MICROSCOPIC, MOLECULAR AUTHENTICATIONS, AND FLAVONOID CONTENTS IN SELECTED *BAUHINIA* SPECIES AND PHARMACOGNOSTIC SPECIFICATIONS OF *BAUHINIA MALABARICA* LEAVES

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ลักษณะทางจุลทรรศน์ อณูโมเลกุล และปริมาณวิเคราะห์ฟลาโวนอยด์ ของพืชสกุลชงโค และมาตรฐานของใบส้มเสี้ยว

น.ส.ปพิชญา เทศนา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ปพิชญา เทศนา : ลักษณะทางจุลทรรศน์ อณูโมเลกุล และปริมาณวิเคราะห์ฟลาโวนอยด์ ของพืชสกุลชงโค และมาตรฐานของใบส้มเสี้ยว. (MICROSCOPIC, MOLECULAR AUTHENTICATIONS, AND FLAVONOID CONTENTS IN SELECTED *BAUHINIA* SPECIES AND PHARMACOGNOSTIC SPECIFICATIONS OF *BAUHINIA MALABARICA* LEAVES) อ.ที่ปรึกษาหลัก : รศ. ดร.ชนิดา พลานุเวช, อ.ที่ปรึกษาร่วม : รศ. ดร.นิจศิริ เรืองรังษี,รศ. ดร.กาญจนา รังษีหิรัญรัตน์

สกุลชงโคพบได้มากกว่า 40 สายพันธุ์ในประเทศไทย พืชสกุลนี้ใช้เป็นสมุนไพรในตำรับยาพื้นบ้านมายาวนาน มีการรายงานว่าพบสารเควอซีติน และเควอซิตรินในพืชสกุลนี้ การศึกษานี้มีวัตถุประสงค์เพื่อประเมินลักษณะทางจุลทรรศน์ และอณูโมเลกุล รวมถึงการหาปริมาณสารเควอซีติน และเควอซิตริน ของพืชสกุลชงโค 20 สายพันธุ์ และจัดทำข้อกำหนดทางเภสัชเวทของใบส้มเสี้ยวที่ใช้ในตำรับยาไทย โดยเก็บใบเพสลาดของพืชสกุลชงโค 20 สายพันธุ์ สายพันธุ์ละ 3 แหล่ง มาศึกษาลักษณะทางจุลทรรศน์ ได้แก่ ค่าคงที่ของแผ่นใบ (จำนวนปากใบ ค่าดัชนีปากใบ อัตราส่วนเซลล์รั้ว จำนวนขน ค่าดัชนีขน และค่าพื้นที่เซลล์ผิว) และภาคตัดขวางของเส้นกลางใบ ประเมินลักษณะทางอณูโมเลกุลโดยใช้เครื่องหมายไอเอสเอสอาร์ วิเคราะห์หาปริมาณสารเควอซิติน และเควอซิตรินในพืชสกุลชงโคทั้ง 20 สายพันธุ์ โดยใช้ชุดสกัด soxhlet สกัดใบแห้งด้วยเอทานอล แยกสารโดยวิธีโครมาโทกราฟีของเหลวสมรรถนะสูง ที่อุณหภูมิ 35 องศาเซลเซียส โดยใช้คอลัมน์ Inersil® ODS-3 C_{18} เป็นเฟสคงที่ และใช้สารละลายของกรดฟอสฟอริก (0.5%) กับเมทานอล ในอัตราส่วน 1 ต่อ 1 เป็นเฟสเคลื่อนที่ ตรวจวัดปริมาณเควอซิติน และเควอซิตรินด้วยดีเทคเตอร์ชนิดโฟโต้ไดโอดอาเรย์ที่ 255 นาโนเมตร จัดทำข้อกำหนดทางเภสัชเวทของใบส้มเสี้ยวที่เก็บจาก 15 แหล่งทั่วประเทศ นำมาวิเคราะห์หาปริมาณน้ำหนักที่หายไปเมื่อทำให้แห้ง ปริมาณเถ้ารวม เถ้าที่ไม่ละลายในกรด ปริมาณความชื้น ปริมาณสิ่งสกัดด้วยน้ำ ปริมาณสิ่งสกัดด้วยเอทานอล ปริมาณสารสำคัญ (เควอซิติน และเควอซิตริน) และจัดทำลายพิมพ์ทางเคมีของสารสกัดเอทานอลของใบส้มเสี้ยวโดยวิธีโครมาโทกราฟีแบบชั้นบาง ผลการศึกษาพืชสกุลชงโคทั้ง 20 สายพันธุ์ทางจุลทรรศน์พบว่า ทุกสายพันธุ์มีปากใบชนิดพาราไซติก ใบไม้สีทอง แสลงพันเถา เถาไฟ ส้มเสี้ยวเถา ชงโค เถากระไดลิง ย่านางแดง เสี้ยวดอกขาว และคิ้วนาง พบปากใบทั้งสองด้าน ชนิดขนที่พบเป็นขนที่ไม่มีต่อมทั้งขนเซลล์เดียวและขนหลายเซลล์ ยกเว้น เถาไฟ แสลงพัน เถากระไดลิง สิรินธรวัลลี ย่านางแดง และคิ้วนางไม่พบขน คิ้วนางพบเซลล์รั้วสองชั้นบนผิวใบด้านบน ชงโคพบปากใบจำนวนมากที่สุด (1120 – 1208 ต่อตารางมิลลิเมตร) และเสี้ยวป่าพบจำนวนขนมากถึง 200 เส้นในหนึ่งตารางมิลลิเมตรของผิวใบด้านล่าง ลักษณะทางกายวิภาคของภาคตัดขวางของเส้นกลางใบแสดงในรูปแบบลายเส้น ซึ่งสามารถนำมาใช้ระบุลักษณะในแต่ละสายพันธุ์ได้ การประเมินทางอณูโมเลกุลด้วยไพรเมอร์ไอเอสเอสอาร์ทั้งหมด 20 ไพรเมอร์ พบ 6 ไพรเมอร์ที่ให้แถบดีเอ็นเอที่แตกต่างกันทุกสายพันธุ์ ซึ่งการศึกษานี้สามารถใช้ระบุสายพันธุ์ของพืชสกุลชงโคได้ โดยเฉพาะส้มเสี้ยว การวิเคราะห์หาปริมาณเควอซิติน และเควอซิตรินในใบของพืชสกุลชงโคทั้ง 20 สายพันธุ์พบว่า ในใบส้มเสี้ยวพบปริมาณของเควอซิติน และเควอซิตรินมากที่สุด ข้อกำหนดทางเภสัชเวทของใบส้มเสี้ยว ได้แก่ น้ำหนักที่หายไปเมื่อทำให้แห้ง ปริมาณเถ้ารวม เถ้าที่ไม่ละลายในกรด ปริมาณความชื้น ไม่มากกว่า 8.00, 7.08, 1.79 และ 8.28 กรัมต่อ 100 กรัมโดยน้ำหนักแห้ง ปริมาณสิ่งสกัดด้วยเอทานอล และปริมาณสิ่งสกัดด้วยน้ำ ไม่น้อยกว่า 16.47 และ 13.78 กรัมต่อ 100 กรัมโดยน้ำหนักแห้ง ตามลำดับ พบปริมาณสารเควอซีตินและเควอซิตรินเท่ากับ 0.1796 ± 0.0678 และ 0.3833 ± 0.2138 กรัมต่อ 100 กรัมโดยน้ำหนักแห้ง

สาขาวิชา	วิทยาศาสตร์สาธารณสุข	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....
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KEYWORD: BAUHINIA SPP., BAUHINIA MALABARICA, QUERCETIN, QUERCITRIN, MICROSCOPIC LEAF MEASUREMENT, MIDRIB ANATOMY, ISSR-PCR, DNA FINGERPRINT, PHARMACOGNOSTIC SPECIFICATION

Paphitchaya Thetsana : MICROSCOPIC, MOLECULAR AUTHENTICATIONS, AND FLAVONOID CONTENTS IN SELECTED *BAUHINIA* SPECIES AND PHARMACOGNOSTIC SPECIFICATIONS OF *BAUHINIA MALABARICA* LEAVES. Advisor: Assoc. Prof. CHANIDA PALANUVEJ, Ph.D. Co-advisor: Assoc. Prof. Nijsiri Ruangrungsi, Ph.D.,Assoc. Prof. KANCHANA RUNGSIHIRUNRAT, Ph.D.

There are more than 40 *Bauhinia* species throughout Thailand. Many pharmacologically active compounds have been reported, particularly flavonoids such as quercetin and its glycoside (quercitrin). This study aimed to characterize 20 *Bauhinia* species throughout Thailand using microscopic, molecular analysis as well as quercetin and quercitrin quantification. The pharmacognostic specification of *B. malabarica* leaf which has been used in Thai's remedies was established. Twenty *Bauhinia* species leaves were collected from 3 places throughout Thailand. The lamina of fresh mature leaves were microscopically evaluated for the stomatal number, stomatal index, palisade ratio, trichome number, trichome index, and epidermal cell area. The anatomy of midrib transverse sections were illustrated. ISSR-PCR was performed to classify these *Bauhinia* species. Preliminary quantification of quercetin and quercitrin in 20 *Bauhinia* species were done using RP-HPLC. Dried mature leaves were exhaustively extracted with 95% ethanol using Soxhlet apparatus. The extracts were injected to Inersil® ODS-3 C_{18} column and eluted by 0.5% phosphoric acid and methanol (1 : 1) at 35 ºC. Photo-diode array detector was set at 255 nm. Pharmacognostic specification of *B. malabarica* dried leaves collected from 15 places throughout Thailand was determined for the contents of loss on drying, water, total ash, acid insoluble ash, extractive matter as well as active compounds (quercetin and quercitrin). Chemical fingerprint of the ethanolic extracts was performed by TLC. All twenty *Bauhinia* species showed paracytic stomata type. *B. aureifolia*, *B. bracteata*, *B. integrifolia*, *B, lakhonensis*, *B. purpurea*, *B. scandens*, *B. strychnifolia*, *B. variegata*, and *B. winitii* were amphistomatic. Unicellular and multicellular nonglandular trichomes were found except *B. integrifolia*, *B. pulla*, *B. scandens*, *B. sirindhorniae*, *B. strychnifolia*, and *B. winitii* contained no trichomes. *B. winitii* showed two layers of palisade cells at upper epidermis. *B. purpurea* displayed the highest numbers of stomata (1120 – 1208 per mm^2). *B. saccocalyx* had trichome number upto 200 per mm^2 of lower epidermis. The anatomical characteristics of the midribs were illustrated. Their uniqueness could be used for species identification. Six ISSR primers produced 100% polymorphic DNA bands. A dendrogram generated by UPGMA could classify *Bauhinia* species in this study especially *B. malabarica*. Phytochemical analysis revealed quercetin and/or quercitrin contents in dried leaves of these 20 *Bauhinia* species. The highest contents of quercetin and quercitrin were found in *B. malabarica*. Pharmacognostic specification of *B. malabarica* dried leaves revealed the loss on drying, total ash, acid insoluble ash, and water contents should be not more than 8.00, 7.08, 1.79, and 8.28 g/100 g while ethanol and water soluble extractive matters should be not less than 13.78 and 16.47 g/100 g of dried leaves respectively. TLC-fingerprint was demonstrated. The contents of quercetin and quercitrin were 0.1796 ± 0.0678 and 0.3833 ± 0.2138 g/100 g of dried leaves, respectively.

