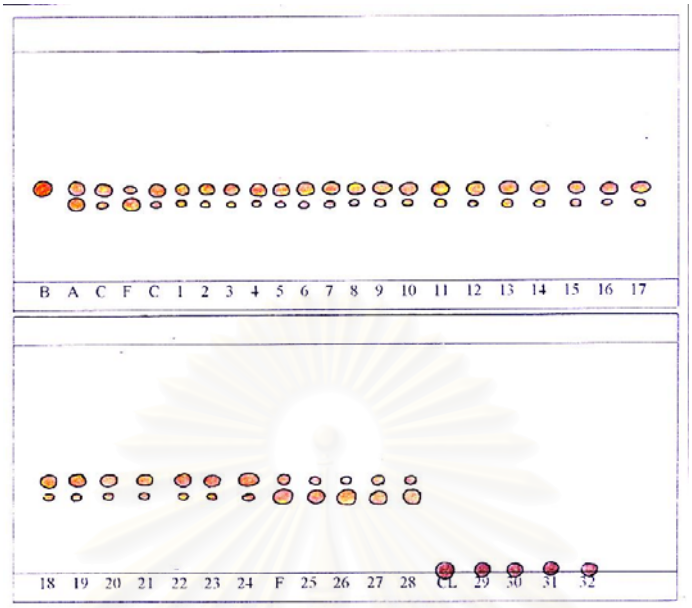

Figure 32. One-dimensional TLC of Khamin khruea stem extract
(system : n-Butanol : acetic acid : water (7:1:2)) (continued)
C. Detection with Dragendorff ’s reagent

Figure 32. One-dimensional TLC of Khamin khruea stem extract
(system : n-Butanol : acetic acid : water (7:1:2)) (continued)
D. Detection with Anisaldehyde-sulphuric acid reagent

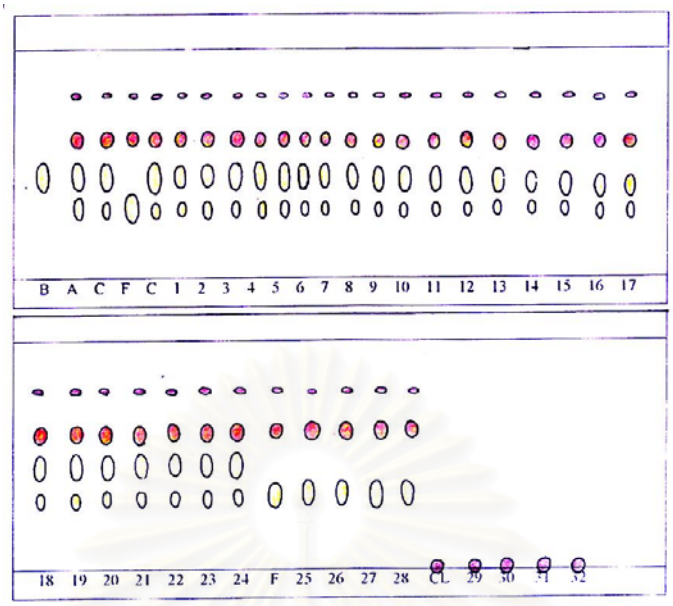

Figure 33. One-dimensional TLC of Khamin khruea stem extract
(system : Methanol : diethylamine : water (8:1:1) and
Cyclohexane : diethylamine (9:1))
A. Visible in daylight.

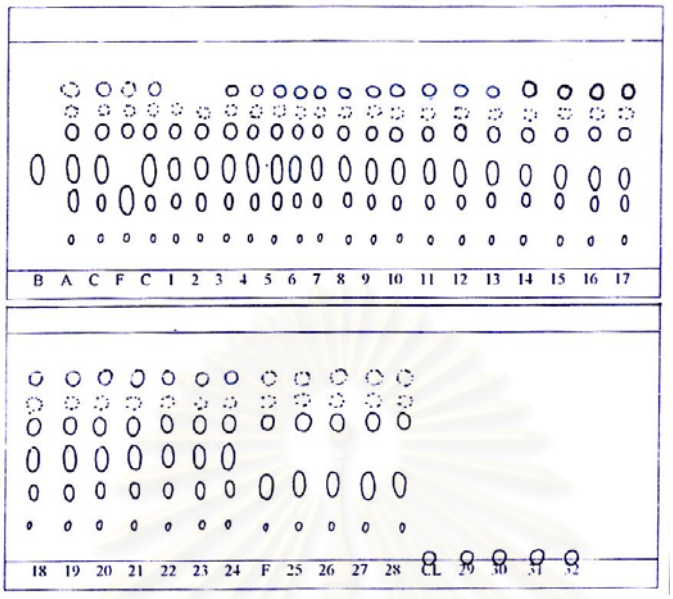

Figure 33. One-dimensional TLC of Khamin khruea stem extract
(system : Methanol : diethylamine : water (8:1:1) and
Cyclohexane : diethylamine (9:1)) (continued)

B. Fluorescence under UV

- - - = Fluorescence under UV 254 nm

..... = Fluorescence under UV 365 nm

____ = Fluorescence under UV both 254 and 365 nm

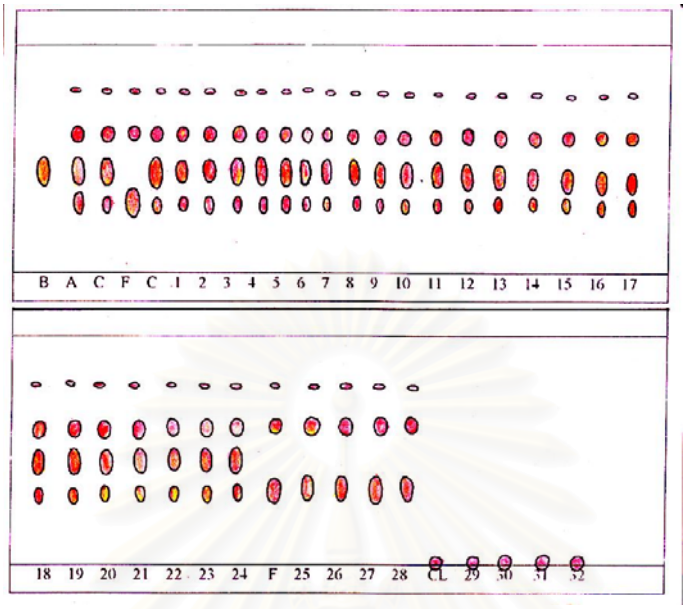

Figure 33. One-dimensional TLC of Khamin khruea stem extract (system : Methanol : diethylamine : water (8:1:1) and Cyclohexane : diethylamine (9:1)) (continued)

C. Detection with Dragendorff 's reagent.

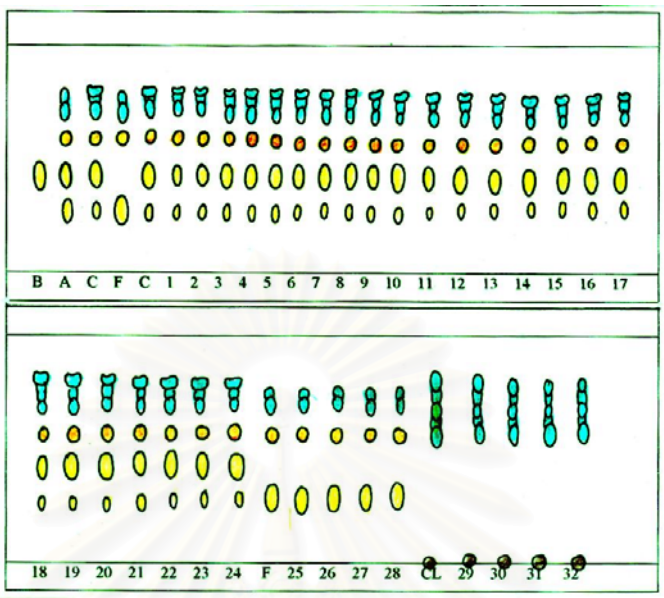

Figure 33. One-dimensional TLC of Khamin khruea stem extract
(system : Methanol : diethylamine : water (8:1:1) and
Cyclohexane : diethylamine (9:1)) (continued)
D. Detection with Anisaldehyde-sulphuric acid reagent.

