CHAPTER I

GENERAL BACKGROUND

1. INTRODUCTION

Khamin Khruea is a folk medicine in Thailand. Leaves are used for emmenagogue. Flowers are used to treat dysentery. Stem has been used to treat orchitis, lymphatic system malfunction and eyes disease. The wood is used to treat fever. In Malaya the plant is used to dress wounds and ulcers, and also as an ingredient in arrow poisons (Forman, 1991).

HAMM is a Khamin Khruea. It is mostly found in North-eastern of Thailand (Nong Khai province, Ubon Ratchathani province). HAMM has been used in a wide variety of diseases. Stem has been used to treat hepatitis, diabetes mellitus, some cancer, decrease cholesterol in blood and hypotension.

The aim of this study was to investigate

1. Isolation of hypoglycemic constituents of water extract from HAMM.

2. Illustration of structure and chemical property of constituents from HAMM with hypoglycemic activity in rat.

2. LITERATURE REVIEW

The name 'Khamin Khruea' has been used for several plants, i.e.; *Arcangelisia flava* (L.) Merr., *Coscinium fenestratum* (Gaertn.) Colebr., *Fibraurea tinctoria* Lour. of Family Menispermaceae and *Combretum latifolium* Blume (*C. extensum* Roxb.) of Family Combretaceae.

2.1 The species of Khamin Khruea found in Thailand

2.1.1 ARCANGELISIA (Forman, 1991)

Lianas. 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 flower sessile or subsessile. Sepals 9-10, glabrous; the outermose, 3-4 minute; larger inner sepals, 3+3. Petal 0. Synandrium a sessile, globose cluster of 9-12 anthers. Female flower : Sepals as in male. Petals 0. Staminodes present. Carpels 3, stigma broad. Infructescences with clubshaped, unbranched carpophores. Drupestransversely subovoid, or subglobose with style-scar lateral, large; endocarp not sculptured but bearing a layer of radially arranged fibres; condyle inconspicuous or absent. Seeds broadly ellipsoidal; endosperm deeply ruminate; cotyledons divergent and much folded.

Two species : one in Hainan, Indochina Peninsular, Thailand, Malaya to New Guinea; the other only in New Guinea.

2.1.1.1 *Arcangelisia flava*

Plant glabrous apart from leaf-domatia; stems with yellow wood

and exuding yellow sap when cut, bearing prominent cup-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 slighly 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-)7-159-20) 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, ca 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; stigma 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.

Distribution : Thailand, Hainan, Indochina, Malaya to New Guinea

Ecology : In evergreen forests; at low altitude.

Thai name : Khamin Khruea (South-eastern), Khamin Ruesi, Hap (Peninsular).

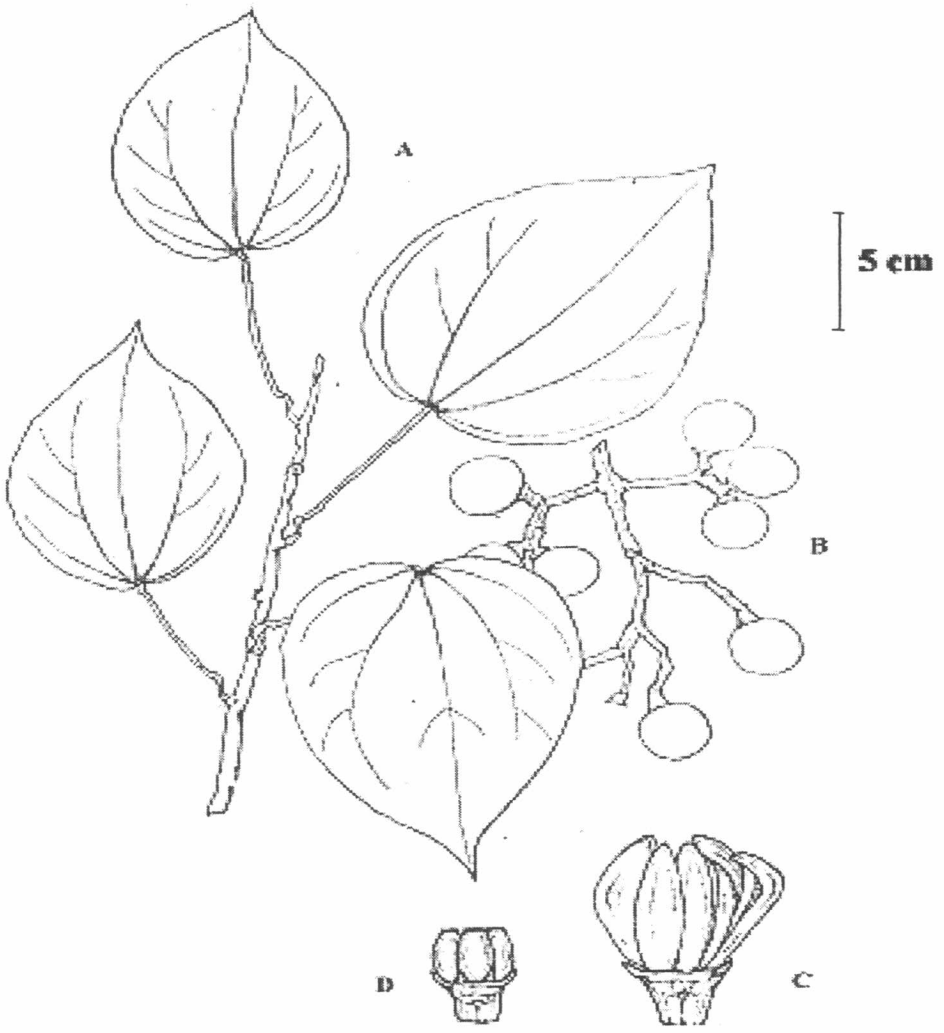

Figure 1. ***Arcangelisia flava*** **(L.) Merr. (** ถนอมหวัง**, 2517)**

A. Leaves B. Fruits

C. Female flower D. Ovaries

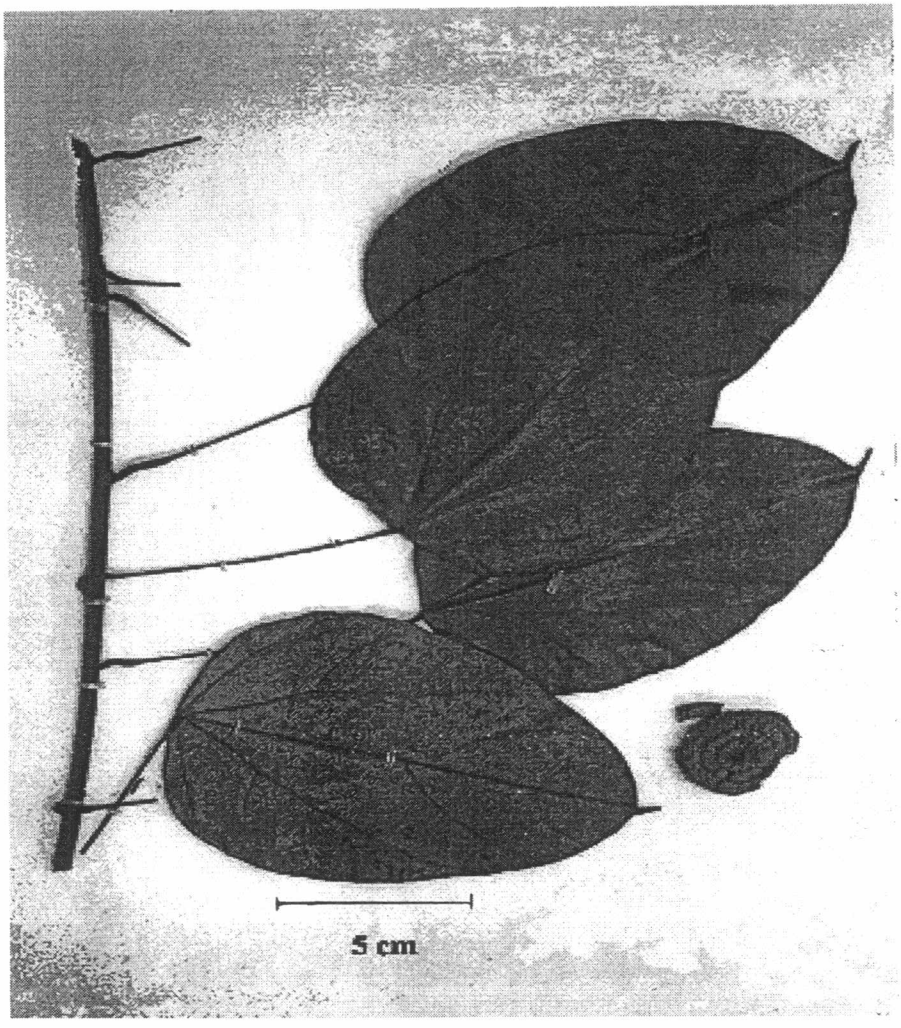

Figure 2. Leaves of *Arcangelisia flava* (L.) Merr.

Locality : Khao chamao-khao wonge National Park, Rayong province.

Collector : Mr. Vilas Panthumapol

Date : November 18, 2000

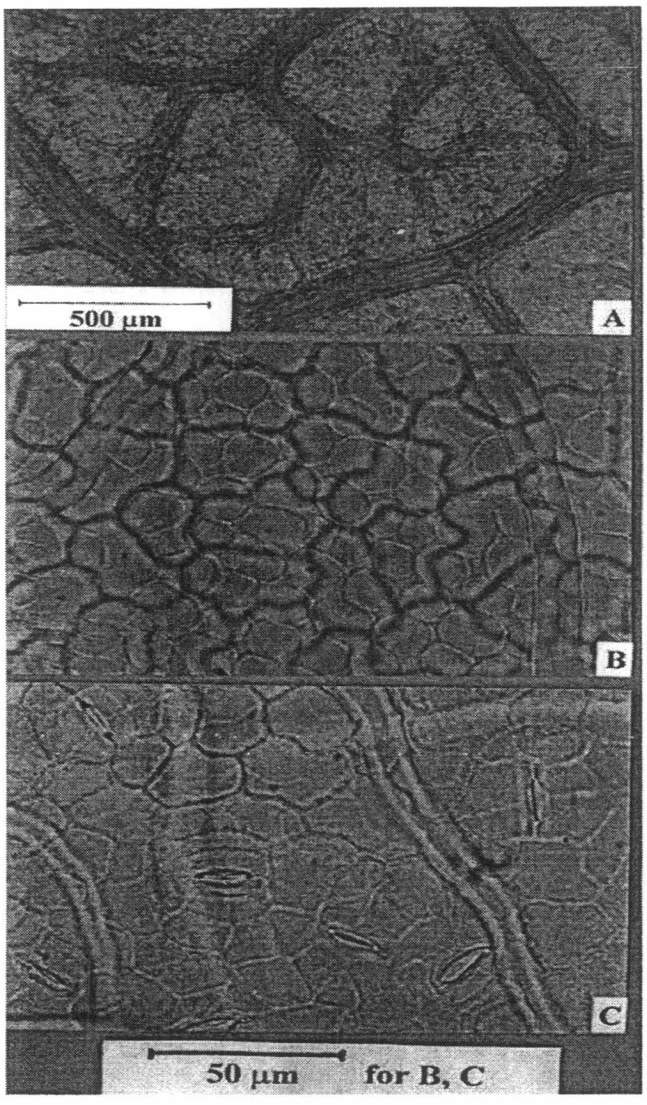

Figure 3. Microscopic illustration of *Arcangelisia flava* (L.) Merr. leaf (Rungsimakan, 2001)