Field of Study:	Public Health Sciences	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....
		Co-advisor's Signature .....
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Paphitchaya Thetsana

TABLE OF CONTENTS

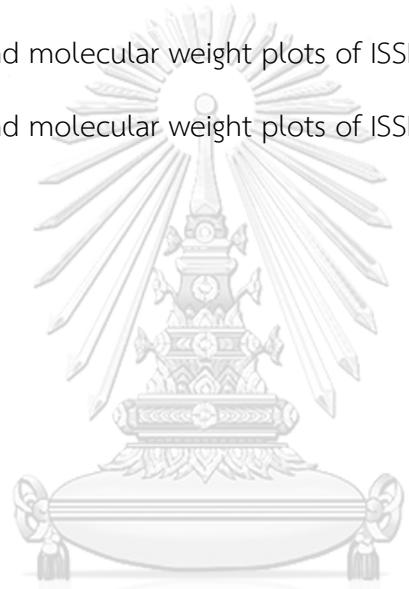
Page

LIST OF TABLES

Page

LIST OF FIGURES

Page



CHAPTER I

INTRODUCTION

Leguminosae is a large angiosperm family of flowering plants. This family includes about 751 genera and some 19,000 known species of trees, shrubs and perennial or annual herbaceous plants.

Bauhinia, a large genus of the family Leguminosae, consists of 250 species of trees, shrubs and climbers which are normally known as 'cow's paw' or 'cow's hoof' because of their leaves shape. They are widely distributed in most warm countries. *Bauhinia* species mostly are ornamental plants because their flowers are beautiful and colorful (Saisaard *et al*., 2015). In Brazil folk medicine, *Bauhinia forficata* has been treated diabetes, infections, pain and inflammatory processes using leaves and stem-barks (Filho, 2009). In Thailand, *Bauhinia malabarica* has been used in traditional medicine for treating many diseases i. e. headache, fever and urinary disorder (Kaewamatawong *et al*. 2008). The bark of *B. purpurea* and *B. variegata* is astringent so it is used for poultice, and the buds of *B. variegata* are used to treat diarrhea. Some other species are used for treat cough. In Philippines, the bark of *Bauhinia tomentosa* and *B. malabarica* are used against dysentery. The leaves of some *Bauhinia* species are edible. They have a sour taste that used as a flavoring. *B. racemosa* leaves are used instead of paper cigarette-wrapper. The young shoots and seeds in some *Bauhinia* species are cooked and edible.

Flavonoids are chemical constituents which commonly found and have been isolated from leaves and other parts of some *Bauhinia* species. Quercitrin (quercetin-3-O-rhamnoside), kaempferol-3-galactoside, kaempferol-3-rhamnoside, quercetin, rutin, apigenin and apigenin-7-O-glucoside have been isolated from leaves of *Bauhinia reticulata*, *Bauhinia variegata*, and *Bauhinia purpurea* (Rabaté, 1938, Rahman & Begum, 1966, Abd-El-Wahab *et al*., 1987, and Spilková & Húbik 1992).

Methoxylated and methylenedioxyflavones were isolated from root of *Bauhinia championii* (Chen *et al.* 1984), naringenin-4'-rhamnoglucoside and Bausplendin (a dimethylenedioxyflavone) were obtained from stem and wood of *Bauhinia variegata* and *Bauhinia splendens* (Gupta *et al*., 1980 and Laux *et al*. 1985), butein-4'-arabinosylgalactoside, kaempferol and agatisflavone were isolated from seeds of *Bauhinia purpurea* and pods of *Bauhinia vahlii* (Bhartiya *et al.* 1979, Bhartiya & Gupta 1981, and Kumar *et al.* (1990).

Bauhinia species were found more than 40 species throughout Thailand. Twenty species were studied in this research for the microscopic and molecular characteristics as well as preliminary quantification of quercetin and quercitrin using RP-HPLC method.

Table 1 Twenty *Bauhinia* species in this research

No.	Scientific Name	Thai Name
1.	*Bauhinia acuminata* Linn.	กาหลง
2.	*B. aureifolia* K. & S. S. Larsen	ย่านดาโอ๊ะ ใบไม้สีทอง
3.	*B. bracteata* (Graham ex Benth.) Baker	แสลงพันเถา เสี้ยวเครือ
4.	*B. galpinii* N. E. Br.	กาหลงดอกแดง
5.	*B. integrifolia* Roxb.	เถาไฟ
6.	*B. lakhonensis* Gagnep.	ส้มเสี้ยวเถา
7.	*B. malabarica* Roxb.	ส้มเสี้ยว
8.	*B. ornata* Kurz	ปอเกี๋ยน
9.	*B. pottsii* G. Don	ชงโคดำ
10.	*B. pulla* Craib.	แสลงพันเถา แสลงพัน
11.	*B. purpurea* Linn.	ชงโค
12.	*B. racemosa* Lam.	ชงโคนา
13.	*B. saccocalyx* Pierre	เสี้ยวป่า

Table 1 (cont.) Twenty *Bauhinia* species in this research

No.	Scientific Name	Thai Name
14.	*B. scandens* Linn.	เถากระไดลิง
15.	*B. siamensis* K. & S. S. Larsen	สร้อยสยาม
16.	*B. sirindhorniae* K. & S. S. Larsen	สิรินธรวัลลี
17.	*B. strychnifolia* Craib	เถาขยัน
18.	*B. tomentosa* Linn.	โยทะกา, ชงโคดอกเหลือง
19.	*B. variegata* Linn.	เสี้ยวดอกขาว
20.	*B. winitii* Craib	อรพิม, คิ้วนาง

B. malabarica leaf is a crude drug in traditional Thai medicine. It is an ingredient of Thai's remedies (คัมภีร์ทิพย์มาลา) for treating cancer and wound healing. In Thai folk medicine, it has been used for diuretic, dysentery and emmenagogue (Kaewamatawong *et al*., 2008). The pharmacognostic parameters with reference to quercetin and quercitrin contents of *B. malabarica* dried leaves were investigated for establishing the specification of this crude drug. This pharmacognostic specification consists of macroscopic evaluation, microscopic evaluation, physico-chemical evaluation, TLC fingerprinting and active chemical compound analysis (WHO, 2011).

Objectives

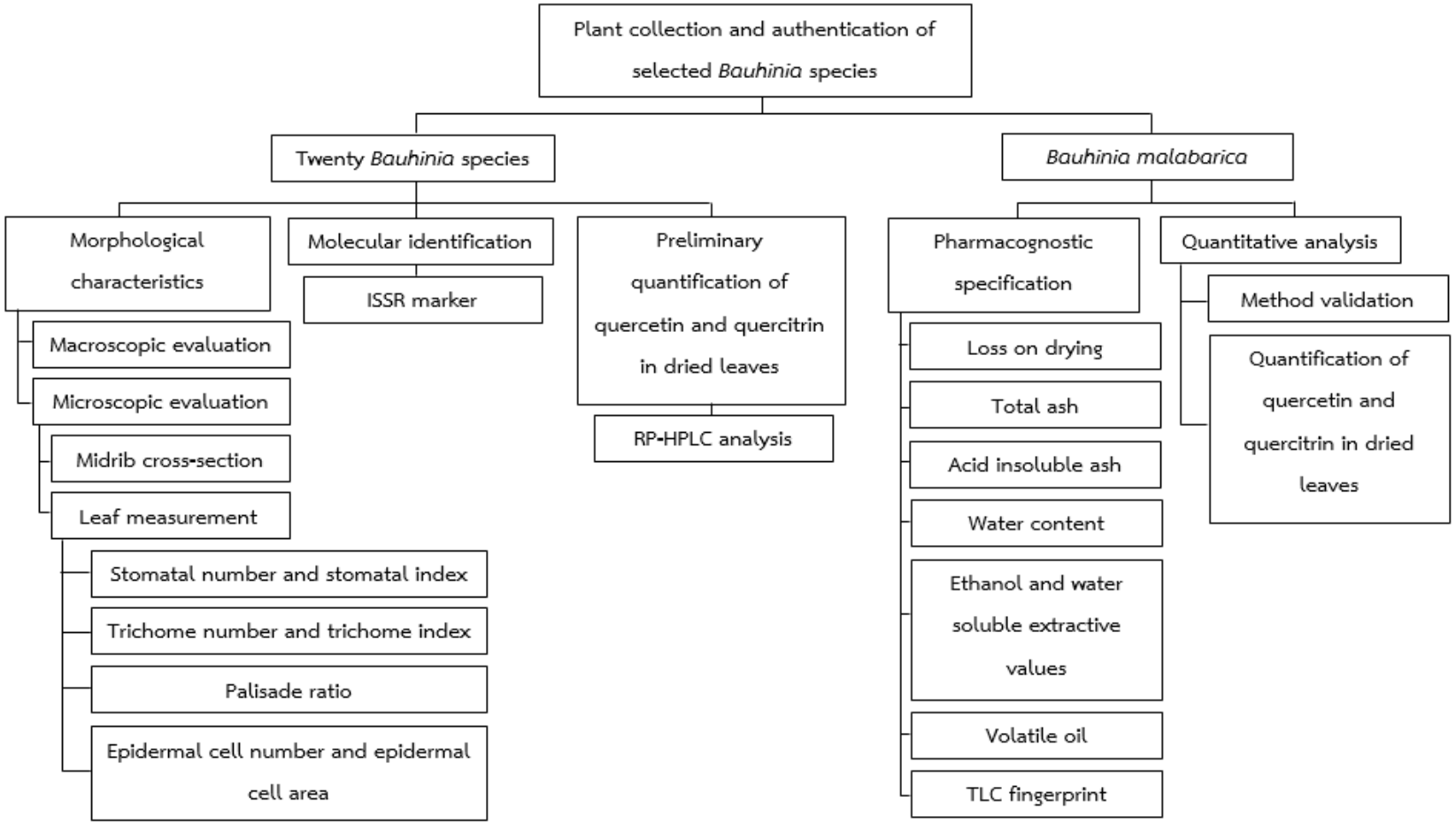
1. To establish the characteristics of *Bauhinia* species by microscopic evaluation.

2. To preliminary quantify quercetin and quercitrin contents in *Bauhinia* species using reversed phase high performance liquid chromatography (RP-HPLC).

3. To establish the pharmacognostic specification of *B. malabarica* leaves.

4. To establish DNA fingerprint and phylogenetic relationship among *Bauhinia* species by ISSR-PCR method.

Conceptual framework



CHAPTER II

LITURATURE REVIEWS

Bauhinia acuminata Linn.