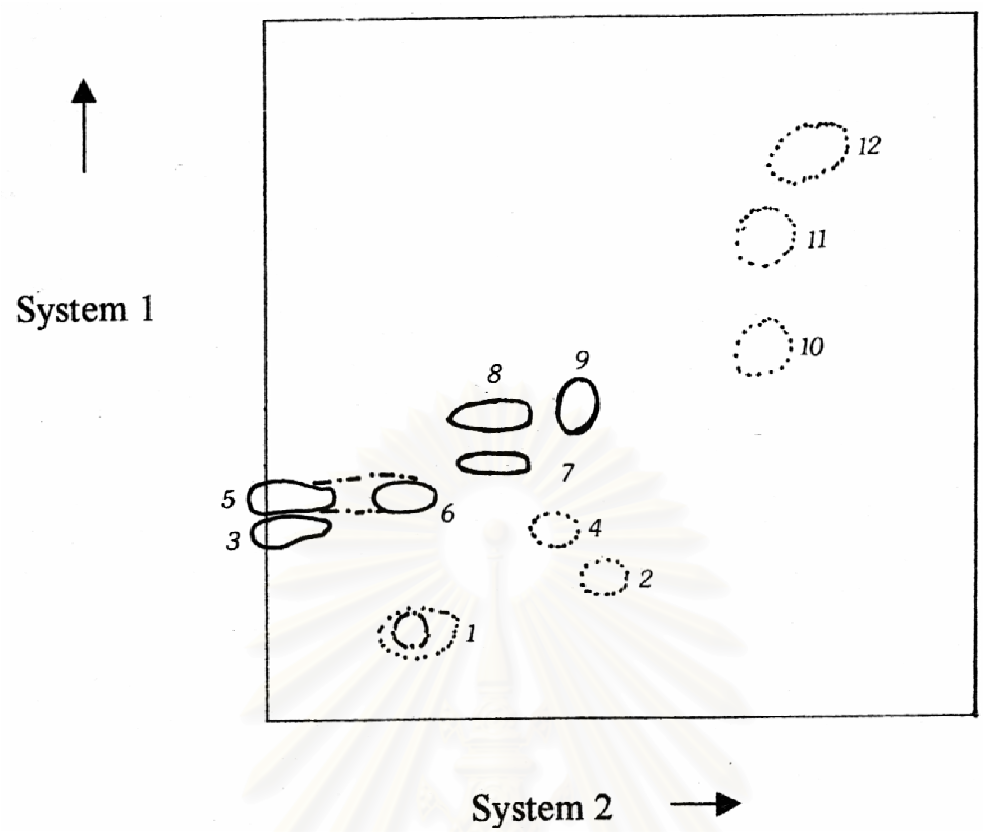

Figure 34. Two-dimensional TLC of *Arcangelisia flava* (L.) Merr. stem extract

- - - = Fluorescence under UV 254 nm

…… = Fluorescence under UV 365 nm

____ = Fluorescence under UV both 254 and 365 nm

System 1: n-Butanol : acetic acid : water (7:1:2))

System 2: Methanol : diethylamine : water (8:1:1)

Table 36. R_f value of two-dimensional TLC of *Arcangelisia flava* (L.) Merr. stem extract

Component	Code of R_f		Code of color (254 nm)		Code of color (365 nm)	
	S1	S2	C1	C2	C1	C2
1	08	18	38	38	60	60
2	18	40	00	00	60	60
3	20	00	30	30	85	38
4	20	20	30	00	85	60
5	28	00	38	38	60	60
6	28	18	38	38	60	60
7	30	38	45	45	45	45
8	38	38	45	45	45	45
9	38	50	45	24	45	85
10	50	68	00	00	40	40
11	68	68	00	00	40	40
12	78	70	00	00	40	40

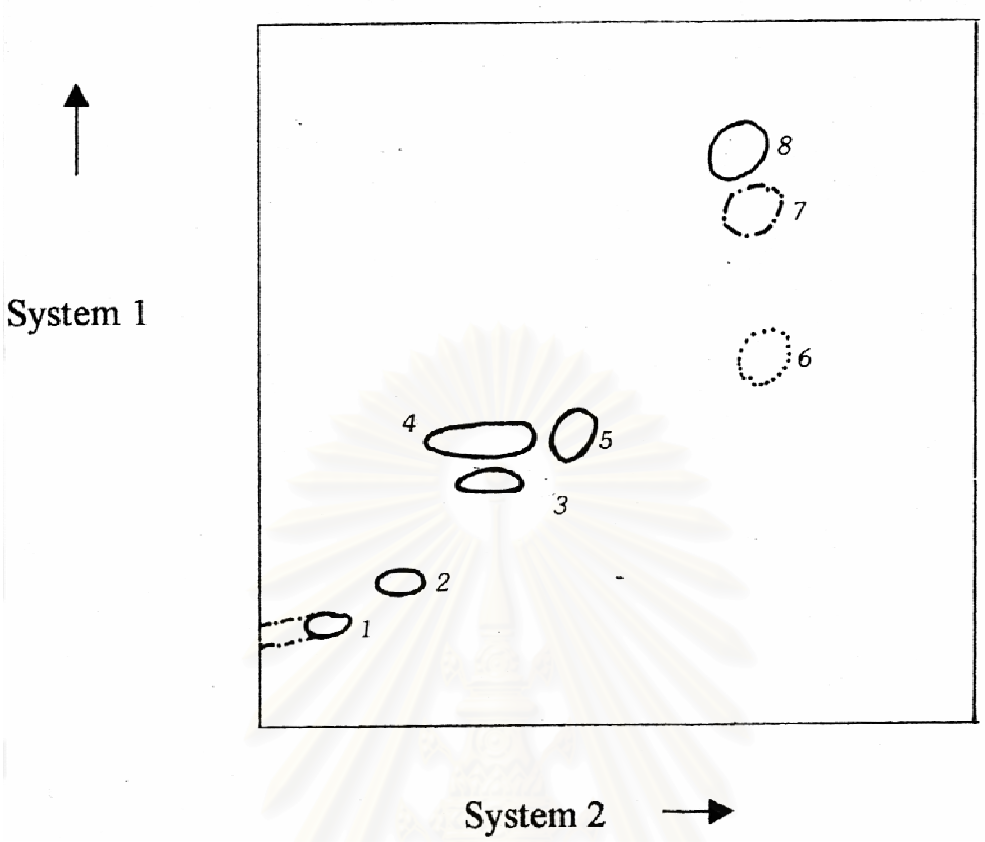

Figure 35. Two-dimensional TLC of *Coscinium fenestratum* (Gaertn.) Colebr. stem extract

..... = Fluorescence under UV 254 nm

..... = Fluorescence under UV 365 nm

____ = Fluorescence under UV both 254 and 365 nm

System 1: n-Butanol : acetic acid : water (7:1:2))

System 2: Methanol : diethylamine : water (8:1:1)

Table 37. R_f value of two-dimensional TLC of *Coscinium fenestratum* (Gaertn.) Colebr. stem extract

Component	Code of R_f		Code of color (254 nm)		Code of color (365 nm)	
	S1	S2	C1	C2	C1	C2
1	10	08	40	85	40	40
2	20	18	40	85	40	40
3	30	38	45	45	45	45
4	38	38	45	45	45	45
5	38	50	45	24	45	85
6	50	68	00	00	40	40
7	68	68	40	85	00	00
8	78	68	38	38	60	60

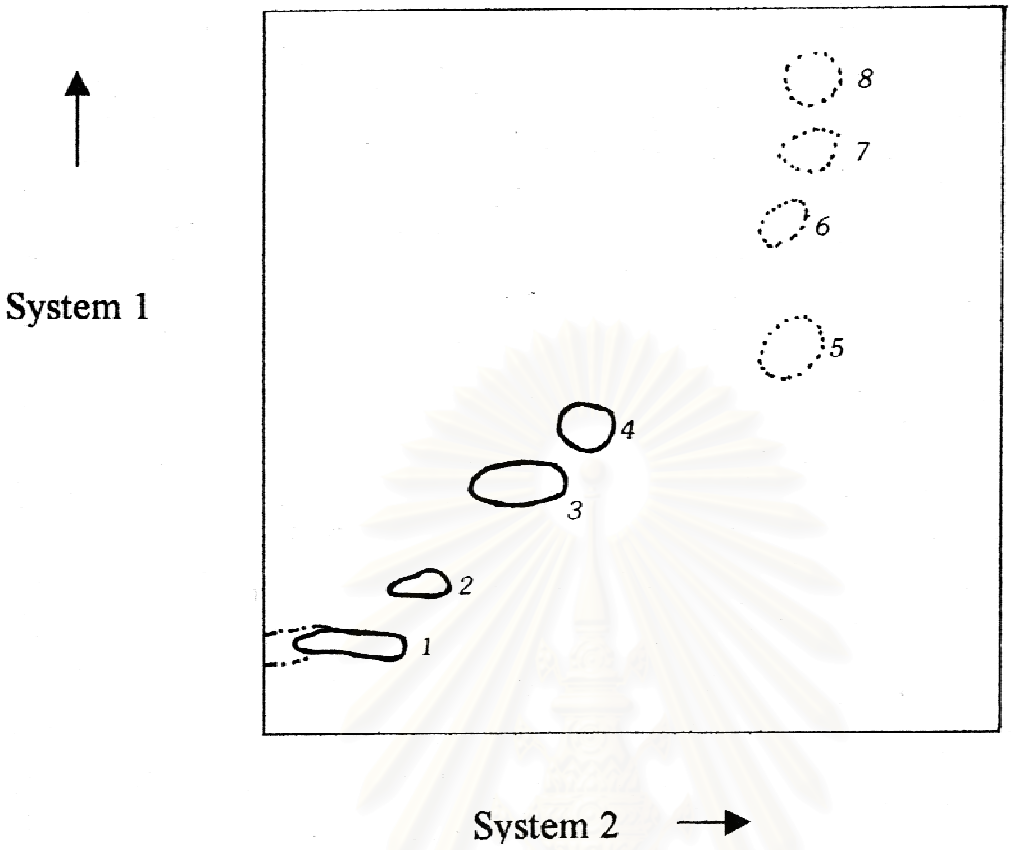

Figure 36. Two-dimensional TLC of *Fibraurea tinctoria* Lour. stem extract

…… = Fluorescence under UV 254 nm

…… = Fluorescence under UV 365 nm

____ = Fluorescence under UV both 254 and 365 nm

System 1: n-Butanol : acetic acid : water (7:1:2))

System 2: Methanol : diethylamine : water (8:1:1)

Table 38. R_f value of two-dimensional TLC of *Fibraurea tinctoria* Lour. stem extract

Component	Code of R_f		Code of color (254 nm)		Code of color (365 nm)	
	S1	S2	C1	C2	C1	C2
1	08	08	38	38	45	45
2	18	18	38	38	45	45
3	30	38	45	45	45	45
4	38	50	45	24	45	85
5	50	68	00	00	40	40
6	68	68	00	00	40	40
7	78	70	00	00	40	40
8	88	60	00	00	40	40

Part 4 The results of phytochemical screening

Alkaloid and tannin tests were investigated to show the differences of chemical constituents among genera. The results are shown as follows (Table 39).