A. Vein-islet and veinlet termination

B. Upper epidermis with underlying palisade cells

C. Lower epidermis with stomata

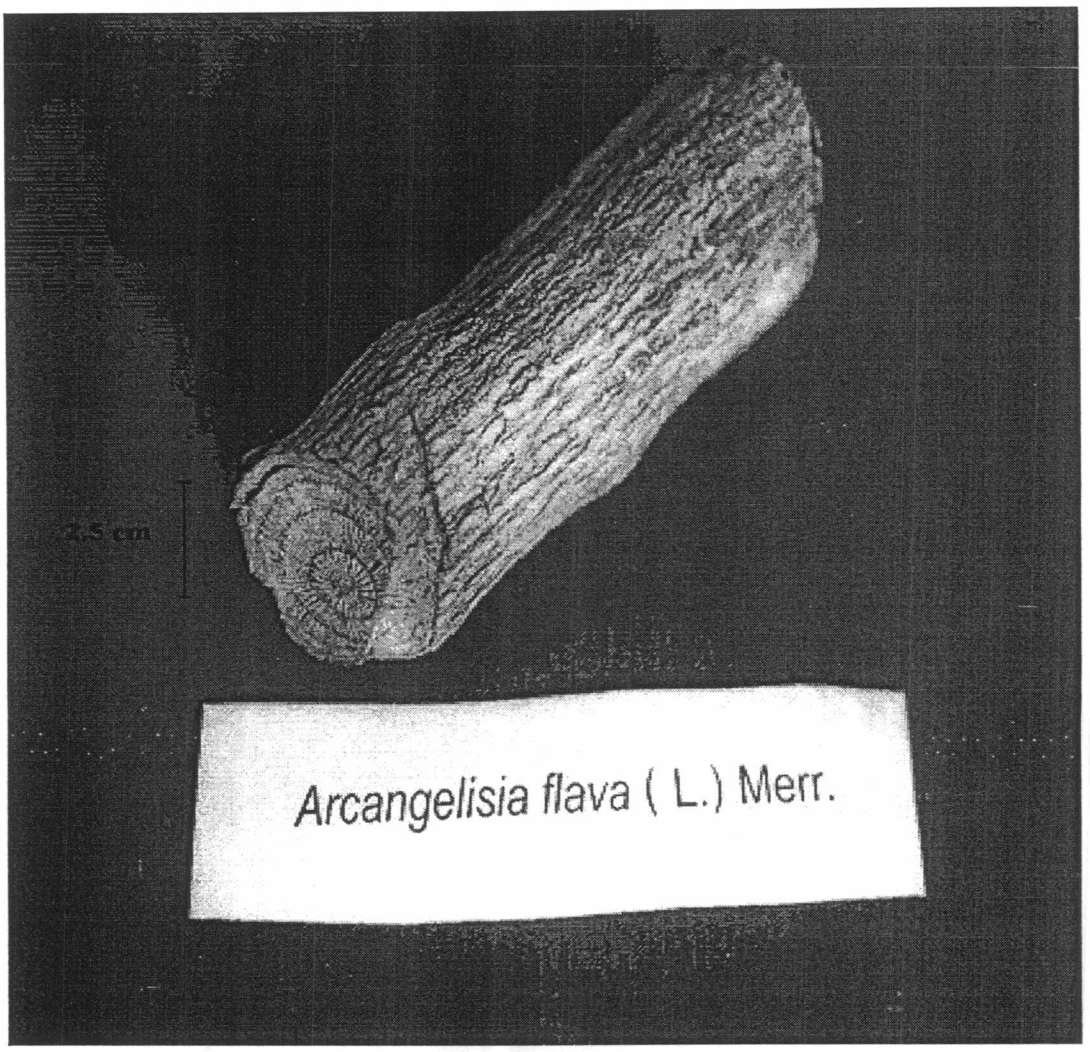

Figure 4. Stem of *Arcangelisia flava* (L.) Merr.

Locality : Khao chamao-khao wonge National Park, Rayong province.

Collector : Mr. Vilas Panthumapol

Date : November 18, 2000

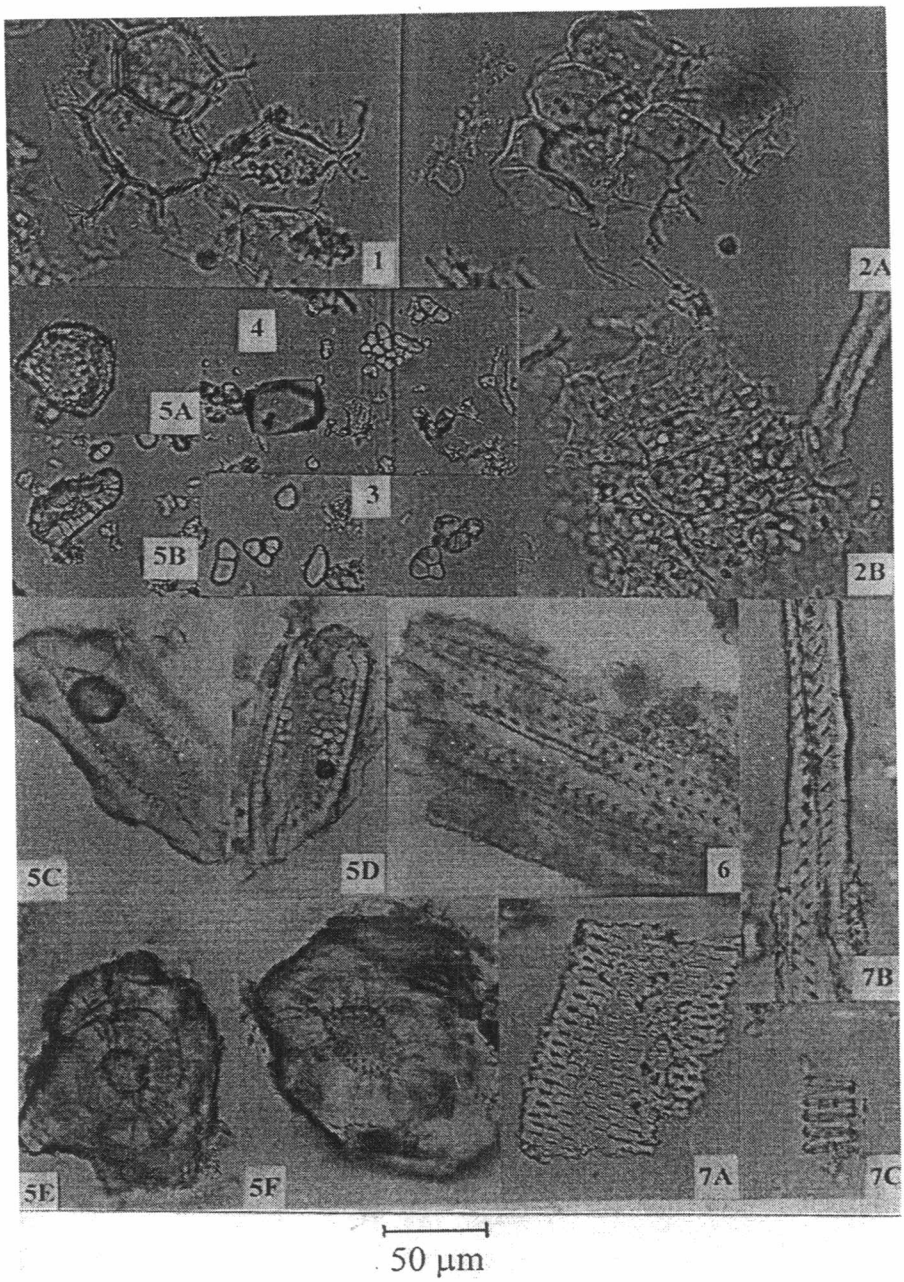

Figure 5. Microscopic characters of *Arcangelisia flava* (L.) Merr. stem (Rungsimakan, 2001)

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

2.1.2 COSCINIUM

Large liana 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 flower: 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. Petal 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 conved with anastomosing fibrous ridges; condyle deeply intrusive, thickly clavate. Seeds subglobose, hollow, enveloping the condyle; endosperm surrounding the divaricate, folded and devided cotyledons.

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

Key to the species

1. Leaves 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, ca 7 mm . 1. *C. fenestratum*

2. Leaves 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, ca 13 mm . 2. *C. blumeanum*

2.1.2.1 *Coscinium fenestratum* (Gaertn.) Colebr.

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 corriaceous; 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., onpeduncles 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 obvate; 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. Infructescences with carpophore globose, tomentellous, 7-8 mm., bearing 1-3 drupes. Drupes subglobose, tomentellous, brown to orange or yellowish, 2.8-3 cm.; pericarp drying woody, ca 1 mm. thick; endocarp bony, 2.2-2.5 cm. ; pericarp drying woody, ca 1 mm. thick; endocarp bony, 2.2-2.5 cm., wall 3 mm. thick covered with anastomosing fibrous ridges; condyle deeply intrusive, thickly clavate. Seeds whitish, subglobose, enveloping the condyle.

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

Ecology : In evergreen forests; at 200 m. altitude.

Thai name : Khruea Hen (North-eastern), Khamin Khruea (South-eastern).

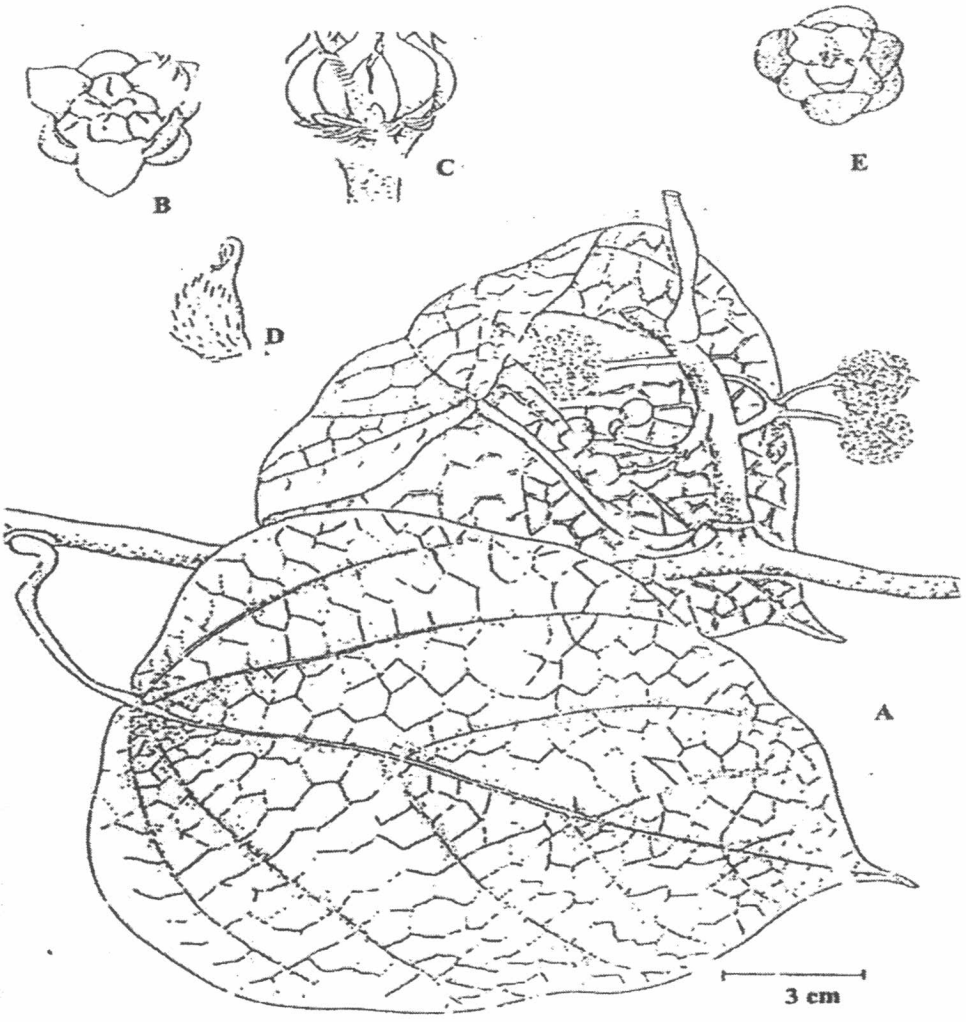

Figure 6. *Coscinium fenestratum* (Gaertn.) Colebr. (Jayaweera, 1982)