Synonym: -

Common name: Dwarf white orchid tree, snowy orchid

Native distribution: Southeast Asia, India, Sri Lanka, and Malaysia

Plant description

"Shrubs or small trees, to 3 m tall. Young branches zigzag, glabrous. Petiole 2.5-4 cm, pubescent; leaf blade ovate-cordate to cordate, 9-12 × 8-12.5 cm, subleathery, abaxially grayish pubescent, adaxially glabrous, primary veins 9-11, secondary and higher order veins protruding, base cordate, apex bifid to 1/3-2/5, lobes acuminate or slightly acute at apex or rarely rounded. Inflorescence a raceme, with few (3-15) flowers, axillary, appearing cymose; peduncle short, pubescent as inflorescence axis; bracts and bracteoles linear, pubescent. Flower buds ca. 2.5 cm, acutely tapering and ending in 5 linear calyx teeth ca. 3 mm. Hypanthium tubular.

Calyx spathe open on one side, shortly 5-toothed. Petals white, obovate-elliptic, 3.5-5 × ca. 2 cm, sessile. Fertile stamens 10 in 2 whorls, subequal, 1.5-2.5 cm, pubescent on lower 1/3; anthers yellow, oblong. Ovary prominently stalked, pubescent or almost glabrous; style 15-20 mm; stigma peltate, ca. 3 mm in diam. Legume straight or slightly curved, linear-oblanceolate, compressed, 6-12 × ca. 1.5 cm, with stalk ca. 1 cm, apex acuminate, beaked; valves leathery, glabrous, sharply ridged near suture. Seeds 5-12, compressed, 8-10 mm in diam."

(http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200011909)

Medicinal uses

Bark – anti-cancer drug, skin diseases, worms, diabetes, gastrointestinal diseases, and respiratory diseases (Chandrashekara & Somashekarappa, 2016)

Flower – headache, and high blood pressure (Prabhu *et al*., 2018)

Root – cough, skin diseases, worms, tumors, and diabetes (Prabhu *et al*., 2018)

Chemical constituents

Quercetin (Padgaonkar *et al*., 2018), caffeic acid, vanillic acid, syringic acid, kaempferol (Gupta *et al*., 2015), apigenin, Quercetin-3-glucoside (Sinha & Singh, 2013), lupeol and ursolic acid (Gupta *et al*., 2018), phytol, β-caryophyllene (Vasudevan *et al*., 2013), vitamin E, α-Tocopherol-β-D-mannoside (Arunachalam *et al*., 2017), hexamethyl cyclotrisiloxane, 1-methly-3-nonyl indane (Bhaskara Rao *et al*., 2015).

Pharmacological activity

Flower – antioxidant activity (Bhaskara Rao *et al*., 2015, and Sanjeev *et al*., 2017)

Stem and bark – hemolytic activity, antifungal activity, and antioxidant activity (Bhaskara Rao *et al*., 2015, Sanjeev *et al*., 2017, and Alharbi *et al*., 2018)

Leaf – anti-diabetic activity, anthelmintic activity, anti-nociceptive activity, PDE5 inhibitory activity, hemolytic activity, antifungal activity, antibacterial activity, and antioxidant activity (Temkitthawon *et al*., 2011, Khan *et al*., 2014, Bhaskara Rao *et al*., 2015, Dej-adisai & Pitakbut, 2015, Pai *et al*., 2016, Sanjeev, 2017, Prabhu *et al*., 2018, Padgaonkar *et al*., 2018, and Alharbi *et al*., 2018)

Toxicity

Acute toxicity

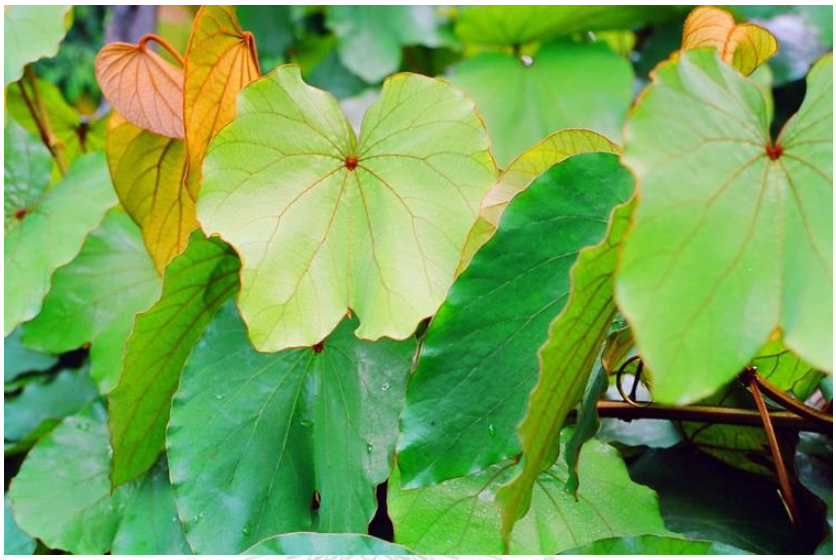
Single oral administration of 5000 mg/kg dose of *B. acuminata* leaves aqueous and alcoholic extract produced no mortality and morbidity in the animals during observation period of 14 days (Padgaonkar *et al*., 2018).

***Bauhinia aureifolia* K. Larsen & S. S. Larsen**

Synonym: *Phanera aureifolia* K. Larsen & S. S. Larsen, *Bauhinia chrysophylla* K.Larsen & S.S.Larsen

Common name: Golden leaf

Native distribution: Thailand



Plant description

"Large, woody, tendrilled liana, 100 cm DBH, main stem angular-terete; young branches rusty pubescent with long persistent indumentum. Leaves broadly ovate to subrotundate up to 19 by 18 cm; nerves 11-13; apex bilobed 1/3 with wide sinus; tip of lobes rounded triangular, base deeply cordate; leaves at young branches reddish pubescent mainly on the nerves; at the flowering branches upper surface with golden-rusty to reddish indumentum or silvery, most often persistent; lower surface rusty velvety. Stipules falcate-auriculate, caduceus, reddish velvety, 1-1.5 cm. Flowers fragrant in dense, corymbose, rusty inflorescences, often 2-3 together. Pedicels ca. 1 cm. Bracts ovate-lanceolate, acuminate, 6-8 mm; bracteoles narrow lanceolate-subulate, 4-5 mm, inserted near the base of the hypanthium. Buds

ellipsoid, ca 1 cm, reddish pubescent; hypanthium short, tubular ca 4 mm. Calyx splitting into 3-5 segments, inside glabrous. Petals white turing creamy, subequal, spatulate with crenulate margin, light rusty hairy outside, glabrous inside, 15-18 mm. Stamens 3 fertile; filaments greenish, glabrous, slightly longer than the petals; anthers elliptic, pinkish, 2-3 mm; staminodes 1-2, minute, subulate. Ovary subsessile, ca 7 mm long, densely reddish-brown lonhhaired; style greenish, slender, glabrous towards the stigma, ca 10 mm; stigma green, peltate. Pods dehiscent, woody, broadly strapshaped, 20-23 by 5-5.6 cm, with 15 mm long stout stalk, brownish velvety. Seeds 4-6, suborbicular, 2.5-2.8 mm diam. (Larsen & Larsen, 1995)"

Pharmacological activity

Leaf – antioxidant activity, antibacterial activity, and anti-diabetic activity (Dej-adisai & Pitakbut, 2015, Chelae *et al*., 2017, and Sangwiman *et al*., 2017)

***Bauhinia bracteata* (Graham ex Benth.) Baker**

Synonym: *Phanera bracteata*

Common name: -

Native distribution: Southeast Asia, India, Sri Lanka, and Malaysia

Plant description

"Woody climbers. Leaves simple, alternate, ovate or orbicular, 3-15 by 3-12 cm; apex deeply emaginate, ca. 1/3 of the leaf length. Inflorescences paniculate, axillary or terminal. Flowers yellowish green with bright red disc. Pods flattened, oblong-lanceolate."

(http://www.qsbg.org/database/botanic_book%20full%20option/search_detail.asp?botanic_id=2745)

Medicinal uses

Stem – skin diseases, heamatonic, and emmenagogue (Pangngern, 2013)

Chemical constituents

4',5,7-trihydroxy-6,8-dimethylflavanone (farresol) and 3',4',- 4trihydroxy chalcone (isoliquiritigenin), and Friedelin (Udomputtimekakul *et al*., 2017).

Pharmacological activity

Stem – hepatoprotective activity (Lee *et al*., 2017)

***Bauhinia galpinii* N. E. Br.**

Synonym: *Bauhinia punctata*

Common name: red *Bauhinia*, red butterfly tree, Pride of the Cape

Native distribution: South Africa, Swaziland, Zimbabwe

Plant description

"Semi-deciduous scandent shrub with leaves resembling a butterfly in shape, able to grow up to about 3 m tall. Flowers are red to orange and borne in clusters near the ends of the branches. Fruit is a narrow, brown pod."

(https://florafaunaweb.nparks.gov.sg/Special-Pages/plant-detail.aspx?id=1722)

Medicinal uses

Leaf – tuber, pneumonia, venereal diseases, diarrhea, epilepsy, and convulsion (Verchaeve & Staden, 2008)

Root – infertility, stomach pains, diarrhea, sexual performance, treating stomach worms, infant food, bloody vomit, tonic, and fontanelle syndrome (Arnold &

Gulumian, 1984, Mabogo, 1990, Samie *et al*., 2009, Bruschi *et al*., 2011, and Mahwasane *et al*., 2013)

Bark – stomach spasm (Arnold & Gulumain, 1984)

Chemical constituents

Quercetin-3-*O*-galactoside, myricetin-3-*O*-galactoside, 2”-*O*-rhamnosylvitexin (Aderogba *et al*., 2007), (Epi)catechin dimer, (Epi)catechin trimer, (Epi)afzelechin-(epi)catechin dimer, luteolin-*C*-hexoside, rutin, apigenin-*C*-hexoside, quercetin-3-*O*-hexoside (isoquercetin), kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, quercetin-3-*O*-rhamnoside, isorhamnetin-3-*O*-hexoside, trihydroxy-octadecadienoic acid, trihydroxy-octadecenoic acid, dihydroxyhexadecanoic acid, and hydroxy-octadecatrienoic acid (Farag *et al*.,2015).

Pharmacological activity

Leaf – anti-mutagenic activity, antibacterial activity, antifungal activity, anti-inflammatory activity, anti-diabetic activity (Reid *et al*., 2006, Aderogba *et al*., 2007, Verchaeve & Staden, 2008, Samie *et al*., 2009, Ahmed *et al*., 2012, Farag *et al*.,2015, and Molele *et al*., 2016)

Bark – antibacterial activity (Samie *et al*., 2009, and Ahmed *et al*., 2012)

Shoot – anti-diabetic activity (Farag *et al*., 2015)

Toxicity

The isolated compounds of *B. galpinii* dried leaf revealed that Quercetin-3-*O*-galactoside, and 2”-*O*-rhamnosylvitexin have no cytotoxic effect using MTT assay (Aderogba *et al*., 2007).