Table 39. The phytochemical screening of alkaloid and tannin groups

Crude drug sample	Alkaloid test		Tannin test		
	Mayer's reagent	Dragendorff's reagent	Gelatin solution	Ferric chloride solution	Bromine water
A	3	3	3	green++	ppt.
C	3	3	3	green+	ppt.
F	3	3	3	green++	ppt.
CL	6	6	3	blue+++	O
1	3	3	3	green++	ppt.
2	3	3	3	green++	ppt.
3	3	3	3	green+	ppt.
4	3	3	3	green+	ppt.
5	3	3	3	green++	ppt.
6	3	3	3	green++	ppt.
7	3	3	3	green+	ppt.
8	3	3	3	green+	ppt.
9	3	3	3	green+	ppt.
10	3	3	3	green+	ppt.
11	3	3	3	green+	ppt.
12	3	3	3	green+	ppt.
13	3	3	3	green+	ppt.
14	3	3	3	green+	ppt.
15	3	3	3	green+	ppt.
16	3	3	3	green++	ppt.
17	3	3	3	green+	ppt.
18	3	3	3	green++	ppt.
19	3	3	3	green+	ppt.

Table 39. The phytochemical screening of alkaloid and tannin groups (continued)

Crude drug sample	Alkaloid test		Tannin test		
	Mayer's reagent	Dragendorff's reagent	Gelatin solution	Ferric chloride solution	Bromine water
20	3	3	3	green+	ppt.
21	3	3	3	green++	ppt.
22	3	3	3	green++	ppt.
23	3	3	3	green+	ppt.
24	3	3	3	green+	ppt.
25	3	3	3	green++	ppt.
26	3	3	3	green++	ppt.
27	6	6	3	blue+++	O
28	6	6	3	blue+++	O
29	6	6	3	blue+++	O
30	6	6	3	blue+++	O
31	6	6	3	blue+++	O
32	6	6	3	blue+++	O

Remark:

3	positive reaction
6	negative reaction
O	not change
+	light color
++	moderate color
+++	dark color
ppt.	precipitation
A	Authentic sample of *Arcangelisia flava* (L.) Merr.
C	Authentic sample of *Coscinium fenestratum* (Gaertn.) Colebr.
F	Authentic sample of *Fibraurea tinctoria* Lour.
CL	Authentic sample of *Combretum latifolium* Blume

Part 5 The results of quality control

According to the results of macroscopic, microscopic characters and TLC pattern compare between authentic drug and crude drugs from traditional drugstore, the quality controls of "Khamin khruea" which were purchased from traditional drugstores are shown in each species among these 5 species (Table 40-44).

Table 40. Loss on drying, total ash, acid insoluble-ash and extractive value of *Arcangelisia flava* (L.) Merr.

Crude drug Sample	Loss on drying (%)	Ash content (%)		Extractive value (%)	
		Total ash	Acid-insoluble ash	Ethanol	Water
a*	6.06	4.59	0.08	4.26	7.56
b*	6.71	4.31	0.06	4.42	8.78
c*	6.24	4.65	0.13	3.34	6.12
mean	6.34	4.52	0.09	4.01	7.49
S.D.	0.34	0.18	0.04	0.58	1.33

* a = Authentic sample from Faculty of Pharmaceutical Science Chulalongkorn University, Bangkok province

b = Authentic sample from Kleang district, Rayong province

c = Authentic sample from Department of Medical Science, Chanthaburi province

Table 41. Loss on drying, total ash, acid insoluble-ash and extractive value of *Coscinium* sp.

Crude drug sample	Loss on drying (%)	Ash content (%)		Extractive value (%)	
		Total ash	Acid-insoluble ash	Ethanol	Water
1	5.97	3.41	0.19	5.28	6.95
2	5.79	3.58	0.26	4.91	5.83
mean	5.88	3.50	0.23	5.10	6.39
S.D.	0.13	0.12	0.05	0.26	0.79

Table 42.. Loss on drying, total ash, acid insoluble-ash and extractive value of *Coscinium fenestratum* (Gaertn.) Colebr.

Crude drug sample	**Loss on drying** (%)	**Ash content** (%)		**Extractive value** (%)	
		Total ash	**Acid-insoluble ash**	**Ethanol**	**Water**
C *	6.33	2.46	0.30	5.81	7.23
3	7.47	4.03	0.77	4.49	5.92
4	7.47	1.92	0.30	5.39	6.62
5	7.34	3.00	0.69	4.95	6.66
6	6.18	3.19	0.62	5.41	7.24
7	6.26	2.44	0.30	4.87	6.34
8	6.65	2.41	0.37	5.22	6.95
9	5.68	2.09	0.14	4.63	7.41
10	5.57	2.58	0.28	4.27	6.31
11	6.41	2.01	0.46	4.48	6.09
12	6.28	2.13	0.14	4.43	5.71
13	6.00	3.23	0.92	4.53	7.08
14	6.3	3.27	0.50	4.32	6.78
15	5.53	3.35	0.71	5.66	7.83
16	6.17	2.52	0.45	5.08	7.03
17	6.26	4.55	1.35	4.03	6.56
18	7.47	2.38	0.60	3.92	5.99
19	5.84	1.97	0.14	3.57	5.95
20	6.09	1.85	0.06	4.55	6.35
21	6.56	3.34	0.80	5.03	6.21
22	6.08	3.59	0.86	3.60	6.08
23	7.27	2.45	0.18	3.98	5.96
24	6.81	2.82	0.41	3.01	5.97
mean	6.44	2.76	0.49	4.58	6.53
S.D.	0.61	0.71	0.31	0.70	0.57

* C = Authentic sample of *Coscinium fenestratum* (Gaertn.) Colebr. from Nong Khai province

Table 43. Loss on drying, total ash, acid insoluble-ash and extractive value of *Fibraurea tinctoria* Lour.

Crude drug sample	Loss on drying (%)	Ash content (%)		Extractive value (%)	
		Total ash	Acid-insoluble ash	Ethanol	Water
25	7.12	6.66	0.17	3.18	5.62
26	6.74	6.21	0.25	4.33	5.94
27	6.71	3.74	0.07	3.90	5.50
28	6.52	4.30	0.11	4.10	5.44
mean	6.77	5.23	0.15	3.88	5.63
S.D.	0.25	1.42	0.08	0.50	0.22

Table 44. Loss on drying, total ash, acid insoluble-ash and extractive value of *Combretum latifolium* Blume

Crude drug sample	Loss on drying (%)	Ash content (%)		Extractive value (%)	
		Total ash	Acid-insoluble ash	Ethanol	Water
a*	5.62	5.71	0.18	4.85	6.55
b*	6.90	4.90	0.23	5.08	7.13
29	7.54	7.08	0.46	5.87	6.28
30	6.15	6.01	0.36	5.50	6.81
31	7.21	5.50	0.39	5.57	7.21
32	6.11	4.75	0.25	4.41	6.83
mean	6.59	5.66	0.31	5.21	6.80
S.D.	0.74	0.85	0.11	0.54	0.35

* a = Authentic sample from Sa nam chai khet district, Chachoengsao province

b = Authentic sample from Sakaeraj district, Nachon Ratchasima province

Discussion

Four species of Khamin khruea from two families (Menispermaceae and Combretaceae) were investigated and the results of this experiment were shown the significant difference. The prominent characters of Menispermaceae are spiral leaves, swollen at base and the transversely cut of stem showed the medullary rays radiating from the center whereas the *Combretum latifolium* Blume (Combretaceae) does not. The three kinds of Khamin khruea from Menispermaceae including *Arcangelisia flava* (L.) Merr., *Cosciniun fenestratum* (Gaertn.) Colebr. and *Fibraurea tinctoria* Lour. have several characters in the lamina of the leaves which are distinguishable. *Coscinium* spp. could be excluded from the other two species. The *Coscinium* leaves are often peltate but not peltate in the other two genera. In *Arcangelisia* and *Fibraurea* have glabrous leaves whereas *Coscinium* has lamina whitish tomentellous below. Only *Coscinium* contains the glandular trichomes on the upper epidermis. Concerning dense tomentose of the *Coscinium* on the lower epidermis which can be seen a lot of unicellular trichomes instead of the stomata, so that the stomata were substituted by glandular trichomes on the upper epidermis. Stomatal number and stomatal index of *Coscinium* were defined by terms of glandular number and glandular index respectively.

From the results of leaf measurements compared by means of statistic using one-way ANOVA (used SPSS program). Since the leaf in the same species was measured more than 2 samples, the 95% confidence interval has been conducted to find the representative information for each measurement of each species. The confidence interval help to estimate the range of data for being reliable information to identify the leaves samples. The data interval is represented the range between minimum and maximum of total data utilized.

1. *Arcangelisia flava* (L.) Merr. (5 samples)

The 5 samples of *A. flava* (L.) Merr. were determined by one-way ANOVA and found the significant differences (p value ≤ 0.05) within species from various places in every measurements (see Appendices). The 95% confidence interval of those leaf measurements are calculated using total data of 5 samples (150 data, Table 9-13, 22-26).

