การประเมินทางเภสัชเวทของใบลิ้นงูเห่า

นางสาว จิรานุช แจ่มทวีกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชเวท ภาควิชาเภสัชเวท
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2544
ISBN 974-17-0677-4

PHARMACOGNOSTIC EVALUATION OF *CLINACANTHUS SIAMENSIS* LEAVES

Miss Jiranuch Jamtaweekul

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy

Department of Pharmacognosy
Faculty of Pharmaceutical Sciences
Chulalongkorn University

Academic Year 2001
ISBN 974-17-0677-4

Thesis Title	Pharmacognostic Evaluation of *Clinacanthus siamensis* leaves
By	Miss Jiranuch Jamtaweekul
Field of Study	Pharmacognosy
Thesis Advisor	Associate Professor Thatree Phadungcharoen, M.Sc. in Pharm.
Thesis Co-advisor	Mr. Khanit Suwanborirux, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree.

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……Thesis Co-advisor

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(Associate Professor Ekarin Saifah, Ph.D.)

…… Member

(Associate Professor Kalaya Pharadai, M. Eng. (Nuclear Tech.))

จิรานุช แจ่มทวีกุล: การประเมินทางเภสัชเวทของใบลิ้นงูเห่า (PHARMACOGNOSTIC EVALUATION OF *CLINACANTHUS SIAMENSIS* LEAVES) อาจารย์ที่ปรึกษา: รศ. ธาตรี ผดุงเจริญ อาจารย์ที่ปรึกษาร่วม: อ. ดร. คณิต สุวรรณบริรักษ์, 128 หน้า.
ISBN 974-17-0677-4

การศึกษาลักษณะทางเภสัชเวทของใบลิ้นงูเห่า สามารถกำหนดค่าคงที่ของใบที่ใช้ในการพิสูจน์เอกลักษณ์ของพืชชนิดนี้ โดยค่าของจำนวนปากใบ ดัชนีปากใบ อัตราส่วนเซลล์รั้ว จำนวนแก่งเส้นใบ และจำนวนปลายเส้นใบ มีค่าเฉลี่ยเท่ากับ 145.66, 12.34, 3.57, 3.29 และ 2.77 ตามลำดับ สำหรับค่าอัตราส่วนเซลล์รั้วของใบลิ้นงูเห่าและของใบพญายอ มีความแตกต่างอย่างเห็นได้ชัด และยังมีความแตกต่างของกระสวนขององค์ประกอบทางเคมีบนโครมาโตแกรมฉาบบางระหว่างสิ่งสกัดจากใบลิ้นงูเห่าและจากใบพญายอ นอกจากนี้ยังพบฤทธิ์การต้านเชื้อไวรัสเริมทั้งชนิดที่ 1 และ ชนิดที่ 2 ของสิ่งสกัดด้วยเอทิลอะซิเตตจากใบลิ้นงูเห่าด้วย

ภาควิชา เภสัชเวท **ลายมือชื่อนิสิต**.....
สาขาวิชา เภสัชเวท **ลายมือชื่ออาจารย์ที่ปรึกษา**.....
ปีการศึกษา 2544 **ลายมือชื่ออาจารย์ที่ปรึกษาร่วม**.....

4376563033: MAJOR PHARMACOGNOSY
KEY WORD: PHARMACOGNOSTIC EVALUATION / *CLINACANTHUS SIAMENSIS* / LIN NGU HAO / ACANTHACEAE
JIRANUCH JAMTAWEEKUL: PHARMACOGNOSTIC EVALUATION OF *CLINACANTHUS SIAMENSIS* LEAVES. THESIS ADVISOR: ASSOCIATE PROFESSOR THATREE PHADUNGCHAROEN, M. Sc. In Pharm. THESIS CO-ADVISOR: Mr. KHANIT SUWANBORIRUX, Ph.D. 128 pp. ISBN 974-17-0677-4

Pharmacognostic study of Lin ngu hao (*Clinacanthus siamensis* Brem.) leaves can be used to determine the values of leaf measurements which are the important property in the identification of this species. The values of stomata number, stomata index, palisade ratio, vein-islet number and veinlet termination are 145.66, 12.34, 3.57, 3.29 and 2.77, respectively. Not only palisade ratio of Lin ngu hao leaves obviously differs from that of Phaya yo leaves, but the TLC patterns of their leaf extracts also show differences. In addition, the ethyl acetate extracts of Lin ngu hao leaves are found to be effective against herpes simplex virus types I and II.

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

ACKNOWLEDGEMENTS

I am very grateful to my thesis advisor, Associate Professor Thatree Phadungcharoen and my thesis co-advisor, Dr. Khanit Suwanborirux, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for their advice and valuable suggestion throughout this course of this study.

I am deeply grateful to Associate Professor Dr. Vimolmas Lipipun, Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for providing anti-herpes simplex virus activity test.

I would like to express my grateful thanks to Drug analysis, the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, for giving me facilities at laboratory unit.

I would like to thank Miss Chitra Chaiwat and my colleague for their kindnesses, helpfuls and friendships.

I would like to thank the thesis committee for their suggestions to improve my thesis.

I wish to convey my thanks to the Graduate School of Chulalongkorn University for granting partial financial support of this study.

Finally, I wish to express my deep appreciation to my parents, for their love, understanding, kindnesses and encouragements

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ABBREVIATIONS

$^{\circ}C$	=	Degree Celsius
cm	=	Centimeter
conc.	=	Concentration
ED_{50}	=	50 % Effective dose
LD_{50}	=	50 % Lethal dose
m	=	Meter
μg	=	Microgram
g	=	Gram
mg	=	Milligram
kg	=	kilogram
ml	=	Milliliter
μm	=	Micrometer
mm	=	Millimeter
mm^2	=	Square millimeter
nm	=	Nanometer
No.	=	Number
S. D.	=	Standard Deviation
TLC	=	Thin-layer chromatography
UV	=	Ultraviolet
Glu	=	Glucose
gl	=	glandular trichome
cys	=	lithocyst

CHAPTER I

INTRODUCTION

Pharmacognostic evaluation was established by detailed studying of the leaf measurement, macroscopic and microscopic characters of the herbal drug, phytochemical screening, chromatographic patterns of chemical constituents and quality control.

The two species of genus *Clinacanthus* in Thailand had been reported by Tem Smitinand in the Thai Plant Names (2001) as follows (Smitinand, 2001) :

- *Clinacanthus nutans* (Burm. f.) Lindau
- *Clinacanthus siamensis* Brem.

1. *Clinacanthus nutans* (Burm. f.) Lindau (Phaya yo) is known in Thailand by the following names: Phaya yo, Phaya plong dam, Phaya plong thong (Central); Phak man kai, Phak lin khait (Chiang Mai); Phaya plong kham (Lampang); Pho-so-chaang (Karen-Mae Hong Son); Salaed pang phon tua mia (Phitsanulok) (Smitinand, 2001). Some of the vernacular names in the Southeast Asian indicate the traditional uses of the plant (Cherdchu, 1977), for example:

Keu lee beng, Chinese name meaning "for snakebite"

Pokok stawa ular, Malaysian name meaning "plant against snake"

Ubat-nga giget ular, Malaysian name meaning "medicine for snakebite"

Clinacanthus nutans (Burm. f.) Lindau is a plant of southern China, Indo-China, and Thailand. It is cultivated in the Malay Peninsula and run wild in Penang. It has been collected in Malacca (Burkill, 1935).

In Thailand, Phaya yo is used as antipyretic, anti-inflammatory, anti-snake venom, anti-dysentery and treatment of aphthous-ulcer, insect bites and rashes (นันทวัน

บุณยะประภัศร และ อรนุช โชค-ชัยเจริญพร, 2542; มหาวิทยาลัยมหิดล. คณะเภสัชศาสตร์. 2538).

In addition, it is used for dysentery in Japan (Burkill, 1935), as an antidote against snake bite and antidysentery (Perry 1980).

Clinacanthus nutans (Burm. f.) Lindau has been recommended as remedies for herpes simplex and varicella zoster virus lesions. In vitro, its virucidal activity against herpes simplex virus (Jayavasu et al., 1992a) and varicella zoster virus (Thawaranantha et al., 1992) have been reported. Clinical trials for the treatment of genital herpes and varicella zoster were reported for *Clinacanthus nutans* preparations (Jayavasu *et al*., 1992b; Sangkitporn et al., 1995; Charuwichitratana *et al*., 1996).

Methanol extracts of *Clinacanthus nutans* (Burm. f.) Lindau did not show any activity against herpes simplex virus type 2 strain G, HSV-2 (G) as determined by plaque inhibition assay (Yoosook *et al*., 1999). The ethanolic extract of C. nutans leaves can not antagonize the action of cobra venom (Thongharb and Tejasen, 1977).

However, a flavonoid compound isolated from butanol extracts of this plant possessed anti-inflammatory activity. (Chuakul, 1986; Kittisiripornkul, 1984 and Tanasomwang, 1986). A clinical trial on the use of an alcohol extract of C. nutans leaves was carried out at the hospital in Pracheenburi, Thailand. It was effective against aphthous ulcers and herpes simplex. The extract accelerated wound healing and lowered the inflammation (Chotikieat and Pitiporn, 1989).

The water extract of *C. nutans* leaves can antagonize the action of *Naja naja siamensis* venom in rat (Utogapachana, 1975; Thongharb, 1976). But it was found that the aqueous extract of *C. nutans* leaves has no effect on the inhibition of neuromuscular transmission produced by purified *Naja naja siamensis* neurotoxin in isolated rat phrenic-nerve diaphragm preparations (Cherdchu *et al*., 1975) and the aqueous extract of C. nutans leaves failed to protect mice treated with lethal dose of sea-snake venom (*Laticauda colubrina*) (Levey, 1969).

Since a number of experiment reported that the leaves extract have antiviral and anti-inflammatory activities. So Phaya yo is a herbal drug which selected as herb of choice in the National List of Essential Drugs A.D. 1999 to be used against herpes simplex virus, varicella- zoster virus, rashes and allergy.

2. *Clinacanthus siamensis* Brem. is known in Thailand by the following name:

Lin ngu hao, Thai name meaning "cobra's tongue"

This plant species is found cultivated or grown wild throughout Thailand such as several medicinal plant garden of many institutes in Bangkok, Nonthaburi Province, Nakorn pathom Province, Rayong Province, Chachuang-chao Province and found naturally grown in Chantaburi Province (Makham) and Rayong Province (Chamao).

There are many research works of *C. nutans* (Burm. f.) Lindau including the Pharmacognostic character in the Specification of Thai Medicinal Plants, Vol. I. (1986). Up to now, there are no reports on pharmacognostic and phytochemical studies of *C. siamensis* Brem. In addition, our preliminary test on anti-herpes simplex virus activity of *C. siamensis* leaves, it was found that the ethyl acetate extract of each parts (stem, young leaves and mature leaves) of *C. siamensis* have activity against herpes simplex virus types I and II. Therefore, this investigation deals with the pharmacognostic characterization and examination of *C. siamensis* leaves collected at regular monthly intervals from the same plant throughout the year which might give some indications of seasoning variation in activities. The results of this work are expected to provide valuable information to inform the standardization of this species.

CHAPTER II

HISTORICAL

Botanical aspect

Plants of the family ACANTHACEAE are herbaceous or climbing, rarely somewhat shrubby; leaves opposite, often with distinct cystoliths; stipules absent; flowers, zygomorphic, often with conspicuous bracts; calyx-segments or lobes 4 or 5, imbricate or valvate, rarely the calyx reduced to a ring; corolla sympetalous, 2-lipped or sometimes 1-lipped, lobes imbricate or contorted; stamens 4, didynamius, or 2, inserted on the corolla-tube and alternate with its lobes; filaments free amongst themselves, or partially connate in pairs; anthers 2-locular or 1-locular by reduction, loculi confluent or separated, sometimes one much smaller than the other, opening lengthwise; disk present; ovary superior, sessile on the disk, 2-locular; style simple; ovules axile, 2 or more in each loculus; fruit a capsule, often club-shaped, mostly elastically dehiscent from the apex downwards, the valves recurved and leaving the central axis; seeds mostly with indurated funicle; endosperm rarely present; embryo large (Bendle, 1952; Hutchison, 1959).

Genus *Clinacanthus*

Clinacanthus Nees is a very small genus in the Acanthaceae. The plants in this genus are found distributing from southern China to Malaysia, young leaves are edible in Vietnam (Mabberly, 1997).

Flowers in dense cymes at the tops of the branches and their branchlets, resupinate; bracts narrow; calyx deeply 5-partite; segments narrow; corolla-tube long, curved, widened upwards, inside above the ovary with a whorl of hairs; limb bilabiate; upper lip forming a prolongation of the corolla-tube, turned downwards, shortly bilobate, innermost in bud, lower one slightly recurved, much broader than the upper

lip, of about the same length, 3-lobed; median lobe outermost in bud; stamens 2, inserted in the throat, subequalling the upper lip; anthers 1-celled, ecalcarate; staminodes none; ovary compressed; ovary-cells 2-ovuled; style filiform, shortly bidentate; capsule basally contracted into a short, solid stalk, oblong, 4-seeded. Leaves opposite, ovate-oblong-lanceolate, with linear cystoliths. Shrub; branchlets erect-drooping or clambering over plants (Backer and Bakhuizen van den Brink, 1965; Benoist, 1935).

According to The Index Kewensis and its supplements, there are only 3 species of this genus as shown below:-

1. *Clinacanthus nutans* (Burm. f.) Lindau (*Clinacanthus Burmanni* Nees) (Figure 1.)

The plant is a shrub with pubescent branches. Leaves are simple, opposite, narrowly elliptic oblong or lanceolate, 2.5-13 cm long, 0.5-1.5 cm wide. The leaves have apex acute or acuminate; margin exsculptate-dentate or subentire; base cuneate, obtuse, rounded or truncate often oblique; pubescent on the nerves; petiole 3-15 mm long. Flowers are in dense cymes at the top of the branches and their branchlets; cymes 5-∝ flowered, often terminating drooping horizontal branches but themselves erect, subsecund, combined into a large lax, leafy panicle. Each flower has calyx densely patently glandular-pubescent, about 1 cm long; corolla glandular-pubescent, about 3.5 cm, dull red with green base; lower lip (turned upwards) with yellow streaks, apically sordidly yellow or greenish yellow; stamens 2, inserted in the throat, more or less appressed against the upper lip. Ovary is compressed, 2-celled, 2 ovules in each cell; having style filiform, shortly bidentate. Capsule is oblong, basally contracted into a short, solid stalk, 4-seeded (Backer and Bakhuizen van den Brink, 1965; Benoist, 1935).

Scandent shrub, erectdrooping, leave: opposite, ovate- or oblong-lanceolate, tapering or long acuminate, exsculptate-dentate or subentire, base often oblique, cuneate, obtuse or rounded, pubescent on the nerves, 2-3 cm wide and 7-9 cm long, yellowish green to dark green; petiole 0.5 mm long: inflorescence in dense cyme, terminal; flowers 3-4 cm long, bilabiate; calyx 5, green, united at the base, glandular-

pubescent, 10-16 mm long; corolla tube long, dull red with a green base, lower lip 3-lobed, with yellow streaks, apically pale yellow or greenish yellow, turn upwards, much broader than the upper lip and about the same length, upper lip turn downwards, shortly bilobate; stamens 2, epipetalous, subequalling the upper lip; ovary 1, compressed, 2-celled; bracteoles 3.5-10 mm long, green: fruit a capsule, basally contracted into a short solid stalk, oblong, 4-seeded (Hooker, 1979 and Ridley, 1967).

2. *Clinacanthus spirei* R.Ben.

Tiges cylindriques, striees longitudinalement, glabres. Feuilles brievement petiolees, lanceolees, arrondies a la base, acuminees au sommet, a bord entier, recourbe en dessous, glabres, mais un peu rudes sur les 2 faces, longues de 6-10 cm. sur 1.5-3 cm Inflorescence: fleurs en epis denses, terminaux, penches; bractees largement lanceolees, a sommet arrondi, obtus, pubescentes-glanduleuses, longues de 20-22 mm. –Sepales 5, lineaires, pubescents-glanduleux, libres des la base, longs de 10 mm. environ. Corolle longue de 35-40 mm., le tube legererement courbe et s elargissant graduellement jusqu a la gorge; levre superieure lanceolee, echancree au sommet; l inferieure brievement trilobee. Etamines 2, a filets poilus. Ovaire et base du style poilus (Hill, 1931-1935; Benoist, 1935).

3. *Clinacanthus siamensis* Brem. (Figure 2.)

Caulis ramique an initio glabri, subteretes, leviter striati; internodia 1-4 cm. Longa et 2.5-3.0 mm. diam. Folia petiolo glabro plerumque 2-3 cm. Longo instructa; lamina lanceolata, plerumque 11-16 cm. Longa et 3-5 cm. Lata, apice acuminata, basi acuta vel subacuta, rarius paulum asymmetrica, costa subtus prominula, nervis utroque latere costae plerumque 5 vel 6. Inflorescentia corynbosa, nutans, rachidibus pedicellisque puberulis. Flores resupinati, bracteis linearibus 5-7 mm. longis suffulti. Pedicelli circ. 2 mm. longi. Calycis lobi lineares, 12 mm. longi, ut bracteae pilis capitatis puberulo-hirtelli. Corolla 5 cm. longa, extus pilis capitatis parce et vix notabile puberula, intus prope basin breviter pubescens, labiis 15 mm. longis, supero deltoideo, ad basin 6 mm. lato, apice obtusis, mediano conduplicato. Granula pollinis globosa, eis generis Pseuderanthemi similiora, plurima parva et sterilia, aliquae tamen

normalia, 46 μ diam. Discus annularis glaber. Ovarium glabrum, 2.5 mm, altum; stylus glaber 4.5 cm. longus; stigma breviter bilobatum. Capsula nondum visa. (Munksgaad, 1961)

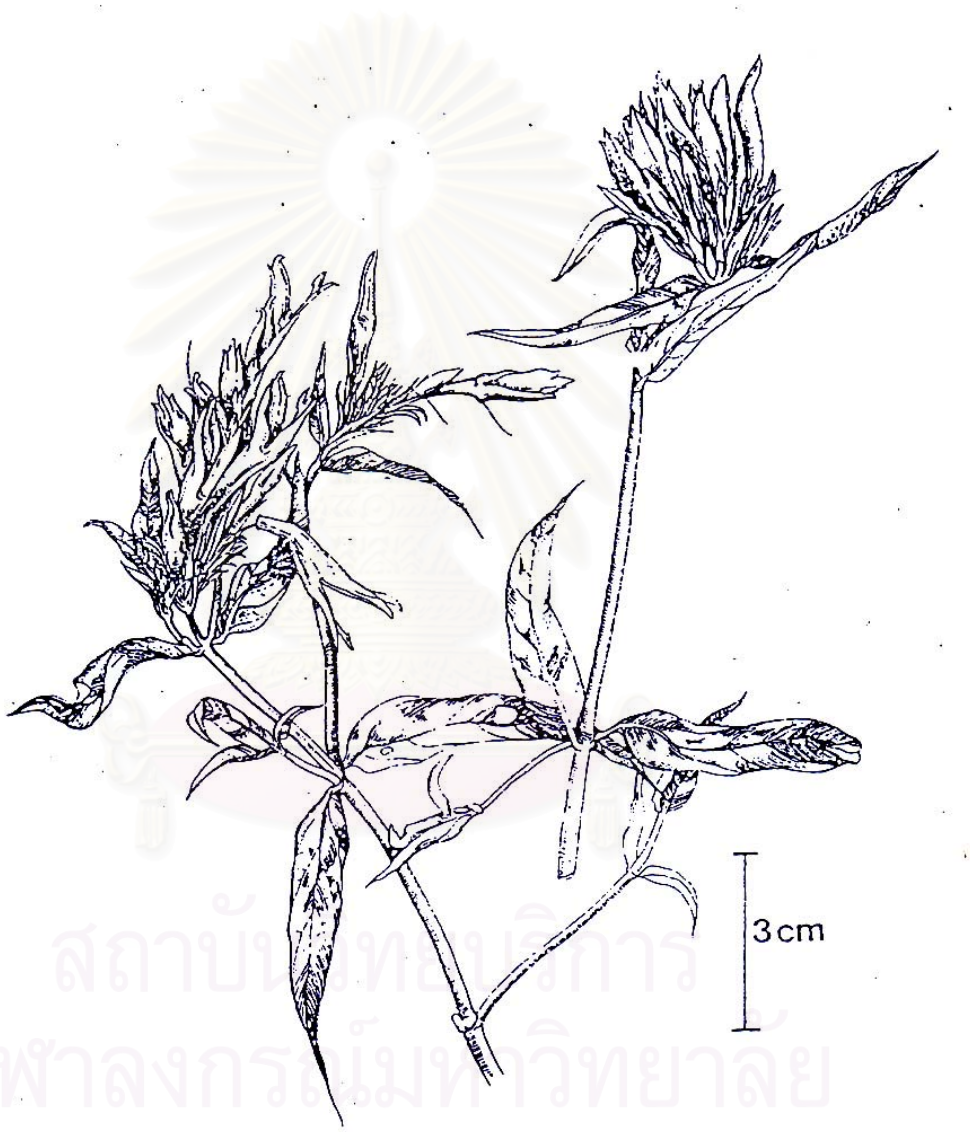


Figure 1. *Clinacanthus nutans* (Burm. f.) Lindau (Somanabandhu, et al. 1986)

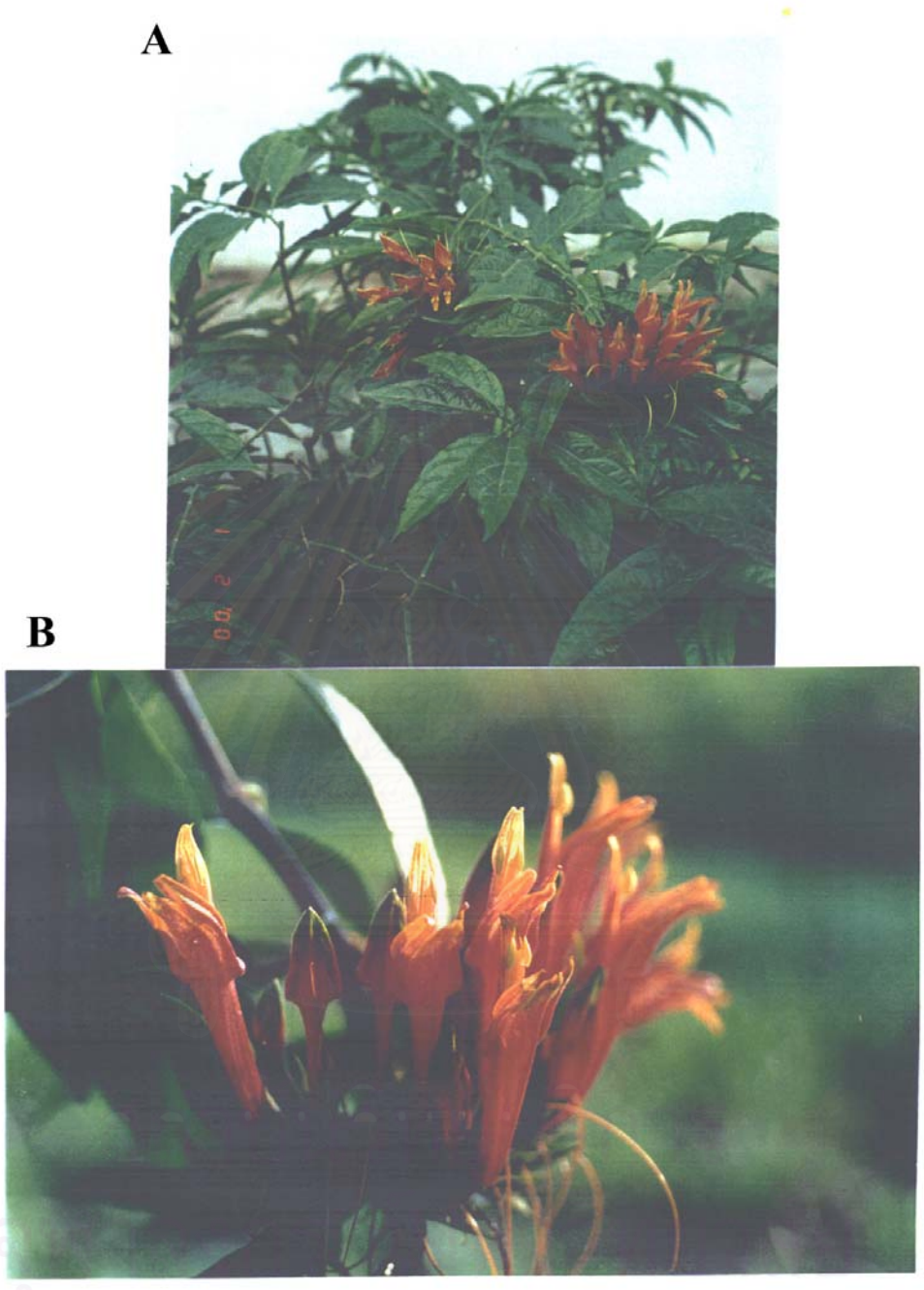


Figure 2. *Clinacanthus siamensis* Brem.