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

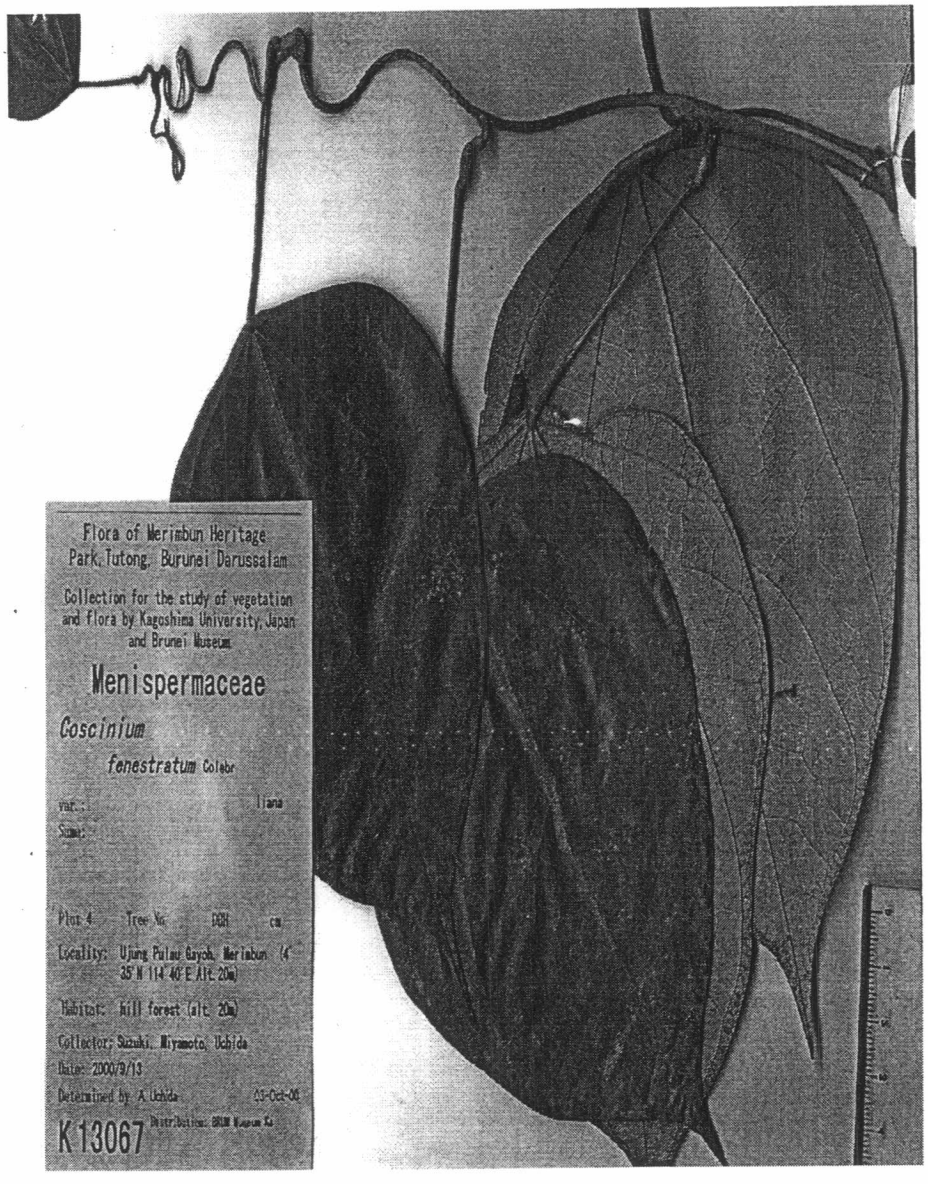

Figure 7. Leaves of *Coscinium fenestratum* (Gaertn.) Colebr.

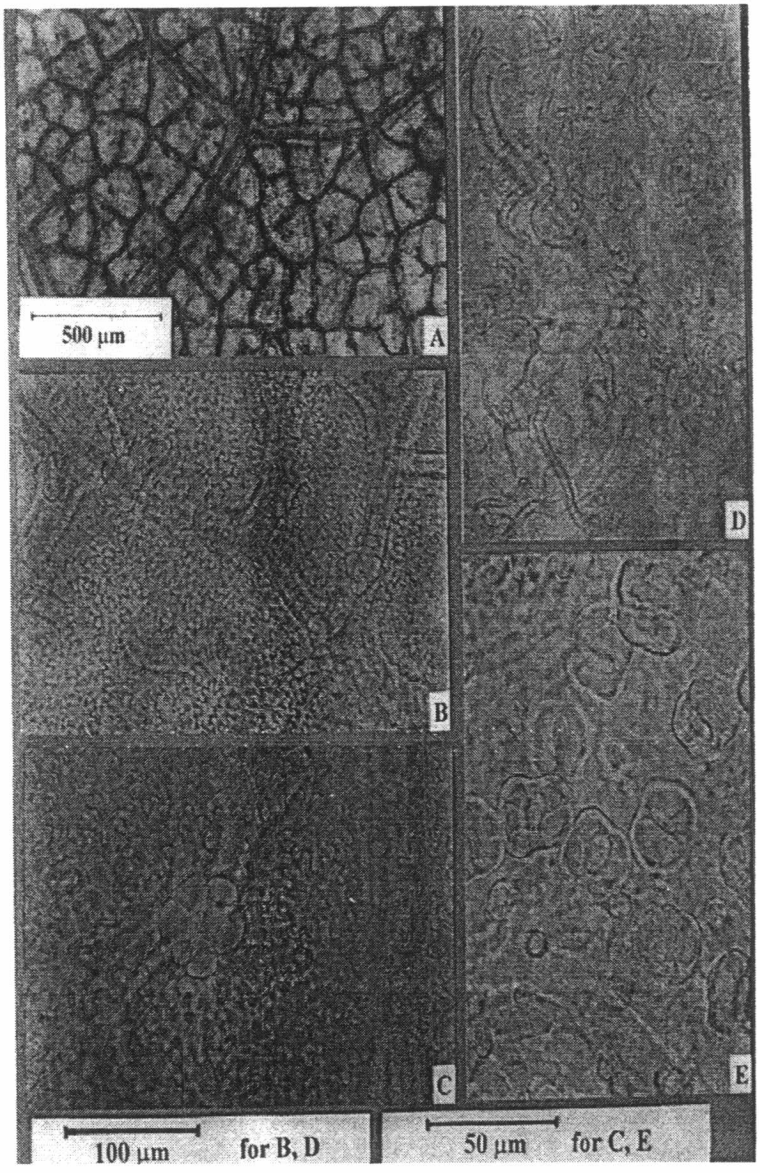

Figure 8. Microscopic illustration of *Coscinium fenestratum* (Gaertn.) Colebr. Leaf (Rungsimakan, 2001)

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

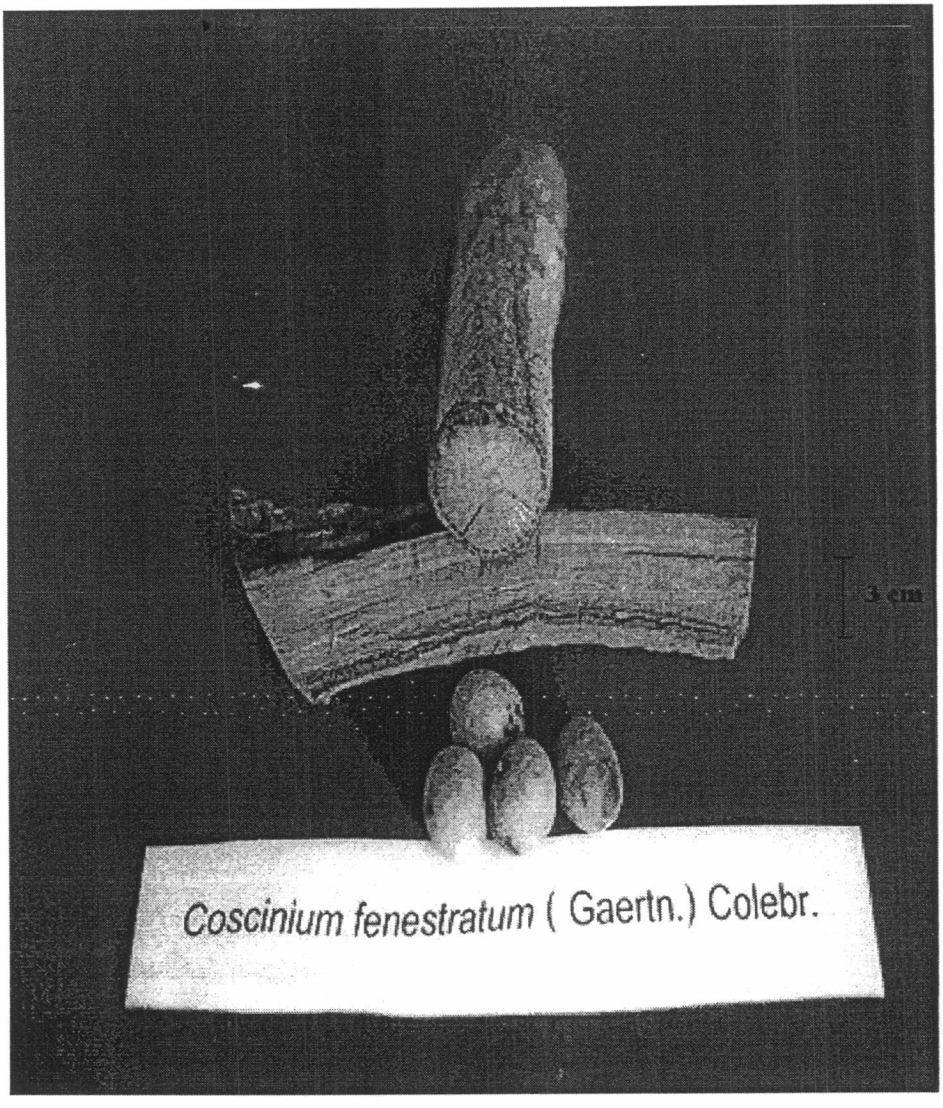

Figure 9. Stems of *Coscinium fenestratum* (Gaertn.) Colebr.

Locality : Beung karn district, Nong Khai province.

Collector : Associate Professor Rapepol Bavovada, Ph.D.