	95 % Confidence interval	Data interval	Mean (S.D.)
Stomatal number	257.53 – 269.34	176.37-388.01	263.43 (36.58)
Stomatal index	8.39 – 8.65	6.86-10.98	8.52 (0.80)
Vein-islet number	2.16 – 2.31	1.50-3.75	2.24 (0.49)
Veinlet termination number	6.52 – 6.96	3.25-9.50	6.74 (1.36)
Palisade ratio	5.47 – 5.70	4.25-7.00	5.58 (0.72)

2. *Coscinium* spp.

The leaf measurements of *Coscinium fenestratum* (Gaertn.) Colebr. are proceeded only 1 sample from Nong Khai province. Those data represent the range between minimum and maximum of each measurements (Table 14, 27).

The 2 samples of other species of *Coscinium* (Trang and Songkhla samples) were shown the different observations such as the number of glandular trichome in unit area, the size of epidermal cells, the vein structure and palisade ratio. The leaf measurements of *Coscinium* sp. were determined by statistic process. They showed the significant differences (p value ≤ 0.05) in three measurements including glandular index, vein-islet number and veinlet termination number between these 2 samples (see Appendices). The 95% confidence interval of those leaf measurements are calculated using total data of 2 samples (60 data, Table 15-16, 28-29).

Coscinium fenestratum (Gaertn.) Colebr.(1 sample)

	Data interval	Mean (S. D.)
Glandular number	52.91-79.37	65.85 (8.89)
Glandular index	2.39-3.56	3.06 (0.32)
Vein-islet number	24.00-36.00	29.54 (3.76)
Veinlet termination number	14.75-19.75	16.72 (1.42)
Palisade ratio	3.75-6.75	5.03 (0.75)

Coscinium sp. (2 samples)

	95 % Confidence interval	Data interval	Mean (S.D.)
Glandular number	24.48-27.55	17.64-35.27	26.02 (5.95)
Glandular index	1.76-2.05	1.01-3.08	1.91 (0.56)
Vein-islet number	12.88-13.67	10.25-16.75	13.28 (1.54)

Veinlet termination number	11.09-12.31	8.25-15.50	11.70 (2.36)
Palisade ratio	11.19-11.87	8.25-14.50	11.53 (1.31)

3. *Fibraurea tinctoria* Lour.(3 samples)

These samples of *F. tinctoria* Lour. were determined for their means and standard deviations of leaf measurements which were shown in Table17-19 and 30-32. The significant differences (p value ≤ 0.05) were found in each sample from various places except palisade ratio (see Appendices). These are the following of 95% confidence interval of this species which are calculated using total data of 3 samples (90 samples in each measurements).

	95 % Confidence interval	Data interval	Mean (S.D.)
Stomatal number	258.66 – 284.95	176.37-396.83	271.80 (62.75)
Stomatal index	8.98 – 9.44	7.19-11.71	9.21 (1.08)
Vein-islet number	1.77 – 1.93	1.25-2.50	1.85 (0.36)
Veinlet termination number	3.75 – 4.03	2.50-5.50	3.89 (0.66)
Palisade ratio	4.22 – 4.41	3.50-5.25	4.32 (0.45)

4. *Combretum latifolium* Blume (2 samples)

The data of leaf measurements from Table 20-21 and 33-34 were calculated by SPSS program. The significant differences (p value ≤ 0.05) were found in 2 measurements between 2 samples from different places including stomatal number and stomatal index (see Appendices). These are the following of 95% confidence interval of this species which are calculated using total data of 2 samples (60 samples in each measurements).

	95 % Confidence interval	Data interval	Mean (S.D.)
Stomatal number	362.85-387.81	291.01-485.01	375.33 (48.31)
Stomatal index	13.56-14.25	11.96-16.67	13.90 (1.33)
Vein-islet number	3.59-3.94	2.50-5.75	3.77 (0.69)
Veinlet termination number	1.89-2.18	1.00-3.00	2.03 (0.57)
Palisade ratio	14.72-15.51	11.50-17.75	15.11 (1.54)

The leaf measurements are used as a character for the identification concerning their constant value in each species. Using SPSS program, the data collected in each measurement showed the significant difference within species from various places. It is interesting to note that the represent data should be used more than 1 sample in each species for reliable information. *Fibraurea tinctoria* Lour. has the non-lignified idioblasts in their leaves which can be seen underneath epidermal cells (Figure 18A). *Combretum latifolium* Blume has obviously seen rosette aggregate crystals of calcium oxalate (Figure 19D).

The *Coscinium* has 2 species in this investigation which undertaken under the same name “Khamin khruea” (or Ham), 1 sample from Nong Khai province is a *Coscinium fenestratum* (Gaertn.) Colebr. and other species of *Coscinium* (Trang and Songkhla sample). They were shown the different observations such as the number of glandular trichome in unit area, the size of epidermal cells, the vein structure, palisade ratio, size of lamina and length of petiole inserted from basal margin. Not only leaves but also the TLC patterns are shown the distinguish among the 2 species (Figure 32, 33). The chemical tests represent alkaloid and tannin (catechol) groups of both species (Table 39). Macroscopic characters of both *Coscinium* spp. in this study are similar but the microscopic characters are different. Only *Coscinium* sp. (Trang sample) do has the elongated quadrilateral parenchyma cells containing starch granules and long lignified thickened wall fibers. Although this genus contains 2 species (*C. fenestratum* and *C. blumeanum*) in Thailand (Forman, 1991) but the conclusion must be considered. The both samples of *Coscinium* sp. (collected from Trang and Songkhla province) obtained only leaves and stems which are not enough taxonomic criteria to species identification. The further study should be recommend to complete this conclusion.

Arcangelisia flava (L.) Merr. shows the prominent successive rings in transverse section which differ from *Coscinium* spp. and *Fibraurea tinctoria* Lour. The main microscopic characters of stem were stratified sclereids and starch granules. The TLC patterns showed minor constituents that distinguish from the others (Figure 32, 33). The chemical tests represented alkaloid and tannin (catechol) groups (Table 39).

Macroscopic character of *Fibraurea tinctoria* Lour. is similar to *Coscinium* spp. in transverse section but the stem do has longitudinal furrow bark which is hardly to distinguish among genera. The main microscopic characters of *Fibraurea tinctoria* Lour. stem were large sclereids and fibrous sclereids. The chemical tests represented alkaloid and tannin (catechol) groups (Table 39). TLC patterns showed the differences from *Arcangelisia flava* (L.) Merr. and *Coscinium* spp. which were berberine and minor constituents (Figure 32, 33). most of previous phytochemical studies had not been found berberine in this species.

The microscopic characterization of *Combretum latifolium* Blume stem are reserved parenchyma, parenchyma with prism of calcium oxalate, medullary ray and starch granules. The chemical tests showed the presence of tannin group which is pyrogallol (blue precipitation with ferric chloride solution and no change with bromine water) and no alkaloid content (Table 39).

The crude drugs samples which were purchased from traditional drugstores throughout Thailand can be distinguishable into 4 species according to the results of macroscopic, microscopic characterizations and TLC patterns. There are *Coscinium fenestratum* (Gaertn.) Colebr., *Coscinium* sp., *Fibraurea tinctoria* Lour. and *Combretum latifolium* Blume. *Coscinium* sp. had 2 samples (No. 1-2) from total 32 samples (6.25%), both of them from the South part. *Coscinium fenestratum* (Gaertn.) Colebr. had 22 samples (No. 3-24) from total 32 samples (68.75%) mostly from the Central, North-Eastern part or the other part which almost distributed by retail traditional drugstores in Bangkok. *Fibraurea tinctoria* Lour. had 4 samples (No. 25-28) from total 32 samples (12.5 %), all of them purchased from the South part. However, the quality controls of authentic sample of *F. tinctoria* Lour. were not proceeded because of its limited amount. *Combretum latifolium* Blume had 4 samples (No. 29-32) from total 32 samples (12.5%) purchased from Bangkok and Ubon Ratchathani provinces. No purchased sample resembled *Arcangelisia flava* (L.) Merr. so that the authentic samples of *A. flava* (L.) Merr. from various places were carried out for its quality controls.

Arcangelisia flava (L.) Merr. (authentic samples: Table 40)

	data interval (%)	mean (%)
Loss on drying	6.06-6.71	6.34
Total ash	4.31-4.65	4.52
Acid-insoluble ash	0.06-0.13	0.09
Ethanol-soluble extractive value	3.34-4.42	4.01
Water-soluble extractive value	6.12-8.78	7.49

Coscinium sp. (purchased sample No. 1,2: Table 41)

	data interval (%)	mean (%)
Loss on drying	5.79-5.97	5.88
Total ash	3.41-3.58	3.50
Acid-insoluble ash	0.19-0.26	0.23
Ethanol-soluble extractive value	4.91-5.28	5.10
Water-soluble extractive value	5.83-6.95	6.39

Coscinium fenestratum (Gaertn.) Colebr. (authentic and purchased samples No. 3-24: Table 42)

	data interval (%)	mean (%)
Loss on drying	5.53-7.47	6.44
Total ash	1.85-4.55	2.76
Acid-insoluble ash	0.06-1.35	0.49
Ethanol-soluble extractive value	3.01-5.81	4.58
Water-soluble extractive value	5.71-7.83	6.53

Fibraurea tinctoria Lour. (No. 25-28: Table 43)

	data interval (%)	mean (%)
Loss on drying	6.52-7.12	6.77
Total ash	3.74-6.66	5.23
Acid-insoluble ash	0.07-0.25	0.15
Ethanol-soluble extractive value	3.90-4.33	3.88

Water-soluble extractive value	5.44-5.94	5.63

Combretum latifolium Blume (authentic and purchased samples No. 29-32: Table 44)

	data interval (%)	mean (%)
Loss on drying	6.11-7.54	6.59
Total ash	4.75-7.08	5.66
Acid-insoluble ash	0.18-0.46	0.31
Ethanol-soluble extractive value	4.41-5.87	5.21
Water-soluble extractive value	6.28-7.21	6.80

Loss on drying is employed in the Pharmacopoeia to control the loss in weight (due to water and other volatile materials) of crude drugs. However, the little volatile materials when drying (105°C) to constant weight, the loss weight is mostly due to water. The excessive content of water in crude drugs and temperature are suitable environment of fungi and bacteria growth which can cause the deterioration. Besides the loss on drying, ash contents are used to control the admixture of foreign inorganic matter due to their storage, container or intentional add to improve the appearance of crude drug. The determination of ethanol- and water-soluble extractive values are used to control the constituents of crude drugs which can inferiority from many factors such as moisture content, temperature, harvesting, drying process, kept duration and storage. The random samplings of crude drugs “Khamim khruea” from traditional drugstores in many provinces are determined and concluded the data as an estimated percentage values in terms “not more than” (for loss on drying, total ash and acid-insoluble ash) and “not less than” (for ethanol- and water-soluble extractive values).