A. Photography by Phadungcharoen, T.,2000 and

B. Photography by Suwanborirux, K., 2002

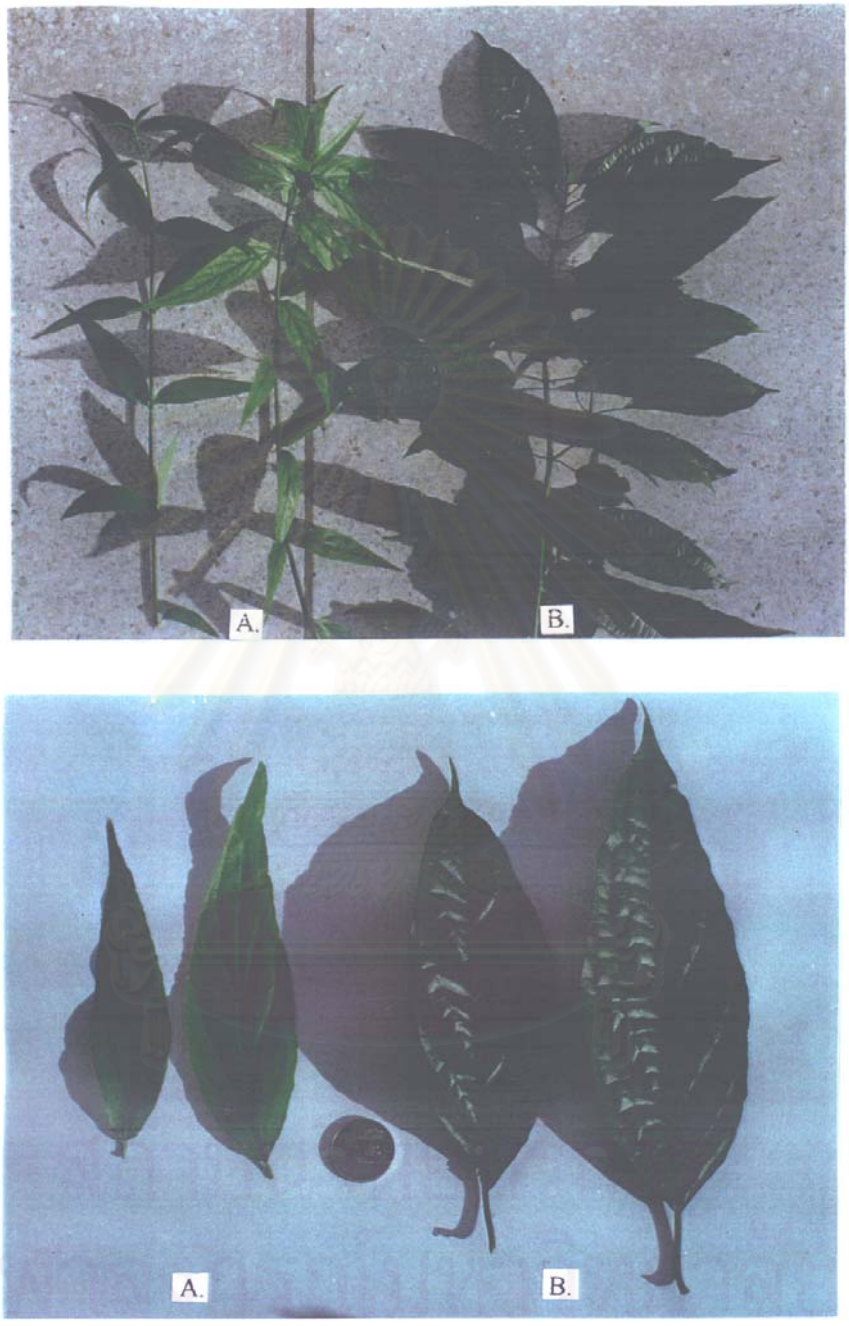


Figure 3. Comparison between A. *Clinacanthus nutans* (Burm. f.) Lindau and B. *Clinacanthus siamensis* Brem.

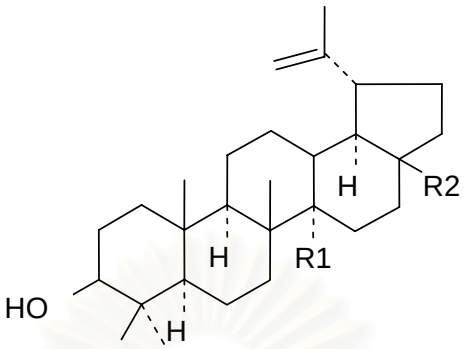
The chemical constituents and biological activities of plants in genus *Clinacanthus*

The chemical constituents

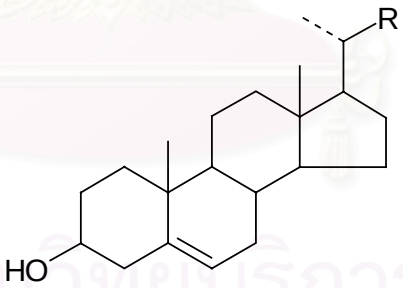
So far phytochemical investigation of plant in this genus has been done mainly in *Clinacanthus nutans* (Burm. f.) Lindau (Phayaa yo) and the results of search investigation are summaried in Table 1.

Table 1. Chemical Constituents of *Clinacanthus nutans* (Burm. f.) Lindau

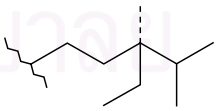
Chemical compound	Category	Plant part	References
Lupeol **[1]**, betulin **[2]**, β-sitosterol **[3]**, stigmasterol **[4]**	Terpenoids	Roots	Lin, et al, 1983
Lup-20 (29)-ene-3-one, lupeol, β-sitosterol, stigmasterol	Terpenoids	Stems and leaves	Dampawan, et al., 1997
C-glycosyl flavones: Vitexin **[5]**, isovitexin **[6]**, shaftoside **[7]**, isomollupentin 7-O-*β*-glucopyranoside **[8]**, Orientin **[9]**, isoorientin **[10]**	Glycosylflavones	Stems and leaves	Teshima, et al., 1997
Sulfur containing glucosides: (E)-3-methylsulfonyl-2-propenyl *β*-D-glucoside (clinacoside A) **[11]**, (E)-3-methylsulfinyl-2-propenyl *β*-D-glucoside (clinacoside B) **[12]** , clinacoside C **[13]**, cycloclinacoside A1**[14]**, cycloclinacoside A2 **[15]**	Glucosides	Stems and leaves	Teshima, et al., 1998



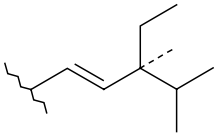
	R1	R2
[1] lupeol	H	CH_3
[2] betulin	CH_3	CH_2OH

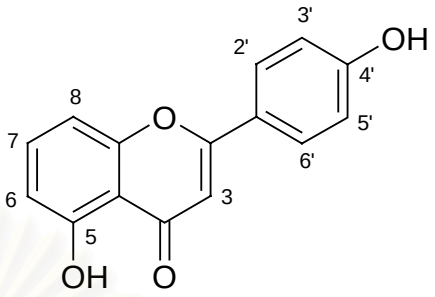


[3] β Sitosterol R =

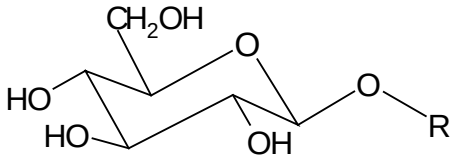


[4] Stigmasterol R =

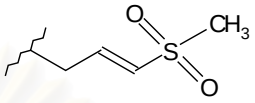


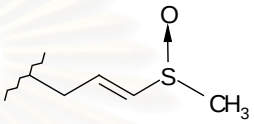


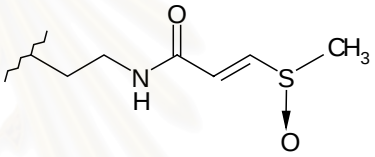
	6	7	8	3’
[5] vitexin	H	OH	Glu	H
[6] isovitexin	Glu	OH	H	H
[7] schaftoside	OH	Glu	H	
[8] isomollupentin7-O-β-gluco pyranoside	Ara	O-Glu	H	H
[9] orientin	H	OH	Glu	OH
[10] isoorientin	Glu	OH	H	OH

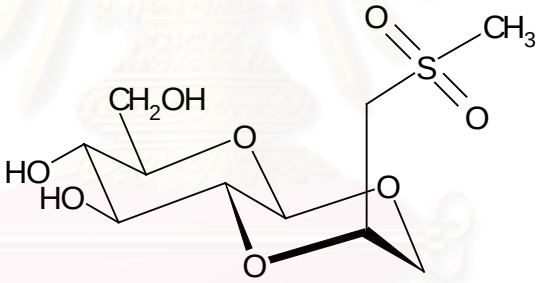


R

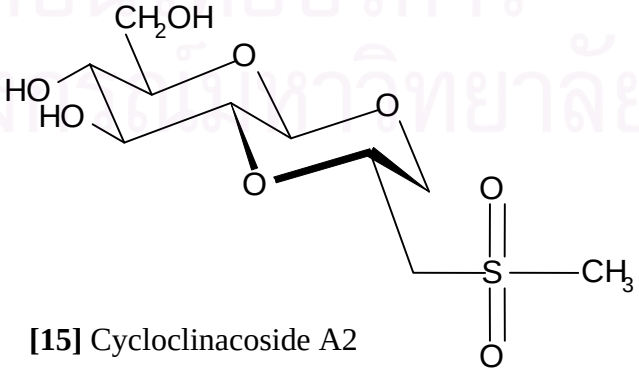
[11] Clinacoside A 

[12] Clinacoside B 

[13] Clinacoside C 



[14] Cycloclinacoside A1



[15] Cycloclinacoside A2

The biological activities

The pharmacological activities of *Clinacanthus nutans* (Burm. f.) Lindau extracts were shown in Table 2.

Table 2. The biological activities of *Clinacanthus nutans* (Burm. f.) Lindau extract

Part used	Activity	References
Leaves (water extract)	Anti-cobra venom activity	Utogapachana, 1975; Tongthab, 1976
Leaves	Anti-herpes simplex type II and anti-herpes zoster	ชื่นฤดี ไชยวสุ และคณะ, 2535 สมชาย แสงกิจพร และคณะ,2536
Leaves (buthanol extract)	Analgesic activity and anti-inflammatory activity	Satayavivad *et al*., 1996

The toxicity of *Clinacanthus nutans* (Burm. f.) Lindau extracts were shown in Table 3.

Table 3. The toxicities of *Clinacanthus nutans* (Burm. f.) Lindau extract

Part used	Toxicity	References
Leaves (95 % ethanolic extract)	Acute toxicity (mices model, LD_{50} of oral dose > 60 g/kg, LD_{50} of subcutaneous injected dose = 20 g/kg and LD_{50} of intraperitoneal = 12 g/kg)	Satayavivad et al., 1996

Table 3 (continued) The toxicities of *Clinacanthus nutans* (Burm. f.) Lindau extract

Part used	Toxicity	References
Leaves (n-buthanol extract)	Sub-chronic toxicity : thymus gland weight were decreased and liver weight were increased (rat model, 1.3 g/kg/day for 6 weeks by oral administration) Gastric ulcerogenicity : 270 and 540 mg/kg/day for 6 weeks by oral administration, size of thymus glands were decreased	Satayavivad *et al*., 1996
Leaves (ethanol extract)	Chronic toxicity : weight and platelet were decreased (mices model, at doses 0.01, 0.1 and 1 g/kg)	ปราณี ชวลิตธำรง เอมมนัส อัตต-วิชญ์ พัช รักษามั่น และ ปราณี จันทเพ็ชร, 2538
Leaves (95 % ethanol extract)	Skin irritation : skin rashes (gazbies model, dose of 100 g % were found to irritrate skin and 200 g % were caused of skin rashes)	Kittisiripornkul, 1984

CHAPTER III

PHARMACOGNOSTIC STUDY

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

Apparatus for microscopic measurements

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

1. Micrometers

Two scales are required, known, respectively, as a stage micrometer and an eyepiece micrometer. The stage micrometer is a glass slide 7.6×2.5 cm with a scale engraved on it. The scale is usually 1 mm long and is divided into 0.1 and 0.01 parts of a millimeter. The eyepiece micrometer may be a linear scale (Figure 4.) or it may be ruled in squares. The value of one eyepiece division is determined for every optical combination to be used, a note being made in each case of the objective eyepiece and length of draw-tube.

Accurate measurement of a stage micrometer and an eyepiece micrometer was to run follow below steps:

1. Unscrew the upper lens of the eyepiece, place the eyepiece micrometer on the ridge inside, and replace the lens.
2. Put the stage micrometer on the stage and focus it in the ordinary way.
3. It will be seen that the two lines of the stage micrometers coincides with the two lines of the eyepiece when the 4 mm objective eyepiece is in use.
4. The distance between two lines on the stage scale is equal to the numbers of the small eyepiece divisions. Then you can calculate the distance of 1 eyepiece division.

   For example: the two micrometer scales now appear as in Figure 4B, when the 7 th line of the stage micrometer coincides with the 0 of the eyepiece, the 10 of the stage coincides with 7.7 of the eyepiece. As the distance between 7 and 10 on the stage scale is 0.3 mm, 77 of the small eyepiece divisions equal 0.3 mm or 300 μm; therefore, 1 eyepiece division equals 300/77 or 3.9 μm.

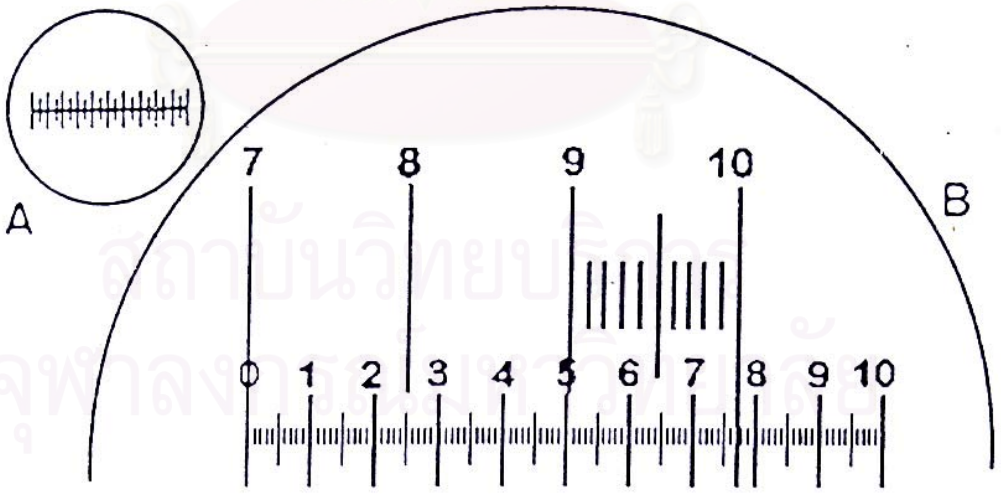


Figure 4. A. Eyepiece micrometer
B. Eyepiece micrometer superimposed on portion of stage micrometer scale (Trease and Evans, 1996)

2. Drawing attachment (Olympus model BH2-DA)

The drawing attachment (Figure 5.) is used to visually superimpose the image of a specimen over the surface image of a drawing paper placed beside the microscope so that the specimen image can be traced on the paper. Different from the ordinary microprojection system where an image is projected on a screen, the model BH2-DA permits drawing in a bright room without any more light intensity than required by ordinary microscopy. If the drawing surface is dark, increase the brightness of the room by additional used of a lamp to illuminate the drawing surface, which makes drawing easier.

Figure 5. Drawing attachment (Olympus, model BH2-DA)

To do this, mount the drawing attachment on the microscope and insert the photo eyepieces into it. Place a drawing paper on the desk just under the mirror. The setting position of the magnification adjustment screw varies depending on the microscope in use. As the distance between the mirror and drawing surface changes, the drawing magnification and area are varied. The magnification adjustment screw is set at position C (Figure 5.), the microscope in used is CH model.

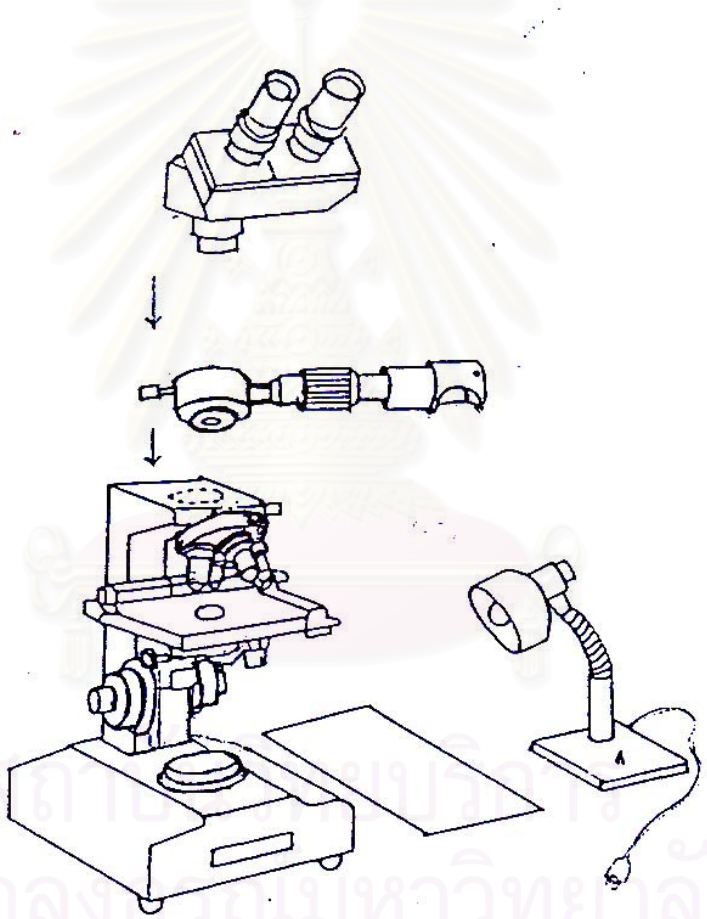


Figure 6. The assembly and operation to mount the drawing attachment on the microscope. A lamp help to illuminate the drawing surface.

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

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

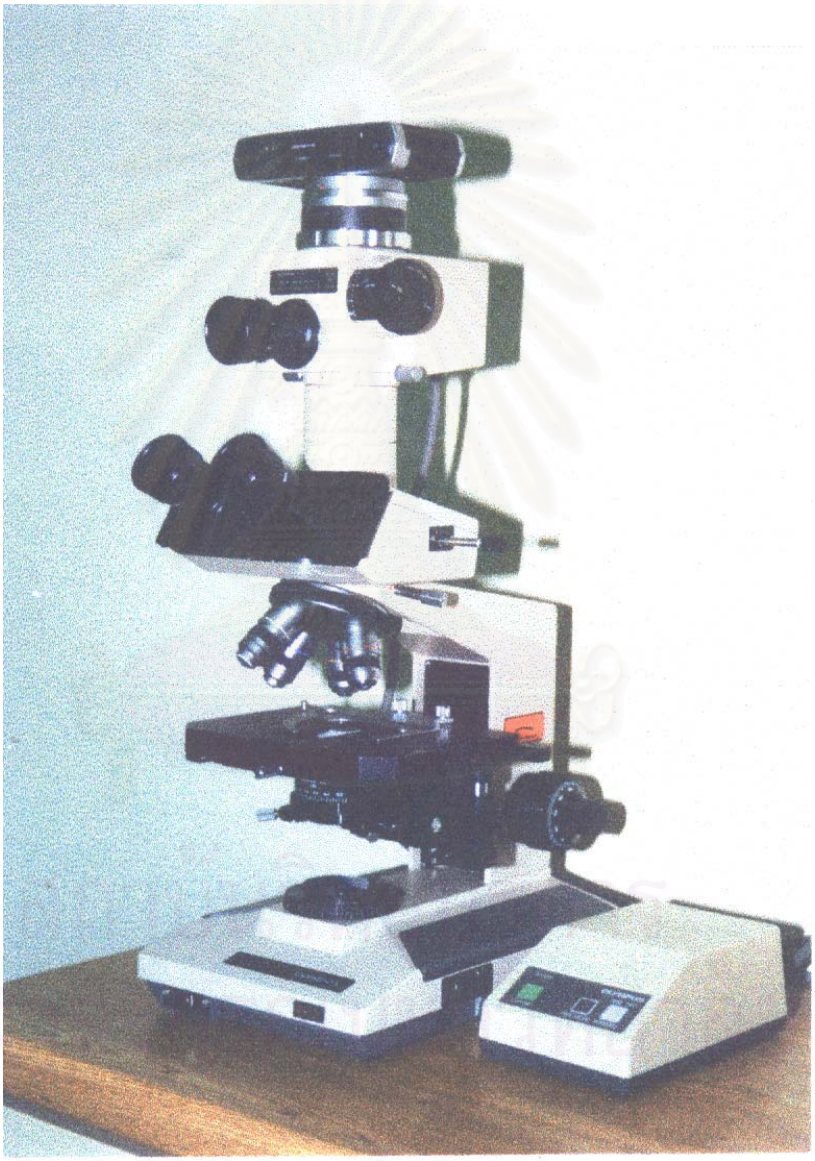


Figure 7. Microscope with Photomicrographic equipment

Leaf measurement

A number of leaf measurements are used to distinguish between some closely related species not easily characterized by general microscopy.

1. Stomatal Number and Stomatal Index
2. Palisade Ratio
3. Vein-islet Number
4. Veinlet Termination Number
5. Glandular trichome Number and Glandular trichome index
6. Lithocyst Number and Lithocyst Index

Stomata

Stomata are openings (the stomatal pores or apertures) epidermis bounded by two specialized epidermal cells, the guard cells, which by changes in shape bring about the opening and closure of the aperture. It is convenient to apply the term stoma to the entire unit, the pore and the two guard cells. The stoma may be surrounded by cells that do not differ from other guard cells of the epidermis. On the other hand, in many plants the stomata are flanked or surrounded by cells that differ in shape and sometimes also in content from the ordinary epidermal cells. These distinct cells are called subsidiary cell of the stoma. The subsidiary cells may or may not be closely related ontogenetically to the guard cells (Esau, 1960; Eames and MacDaniels, 1974).

