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PHARMACOGNOSTIC PROPERTIES OF *ACANTHUS EBRACTEATUS* Vahl

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ภัทรกร มานะสมบูรณ์ : ลักษณะทางเภสัชเวทของต้นเหงือกปลาหมอ (PHARMACOGNOSTIC PROPERTIES OF *ACANTHUS EBRACTEATUS* Vahl) อ. ที่ปรึกษา : ผศ.สุนทรี วิทยานารถไพศาล, อ. ที่ปรึกษาร่วม : รศ. ดร. รพีพล ภโววาท 121 หน้า. ISBN 974-17-6048-5

ศึกษาจัดทำข้อมูลจำเพาะทางเภสัชเวทของต้นเหงือกปลาหมอชนิดดอกขาวและชนิดดอกม่วง โดยการศึกษาทางสัณฐานวิทยา ค่าคงที่ของใบ ทางจุลทรรศนลักษณะของผงสมุนไพร ศึกษาทางโครมาโตกราฟฟีชนิดผิวบาง โดยวิธี 1 มิติ (one-dimensional) และ สองมิติ (two-dimensional)

ข้อมูลจำเพาะทางเภสัชเวทของเหงือกปลาหมอแต่ละชนิด แสดงค่าที่แตกต่างด้วยภาพและตาราง ซึ่งเป็นข้อมูลที่นำไปใช้ในการจำแนกชนิดของเหงือกปลาหมอที่ได้จากร้านขายยา

ภาควิชา…… ลายมือชื่อนิสิต……...

สาขาวิชา ……. ลายมือชื่ออาจารย์ที่ปรึกษา ……..

ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……..

MAJOR : PHARMACEUTICAL BOTANY

KEY WORD : *ACANTHUS EBRACTEATUS* Vahl/ PHARMACOGNOSTIC PROPERTIES

PATARAKORN MANASOMBOON : PHARMACOGNOSTIC PROPERTIES OF *ACANTHUS EBRACTEATUS* Vahl. THESIS ADVISOR : ASSISTANT PROFESSOR SUNTAREE VITAYANATPAISAN, THESIS CO-ADVISOR : ASSOCIATE PROFESSOR RAPEPOL BAVOVADA, Ph. D., 121 PP. ISBN 974-17-6048-5

An investigation crude drugs offer identification as well as establishment of pharmacognostic specification of ngueak plaa mo. Theses are *A. ebracteatus* Vahl and *Acanthus ilicifolius* L. Pharmacognostical specification were established by detailed studying of each kind of ngueak plaa mo included morphology, leaf measurement, microscopical study concerning character of powder drug, chromatographic study of their extracts one-dimensional and two-dimensional thin-layer chromatography.

Pharmacognostic studies of each kind of crude drug of ngueak plaa mo revealed specific comparative data of which displayed in the form of tables. The result of this investigation offer valuable tool for the identification of each crude drug of ngueak plaa mo from various Thai traditional drugstores.

Department……Student's signature…….

Field of study……Advisor's signature…….

Academic year……..Co-advisor's signature…….

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ABBREVIATIONS

°C	=	Degree of Celsius
CCl_4	=	Carbon tetrachloride
$CHCl_3$	=	Chloroform
cm	=	Centimeter
cm^2	=	Square centimeter
EtOAc	=	Ethyl acetate
EtOH	=	Ethanol
g	=	Gram
H_2SO_4	=	Sulphuric acid
IC_{50}	=	50% Inhibition concentration
kg	=	Kilogram
LD_{50}	=	50% Lethal dose
m	=	Meter
MeOH	=	Methanol
mg	=	Milligram
ml	=	Milliliter
mm	=	Millimeter
mm^2	=	Square millimeter
nm	=	Nanometer
TLC	=	Thin Layer Chromatogram
µg	=	Microgram
µm	=	Micrometer
UV	=	Ultraviolet

CHAPTER I

INTRODUCTION

Acanthus L. is a genus belongs to the family Acanthaceae, in the order Scrophulariales, class Magnoliopsida. There are about 50 species mainly native in tropical Asia and Africa but with a center of diversity in the Mediterranean region including southern Europe.[1] The characteristic features of the plants in this genus are described as follows:- Flowers spicate; spikes terminal, sometimes moreover placed in the upper leaf-axils; flowers solitary in the axil of an often fugacious bract; bracteoles 2 or none; **calyx** deeply 4-partite; anterior and posterior segment largest; **corolla-tube** short; upper lip none, **lower lip** large, ovate-obovate, 3-lobed; **stamens** 4, in the throat, subequal; filaments thick; **anthers** appressed against each other, medifixed, longitudinally hairy, 1-celled; **ovules** in each ovary-cell 2, superposed; **style** bidentate; capsule ovoid-oblong, compressed, coriaceous, shining; retinacula robust; **seeds** 2-4, flat, tuberculate-rugose, glabrous. **Stem** not thickened above the nodes; **leaves** opposite, entire or sinuately dentate-pinnatifid, with spiny teeth, coriaceous, shining, glabrous, not connected by transverse ridges, without cystoliths. Undershrub, often with a thistle-like habit. [2]

Two species are circumscribed as indicated in the accompanying key. [2]

1a. Bracteoles subtending the calyx 2, semi-persistent, 6-8 mm long; calyx 1 ¼ - 1 ½ cm; corolla 3- 4 ½ cm; tube ¾ -1 cm, on the inside of the top with a ring of hairs; lip usually violet with a yellow median band, rarely white, 2 ¼ - 3 ¼ cm; filaments 13-16 mm; sytle 2 ¼ - 2 ½ cm; capsule 2 ¼ - 3 cm; seeds reniform; spikes 6-30 cm, not very dense; few flowers open at the same time; bracts caducous at or before the beginning of anthesis, ovate, entire, glabrous, 7-9 mm long. Stem terete, often provided with aerial

roots; 2 sharp spines present, next to each leaf; leaves oblong or lanceolate, always with an apical spine, often moreover with marginal spines, 9-30 cm by 4-12 cm; petiole 3-15 mm. Erect, ascending or sometimes scandent (not twining), much tufted. 0.50-3.00; W.C.E., Mad., especially in and near mangrove; in C.E. (up to 450) also along fresh water……...…….*A. ilicifolius* L.

b. Bracteoles subtending the calyx absent or fugacious, 3-4 mm by 1-2 mm; calyx ¾ - 1 ¼ cm; corolla 2-3 cm; tube ½ - 4/5 cm, on the inside of the top with a ring of hairs; lower lip white, 1 ½ - 2 ¼ cm; filaments ¾ - 1 ¼ cm; style 1-2 cm; capsule 2 cm by ¾ - 1 cm; spikes many-flowered (18-30 flower-pairs), lax to dense; few flowers open at the same time; bracts minutely diliate, glabrous on dorsal side. Stem spiny or not; leaves oblong, entire or not, with or without marginal spines, always with an apical spine, 6-23 cm by 2 ½ - 7 ½ cm; petiole 10-25 mm. Erect, VI; once in W. near Bantam; ½ ; bank of harbour-canal (*A. ilicifolius* L. var. *ebracteatus* (Vahl) R. Benoist)…….*A. ebracteatus* Vahl

Some species of *Acanthus* are used medicinally. Different parts of the plant have been used in ethnomedical practices in many countries. The boiled seeds of *A. ebracteatus* are commonly used in Peninsular Malaysia, as an ingredient of a cough medicine. The seeds are also used for poulticing boils, or the decoction is drunk against boils. [3, 4]

A. ilicifolius is employed in traditional medicine in China: the root is used for coughs and asthma. The tender shoot and leaves are used in India as a snake-bite cure. In Goa, the leaves are employed as an emollient fomentation for rheumatism and neuralgia. Indo-Chinese consider the roots to be useful in paralysis and asthma. In the Philippines, the leaves and roots are used in the form of a decoction as an anti-

asthmatic. In China, the stem and roots are useful as anticancer. The roots are regarded as a remedy to treat chronic fever. [3, 5]

In Malaysia and Indonesia, *A. ebracteatus* and *A. ilicifolius* are often uses in the same way, mainly for the treatment of boils, and as an antiphlogistic and expectorant. [4]

In Greece the roots of *A. mollis* (bear's breech) are recommended in the form of a plaster to treat burns and to wrap around dislocated joints. As an infusion, it was thought to be diuretic. It was also used to relieve gas, spasms, and digestive upsets, and to soothe damaged nerves and alleviate tension. [6]

A. montanus has various medicinal uses in Nigeria, chiefly as a cough medicine. Use against cough is recorded in Gabon and Congo either as a leaf-infusion or cooked with vegetable, and in Cameroun for cough and chest-complaints. A decoction of leafy-twigs is taken in Congo as a purgative. A leaf-macerate is given to children in Gabon as an emetic and the fresh young growths are taken for heart troubles. The young shoots cooked with groundnuts or the kernel-butter of *Irvingia gabonensis* Baill. (Ixonanthaceae) are taken to settle upset-tummy and to counteract 'morning-sickness' in pregnant women. The pounded leaves cooked with pepper and salt to eat with fish for rheumatism. Diuretic action is claimed in Congo where the plant is pounded up with a stem of *Costus* and a young pineapple fruit and then soaked in palm-wine: this is held to be a good remedy for urethral discharge. A shoot-macerate enters into a Gabon treatment for syphilis, and the leaf-spines are used to make scarifications in treatment and area of rheumatic pain which precedes yaws. An alcohol extract of roots of in reputed to give fast relief, when taken orally in cases of dysmenorrhoea in Nigeria. [7]

In Thailand both *A. ebracteatus* and *A. ilicifolius* have the same vernacular name as "ngueak plaa mo". The whole plant is boiled in water for bath in order to heal rash and skin diseases. The fresh plant is crushed and applied as a poultice on boils or taken orally as depurative. The fruits are taken orally to ease menstrual disorders.[8] According to the official Thai traditional medicine book called Pad-sard-songkroh, the leaves are cooked as soup and is taken to alleviate symptoms of debility. [9]

There has been a tendency to treat them as one single variable species. They do not seem to differ in any consistent vegetative feature. The chemical analysis of *A. ilicifolius* L. and *A. ebracteatus* Vahl have been done previously.[10-20] According to the data the diagnostic difference between both plants are the color of corolla, decidousness of bracteoles, and chemical constituents. The pharmacognostic investigations about the macroscopical and microscopical characters of the aerial parts of *A. ilicifolius* L. and *A. ebracteatus* Vahl have not been undertaken. The present investigation deals with the macroscopical characters, microscopic characters, leaf measurement, quality control of crude drugs and the chromatograms of some chemical constituents in the crude extracts of the two kind of ngueak plaa mo. The results of this work are expected to provide valuable information of the pharmacognostic standardization among *A. ebracteatus* L. and *A. ilicifolius* Vahl.

CHAPTER II

LITERATURE REVIEW

Acanthus L.

Major species

Acanthus ebracteatus Vahl, *A. ilicifolius* L.

Origin and geographic distribution

Acanthus comprises to 50 species, distributed mainly in the tropics and subtropics of the Old World, but also with a center of diversity in the Mediterranean region. [4]

Ecology

Acanthus species from India and South-East Asia are mangrove and salt-marsh plants, very common along banks of estuaries and lagoons close to seashore. They grow well on fine silt or mud with high salt content and high water level. Diurnal fluctuations in inundation can be tolerated but not continuous water logging. [4]

Growth and development

Growth of *A. ebracteatus* and *A. ilicifolius* is continuous in the sense that there are no resting terminal buds. There is a lot of variation in leaf form in both species, they mostly have a sinuous dentate and spinous margin, but can also be spineless and entire. Lack of spines and undulate margins seems to be a juvenile character, which can also occur just below the inflorescence. The spininess seems to be accentuated with water stress, which is related to salinity, seasonality and light intensity. The epidermal glands of *A. ilicifolius* are the source of secreted salt, which gives the upper leaf surface

a greasy feel. In South-East Asia flowering and fruiting are non-seasonal. Flowers are pollinated by both sunbirds and insects. The weak protandry restricts self-pollination. Flowers usually last 2 days, only a few opened flowers are found at a time on a spike. [4]

Propagation and planting

Acanthus is propagated by seed. Release of the seed is explosive, with the capsule splitting violently, dispersing the seeds up to 2 m away. *A. ebracteatus* and *A. ilicifolius* grow in clumps in the wild, and division of these clumps is also a means of propagation. [4]

Diseases and pests

A. ebracteatus and *A. ilicifolius* are normally free from diseases and pests. [4]

Harvesting

Harvesting of *Acanthus* from the wild can be done throughout the year. When dug up for the roots, plants should be replanted with some small roots left. [4]

Handling after harvest

Fruits of *Acanthus* harvested for the seeds are sold fresh in Malaysia. Fruits and roots should be dried and kept as stock. [4]

Production and international trade

Plants of both *Acanthus* species are generally collected from the wild for use within the region. International trade exists within the Chinese herbal medicine network, but export from South-East Asian countries is not known to exist. [4]

Acanthus ebracteatus Vahl

Family Acanthaceae

Synonym

Acanthus ilicifolius Lour. [21]

Dilivaria ebracteata Pers. [21]

Vernacular names

Sea Holly (English) [4, 22]

Indonesia: juruju (Sumatra), daruju (Javanese) [4, 22]

Malaysia: beruju, jeruju hitam (Peninsular) [4, 22]

Thailand: ngueak plaa mo (general) [4]

Vietnam: [oo] r[oo] [4]

Botanical description

An erect or reclining, smooth herb, up to 1 m tall, scarcely branched, with adventitious aerial roots; leaves oblong, 12-20 cm x 3-5 cm; spike up to 10 cm long, many-flowered, bracts ovate, 6-8 mm long, bracteoles early caducous, calyx lobes ovate, corolla lobe elliptical-oblong, 2.5 cm x 2 cm, white, rarely blueish. *A. ebracteatus* is gregarious and very common in tidal rivers. [4]

Distribution

A. ebracteatus Vahl is distributed from South-East Asia to northern Australia, very common in Malaysia, but less common in Indonesia. [4]

Traditional use

In the Malay Peninsula: The seeds are boiled along with the flowers of *Averrhoa* and black sugar-cane, adding cinnamon and crystalline sugar for flavoring.[22] This decoction is drunk as a cough remedy; they may be crushed and applied as a poultice on boils, or roasted and pulverized, they are taken as depurative by people afflicted with boils.[3, 4, 22] Two or three seeds may be given to children as anthelmintic.[3, 22] The juice from leaves is used as an application to the head to prevent the hair loosing.[22] In Thailand, the roots and stem are used for skin diseases and for longevity.[4] The roots are part of a decoction drunk as a remedy for shingles.[3, 22]

The compounds which found in *A. ebracteatus* Vahl were shown in Table 1.

Table 1 Chemical constituents of *A. ebracteatus* Vahl aerial part [10]

Category	Chemical constituent
Flavonoids	Vicenin-2 Schaftoside Luteolin-7-O-β-D-glucuronide Apigenin-7-O-β-D-glucuronide
Phenylpropanoids	Verbascoside β-hydroxyacteoside Isoverbascoside Leucosceptoside A Martynoside

Table 1 Chemical constituents of *A. ebracteatus* Vahl aerial part (continued)

Category	Chemical constituent
Sesquiterpenoids	Plucheoside B Alangionoside C Ebracteatoside A Premnaionoside
Lignans	Magnolenin C (+)-lyoniresinol 3α-O-β-D-glucopyranoside (-)-lyoniresinol 3α-O-β-D-glucopyranoside (8R,7'S,8'R)-5,5'-dimethoxylariciresinol 4'-O -β-D-glucopyranoside (+)-syringaresinol-4-O-β-D-apiofuranosyl -(1→2)-O-β-D-glucopyranoside
Miscellaneous	Ebracteatoside B Ebracteatoside C Ebracteatoside D 8-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside 7-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside (zizybeoside I) (*2R*)-2-*O*-β-D-glucopyranosyl-2*H*-1,4-benzoxazin-3(4*H*)-one (HBOA-Glc, blepharin)

Table 1 Chemical constituents of *A. ebracteatus* Vahl aerial part (continued)

Category	Chemical constituent
Miscellaneous	(*2R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc) 7-chloro-(*2R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (7- Cl-DIBOA-Glc) Adenosine

Medicinal and pharmacological activities

***Substrates for microbial protein production*:** *A. ebracteatus* was investigated for its feasibility in becoming the substrates for microbial growth. The plant could be used successfully by the organisms, *Cellulomonas* A 1 that produce protein. The protein content was relatively high, 39%, likewise protein yield was 23.0 mg/g of initial substrates. The research in this field is invaluable to mankind, it requires greater cooperation among different fields of scientists in order to produce microbial protein of good quality for human consumption. [23]

Larvicidal activity: The ethanol crude-extract from leaves of *A. ebracteatus* show larvicidal effect on tick larvae (*Boophilus microplus*). It caused 90.97% mortality of larvae after contact with 1.14 mg/cm^2 of crude-extract. [24]

Light-mediated antimicrobial activity: The ethanol extract of dried *A. ebracteatus* exhibit light-mediated biological activity against *Staphylococcus aureus* K 147 methicillin-sensitive both in the presence and absence of UV light. [25]

***Anti-inflammatory activity*:** The inhibitory effect on 5-lipoxygenase activity indicated by a significant reduction in LTB_4 production was shown by the *A. ebracteatus* (64% for 500 μg/ml ethanol extract, 44% for 500 μg/ml water extract) in the *in vitro* test for eicosanoid synthesis inhibiton. The result provided slight indication of activity which could explain the use of *A . ebracteatus* in treating arthritis. [26]

The compounds which found in *A. ebracteatus* Vahl showed biological activity were shown in Table 2.