The acetone extract of *B. galpinii* bark showed high level of cytotoxicity of the monkey kidney Vero cells (Samie *et al*., 2009).

***Bauhinia integrifolia* Roxb.**

Synonym: *Bauhinia cumingiana* (Benth.) Fern.-Vill., *B. flammifera* Ridley, *Phanera integrifolia*

Common name: -

Native distribution: Southern Thailand, Peninsular Malaysia, Sumatra, Borneo and The Philippines

Plant description

"A large-tendrilled liana with rusty-woolly, grooved young branches. Leaves alternate; stipules minute, early caducous; petiole 1-5 cm long; blade ovate to orbicular, 6.8-12.4 cm × 7.5-12.5 cm, base deeply cordate, apex entire, emarginate, shallowly or deeply bifid, 9-11-veined. Inflorescence a more or less dense panicle composed of corymbose racemes; flower buds globose, ovoid or ellipsoid, hypanthium tubular; flowers bisexual; calyx splitting into 2(-3) lobes; petals 5, obovate, 8-15 mm long, claw short, orange turning red; stamens 3, staminodes 2,

minute; ovary subsessile, rusty-woolly. Fruit a legume, oblong, up to 20 cm × 5 cm, glabrescent, 5-8seeded, dehiscent. Seed ovateorbicular, about 2 cm in diameter."

(https://uses.plantnet-project.org/en/Bauhinia_integrifolia_(PROSEA))

Medicinal uses

Stem – wound healing, dysentery, diarrhea, abortifacient and birth control, and tonic (Allado-Ombat & Teves, 2015)

Leaf – stomach disorders (https://uses.plantnetproject.org/en/Bauhinia _integrifolia_(PROSEA))

Root – treatment of syphilis, and cough (https://uses.plantnetproject.org/ en/Bauhinia_integrifolia _(PROSEA))

Pharmacological activity

Stem – antibacterial activity, and anti-angiogenetic activity (Allado-Ombat & Teves, 2015)

Toxicity

From the cytotoxic activity, both acetone and aqueous *B. integrifolia* stem extract revealed that they have nontoxic effect to brine shrimp nauplii (Allado-Ombat & Teves, 2015).

***Bauhinia lakhonensis* Gagnep.**

Synonym: *Phanera lakhonensis* (Gagnep.) A. Schmitz, *Cheniella lakhonensis* (Gagnep.) R.Clark & Mackinder

Common name: -

Native distribution: Laos, Thailand, and Vietnam

Plant description

"Leaf bilobed, upper surface glabrous or sparsely ferruginous pilose, lower surface sparsely to moderately ferruginous pilose. Stipules ca 2 mm. Inflorescence a raceme, becoming corymbose at the apex, axis 3.0–9 cm. Pedicel 0.7–1.7 cm. Bracts 3–4(–6) mm. Bracteoles 4–5 mm. Hypanthium 2.0–2.8 cm, sparsely to moderately ferruginous pilose. Petals white, claws pink, 8–14 × 4–9 mm, sparsely pilose/tomentose on outer surface, claw 1–3 mm. Fertile stamens 3, each ca 8–10 mm. Staminodes 7, of which 2 are narrow, inserted individually between the

stamens, 5 mounted on a raised fleshy disc that is open on the side where the stamens are inserted, disc 0.5 × 1 mm, staminodes 2–3 mm. Ovary glabrous. Fruit oblong, rounded at the apex, base cuneate, 10–12.5 × 1.7–2.3. Seeds ca 15–21 per fruit. (Larsen *et al*., 1984)"

Medicinal uses

Leaf and stem – arthropathy, inflammatory disorder, and pain (Duong *et al*., 2017)

Root – tonic, and anti-diarrheal (Promprom, 2015)

Pharmacological activity

Leaf and stem – xanthine oxidase (OX) inhibitory activity (Duong *et al*., 2017)

***Bauhinia malabarica* Roxb.**

Synonym: -

Common name: mountain ebony, malabar orchid

Native distribution: Australia, India, Indonesia, Malaysia, Myanmar, Philippines, Thailand

Plant description

"Small or moderate sized deciduous tree. Bark is rough brown, peeling in linear flakes, fibrous, red inside. Leaves are broader than long, 1.5-4 inches long, 2-5 inches broad, divided through 1/3 of the length, 7-9 nerved, slightly heart-shaped at base, rigidly leathery, glaucous and smooth beneath. Flowers are borne in stalkless racemes in leaf axils, 1.5-2 inches long, often 2-3 together. Flowers are 1/2-inch-long, dull-white, often uni-sexual, on very slender stalks, which are 1 in. long. Male and female flowers are usually on different stems. Sepal cup has 5 equal triangular teeth. Petals are spade-shaped, equal. Pod is 7-12 inches long, 2-2.5 cm broad, on a stalk 1

in. long, flat flexible, many-seeded, more or less straight reticulate veins, which starting diagonally from both sutures meet in the middle. Seeds are 20-30."

(https://www.flowersofindia.net/catalog/slides/Malabar%20Bauhinia.html)

Medicinal uses

Stem – cure abdominal pain, dysentery, diarrhea, jaundice, diabetes, and wound healing (Larsen *et al*., 1984, Maheshwari *et al*., 1986, Narayanan *et al*., 2011, and Thenmozhi *et al*., 2013)

Leaf – fever, wound healing, diuretic, dysentery, headache, emmenagogue, flavoring for food, and aphrodisiac (Larsen *et al*., 1984, Boonyapraphatsara & Chokchaicharoenporn, 1996, Manandhar, 2002, and Sharma *et al*., 2014)

Root – liver diseases, cholera, diuretic, and dysentery (Sharma *et al*., 2014)

Shoot – treatworm infestations, leprosy, wounds, menorrhagia, gout, scrofula, wasting diseases, cough, hemorrhage, urinary disorders, glandular swelling, goiter, and food (Thenmozhi *et al*., 2013)

Flower – dysentery, and abdominal pain (Larsen *et al*., 1984, and Manandhar, 1991)

Fruit – antitussive, and sore throat (Sakong *et al*., 2011)

Chemical constituents

Racemosol, demethylracemosol, preracemosol A, and preracemosol B (Kittakoop *et al*., 2000), β-sitosterol, quercetin, 6,8-C-dimethyl kaempferol-3-*O*-rhamnopyranoside, hyperin, and 6,8-C-dimethyl kaempferol-3-methyl ether (Park *et al*., 2014), tartaric acid (Stafford, 1959), 6,8-di-C-methylkaempferol 3-methyl ether, kaempferol, afzelin, isoquercitrin, quercitrin, and hyperoside (Kaewamatawong, 2008)

Pharmacological activity

Root – antimalarial activity (Kittakoop *et al*., 2000)

Pod – antioxidant activity (Sakong *et al*., 2011, Thenmozhi *et al*., 2013, and Sharma *et al*., 2014)

Leaf – anti-diabetic activity, antifungal activity, and antioxidant activity (Sakong *et al*., 2011, Thenmozhi *et al*., 2013, Sharma *et al*., 2014, and Dej-adisai & Pitakbut, 2015)

Stem and bark – hepatoprotective activity, anti-hemolytic activity, and antioxidant activity (Sakong *et al*., 2011, Thenmozhi *et al*., 2013, Sharma *et al*., 2014, and Thenmozhi *et al*., 2018)

Toxicity

Acute toxicity

The oral administration of aqueous methanolic extract (50%) of *B. malabarica* stem bark revealed the nontoxic effect in the CCl_4 induced liver damage method in rats (Thenmozhi *et al*., 2018).

***Bauhinia ornata* Kurz**

Synonym: *Bauhinia bakeriana* S.S.Larsen, *Phanera ornata* Kurz

Common name: -

Native distribution: China, Vietnam, Lao, Thailand, Myanmar and north east India

Plant description

"Large tendrilled climber. Leaf 19.5 × 18 cm, broadly ovate. 13-nerved, bifid c. 1/3 their length into subacute lobes at apex, cordate at base, glabous above, glabrescent below along nerves; Petiole 12.3 cm long, pubescent. Stipules c. 0.6 × 0.4 cm, oblong, obtuse at apex, densely pubescent. Inflorescence subumbelliform, 10 × 9 cm. pedicles c. 3 cm long, pubescent. Bracts subulate; bracteoles minute, situated high up on pedicel. Receptacle c. 0.5 cm long, turbinate, pubescent. Calyx c.

0.6 cm long, 2-3-lobed, pubescent. Stripe c. 0.1 cm long; ovary c. 0.6 cm long, densely ferruginous pubescent; style c. 0.8 cm long, glabrous towards the minutely peltate stigma (Bandyopadhyay & Sharma, 1993).”

***Bauhinia pottsii* G. Don**

Synonym: *Bauhinia elongata* Korth, *Phanera elongata* (Korth.) Benth

Common name: -

Native distribution: Myanmar, Cambodia, Indonesia, Java, Kalimantan, Peninsular Malaysia, Sumatra, and Thailand.

Plant description

"A shrub, tree or straggling tree with glabrescent young branches. Leaves alternate; stipules minute, early caducous; petiole 3-6 cm long; blade ovate to rotundate, 9-14 cm × 10-15 cm, base cordate, apex bifid up to half of the blade length, lobes rounded, 11-15-veined. Inflorescence a lateral or terminal raceme; flower buds elongate, 3-4 cm long, hypanthium tubular; flowers bisexual; calyx splitting into 2-5 segments; petals 5, 4-6 cm long; stamens 3, staminodes few; ovary 1-1.5 cm long, stipe 1-2 cm long. Fruit a strapshaped legume, broadest towards apex,

4-6-seeded, dehiscent. Seed orbicular, up to 1.5 cm in diameter. Based mainly on length of the petal claw, petal colour and hairiness of leaf and ovary, 5 varieties are distinguished. *B. pottsii* occurs at lower altitudes, usually along forest margins, rivers, ditches and in swamps."