CHAPTER VI

CONCLUSION

Khamin khruea is fairly used as folkloric medicine in Thailand. It is referred to several plants including *Arcangelisia flava* (L.) Merr., *Coscinium fenestratum* (Gaertn.) Colebr., *Fibraurea tinctoria* Lour. and *Combretum latifolium* Blume. Previously, this name is questionable to defined the scientific name whereas people do not differentiate between these plants. The results of this investigation clearly indicated that the macroscopic and microscopic characters of stem, leaf measurements, thin-layer chromatographic patterns and phytochemical screening can be effectively used together as an important role in species identification of Khamin khruea. The statistic process of leaf measurements showed significant differences in each sample of each species. The reliable information of each measurement must be determine more than 1 sample.

Under the name Khamin khruea, the purchased samples from traditional drugstores throughout Thailand can separate into 4 species including *Coscinium fenestratum* (Gaertn.) Colebr., *Coscinium* sp., *Fibraurea tinctoria* Lour. and *Combretum latifolium* Blume. No purchased sample resembled *Arcangelisia flava* (L.) Merr. so that the authentic samples of this species from various places would be carried out for its quality controls. The results of quality controls of these Khamin khruea can inform the standardization of each species as shown belows.

	not more than (%)			not less than (%)	
	Loss on drying	Ash content		Extractive value	
Khamin khruea		Total ash	Acid-insoluble ash	Ethanol	Water
Arcangelisia flava	7.5	5.0	0.5	3.5	6.5
Coscinium fenestratum	7.5	3.5	1.0	4.0	6.0
Coscinium sp.	7.5	4.0	0.5	4.0	5.5
Fibraurea tinctoria.	7.5	6.0	0.5	3.5	5.0
Combretum latifolium	7.5	6.5	0.5	4.5	6.0

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APPENDICES

A. Reagents

Anisaldehyde-sulphuric acid reagent

The modified anisalehyde-sulphuric acid spray solution composed of 0.5 ml anisaldehyde, 9 ml ethanol, 0.5 ml 97% sulphuric acid and 0.1 ml glacial acetic acid. The sprayed chromatogram was heated 5-10 minutes at 90-100°C until maximal visualization of the spots. Phenols, terpenes, sugars and steroids would turn violet, blue, red, gray or green.

Bromine TS (Bromine water)

A saturated solution o bromine, prepared by agitating 2 to 3 ml of bromine with 100 ml of cold water in a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum. Store in a cool place, protected from light.

Chloroform water

Dissolve 2.5 ml of chloroform in the purified water by shaking. Purified water, freshly boiled and cooled sufficient to produce 1000 ml.

Chloral hydrate solution BP

Dissolve chloral hydrate 80 g in 20 ml of water, using gentle heat if necessary.

Dragendorff's TS, modified

Dissolve 1.7 g of bismuth oxynitrate in a mixture of 80 ml of water and 20 ml of glacial acetic acid, warming if necessary. Cool, add 100 ml of a 50 percent w/v solution of potassium iodide, and mix. Refrigerate this stock solution with water to 100 ml, add 10 ml of glacial acetic acid and mix. Then add 120 mg of iodine and shake until the iodine has completely dissolved. Store refrigerated and discard after 2 weeks.

Ferric chloride TS

Dissolve 9 g of iron (III) chloride in 100 ml of water.

Gelatin solution

Dissolve 10 g of gelatin in 1000 ml of water.

Iodine solution

Mix 2 g of iodine and 3 g of potassium iodide and add about 5 ml of water, agitate until dissolve, slowly dilute with water to 100 ml.

Mercuric- potassium iodide TS (Mayer's reagent)

Dissolve 1.358 g of mercury (II) chloride in 60 ml of water. Dissolve 5 g of potassium iodide in 10 ml of water. Mix the two solutions, and dilute with water to 100 ml.

Phloroglucinol solution

Dissolve 1 g of phloroglucinol in 100 ml of 95% Alcohol

B. The results of data analysis by SPSS program

The One-Way Analysis of Variance (One-Way ANOVA)

1. *Arcangelisia flava* (L.) Merr.
2. *Fibraurea tinctoria* Lour.

Testing Two Sample Mean (T-Test)

1. *Coscinium* sp.
2. *Combretum latifolium* Blume

The One-Way Analysis of Variance (One-Way ANOVA)

1. *Arcangelisia flava* (L.) Merr. (5 samples: Table 9-13, 22-26)

1.1 Stomatal number (SN)

Oneway

Descriptives

							95% Confidence Interval for Mean			
			N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
SN	VAR00002	1.00	30	292.76897	32.61922	5.95543	280.58875	304.94918	238.095	388.007
		2.00	30	214.57987	19.55904	3.57097	207.27640	221.88333	176.367	255.732
		3.00	30	258.37740	22.23054	4.05872	250.07638	266.67842	220.459	317.460
		4.00	30	283.36277	24.91328	4.54852	274.06000	292.66554	238.095	343.915
		5.00	30	268.07767	22.28477	4.06862	259.75640	276.39894	229.277	317.460
		Total	150	263.43333	36.58493	2.98715	257.53069	269.33597	176.367	388.007

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SN	Between Groups	110746.656	4	27686.664	45.269	.000
	Within Groups	88683.407	145	611.610		
	Total	199430.063	149			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: SN

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	78.18910*	6.385	.000	60.77100	95.60720
		3.00	34.39157*	6.385	.000	16.97347	51.80966
		4.00	9.40620	6.385	.580	-8.01190	26.82430
		5.00	24.69130*	6.385	.001	7.27320	42.10940
	2.00	1.00	-78.18910*	6.385	.000	-95.60720	-60.77100
		3.00	-43.79753*	6.385	.000	-61.21563	-26.37944
		4.00	-68.78290*	6.385	.000	-86.20100	-51.36480
		5.00	-53.49780*	6.385	.000	-70.91590	-36.07970
	3.00	1.00	-34.39157*	6.385	.000	-51.80966	-16.97347
		2.00	43.79753*	6.385	.000	26.37944	61.21563
		4.00	-24.98537*	6.385	.001	-42.40346	-7.56727
		5.00	-9.70027	6.385	.550	-27.11836	7.71783
	4.00	1.00	-9.40620	6.385	.580	-26.82430	8.01190
		2.00	68.78290*	6.385	.000	51.36480	86.20100
		3.00	24.98537*	6.385	.001	7.56727	42.40346
		5.00	15.28510	6.385	.117	-2.13300	32.70320
	5.00	1.00	-24.69130*	6.385	.001	-42.10940	-7.27320
		2.00	53.49780*	6.385	.000	36.07970	70.91590
		3.00	9.70027	6.385	.550	-7.71783	27.11836
		4.00	-15.28510	6.385	.117	-32.70320	2.13300
LSD	1.00	2.00	78.18910*	6.385	.000	65.56851	90.80969
		3.00	34.39157*	6.385	.000	21.77098	47.01215
		4.00	9.40620	6.385	.143	-3.21439	22.02679
		5.00	24.69130*	6.385	.000	12.07071	37.31189
	2.00	1.00	-78.18910*	6.385	.000	-90.80969	-65.56851
		3.00	-43.79753*	6.385	.000	-56.41812	-31.17695
		4.00	-68.78290*	6.385	.000	-81.40349	-56.16231
		5.00	-53.49780*	6.385	.000	-66.11839	-40.87721
	3.00	1.00	-34.39157*	6.385	.000	-47.01215	-21.77098
		2.00	43.79753*	6.385	.000	31.17695	56.41812
		4.00	-24.98537*	6.385	.000	-37.60595	-12.36478
		5.00	-9.70027	6.385	.131	-22.32085	2.92032
	4.00	1.00	-9.40620	6.385	.143	-22.02679	3.21439
		2.00	68.78290*	6.385	.000	56.16231	81.40349
		3.00	24.98537*	6.385	.000	12.36478	37.60595
		5.00	15.28510*	6.385	.018	2.66451	27.90569
	5.00	1.00	-24.69130*	6.385	.000	-37.31189	-12.07071
		2.00	53.49780*	6.385	.000	40.87721	66.11839
		3.00	9.70027	6.385	.131	-2.92032	22.32085
		4.00	-15.28510*	6.385	.018	-27.90569	-2.66451

*. The mean difference is significant at the .05 level.