Table 2 Summary on bioactive compounds

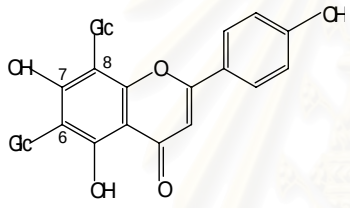
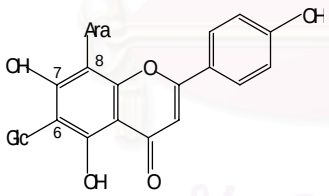
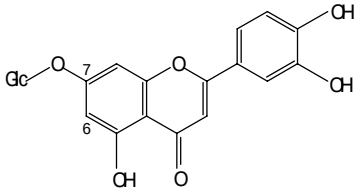
Compounds	Biological activity
 Vicenin-2 (Apigenin 6,8-di-C-glucoside)	Oviposition stimulant [27]
 Schaftoside (Apigenin 8-C-arabinoside)	Antifeedant [27,28]
 Luteolin-7-O-β-D-glucuronide	Antigonadotropic [29, 30] Lens aldose reductase inhibitor [31]

Table 2 Summary on bioactive compounds (continued)

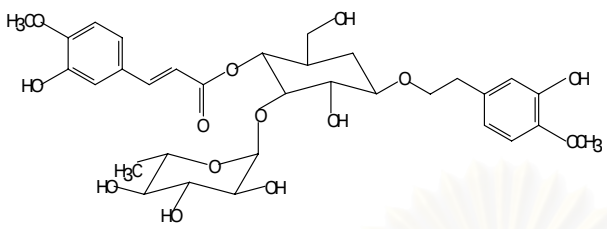
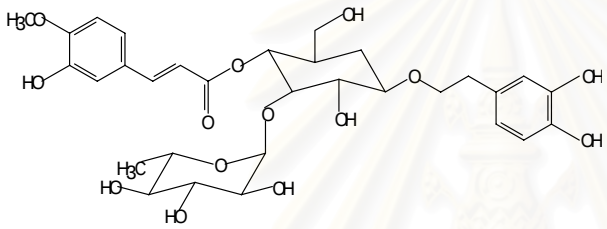
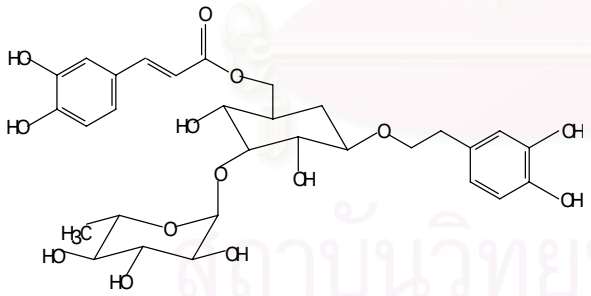
Compounds	Biological activity
 Martynoside	Antioxidant [32-36] and antiproliferative [38]
 Leucosceptoside A	Antioxidant [32-34, 38] ; antiproliferative [37] ; PKCα-inhibitor [39] ; antibacterial [40] and analgesic [41]
 Isoacteoside	Antioxidant [33, 35, 42-45]; antiproliferative [37] inducing differentiation in human hepatocarcinoma cells [46]; proliferation and differentiation of human gastric cancer cell [47]; antineoplastic [48]; antiviral [49]; hepatoprotective [50-51]; immunosuppressive [52] and analgesic [41]

Table 2 Summary on bioactive compounds (continued)

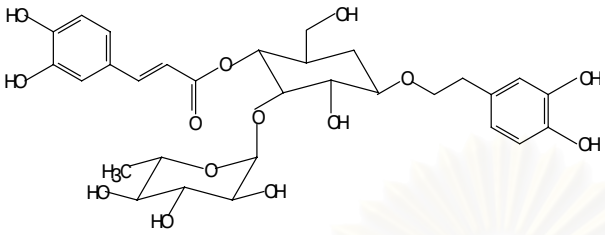
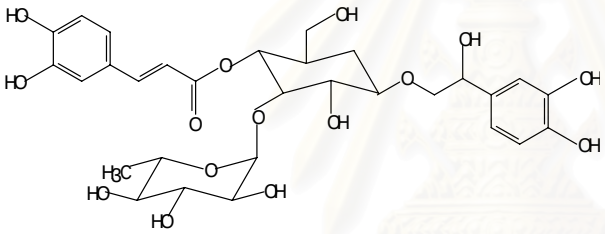
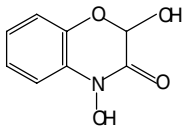
Compounds	Biological activity
 β-hydroxyacteoside (Campneoside II) [54]	Antibacterial [53]; inhibitor of 5-HETE formation [54]
 Acteoside (Kusaginin; Verbascoside; Orobanchin) [53]	Antioxidant [32-35, 38, 42-45, 55-61]; antiproliferative [37, 62]; PKCα-inhibitor [39, 63]; antineoplastic [48]; cytotoxic [64]; telomerase inhibitor [65]; differentiation of human gastric adenocarcinoma cell line [66]; antimetastatic [67]; antibacterial [68-70]; antiviral [49, 71-72]; hepatoprotective [50-51, 81]; immunosuppressive [52]; analgesic [41]; sedative [41]; antihypertensive [73]; cardioactive [74-75]; aortic rings relaxant [76]; enhancement of contraction of rat mesenteric artery [77]; neuroprotective [78]; immunomodulatory [79]; anti-inflammatory [54, 80] and lens aldose reductase inhibitor [82]

Table 2 Summary on bioactive compounds (continued)

Compounds	Biological activity
 2, 4-dihydroxy-1, 4-benzoxazin-3-one (DIBOA)	Mycelial growth inhibitor [83]; insectiside [83]; antigermination [83] ; allelopathy [84, 87]; insect antifeedant [83, 85];prostate cell growth inhibitor [86]; auxin inhibitor [87]; mutagenicity [87]

Acanthus ilicifolius L.

Family Acanthaceae

Synonym

Acanthus doloarius Blanco. [21]

Dilivaria ilicifolia Nees. [21]

D. ilicifolia Juss. [3]

Vernacular names

Sea Holly (English) [88]

China: lao shu le [5]

India: Harkuchkanta (Hindi, Bengal), Harikusa (Sanskrit), Attumulli, Kaludaimulli, Kolimulli, Uppukkarinimulli (Tamilnadu) [88, 89]

Nivagur (Bombay) [88]

Indonesia: jeruju (Sumatra), daruju (Javanese) [4]

Malaysia: jeruju, jeruju puteh (Peninsular) [4]

Philippines: daguari, diluariu (Tagalog), kasumba (Iloko) [4]

Papua New Guinea: kikia (Kavataria, Trobriand Island, Milne Bay Province) [4]

Thailand: kaem mo (peninsular), cha kreng (central), ngueak plaa mo namngoen (general) [4]

Vietnam: [oo] r[oo], n[uw] [ows]c, l[ax]o th[uwr] c[aa]n [4]

Botanical description

A stout, erect or reclining shrub, up to 1.5 m tall, scarcely branched, glabrous, with acventitious aerial roots; leaves oblong, 6.5-11 cm x 4-6 cm; spike up to 16.5 cm long, dense or interrupted, bracts lanceolate, 10 mm long, bracteoles in 2 pairs, oblong-lanceolate, up to 1.5 cm long, calyx lobes obovate-oblong, ciliolate, corolla lobe

obovate, 3 cm x 2.5 cm, pale to bright blue, corolla tube white. *A. ilicifolius* is gregarious and very common along banks of estuaries and lagoons, and in marshy land and mangroves close to the seashore. It is rarely found inland.[4]

Distribution

Distributed from South India and Sri Lanka to Indo-China, Indonesia, the Philippines and northern Australia, but rather scarce in Malaysia.[4]

Traditional use

In China: The roots are regarded as a remedy to treat chronic fever[3] and also prescribed with other plants in cancer, hepatosplenomegaly, hepatitis, scrofula and lymphadenitis.[90-91] Indo-China: The plant is used as diuretic. The leaves are mucilaginous, resolvent, emollient; they are used in fomentations to treat rheumatism, neuralgia; the plant is employed to make a cordial given in cases of paralysis and asthma.[3] Indonesia: The roots are chewed and laid on wounds caused by poisoned arrows; ground with a little ginger, they are used to poultice swollen legs, and a little of this paste may be taken to treat colic and a stitch in the side; another remedy for colic was chewing the young leaves with cinnamon bark. A poultice may be applied to treat rheumatic pain; sometimes the stem and leaves have been used as purgative.[3] Burma and India: The shoots are used to treat snake bite; the leaves are used in treating rheumatism.[3, 92] Philippines : The roots and leaves are used in decoction as an antiasthmatic.[3] The roots, boiled in milk, is largely used in leucorrhoea and general debility. A decoction of the leaves is considered as emollient.[92]

The compounds which found in *A. ilicifolius* L. were shown in Table 3.

Table 3 Chemical constituents of *A. ilicifolius* L.

Plant part	Category	Chemical constituent
-	Alkaloid	Acanthicifoline [11]
Aerial part	Lignans	(+)-lyoniresinol 3a-[2-(3,5-dimethoxy-4-hydroxy)-benzoyl]- *O*-β-glucopyranoside [18] Dihydroxymethyl-bis(3,5-dimethoxy-4-hydroxyphenyl) tetrahydrofuran-9-(or 9')-*O*-β-glucopyranaside [18] (+)-lyoniresinol 3a-*O*-β-D-glucopyranoside [18, 20] (-)-lyoniresinol 3a-*O*-β-glucopyranoside [18] Alangilignoside C [18] (8R,7'S,8'R)-5,5'-dimethoxylariciresinol 4-*O*-β-glucopyranoside [18] (+)-syringaresinol-*O*-β-glucopyranoside [18]
	Phenylpropanoids	Verbascoside [18, 20] β-hydroxyacteoside [18] Campneoside I [20] Cistanoside E [20] Cistanoside F [20] Ilicifoliosides A [20] Phenylethyl-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside [20]

Table 3 Chemical constituents of *A. ilicifolius* L. (continued)

Plant part	Category	Chemical constituent
Aerial part	Quinones	2,6-dimethoxy-*p*-hydroquinone 1-*O*-β-glucopyranoside [19]
	Sesquiterpenoids	Plucheoside B [18]
Leaves	Steroids	Cholesterol [13-14] Campesterol [13-14] Sitosterol [13-14] 28-Isofucosterol [13] Stigmast-7-en-3β-ol [14] Stigmasteryl-β-D-glucopyranoside [15]
Leaves, root	Steroids	Stigmasterol [13-15]
Leaves	Triterpenoids	α-Amyrin [14] β-Amyrin [14] Lupeol [14] Oleanolic acid [14] Ursolic acid [14]

Table 3 Chemical constituents of *A. ilicifolius* L. (continued)

Plant part	Category	Chemical constituent
Root	Triterpenoids	[α-L- arabinofuranosyl-(1→4)-β-D-glucuronopyranosyl (1→3)]-3β-hydroxy-lup-20 (29)-ene [12]
Leaves	Flavonoids	Methylapigenin 7-*O*-β-D-glucopyranuronate [16] Apigenin-7-O-glucuronide [16]
Aerial part	Miscellaneous	(*2R*)-2-*O*-β-D-glucopyranosyl-2*H*-1,4-benzoxazin-3(4*H*)-one (HBOA-Glc, blepharin) [19-20] (*2R*)-2-*O*-β-D-glucopyranosyl-5-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one [19] (*2R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc) [19] (*2R*)-2-*O*-β-D-glucopyranosyl-7-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DHBOA-Glc) [19] 7-Chloro-(*2R*)-2-*O*-β-D-glucopyranosyl-2*H*-1,4-benzoxazin-3(4*H*)-one [19] Syringic acid β-glucopyranosyl ester [19] Adenosine [19-20] Ilicifoliosides B [20]

Table 3 Chemical constituents of *A. ilicifolius* L. (continued)

Plant part	Category	Chemical constituent
Root	Miscellaneous	Benzoxazoline-2-one [15]
		Octacosyl alcohol [15]
Leaves		5,5' bis-benzoxazoline-2,2'-dione [17]

Medicinal and pharmacological activities

Analgesic anti-inflammatory activity: The methanol extract of *A. ilicifolius* exhibited marked analgesic effect. The extract, also showed significant anti-inflammatory activity against the proliferative phase of inflammation induced by carrageenin. Acute toxicity in mice by intraperitoneal administration showed that the LD_{50} was more than 1 g/kg. [93]

Antileukemic activity: The toxicity and effect of the aqueous extract of roots of *A. ilicifolius* in treatment of leukemic Swiss mice induced by Friend leukemia virus were studied. The extract was not toxic to Swiss mice in the dose used for the treatment. The survival rate of the treated leukemic mice increased 70% as compared to the control group. [94-95]

Toxicity: To study the acute and subacute toxicities of *A. ilicifolius* in Swiss mice, the aqueous extract of leaves and roots were used seperatly in different doses. The results indicated no acute toxicity but using high doses for a long period of time might cause abnormalities to the urinary system. [96]

Killing effect against the mosquito: Ten mosquito coil formulations were prepared using ten mangrove plants samples separately. The smoke from the coils were tested against biting of female mosquitoes of *Aedes aegypti*. Among the samples tested, the leaf of *Acanthus ilicifolius* was found most effective against the biting activity and also reduced the mosquito population in F1 generation. [97]

Antioxidant and hepatoprotective effect: The alcoholic extract of *Acanthus ilicifolius* leaves inhibited the formation of oxygen derived free radicals (ODFR) *in vitro* with IC_{50} of 550 μg/ml, 2750 μg/ml, 670μg/ml and 600 μg/ml (Fe^{2+} / ascorbate system), 980 μg/ml (Fe^{3+} / ADP/ ascorbate system) for superoxide radical production, hydroxyl radical generation, nitric oxide radical formation and lipid peroxide formation, respectively. The oral administration of the extract (250 and 500 mg/ kg) significantly reduced CCl_4 induced hepatotoxicity in rats, as judged from the serum and tissue activity of marker enzymes [glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP)]. These results were comparable with those obtained with curcumin (100 mg/ kg, p.o.). [98]

Tumour reducing and anticarcinogenic activity: Alcoholic extract of *A. ilicifolius* was found to be effective against tumour progression and carcinogen induced skin papilloma formation in mice. The extract was found to be cytotoxic towards lung fibroblast (L-929) cells in 72 h MTT assay and the concentration required for 50% cell death was 18 μg/ml. Oral administration of the extract (500 mg/kg b wt) reduced the tumour volume and administration of the same concentration increased the life span by 75% in ascites tumour (EAC cells) harbouring animals. The extract also significantly delayed the onset of dimethylbenzanthrazene DMBA/ Croton oil induced skin papilloma in mice in a dose dependent manner. [99]

The compounds which found in *A. ilicifolius* L. showed biological activity were shown in Table 4.