Date : August 16, 2000

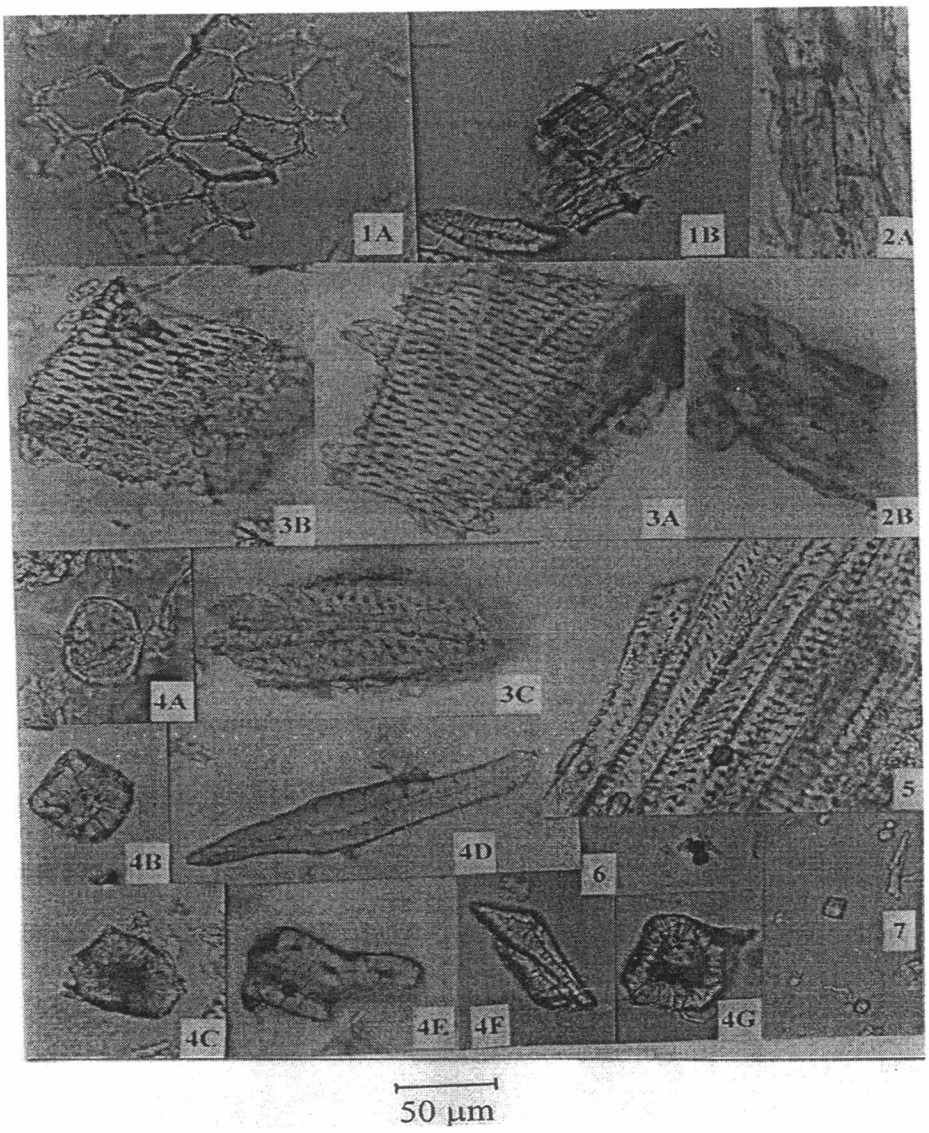

Figure 10. Microscopic characters of *Coscinium fenestratum* (Gaertn.) Colebr. stem (Rungsimakan, 2001)

1 Cork (A. surface view, B. sectional view and sclereid)

2. Parenchyma

3. Vessels

4. Sclereids

5. Fibers

6. Starch granules

7. Starch granules and a prism of calcium oxalate

2.1.2.2 *Coscinium blumeanum* Miers

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 coriceous; 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. 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-obvate, 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.

Distribution : Thailand, Malay peninsula.

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

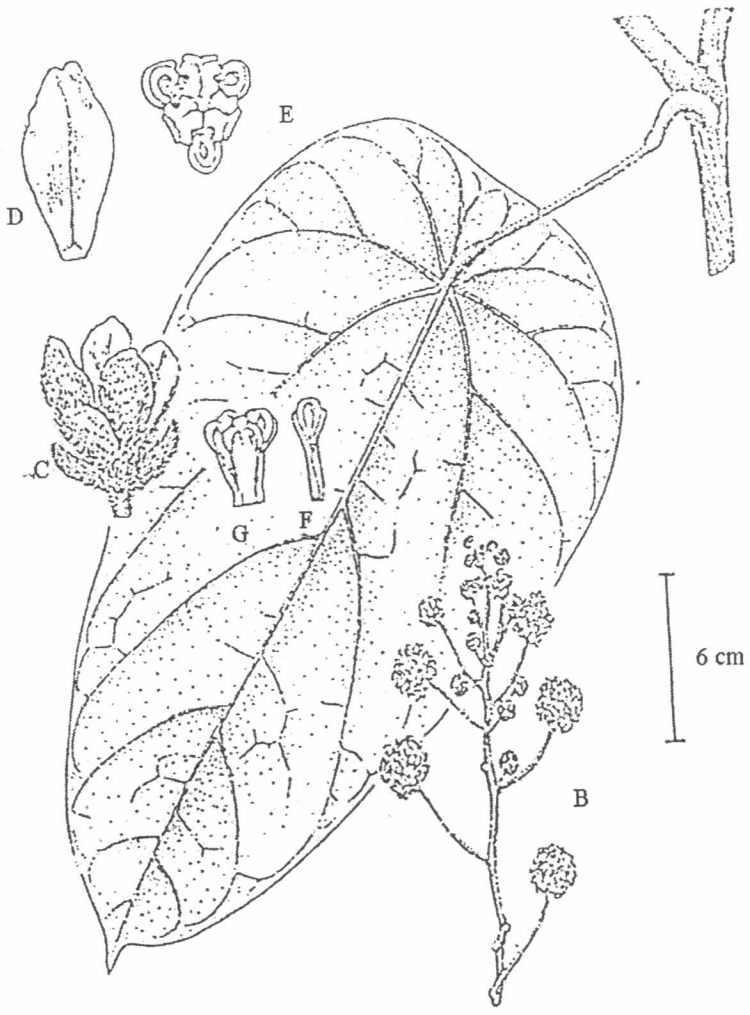

Figure 11. ***Coscinium blumeanum*** **Miers (Forman, 1991)**

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

2.1.3 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 flower : main sepals 6, with 2-3 minute outer ones. Petals 0. Stamens 3 or 6; the filament thick with a prominent collar around the base of the anthers, dehiscence longitudinal to oblique. Female 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.

A genus of two species : one widespread from the Nicobar, Burma, Peninsular Thailand, Indochina, South China, and from Sumatra and Malaya to Celebes and Philippines; the other only in South China, Vietnam and Cambodia.

Key to the species

1. Stamens 3. Wall of endocarp very thin, firmly crustaceous, less than 0.5 mm. thick (S. China, Vietnam and Cambodia) 1. *F. recisa* Pierre

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

2.1.3.1 *Fibraurea tinctoria* Lour.

Large woody climber, up to ca 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, ca 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 columna, incurved. Female flower : 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 ca 55 cm. Drupes yellow to orange on pedicels, 6-15 mm.; endocarp 2-2.5 cm. long, wall ca 1 mm. thick, hard and rigid.

Distribution : North-East India (Manipur, Nicobar IS.) Burma to Indochina, Malay Peninsula and Sumatra to Celebes and Philippines.

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

Thai name : Khamin Ruesi, Khamin Khruea, Man Miat (Peninsular; Thaowan Thong (South-western); Kamphaeng Chet Chan (Central)

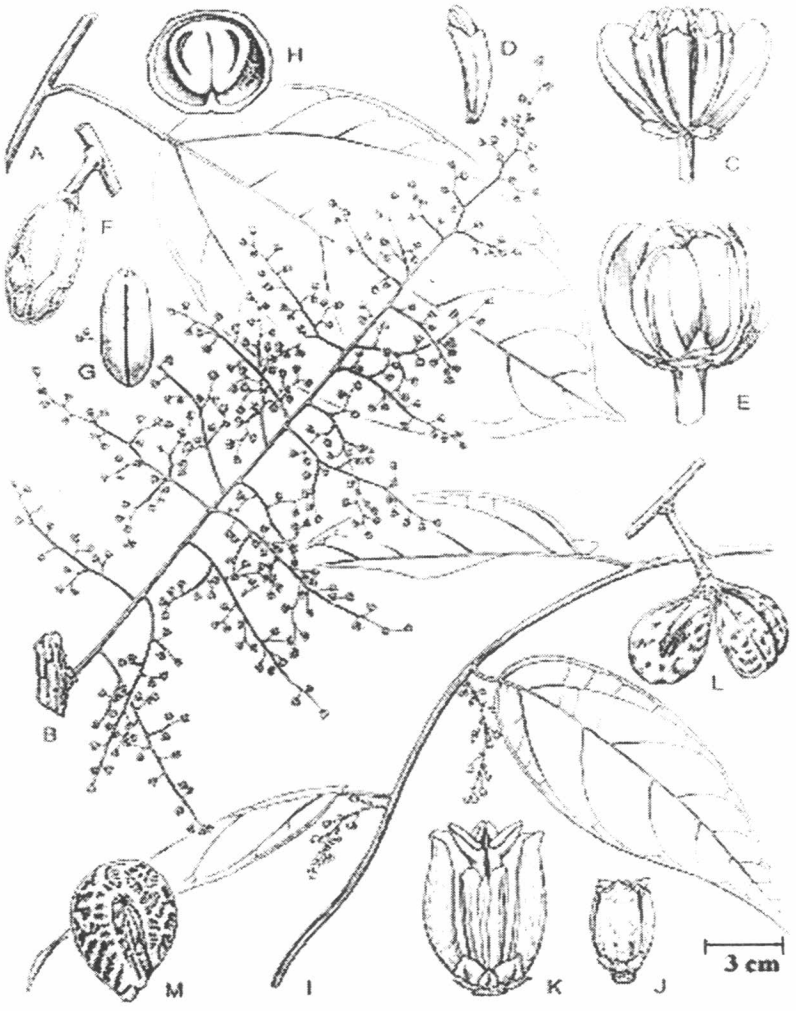

Figure 12. ***Fibraurea tinctoria*** **Lour. (Forman, 1991)**

A. Leaf	B. Male inflorescence
C. Male flower	D. Stamen
E. Female flower	F. Drupe
G. Endocarp	H. Endocarp (transverse section)

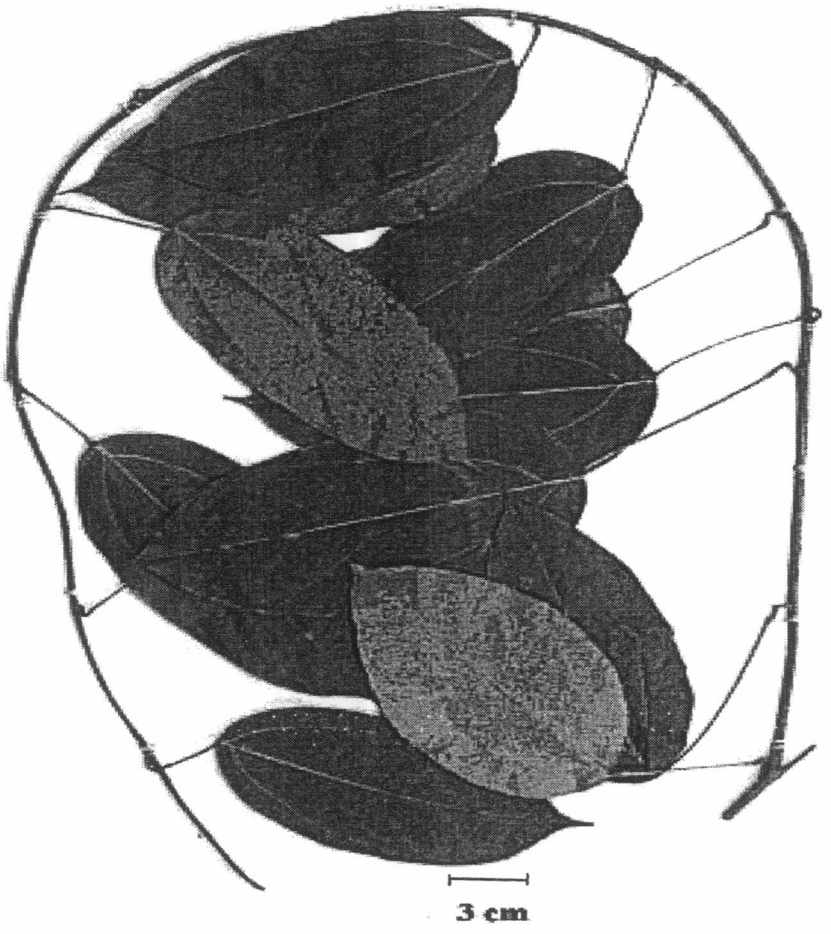

Figure 13. Leaves of *Fibraurea tinctoria* Lour.