(https://uses.plantnet-project.org/en/Bauhinia_pottsii_(PROSEA))

Medicinal Uses

Stem – tonic, and fever (Neamsuvan *et al*., 2014)

***Bauhinia pulla* Craib**

Synonym: *Lasiobema pullum* (Craib) A.Schmitz

Common name: -

Native distribution: Cambodia, Thailand, Myanmar, and Cambodia

Plant description

"Habit Desc.: Tendrilled climber, up to 5 m high; young branches greyish pubescent, Leaf Desc.: Simple, alternate, ovate, bifid; stipules falcate, Flower Desc.: Inflorescence in terminal or axillary raceme, up to 20 cm long; flowers greenish, Fruit Desc.: Pod flat, woody-valved, dehiscent, velvety, strap-shaped"

(https://www.pharmacy.mahidol.ac.th/newspdf/sirimedinfo/240_en.pdf)

Medicinal uses

Stem – emmenagogue, and hematonic (https://www.pharmacy.mahidol.ac.th/newspdf/sirimedinfo/240_en.pdf)

Seed – fever, and anthelmintics (https://www.pharmacy.mahidol.ac.th/newspdf/sirimedinfo/240_en.pdf)

Root – ulcers, tumors, celebral palsy, asthma, constipation, chest pain, and toothache (Chhem, 2004, and Prachuabaree, 2008)

Pharmacological activity

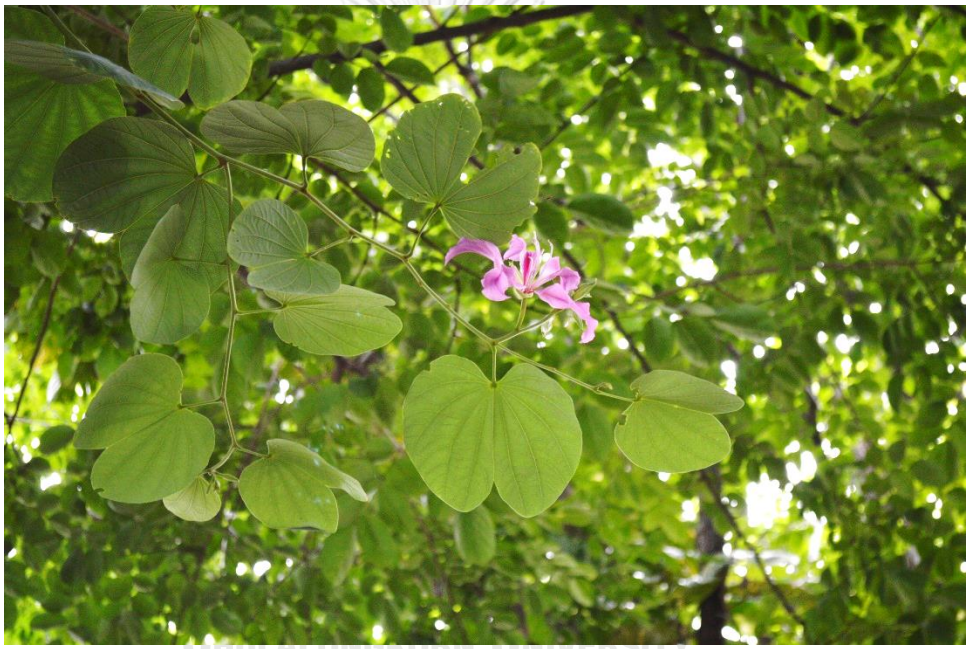
Bark – neuroprotective activity (Keo *et al*., 2012)

***Bauhinia purpurea* Linn.**

Synonym: *Bauhinia castrata* Blance, *Phanera purpurea* Benth., *Phanera purpurea* (L.) Benth.

Common name: orchid tree, purple *Bauhinia*, purple orchid tree, butterfly tree

Native distribution: Tropical Asia, probably only in the continental monsoon area northwest to Nepal.



Plant description

"Trees or erect shrubs, 7-10 m tall. Bark grayish to dark brownish, thick, smooth; branches puberulent when young, later glabrous. Petiole 3-4 cm; leaf blade suborbicular, 10-15 × 9-14 cm, stiffly papery, abaxially almost glabrous, adaxially glabrous, primary veins 9-11, secondary and higher order veins protruding, base shallowly cordate, apex bifid to 1/3-1/2, lobes slightly acute or rarely rounded at apex. Inflorescence a raceme with few flowers, or a panicle with up to 20 flowers, axillary or terminal. Flower buds fusiform, 4- or 5-ridged, with an obtuse apex.

Pedicel 7-12 mm. Calyx open as a spathe into 2 lobes, one with 2 teeth and other 3-toothed. Petals light pink, oblanceolate, 4-5 cm, clawed. Fertile stamens 3; filaments ca. as long as petals. Staminodes 5 or 6, 6-10 mm. Ovary stalked, velvety; style curved; stigma slightly enlarged, peltate. Legume linear, flat, 12-25 × 2-2.5 cm; valves woody. Seeds compressed, suborbicular, 12-15 mm in diam."

(http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200011953)

Medicinal uses

Root – carminative, abdominal pains, hemorrhoids, goiter, and vitiligo (Chatterjee & Pakrashi, 1992, Kurian, 2004, and Kamble *et al*., 2010)

Bark – diarrhea, wash solution for ulcers, prescribe in enlarge cervical glands, goiters, scrofulous tumors, lymphadenitis, asthma and respiratory disorders, anti-inflammatory agent, emmenagogue, leukorrhea, skin diseases, and dysentery (Yadav & Bhadoria, 2001, Kurian, 2004, Pawar & Patil, 2007, Patil *et al.*, 2008, and Das *et al*.,2008)

Flower – mix with bark or root for treat boils and abscesses, laxative, dysentery, and anthelmintic (Wassel *et al*., 1986, Kalakoti & Pangtey, 1988, Kurian, 2004, and Shiddamallayya *et al*., 2010)

Leaf – cough, diuretic, and abscesses and sores (https://medthai.com/ชงโค/)

Whole plant – dropsy, pain, rheumatism, convulsions, delirium, septicemia (Asolker *et al*., 2000)

Chemical constituents

Bauhiniastatins1, bauhiniastatins2, bauhiniastatins3, pacharin (Pettit *et al*., 2006), flavone glycoside (5,6-dihydroxy-7-methoxyflavone 6-O-b-D-xylopyranoside) (Yadav & Tripathi, 2000), 2,3-dihydroxypropyl oleate, 2,3-dihydroxypropyl linoleate,

2,3-dihydroxypropyl 16-hydroxy-decanoate, 6-buthyl-3-hydroxyflavone, 6-(3"-oxobutyl)-taxifolin (Kuo *et al*., 1998), two dimeric flavonoids (bis[3',4'-dihydroxy-6-methoxy-7,8-furano-5',6'-monomethlyalloxy]-5-C-5-biflavonyl and (4'-hydroxy-7-methly3-C-α-L-rhamnopyranosyl)-5-C-5-(4'-hydroxy-7-methyl-3-C-α-D-glucopyranosyl) biflavonoid) (Yadav & Bhadoria, 2005), leutin, β-sitosterol (Ragasa *et al*., 2004), α-amyrin caprylate (Verma & Chandrashekar, 2009), monoterpenes (a-terpinen, limonene, myrcene, linalool, citronellyl acetate), eugenol (Wassel *et al*., 1986), quercetin, isoquercitrin, astragalin, kaempferol, isorhamnoside (Ramchandra & Joshi, 1967, Salatino, *et al*., 1999), butein 4' O-β-L-arabinopyranosyl-O-β-D-galactoside (Bharatiya *et al*., 1979), 3,4-dihydroxychalcone 4-O-β-L-arabinopyranosyl-O-β-D-galactopyranoside (Bharatiya & Gupta, 1981), a novel glycoside (glycoside-6-4'-Diahydroxy-3'-prenyl-3,7,5,7'-Tetramethoxy Flavone-6-O-α-L-rhamnopyranoside) (Yadav & Sodhi, 2001), bauhinoxepin C-J, bauhinobenzofurin A, bauhispirorin A, bauhinol E, (-)-strobopinin, demethoxymatteucinol, five known bibenzyls (Boonphong *et al*., 2007), phenylalanine, methionine, leucine (Sharanabasappa *et al*., 2007), caffeic acid, syringic acid, vanillic acid (Gupta *et al*., 2015).

Pharmacological activity

Seed – hemagglutination inhibition (HI) assays, and antibacterial activity (Codington *et al*., 1975, and Sakthivel *et al*., 2016)

Leaf – anti-diarrhea activity, anti-nociceptive activity, anti-inflammatory activity, analgesic activity, antipyretic activity, antioxidant activity, nephroprotective activity, wound healing activity, gastroprotective activity, antibacterial activity, antiulcer activity, hepatoprotective activity, and antidiabetic activity (Abd-El-Wahab, 1987, Mukherjee *et al*., 1998, Zakaria *et al*., 2007, Zakaria *et al*., 2009, Joshi *et al*., 2009, Lakshmi *et al*., 2009, Ananth *et al*., 2010, Murugan & Mohan, 2011, Zakaria *et*

al., 2011, Abdul Hisam *et al*., 2012, Kamarolzaman *et al*., 2014, Dej-adisai & Pitakbut, 2015, and Zakaria *et al*., 2016)

Stem and bark – anti-inflammatory activity, analgesic activity, anti-diabetic activity, cardiotonic activity, hormone regulation, hepatoprotective effect, antioxidant activity, antibacterial activity, antifungal activity, anti-lipidemic activity, and anti-obesity activity (Panda & Kar, 1999, Panda *et al*., 2003, Muralikrishna *et al*., 2008, Shreedhara *et al*., 2009, Chandrashekar *et al*., 2009a, Chandrashekar *et al*., 2009b, Jatwa & Kar, 2009, Ramgopal *et al*., 2010, Murugan & Mohan, 2011, Chaturvedi *et al*., 2011, Sardessai *et al*., 2013, Krishnaveni, 2015, and Dej-adisai & Pitakbut, 2015)

Root – anti-mycobacterial activity, antimalarial activity, antifungal activity, cytotoxic activity, and anti-inflammatory activity (Boonphong *et al*., 2007)

Pod – nephroprotective activity, and anti-diabetic activity (Lakshmi *et al*., 2009, and Dej-adisai & Pitakbut, 2015)

Flower – antioxidant activity (Dej-adisai & Pitakbut, 2015)

Toxicity

Acute toxicity

Aqueous extract of *B. purpurea* bark showed non acute toxicity effect at the dose 50 up to 500 mg/kg^{-1} in male albino rats (Chaturvedi *et al*., 2011).

In addition, chloroform extract of *B. purpurea* leaf showed no symptom or sign of toxicity effect at the dose of 5000 mg/kg in male Sprague – Dawley rats until the end of experiment (Abdul Hisam *et al*., 2012).

***Bauhinia racemosa* Lam.**

Synonym: *Bauhinia parviflora* Vahl, *Piliostigma racemosa* (Lam.) Benth., *Piliostigma racemosum* (Lam.)Benth.

Common name: Burmese Silk Orchid

Native distribution: India, Myanmar, China, Cambodia, Thailand

Plant description

"Trees, deciduous, small, to 15 m tall. Bark blackish, rough; branches spreading or pendulous, zigzag, slender, glabrous. Stipules caducous; petiole 0.8-1.2 cm; leaf blade broadly orbicular, 1.5-4 × 2.2-6 cm, 7-9-veined, leathery, abaxially pubescent or glabrous, adaxially glabrous, base cordate, apex bifid to ca. 1/3, lobes rounded at apex. Inflorescence a lateral or terminal raceme, ca. 20-flowered; peduncle short; bracts and bracteoles linear. Flower buds obovoid, puberulent, apex protruding. Hypanthium turbinate, short. Calyx split spathaceously at anthesis. Petals

yellowish, subequal, oblanceolate, 8-10 mm, subsessile. Fertile stamens 10, unequal; filaments 6-7 mm; anthers small, ca. 3 mm. Ovary stalked, glabrous; stigma subsessile, peltate, small. Legume linear-cylindric, 15-20 × 1.8-2.2 cm; valves woody, glabrous. Seeds 12-20, dark brownish, ellipsoid, 8-10 mm in diam."