Table 4 Summary on bioactive compounds

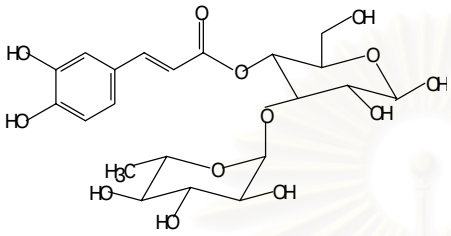
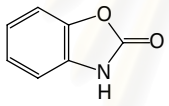
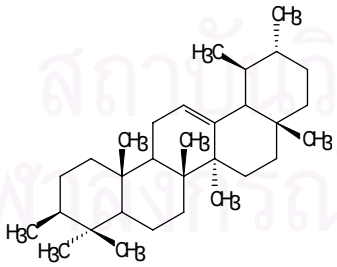
Compounds	Biological activity
Acteoside and β-hydroxyacteoside	See Table 2
 Cistanoside F	Antioxidant [46] Immunosuppressive [53]
 2-Benzoxazolinone (BOA)	Mycelial growth inhibitor [84]; plant growth inhibitor [84]; anticonvulsant [84]; allelopathy [84-85, 88]; auxin inhibitor [88]; antiinflammatory [100]; leishmanicidal [101] and antimicrobial [102]
 α-Amyrin	Antiinflammatory [103-104]; gastroprotective [105]

Table 4 Summary on bioactive compounds (continued)

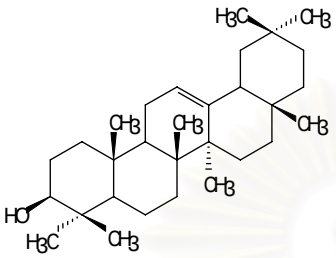
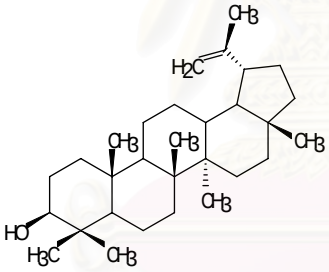
Compounds	Biological activity
 β-Amyrin	Antiinflammatory [103-104, 107]; gastroprotective [105] and cytotoxic [106]
 Lupeol	Antiinflammatory [103-104, 107, 110-111, 115]; antiplasmodial [108]; cytotoxic [109]; antilithiatic [112, 114]; cytoprotective [113]; hepatoprotective [116]; antitumor [117] and antiangiogenic [118]

Table 4 Summary on bioactive compounds (continued)

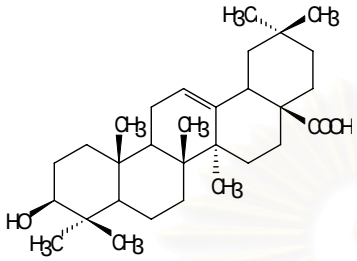
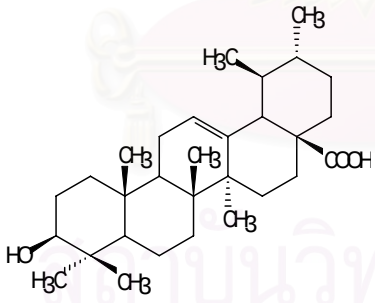
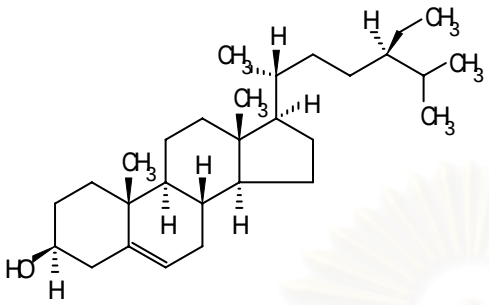
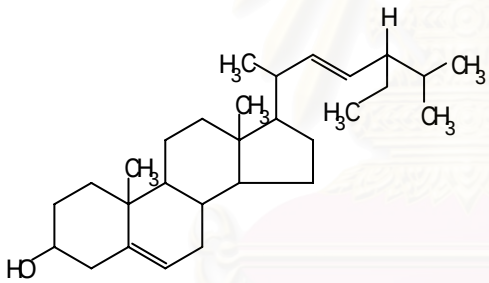
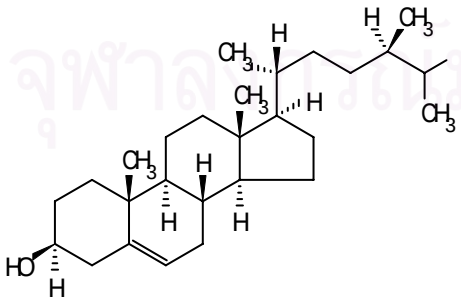
Compounds	Biological activity
 Oleanolic acid	Anticariogenic; antifertility; antifungal; antihyperglucemia; inhibition of lipid peroxidation and protection against adriamycin toxicity; inhibition of mutagenicity by B[a]P [119]; antiinflammation [103, 119-121]; antiangiogenic [122]; antitumour [123-124]; hepatoprotective [125-126]; cytotoxic [127-128]; gastroprotective [129]; antihypertensive [130] and antioxidative [134]
 Ursolic acid	Antimicrobial; hepatoprotective; inhibition of lipid peroxidation and protection against adriamycin toxicity; inhibition of lipoxygenase and cyclooxygenase in HL60 leukemic cells; inhibition of mutagenesis in bacteria; antihistamine; inhibition of mouse skin tumorigenesis [119]; antitumour promotion [119, 123]; cytotoxic [119, 127]; antiinflammation [103, 119, 131]; antiangiogenic [122]; hypoglycemic; antihyperlipidemic; antioxidative [130, 134]; antifeedant [132]

Table 4 Summary on bioactive compounds (continued)

Compounds	Biological activity
 β-sitosterol	Gastroprotective [105]; antiviral [133]; antioxidative [134, 146]; anti-inflammatory [135, 139, 147]; antipyretic [135]; anticomplementary [136]; antifungal [137]; antifertility [138]; estrogen-like effect [140]; antibacterial [141]; decrease reproductive steroids [143]; combination treatment of severe hypercholesterolemia [144]; and immunomodulatory [145]
 Stigmasterol	Antiviral [133]; antioxidative [134]; anticomplementary [136]; antibacterial [141]; and suppress prostate-cell metabolism and growth [142]
 Campesterol	Anticomplementary [136]; and suppress prostate-cell metabolism and growth [142]

CHAPTER III

MATERIALS AND METHODS

Scopes of Investigation

1. Study of the macroscopical characters of the aerial parts in each species.
2. Study of the microscopical characters of the aerial part powder to determine the main characteristics of each species.
3. Leaf measurements : stomatal number, stomatal index, palisade ratio, vein-islet number and veinlet termination number in each species.
4. Illustration of the two dimensional thin layer chromatographic chemical pattern of the aerial part extracts as a standard information of each species for comparative studies.
5. Qualitative determination of crude drugs according to the Pharmacopoeia: total ash, acid-insoluble ash and water determination .

Part I Macroscopic Identification

1. Materials

1.1 Fresh aerial part authentic samples

Acanthus ebracteatus Vahl was collected from Samut Songkhram Province in October 2002.

A. ilicifolius L. was collected from Samut Sakhon Province in September 2002.

1.2 Ten samples of crude drugs which are called nguaek plaa mo were purchased randomly from traditional drugstores in five regions of Thailand, i.e.

Wassana Bansamunpri, Jaroadwitheethong road, Muang district, Sukhothai. 12/11/45

Wong-hem-foh, Chotana road, Mae-rim district, Chiang Mai. 15/11/45

Kang-leng-tung, Suriyadejbumrung road, Muang district, Roi Ed. 28/11/45

E-sae, Na-muang road, Muang district, Khon Kaen. 27/11/45

Ung-guang-un, Thedsabaan road, Kloong district, Chanthaburi. 26/2/46

Jee Un Bhesaj, Srisothorn road, Muang district, Chachoengsao. 26/2/46

Vej-ja-pong, Chakkrawad road, Sampanthawong district, Bangkok. 13/12/45

Mae-jang, Sukapibaan road, Muang district, Samut Sakhon. 13/12/45

Saiburi Osoth, Saiburi road, Muang district, Songkhla. 15/3/46

Peng-un-teung, Raadrudee road, Muang district, Surat Thani. 23/1/46

2. Method

Fresh aerial part authentic samples were chopped into small pieces and dried in a hot air oven at 50 °C.

The shape, size, color and taste of authentic and crude drug samples were determined by organoleptic method. Photographs of crude drugs were taken for the record.

Part II Microscopic identification

1. Material

Dried aerial part authentic samples from part I.

2. Apparatus

- laboratory mill

- sieve no. 60
- slide and cover slips
- stage micrometer
- compound microscope Zeiss model Axiostar attached with digital camera Sony Cyber-shot DSC-S85

3. Chemical reagents

- mountants :

Chloral hydrate solution BP (chloral hydrate 80g, water 20 ml).

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, flowers, pollen grains, etc. It does not dissolve calcium oxalate and is therefore a good clearing reagent of plant tissues for observing these crystals.

Phloroglucinol solution

A 1% solution in 90% ethanol with hydrochloric acid as a test for lignin. Mount the section in a 1% solution of phloroglucinol in ethanol (90%) and allow to stand for about 2 minutes; remove any alcohol which has not evaporated with a piece of filter paper; add concentrated hydrochloric acid, cover and examine. All lignified walls stain pink or red.

4. Method

Dried aerial part authentic samples were ground and passed through a sieve with mesh number 60. Then were kept in a well-closed container for microscopic study as the following step:-

(a) The powdered samples were mounted with a suitable mountant and examined under the microscope.

(b) The characteristic cells and tissues were photographed using digital camera.

Figure 1 Compound microscope Zeiss model Axiostar attached with digital camera Sony Cyber-shot DSC-S85

Part III Leaf measurements

1. Material
    - Fresh leaves of authentic samples
2. Apparatus
    - hot plate
    - beaker
    - stirring rod
    - forcept
    - compound microscope Zeiss model Axiostar attached with digital camera Sony Cyber-shot DSC-S85
3. Chemical reagents
    - chloral hydrate solution B.P.
    - glycerin solution U.S.P.
4. Method

Stomatal number

A stomata consists of two similar cells, the guard cells, placed with their long axis parallel and having a small cellular space, the porous between them. The two guard cells and the porous counted as 1 cell stomata.

The average number of stomata per square millimeter of epidermis is termed the stomatal number.

1. Fragments of cleaned leaf in the region of midway between the midrib and the margin of the lamina are cleared by warming in a cholral hydrate solution (4 g/ml in distilled water). This solution should be frequently shaken and changed for rapid removing of chlorophyll. When the leaf fragment were cleared, they were washed with distilled water then kept in glycerin solution in order to maintain the structure.

2. Counted the number of stomata in the circle field of view and incomplete part of the cells in one semicircle. The incomplete part of the cells in the other semicircle not to be counted. There are 30 fields to be determined for each sample and from a knowledge of the area of the circle field was able to calculate the stomatal number.

$$\text{Stomatal number} = \frac{\text{number of stomatal}}{\text{area of epidermal cell } (mm^2)}$$

Stomatal index

The percentage proportion of the ultimate divisions of the epidermis of a leaf which have been converted into stomata is termed the stomatal index:

$$I = \frac{S}{E+S} \times 100$$

Where S = number of stomata per unit area

E = number of ordinary epidermal cells in the same unit area

1. Pieces of leaf are cleared by boiling with chloral hydrate solution, mounted and the lower surface examined by means of a microscope with a 4 mm objective.
2. Counts are made of the numbers of epidermal cells and of stomata (the two guard cells and ostiole being considered as one unit) within the square grid. (Figure 2)

Palisade ratio

The average number of palisade cells beneath each upper epidermal cell is termed the palisade ratio.

1. Pieces of fresh leaf are cleared by boiling with chloral hydrate solution, mounted and examined with a 4 mm objective.

2. A number of groups each of four epidermal cells are traced then the palisade cells in each group are focused and traced.

3. The palisade cells in each group are counted, those being included in the count which are more than half-covered by the epidermal cells; the figure obtained divided by 4 gives the palisade ratio of that group . (Figure 3)

Vein-islet and veinlet termination 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 millimeters in the central part of the lamina, midway between the midrib and the margin, is termed the vein-islet number.

The term ‘ veinlet termination number’ defined 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.

1. Leaves are cleared by boiling with chloral hydrate solution.

2. A projection apparatus is set up and by means of a stage micrometer is the replaced by the cleared preparation and the veins are traced in four contiguous squares, either in a square 2 mm × 2 mm.

3. Each numbered area must be completely enclosed by veins, and those which are incomplete are excluded from the count if cut by the top and left-hand sides of the square but included if cut by the other two sides. (Figure 4-5)

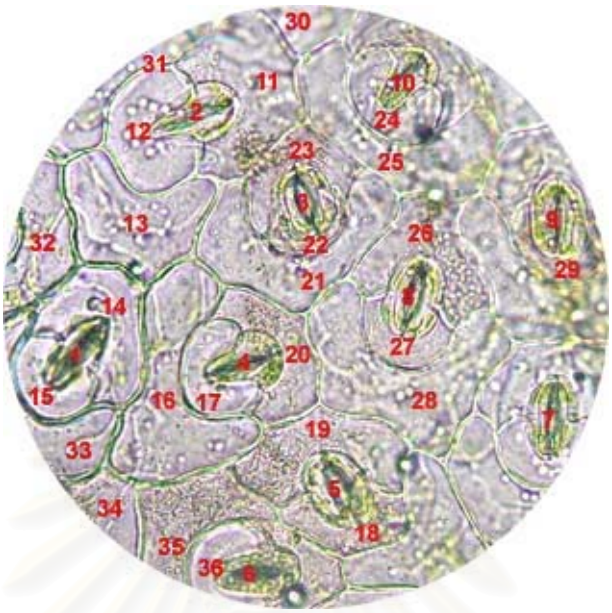


Figure 2 Lower epidermis of *A. ilicifolius* L.