With regard to the subsidiary cells, the dicotyledons show four principal stomatal types: type A (Figure 8., A), no subsidiary cells are present, several ordinary epidermal cells irregularly surround the stoma (anomocytic or irregular-celled type); type B (Figure 8., B), three subsidiary cells, one distinctly smaller than the other two, surround the stoma (anisocytic or unequal-celled type); type C (Figure 8., C), one or more subsidiary cells flank the stoma paralled with the long axis of the guard cells (paracytic or paralled-celled type); type D (Figure 8., D), on pair of subsidiary cells,

with their common walls at right angles to the long axis of the guard cells, surrounds the stoma (diacytic or cross-celled type) (Esau, 1960).

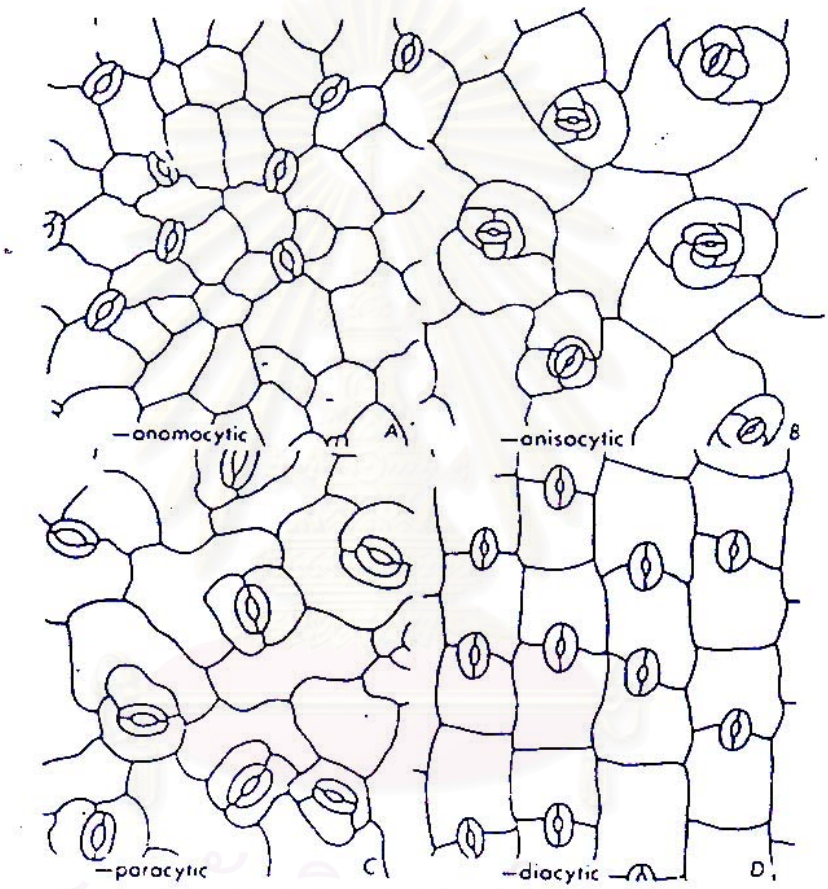


Figure 8. Epidermis in surface view illustrating patterns formed by guard cells and surrounding cells.

A. anomocytic
B. anisocytic
C. paracytic
D. diacytic

Stomatal Number

The significance of the number of stomata per unit area of leaf was investigated by Timmerman in 1927 (Youngken, 1948). The actual number of stomata per square millimeter is variable for the same plant, this brings especially noticeable if records are made for different years (Wallis, 1960). The average number of stomata per square millimeter of epidermis is termed the stomata number. In recording result the range as well as the average value should be recorded for each surface of the leaf and the ratio of values for the two surfaces. In certain cases this ratio may be of diagnostic importance (Trease and Evans, 1996).

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

Stomatal Index

The significance of the number of stomata per unit area of leaf was investigated by Timmerman in 1927. Salisbury showed that a high correlation coefficient exists between the number of stomata and the number of epidermal cells per unit area of leaf surface of a given species (Youngken, 1948). The stomatal index expresses 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 and I = stomatal index. While stomatal number varies considerably with the age of the leaf, stomatal index is highly constant for a given species and may be determined on either entire or powdered samples (Trease and Evans, 1996).

Palisade Cells

Palisade cells are a type of photosynthetic cells of the mesophyll of a leaf occurring mostly just beneath the upper epidermal surface layer (Esau,1972). The cells are elongated and more or less cylindrical and arranged in one or more rather regular, relatively compact layers near the ventral, or upper side of the leaf with the long axis of the cells perpendicular to the leaf surface (Eames and MacDaniels, 1974).

Palisade Ratio

The palisade cells of the mesophyll bear a definite relation of the epidermal cells (Wallis, 1960).The term “palisade ratio” was introduced by two British pharmacognosists, T.E. Wallis and T. Dewar, in 1933. It represents a figure obtained by counting the total number of palisade cells beneath four upper epidermal cells and dividing the number by four.(Youngken, 1948). The average number of palisade cells beneath each upper epidermal cell is termed the palisade ratio. Quite fine powders can be used for the determination (Trease and Evans, 1996).

Vein-islet Number

Vein-islets are divisions of green leaf tissue formed by the ultimate divisions of the conducting strands of vascular bundles which either completely or partially surround areas of the chlorenchyma. The islets increase in size as the leaf matures, the full grown leaf showing a constancy in vein-islet number (Youngken, 1948). 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/mm^2 calculated from four contiguous square millimetres in the central part of the lamina, midway between the midrib and the margin, is termed the vein-islet number. When determined on whole leaves, the area examined should be from the central part of the lamina, midway between the margin and midrib (Trease and Evans, 1996).

Veinlet Termination Number

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

Thin-layer chromatography (TLC)

TLC, which together with paper chromatography comprise “ planar” or “flatted” chromatography, is the simplest of all of the widely used chromatographic methods to perform. A suitable closed vessel containning solvent and a coated plate are all that are required to carry out separations and qualitative and semiquantitative analysis. With optimization of techniques and materials, highly efficient separations and accurate and precise quantification can be achieved. TLC can be used also for preparative scale separations by employing specialized apparatus and techniques.

Basic TLC is carried out as follows. An initial zone of mixture is placed near one end of the stationary phase, a thin layer; the sample is dried; and the end of the stationary phase with the initial zone is placed into a mobile phase, usually a mixture of pure solvents, inside a closed chamber. The components of the mixture migrate at different rates during movement of the mobile phase through the stationary phase, which is termed the development of the chromatogram. When the mobile phase has moved an appropriate distance, the stationary phase is removed, the mobile phase is rapidly dried, and the zones are detected by application of a suitable visualization reagent.

Differential migration is the result of varying degrees of affinity of the mixture components of the stationary and mobile phases. Different separation mechanisms are involved, the predominant forces depending on the exact nature of the two phases and the solutes. The interactions involved in determining chromatographic retention and

selectivity include hydrogen bonding, electron-pair donor/electron-pair acceptor (charge transfer), ion-ion, ion-dipole, and van der Waals interactions. Among the latter are dipole-dipole, dipole-induced dipole, and instantaneous dipole-induced dipole interactions.

Sample collection, preservation, and purification are problems common to TLC and all other chromatographic methods. For complex samples, the TLC development will usually not completely resolve the analyte (the substance to be determined) from interferences unless a prior purification is carried out. This is most often done by selective extraction and column chromatography. In some cases, substances are converted, prior to TLC, to a derivative that is more suitable for separation, detection, and/or quantification than the parent compound.

Detection is most simple when the compounds of interest are naturally colored or fluorescent or absorb ultraviolet (UV) light. However, application of a location or visualization reagent by spraying or dipping is usually required to produce color or fluorescence for most compounds. Absorption of UV light is common for many compounds, e.g., aromatics and those with conjugated double bonds. This leads to a simple, rather universal detection method on layers impregnated with a fluorescence indicator (fluorescence quench detection).

Compound identification in TLC is based initially on R_f values compared to authentic standards. R_f values are generally not exactly reproducible from laboratory to laboratory or even in different runs in the same laboratory, so they should be considered mainly as guides to relative migration distances and sequences. Factors causing R_f values to vary include: dimensions and type of the chamber, nature and size of the layer, direction of mobile phase flow, the volume and composition of the mobile phase, equilibration conditions, humidity, and sample preparation methods preceding chromatography (Sherma,1991).

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Phytochemical screening

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

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

Quality Control

Loss on drying

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

Ash content

The determination of total ash values to carbon must be removed at as low a temperature (450 °C) as possible because alkali chlorides, which may be volatile at high temperatures, would otherwise by lost. If carbon is still present after heating at a moderate temperature, the water-soluble ash may be separated and the residue again ignited as described in the *BP*, or the ash may be broken up, with the addition of alcohol, and again ignited (see *USP*). The total ash usually consists mainly of carbonates, phosphates, silicates and silica. To produce a more consistent ash the *EP* and *BP* use a sulphated ash, which involves treatment of the drug with dilute sulphuric acid before ignition. In this all oxides and carbonates are converted to sulphates and the ignition is carried out at a higher temperature (600 °C) (Trease and Evans, 1996).

Extractive values

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

CHAPTER IV

EXPERIMENTAL

Scopes of investigation

1. Microscopical determination of leaf measurements which may usefully be made in certain cases are stomatal number, stomatal index, palisade ratio, vein-islet number and veinlet termination number.
2. Study of the macroscopic and microscopic characters of *Clinacanthus siamensis* leaves.
3. Study of anti-herpes simplex virus of ethyl acetate extracts of *Clinacanthus siamensis* leaves in each month.
4. Thin-layer chromatographic patterns of methanol extracts of *Clinacanthus siamensis* leaves from several sources.
5. Thin-layer chromatographic patterns of ethyl acetate extracts of *Clinacanthus siamensis* leaves.
6. Phytochemical screening
7. Quality controls of crude drugs which were collected from several sources according to the Pharmacopoeia: loss on drying, total ash, acid-insoluble ash and extractive value.

Part I Leaf Measurements

1. Materials

a) The *Clinacanthus siamensis* leaves (mature leaves) were collected from several sources and were authenticated by comparison with the herbarium specimens of the Forest herbarium, Royal forest department, Bangkok and at herbarium of the Botany and Weed Division, Department of Agriculture,

Ministry of Agriculture and Co-operation, Bangkok. Eight locations have been selected for plant collection as follows:

- The Faculty of Pharmaceutical Sciences, Chulalongkorn University (November, 2001)
- The Department of Medical Sciences, Ministry of Public Health, Nonthaburi province , the leaves were collected during the fourth week of every month between march 2001 and February 2002
- The Khao Hin Chon, Chachongshao Province (November, 2001)
- The Somdej Phra Thepratanarajasuda Medicinal Plants Garden, Petroleum Authority of Thailand, Rayong Province (November, 2001)
- Khao Chamao, Waterfall, Khao Chamao-Kao Wonge National Park, Rayong Province (October, 2001)
- Kasetsart University, Bangkok (January, 2002)
- Khanit's garden, Buddhamonthon sai 3, Bangkok (January, 2002)
- The Sireerukachart Garden, Mahidol University, Salaya Campus, Nakhon Pathom Province (January, 2002)

b) *Clinacanthus nutans* leaves were collected from two locations:

- The Faculty of Pharmaceutical Sciences, Chulalongkorn University (November, 2001)
- The Department of Medical Sciences, Ministry of Public Health, Nonthaburi province (January, 2002)

c) Chloral hydrate B.P.

d) Distilled water

e) Glycerin U.S.P.

2. Apparatus

a) Microscope (Olympus, model CH)

b) Drawing attachment (Olympus, model BH-2DA)

c) Day-light lamp

3. Procedure

a) Preparation of leaves

The pieces of leaf from the middle of the lamina about 25 mm square were cleared by gently warming in chloral hydrate solution (4 g/ml in distilled water). This solution was frequently shaken and changed for rapid removing of chlorophyll from the leaf fragments. When the leaf fragments were cleared, they were rinsed off in distilled water at least 2 times and finally kept in glycerin to maintain the structure and moisture of the cells.

b) Method for Stomatal number and Stomatal index

Prior to the determination, the drawing attachment has already set for the microscope and the stage micrometer was used for measuring the diameter of the drawing magnification on the paper surface. The area of this circle was calculated in square millimeter and this area was specific for each magnification of lens. If the lens and the magnification wear changed, the area must be calculated again. The ordinary epidermis and the stomata wear traced and counted. The trichome or its cicatrix was also counted as 1 ordinary epidermal cell. Counted incomplete cell of epidermis and stoma on the bordered of circle as 1 cell from only one side of half circle. Concerning the covering trichomes that were difficult to focused and traced on the circled area of paper. Thus, the stomatal number and the stomatal index were determined using glandular trichomes instead of stomata and defined in terms of glandular number and glandular index respectively.

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

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

S = the total numbers of stomata

E = the total numbers of epidermal cells

c) Method for Palisade ratio

First a number of groups each of four epidermal cells are traced and their outlines inked in to make them more conspicuous. The palisade cells lying beneath each group are counted, those being included in the count which are more then half-covered by the epidermal cells; the figure obtained divided by 4 gives the palisade ratio of that group.

d) Method for Vein-islet number and Veinlet termination number

A drawing attachment apparatus is set up and by means of a stage micrometer the paper is divided into squares of 4 mm^2. The stage micrometer is then replaced by the cleared preparation and the veins are traced in a square 2 mm × 2 mm. Counted the number of the vein-islets that completely enclosed by veins and counted the incomplete vein-islets which were cut by a two adjacent sides. The total numbers of vein-islet divided by 4 is termed “vein-islet number”. For the free veinlet termination were counted in the same method. The total numbers of veinlet termination divided by 4 is termed “veinlet termination number”.

d) Method for Glandular trichome number and Glandular trichome index

The area of this circle was calculated in square millimeter and this area was specific for each magnification of lens. The ordinary epidermis, the glandular trichomes were traced and counted. The lithocyst was also counted as 1 ordinary epidermal cell and stoma was counted as 2 ordinary epidermal cell. Counted incomplete cell of epidermis and glandular trichome on the bordered of circle as 1 cell from only one side of half circle.

$$\text{Glandular trichome number} = \frac{G}{1\ mm^2}$$

$$\text{Glandular trichome index} = \frac{G}{G + E} \times 100$$

G = the total numbers of glandular trichomes

E = the total numbers of epidermal cells

f) Method for Lithocyst number and Lithocyst index

The area of this circle was calculated in square millimeter and this area was specific for each magnification of lens. The ordinary epidermis, the lithocyst were traced and counted. The glandular trichome was also counted as 1 ordinary epidermal cell and stoma was counted as 2 ordinary epidermal cells. Counted incomplete cell of epidermis and glandular trichome on the bordered of circle as 1 cell from only one side of half circle.

$$\text{Lithocyst number} = \frac{L}{1\ mm^2}$$

$$\text{Lithocyst index} = \frac{L}{L + E} \times 100$$

L = the total numbers of lithocysts

E = the total numbers of epidermal cells

Part II The macroscopic and microscopic characterization of *Clinacanthus siamensis* leaves

Materials

a) The fresh mature leaves (Lin ngu hao) were collected from eight locations as described in Part I.

b) Chloral hydrate B.P.

c) Distilled water

Procedure

The fresh leaves were dried in a hot air oven at 50 °C. Kept in a well-closed container for macroscopic and microscopic study as the following steps:

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

2.2 The microscopic method: determine the characteristic cells and tissue were traced using a drawing attachment.

After the fresh leaves were dried, then ground and passed through a sieve with mesh number 60. The powdered sample was mounted in water to determine the characteristic cell and tissue. For uncleared fragments, chloral hydrate solution was dropped into the powdered sample and then powder sample was mounted in glycerine to prevent the formation of chloral hydrate crystals during the examination. The tissue and cell inclusion were photographed by Photomicrographic equipment which is attached to microscope.

Part III Extraction for anti-herpes simplex virus activity test

Materials

a) The plant materials, Lin ngu hao leaves, were collected from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, the leaves were collected during the fourth week of every month between March 2001 and February 2002
b) Methanol
c) Ethyl acetate
d) Distilled water

Procedure

Twenty-five grams of dried coarse powdered of *Clinacanthus siamensis* leaves were macerated twice for three-day period, with methanol (200 ml) and filtered through Whatman filter paper No.1. Then the filtrate was evaporated under reduced pressure at 40-50 °C until dryness.

The crude methanol extract was dissolved in 10 % v/v methanol and partitioned with ethyl acetate. The ethyl acetate extract was separated and evaporated to dryness under reduced pressure to yield ≅ 100 mg of green mass (CS). Then it was dried under vacuum and 2 milligrams of CS was sent for anti-herpes simplex virus activity test.

Part IV Thin-layer chromatographic patterns of leaf extracts

Materials

a) *Clinacanthus siamensis* leaves were collected from eight locations as described in Part I. and *Clinacanthus nutans* leaves was collected from the

Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province.

b) Methanol
c) Chloroform
d) Acetone
e) Distilled water

Procedure

The fresh leaves were dried in a hot air oven at 50 °C, then ground and kept in a well-closed container.

Technique for Thin-layer chromatography (TLC)

The conditions used for the analytical TLC used in this work are as follows:

Technique : One-dimentional TLC
Adsorbent : Silica gel 60 F_{254} (E. Merck) precoated plate
Solvent system : System 1 —— Chloroform : methanol (4 : 1)
System 2 —— Chloroform : acetone (1 : 1)
Layer thickness : 0.2 mm
Distance : 8.5 cm
Temperature : Laboratory Temperature (25-28 °C)
Detection : Visible daylight, UV 254 nm, anisaldehyde-sulfuric acid TS

The detail of each step is described below:

1. Five grams of dried leaves powdered drug were macerated in 25 ml of methanol for 24 hours, then filtered through filter paper (Whatman No.1) and kept in well-closed container prior to spot on TLC plate.
2. Selected suitable solvent system that can provide separation and identification.

System 1—— Chloroform : Methanol (4 : 1)

System 2—— Chloroform : Acetone (1 : 1)

3. The amount of 6 μl per each sample was spotted on TLC plate by micropipetted, allowed to dry in the air.
4. The spotted TLC plate was developed in solvent system saturating in chamber. After the solvent ascended 15 cm, the plate was removed from the chamber, allowed to dry in the air.

For TLC pattern of ethyl acetate extract from Lin ngu hao leaves, the detail of each step is described above.

Part V Phytochemical screening

Materials

a) The plant materials, Lin ngu hao leaves, were collected from eight locations as described in Part I.
b) Ethanol
c) Chloroform
d) Distilled water
e) Sulphuric acid 95-97 %
f) Ferric chloride
g) Mercuric chloride
h) Iodine
i) Potassium iodine
j) glacial acetic acid,
k) Hydrochloric acid
l) Acetic anhydride
m) Bismuth oxynitrate
m) Cupric sulfate
n) Potassium sodium tartrate
o) Sodium hydroxide

Procedure

The fresh plant or dried coarce powdered drug was tested with chemical method such as Froth test, Shinoda's test, Alkaloid test, Liebermann-Burchard test, Ferric chloride TS, Iodine TS and Fehling TS.

Flavonoids test (Shinoda'test, Cyanidin reduction test)

1. Five grams of dried coarse powdered drug was macerated in 50 ml of 95 % ethanol for 24 hours, filtered and then evaporated to dryness on water-bath.
2. Mix residue and petroleum ether, decanted, 2 times.
3. Dissolved the residue in 50 % ethanol, filtered and separated the filtrate into 2 portions:
   portion 1: As control
   portion 2: added conc. HCl and 2-3 pieces of Magnesium ribbon, positive test with flavonoids must be observed pink to red solution in 1-2 minutes.

Alkaloid test

1. Twenty grams of dried coarse powdered drug was macerated in 50 ml of 95 % ethanol for 24 hours, filtered and then evaporated to dryness on water-bath.
2. Dissolved the crude extract in 10 ml of dilute sulfuric acid (2 %), filtered and separated the filtrate into 2 portions:
   Portion 1: added a few drops of Mayer's reagent, an alkaloid-positive reaction must be observed white precipitation.
   Portion 2: added a few drops of Dragendorff's reagent, an alkaloid-positive reaction must be observed orange precipitation.
3. The observed precipitation must not dissolve in alcohol.

Froth test

The two grams of dried coarse powdered drug was warmed in 10 ml of distilled water, filtered, separated the filtrate into 2 tubes:

Tube 1: Shake rapidly and then allowing to stand.
-positive test with honeycomb froth which persists for at least 30 minutes

Tube 2: Boiled in sulfuric acid and shaked, positive test with no froth.

Liebermann-Burchard test for detecting triterpene and steroidal group

The 0.5 grams of the pulverized sample was warmed in 2 ml of acetic anhydride on a water bath for 2 minutes while shaking, then filter, and to the filtrate add carefully 1 ml of sulfuric acid to make two layers: a brownish red color develops at the zone of contact.

Part VI Quality controls

Materials

a) The plant materials, Lin ngu hao leaves, were collected from seven locations as described in Part I excluding sample from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.
b) Ethanol
c) Chloroform-water

Procedure

The fresh leaves were dried in a hot air oven at 50 °C, then ground and kept in a well-closed container.

Loss on drying

As directed in the monograph, conduct the determination on 1 to 2 g of the substance (2 to 5 g in case of crude drugs), previously mixed and accurately weighed. Tare a glass-stoppered bottle that has been dried for 30 minutes under the same conditions to be employed in the determination. Put the test specimen in the bottle, replace the cover, and accurately weigh the bottle and the contents. Dried the test specimen at the temperature and for the time specified in the monograph. Place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber. Upon opening the chamber, close the bottle promptly and allow it to come to room temperature in a desiccator before weighing. Calculated the percentage of loss on drying with reference to the air-dried substance (Thai Pharmacopoeia, 1987).

Total ash

Place a 2 to 4 g sample of the ground substance, accurately weighed, or the quantity specified in the monograph, in a suitable tared crucible (usually of platinum or silica), previously ignited, cooled and weighed. Incinerate the sample by gradually increasing the temperature, not exceeding 450 °C, 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 a hot plate and incinerate to constant weight. Calculate the percentage of total ash with reference to the air-dried substance (Thai Pharmacopoeia, 1987).

Acid-insoluble ash

Boil the total ash for 5 minutes with 25 ml of dilute hydrochloric acid, collect the insoluble matter on an ashless filter paper, wash with hot water until the filtrate is neutral, and ignite at about 500 °C. Calculate the percentage of acid-insoluble ash with reference to the air-dried substance (Thai Pharmacopoeia, 1987).

Extractive value

Ethanol-soluble Extractive

Macerate 5 g of the air-dried drug, coarsely powdered and accurately weighed, with 100.0 ml of ethanol of the specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and then allowing to stand for 18 hours. Filter rapidly, taking precautions against loss of ethanol, evaporate 20.0 ml of the filtrate to dryness in a tared, flat-bottomed, shallow dish and dry at 105 °C to constant weight. Calculate the percentage of ethanol-soluble extractive with reference to the air-dried drug (British Pharmacopoeia, 1993).

Water-soluble Extractive

Proceed as directed in Ethanol-soluble Extractive but using chloroform water in place of ethanol (British Pharmacopoeia, 1993).

CHAPTER V

RESULTS AND DISCUSSION

Part I The results of leaf measurements

The leaf measurements data are shown in Tables 4. - 43.

Tables 4. - 11. Stomatal number and stomatal index of *Clinacanthus siamensis* Brem.

Tables 12. - 19. Glandular number and glandular index of *Clinacanthus siamensis* Brem.

Tables 20. - 27. Lithocyst number and lithocyst index of *Clinacanthus siamensis* Brem.

Tables 28. - 35. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus siamensis* Brem.