1.2 Stomatal index (SI)

Oneway

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
SI	VAR00002	1.00	30	8.7283	.6956	.1270	8.4686	8.9881	7.63	10.28
		2.00	30	7.6533	.3960	7.230E-02	7.5055	7.8012	7.14	8.67
		3.00	30	8.8527	.6524	.1191	8.6091	9.0963	7.72	10.13
		4.00	30	9.2557	.5568	.1016	9.0478	9.4636	8.54	10.98
		5.00	30	8.0907	.4687	8.558E-02	7.9156	8.2657	6.86	8.95
		Total	150	8.5161	.7993	6.527E-02	8.3872	8.6451	6.86	10.98

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SI	Between Groups	48.919	4	12.230	38.314	.000
	Within Groups	46.283	145	.319		
	Total	95.203	149			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: SI

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Tukey HSD	1.00	2.00	1.0750*	.146	.000	.6771	1.4729
		3.00	-.1243	.146	.914	-.5222	.2736
		4.00	-.5273*	.146	.003	-.9252	-.1294
		5.00	.6377*	.146	.000	.2398	1.0356
	2.00	1.00	-1.0750*	.146	.000	-1.4729	-.6771
		3.00	-1.1993*	.146	.000	-1.5972	-.8014
		4.00	-1.6023*	.146	.000	-2.0002	-1.2044
		5.00	-.4373*	.146	.023	-.8352	-3.9417E-02
	3.00	1.00	.1243	.146	.914	-.2736	.5222
		2.00	1.1993*	.146	.000	.8014	1.5972
		4.00	-.4030*	.146	.045	-.8009	-5.0834E-03
		5.00	.7620*	.146	.000	.3641	1.1599
	4.00	1.00	.5273*	.146	.003	.1294	.9252
		2.00	1.6023*	.146	.000	1.2044	2.0002
		3.00	.4030*	.146	.045	5.083E-03	.8009
		5.00	1.1650*	.146	.000	.7671	1.5629
	5.00	1.00	-.6377*	.146	.000	-1.0356	-.2398
		2.00	.4373*	.146	.023	3.942E-02	.8352
		3.00	-.7620*	.146	.000	-1.1599	-.3641
		4.00	-1.1650*	.146	.000	-1.5629	-.7671
LSD	1.00	2.00	1.0750*	.146	.000	.7867	1.3633
		3.00	-.1243	.146	.395	-.4127	.1640
		4.00	-.5273*	.146	.000	-.8157	-.2390
		5.00	.6377*	.146	.000	.3493	.9260
	2.00	1.00	-1.0750*	.146	.000	-1.3633	-.78 7
		3.00	-1.1993*	.146	.000	-1.4877	-.9110
		4.00	-1.6023*	.146	.000	-1.8907	-1.3140
		5.00	-.4373*	.146	.003	-.7257	-.1490
	3.00	1.00	.1243	.146	.395	-.1640	.4127
		2.00	1.1993*	.146	.000	.9110	1.4877
		4.00	-.4030*	.146	.006	-.6913	-.1147
		5.00	.7620*	.146	.000	.4737	1.0503
	4.00	1.00	.5273*	.146	.000	.2390	.8157
		2.00	1.6023*	.146	.000	1.3140	1.8907
		3.00	.4030*	.146	.006	.1147	.6913
		5.00	1.1650*	.146	.000	.8767	1.4533
	5.00	1.00	-.6377*	.146	.000	-.9260	-.3493
		2.00	.4373*	.146	.003	.1490	.7257
		3.00	-.7620*	.146	.000	-1.0503	-.4737
		4.00	-1.1650*	.146	.000	-1.4533	-.8767

*. The mean difference is significant at the .05 level.

1.3 Vein-islet number (VI)

Oneway

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
VI	VAR00002	1.00	30	2.1417	.3516	6.420E-02	2.0104	2.2730	1.50	3.00
		2.00	30	1.9917	.3113	5.684E-02	1.8754	2.1079	1.50	2.75
		3.00	30	2.6000	.5746	.1049	2.3854	2.8146	1.75	3.75
		4.00	30	2.4667	.5241	9.569E-02	2.2710	2.6624	1.50	3.50
		5.00	30	1.9750	.2962	5.407E-02	1.8644	2.0856	1.50	2.75
		Total	150	2.2350	.4926	4.022E-02	2.1555	2.3145	1.50	3.75

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
VI	Between Groups	9.672	4	2.418	13.241	.000
	Within Groups	26.481	145	.183		
	Total	36.154	149			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: VI

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	.1500	.110	.654	-.1510	.4510
		3.00	-.4583*	.110	.000	-.7593	-.1573
		4.00	-.3250*	.110	.027	-.6260	-2.4012E-02
		5.00	.1667	.110	.556	-.1343	.4677
	2.00	1.00	-.1500	.110	.654	-.4510	.1510
		3.00	-.6083*	.110	.000	-.9093	-.3073
		4.00	-.4750*	.110	.000	-.7760	-.1740
		5.00	1.667E-02	.110	1.000	-.2843	.3177
	3.00	1.00	.4583*	.110	.000	.1573	.7593
		2.00	.6083*	.110	.000	.3073	.9093
		4.00	.1333	.110	.747	-.1677	.4343
		5.00	.6250*	.110	.000	.3240	.9260
	4.00	1.00	.3250*	.110	.027	2.401E-02	.6260
		2.00	.4750*	.110	.000	.1740	.7760
		3.00	-.1333	.110	.747	-.4343	.1677
		5.00	.4917*	.110	.000	.1907	.7927
	5.00	1.00	-.1667	.110	.556	-.4677	.1343
		2.00	-1.6667E-02	.110	1.000	-.3177	.2843
		3.00	-.6250*	.110	.000	-.9260	-.3240
		4.00	-.4917*	.110	.000	-.7927	-.1907
LSD	1.00	2.00	.1500	.110	.176	-6.8086E-02	.3681
		3.00	-.4583*	.110	.000	-.6764	-.2402
		4.00	-.3250*	.110	.004	-.5431	-.1069
		5.00	.1667	.110	.133	-5.1419E-02	.3848
	2.00	1.00	-.1500	.110	.176	-.3681	6.809E-02
		3.00	-.6083*	.110	.000	-.8264	-.3902
		4.00	-.4750*	.110	.000	-.6931	-.2569
		5.00	1.667E-02	.110	.880	-.2014	.2348
	3.00	1.00	.4583*	.110	.000	.2402	.6764
		2.00	.6083*	.110	.000	.3902	.8264
		4.00	.1333	.110	.229	-8.4753E-02	.3514
		5.00	.6250*	.110	.000	.4069	.8431
	4.00	1.00	.3250*	.110	.004	.1069	.5431
		2.00	.4750*	.110	.000	.2569	.6931
		3.00	-.1333	.110	.229	-.3514	8.475E-02
		5.00	.4917*	.110	.000	.2736	.7098
	5.00	1.00	-.1667	.110	.133	-.3848	5.142E-02
		2.00	-1.6667E-02	.110	.880	-.2348	.2014
		3.00	-.6250*	.110	.000	-.8431	-.4069
		4.00	-.4917*	.110	.000	-.7098	-.2736

*. The mean difference is significant at the .05 level.

1.4 Veinlet termination number (VT)

Oneway

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
VT	VAR00002	1.00	30	7.2583	1.0096	.1843	6.8813	7.6353	5.50	9.00
		2.00	30	5.0750	.8836	.1613	4.7450	5.4050	3.25	7.50
		3.00	30	7.2167	1.0437	.1906	6.8269	7.6064	5.00	8.75
		4.00	30	7.8167	.7250	.1324	7.5460	8.0874	6.25	9.50
		5.00	30	6.3167	1.1762	.2147	5.8775	6.7558	4.25	8.75
		Total	150	6.7367	1.3643	.1114	6.5165	6.9568	3.25	9.50

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
VT	Between Groups	138.194	4	34.549	36.000	.000
	Within Groups	139.154	145	.960		
	Total	277.348	149			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: VT

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	2.1833*	.253	.000	1.4934	2.8733
		3.00	4.167E-02	.253	1.000	-.6483	.7316
		4.00	-.5583	.253	.177	-1.2483	.1316
		5.00	.9417*	.253	.002	.2517	1.6316
	2.00	1.00	-2.1833*	.253	.000	-2.8733	-1.4934
		3.00	-2.1417*	.253	.000	-2.8316	-1.4517
		4.00	-2.7417*	.253	.000	-3.4316	-2.0517
		5.00	-1.2417*	.253	.000	-1.9316	-.5517
	3.00	1.00	-4.1667E-02	.253	1.000	-.7316	.6483
		2.00	2.1417*	.253	.000	1.4517	2.8316
		4.00	-.6000	.253	.123	-1.2900	8.997E-02
		5.00	.9000*	.253	.003	.2100	1.5900
	4.00	1.00	.5583	.253	.177	-.1316	1.2483
		2.00	2.7417*	.253	.000	2.0517	3.4316
		3.00	.6000	.253	.123	-8.9966E-02	1.2900
		5.00	1.5000*	.253	.000	.8100	2.1900
	5.00	1.00	-.9417*	.253	.002	-1.6316	-.2517
		2.00	1.2417*	.253	.000	.5517	1.9316
		3.00	-.9000*	.253	.003	-1.5900	-.2100
		4.00	-1.5000*	.253	.000	-2.1900	-.8100
LSD	1.00	2.00	2.1833*	.253	.000	1.6834	2.6833
		3.00	4.167E-02	.253	.869	-.4583	.5416
		4.00	-.5583*	.253	.029	-1.0583	-5.8407E-02
		5.00	.9417*	.253	.000	.4417	1.4416
	2.00	1.00	-2.1833*	.253	.000	-2.6833	-1.6834
		3.00	-2.1417*	.253	.000	-2.6416	-1.6417
		4.00	-2.7417*	.253	.000	-3.2416	-2.2417
		5.00	-1.2417*	.253	.000	-1.7416	-.7417
	3.00	1.00	-4.1667E-02	.253	.869	-.5416	.4583
		2.00	2.1417*	.253	.000	1.6417	2.6416
		4.00	-.6000*	.253	.019	-1.0999	-.1001
		5.00	.9000*	.253	.001	.4001	1.3999
	4.00	1.00	.5583*	.253	.029	5.841E-02	1.0583
		2.00	2.7417*	.253	.000	2.2417	3.2416
		3.00	.6000*	.253	.019	.1001	1.0999
		5.00	1.5000*	.253	.000	1.0001	1.9999
	5.00	1.00	-.9417*	.253	.000	-1.4416	-.4417
		2.00	1.2417*	.253	.000	.7417	1.7416
		3.00	-.9000*	.253	.001	-1.3999	-.4001
		4.00	-1.5000*	.253	.000	-1.9999	-1.0001

*. The mean difference is significant at the .05 level.