Area determination 0.031429 mm^2

Stomatal number = $\frac{10}{0.031429}$ = 318.18

Stomatal index = $\frac{10}{10 + 36}$ X100 = 21.74

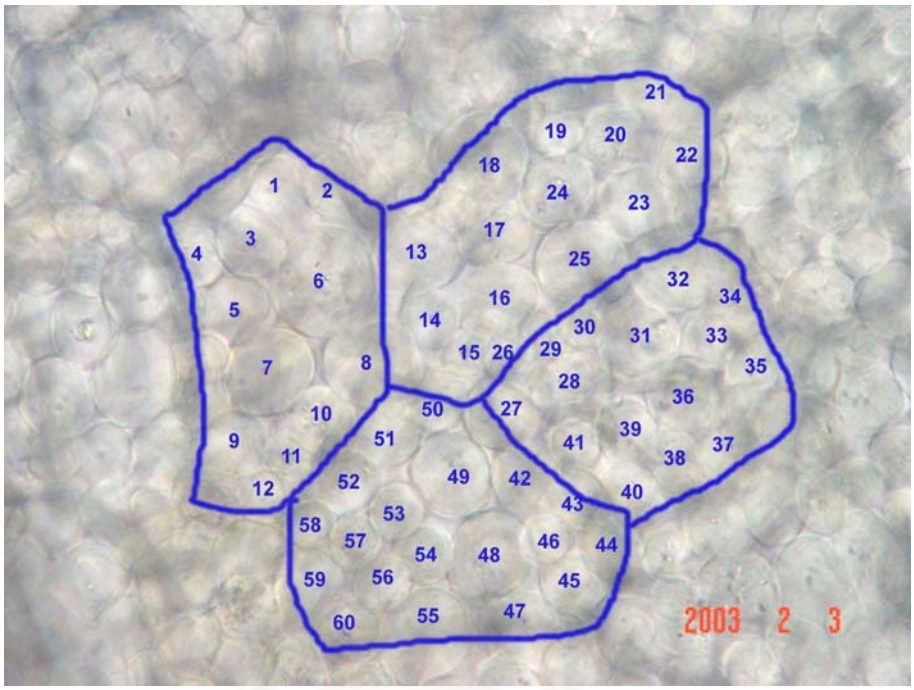


Figure 3 Upper palisade of *A. ebracteatus* Vahl

$$\text{Palisade ratio} = \frac{60}{4} = 15$$

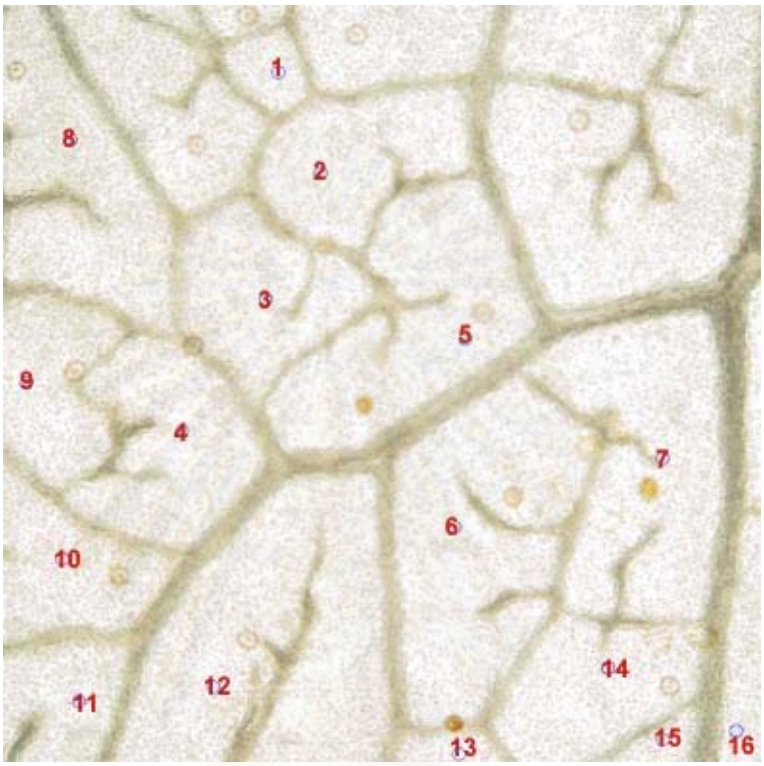


Figure 4 Vein of *A. ilicifolius* L.

$$\text{Vein-islet number} = \frac{16}{4} = 4$$

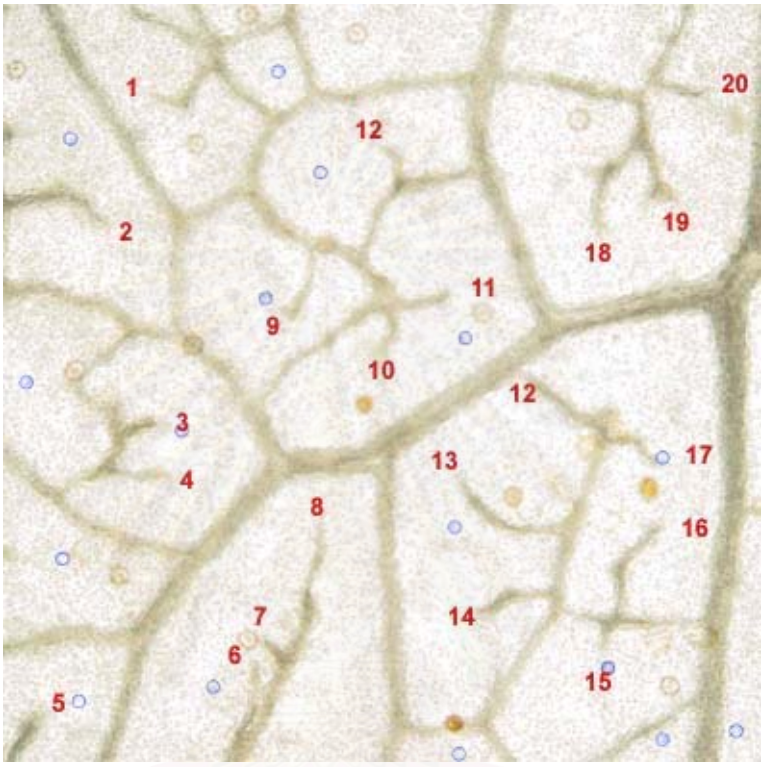


Figure 5 Vein of *A. ilicifolius* L.

$$\text{Veinlet termination number} = \frac{20}{4} = 5$$

Part IV Two-dimension thin-layer chromatographic

1. Materials
   - Dried aerial part authentic samples
   - Crude drugs which were purchased from traditional drugstores

2. Apparatus
   - erlenmeyer flasks
   - capillary tube
   - TLC Aluminum sheets of precoated silica gel 60 F_{254} Merck^{R} , 0.2 mm thick (10×10 cm)
   - Developing chamber
   - Ultraviolet light source
   - reagent sprayer

3. Chemical reagents
   - solvent : hexane, chloroform and methanol
   - spraying reagents : 10% sulfuric acid in ethanol

4. Method

   4.1 Preparation of crude extracts: 100 g of powder of 2 authentic plants and 10 samples were extracted with hexane, chloroform and methanol in Sohxlet apparatus respectively.

   4.2 Selection of suitable solvent system

   methanol crude extract part

   - The first dimension was chloroform : methanol (6 : 4)
   - The second dimension was ethyl acetate : ethanol : water (7 : 3 : 0.3)

chloroform crude extract

- The first dimension was chloroform : methanol (9 : 1)
- The second dimension was ethyl acetate : ethanol (8 : 2)

4.3 Application of the extract by capillary tube on the left side angle of TLC plate, allowed to dry.

4.4 Developing of the chromatogram after first solvent system saturated in TLC tank. The developed distance is 6 cm, removed the plate from the tank and allowed to dry. Re-develope the same plate in the second direction commenced in the perpendicular direction with the second solvent system until ascended 6 cm, removed the plate and allowed to dry.

4.5 Detection of the chromatogram

- visible in daylight
- fluorescence under UV 254 and 365 nm
- spray with 10% H_2SO_4 in ethanol reagent then heat 110 ºC for 2-3 minutes

4.6 Record R_f value.

Part V Quality control

Total ash

1. Material

   Powder authentic and crude drug samples

2. Apparatus

   - crucible
   - hot plate
   - forcept

- muffle furnace Gallenkamp Size 2
- analytical balance Mettler AJ 180

3. Method

3.1 Place 3 g sample of the ground substance, accurately weighed in a suitable tared crucible (usually of platinum or silica), previously ignited, cooled and weighed.

3.2 Incinerate the sample in muffle furnace by gradually increasing the temperature, not exceeding 450 ºC. until free from carbon; cool and weigh. If a carbon-free ash cannot be obtained in this way, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate. Dry on a water-bath and then on hot plate and incinerate to constant weight.

3.3 Calculate the percentage of total ash with reference to the air-dried substance.

Acid-insoluble ash

1. Material

The total ash of each sample

2. Apparatus

- Beaker
- Glass funnel
- ashless filter paper Whatman^{R}
- pH paper
- muffle furnace Gallenkamp Size 2

3. Chemical reagent

   - hydrochloric acid

4. Method

   4.1 Boil the total ash for 5 minutes with 25 ml of dilute hydrochloric acid.

   4.2 Collect the insoluble matter on an ashless filter paper, wash with hot water until the filtrate is neutral, and ignite in muffle furnace at about 500 ºC.

   4.3 Calculate the percentage of acid-insoluble ash with reference to the air-dried substance.

Figure 6 Muffle furnace Gallenkamp Size 2

Determination of water : Azeotropic Distillation Method

1. Material

   Powder authentic and crude drug samples

2. Apparatus

   The apparatus (see figure 7) consists of a glass flask (A) connected by a tube (D) to a cylindrical tube (B) fitted with a graduated receiving tube (E) and a reflux condenser (C). The receiving tube (E) is graduated in 0.1 ml sub-divisions so that the error of reading is control or an oil-bath. The upper portion of the flask and the connecting tube may be insulated with asbestos.

3. Chemical reagents

   - toluene

4. Method

   4.1 Clean the receiving tube and the condenser of the apparatus by a suitable method, thoroughly rinse with water, and dry.

   4.2 Introduce 200 ml of toluene and about 2 ml of water into the dry flask. Distill for about 2 hours, allow to cool to room temperature and read the water volume to a accuracy of 0.05 ml.

   4.3 Place in the flask a quantity of the substance, weighed to the nearest centigram, expected to give about 2 to 3 ml of water. Add a few pieces of porous material and heat the flask gently for 15 minutes.

   4.4 When the toluene begins to boil, distil at the rate of 2 drops per second until most of the water has distilled over, and then increase the rate of distillation to about 4 drops per second.

4.5 When the water has all distilled over, rinse the inside of the condenser tube with toluene. Continue the distillation for 5 minutes, remove the heat, allow the receiving tube to cool to room temperature, and dislodge any droplets of water which adhere to the walls of the receiving tube.

4.6 When the water and toluene have completely separated, read the volume of water and calculate the percentage present in the substance using the formula

$$\frac{100\ (n' - n)}{p}$$

where p = the weight in g of the substance to be examined,

n = the volume in ml of water obtained in the first distillation, and

n' = the total volume in ml of water obtained in the two distillations.

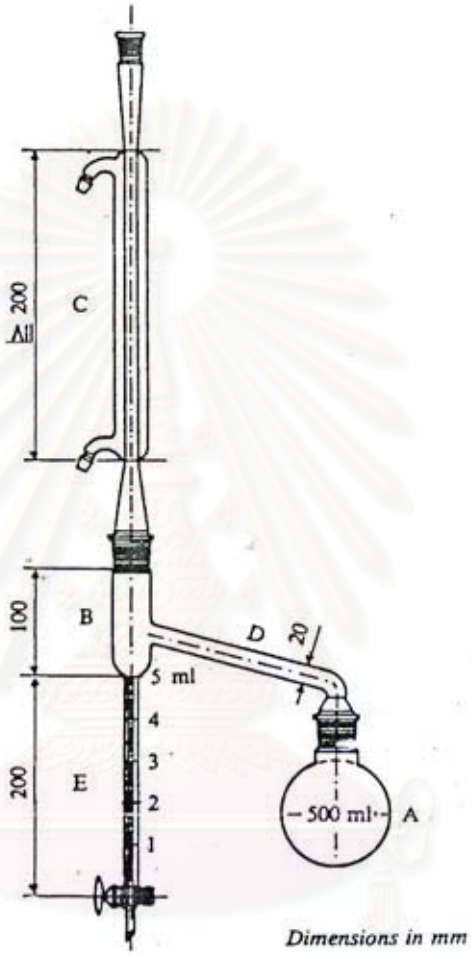


Figure 7 Apparatus for determination of water by Azeotropic Distillation Method

CHAPTER IV

RESULTS AND DATA

Crude drug randomization

1. Ten samples of crude drugs which are called ngueak plaa mo were purchased randomly from traditional drugstores in five regions of Thailand are shown as follow. (Figure 8-17)
2. Study of all samples on macroscopic and Thin-layer chromatography characteristic were carried out. Comparison of morphology (Table 5) and Thin-layer chromatogram (Figure 18) of each crude drug revealed the nine samples are all similar except the one from Samut Sakhon.

Figure 8 Morphology of the crude drug from Bangkok

Figure 9 Morphology of the crude drug from Samut Sakhon

Figure 10 Morphology of the crude drug from Chiang Mai

Figure 11 Morphology of the crude drug from Sukhothai

Figure 12 Morphology of the crude drug from Khon Kaen

Figure 13 Morphology of the crude drug from Roi Ed

Figure 14 Morphology of the crude drug from Chanthaburi

Figure 15 Morphology of the crude drug from Chachoengsao

Figure 16 Morphology of the crude drug from Songkhla

Figure 17 Morphology of the crude drug from Surat Thani

Table 5 Macroscopic characters of purchased ngueak plaa mo

Source of samples	Macroscopic character
Bangkok	The chopped drug consists of yellowish brown , cylindrical stems 0.5-0.7 cm in diameter and pieces of greenish brown leaf with spiny margin. The odor is faint, the taste is salty.
Samut Sakhon	The cut drug is made up of brown pieces of stem, 0.5-0.6 cm in diameter and yellow to greenish brown small pieces of leaf. Fragments with the leaf margin show spine. The odor is faint, the taste is salty.
Chiang Mai	The drug contains round, yellowish brown pieces of stem 0.5-0.6 cm in diameter and brown much crumpled leaf fragment with entire margin. The odor is faint, the taste is salty.
Sukhothai	The cut drug is made up of cylindrical, brown stem with 0.7-0.8 cm in diameter and some have spine at node position. The fragment of yellowish brown leaf with the leaf margin show spine. The odor is faint, the taste is salty.
Khon Kaen	The drug consists of small pieces greenish brown stem with rough fissured bark, 0.4-0.7 cm in diameter and yellowish brown fragment of leaf with spiny margin, some entire. The odor is faint, the taste is salty.
Roi Ed	Pieces of the roundish stem are green to brown , 0.5-0.7 cm in diameter and some also have node. Fragments of the leaf margin including the apexes broadly tridentate including a apical spine. The odor is faint, the taste is salty.
Chanthaburi	The chopped drug is made up of oblique slice stem, 0.3-0.7 cm in diameter thickness and yellowish brown leaf fragments with the spiny margin. The odor is faint, the taste is salty.
Chachoengsao	The chopped drug contains round, yellowish brown pieces of stem 0.5-0.8 cm in diameter and greenish brown much crumpled leaf fragment with entire or spiny margin. Some fragments of stem remains of spine pairs in node position. The odor is faint, the taste is salty.
Surat Thani	The cut drug consists of round yellowish brown stem, 0.5-0.7 in diameter and small pieces greenish brown of leaf. The odor is faint, the taste is salty.
Songkhla	The cylindrical, yellowish brown pieces of stem, 0.5-0.6 cm in diameter with pair of spines at the node position. Leaf fragments are dark brown with spiny margin. The odor is faint, salty taste.

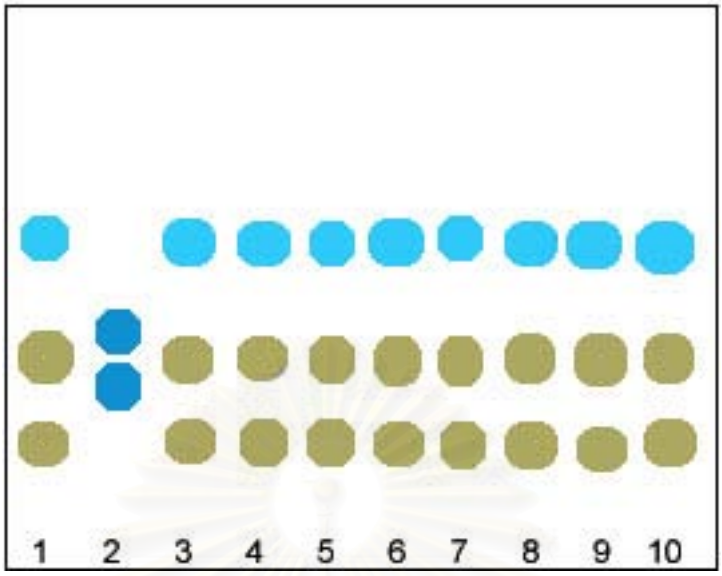


Figure 18 Thin-layer Chromatogram of methanol extract of purchased samples

System $CHCl_3$: MeOH (6 : 4)

Detection under UV light (365 nm) after spraying with 10%H_2SO_4 and heated

1 = crude drug from Bangkok

2 = crude drug from Samut Sakhon

3 = crude drug from Chiang Mai

4 = crude drug from Sukhothai

5 = crude drug from Khon Kaen

6 = crude drug from Roi Ed

7 = crude drug from Chanthaburi

8 = crude drug from Chachoengsao

9 = crude drug from Songkhla

10 = crude drug from Surat Thani

Plant identification

1. Authentic samples

   Identification of plants were carried out by comparisons of the characters of stems, leaves, flowers, fruits and seeds of each authentic samples with the herbarium specimens deposit in the Royal Forestry Department of Thailand, Ministry of Natural Resources and Environment.

2. Specification of ngueak plaa mo

   The specification of each kind of ngueak plaa mo was investigated by using pharmacognostic, and chromatographic methods. The results will be described separately in the following sections.