Locality : Faculty of Pharmacy, Prince of Songkla University, Songkla province.

Collector : Niwat Keawpradub, Ph.D.

Date : January 15, 2001.

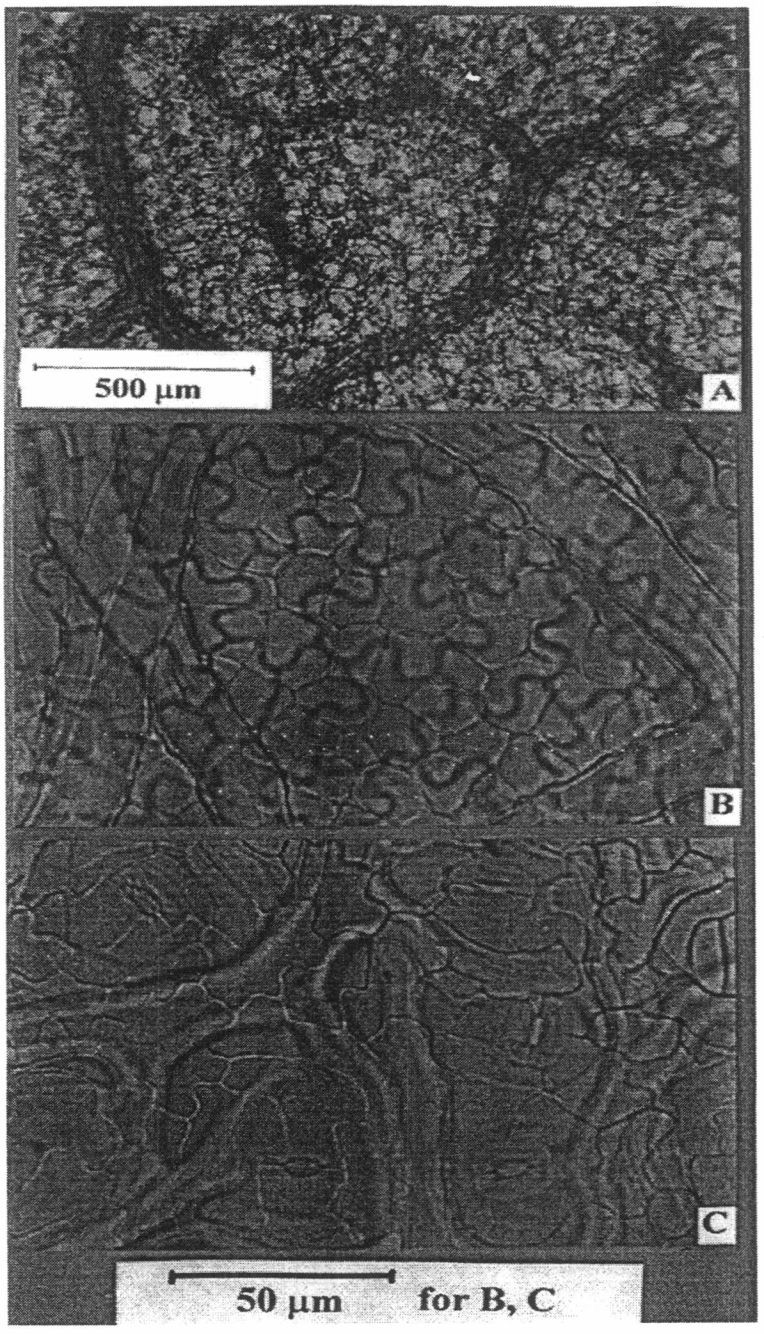

Figure 14. Microscopic illustration of *Fibraurea tinctoria* Lour. leaf (Rungsimakan, 2001)

A. Vein-islet and veinlet termination

B. Upper epidermis with underlying palisade cells

C. Lower epidermis with stomata

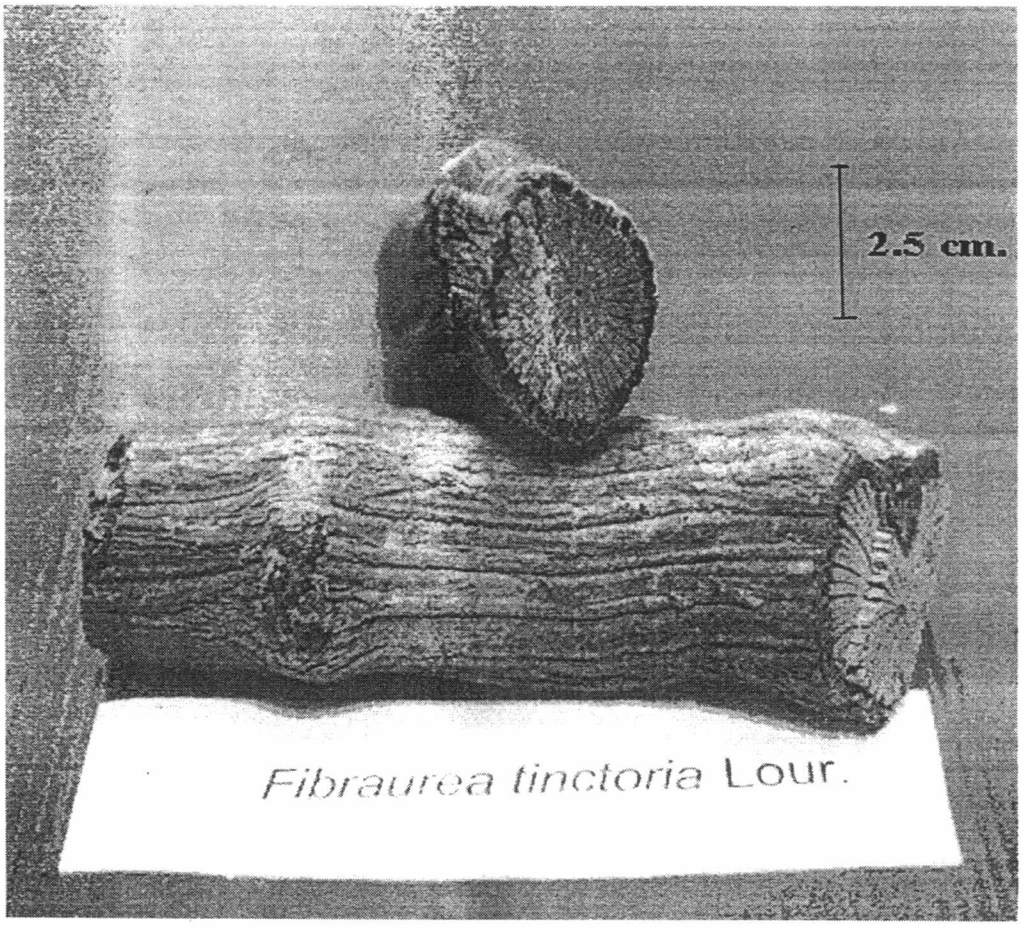

Figure 15. Stem of *Fibraurea tinctoria* Lour.

Locality : Faculty of Pharmacy, Prince of Songkla University, Songkla province.

Collector : Niwat Keawpradub, Ph.D.

Date : January 15, 2001.

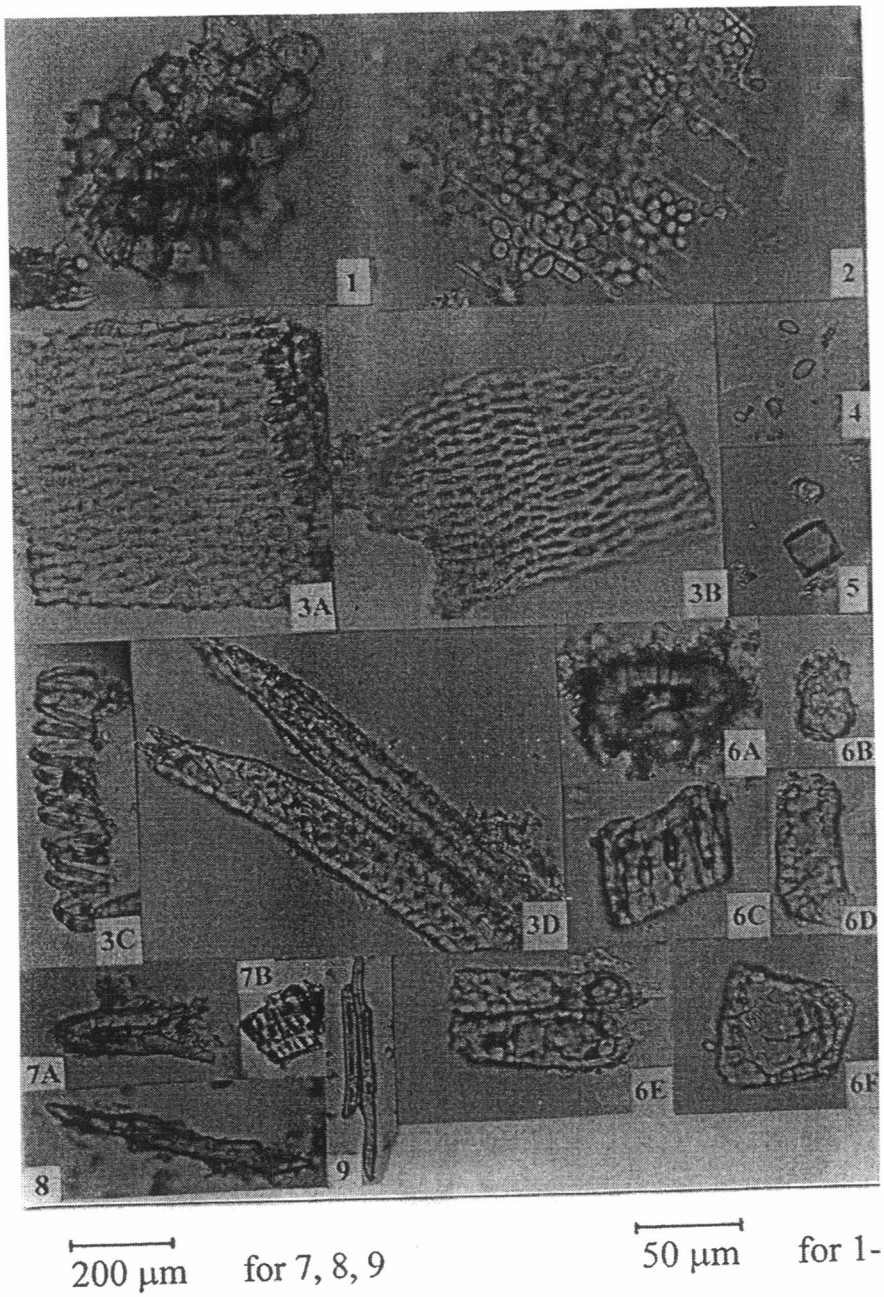

Figure 16. Microscopic characters of *Fibraurea tinctoria* Lour. stem

(Rungsimakan, 2001)

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

2.2 The chemical constituents of Khamin Khruea.

Constituents of *Coscinium fenestratum* Colebrooke. Dried stems *of Coscinium fenestratum* (Gaertn.) Colebr. were collected in Thailand, South-Kalimantan, Indonesia, of which berberine is the main alkaloidal constituent. Smaller amounts are the quaternary alkaloids : jatrorrhizine, palmatine, berberrubine, thalifendine, N, N-dimethyllindcarpine (Siwon et al., 1980; Garcia, 1970), oxyberberine, oxypalmatine, (-)-8-oxocanadine, (-)-8-oxotetrahydrothalifendin, (-)-oxoisocorypalmine and (-)-8-oxothaicanine or (-)-8-oxo-3-hydroxy-2, 4, 9, 10-tetramethoxyberbine, with oxypalmatine, (-)-8-oxotetrahydrothalifendine, (-)-oxoisocorypalmine (Pinho et al, 1992), 12, 13-dihydro-8-oxo-berberine, berberine, oxyberberine (berlambine), tetrahydroberberine (canadine), tetrahydropalmatine; aporphine alkaloids : crebanine (Keawpradub, 1992); steroid : sitosterol and stigmasterol (Malhotra et al., 1989).