Tables 36. - 37. Stomatal number and stomatal index of *Clinacanthus nutans* (Burm. f.) Lindau

Tables 38. - 39. Glandular number and glandular index of *Clinacanthus nutans* (Burm. f.) Lindau

Tables 40. - 41. Lithocyst number and lithocyst index of *Clinacanthus nutans* (Burm. f.) Lindau

Tables 42. - 43. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus nutans* (Burm. f.) Lindau

The outline drawing of each species of leaf measurements including stomata, glandular trichomes, lithocyst, vein structure and four epidermal cells with underlying palisade cells are shown in Figures 9-12 and microscopic illustration of their leaves are shown in Figures 13-14.

Table 4. Stomatal number and stomatal index of *Clinacanthus siamensis* Brem.[1]

Area of determination = 0.1075 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
14	125	130.23	10.07
18	116	167.44	13.43
17	110	158.14	13.39
15	114	139.53	11.63
16	124	148.84	11.43
16	130	148.84	10.96
18	117	167.44	13.33
19	112	176.74	14.50
17	120	158.14	12.41
18	147	167.44	10.91
17	145	158.14	10.49
15	127	139.53	10.56
20	154	186.05	11.49
14	131	130.23	9.66
14	128	130.23	9.86
19	168	176.74	10.16
16	142	148.84	10.13
16	154	148.84	9.41
15	117	139.53	11.36
16	107	148.84	13.01
13	155	120.93	7.74
15	134	139.53	10.07
15	120	139.53	11.11
19	148	176.74	11.38
21	139	195.35	13.13
16	132	148.84	10.81
14	127	130.23	9.93
16	134	148.84	10.67
18	147	167.44	10.91
16	127	148.84	11.19
	mean	152.87	11.17
	S.D.	18.23	1.45

1 = sample from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.

Table 5. Stomatal number and stomatal index of *Clinacanthus siamensis* Brem.[2]

Area of determination = 0.1075 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
14	118	130.23	10.61
14	112	130.23	11.11
15	110	139.53	12.00
14	120	130.23	10.45
13	124	120.93	9.49
15	102	139.53	12.82
14	123	130.23	10.22
18	119	167.44	13.14
16	97	148.84	14.16
16	125	148.84	11.35
14	112	130.23	11.11
17	116	158.14	12.78
17	120	158.14	12.41
14	117	130.23	10.69
16	110	148.84	12.70
16	113	148.84	12.40
16	116	148.84	12.12
12	107	111.63	10.08
15	107	139.53	12.30
16	115	148.84	12.21
16	111	148.84	12.60
14	118	130.23	10.61
16	112	148.84	12.50
23	127	213.95	15.33
17	105	158.14	13.93
17	106	158.14	13.82
18	104	167.44	14.75
15	100	139.53	13.04
11	87	102.33	11.22
15	124	139.53	10.79
	mean	143.88	12.09
	S.D.	20.11	1.45

2 = sample from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province

Table 6. Stomatal number and stomatal index of *Clinacanthus siamensis* Brem.[3]

Area of determination = 0.1075 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
16	122	148.84	11.59
14	102	130.23	12.07
17	104	158.14	14.05
17	116	158.14	12.78
13	100	120.93	11.50
13	97	120.93	11.82
12	100	111.63	10.71
18	93	167.44	16.22
13	98	120.93	11.71
13	110	120.93	10.57
14	106	130.23	11.67
15	111	139.53	11.90
14	99	130.23	12.39
11	99	102.33	10.00
14	90	130.23	13.46
13	95	120.93	12.04
11	89	102.33	11.00
13	95	120.93	12.04
15	100	139.53	13.04
15	91	139.53	14.15
14	104	130.23	11.86
14	112	130.23	11.11
12	91	111.63	11.65
14	90	130.23	13.46
12	95	111.63	11.21
16	102	148.84	13.56
13	90	120.93	12.62
15	93	139.53	13.89
13	96	120.93	11.93
15	115	139.53	11.54
	mean	129.92	12.25
	S.D.	15.92	1.29

3 = sample from Khao Hinshon, Chachoengsao Province

Table 7. Stomatal number and stomatal index of *Clinacanthus siamensis* Brem.[4]

Area of determination = 0.1075 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
15	82	139.53	15.46
15	88	139.53	14.56
15	94	139.53	13.76
14	86	130.23	14.00
19	102	176.74	15.70
13	92	120.93	12.38
16	92	148.84	14.81
14	95	130.23	12.84
18	94	167.44	16.07
11	75	102.33	12.79
18	112	167.44	13.85
14	82	130.23	14.58
17	97	158.14	14.91
13	99	120.93	11.61
15	95	139.53	13.64
14	86	130.23	14.00
15	95	139.53	13.64
14	92	130.23	13.21
16	85	148.84	15.84
20	109	186.05	15.50
15	93	139.53	13.89
17	97	158.14	14.91
18	98	167.44	15.52
17	109	158.14	13.49
16	90	148.84	15.09
19	93	176.74	16.96
15	90	139.53	14.29
16	89	148.84	15.24
14	86	130.23	14.00
18	93	167.44	16.22
	mean	146.05	14.43
	S.D.	19.26	1.23

4 = sample from the Somdej Phra Thepratanarajasuda Medicinal Plants Garden, Petroleum Authority of Thailand, Rayong Province

Table 8. Stomatal number and stomatal index of *Clinacanthus siamensis* Brem.[5]

Area of determination = 0.1075 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
12	84	111.63	12.50
15	81	139.53	15.63
13	93	120.93	12.26
13	86	120.93	13.13
13	77	120.93	14.44
14	77	130.23	15.38
14	82	130.23	14.58
16	90	148.84	15.09
16	110	148.84	12.70
13	111	120.93	10.48
14	104	130.23	11.86
13	106	120.93	10.92
13	105	120.93	11.02
13	100	120.93	11.50
12	107	111.63	10.08
13	89	120.93	12.75
13	101	120.93	11.40
12	99	111.63	10.81
15	105	139.53	12.50
12	102	111.63	10.53
11	104	102.33	9.57
14	101	130.23	12.17
11	93	102.33	10.58
12	103	111.63	10.43
15	103	139.53	12.71
13	100	120.93	11.50
15	100	139.53	13.04
13	100	120.93	11.50
13	112	120.93	10.40
13	104	120.93	11.11
	mean	123.72	12.09
	S.D.	12.01	1.64

5 = sample from Khao Chamao, Waterfall, Khao Chamao-Kao Wonge National Park, Rayong Province

Table 9. Stomatal number and stomatal index of *Clinacanthus siamensis* Brem.[6]

Area of determination = 0.1075 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
20	143	186.05	12.27
19	146	176.74	11.52
16	125	148.84	11.35
22	145	204.65	13.17
18	153	167.44	10.53
21	164	195.35	11.35
17	132	158.14	11.41
21	143	195.35	12.80
16	153	148.84	9.47
22	143	204.65	13.33
17	135	158.14	11.18
18	147	167.44	10.91
23	153	213.95	13.07
22	159	204.65	12.15
19	155	176.74	10.92
18	173	167.44	9.42
23	150	213.95	13.29
20	138	186.05	12.66
16	143	148.84	10.06
21	138	195.35	13.21
19	137	176.74	12.18
21	147	195.35	12.50
21	160	195.35	11.60
23	153	213.95	13.07
22	139	204.65	13.66
19	120	176.74	13.67
18	142	167.44	11.25
26	154	241.86	14.44
20	142	186.05	12.35
17	136	158.14	11.11
	mean	184.50	12.00
	S.D.	23.06	1.27

6 = sample from Kasetsart University, Bangkok

Table 10. Stomatal number and stomatal index of *Clinacanthus siamensis* Brem.[7]

Area of determination = 0.1075 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
18	122	167.44	12.86
17	123	158.14	12.14
18	117	167.44	13.33
12	111	111.63	9.76
19	121	176.74	13.57
18	124	167.44	12.68
17	110	158.14	13.39
17	106	158.14	13.82
17	118	158.14	12.59
16	107	148.84	13.01
19	127	176.74	13.01
19	127	176.74	13.01
19	125	176.74	13.19
16	108	148.84	12.90
16	118	148.84	11.94
20	118	186.05	14.49
20	120	186.05	14.29
13	100	120.93	11.50
13	112	120.93	10.40
23	124	213.95	15.65
14	104	130.23	11.86
19	107	176.74	15.08
17	106	158.14	13.82
19	121	176.74	13.57
17	114	158.14	12.98
16	114	148.84	12.31
15	104	139.53	12.61
16	106	148.84	13.11
15	124	139.53	10.79
16	117	148.84	12.03
	mean	158.45	12.86
	S.D.	21.92	1.26

7 = sample from Khanit's garden, Buddhamonthon sai 3, Bangkok

Table 11. Stomatal number and stomatal index of *Clinacanthus siamensis* Brem.[8]

Area of determination = 0.1075 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
15	110	139.53	12.00
19	118	176.74	13.87
13	84	120.93	13.40
14	88	130.23	13.73
14	111	130.23	11.20
12	102	111.63	10.53
12	97	111.63	11.01
12	100	111.63	10.71
13	92	120.93	12.38
12	84	111.63	12.50
15	109	139.53	12.10
15	101	139.53	12.93
14	113	130.23	11.02
16	108	148.84	12.90
11	100	102.33	9.91
15	111	139.53	11.90
12	98	111.63	10.91
13	95	120.93	12.04
13	99	120.93	11.61
12	101	111.63	10.62
13	100	120.93	11.50
14	104	130.23	11.86
11	95	102.33	10.38
12	109	111.63	9.92
14	111	130.23	11.20
14	101	130.23	12.17
20	114	186.05	14.93
11	87	102.33	11.22
12	84	111.63	12.50
13	95	120.93	12.04
	mean	125.89	11.83
	S.D.	19.50	1.19

8 = sample from the Sireerukachart Garden, Mahidol University, Salaya Campus, Nakhon Pathom Province

Table 12. Glandular trichome number and glanular trichome index of *Clinacanthus siamensis* Brem.[1]

Area of determination = 0.1075 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular trichome number	Glandular trichome index
0	153	0.00	0.00
1	151	9.30	0.66
1	143	9.30	0.69
1	143	9.30	0.69
1	155	9.30	0.64
1	161	9.30	0.62
1	152	9.30	0.65
1	149	9.30	0.67
1	154	9.30	0.65
1	182	9.30	0.55
1	178	9.30	0.56
0	157	0.00	0.00
2	192	18.60	1.03
1	158	9.30	0.63
2	154	18.60	1.28
2	204	18.60	0.97
2	172	18.60	1.15
2	184	18.60	1.08
0	147	0.00	0.00
0	139	0.00	0.00
2	179	18.60	1.10
1	163	9.30	0.61
2	148	18.60	1.33
1	185	9.30	0.54
1	180	9.30	0.55
1	163	9.30	0.61
1	154	9.30	0.65
1	165	9.30	0.60
2	181	18.60	1.09
2	157	18.60	1.26
	mean	10.85	0.70
	S.D.	6.03	0.37

1 = sample from the Faculty of Pharmaceutical Sciences, Chulalongkorn University Bangkok

Table 13. Glandular trichome number and glanular trichome index of *Clinacanthus siamensis* Brem.[2]

Area of determination = 0.1075 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular trichome number	Glandular trichome index
1	145	9.30	0.68
1	139	9.30	0.71
1	139	9.30	0.71
2	146	18.60	1.35
1	149	9.30	0.67
1	131	9.30	0.76
1	150	9.30	0.66
1	154	9.30	0.65
1	128	9.30	0.78
2	155	18.60	1.27
1	139	9.30	0.71
1	149	9.30	0.67
1	153	9.30	0.65
1	144	9.30	0.69
3	139	27.91	2.11
1	144	9.30	0.69
0	148	0.00	0.00
2	129	18.60	1.53
1	136	9.30	0.73
1	146	9.30	0.68
1	142	9.30	0.70
2	144	18.60	1.37
2	142	18.60	1.39
1	172	9.30	0.58
2	137	18.60	1.44
1	139	9.30	0.71
1	139	9.30	0.71
2	128	18.60	1.54
1	108	9.30	0.92
2	152	18.60	1.30
	mean	12.09	0.91
	S.D.	5.54	0.42

2 = sample from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province

Table 14. Glandular trichome number and glanular trichome index of *Clinacanthus siamensis* Brem.[3]

Area of determination = 0.1075 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular trichome number	Glandular trichome index
2	152	18.60	1.30
2	128	18.60	1.54
1	137	9.30	0.72
1	149	9.30	0.67
1	125	9.30	0.79
1	122	9.30	0.81
2	122	18.60	1.61
1	128	9.30	0.78
1	123	9.30	0.81
1	135	9.30	0.74
1	133	9.30	0.75
1	140	9.30	0.71
1	126	9.30	0.79
1	120	9.30	0.83
0	118	0.00	0.00
0	121	0.00	0.00
1	110	9.30	0.90
1	120	9.30	0.83
1	129	9.30	0.77
0	121	0.00	0.00
1	131	9.30	0.76
1	139	9.30	0.71
1	114	9.30	0.87
1	117	9.30	0.85
1	118	9.30	0.84
1	133	9.30	0.75
1	115	9.30	0.86
0	123	0.00	0.00
2	120	18.60	1.64
2	143	18.60	1.38
	mean	9.61	0.80
	S.D.	5.17	0.42

3 = sample from Khao Hinshon, Chachoengsao Province

Table 15. Glandular trichome number and glanular trichome index of *Clinacanthus siamensis* Brem.[4]

Area of determination = 0.1075 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular trichome number	Glandular trichome index
0	112	0.00	0.00
1	117	9.30	0.85
0	124	0.00	0.00
0	114	0.00	0.00
0	140	0.00	0.00
1	117	9.30	0.85
1	123	9.30	0.81
1	122	9.30	0.81
1	129	9.30	0.77
2	115	18.60	1.71
1	147	9.30	0.68
0	110	0.00	0.00
3	128	27.91	2.29
2	123	18.60	1.60
1	124	9.30	0.80
1	113	9.30	0.88
2	123	18.60	1.60
0	120	0.00	0.00
1	116	9.30	0.85
2	147	18.60	1.34
1	122	9.30	0.81
1	130	9.30	0.76
1	133	9.30	0.75
1	142	9.30	0.70
1	121	9.30	0.82
0	131	0.00	0.00
0	120	0.00	0.00
1	120	9.30	0.83
1	113	9.30	0.88
0	129	0.00	0.00
	mean	8.37	0.71
	S.D.	7.06	0.59

4 = sample from the Somdej Phra Thepratanarajasuda Medicinal Plants Garden, Petroleum Authority of Thailand, Rayong Province

Table 16. Glandular trichome number and glanular trichome index of *Clinacanthus siamensis* Brem.[5]

Area of determination = 0.1075 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular trichome number	Glandular trichome index
1	107	9.30	0.93
1	110	9.30	0.90
2	117	18.60	1.68
1	111	9.30	0.89
1	102	9.30	0.97
0	105	0.00	0.00
1	109	9.30	0.91
0	122	0.00	0.00
1	141	9.30	0.70
1	136	9.30	0.73
1	131	9.30	0.76
1	131	9.30	0.76
1	130	9.30	0.76
0	126	0.00	0.00
1	130	9.30	0.76
0	115	0.00	0.00
0	127	0.00	0.00
1	122	9.30	0.81
0	135	0.00	0.00
1	125	9.30	0.79
1	125	9.30	0.79
1	128	9.30	0.78
0	115	0.00	0.00
0	127	0.00	0.00
0	133	0.00	0.00
1	125	9.30	0.79
0	130	0.00	0.00
1	125	9.30	0.79
1	137	9.30	0.72
2	128	18.60	1.54
	mean	6.82	0.59
	S.D.	5.43	0.47

5 = sample from Khao Chamao, Waterfall, Khao Chamao-Kao Wonge National Park, Rayong Province

Table 17. Glandular trichome number and glanular trichome index of *Clinacanthus siamensis* Brem.[6]

Area of determination = 0.1075 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular trichome number	Glandular trichome index
3	180	27.91	1.64
2	182	18.60	1.09
2	155	18.60	1.27
1	188	9.30	0.53
1	188	9.30	0.53
1	205	9.30	0.49
1	165	9.30	0.60
1	184	9.30	0.54
1	184	9.30	0.54
2	185	18.60	1.07
1	168	9.30	0.59
2	181	18.60	1.09
2	197	18.60	1.01
3	200	27.91	1.48
1	192	9.30	0.52
2	207	18.60	0.96
1	195	9.30	0.51
2	176	18.60	1.12
1	174	9.30	0.57
0	180	0.00	0.00
1	174	9.30	0.57
2	187	18.60	1.06
1	201	9.30	0.50
1	198	9.30	0.50
2	181	18.60	1.09
1	157	9.30	0.63
2	176	18.60	1.12
2	204	18.60	0.97
1	181	9.30	0.55
1	169	9.30	0.59
	mean	13.64	0.79
	S.D.	6.34	0.36

6 = sample from Kasetsart University, Bangkok

Table 18. Glandular trichome number and glanular trichome index of *Clinacanthus siamensis* Brem.[7]

Area of determination = 0.1075 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular trichome number	Glandular trichome index
1	157	9.30	0.63
1	156	9.30	0.64
1	152	9.30	0.65
1	134	9.30	0.74
0	159	0.00	0.00
1	159	9.30	0.63
0	144	0.00	0.00
0	140	0.00	0.00
1	151	9.30	0.66
1	138	9.30	0.72
2	163	18.60	1.21
2	163	18.60	1.21
1	162	9.30	0.61
1	139	9.30	0.71
1	149	9.30	0.67
1	157	9.30	0.63
1	159	9.30	0.63
1	125	9.30	0.79
1	137	9.30	0.72
2	168	18.60	1.18
1	131	9.30	0.76
0	145	0.00	0.00
1	139	9.30	0.71
1	158	9.30	0.63
1	147	9.30	0.68
1	145	9.30	0.68
1	133	9.30	0.75
1	137	9.30	0.72
1	153	9.30	0.65
1	148	9.30	0.67
	mean	8.99	0.64
	S.D.	4.56	0.30

7 = sample from Khanit's garden, Buddhamonthon sai 3, Bangkok

Table 19. Glandular trichome number and glanular trichome index of *Clinacanthus siamensis* Brem.[8]

Area of determination = 0.1075 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular trichome number	Glandular trichome index
1	139	9.30	0.71
1	155	9.30	0.64
1	109	9.30	0.91
1	115	9.30	0.86
1	138	9.30	0.72
1	125	9.30	0.79
1	120	9.30	0.83
2	122	18.60	1.61
1	117	9.30	0.85
1	107	9.30	0.93
1	138	9.30	0.72
1	130	9.30	0.76
2	139	18.60	1.42
2	138	18.60	1.43
2	120	18.60	1.64
2	139	18.60	1.42
1	121	9.30	0.82
1	120	9.30	0.83
1	124	9.30	0.80
1	124	9.30	0.80
1	125	9.30	0.79
1	131	9.30	0.76
1	116	9.30	0.85
2	131	18.60	1.50
1	138	9.30	0.72
1	128	9.30	0.78
1	153	9.30	0.65
1	108	9.30	0.92
1	107	9.30	0.93
1	120	9.30	0.83
	mean	11.16	0.94
	S.D.	3.78	0.30

8 = sample from the Sireerukachart Garden, Mahidol University, Salaya Campus, Nakhon Pathom Province

Table 20. Lithocyst number and lithocyst index of *Clinacanthus siamensis* Brem.[1]

Area of determination = 0.1075 mm^2

Number of lithocyst	Number of epidermal cells	Lithocyst number	Lithocyst index
4	149	37.21	2.61
4	148	37.21	2.63
3	141	27.91	2.08
1	143	9.30	0.69
4	152	37.21	2.56
2	160	18.60	1.23
3	150	27.91	1.96
2	148	18.60	1.33
1	154	9.30	0.65
3	180	27.91	1.64
3	176	27.91	1.68
3	154	27.91	1.91
3	191	27.91	1.55
2	157	18.60	1.26
2	154	18.60	1.28
1	205	9.30	0.49
2	172	18.60	1.15
2	184	18.60	1.08
2	145	18.60	1.36
2	137	18.60	1.44
3	178	27.91	1.66
2	162	18.60	1.22
1	149	9.30	0.67
2	184	18.60	1.08
1	180	9.30	0.55
2	162	18.60	1.22
3	152	27.91	1.94
2	164	18.60	1.20
3	180	27.91	1.64
2	157	18.60	1.26
	mean	21.71	1.43
	S.D.	8.22	0.57

1 = sample from the Faculty of Pharmaceutical Sciences, Chulalongkorn University Bangkok

Table 21. Lithocyst number and lithocyst index of *Clinacanthus siamensis* Brem.[2]

Area of determination = 0.1075 mm^2

Number of lithocyst	Number of epidermal cells	Lithocyst number	Lithocyst index
3	143	27.91	2.05
2	138	18.60	1.43
1	139	9.30	0.71
2	146	18.60	1.35
3	147	27.91	2.00
1	131	9.30	0.76
1	150	9.30	0.66
1	154	9.30	0.65
1	128	9.30	0.78
2	155	18.60	1.27
0	140	0.00	0.00
0	150	0.00	0.00
4	150	37.21	2.60
3	142	27.91	2.07
3	139	27.91	2.11
3	142	27.91	2.07
3	145	27.91	2.03
3	128	27.91	2.29
3	134	27.91	2.19
1	146	9.30	0.68
1	142	9.30	0.70
2	144	18.60	1.37
2	142	18.60	1.39
3	170	27.91	1.73
2	137	18.60	1.44
3	137	27.91	2.14
1	139	9.30	0.71
2	128	18.60	1.54
1	108	9.30	0.92
2	152	18.60	1.30
	mean	18.29	1.36
	S.D.	9.61	0.70

2 = sample from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province

Table 22. Lithocyst number and lithocyst index of *Clinacanthus siamensis* Brem.[3]

Area of determination = 0.1075 mm^2

Number of lithocyst	Number of epidermal cells	Lithocyst number	Lithocyst index
2	152	18.60	1.30
2	128	18.60	1.54
2	136	18.60	1.45
1	149	9.30	0.67
1	125	9.30	0.79
2	121	18.60	1.63
2	122	18.60	1.61
0	129	0.00	0.00
2	122	18.60	1.61
2	134	18.60	1.47
2	132	18.60	1.49
2	139	18.60	1.42
1	126	9.30	0.79
2	119	18.60	1.65
3	115	27.91	2.54
1	120	9.30	0.83
3	108	27.91	2.70
2	119	18.60	1.65
2	128	18.60	1.54
2	119	18.60	1.65
2	130	18.60	1.52
1	139	9.30	0.71
1	114	9.30	0.87
2	116	18.60	1.69
1	118	9.30	0.84
3	131	27.91	2.24
2	114	18.60	1.72
2	121	18.60	1.63
2	120	18.60	1.64
2	143	18.60	1.38
	mean	16.74	1.42
	S.D.	6.18	0.56

3 = sample from Khao Hinshon, Chachoengsao Province

Table 23. Lithocyst number and lithocyst index of *Clinacanthus siamensis* Brem.[4]

Area of determination = 0.1075 mm^2

Number of lithocyst	Number of epidermal cells	Lithocyst number	Lithocyst index
1	111	9.30	0.89
3	115	27.91	2.54
2	122	18.60	1.61
1	113	9.30	0.88
1	139	9.30	0.71
2	116	18.60	1.69
1	113	9.30	0.88
1	122	9.30	0.81
1	129	9.30	0.77
1	96	9.30	1.03
3	145	27.91	2.03
3	107	27.91	2.73
0	131	0.00	0.00
2	123	18.60	1.60
3	122	27.91	2.40
2	112	18.60	1.75
2	123	18.60	1.60
3	117	27.91	2.50
1	116	9.30	0.85
2	147	18.60	1.34
1	122	9.30	0.81
3	128	27.91	2.29
2	132	18.60	1.49
1	142	9.30	0.70
2	120	18.60	1.64
1	130	9.30	0.76
0	120	0.00	0.00
2	119	18.60	1.65
1	113	9.30	0.88
1	128	9.30	0.78
	mean	15.19	1.32
	S.D.	8.28	0.72