(http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200011956)

Medicinal uses

Root – deobstruent, cooling, and liver diseases (Chopra *et al*., 1956, Granner, 1996, and Kumar *et al*., 2005)

Fruit – cough, asthma, and diarrhea (Chopra *et al*., 1956)

Seed – opthalmia, tonic, and aphrodisiac (Chopra *et al*., 1956, Bailey & Day, 1989, and Nirmal *et al*., 2011)

Stem and bark - anthelminthic drug, fish poison, tonic, Insecticidal, snake repellent, ulcer wash solution, astringent, headache, fever, skin diseases, tumors, blood diseases, dysentery, diarrhea, abdominal pain, swelling, wound, and mouth ulcer (Chopra *et al*., 1956, Kirtikar & Basu, 1975, Desai *et al*., 1975, Jagtap *et al*., 2006, and Karuppusamy, 2007)

Leaf – scorpion bite, and dysentery (Jagtap *et al*., 2006, Granner, 1996, and Kumar *et al*., 2005)

Flower – diuretic (Granner, 1996, and Kumar *et al*., 2005)

Whole plant – veterinary medicine (Nirmal *et al*., 2011)

Chemical constituents

Phosphatidylinositol, lysophosphotidyl ethanolamine, phosphotidylcholine, lysine, phenylalanine, methionine, leucine (Sharanabasappa *et al*., 2007), 1,3-

Dioxolane-2-methanol, 2-Butanone, 3-methoxy-3-methyl-, Myo-Inositol, 4-C-methyl-, 1,3-Dioxolane, 2-(2-propenyl), Pentane, 1,3-epoxy-4-methyl, Butane, 1-bromo-2-methyl, 4-Methyl-2,4-bis(40trimethylsilyloxyphenyl)pentene-1, Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester (Panda *et al*., 2018), α-d-glucopyranosyl-(4⟶1′)-O-α-d-glucopyranoside, lauryl-O-α-d-glucopyranoside, α-d-glucopyranosyl-(4 ⟶ 1′)-O-α-d-glucopyranosyl-(4′ ⟶ 1″)-O-α-d-glucopyranoside, α-d-glucopyranosyl-(6⟶1′)-O-α-d-glucopyranosyl-(6′⟶1″)-O-α-d-glucopyranosyl-(6″⟶1‴)-O-α-d-glucopyranoside, Linoleiyl-O-α-d-glucopyranosyl-(4′⟶1″)-O-α-d-glucopyranoside, linoleiyl-O-β-d-arabinopyranoside (Rahman & Akhtar, 2016), kaempferol, quercetin, coumarins (scopoletin and scopolin), β-sitosterol, β-amyrin, stilbene, tetra cyclic lupeol, botulin, tetracyclic 2, 2-dimethlychroman (Prakash & Khosa 1976, Prabhakar *et al*., 1994, and El-Hossary *et al*., 2000), kaempferol-3-O-α-rhamnoside, kaempferol-3-O-β-galactoside, quercetin-3-O-β-galactoside, kaempferol 4'-methyl ether, quercetin 5,7,3',4'-tetramethyl ether (Anjaneyulu *et al*., 1986), racemosolone, n-tetracosane, β-sitosteryl stearate, eicosanoic acid, stigmasterol, octacosyl ferulate, de-*O*-methyl racemosol, lupeol, 1,7,8,12b-tetrahydro-2,2,4-trimethyl-2H-benzo[6,7]cyclohepta [1,2,3-*de*] (Jain *et al*., 2013).

Pharmacological activity

Leaf – analgesic activity, anti-inflammatory activity, antipyretic activity, antiulcer activity, antimicrobial activity, antispasmodic acitivity, antihistaminic activity, anti-diabetic activity, anti-obesity activity, anti-hyperlipidemic activity, tyrosinase inhibitory activity, and thrombolytic activity (Bhaskara Rao *et al*., 2010, Dahikar *et al*., 2011, Nirmal *et al*., 2011, Timane *et al*., 2016, Kumar *et al*., 2017, and Panda *et al*., 2018)

Stem bark – antimicrobial activity, analgesic activity, anti-inflammatory activity, antipyretic activity, antioxidant activity, anti-filarial activity, anthelmintic activity, antimalarial activity, abortifacient, aanti-hepatoprotective activity, antitumor activity, and hepatoprotective activity (Gupta *et al*., 2004, Gupta *et al*., 2005, Kumar *et al*., 2005, Sashidhara *et al*., 2012, Chavan & Kadam, 2012, and Rahman *et al*., 2016)

Flower bud – antiulcer activity (Akhtar & Ahmad, 1995)

Fruit – antiulcer activity (Borika *et al*., 2009)

Whole plant – anthelmintic activity (Kumar *et al*., 2011)

Aerial part – antimicrobial activity, and antioxidant activity (Rashed & Butnariu, 2013)

Toxicity

Acute toxicity

The ethanolic extract of *B. racemosa* leaf showed that the extract up to 2000 mg/kg has nontoxic effect in both male and female Wister Albino rats (Timane *et al*., 2016)

Methanolic *B. racemosa* bark extract revealed the potent cytotoxic activity against a human cancer cell line (HeLa) and induces apoptosis (Rahman *et al*., 2016).

Bauhinia saccocalyx **Pierre**

Synonym: -

Common name: -

Native distribution: Thailand and Indochina

Plant description

"Shrub or straggling shrub, 4-8 m high, normally dioecious. Leaves simple, broadly ovate, bifid to 1/3-1/2, tip of lobes triangular-acute, 6-10 cm long by 5-9 cm wide, upper surface glabrous; secondary nerves 9-11; stipules minute, early caducous. Inflorescence panicle with dense flowered, up to ca 7 cm long. Flowers white to pinkish white, 1-1.4 cm across; calyx spathaceous or splitting in two; corolla 5, obovate, apex rounded. Male flower with 10 fertile stamens. Female flower with 10 filiform staminodes; ovary hairy. Pod dehiscent, strap-shaped, apex with curved beak, glabrous, 7-14 cm long. Seeds 3-5."

(http://www.qsbg.org/Database/BOTANIC_Book%20full%20option/search_detail.asp?botanic_id=1420)

Medicinal uses

Leaf – blood purifier medicines (Sapcharoen, 2006)

Chemical constituents

Bauhinoxepin A (3,3,5-trimethylbenzo[b]pyrano[g][1]benzoxepin-6,11-diol), bauhinoxepins B (6-methoxy-7-methyl-2-(3-methylbut-2-enyl)dibenzo[b,f]oxepine-1,8-diol) (Kittakoop *et al*., 2004), bauhinol A, bauhinol B, bauhinol C, bauhinol D, bibenzyls 5, bibenzyl 6 (Apisantiyakom *et al*., 2004).

Pharmacological activity

Root – antimycobacterial activity, antimalarial activity, and cytotoxicity activity (Kittakoop *et al*., 2004, Apisantiyakom *et al*., 2004)

Toxicity

The isolated compounds from *B. saccocalyx* root, bauhinol A, bauhinol B, and bibenzyl 6 have been reported that they revealed the cytotoxicity effect against small-cell lung cancer (NCI-H187), breast cancer (BC), and oral-cavity cancer (KB) (Apisantiyakom *et al*., 2004).

***Bauhinia scandens* Linn.**

Synonym: *Lasiobema scandens* (L.) de Wit, *Bauhinia divergens* Baker

Common name: -

Native distribution: India, Bangladesh, Bhutan, China, Cambodia, Indonesia, Malaysia, Myanmar, Nepal, Vietnam, and Thailand

Plant description

"Lianas, large, woody. Branches cylindric when young, applanate when old, forming "monkey ladders," puberulent, later glabrous; tendrils in pairs, puberulent. Stipules caducous; petiole 2-4 cm, slender; leaf blade ovate to broadly ovate, 5-9 × 4-8 cm, papery, both surfaces glabrous, primary veins usually 7-9, base shallowly cordate to truncate, entire on flowering branches, apex bifid to more than 1/2 in sterile or juvenile branches, lobes with obtuse or acuminate apices. Inflorescence an elongated raceme, 10-15 cm, many flowered, or several joined in a panicle 15-25 cm, terminal, puberulent; bracts and bracteoles linear. Pedicel 3-4 mm, slender. Flower

buds ovoid, 1.8-2 mm in diam., apex open. Calyx lobes 5, triangular, outside pubescent. Petals white, subequal, obovate to oblanceolate, ca. 3 mm, shortly clawed. Fertile stamens 3; filaments glabrous. Staminodes 2. Floral disk fleshy, swollen. Ovary shortly stalked, oblique, glabrous; style stout; stigma small. Legume rhombic to oblong, 1.8-3 × 1-1.6 cm, indehiscent or tardily dehiscent; valves thin, reticulate veined. Seeds 1 or 2(-4), ellipsoid to obovoid-orbicular, ca. 8 mm in diam."

(http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200011958)

Medicinal uses

Leaf – anemia, menstrual disorder, abortion, and fever (Islam *et al*., 2010, Islam *et al*., 2010, www.medplant.mahidol.ac.th)

Stem and bark – fever, abscesses, cough, dysentery, skin diseases, abortion and birth control, and joint pain (Singh *et al*., 2010, www.rspg.or.th/plants_data/, www.saiyathai.com, www.medplant.mahidol.ac.th, and www.thairath.co.th)

Root – antitoxin (www.medplant.mahidol.ac.th)

Seed – anthelmintic, fever, and antitoxin (www.medplant.mahidol.ac.th, and www.thairath.co.th)

Chemical constituents

1-*O*-alkyl glycerol (Hazra & Chatterjee, 2007), gallic acid, ellagic acid, rosmarinic acid, vanillin, vanillic acid acid, syringic acid (Hossain *et al*., 2017), tetradecane, hexadecane, octadecane, nonadecane, eicosane, heneicosane, docasane, tricosane, tetracosane, pentacosane, hexacosane, heptacosane (Poonsri *et al*., 2014).

Pharmacological activity

Leaf – antitumor activity (Hazra & Chatterjee, 2008)

Whole plant – antioxidant activity (Hossain *et al*., 2016)

Stem – insecticidal activity (Poonsri *et al*., 2015)

Toxicity

The major compounds, heptacosane and hexacosane of *B. scandens* stem ethanolic extract have been reported that they have toxic effect with *Plutella xytostella* larvae (LD_{50} =2.76μg/larva) (Poonsri *et al*., 2015).