Coscinium blumeanum (Wall.) Miers. alkaloids : palmatine, berberine and jatrorrhizine (Thornber, 1970).

Arcangelisia flava (L.) Merr. alkaloids : berberine, palmatine, columbamine, jatrorrhizine (Thornber, 1970), thalifendine, dehydrocorydalmine, pycnarrhine and three tertiary alkaloids; hydroxy-berberine, limacine and homoaromoline (Verpoorte et al., 1981)

Fibraurea tinctiria Lour. alkaloids : palmatine, jatrorrhizine (Thornber, 1970 and Boonyaprakorn, 1983), magnoflorine, pseudocolumbamine, dehydro-corydalmine, palmatrubine, berberine, berberubine (Siwon et al., 1981); furanoditerpenes : fibleucin , fibraurin, chasmanthin and palmarin (Itokawa et al., 1986 and Zakaria et al., 1989); furanoditerpene glucosides : tinophylloloside, fibleucinoside and fibraurinoside (Itokawa et al., 1986).

2.3 Pharmacological effect and toxicity of Khamin Khruea

Biological activities of *Arcangelisia flava* extracts are attributable to its berberine content which is claimed to be the principal alkaloid contained in the plant. The finding did not sattle the issue, but the following conclusions are made. Intravenous doses of *Arcangelisia flava* extracts in the dog caused hypotention, bradycardia and increased myocardial contractions. *Arcangelisia flava* extracts administerd intravenously to dogs caused stimulation of respiratory rate an initial increase in amplitude of respiration followed by a longer depression but no significant changes in ileal air. There was also an increase in ileal amplitude and tone. Intraarterially, *Arcangelisia flava* Merr. extracts produced paralysis of gastrocnemius-soleus contractions of the dog. No effects were seen on guinea pig non-pregnant isolated uterine strips and pregnant and non-pregnant dog uteri in situ (Estrada et al., 1963). Ethanol extract were precipitated in form of hydrochloride and recrystallized with diluted ethanol. Berberine hydrochloride has been used in the treatment of diarrhea in adult dose of 0.66 to 1.33 mg/kg. The LD_{50} of isoquinoline alkaloids of *Arcangelisia flava* Merr. which were 1.1085 and 1.1150 g/kg in female and male respectively were found to be higher than therapeutic dose of berberine hydrochloride as antidiarrhea (Utaipatana, 1987).

Effect of CCl_4 extract of *Arcangelisia flava* showed to increase pentobarbital activity, but did not have hepatotoxic effect (Chitcharoenthum, 1990).

In Thailand, biological activity of *Arcangelisia flava* Merr. has been investigated, Avirutnant et al., 1983 studied antimicrobial and anti-fungal activity of *Arcangelisia flava* Merr. Alcohol extracts were tested for their antimicrobial activity against 6 microorganisms; *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhimurium* and *Candida albicans*, by the disk

diffusion method. Alkaloid extract of *Arcangelisia flava* Merr. demonstrated inhibitory effect against *S. aureus, Sh. dysenteriae* and *C. albicans* and antifungal activity of *Arcangelisia flava* Merr. were studied by the agar dilution method against *Trichophyton rubrum, Epidermophyton floccosum* and *Microsporum gypseum*. It was found that alcohol extract showed antifungal activity against *E. floccosum* and *M. gypseum*.

Methanol and methanol-water (1:1) extracts of *C. fenestratum* exhibited antiproliferative activites in a concentration-dependent manner, where it showed selective activity against lung carcinoma and/or lung metastatic cell lines, A549, LLC and B16-BL6 (Ueda et al, 2002). A 50% ethanol extract of *C. fenestratum* stem material (AECF) has been found to possess hypotensive action in anaesthetised dogs, rats and guinea pigs in a dose-related pattern. AECF failed to exhibit any hypotensive activity when administered via cannula into the lateral cerebral ventricle (Singh et al, 1990).

2.4 Chemical structure of some constituents in Khamin Khruea.

2.4.1 Berberine ($C_{20}H_{18}O_4N^+$)

Berberine was isolated from *Xanthoxylum clava* Herculis under the name of Xanthopicrit and obtained independently from *Berberis vulgalis*. The base occurs in several plants including those of the Ranunculaceae (*Coptis japonica, C. trifolia, C. occidentalis* and *Thalictrum foliosum*), the Berberidaceae (*Berberis buxifolia* Lam., *B. darwinii* Hook, *B. glauca* DC., *B. nervosa*, *Mahonia aquifolium* Nutt., *M. trifoliate* Fedde and *Nandina domestica* Thunb.), the Anonaceae (*Coelocline polycarpa* DC), the Menispermaceae (*Arcangelisia flava* (L.) Merr., *Coscinium*

blumeanum Miers, *C. fenestratum* (Gaertn.)Colebr.), the Papaveraceae (*Argemon mexicana* L., *Chelidonium majus* L., *Corydalis cheilantheifolia* Hemsl., *C. ophiocarpa* Hook.) and the Rutaceae (*Evodia meliifolia* Benth, *Phellodendron amurense* Rupr., *Toddalia aculeate* Pers. and *Zanthoxylum caribaeum* Lam.).

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

When berberine is the chief alkaloid in a plant extract, its isolation is conveniently effected by making use of the sparing solubility of its sulfate in dilute sulfuric acid. The hydrochloride, hydroiodide and nitrate are easily recrystallized from water or precipitated from acetic acid solutions on the addition of the appropriate ions in the form of salts.

Since canadine is easily oxidized by atmospheric oxygen to berberine there is little doubt that berberine is a constituent of all plants which contain canadine However, there are many occurrences of berberine unaccompanied by canadine, particularly in plants of the Berberidaceae and Menispermaceae, and it would seem that there is present in these plants a specific oxidative system which converts the presumably intermediate tetrahydro bases into the quaternary compounds.

Many of the older records of the occurrence of berberine are of doubtful authenticity because the characterization was based largely upon color reactions. It is now known that all of the dehydro compounds behave in essentially

the same way, and the only certain method of identifying berberine is by reduction to the tetrahydro base and proper characterization of this (Manske, 1954).

The UV spectrum made in ethanol showed maxima at 350, 264 and 228 nm under neutral conditions. Under basic conditions the maxima were at 354, 278 nm, whereas under acid conditions the maxima were at 350, 264 and 228 nm.

The ^{1}H-NMR spectra were made in CD_3OD at 100 MHz with TMS as internal standard. The ^{1}H-NMR showed characteristic signals at (δ in p.p.m.) : 8.68 (s, H_{13}), 8.11 (d, H_{11}, $J_{11\text{-}12}$= 8.9 Hz), 7.99 (d, H_{12}, $J_{11\text{-}12}$= 8.9 Hz), 7.66 (s, H_1), 6.94 (s, H_1), 6.12 (s, $C_{2\text{-}3}$)O-CH_2-O, 4.23 (s, C_9)OCH_3, 4.12 (s, C_{10})OCH_3 (Siwon et al, 1980).

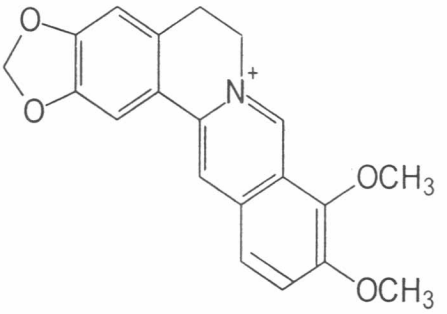

Figure 17. Structure of Berberine

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

Palmatine occurs frequently in the Rhoeadales, being found in *Coptis japonica* Mak. (Ranunculaceae); *Berberis heteropoda* Schrenk; *B. vulgalis* L (Berberidaceae); *Coscinium blumeanum* Miers; *Fibrauria chloroleuca* Miers; *Jatrorrhiza palmata* (Lam.) Miers. (Mernispermaceae); and *Phellodendron amurense* Rupr. (Rutcaeae). The base is usually obtained as the iodide dihydrate forming orange-yellow needles from water, melting point 241^{0}C. Other salts that have been prepared include the chloride, green–yellow needles from water, melting point 205^{0}C; nitrate, yellow needles, from water, melting point 239^{0}C; perchlorate, melting point

point 262^{0}C; sulphate, melting point 250^{0}C; platinichloride, melting point 236^{0}C and the thiocyanate, melting point 210^{0}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).

In many of its source plants it is associated with one or two of its O-desmethyl ethers; namely, jatrorrhizine and columbamine, which form sparingly soluble iodides along with that of palmatine. The mixed iodides are digested with aqueous alkaline in which the phenolic compounds dissolve leaving the sparingly soluble palmatine iodide which may be purified by recrystallization from much boiling water.

Reduction of palmatine yields dl-tetrahydropalmatine, and this can readily be reoxidized to palmatine. It differs from berberine in having four methoxyls, the methylenedioxy group of the latter being replaced by two methoxyls in the former (Manske, 1954).

The UV spectrum made in ethanol showed maxima under neutral conditions at 338, 265 and 220 nm. Under basic conditions the maxima were at 353, 275 and 224 nm. Under acid conditions the maxima were at 340, 270 and 224 nm (Siwon et al., 1980).

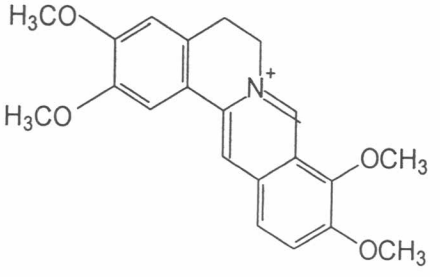

Figure 18. Structure of Palmatine

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

Jatrorrhizine is unknown in the free state and is obtained as the iodide, yellow-red needles of the monohydrate, melting point 208-210^{0}C or the chloride, yellow needles, again of the monohydrate, melting point 206^{0}C. The alkaloid occurs both in the Berberidaceae, *Berberisheteropoda*, Schrenk., *B. thunbergii* DC var. Maximowiczii, *B. vulgaris* L. and *Mahonia philippinensis* Nutt. and in the Menispermaceae, *Archangelisia flava* (L.) Merr., *Coscinium blumeanum* Miers, *Fibrauria chloroleuca* Miers., and *Jateorhiza palmata* Lam. (Miers).

A crystalline nitrate, melting point 225^{0}C (dec.) and apicrate, melting point 217-220^{0}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^{0}C, the d-form of which is corypalmatine (Glasby, 1975).

The UV spectrum showed maxima under neutral and acid conditions at 345, 264 and 225 nm. Under basic conditions the maxima were at 385 and 245 nm. The ^{1}H-NMR made in CD_3OD at 100 MHz showed characteristic signals at (δ in p.p.m.) 9.58 (s, H_8), 8.56 (s, H_{13}), 8.02 (d, H_{11}, $J_{11\text{-}12}$ = 9.2Hz), 7.88 (d, H_{12}, $J_{11\text{-}12}$ = 9.2Hz), 7.45 (s, H_1), 6.65 (s, H_4), 4.17 (s, C_9)OCH_3, 4.07 (s, C_{10})OCH_3, 3.94 (s, C_2)OCH_3 (Siwon et al., 1980).