Acanthus ebracteatus Vahl

Macroscopic character

Morphology of plant

Morphology of authentic sample is a spiny herb with thick stems to a height of 1 m. Leaves decussate mostly have serrate margins armed with spines, but can also be spineless and entire. Inflorescences terminal, forming up to 14 pairs, corolla white. Bract shorter than the calyx; deciduous before flowering. Bracteoles usually present but early deciduous. Ripe fruit are capsule, green and oblong. (Figure 19)

Description of crude drug

The cut drug consists of pieces of stems and leaves. Fragments of the leaf show either broadly lanceolate with an entire margin, or more usually with a sinuous, spiny margin. The small pieces of stems with spine pairs at the insertion of each leaf. (Figure 20)

Figure 19 *Acanthus ebracteatus* Vahl

1. inflorescence
2. fruits

Figure 20 Morphology of the crude drug of *Acanthus ebracteatus* Vahl

Microscopic character

Powdered drug

The powder drug of *A. ebracteatus* Vahl is dark green color. It has slight and characteristic odor. Salty taste. The microscopic character are listed as follows:

a) The lignified vessels, frequently found fragmented of large vessels with reticulate vessel (1), spiral vessel (2), rarely found pit (3) and bordered pits (4).

b) The fragment of epidermis cells (5), which are polygonal in surface view, occasionally found in various sizes.

c) The very abundant fibers (6), which are found in groups of two or more cells. The wall are lignified and strongly thicken.

d) The occasional collasped trichome (8).

e) The fairly occasional bast fiber (7), which are found singly. They are rather short and broad with bluntly pointed end. The wall are strongly thicken.

f) The abundant glandular trichome in side view (9).

g) The fragment of lower epidermis in surface view, showing diacytic stomata (10).

h) The non glandular unicellular trichome (11), which occur singly.

i) Thin-walled, non-lignified xylem parenchyma, elongated rectangular and containing calcium oxalate microcrystals(12).

j) The rosette aggregates of calcium oxalate (13) which are abundant in the cell of spongy mesophyll.

k) Prism of calcium oxalate crystals (14) are found scatted

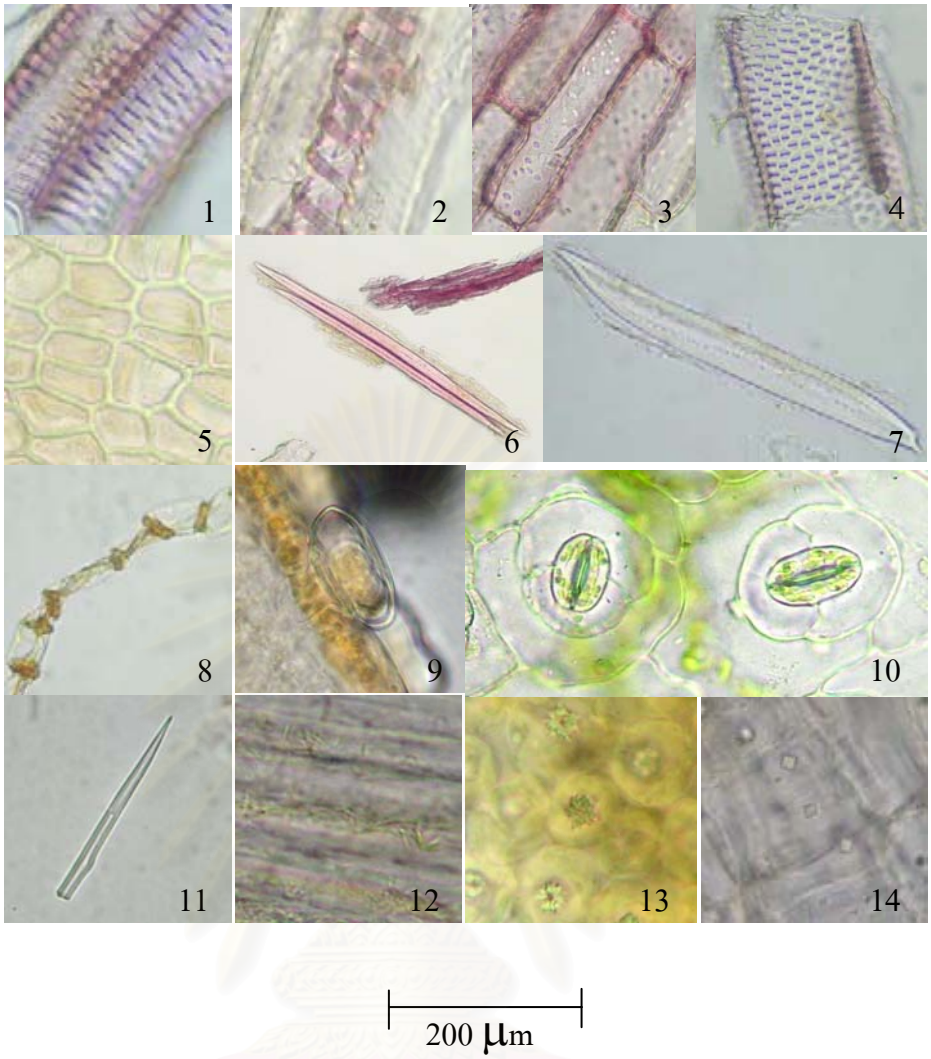


Figure 21 Powdered drug of the leaf and stem of *Acanthus ebracteatus* Vahl

1 Reticulate vessel
2 Spiral vessel
3 Parenchyma
4 Bordered pitted vessel
5 Upper epidermis
6 Fiber with lignified wall
7 Bast fiber
8 Collapsed trichome
9 Glandular trichome
10 Diacytic stomata
11 Trichome
12 Acicular crystals
13 Rosette crystal
14 Prism crystal

The result of leaf measurement

Table 6 Stomatal number and stomatal index of *Acanthus ebracteatus* Vahl

Area determination = 0.031429 mm^2

Number of Stomata	Number of epidermal cells	Stomatal number	Stomatal index
8	27	254.55	22.86
10	29	318.18	25.64
8	27	254.55	22.86
8	26	254.55	23.53
8	26	254.55	23.53
9	28	286.36	24.32
8	27	254.55	22.86
9	27	286.36	25.00
9	27	286.36	25.00
9	31	286.36	22.50
8	30	254.55	21.05
8	26	254.55	23.53
9	32	286.36	21.95
9	31	286.36	22.50
9	31	286.36	22.50
9	31	286.36	22.50
8	28	254.55	22.22
8	30	254.55	21.05
9	29	286.36	23.68
8	28	254.55	22.22
9	28	286.36	24.32
9	29	286.36	23.68
10	33	318.18	23.26
9	31	286.36	22.50
9	32	286.36	21.95
9	31	286.36	22.50
9	30	286.36	23.08
9	31	286.36	22.50
10	29	318.18	25.64
9	28	286.36	24.32
	mean	**278.94**	**23.17**
	S.D.	**19.92**	**1.19**

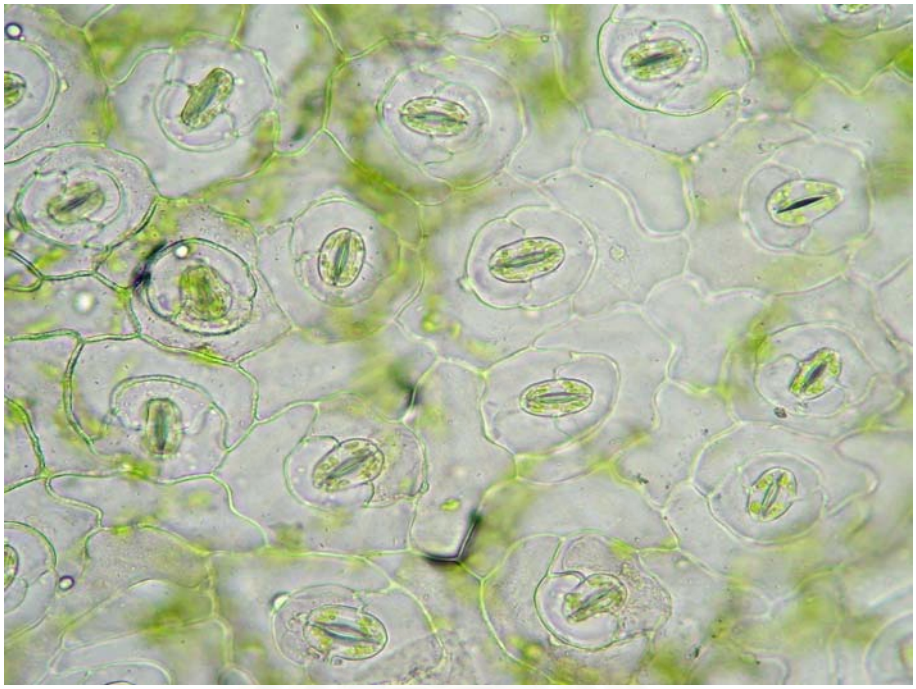


Figure 22 Lower epidermis of the leaf of *Acanthus ebracteatus* Vahl

Table 7 Palisade ratio, vein-islet number and veinlet termination number of *Acanthus ebracteatus* Vahl

Palisade cell		Vein-islet		Veinlet termination	
Number beneath 4 epidermal cells	Palisade ratio	Count in 4 mm^2	Vein-islet number	Count in 4 mm^2	Veinlet termination number
57	14.25	16	4.00	24	6.00
61	15.25	11	2.75	27	6.75
55	13.75	12	3.00	27	6.75
59	14.75	11	2.75	31	7.75
69	17.25	15	3.75	30	7.50
70	17.50	14	3.50	32	8.00
77	19.25	14	3.50	29	7.25
58	14.50	12	3.00	30	7.50
58	14.50	17	4.25	33	8.25
53	13.25	18	4.50	33	8.25
47	11.75	18	4.50	29	7.25
65	16.25	15	3.75	30	7.50
58	14.50	15	3.75	33	8.25
59	14.75	16	4.00	32	8.00
58	14.50	18	4.50	24	6.00
78	19.50	17	4.25	27	6.75
69	17.25	19	4.75	30	7.50
68	17.00	19	4.75	30	7.50
51	12.75	15	3.75	29	7.25
54	13.50	18	4.50	32	8.00
51	12.75	17	4.25	24	6.00
56	14.00	19	4.75	24	6.00
55	13.75	19	4.75	32	8.00
66	16.50	16	4.00	27	6.75
59	14.75	16	4.00	30	7.50
58	14.50	15	3.75	29	7.25
59	14.75	15	3.75	29	7.25
52	13.00	19	4.75	30	7.50
45	11.25	17	4.25	29	7.25
51	12.75	18	4.50	24	6.00
Mean	**14.80**	**Mean**	**4.01**	**Mean**	**7.25**
S. D.	**2.01**	**S. D.**	**0.60**	**S. D.**	**0.71**

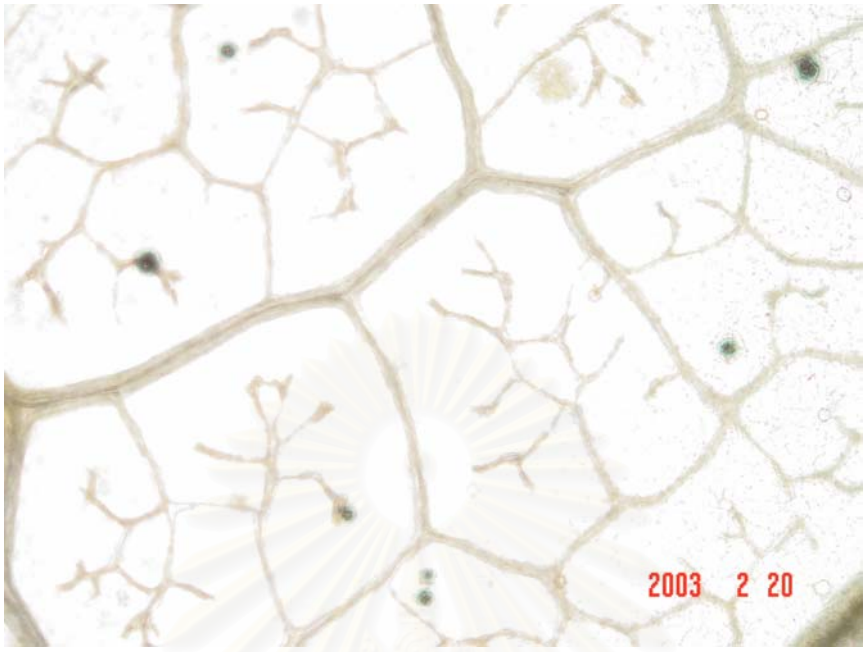
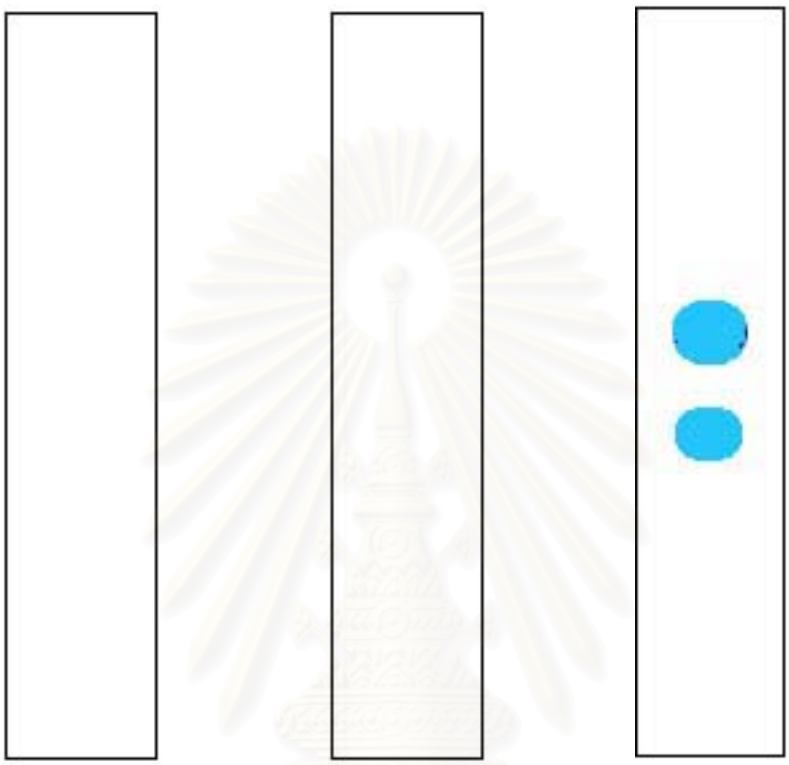


Figure 22 Vein-islet and veinlet termination of leaf of *Acanthus ebracteatus* Vahl

Chromatographic characteristics

Methanol extract

One-dimensional TLC system 1 ($CHCl_3$: MeOH 6:4)



I II III

Figure 23 One-dimensional TLC of the methanolic extracts of *A. ebracteatus* Vahl.

I = detection under UV light (254 nm)

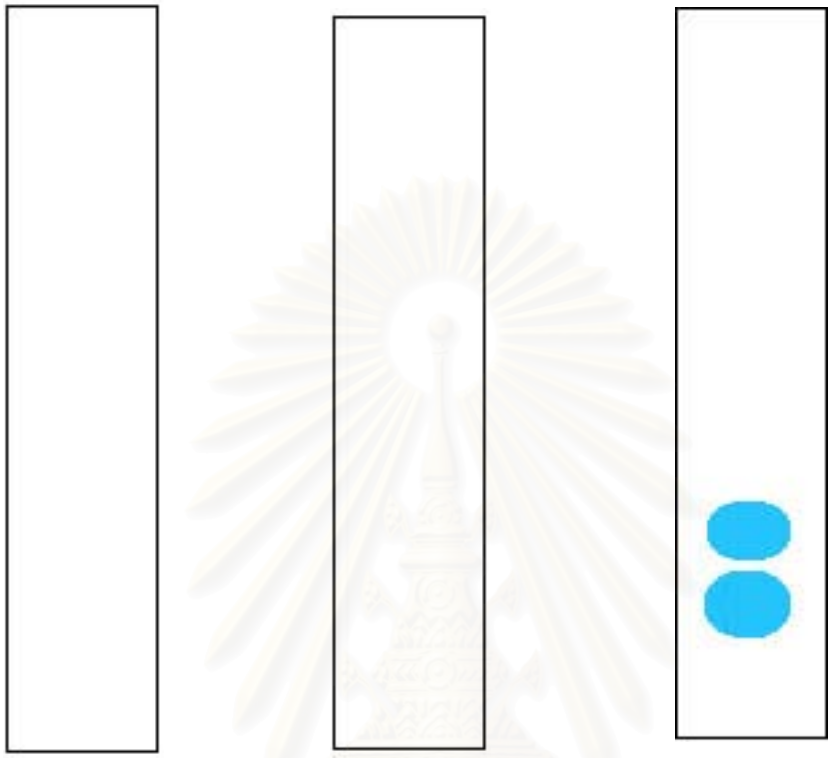
II = detection under UV light (365 nm)

III = detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated

Table 8 R_f value of components in methanol extract of the aerial part of the *A. ebracteatus* Vahl system 1

Spot	R_f value	UV 254	UV 365	10 % H_2SO_4 in Ethanol
1	0.33 - 0.40	-	-	Blue
2	0.50 - 0.67	-	-	Blue

One-dimensional TLC system 2 (EtOAc : EtOH 7:3)



I II III

Figure 24 One-dimensional TLC of the methanolic extracts of *A. ebracteatus* Vahl.