4 = sample from the Somdej Phra Thepratanarajasuda Medicinal Plants Garden, Petroleum Authority of Thailand, Rayong Province

Table 24. Lithocyst number and lithocyst index of *Clinacanthus siamensis* Brem.[5]

Area of determination = 0.1075 mm^2

Number of lithocyst	Number of epidermal cells	Lithocyst number	Lithocyst index
2	106	18.60	1.85
2	109	18.60	1.80
1	118	9.30	0.84
2	110	18.60	1.79
1	102	9.30	0.97
1	104	9.30	0.95
2	108	18.60	1.82
2	120	18.60	1.64
1	141	9.30	0.70
2	135	18.60	1.46
1	131	9.30	0.76
2	130	18.60	1.52
2	129	18.60	1.53
2	124	18.60	1.59
1	130	9.30	0.76
3	112	27.91	2.61
2	125	18.60	1.57
0	123	0.00	0.00
2	133	18.60	1.48
2	124	18.60	1.59
1	125	9.30	0.79
1	128	9.30	0.78
1	114	9.30	0.87
2	125	18.60	1.57
1	132	9.30	0.75
1	125	9.30	0.79
4	126	37.21	3.08
1	125	9.30	0.79
1	137	9.30	0.72
1	129	9.30	0.77
	mean	14.57	1.27
	S.D.	7.20	0.64

5 = sample from Khao Chamao, Waterfall, Khao Chamao-Kao Wonge National Park, Rayong Province

Table 25. Lithocyst number and lithocyst index of *Clinacanthus siamensis* Brem.[6]

Area of determination = 0.1075 mm^2

Number of lithocyst	Number of epidermal cells	Lithocyst number	Lithocyst index
1	182	9.30	0.55
4	180	37.21	2.17
1	156	9.30	0.64
2	187	18.60	1.06
3	186	27.91	1.59
2	204	18.60	0.97
1	165	9.30	0.60
1	184	9.30	0.54
1	184	9.30	0.54
2	185	18.60	1.07
4	165	37.21	2.37
2	181	18.60	1.09
1	198	9.30	0.50
2	201	18.60	0.99
2	191	18.60	1.04
1	208	9.30	0.48
1	195	9.30	0.51
2	176	18.60	1.12
3	172	27.91	1.71
1	179	9.30	0.56
3	172	27.91	1.71
2	187	18.60	1.06
3	199	27.91	1.49
3	196	27.91	1.51
4	179	37.21	2.19
3	155	27.91	1.90
2	176	18.60	1.12
1	205	9.30	0.49
3	179	27.91	1.65
2	168	18.60	1.18
	mean	19.53	1.15
	S.D.	9.25	0.57

6 = sample from Kasetsart University, Bangkok

Table 26. Lithocyst number and lithocyst index of *Clinacanthus siamensis* Brem.[7]

Area of determination = 0.1075 mm^2

Number of lithocyst	Number of epidermal cells	Lithocyst number	Lithocyst index
3	155	27.91	1.90
1	156	9.30	0.64
1	152	9.30	0.65
2	133	18.60	1.48
2	157	18.60	1.26
1	159	9.30	0.63
2	142	18.60	1.39
2	138	18.60	1.43
2	150	18.60	1.32
1	138	9.30	0.72
3	162	27.91	1.82
0	165	0.00	0.00
2	161	18.60	1.23
2	138	18.60	1.43
2	148	18.60	1.33
1	157	9.30	0.63
3	157	27.91	1.88
1	125	9.30	0.79
2	136	18.60	1.45
2	168	18.60	1.18
1	131	9.30	0.76
2	143	18.60	1.38
2	138	18.60	1.43
2	157	18.60	1.26
1	147	9.30	0.68
3	143	27.91	2.05
1	133	9.30	0.75
2	136	18.60	1.45
2	152	18.60	1.30
2	147	18.60	1.34
	mean	16.43	1.18
	S.D.	6.77	0.47

7 = sample from Khanit's garden, Buddhamonthon sai 3, Bangkok

Table 27. Lithocyst number and lithocyst index of *Clinacanthus siamensis* Brem.[8]

Area of determination = 0.1075 mm^2

Number of lithocyst	Number of epidermal cells	Lithocyst number	Lithocyst index
3	137	27.91	2.14
2	154	18.60	1.28
1	109	9.30	0.91
1	115	9.30	0.86
2	137	18.60	1.44
2	124	18.60	1.59
1	120	9.30	0.83
1	123	9.30	0.81
1	117	9.30	0.85
1	107	9.30	0.93
2	137	18.60	1.44
1	130	9.30	0.76
3	138	27.91	2.13
1	139	9.30	0.71
2	120	18.60	1.64
2	139	18.60	1.42
0	122	0.00	0.00
2	119	18.60	1.65
2	123	18.60	1.60
0	125	0.00	0.00
0	126	0.00	0.00
4	128	37.21	3.03
3	114	27.91	2.56
2	131	18.60	1.50
1	138	9.30	0.72
2	127	18.60	1.55
3	151	27.91	1.95
2	107	18.60	1.83
1	107	9.30	0.93
2	119	18.60	1.65
	mean	15.50	1.29
	S.D.	8.92	0.71

8 = sample from the Sireerukachart Garden, Mahidol University, Salaya Campus, Nakhon Pathom Province

Table 28. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus siamensis* Brem.[1]

Vein-islet		Veinlet termination		Palisade cell	
Count in 4 mm^2	Vein-islet number	Count in 4 mm^2	Veinlet termination number	Number beneath 4 epidermal cells	Palisade ratio
11	2.75	8	2	15	3.75
12	3	11	2.75	16	4
13	3.25	9	2.25	14	3.5
9	2.25	12	3	14	3.5
13	3.25	8	2	15	3.75
13	3.25	12	3	15	3.75
14	3.5	11	2.75	14	3.5
12	3	7	1.75	14	3.5
12	3	6	1.5	13	3.25
12	3	8	2	15	3.75
13	3.25	7	1.75	14	3.5
12	3	8	2	15	3.75
11	2.75	11	2.75	15	3.75
10	2.5	12	3	14	3.5
15	3.75	10	2.5	15	3.75
20	5	11	2.75	15	3.75
19	4.75	8	2	15	3.75
14	3.5	14	3.5	14	3.5
18	4.5	13	3.25	15	3.75
18	4.5	14	3.5	13	3.25
16	4	10	2.5	16	4
11	2.75	11	2.75	13	3.25
11	2.75	12	3	16	4
14	3.5	8	2	14	3.5
11	2.75	9	2.25	16	4
10	2.5	8	2	14	3.5
13	3.25	13	3.25	15	3.75
12	3	7	1.75	14	3.5
18	4.5	13	3.25	16	4
10	2.5	8	2	14	3.5
mean	3.31	mean	2.49	mean	3.65
S.D.	0.72	S.D.	0.58	S.D.	0.22

1 = sample from the Faculty of Pharmaceutical Sciences, Chulalongkorn University Bangkok

Table 29. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus siamensis* Brem.[2]

Vein-islet		Veinlet termination		Palisade cell	
Count in 4 mm^2	Vein-islet number	Count in 4 mm^2	Veinlet termination number	Number beneath 4 epidermal cells	Palisade ratio
14	3.5	13	3.25	15	3.75
16	4	11	2.75	17	4.25
12	3	11	2.75	14	3.5
12	3	13	3.25	16	4
11	2.75	14	3.5	15	3.75
14	3.5	12	3	14	3.5
15	3.75	12	3	16	4
19	4.75	6	1.5	14	3.5
17	4.25	12	3	15	3.75
15	3.75	15	3.75	17	4.25
8	2	11	2.75	17	4.25
14	3.5	11	2.75	15	3.75
15	3.75	13	3.25	13	3.25
16	4	8	2	13	3.25
10	2.5	7	1.75	13	3.25
16	4	10	2.5	15	3.75
15	3.75	11	2.75	14	3.5
11	2.75	11	2.75	14	3.5
13	3.25	12	3	14	3.5
15	3.75	12	3	16	4
14	3.5	12	3	15	3.75
14	3.5	12	3	16	4
13	3.25	9	2.25	13	3.25
15	3.75	14	3.5	13	3.25
12	3	14	3.5	13	3.25
17	4.25	16	4	13	3.25
19	4.75	16	4	14	3.5
15	3.75	10	2.5	15	3.75
12	3	11	2.75	14	3.5
12	3	15	3.75	14	3.5
mean	3.51	mean	2.95	mean	3.64
S.D.	0.62	S.D.	0.60	S.D.	0.32

2 = sample from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province

Table 30. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus siamensis* Brem.[3]

Vein-islet		Veinlet termination		Palisade cell	
Count in 4 mm^2	Vein-islet number	Count in 4 mm^2	Veinlet termination number	Number beneath 4 epidermal cells	Palisade ratio
13	3.25	13	3.25	13	3.25
12	3	11	2.75	15	3.75
10	2.5	7	1.75	14	3.5
11	2.75	9	2.25	16	4
16	4	10	2.5	14	3.5
18	4.5	12	3	13	3.25
16	4	10	2.5	15	3.75
17	4.25	14	3.5	13	3.25
13	3.25	11	2.75	15	3.75
11	2.75	12	3	16	4
14	3.5	10	2.5	12	3
14	3.5	8	2	15	3.75
13	3.25	12	3	12	3
13	3.25	9	2.25	17	4.25
14	3.5	14	3.5	14	3.5
15	3.75	9	2.25	15	3.75
17	4.25	16	4	15	3.75
12	3	13	3.25	16	4
15	3.75	8	2	15	3.75
16	4	11	2.75	13	3.25
11	2.75	13	3.25	17	4.25
11	2.75	12	3	17	4.25
12	3	11	2.75	16	4
12	3	10	2.5	16	4
16	4	16	4	13	3.25
11	2.75	16	4	15	3.75
18	4.5	10	2.5	16	4
14	3.5	10	2.5	14	3.5
10	2.5	14	3.5	12	3
14	3.5	15	3.75	12	3
mean	3.41	mean	2.88	mean	3.63
S.D.	0.59	S.D.	0.62	S.D.	0.39

3 = sample from the Khao Hinshon, Chachoengsal Province

Table 31. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus siamensis* Brem.[4]

Vein-islet		Veinlet termination		Palisade cell	
Count in 4 mm^2	Vein-islet number	Count in 4 mm^2	Veinlet termination number	Number beneath 4 epidermal cells	Palisade ratio
10	2.5	7	1.75	16	4
12	3	5	1.25	20	5
10	2.5	12	3	17	4.25
12	3	9	2.25	12	3
10	2.5	10	2.5	13	3.25
11	2.75	7	1.75	14	3.5
12	3	8	2	13	3.25
10	2.5	8	2	16	4
9	2.25	7	1.75	12	3
13	3.25	10	2.5	10	2.5
11	2.75	15	3.75	13	3.25
8	2	8	2	17	4.25
11	2.75	5	1.25	12	3
9	2.25	9	2.25	13	3.25
9	2.25	16	4	13	3.25
11	2.75	9	2.25	15	3.75
14	3.5	12	3	17	4.25
10	2.5	9	2.25	14	3.5
13	3.25	13	3.25	17	4.25
14	3.5	8	2	11	2.75
8	2	11	2.75	16	4
9	2.25	8	2	12	3
7	1.75	9	2.25	11	2.75
11	2.75	7	1.75	16	4
10	2.5	10	2.5	14	3.5
9	2.25	7	1.75	15	3.75
8	2	14	3.5	15	3.75
9	2.25	16	4	12	3
11	2.75	16	4	12	3
9	2.25	15	3.75	12	3
mean	2.58	mean	2.50	mean	3.5
S.D.	0.45	S.D.	0.82	S.D.	0.58

4 = sample from the Somdej Phra Thepratanarajasuda Medicinal Plants Garden, Petroleum Authority of Thailand, Rayong Province

Table 32. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus siamensis* Brem.[5]

Vein-islet		Veinlet termination		Palisade cell	
Count in 4 mm^2	Vein-islet number	Count in 4 mm^2	Veinlet termination number	Number beneath 4 epidermal cells	Palisade ratio
8	2	5	1.25	11	2.75
7	1.75	6	1.5	12	3
8	2	5	1.25	10	2.5
8	2	9	2.25	13	3.25
11	2.75	9	2.25	18	4.5
11	2.75	10	2.5	12	3
12	3	8	2	13	3.25
8	2	11	2.75	13	3.25
8	2	6	1.5	11	2.75
6	1.5	7	1.75	9	2.25
8	2	10	2.5	11	2.75
5	1.25	8	2	14	3.5
6	1.5	10	2.5	14	3.5
10	2.5	8	2	13	3.25
8	2	8	2	10	2.5
7	1.75	7	1.75	16	4
6	1.5	8	2	13	3.25
8	2	7	1.75	14	3.5
12	3	11	2.75	14	3.5
11	2.75	10	2.5	14	3.5
8	2	7	1.75	11	2.75
9	2.25	6	1.5	10	2.5
9	2.25	7	1.75	13	3.25
10	2.5	7	1.75	11	2.75
7	1.75	10	2.5	12	3
8	2	8	2	12	3
8	2	7	1.75	14	3.5
7	1.75	7	1.75	12	3
9	2.25	7	1.75	14	3.5
8	2	11	2.75	12	3
mean	2.09	mean	2.0	mean	3.13
S.D.	0.44	S.D.	0.44	S.D.	0.47

5 = sample from Khao Chamao, Waterfall, Khao Chamao-Khao Wonge National Park, Rayong Province

Table 33. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus siamensis* Brem.[6]

Vein-islet		Veinlet termination		Palisade cell	
Count in 4 mm2	Vein-islet number	Count in 4 mm2	Veinlet termination number	Number beneath 4 epidermal cells	Palisade ratio
14	3.5	15	3.75	14	3.5
15	3.75	15	3.75	10	2.5
14	3.5	9	2.25	15	3.75
14	3.5	12	3	16	4
15	3.75	12	3	14	3.5
16	4	7	1.75	16	4
15	3.75	9	2.25	10	2.5
13	3.25	11	2.75	15	3.75
11	2.75	14	3.5	12	3
17	4.25	20	5	12	3
17	4.25	18	4.5	10	2.5
17	4.25	18	4.5	14	3.5
18	4.5	10	2.5	11	2.75
18	4.5	16	4	16	4
16	4	17	4.25	14	3.5
16	4	18	4.5	15	3.75
18	4.5	14	3.5	17	4.25
16	4	17	4.25	14	3.5
18	4.5	19	4.75	11	2.75
13	3.25	13	3.25	15	3.75
12	3	16	4	15	3.75
17	4.25	17	4.25	15	3.75
16	4	17	4.25	13	3.25
10	2.5	15	3.75	14	3.5
13	3.25	13	3.25	14	3.5
13	3.25	11	2.75	10	2.5
12	3	7	1.75	12	3
17	4.25	19	4.75	14	3.5
11	2.75	14	3.5	15	3.75
14	3.5	7	1.75	12	3
mean	3.72	mean	3.5	mean	3.38
S.D.	0.58	S.D.	0.95	S.D.	0.51

6 = sample from Kasetsart University, Bangkok

Table 34. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus siamensis* Brem.[7]

Vein-islet		Veinlet termination		Palisade cell	
Count in	Vein-islet number	Count in	Veinlet termination	Number beneath	Palisade
17	4.25	16	4	13	3.25
18	4.5	11	2.75	16	4
16	4	16	4	14	3.5
20	5	12	3	14	3.5
20	5	11	2.75	14	3.5
16	4	11	2.75	14	3.5
12	3	17	4.25	20	5
17	4.25	11	2.75	16	4
18	4.5	11	2.75	15	3.75
15	3.75	10	2.5	14	3.5
19	4.75	10	2.5	14	3.5
12	3	11	2.75	14	3.5
13	3.25	12	3	19	4.75
16	4	15	3.75	14	3.5
16	4	8	2	14	3.5
14	3.5	9	2.25	17	4.25
10	2.5	18	4.5	15	3.75
16	4	17	4.25	15	3.75
13	3.25	10	2.5	15	3.75
10	2.5	11	2.75	22	5.5
13	3.25	12	3	13	3.25
13	3.25	10	2.5	19	4.75
14	3.5	12	3	15	3.75
15	3.75	9	2.25	17	4.25
19	4.75	13	3.25	16	4
14	3.5	15	3.75	15	3.75
11	2.75	11	2.75	16	4
14	3.5	12	3	14	3.5
15	3.75	8	2	15	3.75
13	3.25	12	3	16	4
mean	3.74	mean	3.01	mean	3.88
S.D.	0.69	S.D.	0.67	S.D.	0.53

7 = sample from Khanit's garden, Buddhamonthon sai 3, Bangkok

Table 35. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus siamensis* Brem.[8]

Vein-islet		Veinlet termination		Palisade cell	
Count in 4 mm^2	Vein-islet number	Count in 4 mm^2	Veinlet termination number	Number beneath 4 epidermal cells	Palisade ratio
12	3	12	3	13	3.25
17	4.25	11	2.75	16	4
17	4.25	11	2.75	17	4.25
16	4	12	3	14	3.5
20	5	9	2.25	16	4
17	4.25	8	2	16	4
12	3	11	2.75	15	3.75
15	3.75	9	2.25	13	3.25
13	3.25	17	4.25	16	4
20	5	17	4.25	13	3.25
15	3.75	12	3	14	3.5
14	3.5	10	2.5	16	4
16	4	8	2	15	3.75
15	3.75	6	1.5	17	4.25
19	4.75	8	2	14	3.5
18	4.5	11	2.75	13	3.25
17	4.25	17	4.25	15	3.75
20	5	13	3.25	15	3.75
16	4	18	4.5	14	3.5
16	4	12	3	18	4.5
18	4.5	6	1.5	19	4.75
15	3.75	8	2	15	3.75
15	3.75	7	1.75	13	3.25
12	3	13	3.25	14	3.5
14	3.5	12	3	16	4
14	3.5	16	4	15	3.75
16	4	15	3.75	14	3.5
17	4.25	11	2.75	14	3.5
17	4.25	13	3.25	16	4
15	3.75	9	2.25	19	4.75
mean	3.98	mean	2.85	mean	3.79
S.D.	0.56	S.D.	0.84	S.D.	0.42

8 = sample from the Sireerukachart Garden, Mahidol University, Salaya Campus, Nakhon Pathom Province

Table 36. Stomatal number and stomatal index of *Clinacanthus nutans* (Burm. f.) Lindau[1]

Area of determination = 0.1075 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
14	114	130.23	10.94
17	112	158.14	13.18
19	120	176.74	13.67
16	128	148.84	11.11
18	106	167.44	14.52
16	104	148.84	13.33
14	105	130.23	11.76
16	98	148.84	14.04
20	125	186.05	13.79
18	133	167.44	11.92
21	147	195.35	12.50
21	144	195.35	12.73
20	139	186.05	12.58
20	111	186.05	15.27
13	105	120.93	11.02
18	118	167.44	13.24
15	111	139.53	11.90
15	111	139.53	11.90
16	127	148.84	11.19
18	109	167.44	14.17
17	104	158.14	14.05
15	107	139.53	12.30
21	128	195.35	14.09
17	117	158.14	12.69
16	119	148.84	11.85
18	114	167.44	13.64
18	129	167.44	12.24
20	122	186.05	14.08
15	102	139.53	12.82
18	114	167.44	13.64
	mean	161.24	12.87
	S.D.	20.92	1.15

Table 37. Stomatal number and stomatal index of *Clinacanthus nutans* (Burm. f.) Lindau[2]

Area of determination = 0.1075 mm^2

Number of stomata	Number of epidermal cells	Stomatal number	Stomatal index
18	118	167.44	13.24
15	111	139.53	11.90
15	111	139.53	11.90
16	127	148.84	11.19
18	109	167.44	14.17
17	104	158.14	14.05
15	107	139.53	12.30
21	128	195.35	14.09
17	117	158.14	12.69
16	119	148.84	11.85
18	114	167.44	13.64
18	129	167.44	12.24
20	122	186.05	14.08
15	102	139.53	12.82
18	114	167.44	13.64
18	118	167.44	13.24
17	121	158.14	12.32
18	115	167.44	13.53
12	108	111.63	10.00
19	119	176.74	13.77
18	122	167.44	12.86
17	108	158.14	13.60
17	104	158.14	14.05
17	115	158.14	12.88
16	105	148.84	13.22
19	122	176.74	13.48
19	125	176.74	13.19
19	122	176.74	13.48
16	105	148.84	13.22
16	115	148.84	12.21
	mean	159.69	12.96
	S.D.	16.76	0.96

2 = sample from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province

Table 38. Glandular trichome and glandular trichome index of *Clinacanthus nutans* (Burm. f.) Lindau[1]

Area of determination = 0.1075 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular trichome number	Glandular trichome index
2	140	18.60	1.41
2	144	18.60	1.37
1	157	9.30	0.63
3	157	27.91	1.88
0	143	0.00	0.00
0	136	0.00	0.00
2	131	18.60	1.50
2	128	18.60	1.54
1	164	9.30	0.61
2	168	18.60	1.18
1	188	9.30	0.53
1	185	9.30	0.54
1	178	9.30	0.56
1	150	9.30	0.66
2	129	18.60	1.53
2	152	18.60	1.30
0	141	0.00	0.00
1	140	9.30	0.71
1	158	9.30	0.63
2	143	18.60	1.38
2	136	18.60	1.45
1	136	9.30	0.73
2	168	18.60	1.18
2	149	18.60	1.32
2	149	18.60	1.32
2	148	18.60	1.33
1	164	9.30	0.61
2	160	18.60	1.23
2	131	18.60	1.50
2	149	18.60	1.32
	mean	13.95	1.00
	S.D.	6.80	0.51

1 = sample from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok

Table 39. Glandular trichome and glandular trichome index of *Clinacanthus nutans* (Burm. f.) Lindau[2]

Area of determination = 0.1075 mm^2

Number of glandular trichomes	Number of epidermal cells	Glandular trichome number	Glandular trichome index
2	152	18.60	1.30
0	141	0.00	0.00
1	140	9.30	0.71
1	158	9.30	0.63
2	143	18.60	1.38
2	136	18.60	1.45
1	136	9.30	0.73
2	168	18.60	1.18
2	149	18.60	1.32
2	149	18.60	1.32
2	148	18.60	1.33
1	164	9.30	0.61
2	160	18.60	1.23
2	131	18.60	1.50
2	149	18.60	1.32
1	157	9.30	0.63
1	156	9.30	0.64
1	152	9.30	0.65
1	134	9.30	0.74
0	159	0.00	0.00
1	159	9.30	0.63
0	144	0.00	0.00
0	140	0.00	0.00
1	151	9.30	0.66
1	138	9.30	0.72
2	163	18.60	1.21
2	163	18.60	1.21
1	162	9.30	0.61
1	139	9.30	0.71
1	149	9.30	0.67
	mean	11.78	0.84
	S.D.	6.43	0.46

2 = sample from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province

Table 40. Lithocyst number and lithocyst index of *Clinacanthus nutans* (Burm. f.) Lindau[1]

Area of determination = 0.1075 mm^2

Number of lithocyst	Number of epidermal cells	Lithocyst number	Lithocyst index
0	142	0.00	0.00
0	146	0.00	0.00
1	157	9.30	0.63
1	159	9.30	0.63
3	140	27.91	2.10
3	133	27.91	2.21
3	130	27.91	2.26
0	130	0.00	0.00
1	164	9.30	0.61
1	169	9.30	0.59
2	187	18.60	1.06
2	184	18.60	1.08
4	175	37.21	2.23
2	149	18.60	1.32
2	129	18.60	1.53
3	151	27.91	1.95
3	138	27.91	2.13
2	139	18.60	1.42
2	157	18.60	1.26
1	144	9.30	0.69
2	136	18.60	1.45
1	136	9.30	0.73
3	167	27.91	1.76
1	150	9.30	0.66
2	149	18.60	1.32
1	149	9.30	0.67
2	163	18.60	1.21
1	161	9.30	0.62
2	131	18.60	1.50
2	149	18.60	1.32
	mean	16.43	1.16
	S.D.	9.36	0.67