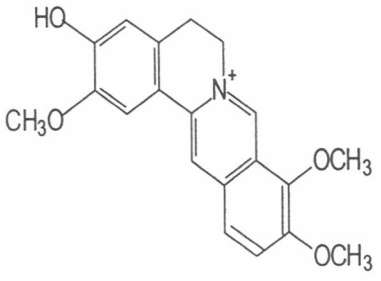

Figure 19. Structure of Jatrorrhizine

2.4.4 Columbamine ($C_{20}H_{21}O_5N^+$)

A quaternary alkaloid which has been isolated from the root bark of various species including *Archangelisia flava* (L.) Merr.; *Berberis heteropoda* Schrenk., *B. lambertii*, *B. japonica* Mak., *B. thunbergii* DC var Maximowiczii, *B. vulgalis* L.; *Coptis japonica* and *Jateorhiza palmata* Lam. (Meirs). The iodide has melting point 223-224^{0}C and is the form in which the alkaloid is normally isolated. When reduced with Zn in AcOH-H_2SO_4, the base yields the tetrahydro derivative, melting point 223^{0}C which furnishes tetrahydropalmatine with CH_2N_2 (Glasby, 1975).

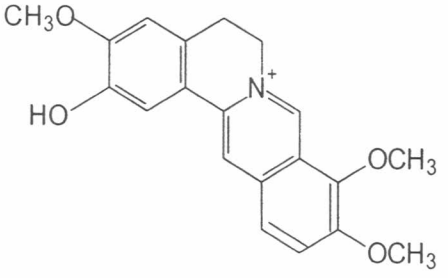

Figure 20. Structure of Columbamine

2.4.5 Dehydrocorydalmine ($C_{21}H_{22}O_4N^+$)

A quaternary alkaloid, this base is isolated from the tubers of *Stephania glabra* as the crystalline chloride. When treated with methylsulphate, this salt gives palmatine chloride (Glasby, 1975).

Yellow powder ($CHCl_3$); m.p. 85-88 ^{0}C; UV λ max/nm (EtOH) (log ε) 228 (4.74), 350 (4.10); IR ν max (neat) cm^{-1} 3500, 2925, 1610; ^{1}H- NMR ($CDCl_3$) δ/ppm 3.27 (2H, t, *J*=6.4 Hz, H_5), 3.94 (3H, s, C_3-OCH_3), 3.99 (3H, s, C_2-OCH_3), 4.15 (3H, s, C_9-OCH_3), 4.92 (2H, t, *J*=6.4 Hz, H-6), 7.05 (1H, s, H_4), 7.65 (1H, s, H_1), 7.78 (1H, d, *J*=8.8 Hz, H_{11}), 7.90 (1H, d, *J*=8.8 Hz, H_{12}), 8.73 (1H, s, H_{13}), 9.95 (1H, s, H_8); EI-MS *m/z* (%) 300 (5), 287 (5), 259 (5), 241 (4), 187 (44), 148 (29), 147 (36) (Chang, 2000).

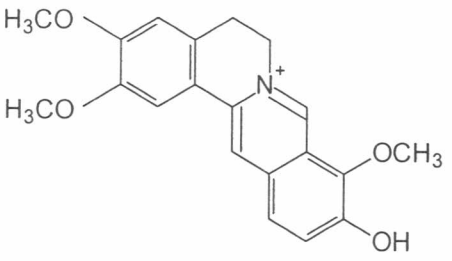

Figure 21. Structure of Dehydrocorydalmine

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

A wide variety of species contain this quaternary alkaloid which is widespread among the families, Aquilegia, Magnolia and Michelia. It has been isolated and characterized as the iodide, melting point 248-249^{0}C; $[\alpha]_D^{15}$+ 200.1^0 (MeOH). The optically inactive base also yields an iodide which crystallizes with 1.5 H_2O, melting point 243^{0}C (Glasby, 1975).

UV spectrum showed maxima at 220, 266 and 300 nm under acidic conditions and at 228, 278 and 310 nm under basic conditions. The mass spectrum showed characteristic fragments at m/z (70eV, 190^{0}C) 342 (9), 341 (36)(M^+), 327 (3), 326 (3), 310 (6), 283 (5), 59 (76) and 58 (100) (Siwon et al., 1981).

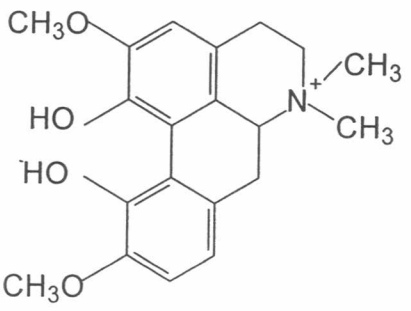

Figure 22. Structure of Magnoflorine

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

A quaternary alkaloid present in *Thalictrum fendleri* Engelm., the base is isolated as the chloride which forms yellow crystals, sintering above 230^{0}C. The ultraviolet spectrum of this salt has absorption maxima at 231, 269 and 348 mμ The structure is similar to that of thalidastine (q.v.) (Glasby, 1975).

The UV spectrum showed maxima at 390, 350, 270 and 230 nm under neutral conditions. Under basic conditions the maxima were at 380, 290, and 230 nm. The ^{1}H-NMR made in CD_3OD at 100 MHz showed characteristic signals at (δ in p.p.m.) : 9.63 (s, H_8), 8.65 (s, H_{13}), 7.89 (d, H_{11}, $J_{11\text{-}12}$ = 8.9 Hz), 7.76 (d, H_{11}, $J_{11\text{-}12}$ = 8.9 Hz), 7.64 (s, H_1), 6.95 (s, H_4), 6.10 (s, $C_{2\text{-}3}$)O-CH_2-O, 4.15 (s, C_9)OCH_3 (Siwon et al., 1980).

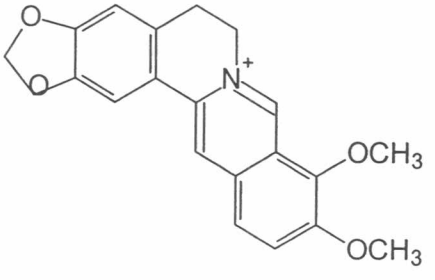

Figure 23. Structure of Thalifendine

2.4.8. Crebanine ($C_{20}H_{21}O_4N$)

This aporphine alkaloid occurs is *Stephania capitata* and *S. sasakii*, forming colourless needles with $[\alpha]_D$ ^{20}-57.50 ($CHCl_3$), melting point 126^{0}C. It contains two methoxyl, one methylene dioxy and one methylimino group in the molecule (Glasby, 1975).

It is non-phenolic and upon oxidation by permanganate afforded hemipinic acid. It was submitted to Hofmann degradation, the nitrogen-free compound oxidized, and the latter ultimately decarboxylated to yield a phenanthrene derivative (melting point 111-112^{0}C) which was stated to be identical with a synthetic specimen of 1, 2-dimethoxy-5, 6-methylene dioxyphenanthrene (Manske, 1954).

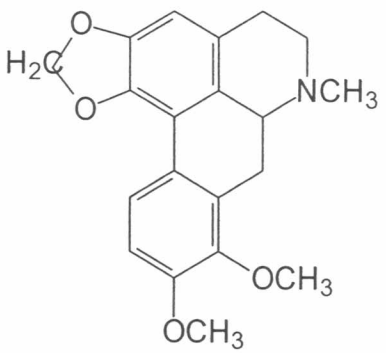

Figure 24. Structure of Crebanine

2.4.9 Homoaromaline ($C_{38}H_{42}O_6N_2$)

This bisbenzyl*isoquinoline* alkaloid occurs in the rhizomes of *Cyclea barbata*, melting point 238-240^{0}C. It forms colourless needles when crystallized from chloroform. Treatment with CH_2N_2 yields O-methoxyacanthine (Glasby, 1975).

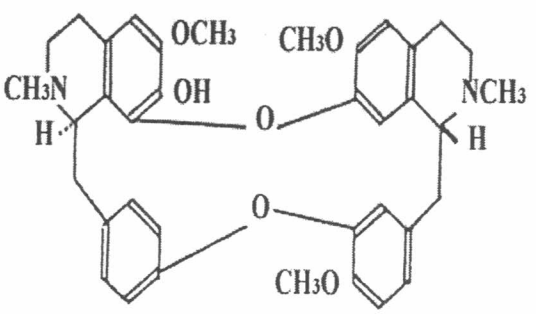

Figure 25. Structure of Homoaromaline

2.4.10 Limacine ($C_{37}H_{40}O_6N_2$)

A bisbenzyl*isoquinoline* alkaloid obtained from *Limacia cuspidate* (Miers.) Hook.f.et Thom., melting point 154-156^{0}C, the base crystallizes from acetone in the form of long, colourless needles. It is strongly laevorotatory with $[\alpha]_D$-212^0 ($CHCl_3$) and has been shown to be the optical antipode of fanchinoline (q.v.) (Glasby, 1975).

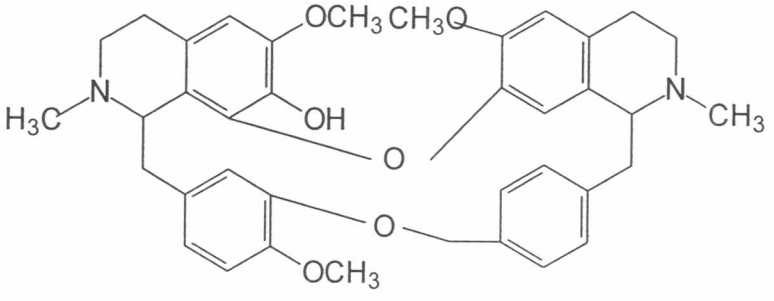

Figure 26. Structure of Limacine

2.4.11 Berberrubine

The UV spectrum showed maxima at 515, 396, 278 and 240 nm under neutraland basic conditions. Under acid conditions the maxima were at 448, 358, 275, and 234 nm (Siwon et al., 1980).

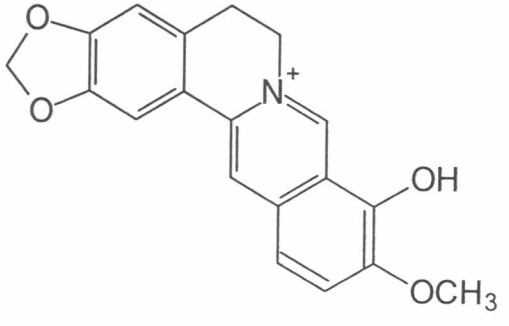

Figure 27. Structure of Berberrubine

2.4.12 (-)-Canadine

The (-) –form of canadine has been obtained from several species, e.g. *Corydalis cheilantheifolia* Hemsl., *Fagara rhoifolia* Lam., *Hydrastis canadensis* L., *Zanthoxylum brachyacanthum* F. Muell. and *Z. veneficum* F.M. Bail. The alkaloid forms silky needles with $[\alpha]_D$- $299^0(CHCl_3)$ or $-432^0(CS_2)$. It is insoluble in H_2O but readily soluble in Et_2O or $CHCl_3$. Both the hydrochloride and nitrate are crystalline, laevorotatory and only slightly soluble in H_2O. When the alkaloid is treated with mercuric acetate, it yields berberine (Glasby, 1975).

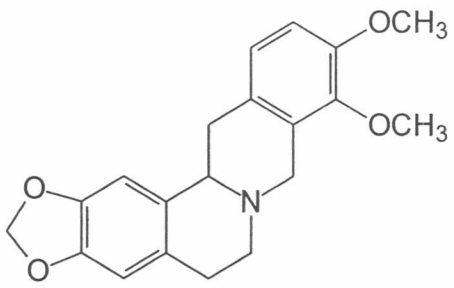

Figure 28. Structure of Canadine

2.4.13 Palmarin ($C_{20}H_{22}O_7$)

MW 374, m.p. 253-258^{0}C $[\alpha]_D$ + 17° (c 1.45, pyridine). Constituent of the roots of *Jateorhiza palmata*. Crystals from Me_2CO-AcOEt. Methyl ether, m.p. 261-263^{0}C, $[\alpha]_D$+500(c 1.71, pyridine). Structure and stereochemistry determined from IR, NMR and MS (Glasby, 1975).