I = detection under UV light (254 nm)

II = detection under UV light (365 nm)

III = detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated

Table 9 R_f value of components in methanol extract of the aerial part of the *A. ebracteatus* Vahl system 2

Spot	R_f value	UV 254	UV 365	10 % H_2SO_4 in Ethanol
1	0.16 - 0.24	-	-	Blue
2	0.24 - 0.32	-	-	Blue

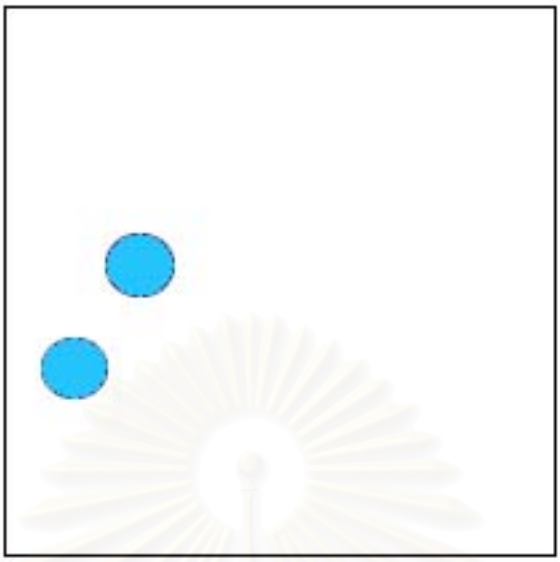


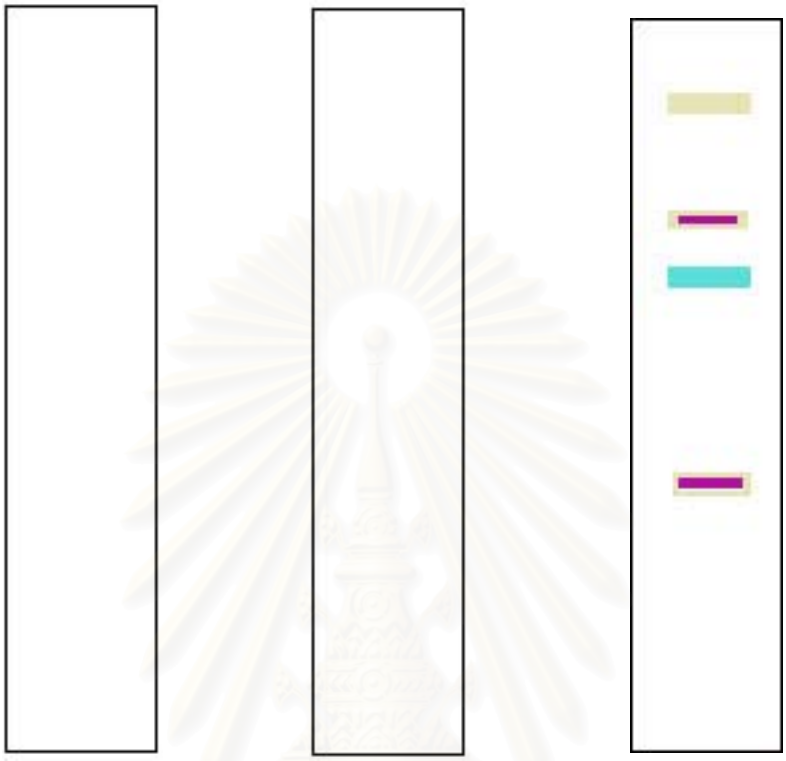
Figure 25 Two-dimensional TLC fingerprint of the methanol extracts of *A. ebracteatus* Vahl

Table 10 R_f value of components in methanol extract of the aerial part of the *A. ebracteatus* Vahl

Spot	R_f value		Color
	X	Y	
1	0.16	0.38	Blue
2	0.28	0.59	Blue

Chlorofroml extract

One-dimensional TLC system 1 ($CHCl_3$: MeOH 9.5: 0.5)



I II III

Figure 26 One-dimensional TLC of the chloroform extracts of *A. ebracteatus* Vahl. system 1

I = detection under UV light (254 nm)

II = detection under UV light (365 nm)

III = detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated

Table 11 R_f value of components in chloroform extract of the aerial part of the *A. ebracteatus* Vahl system 1

Spot	R_f value	UV 254	UV 365	10 % H_2SO_4 in Ethanol
1	0.33 - 0.36	-	-	Pale yellow
2	0.56 - 0.60	-	-	Light blue
3	0.65 - 0.69	-	-	Pale yellow
4	0.82 - 0.85	-	-	Pale yellow

One-dimensional TLC system 2 ($CHCl_3$: Acetone 9: 1)

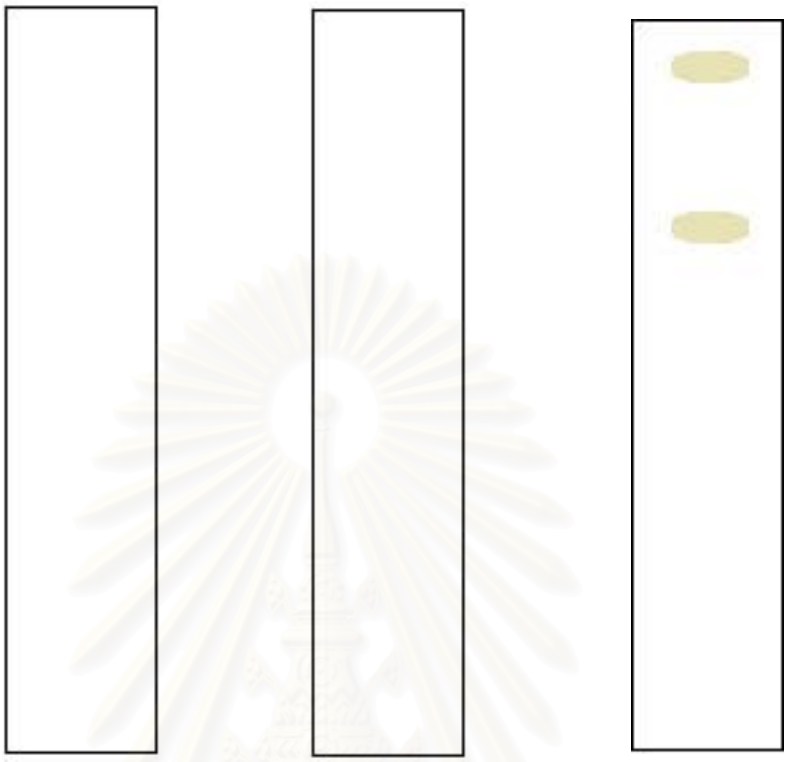


Figure 27 One-dimensional TLC of the chloroform extracts of *A. ebracteatus* Vahl. system 2

I = detection under UV light (254 nm)

II = detection under UV light (365 nm)

III = detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated

Table 12 R_f value of components in chloroform extract of the aerial part of the *A. ebracteatus* Vahl system 2

Spot	R_f value	UV 254	UV 365	10 % H_2SO_4 in Ethanol
1	0.64 - 0.69	-	-	Pale yellow
2	0.86 - 0.92	-	-	Pale yellow

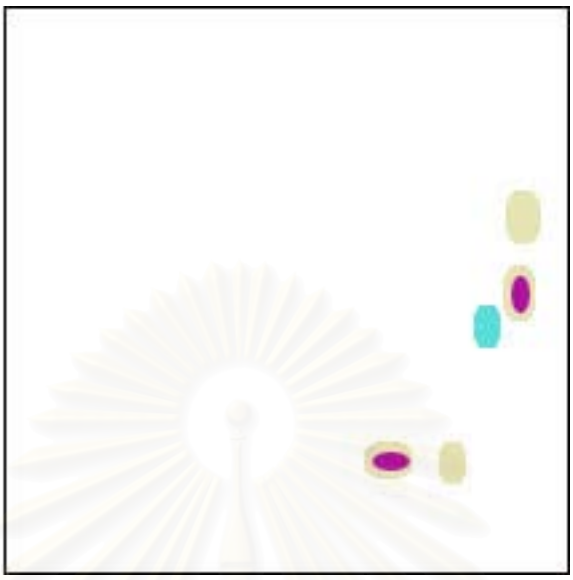


Figure 28 Two-dimensional TLC fingerprint of the chloroform extracts of *A. ebracteatus* Vahl

Table 13 R_f value of components in chloroform extract of the aerial part of the *A. ebracteatus* Vahl

Spot	R_f value		Color
	X	Y	
1	0.61	0.24	Pale yellow-violet
2	0.70	0.22	Pale yellow
3	0.78	0.50	Light green
4	0.84	0.51	Pale yellow-violet
5	0.84	0.67	Pale yellow-violet

A. ilicifolius L.

Macroscopic character

Morphology of plant

Morphology of authentic sample is sprawling herb, to a height of 2 m and robust with spiny to very spiny leaves. Leaves decussate, usually with a pair of spines at the insertion of leaf. The leaves are glossy, stiff yellow-green with a margin that is usually but not always serrate. Inflorescences terminal, forming up to 20 pairs, the bract below each flower often caducous; lateral bracteoles 2, conspicuous and persistent. The flowers in part light blue or violet. Fruit a capsule 2 to 3 cm long and 1 cm wide. (Figure 10)

Description of crude drug

The cut drug consists of oblique slice, round, brown pieces of stem, 0.6-0.7 cm in diameter and greenish brown to brown fragments of the leaf. The leaf margin show the apex broadly tridentate including a apical spine. The odor is faint, the taste is salty. (Figure 11)

Figure 29 *Acanthus ilicifolius* L.

1 aerial part

2 inflorescence

3 fruits

Figure 30 Morphology of the crude drug of *Acanthus ilicifolius* L.

Microscopic character

Powdered drug

Powder drug of *A. ilicifolius* L. is yellowish green. It has mild characteristic odor and salty taste. The microscopic characters are listed as follows:

a) The abundant fragments of upper epidermis (1) in surface view composed polygonal cells.
b) The abundant fragments of non-lignified thick walled fibers (5) occur in groups and some associated with vessels.
c) The lignified vessels, frequently found fragmented of reticulate vessel (2) and spiral vessel (3).
d) The rarly found phloem (6), thin-wall containing acicular crystals of calcium oxalate.
e) The fragments of lignified parenchyma (4).
f) The occasional fragments of the epidermis of stem (7), composing of thin, yellowish brown –walled and elongated epidermis cells.
g) The abundant glandular trichomes in surface view (8) and side view (9).
h) Fragment of multicellular trichomes, showing apical cell (10) and basal cell (11).
i) The fairly occasional lignified wall, collasped trichome (12).
j) The fragment of lower epidermis in surface view, showing diacytic stomata (13).

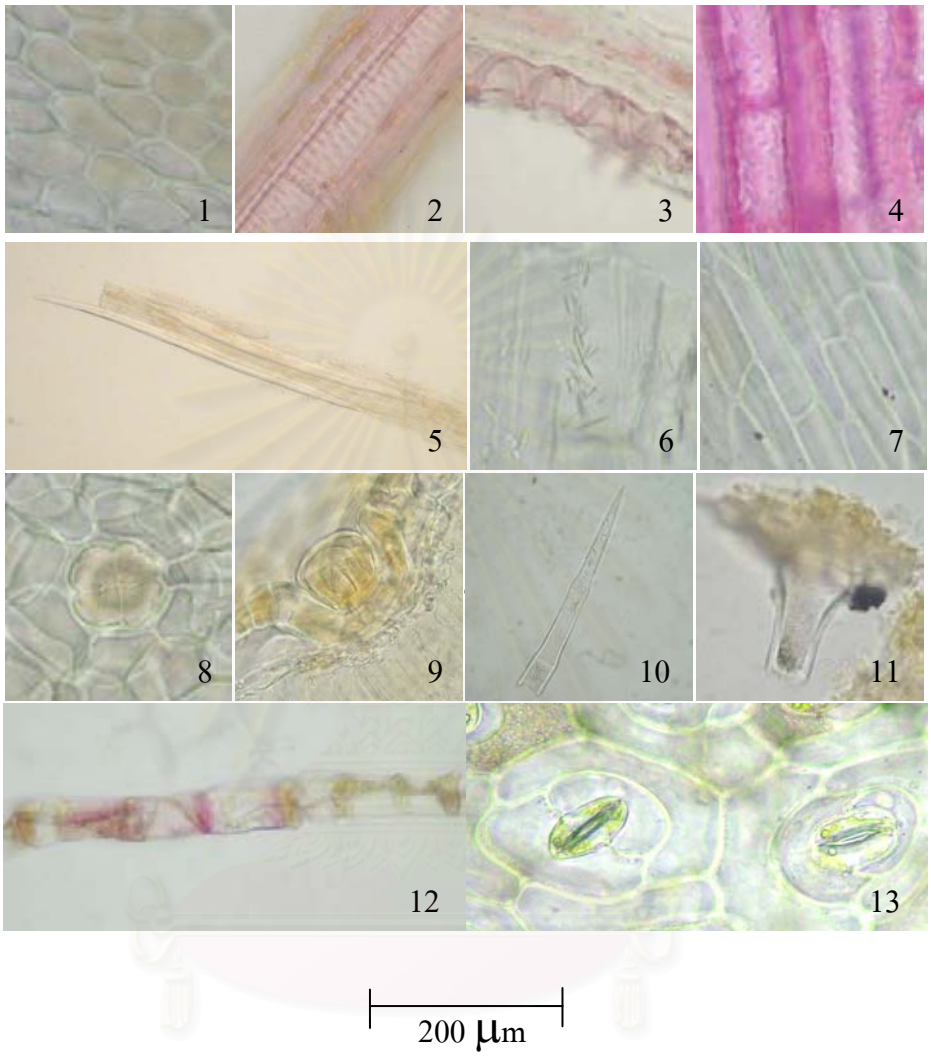


Figure 31 Powdered drug of the leaf and stem of *A. ilicifolius* L.

1. Upper epidermis
2. Reticulate vessel
3. Spiral vessel
4. Parenchyma
5. Fiber
6. Acicular crystal
7. Epidermis of stem
8.-9. Glandular trichomes
10.-11. Multicellular trichomes
12. Collasped trichomes
13. Diacytic stomata

The result of leaf measurement

Table 14 Stomatal number and stomatal index of *Acanthus ilicifolius* L.