Bauhinia siamensis **K. & S. S. Larsen**

Synonym: -

Common name: -

Native distribution: Thailand

Plant description

"Liana; very young branches reddish-brown hairy, soon glabrous. Leaves: stipules rotundate to obovate with truncate base, 6-9 mm long, finely puberulous outside. Pubescent inside; petioles glabrescent. 1-3 cm long, thickened at both ends; lamina chartaceous, ~ ovate, 4-7.5 by 4-7 cm, apex bifid by 1/3 to 2/5, sinus broad, tips of lobes rounded. Base cordate, nerves 7-9, hairy on both sides when very young, later glabrous on the upper surface. Lower surface puberulous mainly on the nerves and at the base. Inflorescence a pendulous, elongate raceme up to 75 cm long, with closely spaced or distant stipules at the base; axis reddish-brown pubescent when young, later subglabrous; bracts lanceolate. 10-12 by 3--4 mm, inside pubescent outside puberulous; pedicels 18-25 mm, pubescent; bracteoles

linear. 4-5 mm long, hairy as the bracts. ~ opposite, inserted around the middle of the pedicel. Buds oblong. finely reddish-brown pubescent, 10-11 by 5-6 mm. apex pointed with 5 minute, free calyx teeth. Hypanthiunz obliquely cup-shaped. Striate when dry, 5-6 by 4-5 mm, hairy as the buds. Calyx splitting into a bilabiate structure at anthesis, finely hairy inside, denser towards apex, upper segment of 2 sepals, lower segment of 3 sepals. Petals 5, pink. Subequal, obovate to elliptic, apex rounded, base attenuate, 15-19 by 8-10 mm, claw 1-2 mm long, upper surface glabrous except at base, lower puberulous along the nerves and at base; a column-like structure (persistent in pod) protruding from the posterior side of the mouth of the hypanthium, 3--4by 2.5-3 mm, apex 3-partite, dorsal side with 2 ridges, ventral side with 2 lateral ridges and a low middle ridge. Stamells: 3 fertile, filaments glabrous, 13-15 mm long, anthers glabrous. oblong, 3-4 by 1-2 mm, dehiscing longitudinally; staminodes 6: 4 posterior inserted at the base of the column, 2 middle ones, 5-6 mm long with minute apical appendages and 2 lateral. Minute, 1-2 mm long, 2 minute between the fertile ones, I-2 mm long. Ovary stipitate, stipe c. 4 mm long, glabrous towards the base; ovary densely appressed golden-hairy, 7-10 mm long; style short c. 1 mm long, glabrous in upper part; stigma punctate-capitate, c. 1 mm broad, sparsely golden-hairy. Pod dehiscent. glabrous, dark brown, narrowly oblong, 16-18 by 3--4em, with a 6 mm long stalk and beak c. 5 mm. Seeds dark brown, flat, ovate, 15-20 mm long. Funicle forked near the hilum into 2 thin branches running almost all the way along the edge of the seed. (Larsen & Larsen, 2002)"

Pharmacological activity

Aerial part – anti-inflammatory activity, analgesic activity, and antipyretic activity (Thammasri *et al*., 2009)

***Bauhinia sirindhorniae* K. & S. S. Larsen**

Synonym: *Phanera sirindhorniae* (K.Larsen & S.S.Larsen) Mackinder & R.Clark

Common name: -

Native distribution: Thailand

Plant description

"Tendrilled liana, 10-20 m long. Very young branches reddish brown hairy, old branches glabrous. Leaves: stipules oblong-elliptic, sometimes slightly falcate, 5-7 mm long, hairy outside, early caducous; petioles 2-6.5 cm, reddish hairy when young later *glabrous, thickened at both ends; lamina coriaceous, ovate, 5-1 8 x 4- 17 cm; apex varying from entire to emarginate or slightly bifid to deeply bifid almost to the base, on sterile shoots often consisting of 2 free leaflets; tip of lobes subacute, acute to acuminate, falcate; base cordate; nerves 9 (-1 1); upper surface glabrous, margin and lower surface at the nerves reddish hairy when young, later glabrous or sometimes puberulous. Inflorescence densely fermgineous pubescent all over,

compound, with dichasial side branches at least in their lower 213; main axis 2-10 cm; bracts hairy outside, glabrous inside, narrowly lanceolate, c 5 mm below diminishing upwards; pedicels pubescent, 1.5-2 cm. Buds ovoid, pointed, finely reddish brown pubescent, 10 x 4 mm. Hypanthium tubular to narrowly funnel-shaped, striate, hairy outside as well as inside, 10- 16 mm long. Calyx erect, spathaceous (splitting one side to the base, opposite side at the tip only), striate, 10-13 mm. Petals yellowish to orange-red, subequal, narrowly to broadly lanceolate, 9-13 x 3-6 mm including the c. 1.5 mm long claw; inner surface glabrous, outer densely reddish brown hairy. Stamens 3 fertile, filaments glabrous, 12-15 mm; anthers glabrous, oblong, 3 x 1 mm, opening by length slits; staminodes 2, triangular, minute (less than 1 mm) on both side of the posterior petal. Ovary reddish brown hairy, 7-10 mm; stipe short, c 1 mm, hairy; style 7-11 mm, hairy; stigma inconspicuous to small capitate. Fruit fermgineous pubescent, strapshaped, 15-18 x 3-4 cm with persistent calyx. Seeds 5-7, dark brown, flat, orbicular, 1.5- 2 cm diam. (Larsen & Larsen, 1997)"

Medicinal uses

Whole plant – muscle relaxants, joint pain, abortifacient (Nithikulworawong, 2012, and https://puechkaset.com/สิรินธรวัลลี/)

Flower – abscesses, blood circulation, diabetes, high blood pressure, and skin swelling

Leaf – diuretic, anthelmintic, and sores

Fruit – diuretic, venereal diseases, anthelmintic, and hemorrhoids

Root – joint pain, diabetes, skin diseases, and insect bite (https://puechkaset.com/สิรินธรวัลลี/)

Stem and bark – tonic, blood circulation, muscle relaxants, skin swelling, sores, and insect bite (Athikomkulchai *et al*., 2005, Chaisri & Laoprom, 2016, and https://puechkaset.com/สิรินธรวัลลี/)

Chemical constituents

Lithospermoside, menisdaurin, (-)-epicatechin, (2*S*)-naringenin, (2*S*)-eriodictyol, (+)taxifolin, luteolin, isoliquiritigenin, 5, 7-dihydroxychromone, 5-hydroxychromone 7-β-D-glucoside, (+)-isolariciresinol 3α-*O*-α-L-rhamnoside, (+)-lyoniresinol 3α-*O*-α-L-rhamnoside, lupeol, glutinol, sitostery-3-*O*-β-D-glucoside, 3, 4, 5-trimethoxyphenolic-1-*O*-β-D-glucoside, protocatechuic acid (Athikomkulchai *et al*., 2004)

Pharmacological activity

Stem, root, leaf, and flower – antibacterial activity, and antioxidant activity (Athikomkulchai *et al*., 2005, Nithikulworawong, 2012, and Chaisri & Laoprom, 2016)

***Bauhinia strychnifolia* Craib**

Synonym: *Lysiphyllum strychnifolium* (Craib) A. Schmitz

Common name: -

Native distribution: Thailand

Plant description

"A climber, it climbs by means of tendrils and can grow up to 5 m in height. The leaves are simple, arranged alternately along the stem and ovate-oblong in shape with a rounded or cordate base. The young leaves are pink, turning green as it matures. Flowers are borne on terminal racemes up to 100 cm long. The flower calyx is pale pink to red, 5 lobed. The petals are obovate in shape and pubescent and range in color from red to dark red. Each flower has 3 stamens and 1 stigma."

(https://florafaunaweb.nparks.gov.sg/special-pages/plant-detail.aspx?id=6503)

Medicinal uses

Leaf – neutralize toxins, laxative, antidote, tonic, and fever relief (Wutthithammavet, 1997, Chuakul *et al*., 2002, Chamratpan & Homchuen, 2005, and tmri.dtam.moph.go.th)

Stem – tonic, fever relief, laxative, antidote, hematonics, and cancer (tmri.dtam.moph.go.th, Wutthithammavet, 1997, and Phalanisong *et al*., 2018)

Root – laxative, fever relief, and hematonics (tmri.dtam.moph.go.th)

Chemical constituents

5,7,3′,5′-Tetrahydroxyflavanone, 3,5,7,3′,5′-Pentahydroxy-flavanonol-3-O-α-L-rhamnopyranoside, 3,5,7-Trihydroxy-chromone-3-O-α-L-rhamnopyranoside, β-sitosterol, stigmasterol (Yuenyongsawad *et al*., 2013) gallic acid (Maitree *et al*., 2018), catechin, myricetin, syringic acid, p-cumaric aicd (Nammatra & Photong, 2018), methyl-phydrozybenzoate, mome inositol, n-Hexadecanoic acid, tetradecanamide, (Z)-9-Octadecanamide, 1,2,3-benzenetriol, methylparaben, 4-(4-Hydroxyphenyl)-2-butanone, ethyl hexadecanoate, Phytol, (Z)-9-Octadecanoic acid and Octadecanoic acid (Sukprasert, 2018)

Pharmacological activity

Stem – anticancer activity, anti-HIV-1 integrase activity, antitumor activity, antimicrobial activity, antioxidant activity, cytotoxicity activity, anti-hyperglycemic activity, and antiviral activity (Bunluepuech & Tewtrakul, 2011, Kaewpiboon *et al*., 2012, Yuenyongsawad *et al*., 2013, Meerungrueang & Panichayupakaranant, 2014, Phalanisong *et al*., 2014, Boon-young *et al*., 2015, Itharat *et al*., 2016, Ekaratchroenchai *et al*., 2017, Kraithep *et al*., 2017, Phalanisong *et al*., 2018, and Sukprasert, 2018)

Leaf – antioxidant activity, Effects on Growth Performance and Survival Rate in catfish, anti-inflammatory activity, antimicrobial activity, antimalarial activity, cytotoxicity activity, antiviral activity, and antihyperuricemic activity (Premkaisorn & Wasupongpun, 2015, Munglue & Dasri, 2015, Boon-young *et al*., 2015, Chutoam *et al*., 2015, Somsak *et al*., 2015, Itharat *et al*., 2016, Panchinda *et al*., 2016, Ekaratchroenchai *et al*., 2017, Thiraworawong *et al*., 2018, Nammatra & Photong, 2018, Sukprasert, 2018, and Sutiyaporn *et al*., 2018

Toxicity

Acute toxicity

The water distillation extract of *B. strychnifolia* leaves revealed nontoxic effects with dose 100 – 6,000 mg/kg of any indication of gross physical or behavioral changes in mice by oral route (Chutoam *et al*., 2015). In addition, ethanolic extract of this plant leaf showed nontoxic effects at the dose up to 3,000 mg/kg in normal mice (Somsak *et al*., 2015).