1 = sample from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok

Table 41. Lithocyst number and lithocyst index of *Clinacanthus nutans* (Burm. f.) Lindau[2]

Area of determination = 0.1075 mm^2

Number of lithocyst	Number of epidermal cells	Lithocyst number	Lithocyst index
3	151	27.91	1.95
3	138	27.91	2.13
2	139	18.60	1.42
2	157	18.60	1.26
1	144	9.30	0.69
2	136	18.60	1.45
1	136	9.30	0.73
3	167	27.91	1.76
1	150	9.30	0.66
2	149	18.60	1.32
1	149	9.30	0.67
2	163	18.60	1.21
1	161	9.30	0.62
2	131	18.60	1.50
2	149	18.60	1.32
3	155	27.91	1.90
1	156	9.30	0.64
1	152	9.30	0.65
2	133	18.60	1.48
2	157	18.60	1.26
1	159	9.30	0.63
2	142	18.60	1.39
2	138	18.60	1.43
2	150	18.60	1.32
1	138	9.30	0.72
3	162	27.91	1.82
0	165	0.00	0.00
2	161	18.60	1.23
2	138	18.60	1.43
2	148	18.60	1.33
	mean	16.74	1.20
	S.D.	7.08	0.50

2 = sample from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province

Table 42. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus nutans* (Burm. f.) Lindau[1]

Vein-islet		Veinlet termination		Palisade cell	
Count in 4 mm^2	Vein-islet number	Count in 4 mm^2	Veinlet termination number	Number beneath 4 epidermal cells	Palisade ratio
7	1.75	9	2.25	29	7.25
11	2.75	7	1.75	25	6.25
8	2	12	3	25	6.25
9	2.25	7	1.75	27	6.75
9	2.25	6	1.5	27	6.75
12	3	5	1.25	28	7
8	2	10	2.5	28	7
12	3	8	2	27	6.75
10	2.5	9	2.25	25	6.25
13	3.25	9	2.25	24	6
13	3.25	6	1.5	25	6.25
8	2	13	3.25	24	6
9	2.25	9	2.25	29	7.25
9	2.25	8	2	24	6
8	2	10	2.5	28	7
11	2.75	10	2.5	24	6
9	2.25	11	2.75	25	6.25
9	2.25	11	2.75	24	6
11	2.75	9	2.25	23	5.75
9	2.25	8	2	25	6.25
7	1.75	8	2	27	6.75
10	2.5	7	1.75	28	7
9	2.25	8	2	24	6
10	2.5	8	2	25	6.25
10	2.5	7	1.75	26	6.5
8	2	9	2.25	28	7
9	2.25	10	2.5	28	7
10	2.5	8	2	26	6.5
8	2	8	2	24	6
7	1.75	8	2	27	6.75
mean	2.36	mean	2.15	mean	6.49
S.D.	0.41	S.D.	0.44	S.D.	0.44

1 = sample from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok

Table 43. Vein-islet number, veinlet termination number and palisade ratio of *Clinacanthus nutans* (Burm. f.) Lindau[2]

Vein-islet		Veinlet termination		Palisade cell	
Count in 4 mm^2	Vein-islet number	Count in 4 mm^2	Veinlet termination number	Number beneath 4 epidermal cells	Palisade ratio
9	2.25	11	2.75	30	7.5
12	3	11	2.75	29	7.25
11	2.75	11	2.75	28	7
13	3.25	11	2.75	25	6.25
12	3	13	3.25	31	7.75
9	2.25	9	2.25	30	7.5
12	3	12	3	30	7.5
8	2	8	2	30	7.5
9	2.25	11	2.75	25	6.25
13	3.25	11	2.75	29	7.25
8	2	15	3.75	24	6
9	2.25	6	1.5	25	6.25
10	2.5	9	2.25	33	8.25
11	2.75	9	2.25	31	7.75
11	2.75	7	1.75	29	7.25
9	2.25	8	2	33	8.25
8	2	12	3	27	6.75
10	2.5	9	2.25	28	7
10	2.5	8	2	29	7.25
11	2.75	13	3.25	32	8
12	3	13	3.25	28	7
10	2.5	11	2.75	29	7.25
10	2.5	10	2.5	27	6.75
11	2.75	13	3.25	30	7.5
12	3	14	3.5	25	6.25
15	3.75	11	2.75	27	6.75
10	2.5	8	2	31	7.75
11	2.75	10	2.5	31	7.75
12	3	15	3.75	31	7.75
11	2.75	13	3.25	32	8
mean	2.66	mean	2.68	mean	7.24
S.D.	0.41	S.D.	0.58	S.D.	0.62

2 = sample from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province

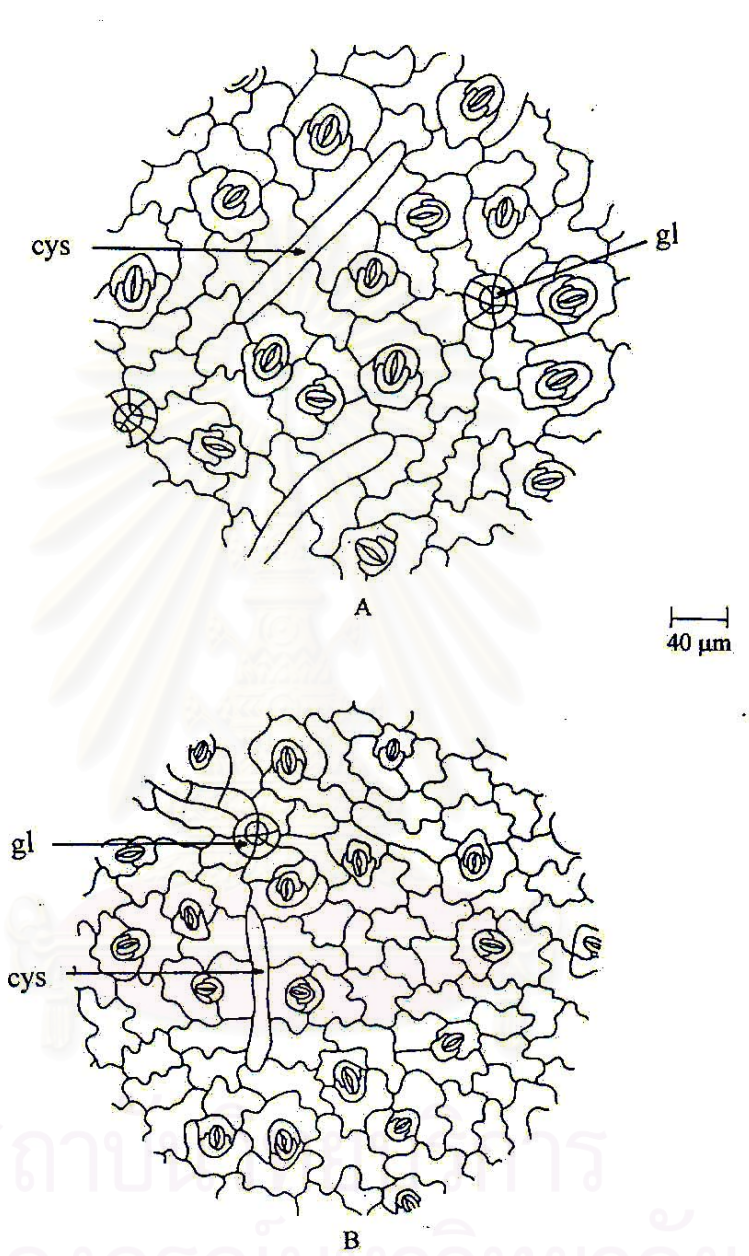

Figure 9. Lower epidermis of the leaves in surface view

A. *Clinacanthus siamensis* Brem.

B. *Clinacanthus nutans* (Burm. f.) Lindau

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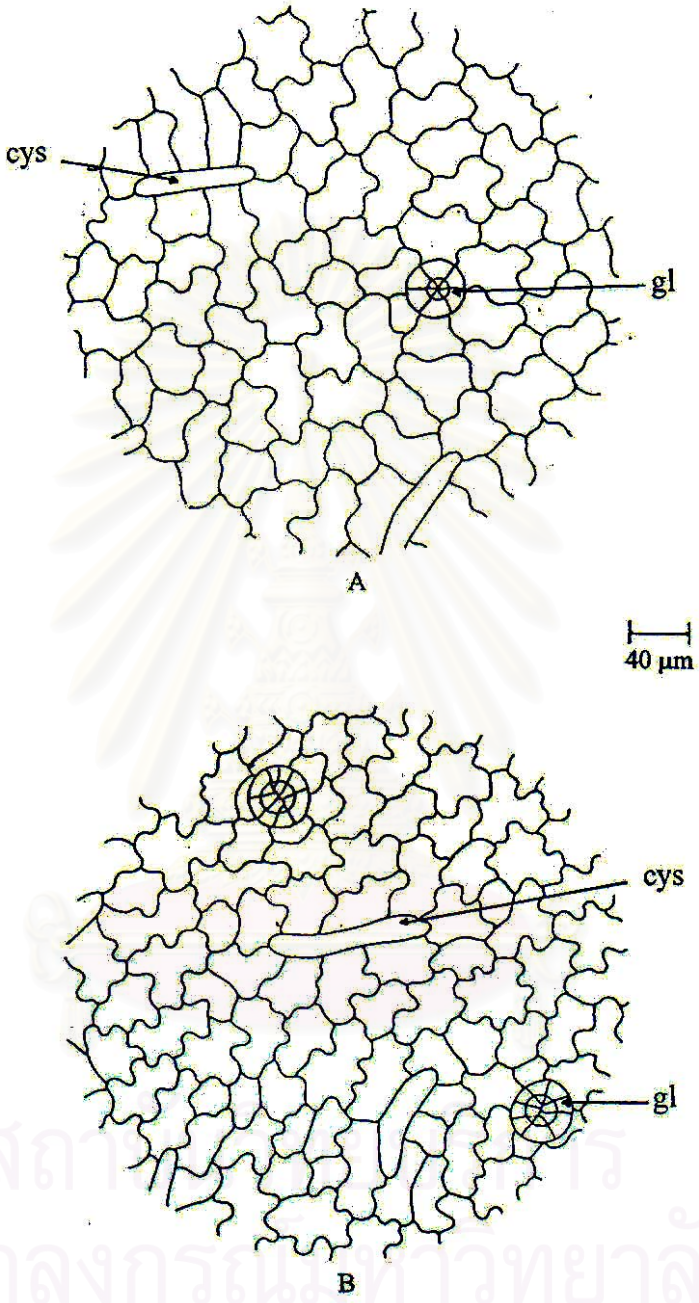

Figure 10. Upper epidermis of the leaves in surface view

A. *Clinacanthus siamensis* Brem.

B. *Clinacanthus nutans* (Burm. f.) Lindau

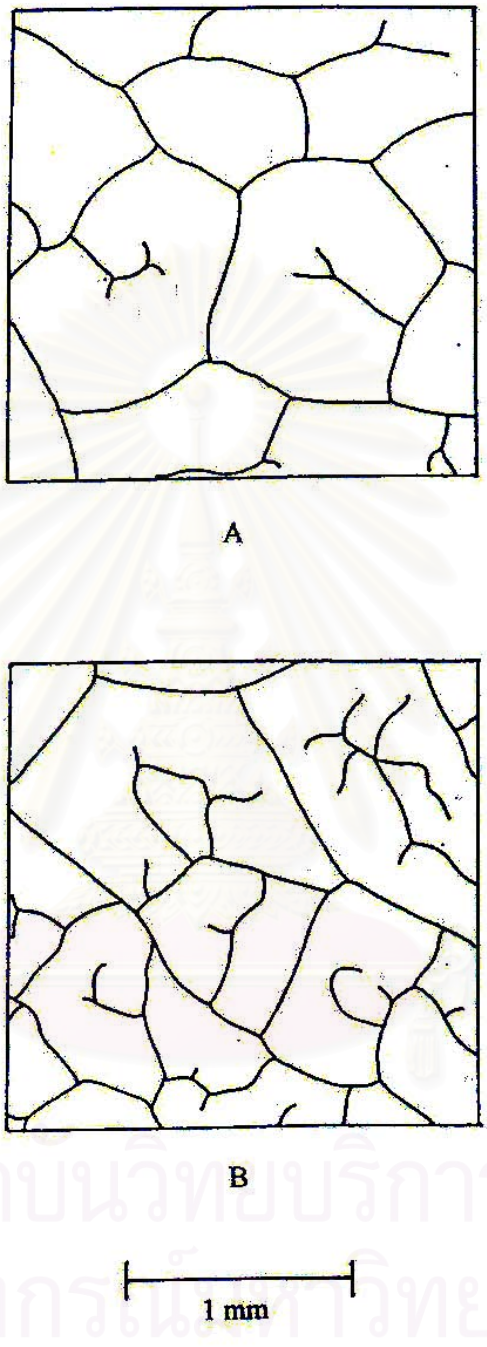

Figure 11. Vein-islet and veinlet termination of the leaves in surface view

A. *Clinacanthus siamensis* Brem.

B. *Clinacanthus nutans* (Burm. f.) Lindau

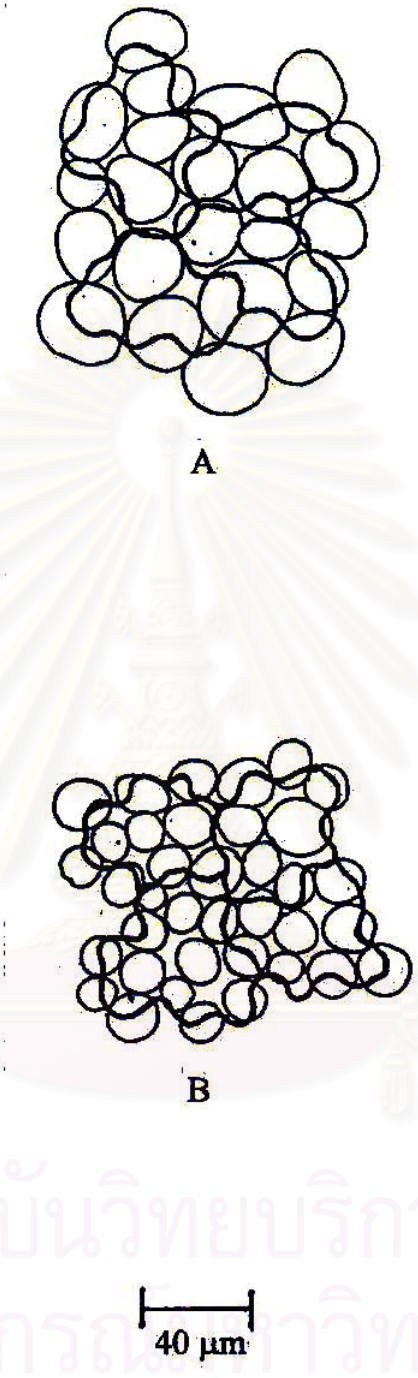

Figure 12. Four upper contiguous epidermal cells with underlying palisade cells in surface view

A. *Clinacanthus siamensis* Brem.

B. *Clinacanthus nutans* (Burm. f.) Lindau

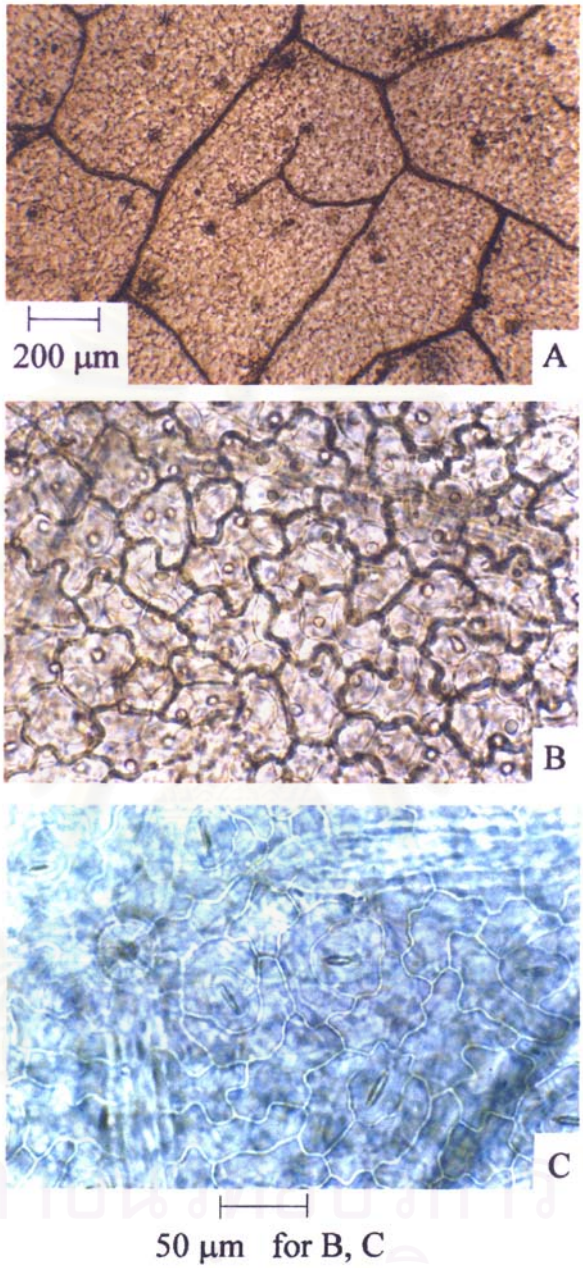

Figure 13. Microscopic illustration of *Clinacanthus siamensis* Brem. leaf

A. Vein-islet and veinlet termination
B. Upper epidermis with underlying palisade cells
C. Lower epidermis with stomata, glandular trichome and lithocyst

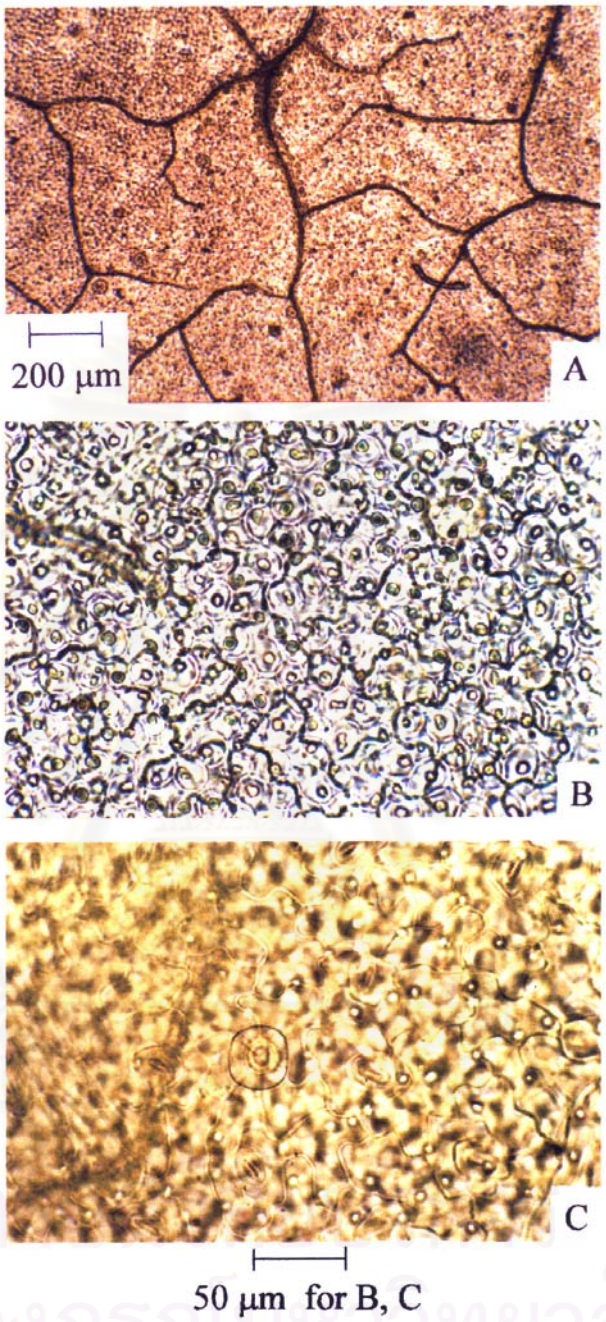

Figure 14. Microscopic illustration of *Clinacanthus nutans* (Burm. f.) Lindau leaf

A. Vein-islet and veinlet termination
B. Upper epidermis with underlying palisade cells
C. Lower epidermis with stomata, glandular trichome and lithocyst

Part II The results of macroscopic and microscopic characterizations of leaves

Macroscopic characters of *Clinacanthus siamensis* l eaves

Form : lanceolate, oblong-lanceolate, ovate, subentire, oblique base
Size : 2.5-4.0 cm wide and 7-12 cm long
Color : green to dark green (freshly leaves) and pale green to yellowish green (dried leaves)
Odor : characteristic
Taste : tasteless

Microscopic characters of *Clinacanthus siamensis* leaves

Anatomy and histology: sectional view of the leaf was shown in Figure 15. The following characteristic features were found. The upper epidermis consisted of slightly wavy walled cells but the stoma was absent. The mesophyll was composed of one layer of palisade cells and 3-6 layers of spongy cells containing chloroplasts. The lower epidermis consisted of the epidermal cells which were similar to those of the upper epidermis with numerous diacytic stomata, the lithocyst, uniseriate multicellular covering trichomes and glandular trichomes with 6 to 8 cells head and short unicellular stalk occur on both epidermises. The midrib consisted three to five layers of collenchymatous cells underneath the epidermis of both sides. The vascular bundle is of collateral type. There are much more covering trichomes on upper surface than the lower surface of the midrib, veins and veinlets.

Powdered drug: The powder is green with a characteristic odor and tasteless. The diagnostic characters are:

1. The abundant fragments of the lamina in surface view. The upper epidermis is composed of slightly wavy walled cells (Figure 16-1A), some underlying with palisade cells (Figure 16-1B).
2. The lower epidermis is composed of wavy walled cells with numerous diacytic stomata. Six- to eight- celled glandular trichomes are found on both epidermises (Figure 16-3A).
3. The occasional fragments of non-glandular uniseriate trichomes are composed of 3-5 cells, 220-370 μm long (Figure 16-7).
4. The fragments of the lamina in sectional view showing the upper epidermis, a single layer of palisade cells, spongy cells, the lower epidermis (Figure 16-3A, 3B) and small vascular bundle in the middle.
5. The fragments of the rectangular petiole epidermis (Figure 16-4).
6. The occasional fragments of the fibers and spiral vessels (Figure 16-5).

The microscopic characters of their leaves were shown in Figures 16-17.