1.5 Palisade ratio (PR)

Oneway

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
PR	VAR00002	1.00	30	5.6500	.6552	.1196	5.4053	5.8947	4.50	6.75
		2.00	30	5.4833	.6596	.1204	5.2370	5.7296	4.25	6.75
		3.00	30	5.9083	.6932	.1266	5.6495	6.1672	4.50	7.00
		4.00	30	5.1000	.6453	.1178	4.8591	5.3409	4.25	6.25
		5.00	30	5.7750	.7232	.1320	5.5049	6.0451	4.25	7.00
		Total	150	5.5833	.7233	5.906E-02	5.4666	5.7000	4.25	7.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PR	Between Groups	11.713	4	2.928	6.409	.000
	Within Groups	66.246	145	.457		
	Total	77.958	149			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: PR

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Tukey HSD	1.00	2.00	.1667	.175	.875	-.3094	.6427
		3.00	-.2583	.175	.575	-.7344	.2177
		4.00	.5500*	.175	.014	7.394E-02	1.0261
		5.00	-.1250	.175	.953	-.6011	.3511
	2.00	1.00	-.1667	.175	.875	-.6427	.3094
		3.00	-.4250	.175	.106	-.9011	5.106E-02
		4.00	.3833	.175	.181	-9.2723E-02	.8594
		5.00	-.2917	.175	.452	-.7677	.1844
	3.00	1.00	.2583	.175	.575	-.2177	.7344
		2.00	.4250	.175	.106	-5.1057E-02	.9011
		4.00	.8083*	.175	.000	.3323	1.2844
		5.00	.1333	.175	.941	-.3427	.6094
	4.00	1.00	-.5500*	.175	.014	-1.0261	-7.3943E-02
		2.00	-.3833	.175	.181	-.8594	9.272E-02
		3.00	-.8083*	.175	.000	-1.2844	-.3323
		5.00	-.6750*	.175	.001	-1.1511	-.1989
	5.00	1.00	.1250	.175	.953	-.3511	.6011
		2.00	.2917	.175	.452	-.1844	.7677
		3.00	-.1333	.175	.941	-.6094	.3427
		4.00	.6750*	.175	.001	.1989	1.1511
LSD	1.00	2.00	.1667	.175	.341	-.1783	.5116
		3.00	-.2583	.175	.141	-.6033	8.660E-02
		4.00	.5500*	.175	.002	.2051	.8949
		5.00	-.1250	.175	.475	-.4699	.2199
	2.00	1.00	-.1667	.175	.341	-.5116	.1783
		3.00	-.4250*	.175	.016	-.7699	-8.0065E-02
		4.00	.3833*	.175	.030	3.840E-02	.7283
		5.00	-.2917	.175	.097	-.6366	5.327E-02
	3.00	1.00	.2583	.175	.141	-8.6602E-02	.6033
		2.00	.4250*	.175	.016	8.006E-02	.7699
		4.00	.8083*	.175	.000	.4634	1.1533
		5.00	.1333	.175	.446	-.2116	.4783
	4.00	1.00	-.5500*	.175	.002	-.8949	-.2051
		2.00	-.3833*	.175	.030	-.7283	-3.8398E-02
		3.00	-.8083*	.175	.000	-1.1533	-.4634
		5.00	-.6750*	.175	.000	-1.0199	-.3301
	5.00	1.00	.1250	.175	.475	-.2199	.4699
		2.00	.2917	.175	.097	-5.3269E-02	.6366
		3.00	-.1333	.175	.446	-.4783	.2116
		4.00	.6750*	.175	.000	.3301	1.0199

*. The mean difference is significant at the .05 level.

2. *Fibraurea tinctoria* Lour. (3 samples: Table 17-19, 30-32)

2.1 Stomatal number (SN_2)

Oneway

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
SN2	VAR00003	1.00	30	212.81613	24.69712	4.50906	203.59408	222.03819	176.367	273.369
		2.00	30	259.25930	27.75097	5.06661	248.89692	269.62168	202.822	317.460
		3.00	30	343.32740	40.63780	7.41941	328.15299	358.50181	273.369	396.825
		Total	90	271.80094	62.75415	6.61487	258.65734	284.94455	176.367	396.825

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SN2	Between Groups	262576.039	2	131288.019	129.924	.000
	Within Groups	87913.365	87	1010.498		
	Total	350489.404	89			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: SN2

	(I) VAR00003	(J) VAR00003	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-46.44317*	8.208	.000	-66.01438	-26.87195
		3.00	-130.51127*	8.208	.000	-150.08248	-110.94005
	2.00	1.00	46.44317*	8.208	.000	26.87195	66.01438
		3.00	-84.06810*	8.208	.000	-103.63931	-64.49689
	3.00	1.00	130.51127*	8.208	.000	110.94005	150.08248
		2.00	84.06810*	8.208	.000	64.49689	103.63931
LSD	1.00	2.00	-46.44317*	8.208	.000	-62.75689	-30.12945
		3.00	-130.51127*	8.208	.000	-146.82499	-114.19755
	2.00	1.00	46.44317*	8.208	.000	30.12945	62.75689
		3.00	-84.06810*	8.208	.000	-100.38182	-67.75438
	3.00	1.00	130.51127*	8.208	.000	114.19755	146.82499
		2.00	84.06810*	8.208	.000	67.75438	100.38182

*. The mean difference is significant at the .05 level.

2.2 Stomatal index (SI2)

Oneway

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
SI2	VAR00003	1.00	30	8.3043	.7440	.1358	8.0265	8.5821	7.19	9.84
		2.00	30	9.2828	.7710	.1408	8.9949	9.5707	8.19	10.80
		3.00	30	10.0430	.9333	.1704	9.6945	10.3915	8.29	11.71
		Total	90	9.2101	1.0817	.1140	8.9835	9.4366	7.19	11.71

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SI2	Between Groups	45.583	2	22.791	33.866	.000
	Within Groups	58.550	87	.673		
	Total	104.133	89			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: SI2

	(I) VAR00003	(J) VAR00003	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.9785*	.212	.000	-1.4836	-.4734
		3.00	-1.7387*	.212	.000	-2.2437	-1.2336
	2.00	1.00	.9785*	.212	.000	.4734	1.4836
		3.00	-.7602*	.212	.002	-1.2652	-.2551
	3.00	1.00	1.7387*	.212	.000	1.2336	2.2437
		2.00	.7602*	.212	.002	.2551	1.2652
LSD	1.00	2.00	-.9785*	.212	.000	-1.3995	-.5575
		3.00	-1.7387*	.212	.000	-2.1597	-1.3177
	2.00	1.00	.9785*	.212	.000	.5575	1.3995
		3.00	-.7602*	.212	.001	-1.1812	-.3392
	3.00	1.00	1.7387*	.212	.000	1.3177	2.1597
		2.00	.7602*	.212	.001	.3392	1.1812

*. The mean difference is significant at the .05 level.

2.3 Vein-islet number (VI2)

Oneway

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
VI2	VAR00003	1.00	30	1.8500	.3511	6.410E-02	1.7189	1.9811	1.25	2.50
		2.00	30	1.9833	.3768	6.879E-02	1.8426	2.1240	1.25	2.50
		3.00	30	1.7167	.3198	5.839E-02	1.5972	1.8361	1.25	2.25
		Total	90	1.8500	.3630	3.826E-02	1.7740	1.9260	1.25	2.50

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
VI2	Between Groups	1.067	2	.533	4.353	.016
	Within Groups	10.658	87	.123		
	Total	11.725	89			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: VI2

	(I) VAR00003	(J) VAR00003	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.1333	.090	.308	-.3488	8.216E-02
		3.00	.1333	.090	.308	-8.2161E-02	.3483
	2.00	1.00	.1333	.090	.308	-8.2161E-02	.3483
		3.00	.2667*	.090	.011	5.117E-02	.4822
	3.00	1.00	-.1333	.090	.308	-.3488	8.216E-02
		2.00	-.2667*	.090	.011	-.4822	-5.1173E-02
LSD	1.00	2.00	-.1333	.090	.144	-.3130	4.629E-02
		3.00	.1333	.090	.144	-4.6293E-02	.3130
	2.00	1.00	.1333	.090	.144	-4.6293E-02	.3130
		3.00	.2667*	.090	.004	8.704E-02	.4463
	3.00	1.00	-.1333	.090	.144	-.3130	4.629E-02
		2.00	-.2667*	.090	.004	-.4463	-8.7040E-02

*. The mean difference is significant at the .05 level.