Area determination = 0.031429 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
9	27	286.36	25.00
9	31	286.36	22.50
9	32	286.36	21.95
10	36	318.18	21.74
8	30	254.55	21.05
9	31	286.36	22.50
8	31	254.55	20.51
9	32	286.36	21.95
8	29	254.55	21.62
9	29	286.36	23.68
10	32	318.18	23.81
9	29	286.36	23.68
10	31	318.18	24.39
10	31	318.18	24.39
8	30	254.55	21.05
10	30	318.18	25.00
9	33	286.36	21.43
9	31	286.36	22.50
9	30	286.36	23.08
9	30	286.36	23.08
9	31	286.36	22.50
10	30	318.18	25.00
9	32	286.36	21.95
8	28	254.55	22.22
9	26	286.36	25.71
10	34	318.18	22.73
9	32	286.36	21.95
10	31	318.18	24.39
9	30	286.36	23.08
10	32	318.18	23.81
	mean	**290.61**	**22.94**
	S.D.	**21.68**	**1.35**

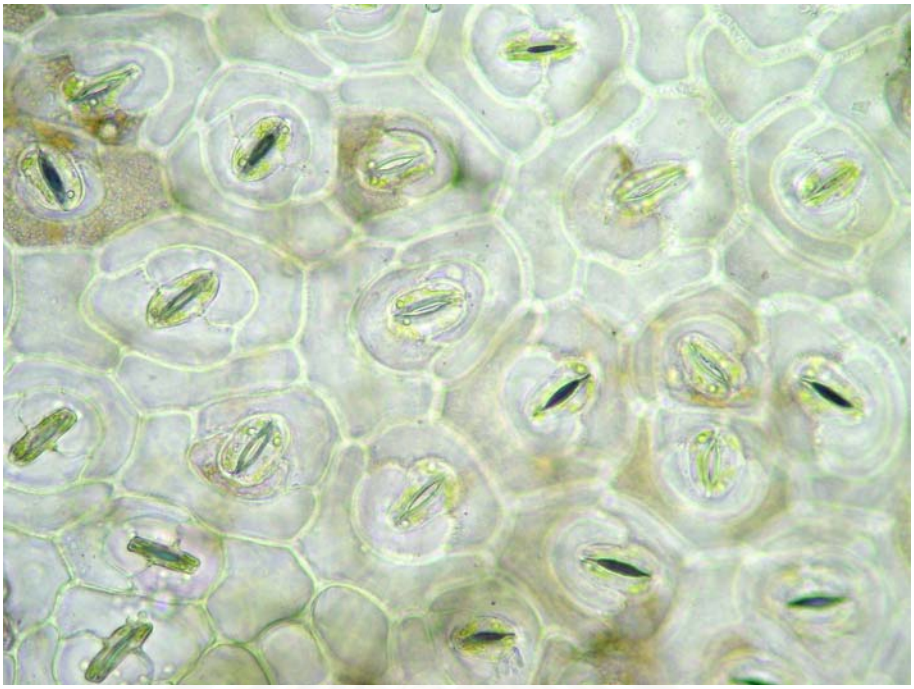


Figure 32 Lower epidermis of the leaf of *Acanthus ilicifolius* Vahl

Table 13 Palisade ratio, vein-islet number and veinlet termination number of *Acanthus ilicifolius* L.

Palisade cell		Vein-islet		Veinlet termination	
Number beneath 4 epidermal cells	Palisade ratio	Count in 4 mm^2	Vein-islet number	Count in 4 mm^2	Veinlet termination number
45	11.25	17	4.25	25	6.25
44	11.00	20	5.00	27	6.75
44	11.00	16	4.00	22	5.50
46	11.50	21	5.25	25	6.25
50	12.50	15	3.75	21	5.25
56	14.00	14	3.50	17	4.25
50	12.50	16	4.00	20	5.00
53	13.25	16	4.00	22	5.50
41	10.25	16	4.00	21	5.25
41	10.25	16	4.00	21	5.25
40	10.00	16	4.00	18	4.50
37	9.25	15	3.75	20	5.00
40	10.00	16	4.00	27	6.75
45	11.25	17	4.25	25	6.25
48	12.00	20	5.00	22	5.50
45	11.25	19	4.75	21	5.25
47	11.75	14	3.50	21	5.25
46	11.50	21	5.25	19	4.75
36	9.00	20	5.00	19	4.75
45	11.25	17	4.25	25	6.25
47	11.75	19	4.75	17	4.25
40	10.00	16	4.00	22	5.50
47	11.75	19	4.75	15	3.75
49	12.25	19	4.75	20	5.00
39	9.75	15	3.75	18	4.50
43	10.75	19	4.75	19	4.75
47	11.75	17	4.25	22	5.50
48	12.00	15	3.75	24	6.00
44	11.00	14	3.50	19	4.75
44	11.00	14	3.50	22	5.50
Mean	**11.23**	**Mean**	**4.24**	**Mean**	**5.30**
S. D.	**1.12**	**S. D.**	**0.55**	**S. D.**	**0.74**

Chromatographic characteristics

Methanol extract

One-dimensional TLC system 1 ($CHCl_3$: MeOH 6:4)

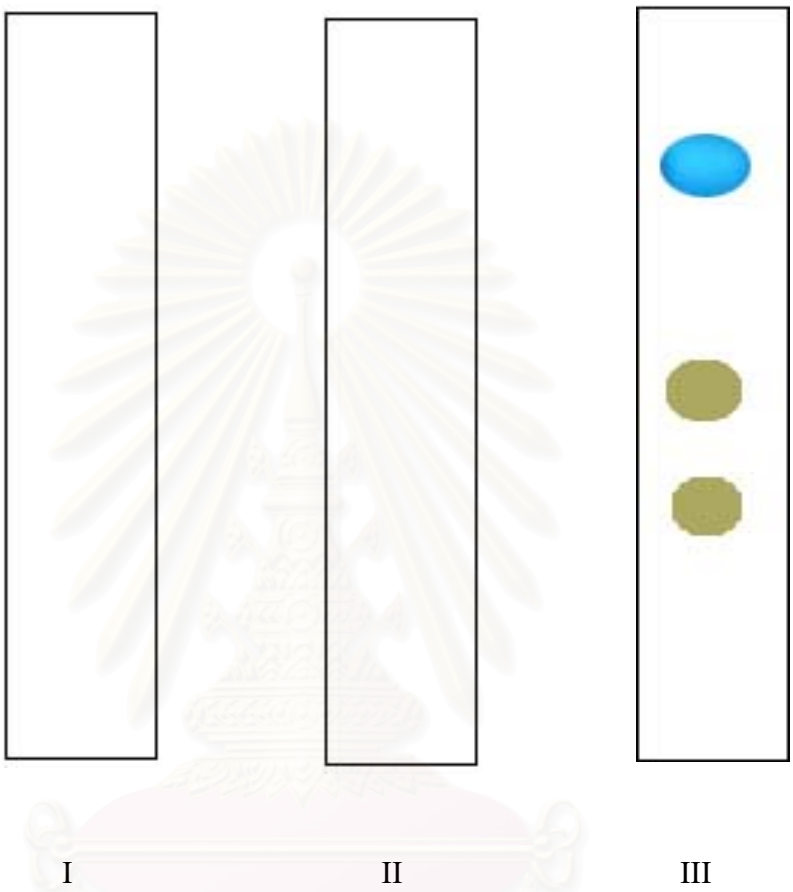


Figure 33 One-dimensional TLC of the methanolic extracts of *A. ilicifolius* L.

I = detection under UV light (254 nm)

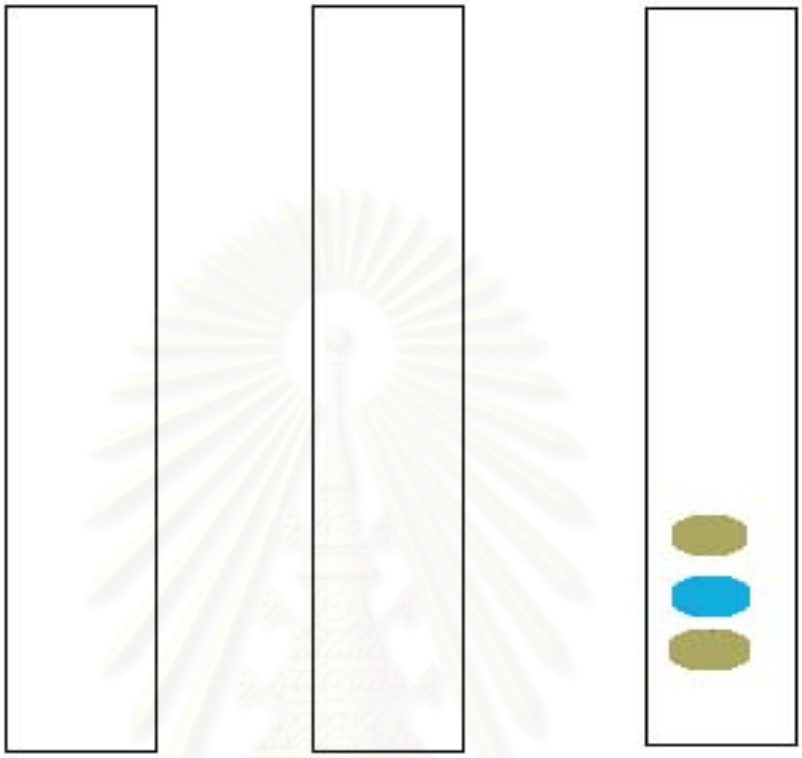
II = detection under UV light (365 nm)

III = detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated

Table 16 R_f value of components in methanol extract of the aerial part of the *A. ilicifolius* L. system 1

Spot	R_f value	UV 254	UV 365	10 % H_2SO_4 in Ethanol
1	0.28 - 0.38	-	-	Yellow
2	0.44 - 0.56	-	-	Yellow
3	0.86 - 0.92	-	-	Light blue

One-dimensional TLC system 2 (EtOAc : EtOH 7:3)



I II III

Figure 34 One-dimensional TLC of the methanolic extracts of *A. ilicifolius* L.

I = detection under UV light (254 nm)

II = detection under UV light (365 nm)

III = detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated

Table 17 R_f value of components in methanol extract of the aerial part of the *A. ilicifolius* L. system 2

Spot	R_f value	UV 254	UV 365	10 % H_2SO_4 in Ethanol
1	0.07 - 0.15	-	-	Yellow
2	0.15 - 0.23	-	-	Light blue
3	0.24 - 0.30	-	-	Yellow

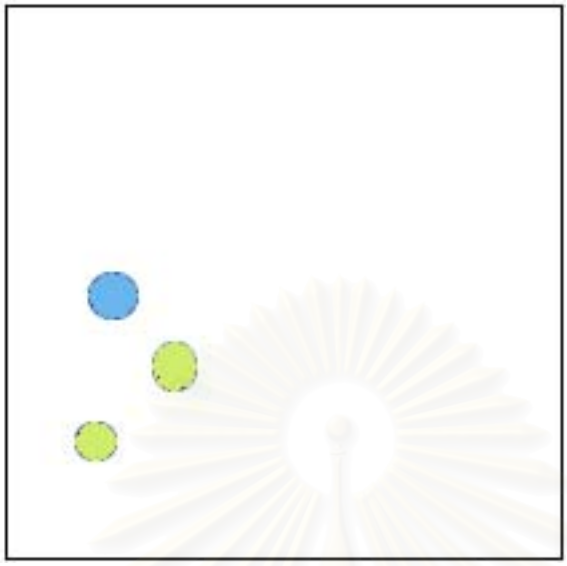


Figure 35 Two-dimensional TLC fingerprint of the methanol extracts of *A. ilicifolius* L.

Table 18 R_f value of components in methanol extract of the aerial part of the *A. ilicifolius* L.

Spot	R_f value		Color
	X	Y	
1	0.16	0.17	Yellow
2	0.32	0.33	Yellow
3	0.19	0.33	Light blue

Chloroform extract

One-dimensional thin layer chromatography system 1 ($CHCl_3$: MeOH 9.5: 0.5)

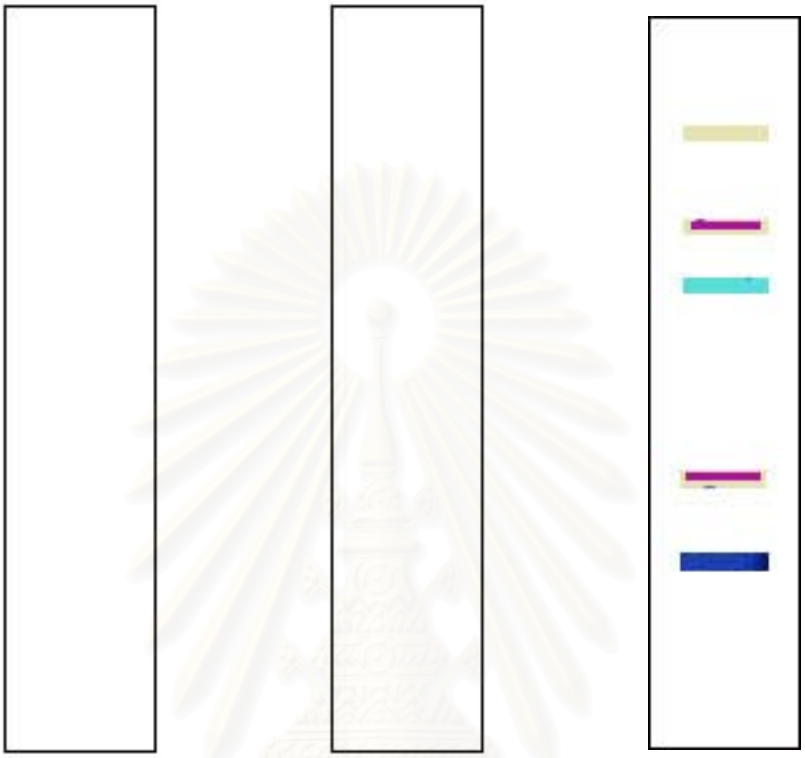


Figure 36 One-dimensional TLC of the chloroform extracts of *A. ilicifolius* L.

I = detection under UV light (254 nm)

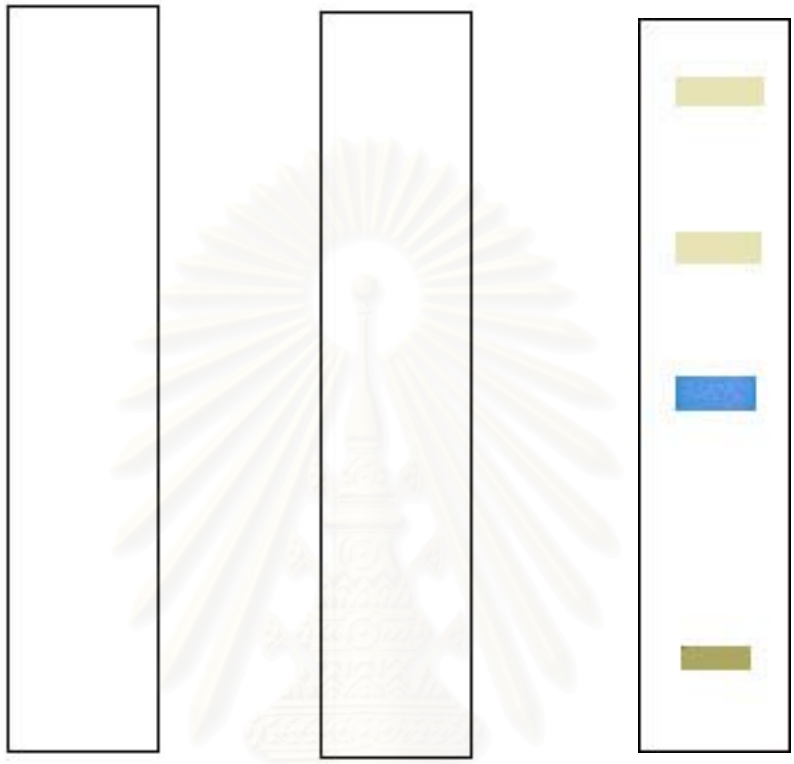
II = detection under UV light (365 nm)

III = detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated

Table 19 R_f value of components in chloroform extract of the aerial part of the *A. ilicifolius* L. system 1

Spot	R_f value	UV 254	UV 365	10 % H_2SO_4 in Ethanol
1	0.22 - 0.26	-	-	Blue
2	0.34 - 0.40	-	-	Pale yellow-violet
3	0.46 – 0.50	-	-	Light blue
4	0.53 - 0.59	-	-	Pale yellow-violet
5	0.67 - 0.70	-	-	Pale yellow

One-dimensional thin layer chromatography system 2 ($CHCl_3$: Acetone 9: 1)



I II III

Figure 37 One-dimensional TLC of the chloroform extracts of *A. ilicifolius* L.