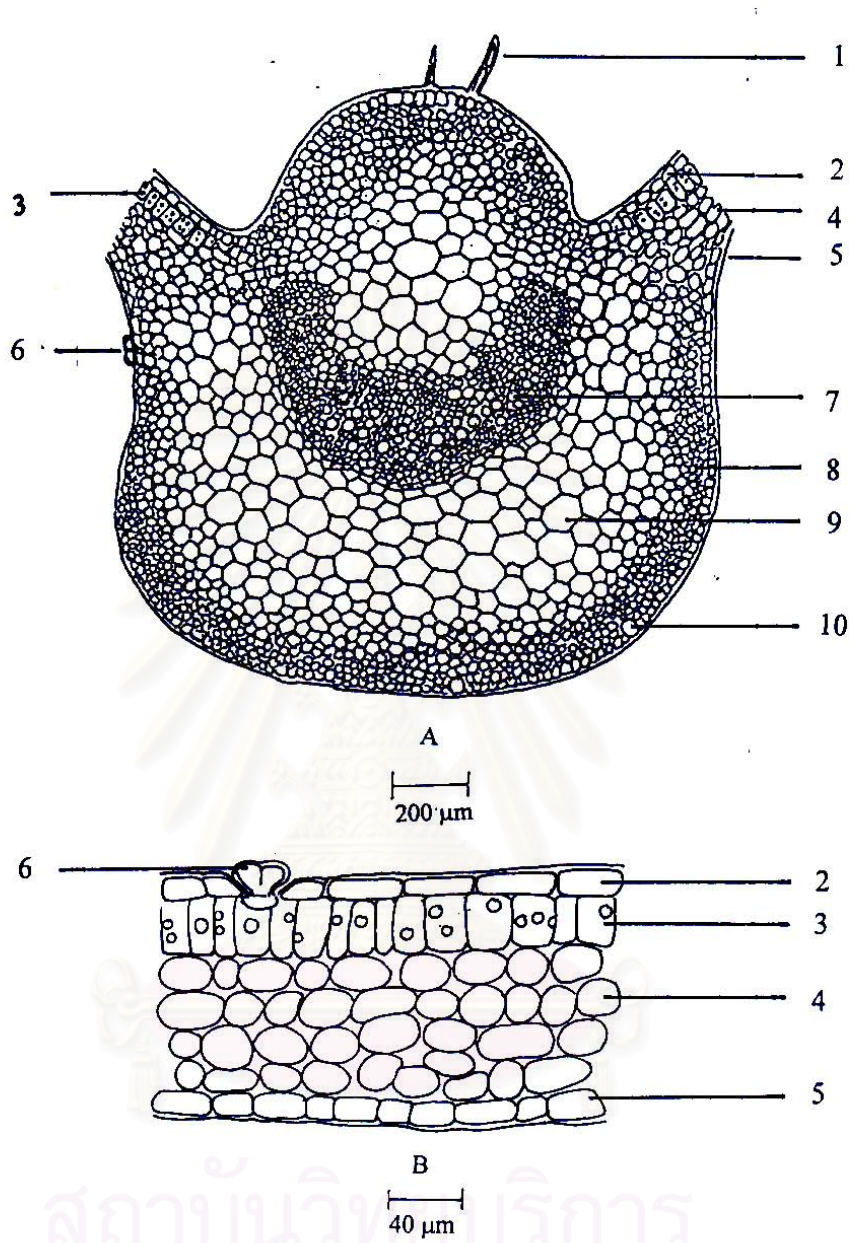

Figure 15. Sectional view of *Clinacanthus siamensis* Brem. leaf

A. mibrib and B. lamina

1. Covering trichome , 2. Upper epidermis, 3. Palisade cell,
4. Spongy cell, 5. Lower epidermis, 6. Glandular trichome,
7. Vessel, 8. Collenchyma, 9. Parenchyma, 10. lithocyst

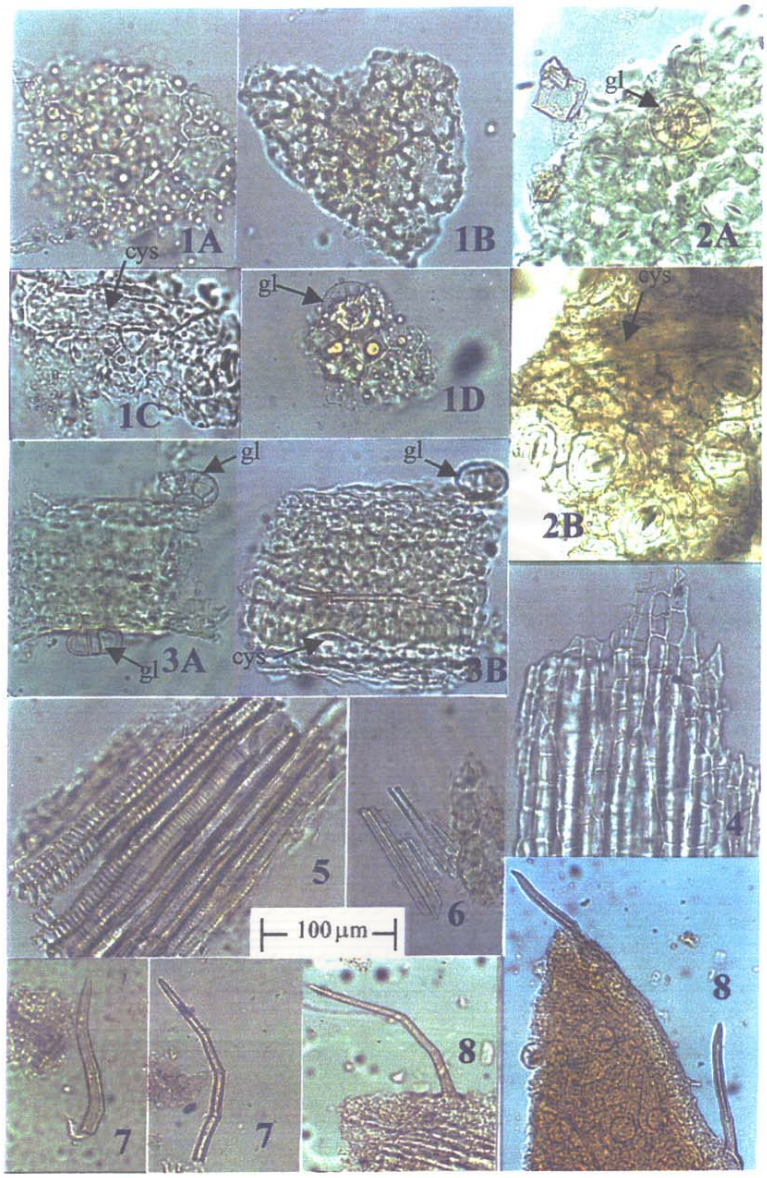

Figure 16. Microscopic characters of powered *Clinacanthus siamensis* Brem. leaves

1. upper epidermis in surface view: A. upper epidermis, B. upper epidermis with underlying palisade cells, C. with lithocyst, D. with glandular trichome
2. lower epidermis in surface view: A. with glandular trichome, B. with glandular trichome
3. sectional view of lamina: A. showing the upper epidermis with glandular trichome, palisade cells, spongy cells and the lower epidermis with glandular trichome; B. showing the lithocyst
4. part of the epidermis of the petiole in surface view
5. the fragment of a group of spiral vessels and pitted
6. the fragment of fiber
7. the fragment of non-glandular uniseriate trichomes
8. surface view of the lamina with non-glandular uniseriate trichome

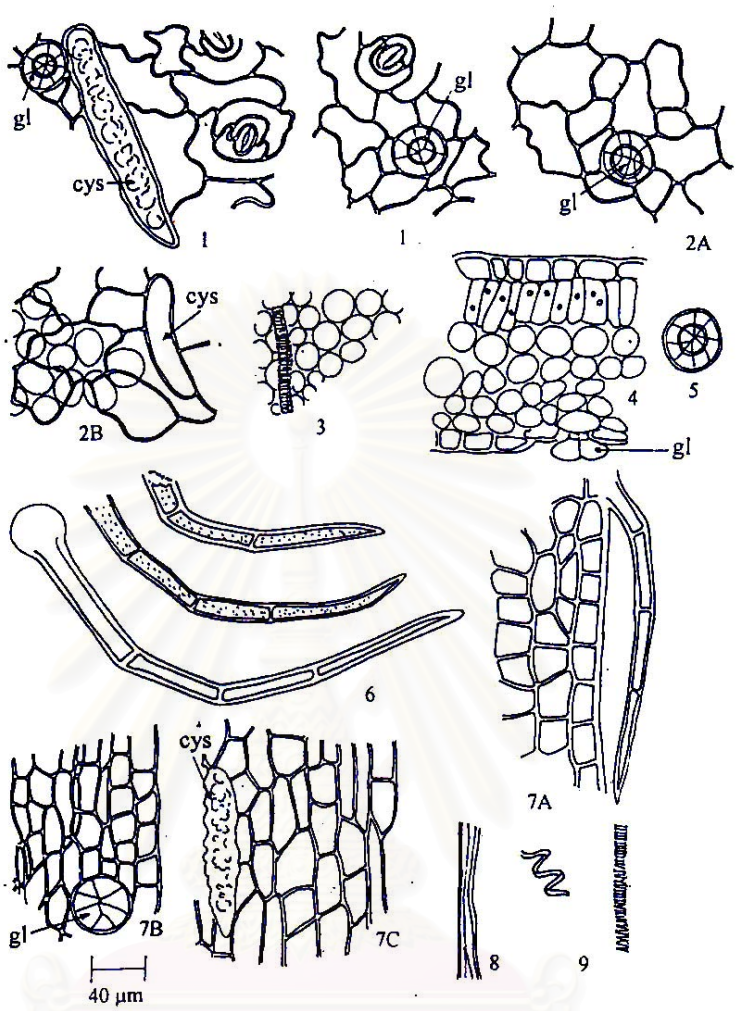

Figure 17. Microscopic characters of powdered *Clinacanthus siamensis* Brem. leaves

1. lower epidermis in surface view showing diacytic stomata, a lithocyst cell (cys) and a glandular trichome (gl)
2. upper epidermis in surface view : A. with a glandular trichome , B. with a lithocyst cell
3. the fragment of mesophyll with part of a veinlet
4. sectional view of the lamina showing the upper epidermis, palisade cells, spongy cells andthe lower epidermis with unicellular stalk and glandular trichome
5. a glandular trichome in surface view
6. the fragments of non-glandular uniseriate trichomes
7. part of the epidermis of the petiole in surface view: A. with an attached non-glandular uniseriate trichome, B. with an attached glandular trichome, C. with a lithocyst cell
8. the fragment of fiber
9. the fragments of a group of spiral vessels

Part III The results of anti-herpes simplex virus activity test of *Clinacanthus siamensis* leaves

The anti-herpes simplex virus activity test of ethyl acetate extracts from mature leaves collected monthly for 1 year showed the potency level against herpes simplex virus as follows in Table 44.

Table 44. The anti-herpes simplex virus activity test of ethyl acetate extracts from *Clinacanthus siamensis* leaves

The extracts in each month	Anti-herpes simplex virus activity (% inhibition)	
	HSV type I	HSV type II
1. January 02	95*	95*
2. February 02	95	80
3. March 01	80	80
4. April 01	80	80
5. May 01	85	80
6. June 01	95*	90*
7. July 01	80	80
8. August 01	90	85
9. September 01	90	90
10. October 01	90	90
11. November 01	90	80
12. December 01	95*	95*

The effective dose at 20 μg/ml and *50 μg/ml.

Part IV The results of Thin-layer chromatographic pattern of leaf extracts

The results of one-dimensional TLC of methanol extracts were shown as follows:

System 1 Chloroform : methanol (4 : 1), Figure 18, Table 45.

System 2 Chloroform : acetone (1 : 1), Figure 19, Table 46.

The results of one-dimensional TLC of ethyl acetate extracts were shown as follows:

System 1 Chloroform : methanol (4 : 1), Figure 20, Table 47.

System 2 Chloroform : acetone (1 : 1), Figure 21, Table 48.

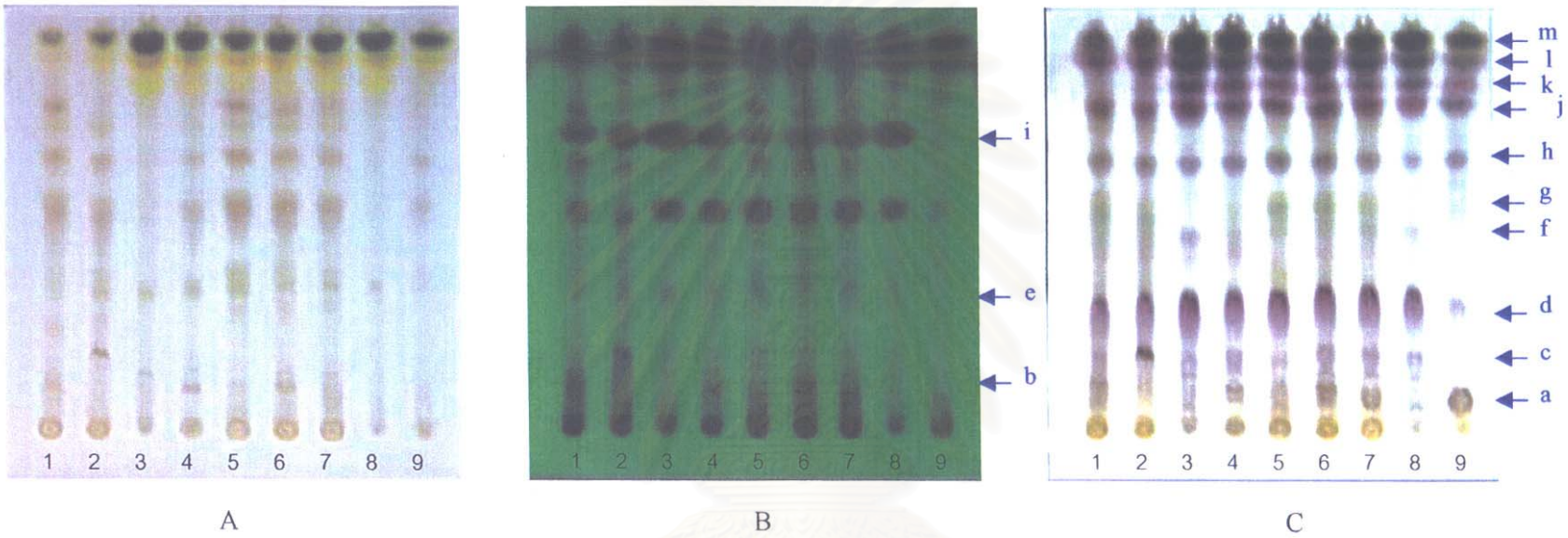

Figure 18. TLC patterns of methanol extracts of *Clinacanthus siamensis* Brem. leaves (spots 1-8) and *C. natans* (Burm. f.) Lindau leaves (spot 9)

Solvent system——Chloroform : methanol (4 : 1)

A. Detection with visible daylight

B. Detection with UV 254 nm

C. Detection with anisaldehyde-sulfuric acid TS

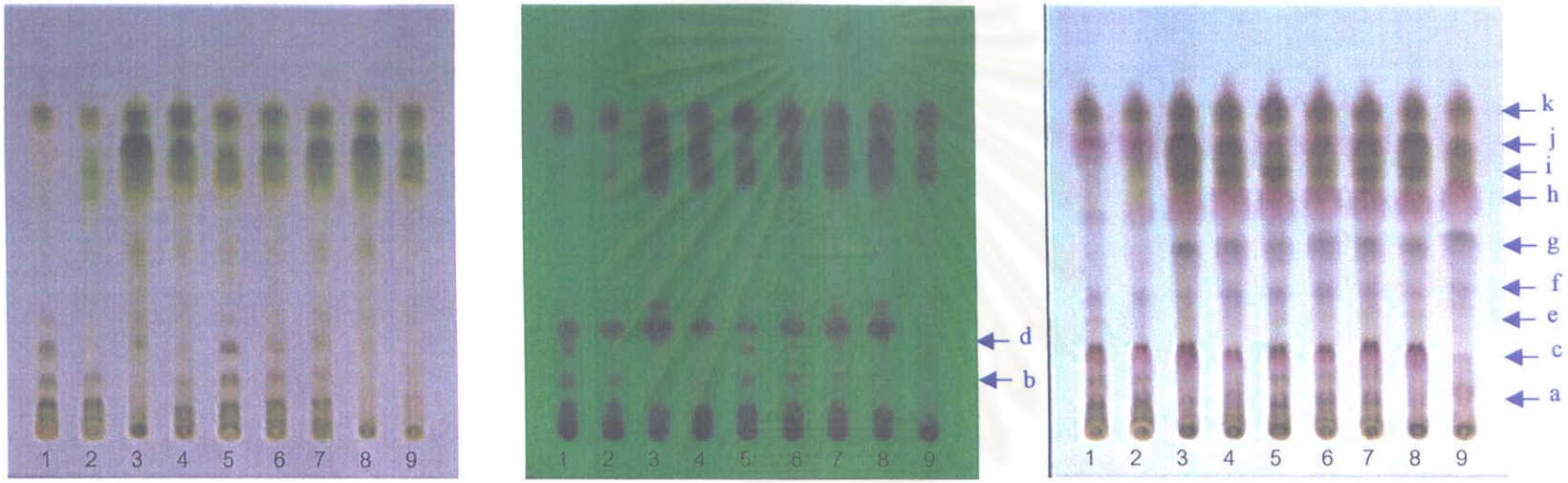

Figure 19. TLC patterns of methanol extracts of *Clinacanthus siamensis* Brem. leaves (spots 1-8) and *C. natans* (Burm. f.) Lindau leaves (spot 9)

Solvent system——Chloroform : acetone (1 : 1)

A. Detection with visible daylight

B. Detection with UV 254 nm

C. Detection with anisaldehyde-sulfuric acid TS

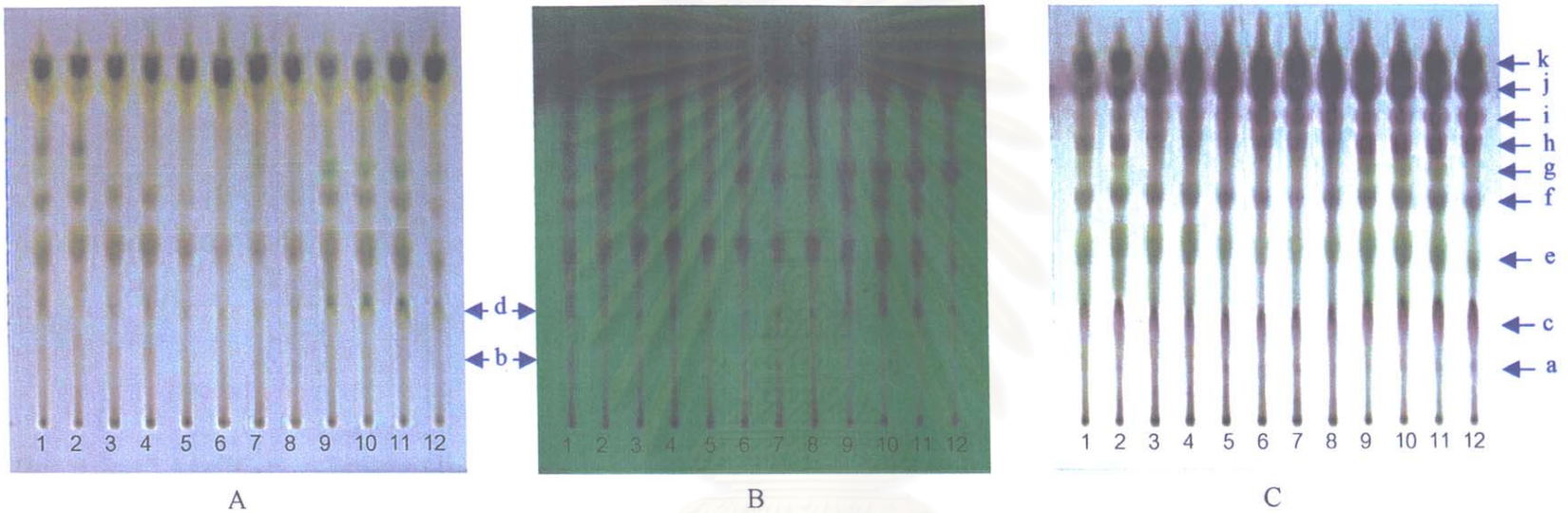

Figure 20. TLC patterns of ethyl acetate extracts of *Clinacanthus siamensis* Brem. leaves

Solvent system—— Chloroform : methanol (4 : 1)

A. Detection with visible daylight

B. Detection with UV 254 nm

C. Detection with anisaldehyde-sulfuric acid TS

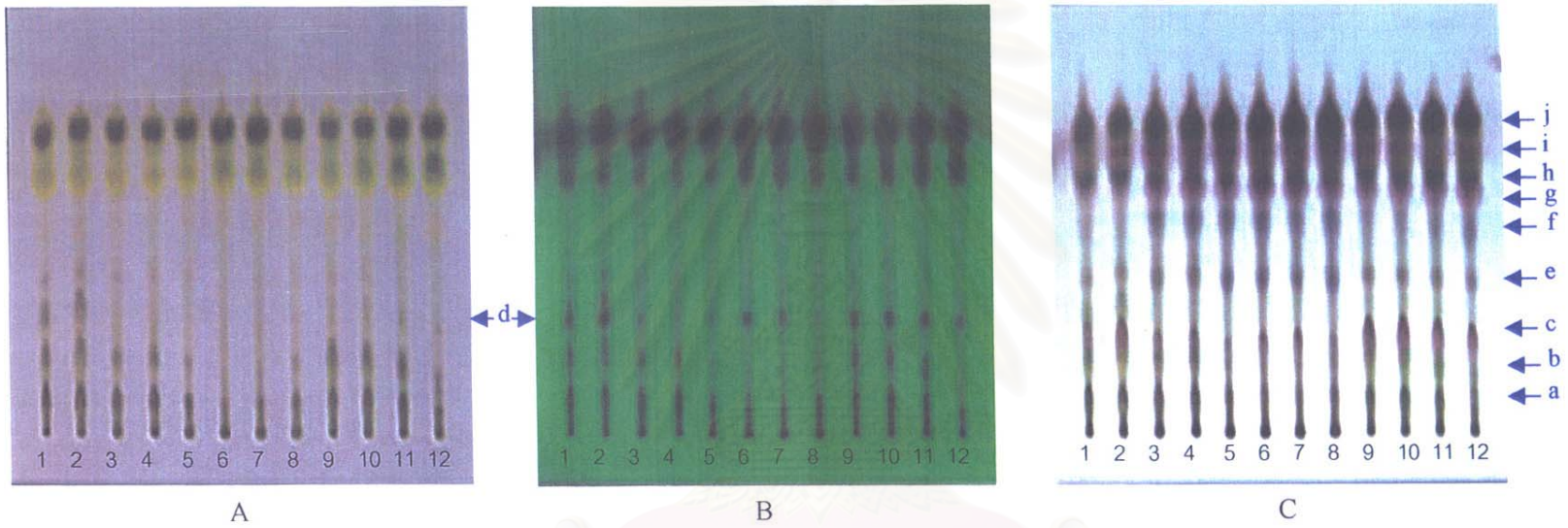

Figure 21. TLC patterns of ethyl acetate extracts of *Clinacanthus siamensis* Brem. leaves

Solvent system—— Chloroform : acetone (1 : 1)

A. Detection with visible daylight

B. Detection with UV 254 nm

C. Detection with anisaldehyde-sulfuric acid TS

Table 45. R_f values of components in methanol extract of the leaves of *Clinacanthus* ***siamensis*** Brem. (Solvent system —— Chloroform : methanol (4 : 1))

Spot	R_f	Detection with		
		Visible day light	UV 254	Anisaldehyde-sulfuric acid TS
a	0.08	-	quenching	yellowish-brown
b	0.11	yellowish-green	quenching	yellowish-brown
c	0.18	-	quenching	pink
d	0.30	-	-	pink
e	0.33	yellowish-green	quenching	-
f	0.46	-	-	light purple
g	0.52	yellowish-green	quenching	yellowish-green
h	0.65	brownish-green	quenching	purple
i	0.71	yellow	quenching	-
j	0.77	brown	quenching	reddish-pink
k	0.83	yellow	-	reddish-pink
l	0.88	yellow	quenching	reddish-pink
m	0.94	green	quenching	green

Table 46. R_f values of components in methanol extract of the leaves of *Clinacanthus* ***siamensis*** Brem. (Solvent system —— Chloroform : acetone (1 : 1))

Spot	R_f	Detection with		
		Visible day light	UV 254	Anisaldehyde-sulfuric acid TS
a	0.06	yellowish-green	quenching	yellowish-brown
b	0.12	brownish-green	quenching	purple
c	0.18	-	-	pink
d	0.20	brownish-green	quenching	reddish-pink
e	0.25	yellowish-green	quenching	yellowish-green
f	0.33	-	-	blue-purple
g	0.42	yellowish-green	quenching	blue-purple
h	0.54	-	-	pink
i	0.61	brownish-green	quenching	green
j	0.68	yellow	-	pink
k	0.76	yellowish-green	quenching	green

Table 47. R_f values of components in ethyl acetate extract of the leaves of *Clinacanthus siamensis* Brem. (Solvent system —— Chloroform : methanol (4 : 1))

Spot	R_f	Detection with		
		Visible day light	UV 254	Anisaldehyde-sulfuric acid TS
a	0.13	-	-	light pink
b	0.17	greenish-brown	quenching	-
c	0.24	-	-	reddish-pink
d	0.27	yellowish-green	quenching	-
e	0.41	greenish-brown	quenching	yellowish-green
f	0.53	greenish-brown	quenching	purple
g	0.60	yellowish-green	quenching	brownish-green
h	0.66	-	quenching	reddish-pink
i	0.73	brownish-green	-	reddish-pink
j	0.79	yellow	quenching	purple
k	0.85	green	quenching	green

Table 48. R_f values of components in ethyl acetate extract of the leaves of *Clinacanthus* ***siamensis*** Brem. (Solvent system —— Chloroform : acetone (1 : 1))

Spot	R_f	Detection with		
		Visible day light	UV 254	Anisaldehyde-sulfuric acid TS
a	0.08	green	quenching	green
b	0.16	greenish-brown	quenching	yellowish-green
c	0.21	-	-	reddish-pink
d	0.26	brownish-green	quenching	-
e	0.36	brown	quenching	purple
f	0.49	yellow	-	blue-purple
g	0.57	-	-	reddish-pink
h	0.62	yellowish-green	quenching	blue-purple
i	0.67	-	-	reddish-pink
j	0.74	green	quenching	green

Part V The results of phytochemical screening

The results of chemical tests of powdered *Clinacanthus siamensis* Brem. leaves were shown in Table 45. It revealed that *Clinacanthus. siamensis* Brem. leaves might contain triterpene and steroid, reducing sugars, phenolic compound, flavonoid.