2.4 Veinlet termination number (VT2)

Oneway

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
VT2	VAR00003	1.00	30	3.7750	.6577	.1201	3.5294	4.0206	2.75	4.75
		2.00	30	4.2417	.5393	9.847E-02	4.0403	4.4431	3.25	5.50
		3.00	30	3.6583	.6515	.1190	3.4150	3.9016	2.50	5.50
		Total	90	3.8917	.6620	6.978E-02	3.7530	4.0303	2.50	5.50

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
VT2	Between Groups	5.717	2	2.858	7.470	.001
	Within Groups	33.290	87	.383		
	Total	39.006	89			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: VT2

	(I) VAR00003	(J) VAR00003	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.4667*	.160	.012	-.8475	-8.5825E-02
		3.00	.1167	.160	.746	-.2642	.4975
	2.00	1.00	.4667*	.160	.012	8.582E-02	.8475
		3.00	.5833*	.160	.001	.2025	.9642
	3.00	1.00	-.1167	.160	.746	-.4975	.2642
		2.00	-.5833*	.160	.001	-.9642	-.2025
LSD	1.00	2.00	-.4667*	.160	.004	-.7841	-.1492
		3.00	.1167	.160	.467	-.2008	.4341
	2.00	1.00	.4667*	.160	.004	.1492	.7841
		3.00	.5833*	.160	.000	.2659	.9008
	3.00	1.00	-.1167	.160	.467	-.4341	.2008
		2.00	-.5833*	.160	.000	-.9008	-.2659

*. The mean difference is significant at the .05 level.

2.5 Palisade ratio (PR2)

Oneway

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
PR2	VAR00003	1.00	30	4.3417	.5272	9.625E-02	4.1448	4.5385	3.50	5.25
		2.00	30	4.2583	.4890	8.928E-02	4.0757	4.4409	3.50	5.25
		3.00	30	4.3500	.3189	5.823E-02	4.2309	4.4691	3.75	4.75
		Total	90	4.3167	.4510	4.754E-02	4.2222	4.4111	3.50	5.25

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PR2	Between Groups	.154	2	7.708E-02	.374	.689
	Within Groups	17.946	87	.206		
	Total	18.100	89			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: PR2

	(I) VAR00003	(J) VAR00003	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	8.333E-02	.117	.758	-.1963	.3630
		3.00	-8.3333E-03	.117	.997	-.2880	.2713
	2.00	1.00	-8.3333E-02	.117	.758	-.3630	.1963
		3.00	-9.1667E-02	.117	.715	-.3713	.1880
	3.00	1.00	8.333E-03	.117	.997	-.2713	.2880
		2.00	9.167E-02	.117	.715	-.1880	.3713
LSD	1.00	2.00	8.333E-02	.117	.479	-.1497	.3164
		3.00	-8.3333E-03	.117	.944	-.2414	.2247
	2.00	1.00	-8.3333E-02	.117	.479	-.3164	.1497
		3.00	-9.1667E-02	.117	.437	-.3247	.1414
	3.00	1.00	8.333E-03	.117	.944	-.2247	.2414
		2.00	9.167E-02	.117	.437	-.1414	.3247

Testing Two Sample Mean (T-Test)

1. *Coscinium* sp. (2 samples, Table 15-16, 28-29)

1.1 Glandular number (GN)

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
GN	VAR00002	1.00	30	27.0453	6.0952	1.1128	24.7693	29.3213	17.64	35.27
		2.00	30	24.9887	5.7104	1.0426	22.8564	27.1210	17.64	35.27
		Total	60	26.0170	5.9468	.7677	24.4808	27.5532	17.64	35.27

T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Mean	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
GN	Equal variances assumed	.001	.982	1.349	58	.183	2.0567	1.5249	-.9958	5.1091
	Equal variances not assumed			1.349	57.755	.183	2.0567	1.5249	-.9960	5.1094

1.2 Glandular index (GI)

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
GI	VAR00002	1.00	30	2.3000	.4516	8.245E-02	2.1314	2.4686	1.57	3.08
		2.00	30	1.5160	.3254	5.942E-02	1.3945	1.6375	1.01	2.07
		Total	60	1.9080	.5555	7.171E-02	1.7645	2.0515	1.01	3.08

T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Mean	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
GI	Equal variances assumed	1.197	.278	7.715	58	.000	.7840	.1016	.5806	.9874
	Equal variances not assumed			7.715	52.723	.000	.7840	.1016	.5801	.9879

1.3 Vein-islet number (VI)

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
VI	VAR00002	1.00	30	12.4250	1.3067	.2386	11.9371	12.9129	10.25	14.75
		2.00	30	14.1250	1.2658	.2311	13.6523	14.5977	12.25	16.75
		Total	60	13.2750	1.5368	.1984	12.8780	13.6720	10.25	16.75

T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Mean	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
VI	Equal variances assumed	.315	.577	-5.118	58	.000	-1.7000	.3322	-2.3649	-1.0351
	Equal variances not assumed			-5.118	57.941	.000	-1.7000	.3322	-2.3649	-1.0351

1.4 Veinlet termination number (VT)

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
VT	VAR00002	1.00	30	9.7000	1.1733	.2142	9.2619	10.1381	8.25	13.25
		2.00	30	13.7000	1.3038	.2380	13.2131	14.1869	11.25	15.50
		Total	60	11.7000	2.3622	.3050	11.0898	12.3102	8.25	15.50

T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Mean	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
VT	Equal variances assumed	1.434	.236	-12.490	58	.000	-4.0000	.3202	-4.6410	-3.3590
	Equal variances not assumed			-12.490	57.367	.000	-4.0000	.3202	-4.6412	-3.3588

1.5 Palisade ratio (PR)

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
PC	VAR00002	1.00	30	11.5167	1.5042	.2746	10.9550	12.0783	8.25	14.50
		2.00	30	11.5417	1.0968	.2002	11.1321	11.9512	10.00	13.75
		Total	60	11.5292	1.3052	.1685	11.1920	11.8663	8.25	14.50

T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Mean	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
PC	Equal variances assumed	2.753	.102	-.074	58	.942	-2.5000E-02	.3399	-.7053	.6553
	Equal variances not assumed			-.074	53.041	.942	-2.5000E-02	.3399	-.7067	.6567

2. *Combretum latifolium* Blume (2 sample, Table 20-21, 33-34)

2.1 Stomatal number (SN)

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
SN	VAR00002	1.00	30	358.3190	51.1830	9.3447	339.2069	377.4311	291.01	485.01
		2.00	30	392.3383	39.1126	7.1410	377.7334	406.9432	335.10	458.55
		Total	60	375.3287	48.3096	6.2367	362.8490	387.8084	291.01	485.01

T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Mean	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SN	Equal variances assumed	1.685	.199	-2.893	58	.005	-34.0193	11.7608	-57.5612	-10.4775
	Equal variances not assumed			-2.893	54.257	.005	-34.0193	11.7608	-57.5958	-10.4429

2.2 Stomatal index (SI)

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
SI	VAR00002	1.00	30	12.9200	.7716	.1409	12.6319	13.2081	11.96	14.71
		2.00	30	14.8860	.9986	.1823	14.5131	15.2589	13.49	16.67
		Total	60	13.9030	1.3287	.1715	13.5598	14.2462	11.96	16.67

T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Mean	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SI	Equal variances assumed	2.965	.090	-8.533	58	.000	-1.9660	.2304	-2.4272	-1.5048
	Equal variances not assumed			-8.533	54.528	.000	-1.9660	.2304	-2.4278	-1.5042

2.3 Vein-islet number (VI2)

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
VI2	VAR00002	1.00	30	3.8320	.8342	.1523	3.5205	4.1435	2.50	5.75
		2.00	30	3.7000	.5102	9.316E-02	3.5095	3.8905	2.75	5.25
		Total	60	3.7660	.6888	8.892E-02	3.5881	3.9439	2.50	5.75

T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Mean	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
VI2	Equal variances assumed	9.258	.004	.739	58	.463	.1320	.1785	-.2254	.4894
	Equal variances not assumed			.739	48.035	.463	.1320	.1785	-.2270	.4910

2.4 Veinlet termination number (VT2)

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
VT2	VAR00002	1.00	30	1.9667	.5282	9.644E-02	1.7694	2.1639	1.00	3.00
		2.00	30	2.1000	.6145	.1122	1.8705	2.3295	1.00	3.00
		Total	60	2.0333	.5721	7.385E-02	1.8856	2.1811	1.00	3.00

T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Mean	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
VT2	Equal variances assumed	.495	.485	-.901	58	.371	-.1333	.1479	-.4295	.1628
	Equal variances not assumed			-.901	56.722	.371	-.1333	.1479	-.4296	.1629

2.5 Palisade ratio (PR2)

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
							Lower Bound	Upper Bound		
PC2	VAR00002	1.00	30	15.0000	1.4870	.2715	14.4447	15.5553	11.50	17.50
		2.00	30	15.2247	1.6021	.2925	14.6264	15.8229	12.75	17.75
		Total	60	15.1123	1.5367	.1984	14.7154	15.5093	11.50	17.75

T-Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Mean	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
PC2	Equal variances assumed	.562	.457	-.563	58	.576	-.2247	.3991	-1.0235	.5742
	Equal variances not assumed			-.563	57.680	.576	-.2247	.3991	-1.0236	.5743

VITA

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