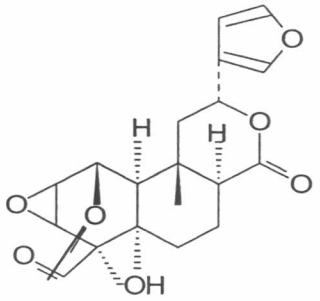

Figure 29. Structure of Palmarin

2.4.14 Chasmanthin ($C_{20}H_{22}O_7$)

MW. 374, m.p. 225-228^{0}C, $[\alpha]_D$ 0^0 (pyridine). Constituent of *Jateorhiza palmata*. Crystals from EtOH. Not obtained completely pure. Structure determined from IR, NMR and MS (Glasby, 1975).

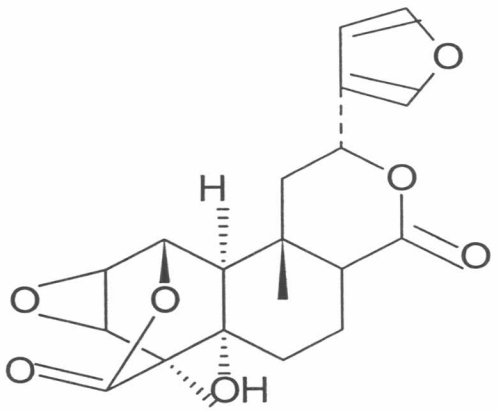

Figure 30. Structure of Chasmanthin

2.4.15 β-Sitosterol & Stigmasterol

White needles (MeOH); m.p. 138-140 ^{0}C; $[\alpha]^{24}_D$-350 (c =0.2, $CHCl_3$); IR ν max (neat) cm^{-1} 3400, 2910, 1625, 1450; ^{1}H- NMR ($CDCl_3$); δ/ppm 0.68 (3H, s, H_{18}), 0.81 (3H, d, *J*=6.8 Hz, H_{26}), 0.84 (3H, d, *J*=6.8Hz, H_{27}), 0.86 (3h, t, *J*=7.0 Hz H_{29}), 0.92 (3H, d, *J*=6.4 Hz, H_{21}), 1.01 (3H, s, H_{19}), 3.53 (1H, m, H_3), 5.02 (1H, dd, *J*=16.1, 8.3 Hz, H_{22}), 5.12 (1H, dd, *J*=16.1, 8.3 Hz, H_{23}), 5.36 (1H, br s, H_6); EI-MS m/z (%) 414 (80, M^+), 412 (30, M^+), 369 (45), 381 (27), 239 (33), 303 (33), 273 (23), 255 (33), 213 (29), 159 (25), 145 (29) (Chang, 2000).

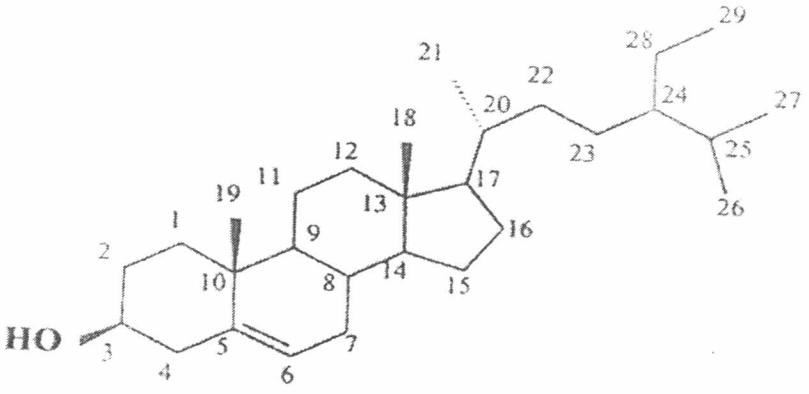

Figure 31. Structure of β- Sitosterol

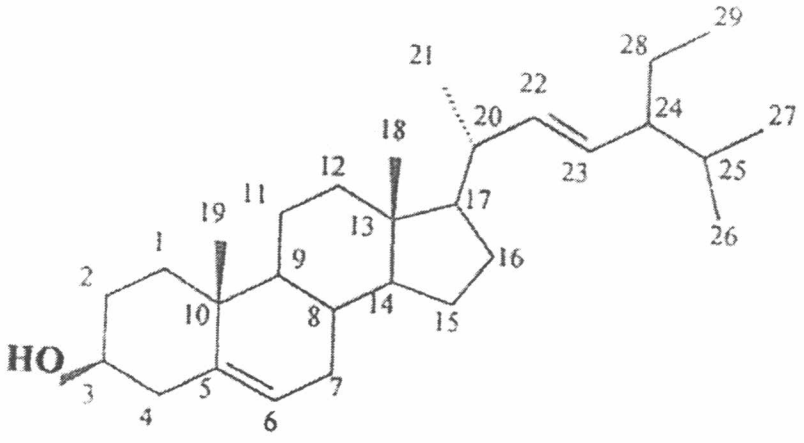

Figure 32. Structure of Stigmasterol

2.5 Pharmacology and toxicology of some constituents in Khamin Khruea.

Berberine is moderately toxic to larger animals causing cardiac damage, dyspnoea, lowered blood pressure and parasis in rabbits. In man large amounts are present in the urine after oral administration. Its main use in Western medicine is as a bitter tonic and stomachic. It has some trypanocidal action and had been used as an adjunct to quinine in the treatment of malaria. The sulphate, in concentrations of 1-3 mg per ml decreases the anticoagulant action of heparin in dog and human blood in vitro (Glasby, 1975). Berberine, extracted from *Arcangelisia flava* (L.) Merr. inhibited telomerase activity in a dose-dependent manner over a range of 30-300 mM indicating that *Plasmodium falciparum* telomerase (Sriwilaijareon et al.,2002). Berberine and palmatine are toxic to insects and vertebrates (Schmeller et al., 1997).

Berberine inhibited the biosyntheses of DNA, RNA, proteins and lipids, as well as the oxidation of glucose [^{14}C] to $^{14}CO_2$, when incubated with S 180 cells *in vitro* . The synthesis of proteins and of RNA was the most sensitive of these parameters to the action of berberine inhibition by berberine of the biosynthesis of macromolecules may reflect such primary actions as inhibition of glucose utilization and interaction with nucleic acid (Creasey, 1979). Berberine sulfate was an effective and safe antisecretory drug for enterotoxigenic *Escherichia coli* diarrhea, whereas the activity against cholera is slight and not additive with tetracycline (Rabbani, 1987).

The effect of protoberberine alkaloids from *Coptis japonica* Makino (COPT) on catecholamine content and tyrosine hydroxylase (TH) activity in PC 12 cells were investigated. The butanol fraction from COPT at a concentration of 40 μg/ml

medium inhibited catecholamine biosynthesis (Lee, 1996). Protoberberine alkaloids such as palmatine, 13-methylpalmatine iodide, 2, 3-methylenedioxy-10, 11 dimethoxy-13-methylprotoberberineiodide, 2, 3-methylenedioxy-9, 10-dimethoxy-13-methylprotoberberine chloride and berberine showed inhibition of reverse transcriptase activity of RNA tumor viruses. These results indicated that the alkaloids caused inhibition of the enzyme activity by interacting with the template primer, particularly of the adenine-thymine base pair (Sethi, 1983). Sethi (1985) studied comparison of inhibition of reverse transcriptase and antileukemic activity exhibited by protoberberine and benzophenanthridine alkaloids and structure-activity relationships. The inhibition of nucleic acid polymerases from calf thymus and bacteria and structure-activity relationships were also presented. The alkaloids with the benzophenanthridine ring system were found to display potent inhibition of reverse transcriptase and antileukemic activities.

2.6 Hypoglycemic effect of some plants

In previous years, several plant extract and folk medicine have been examined for hypoglycemic activity in many countries. In 1991, Perfumi and et al. studied hypoglycemic activity of *Salvia fruticosa* Mill. from Cyprus. These data strongly suggest that *S. fruticosa* treatment produces hypoglycemia mainly by reducing intestinal absorption of glucose. In Iraq, oral administration of 0.39 g/kg body weight of the aqueous extract of the leaves or barks produced a significant reduction in blood glucose level (Al-khazraji et al., 1993).

In 1996, El-Fiky et al. studied effect of ethanolic extracts of *Luffa aegyptiaca* (seeds) and *Carissa edulis* (leaves) on blood glucose. Treatment with both extracts

significantly reduced the blood glucose level in streptozotocin-diabetic rats during the first three hours of treatment. On the other hand, in normal rats, both treatments produced insignificant changes in blood glucose levels compared to glibenclamide treatment.

In 1997, Abdel-Barry et al studied hypoglycemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan-induced diabetic rats. These results suggest that the aqueous extract of *Trigonella foenum-graecum* leaves given both orally and intraperitoneally possesses a hypoglycemic effect in normoglycemic and alloxan-induced hyperglycemic rats.

Momordica cymbalaria at a dosage of 0.5 g/kg body weight is showing maximal blood glucose lowering effect in diabetic rats. The same dosage did not produce any hypoglycemic activity in normal rats (Rao, 2001). *Enicostemma littorale* Blume has been reported on its blood glucose lowering potential in alloxan-induced diabetic rats. A single dose of aqueous extract of *E. littorale* (15 g dry plant equivalent extract/ kg) had shown significant increase in the serum insulin levels in alloxan-induced diabetic rats at 8 hours (Maroo et al, 2002). Single and repeated oral administration of the extract of *Ajuga iva* L. (AI) at a dose of 10 mg/kg produced a slight significant decrease in plasma glucose levels in normal rats 6 hours after administration and after 3 weeks of treatment. AI reduced plasma glucose levels of streptozotocin-diabetic rats from 337 ± 9.3 to 102.2 ± 17.7 mg/dl after 6 hours of oral administration (Hilaly and Lyoussi, 2002). 75% Methanolic extract of *Terminalia chebula, Terminalia belerica, Emblica officinalis* and their combination named 'Triphala' (equal proportion of above three plant extracts) are being used extensively in Indian system of medicine. Oral administration of the extracts (100 mg/kg body weight) reduced the blood glucose level in normal and in alloxan- (120

mg/kg) diabetic rats significantly within 4 hours (Sabu and Kuttan, 2002). *Aegle marmelos* Corr. (Rutaceae) (Kamalakkannan and Stanely Mainzen Prince, 2003) and *Eugenia jambolana* (Sharma et al., 2003) are widely used in Indian Ayurvedic medicine for the treatment of diabetes mellitus In Nigeria, *Ceiba pentandra* L. Gaertn (Bombacaceae) barks extract has antidiabetic properties (Ladeji et al., 2003) and *Psacalium decompositum* in Mexico (Alarcon-Aguilar et al., 2000).

In Thailand, many medicine plants had been studied for hypoglycemic activity. In 1998, Peungvicha et al. studied hypoglycemic effect of the water extract of the whole plant of *Piper sarmentosum* Roxb. (Piperaceae, Thai name : Chaplu).

A single oral administration of the water extract at doses of 0.125 and 0.25 g/kg significantly lowered the plasma glucose level in normal rats, but not significantly in diabetic rats. However, repeated oral administration of the water extract at a dose of 0.125 g/kg for 7 days produced a significant hypoglycemic effect in the diabetic rats. Oral administration of alcoholic extract of *Coccinia indica* at doses 2.5 and 5 g/kg reduced blood glucose levels in rabbits with alloxan-diabetes (Choradol, 1972). *Mimosa pudica* Linn. (Dechativong, 1988) and *Pluchea indica* (Peungvicha et al., 1999) had hypoglycemic activity.