I = detection under UV light (254 nm)

II = detection under UV light (365 nm)

III = detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated

Table 20 R_f value of components in chloroform extract of the aerial part of the *A. ilicifolius* L. system 2

Spot	R_f value	UV 254	UV 365	10 % H_2SO_4 in Ethanol
1	0.10 - 0.15	-	-	Yellow
2	0.46 - 0.52	-	-	Light blue
3	0.65 - 0.70	-	-	Pale yellow
4	0.86 - 0.70	-	-	Pale yellow

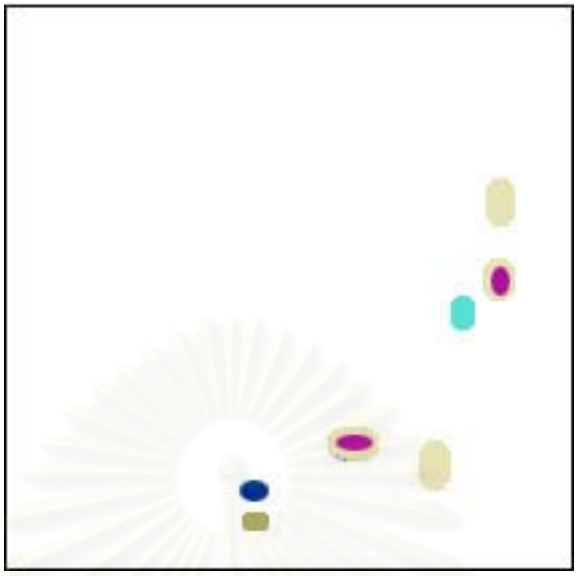


Figure 38 Two-dimensional thin layer fingerprint characteristic of chloroform extract of *A. ilicifolius* L.

Table 21 R_f value of components in chloroform extract of the aerial part of the *A. ilicifolius* L.

Spot	R_f value		Color
	X	Y	
1	0.43	0.07	Yellow
2	0.43	0.13	Blue
3	0.61	0.24	Pale yellow-violet
4	0.70	0.22	Pale yellow
5	0.78	0.46	Light blue
6	0.84	0.51	Pale yellow-violet
7	0.84	0.67	Pale yellow

Comparison chromatographic characters of authentic and purchased samples

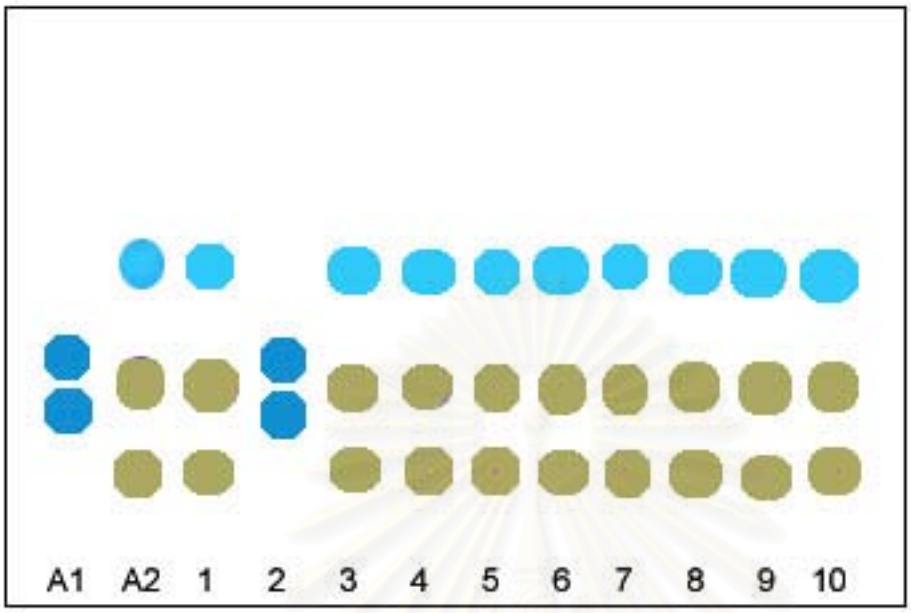


Figure 39 One-dimension TLC of methanol extracts system 1 ($CHCl_3$:MeOH 6:4) detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated

A1 = Authentic sample of *A. ebracteatus* Vahl

A2 = Authentic sample of *A. ilicifolius* L.

1 = Purchased sample from Bangkok

2 = Purchased sample from Samut Sakhon

3 = Purchased sample from Chiang Mai

4 = Purchased sample from Sukhothai

5 = Purchased sample from Khon Kaen

6 = Purchased sample from Roi Ed

7 = Purchased sample from Chanthaburi

8 = Purchased sample from Chachoengsao

9 = Purchased sample from Songkhla

10 = Purchased sample from Surat Thani

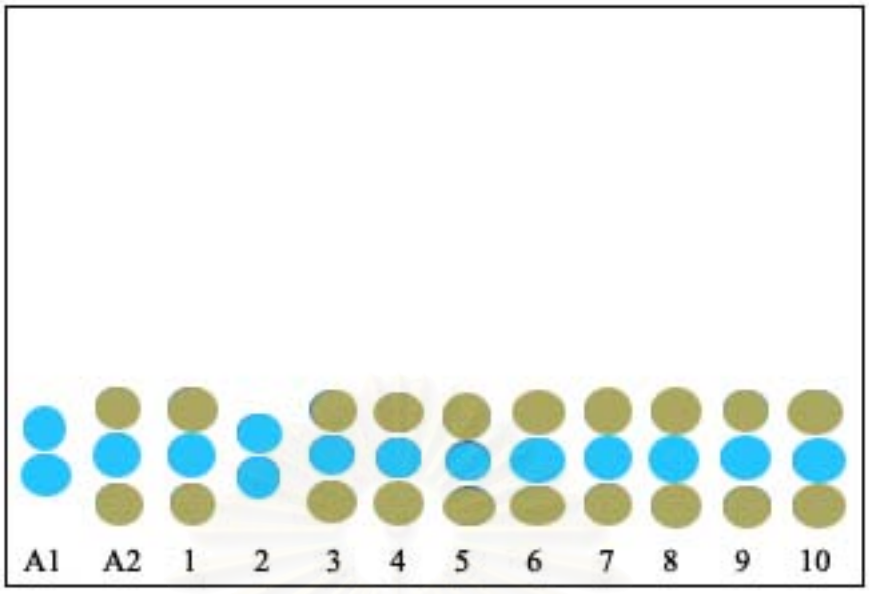


Figure 40 One-dimension TLC of methanol extracts system 2 (EtOAc: EtOH 7:3) detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated

A1 = Authentic sample of *A. ebracteatus* Vahl

A2 = Authentic sample of *A. ilicifolius* L.

1 = Purchased sample from Bangkok

2 = Purchased sample from Samut Sakhon

3 = Purchased sample from Chiang Mai

4 = Purchased sample from Sukhothai

5 = Purchased sample from Khon Kaen

6 = Purchased sample from Roi Ed

7 = Purchased sample from Chanthaburi

8 = Purchased sample from Chachoengsao

9 = Purchased sample from Songkhla

10 = Purchased sample from Surat Thani

Qualitative determination of commercial crude drugs

The crude drug samples which were purchased from traditional drugstores throughout Thailand can be distinguishable into 2 species according to the results of TLC patterns (Figure 39 - 40). *A. ebracteatus* Vahl had 1 sample which was purchased from Samut Sakhon province and the rest 9 samples are *A. ilicifolius* L. According to the above results commercial drugs could be identified and carried out further qualitative analysis as follow.

A. ebracteatus Vahl (authentic sample and purchased sample from Samut Sakhon)

	data interval (%)	mean (%)
Loss on drying	7.83 – 10.70	9.27
Total ash	10.71 – 12.62	11.67
Acid-insoluble ash	13.32 – 16.16	14.74

A. ilicifolius L.

	data interval (%)	mean (%)
Loss on drying	6.50 – 13.80	9.97
Total ash	8.42 – 12.68	10.43
Acid-insoluble ash	3.60 – 17.41	9.39

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 random sampling of crude drugs " ngueak plaa mo" from traditional

drugstores in many provinces are determined and concluded the data as an estimate percentage values in terms “ not more than” for loss on drying, total ash and acid-insoluble ash.

CHAPTER V

DISCUSSION AND CONCLUSION

Discussion

1. The morphology of the aerial part of *A. ebracteatus* Vahl and *A. ilicifolius* L. are alike. The characters used to describe distinct entities, namely bracteoles and corolla color. The diagnostic differences between *A. ebracteatus* Vahl and *A. ilicifolius* L. are as follow: The corolla of *A. ilicifolius* L. possesses and bracteoles subtending the persistent calyx. The corolla of *A. ebracteatus* Vahl on the other hand, is always white, and bracteoles are absent.

2. Leaf measurements

The specific values of the leaf measurements of *A. ebracteatus* Vahl and *A. ilicifolius* L. are individually proceeded and recorded. The minimum, mean and maximum of specific values are shown as follow: -

	Acanthus ebracteatus Vahl	*A. ilicifolius* L.
Stomatal number	254.5 to **278.9** to 318.2	254.5 to **290.6** to 318.2
Stomatal index	21.1 to **23.2** to 25.6	20.5 to **22.9** to 25.7
Palisade ratio	11.3 to **14.8** to 19.5	9.0 to **11.2** to 14.0
Vein-islet number	2.8 to **4.0** to 4.8	3.5 to **4.2** to 5.3
Veinlet termination number	6.0 to **7.3** to 8.3	3.8 to **5.3** to 6.8

By the results of leaf measurements, the values are very less different so it could not be used to distinguish between leaves of both ngueak plaa mo.

3. The main microscopic characters of *A. ebracteatus* Vahl are recognized as lignified fibers and vessels. Calcium oxalate of different shapes are found in the parenchyma cells of leaves and stems. Distinctively, calcium oxalate of *A. ilicifolius* L. on the other hand, are only found as acicular crystals.

4. One and two dimensional TLC patterns of methanol and chloroform extracts of both of ngueak plaa mo were probably used appropriately to differentiate between both drugs.

   For one-dimensional TLC (Figure 41), the methanol extract of *A. ebracteatus* Vahl gave two prominent spots instead of three spots given by *A. ilicifolius* L. Moreover, all Rf values and colors were not identical. TLC of chloroform extracts (Figure 41) showed four equivalent spots for both drugs while an extra spot was only found in *A. ilicifolius* L.

   For two-dimensional TLC patterns of chloroform and methanol extracts of both drugs were proved to be very differents. (Figure 42 and Figure 43)

   Therefor TLC patterns both one- and two-dimensional could be used to determined the identity of each kind of ngueak plaa mo with certainly.

5. The results of quality controls of these ngueak plaa mo can inform the standardization of each species as shown below.

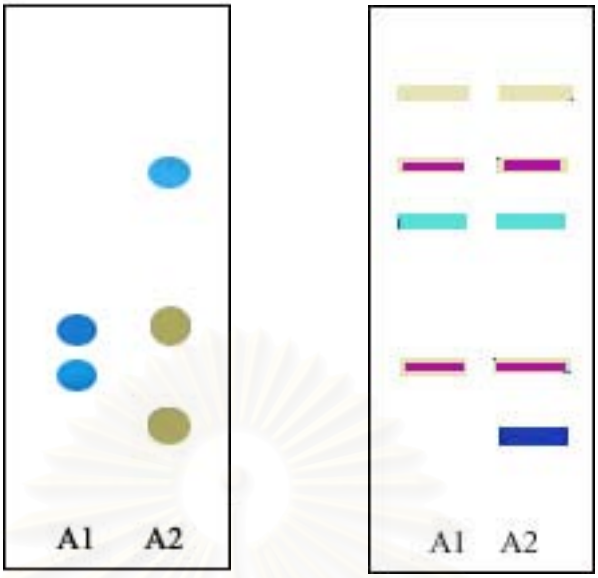
	Not more than (%)		
ngueak plaa mo	Loss on drying	Ash contents	
		Total ash	Acid-insoluble ash
A. ebracteatus Vahl	10.70	12.62	16.16
A. ilicifolius L.	13.80	12.68	17.41

6. The TLC patterns of nine samples extracted from ten crude drugs, purchased from various traditional drugstores throughout Thailand, showed the same pattern of *A. ilicifolius* L. Only one sample from Samut Sakhon is the same of *A. ebracteatus* Vahl meanwhile the authentic sample from Samut Sakhon collected in the study is *A.ilicifolius* L.

Bangkok is the central market of traditional crude drug in Thailand with only few big herbal suppliers. According to all traditional drugstores known, the herb, ngueak plaa mo, was bought from the main herbal stores in Bangkok, including Jao khrom pur, Vej ja pong. Suppliers have no interest in whether there are varieties in the types of ngueak plaa mo or not, some never even acknowledged whether the varieties do exists. The use of ngueak plaa mo has been shown to be non-specific in its varieties, which does follow the Thai herbal doctrine. However, from the study on the chemical compositions in different varieties of ngueak plaa mo, different chemical compositions were found. Therefore, there should also be a difference in the properties of ngueak plaa mo varieties. The further more pharmacological study should be done on each ngueak plaa mo varieties and collect the sample from its original source since it is not know whether the one sold in the market might not be the varieties in concern.

Conclusion

The results of this investigation clearly indicated that the macroscopic, microscopic characters and TLC patterns can be effectively used together as an important role in varieties identification both of ngueak plaa mo.

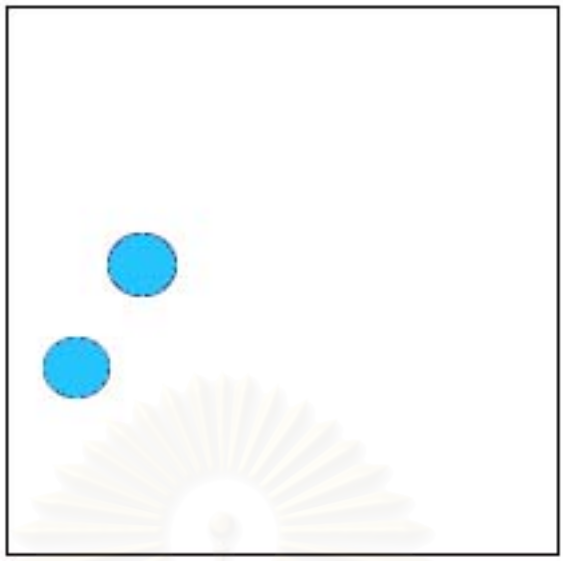


Methanol extract Chloroform extract

Figure 41 One-dimensional TLC of the extracts of ngueak plaa mo
Detection under UV light (365 nm) after spraying with 10 %
H_2SO_4 and heated

A1 = Authentic sample of *A. ebracteatus* Vahl

A2 = Authentic sample of *A. ilicifolius* L.

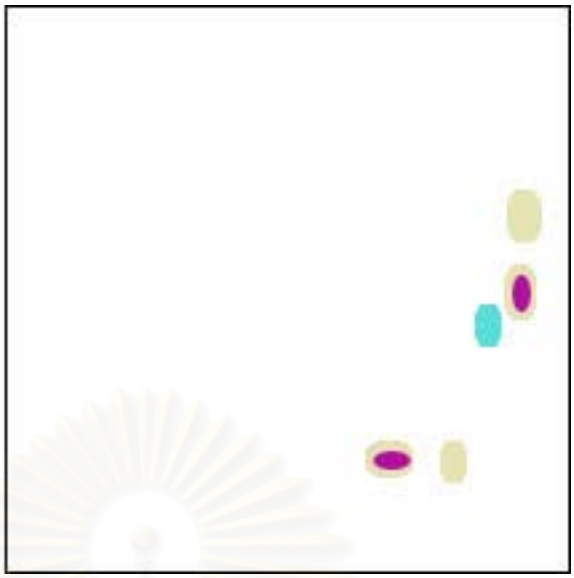


A. ebracteatus Vahl

A. ilicifolius L.

Figure 42 Two-dimensional TLC characteristics of the methanol extracts of *A. ebracteatus* Vahl and *A. ilicifolius* L.

Detection under UV light (365 nm) after spraying with 10 % H_2SO_4 and heated



A. ebracteatus Vahl

A. ilicifolius L.

Figure 43 Two-dimensional TLC characteristics of the chloroform extract of *A. ebracteatus* Vahl and *A. ilicifolius* L.

Detection under UV light (365 nm) after spraying with 10% H_2SO_4 and heated

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ในหนูขาวพันธุ์สวิสที่ทำให้เกิดอีริโทรลิวคีเมียด้วยเฟรนด์ลิวคีเมียไวรัส. วิทยานิพนธ์ปริญญามหาบัณฑิต ภาควิชาสรีรวิทยา บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย.

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