Table 49. Chemical tests of powdered *Clinacanthus siamensis* Brem. leaves

Detection method	Positive test	Results
Froth test	oney comb froth which persists for at least 30 min	Negative (less than 30 min)
Ferric chloride TS	Green or blue precipitate	Negative (Brown precipitate)
Iodine TS	Blackening precipitate	Negative (Brown precipitate)
Fehling's TS	Red precipitate	Positive (Brownish red precipitate)
Shinoda's test	Pink to red solution	Positive (Orange solution)
Alkaloid test	Orange precipitate (Dragendorff's reagent) and white precipitate (Mayer's reagent)	Negative
Liebermann-Burchard test	Red, pink, purple or violet	Positive (Brownish-red)

Part VI The results of quality controls

The quality controls of *Clinacanthus siamensis* leaves which were collected from several sources are shown in Table 50.

Table 50. Loss on drying, total ash, acid insoluble ash and extractive value of *Clinacanthus siamensis* Brem.

Sample*	Loss on drying	Ash content (%)		Extractive value (%)	
		Total ash	Acid-insoluble	Ethanol	Water
1a	8.16	14.71	0.51	14.76	30.76
1b	8.11	14.77	0.63	14.34	30.88
2a	6.44	18.05	0.88	8.07	28.41
2b	6.38	18.08	0.90	8.07	27.22
3a	6.24	17.22	0.56	7.74	28.57
3b	6.18	17.13	0.75	7.63	28.68
4a	6.93	14.22	0.43	7.57	28.37
4b	6.74	14.35	0.47	8.38	28.40
4c	7.59	16.89	1.10	16.94	34.22
4d	7.44	16.81	1.01	16.72	34.24
5a	6.83	17.07	0.58	13.62	26.93
5b	6.73	17.04	0.59	13.30	26.87
6a	7.91	19.29	0.21	12.16	28.46
6b	7.69	19.28	0.22	12.13	28.56
7a	10.31	15.63	0.34	12.33	27.77
7b	10.28	15.57	0.29	12.14	27.74
Mean	**7.50**	**16.63**	**0.59**	**11.62**	**29.13**
S.D.	**1.27**	**1.62**	**0.27**	**3.29**	**2.28**

1 = sample from Khanit's garden, Buddhamonthon sai 3, Bangkok.

2 = sample from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province.

3 = sample from Khao Chamao, Waterfall, Khao Chamao-Kao Wonge National Park, Rayong Province.

4 = sample from Khao Hinshon, Chachoengsao Province.

5 = sample from Kasetsart University, Bangkok.

6 = sample from the Somdej Phra Thepratanarajasuda Medicinal Plants Garden, Petroleum Authority of Thailand, Rayong Province.

7 = sample from the Sireerukachart Garden, Mahidol University, Salaya Campus, Nakhon Pathom Province.

Discussion

This study dealed with the investigation of the pharmacognostic characters of *Clinacanthus siamensis* Brem. (Lin ngu hao) leaves. Leaf measurements were determined from 8 samples and statistic process was used for discussion of those particular data. The 95 % confidence interval of those leaf measurements were calculated using total data of 8 samples (240 data, Tables 4 -35) and the representative information of them are shown in Table 51.

Table 51. Leaf measurements of *Clinacanthus siamensis* Brem.

	95 % Confidence interval	Data interval	Mean (S.D.)
Stomatal number	142.29 - 149.03	102.33 - 241.86	145.66 (26.63)
Stomatal index	12.14 - 12.54	7.74 - 16.96	12.34 (1.61)
Palisade ratio	3.15 - 3.64	2.25 - 5.50	3.57 (0.49)
Vein-islet number	3.19 - 3.40	1.25 - 5.00	3.29 (0.83)
Veinlet termination	2.67 - 2.88	1.25 - 5.00	2.77 (0.81)
Glandular number	9.45 - 10.94	0 - 27.91	10.19 (5.86)
Glandular index	0.71 - 0.82	0 - 2.29	0.76 (0.43)
Lithocyst number	16.19 - 18.30	0 - 37.21	17.25 (8.34)
Lithocyst index	1.23 - 1.38	0 - 3.08	1.30 (0.62)

Furthermore, two samples of *Clinacanthus nutans* (Burm. f.) Lindau were studied in leaf measurements. The 95 % confidence interval of those leaf measurements were calculated using total data of 2 samples (60 data, Tables 36-43) and the representative information of them are shown in Table 52.

Table 52. Leaf measurements of *Clinacanthus nutans* (Burm. f.) Lindau

	95 % Confidence interval	Data interval	Mean (S.D.)
Stomatal number	155.71 - 165.22	111.63 - 195.35	160.47 (18.81)
Stomatal index	8.16 - 13.18	10.0 - 15.27	12.92 (1.05)
Palisade ratio	6.70 - 7.03	5.75 - 8.25	6.87 (0.65)
Vein-islet number	2.40 - 2.62	1.75 - 3.75	2.51 (0.44)

Table 52. (continued) Leaf measurements of *Clinacanthus nutans* (Burm. f.) Lindau

	95 % Confidence interval	Data interval	Mean (S.D.)
Veinlet termination	2.27 - 2.56	1.25 - 3.75	2.42 (0.58)
Glandular number	11.19 - 14.55	0 - 27.91	12.87 (6.65)
Glandular index	0.79 - 1.04	0 - 1.88	0.92 (0.49)
Lithocyst number	14.51 - 18.67	0 - 37.21	16.59 (8.23)
Lithocyst index	1.03 - 1.33	0 - 2.26	1.18 (0.59)

The leaf measurements are used as character for the identification concerning their constant value in each species. It is interesting to note that the represent data should be used more than 1 sample in each species for reliable information.

The characteristic macroscopic features of the *C. siamensis* leaves were simple, opposite, green to dark green (freshly) and pale green to yellowish green (dried leaves), lanceolate to oblong-lanceolate, ovate, subentire, oblique base, 2.5-4.0 cm wide, 7-12 cm long, characteristic odor and tasteless. In this study, it was found that the leaves sizes were variable according to light approach. If this plant is cultivated in the shade of a tree, the leaves will be bigger than in the open field.

The characteristic microscopic features of the leaves were the upper epidermis consisted of slightly wavy walled cells but the stoma was absent. The mesophyll was composed of one layer of palisade cells and 3-6 layers of spongy cells containing chloroplasts. The lower epidermis consisted of the epidermal cells which were similar to those of the upper epidermis with numerous diacytic stomata, the lithocyst, uniseriate multicellular covering trichomes and glandular trichomes with 6 to 8 cells head and short unicellular stalk occur on both epidermises. The midrib consisted three to five layers of collenchymatous cells underneath the epidermis of both sides. The vascular bundle is of collateral type. There are much more covering trichomes on upper surface than the lower surface of the midrib, veins and veinlets.

C. siamensis leaves are similar to *C. nutans* (Phaya yo) leaves but they are shown the different observations such as the size, length of petiole and color of

C. siamensis leaves are larger, longer and darker, respectively. Not only the leaves but also the TLC patterns of the extracts are shown the distinguish between the 2 species (Figures 18-19). Various chemical tests on the powdered sample of *C. siamensis* leaves were done. It revealed that this plant material gave coloring reaction with many reagents which demonstrated that *C. siamensis* Brem. leaves might contain triterpenes, steroids, reducing sugars and flavonoids (Table 49).

The microscopic characters of powdered *C. siamensis* leaves are similar to those of *C. nutans* leaves, except the number of palisade cells beneath 1 epidermal cell. The palisade ratio of *C. nutans* (Burm. f.) Lindau is obviously much more than palisade ratio of *C. siamensis* Brem. (Table 53).

Table 53. Comparison between leaf measurements of *Clinacanthus siamensis* Brem. and *C. nutans* (Burm. f.) Lindau leaves

	Mean [1]	Mean[2]
Stomatal number	145.66	160.47
Stomatal index	12.34	12.92
Palisade ratio	3.57	6.87
Vein-islet number	3.29	2.51
Veinlet termination number	2.77	2.42
Glandular number	10.19	12.87
Glandular index	0.76	0.92
Lithocyst number	17.25	16.59
Lithocyst index	1.30	1.18

1 = *Clinacanthus siamensis* Brem. leaves

2 = *Clinacanthus nutans* (Burm. f.) Lindau leaves

The powdered leaves were used as crude drugs for loss on drying, total ash, acid-insoluble ash, ethanol soluble extractive value and water soluble extractive value.

Loss on drying is employed in the Pharmacopoeia to control the loss in weight (due to water and other volatile materials) of crude drugs. The excessive content of water in crude drugs and temperature are suitable environment of fungi and bacteria growth which can cause the deterioration. Besides the loss on drying, ash contents are used to control the admixture of foreign inorganic matter due to their storage, container or intentional add to improve the appearance of crude drug.

The determination of ethanol- and water – soluble extractive values are used to control the constituents of crude drugs which can inferiority from many factors such as moisture contents, temperature, harvesting, drying process, kept duration and storage.

C. siamensis Brem. leaves from several locations are determined and concluded the data as an estimated percentage values in terms “not more than” (for loss on drying, total ash and acid-insoluble ash and “not less than” (for ethanol- and water-soluble extractive values). The results of quality controls *Clinacanthus siamensis* Brem. leaves can inform the standardization of this species as shown in Table 54.

Table 54. The quality controls of *Clinacanthus siamensis* Brem. leaves

	Data interval (%)	Mean (%)
Loss on drying	6.18 - 10.31	7.50 (not more than 9%)
Total ash	14.22 - 19.29	16.63 (not more than 20%)
Acid-insoluble ash	0.21 - 1.10	0.59 (not more than 1%)
Ethanol-soluble extractive	7.57 - 16.94	11.62 (not less than 9%)
Water-soluble extractive value	26.87 - 34.24	29.13 (not less than 24%)

In addition, the ethyl acetate extracts of *C. siamensis* Brem. leaves are effective against herpes simplex virus types I and II. The anti-herpes simplex virus activity (*in vitro*) of the ethyl acetate extract could inactivate herpes simplex virus types I and II (80-95 % inhibition, at 20-50 μg/ml by plaque reduction assay (pretest)). However, the potency of the leaf extracts collected from March to July show slightly less than the plant extract collected from August to February.

CHAPTER VI

CONCLUSION

Clinacanthus siamensis Brem. leaves are used as folkloric medicine in Thailand. The results of this investigation clearly indicated that the macroscopic and microscopic characters of leaf measurements, thin layer chromatographic patterns can be effectively used together as an important property in species identification between *C. siamensis* Brem. and *C. nutans* (Burm. f.) Lindau.

Clinacanthus siamensis Brem. has been tested *in vitro* for anti-herpes simplex virus activity. The ethyl acetate extract of *C. siamensis* leaves has been found to have activity against herpes simplex virus types I and II. Furthermore, cultivation of *C. siamensis* is quite easy; rapidly growth, it can be cultivated throughout the country. So the use of this plant in the primary health care should be encouraged with confidence in its therapeutic efficacy. Further research is necessary to investigate the toxicity of *C. siamensis* leaves and its chemical constituents.

REFERENCES

Thai

นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร. 2542. สมุนไพรไม้พื้นบ้าน (3). กรุงเทพมหานคร: สำนักพิมพ์ประชาชน.

มหาวิทยาลัยมหิดล. คณะเภสัชศาสตร์. ภาควิชาเภสัชพฤกษศาสตร์. 2538. สยามไภษัชยพฤกษ์ภูมิปัญญาของชาติ. กรุงเทพมหานคร: อัมรินร์พริ้นติ้ง แอนด์ พับลิชชิ่ง.

ชื่นฤดี ไชยวสุ, ทวีผล เดชาติวงศ์ ณ อยุธยา, เครือวัลย์ พลจันทร, ปราณี ชวลิตธำรง, สุทธิโชค จงตระกูลศิริ. 2535. การศึกษาฤทธิ์ของสารสกัดจากใบเสลดพังพอนและใบพญายอต่อเชื้อ Herpes Simplex Virus Type-2 ในหลอดทดลอง. วารสารกรมวิทยาศาสตร์การแพทย์ 34(4): 153-8.

ปราณี ชวลิตธำรง, เอมมนัส อัตตวิชญ์, พัช รักษามั่น และ ปราณี จันเพ็ชร. 2538. การศึกษาพิษของสารสกัดจากใบพญายอ. วารสารกรมวิทยาศาสตร์การแพทย์ 37(4): 323-338.

สมชาย แสงกิจพร, เครือวัลย์ พลจันทร, ปราณี ธวัชสุภา, มาลี บรรจบ และ ปราณี ชวลิตธำรง. 2536. การรักษาผู้ป่วยโรคเริมที่อวัยวะสืบพันธุ์ชนิดเป็นซ้ำ ด้วยยาจากสารสกัดชองใบพญายอ. วารสารกรมวิทยาศาสตร์การแพทย์ 18(5): 226-231.

English

Backer, C. A. and Bakhuizen van den Brink, R. C. 1965. Flora of Java. Vol. II. Netherlands: Wolters Noordhoff N.V., Groningen.

Benoist, R. 1935. Acanthaceae. Flora Genera of Indo-chine. pp. 763-764.

Burkill, I. H. 1935. A Dictionary of The Economic Products of the Malay Penisula. Vol. I. Great Britain: the University Press, Oxford.

Charuwichitratana, S., Wongrattanapasson, N., Timpatanapong, P. and Bunjob, M. 1996. Herpes zoster: treatment with *Clinacanthus nutans* cream. International Journal of Dermatology 35(9): 665-666.

Cherdchu, C., Poopyruchpong, N., Adchariya, R., Patanabanangkoon, S. and Patanabanangkoon, K. 1977. The absence of antagonism between extracts of *Clinacanthus nutans* Burm. and Naja Naja siamensis venum. Southeast Asian J. Trop Med. Public Health 8(2): 249-254.

Chotikieat, U. and Pitiporn, S. 1989. Clinical trials on the treatment of Herpes simplex, Herpes zoster and apthous ulcer with *Clinacanthus nutans* (Burm. f.) Lindau. Workshop "Pharmacist and Development of Medicinal Plants"

Chuakul, W. 1986. Chemical study of the antiinflammatory agents from the leaves of Phayaa Plong Thong (*Clinacanthus nutans* (Burm. f.) Lindau). Master's Thesis, Faculty of Pharmacy, Mahidol University.

Dampawan, P., Huntrakul, C., Reutrakul, V., Raston, C. L. and White, A. H. 1977. Constituents of *Clinacanthus nutans* and the crystal structure of lup-20(29)-ene-3-one. J. Sci. Soc. Thailand 3(1): 14.

Ministry of Public Health. Department of Medical Sciences. 1987. Thai Pharmacopoeia. Vol. I. Bangkok: Text & Journal Publication.

Eames, A. J. and MacDaniels, L. H. 1974. An Introduction to Plant Anatomy. 2 nd ed. New York: McGraw-Hill.

Esau, K. 1960. Anatomy of Seed Plants. USA: John Wiley & Sons.

Farnsworth, N. R. 1966. Biological and Phytochemical Screening of Plants. J. Pharm. Sci. 55: 225-275.

Her Majesty's Stationary office. 1993. British Pharmacopoeia. Vol. II. London: HMSO.

Hill, A. W. 1931-1935. Index Kewensis. Supplement IX. London: Oxford university press.

Hooker, J. D. 1979. The Flora of British India. Vol. II. Ashford: Reeve.

Hutchinson, J. 1959. Families of Flowering Plants. Vol. I. 2 nd ed. Great Britain: the University Press, Oxford.

Jayavasu, C., Detchatiwongse Na Ayudhaya, T., Balachandra, K., Chawalittumrong, P. and Jongtrakulsiri, S. 1992a. The virucidal activity of *Clinacanthus nutans* Lindau extracts against herpes simplex virus type 2: an in vitro study. Bull. Depart. Med. Sci. (Thailand) 34: 153-158.

Jayavasu, C., Balachandra, K., Sangkitporn, S., Maharungraungrat, A., Thavatsupa, P., Bunjob, M., Chavalittumrong, P., Dechatiwongse, T. and Sittisomwong, N. 1992b. Clinical trial in the treatment of genital herpes patients. Communicable Disease Journal (Thailand) 18(3): 152-161.

Kittisiripornkul, S. 1984. The antiinflammatory action and toxicological studies of extracts from *Clinacanthus nutans*. Master's Thesis, Mahidol University.

Lin, J., Li, H. M. and Yu, J. G. 1983. Studies on the chemical constituents of niu xu hua (*Clinacanthus nutans*). Chung Ts'ao Yao 14(8): 337-340.

Mabberley, D. J. 1997. The Plant-Book. 2 nd ed. United Kingdom: University Press, Cambridge.

Munksgaard, E. 1961. Dansk botanisk arkiv.

Perry, L. M. 1980. *Clinacanthus nutans* (Burm. f.) Lindau. Medicinal Plants of East and Southeast Asia. London: The MIT Press.

Rendle, A. B. 1952. The Classification of Flowering Plants. Vol. II. 1 st ed. Great Britain: the University Press, Cambridge.

Ridley, H. N. 1967. The Flora of the Malay Peninsula. Vol. II. Amsterdam: A. Asher.

Sangkitporn, S., Chaiwat, S., Balachandra, K., Dechatiwongse, Na-Ayudhaya, T., Bunjob, M. and Jayavasu, C. 1995. Treatment of herpes zoster with Clinacanthus nutans (bi phaya yaw) extract. J. Med. Assn. Thailand 78(11): 624-627.

Satayavivad, J., Bunyapraphatsara, N., Kitisiripornkul, S. and Tanansomwang. W. 1996. Analgesic and anti-inflammatory activities of extract of *Clinacanthus nutans* (Burm. f.) Lindau. Thai J Phytopharmacy 3(1): 7-17.

Sherma, J. 1991. Basic Techniques, Materials and Apparatus. In Sherma, J. and Fried, B. (eds.) Handbook of Thin-Layer Chromatography, pp. 3-41. New York: Marcel Dekker.

Smitinand, T. 2001. Thai Plant Names. 2 nd ed. Bangkok: Prachachon.

Somanabandhu, A., et al. 1986. Specification of Thai Medicinal Plant. Vol. I. 1 st ed. Bangkok: Aksornsampan Press.

Tanasomwang, W. 1986. The screening of anti-inflammatory action of *Clinacanthus nutans* (Burm. f.) Lindau: a critical evaluation of carrageenan-induced hind paw edema model. Master's Thesis, Mahidol University.

Teshima, K., Kameko, T., Ohtani, K., Kasai, R., Lhieochaiphant, S., Picheansoonthon, C. and Yamasaki, K. 1998. Sulfur-containing glucosides from *Clinacanthus nutans*. Phytochemistry 43: 831-835.

Thawaranantha, D., Balachandra, K., Jongtrakulsiri, S., Chavalittumrong, P., Bhumiswasdi, J. and Jayavasu, C. 1992. In vitro antiviral activity of *Clinacanthus nutans* on varicella-zoster virus. Siriraj Hosp Gaz. 44(4): 285-291.

Thongtharb, C. 1976. Study of the antagonistic effect of slaed pang porn (*Clinacanthus nutans*) against Thailand cobra venom (Naja Naja Siamensis). Master's Thesis, Faculty of Medicine.

Thongtharb, C. and Tejasen, P. 1977. The effect of Slaed Pang Porn (*Clinacanthus nutans*) on Thailand cobra venom (*Naja Naja siamensis*). Thai J Pharm Sci. 2(6): 1057.

Trease, G. E. and Evans, W. C. 1996. Pharmacognosy. 14 th ed. London: WB Saunders.

Utogapachana, C. 1975. The Medicinal Herbs and the Tropical Zone Disease. Bangkok: Prae Pittaya.

Wallis, T. E. 1960. Textbook of Pharmacognosy. 14 th ed. London: J. & A. Churchill.

Yoosook, C., Panpisutchai, Y., Chaichana, S., Santisuk, T. and Reutrakul, V. 1999. Evaluation of anti-HSV-2 activities of *Barleria lupulina* and *Clinacanthus nutans*. Journal of Ethnopharmacol 67(2): 179-187.

Youngken, H. W. 1948. Textbook of Pharmacognosy. 6 th ed. The united state of America: The Blakiston Division, Mcgraw-Hill.

APPENDIX

Reagents

Anisaldehyde-sulfuric acid TS

Mix, in order, 0.5 ml of anisaldehyde, 10 ml of glacial acetic acid, 85 ml of methanol, and 5 ml of sulfuric acid.

Chloral hydrate solution BP

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

Chloroform water

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

Dragendorff's TS, modified

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

Fehling's reagent

The copper solution (A): Dissolve 34.66 g of carefully selected, small crystals of cupric sulfate, showing no trace of efforescence or of adhering moisture in water to make 500 ml. Store this solution in small, tight container.

The alkaline Tartrate Solution (B): Dissolve 173 g of crystallized potassium sodium tartrate and 50 g of sodium hydroxide in water to make 500 ml. Store this solution in small, alkali-resistant containers.

For use, mix exactly equal volumes of solutions A and B at the time required.

Ferric chloride TS

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

Iodine solution

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

Mercuric-Potassium Iodide TS (Mayer's reagent)

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

VITA

Miss Jiranuch Jamtaweekul was born on Febuary 2, 1974 in Bangkok, Thailand. She received her Bachelor's degree of Science in Pharmacy in 1998 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. At present, she is civil official in the Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province.