***Bauhinia tomentosa* Linn.**

Synonym: -

Common name: St. Thomas tree, yellow *Bauhinia*, bell *Bauhinia*, yellow orchid tree

Native distribution: Angola, Ethiopia, Kenya, Somalia, South Africa, Tanzania, Zaire, Zambia, Zimbabwe, Bangladesh, India and Sri Lanka

Plant description

"Shrubs, erect, to 4 m tall. Young branches puberulent. Stipules linear, ca. 1 cm; petiole 1.5-3 cm, slender; leaf blade suborbicular, 3-7 × 4-8 cm, papery, abaxially tomentose, adaxially glabrous, base cordate, 7-9-veined, apex bifid to ca. 1/2, lobes rounded at apex. Inflorescence a lateral raceme, 1-3-flowered; pedicel short; bracts

and bracteoles linear, 4-7 mm. Flower buds fusiform, ca. 2 cm, puberulent. Hypanthium turbinate, ca. 5 mm. Calyx split spathaceously at anthesis. Petals light yellowish, subequal, broadly obovate, 4-5.5 × 3-4 cm, subsessile. Fertile stamens 10, unequal; filaments 1-2 cm, puberulent at base. Ovary stalked, tomentose; style slender, glabrous; stigma peltate, small. Legume flat, linear, 7-15 × 1.2-1.5 cm, sutures not ridged; valves leathery, velutinous. Seeds brownish, suborbicular, compressed, 6-8 mm in diam."

(http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200011960)

Medicinal uses

Root – liver diseases, and anthelmintic drug (Rhama & Madhavan, 2012, Sharma & Kumar, 2012)

Stem and bark – astringent gargle, tumors, and wounds (Kirtiker & Basu, 1935, Rhama & Madhavan, 2012, and Sharma & Kumar, 2012)

Leaf – abscesses, dysentery, and fodder (Agbede, 2007, and Rhama & Madhavan, 2012)

Flower – dysentery (Rhama & Madhavan, 2012, and Sharma & Kumar, 2012)

Fruit – diuretic (Rhama & Madhavan, 2012)

Seed – tonic, aphrodisiac, insect bite, and fodder (Agbede, 2007, and Rhama & Madhavan, 2012

Chemical constituents

1-(2′-hydroxy-4′-methoxyphenyl)-3-(4″-methoxyphenyl)-2-hydroxypropane-1,3-dione, 5-hydroxyflavone, 3,5,7,3′,4′-pentahydroxyflavone, 3,5,7,2′,4′-pentahydroxyflavone and 5,7,3′,4′-tetrahydroxyflavone-3-*O*-rhamnoside (Radha *et al*.,

2016), kaempferol-7-*O*-rhamnoside, kaempferol-3-*O*-glucoside, quercetin-3-*O*-glucoside, (Aderogba *et al*., 2008), quercetin, quercetin-3-O-rutinoside (Row & Viswanadham, 1954), linoleic, stearic, vernolic acids (Daulatabad *et al*., 1991), tannin, phytin, phytin-P, lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cystine, methionine, valine, isoleucine, leucine, tyrosine, phenylalanine (Agbede, 2007), phthalic acid, ethyl pentyl ester, 3-*O*-methyl-D-glucose, 2-butanone, 3-methoxy-3-methyl, 2,2-dimethylpropionic acid, cyclopentyl ester, 2-hexen-1-ol, 2-ethyl, 5-hydroxy-2,2-dimethylhexan-3one, pentanoic acid, 2-methyl, butane, 1-bromo-2-methyl (Panda *et al*., 2018).

Pharmacological activity

Root – anti-diabetic activity, antioxidant, and antimicrobial activity (Aderogba *et al*., 2008, Dugasani *et al*., 2010, and Kaur *et al*., 2011)

Stem and bark – antibacterial activity, and anti-diabetic activity (Gopalakrishnan & Vadivel, 2011, and Tiwari & Singh, 2013)

Leaf – antimicrobial activity (Aderogba *et al*., 2008, and Dugasani *et al*., 2010)

Flower – antibacterial activity (Sathya *et al*., 2013)

Aerial part – antioxidant activity, and nephroprotective activity (Akhitha *et al*., 2019)

Toxicity

Acute toxicity

Ethanolic and aqueous extract of *B. tomentosa* stem indicated the safety effects of the doses up to 2,500 mg/kg in mice (Tiwari & Singh, 2013).

***Bauhinia variegata* Linn.**

Synonym: *Bauhinia alba* Wall., *Phanera variegata* Linn.

Common name: mountain ebony, Kanchnar

Native distribution: Myanmar, China, Hainan, Hong Kong, Laos, Pakistan, Thailand and Vietnam

Plant description

"Trees, deciduous, to 15 m tall. Bark dark brownish, nearly smooth; branches gray puberulent when young, later glabrous. Petiole 2.5-3.5 cm; leaf blade suborbicular or broadly ovate, 5-9 × 7-11 cm, subleathery, abaxially almost glabrous, adaxially glabrous, primary veins 9-13, secondary and higher order veins protruding, base shallowly to deeply cordate, apex bifid to 1/3, lobes rounded at apex. Inflorescence a raceme, few flowered, sometimes corymblike, axillary or terminal.

Flower buds fusiform, smooth, subsessile. Calyx open as a spathe into 2 lobes. Petals white, or with pink or purplish spots, obovate or oblanceolate, 4-5 cm, clawed. Fertile stamens 5; filaments ca. as long as petals, slender. Staminodes 1-5 and small, or absent. Ovary stalked, puberulent; style curved; stigma small. Legume linear, flat, 15-25 × 1.5-2 cm; valves woody. Seeds 10-15, compressed, suborbicular, ca. 10 mm in diam."

(http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200011962)

Medicinal uses

Stem and bark – astringent, alliterative, anti-diabetic, antitumor, tonic, anthelmintic, obesity, washing ulcer, skin diseases, scrofula, diarrhea, leprosy, dysentery, tuberculosis, skin diseases, malaria, antidote to snake poison, menorrhagia, asthma, gall bladder, kidney stone, and piles (The Wealth of India, 1959, Ram *et al*., 1980, Vaidyaratnam, 1994, Ambasta, 1998, Kurien, 2001, Rajkapoor *et al*., 2003, Rajkapoor *et al*., 2006, Mali *et al*., 2009, Prashar *et al*., 2010, Sinha *et al*., 2012, Gautam, 2012, and Singh *et al*., 2013)

Leaf – laxative, piles, fodder, gall bladder, and kidney stone (Asima, 1992, Gautam, 2012, and Singh *et al*., 2013)

Root – antidote, dyspepsia, and reducing corpulence (Tomar *et al*., 2009)

Flower and flower bud – piles, cough, eye diseases, liver diseases, menorrhagia, diarrhea, dysentery, tumors, gall bladder, kidney stones, and anthelmintic (Asima, 1992, Mali *et al*., 2009, and Singh *et al*., 2013)

Chemical constituents

5,7,3',4'tetrahydroxy-3-methoxy-7-*O*-α-L-rhamnopyranosy1(1⟶3)-*O*-β-D-galactopyranoside, 3-methoxy, 5,7,3',4'-tetrahydroxy flavone, 3,5,7,3',4'-

pentamethoxy flavone, 2,4,6,-tri-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-L-rhamnose, (Duret & Paris, 1977, Gupta *et al*., 1979, Gupta *et al*., 1980, Yadava & Reddy, 2001, Yadava & Reddy, 2003) (2S)-5,7-dimethoxy-3',4'-methylenedioxyflavone, 5,6-dihydro-1,7-dihydroxy-3,4-dimethoxy-2-methyldibenz [b,f]oxepin (dihydrodibenzoxepin) (Reddy *et al*., 2003), quercetin 7-methyl ether (Barbera *et al*., 1986), cyaniding, malvidin, peonidin (Rajani *et al*., 2009), rhamnocitrin (bodakhe *et al*., 2012), lupeol, β-sitosterol, quercetin, 3,3'-dimethoxy quercetin, 3,3',6-trimethoxy quercetin, quercetin 3-*O*-β-D-$^{4}C_{1}$-glucopyranoside, quercetin 3-*O*-β-D-$^{4}C_{1}$-galacturonopyranoside, quercetin 3-O-α-L-$^{1}C_{4}$-rhamnopyranosyl (1'''⟶2'')-*O*-β-D-$^{4}C_{1}$-glucopyranoside (Harborne, 1982), caffeic acid, ferulic acid (Lu *et al*., 2002) kaempferol (Jash *et al*., 2014), quercitrin, kaempferol-3-glucoside, apigenin-7-*O*-glucoside, amides, vitamin C, (Dhale, 2011, The Wealth of India, 1998, Sharma *et al*., 1966, Spilkova & Hubik, 1992) ombuin, kaempferol 7,4′-dimethyl ether 3-*O*-β-D-glucopyranoside (Kumar *et al*., 1985), kaempferol 3-*O*-β-D-glucopyranoside (Hari Kishore *et al*., 2003), isorhamnetin 3-*O*-β-D-glucopyranoside, hesperidin, triterpene caffeate (Rao *et al*., 2008), triterpene saponin (23-hydroxy-3α-[O-α-L-$^{1}C_{4}$rhamnopyranosyl-(1''⟶4')-*O*-α-L-$^{4}C_{1}$-arabinopyranosyl-oxy]olean12-en-28-oic acid *O*-α-L-$^{1}C_{4}$-rhamnopyranosyl-(1''''⟶4'''')-*O*-β-D-$^{4}C_{1}$-glucopyranosyl-(1''''⟶6'''')-*O*-β-D-$^{4}C_{1}$-glucopyranosyl ester) (Mohamed *et al*., 2009), syringic acid, vanillic acid (Gupta *et al*., 2015), apigenin, rutin, luteolin (Bhandari *et al*., 2007).

Pharmacological activity

Leaf – antimicrobial activity, anti-diabetic activity, anti-pathogenic activity, larvicidal activity, antitumor activity, antianxiety activity, neuroprotective activity, antimalarial activity, anti-inflammatory activity, anti-nociceptive activity, anti-carcinogen activity, anti-mutagenic activity, and antioxidant activity (Azevedo *et al*., 2006, Mohamed *et al*., 2009, Agrawal & Pandey, 2009, Dhale, 2011, Saha *et al*., 2011,

Bach *et al*., 2012, Mishra *et al*., 2013, Rashid, 2014, Banyal *et al*., 2015, Pandey, 2015, Trivedi *et al*., 2015, Kulkarni & garud, 2016, Shanmugapriya *et al*., 2015, Khare *et al*., 2016, and Mohsin & Akhtar, 2017)

Stem and bark – antimicrobial activity, immunomodulatory activity, antioxidant activity, anthelmintic activity, nephroprotective activity, cytotoxic activity, anti-stress/adaptogenic activity, hepatoprotective activity, anti-ulcer activity, **α**-glucosidase inhibitory activity, and anticataract activity (Rajkapoor *et al*., 2006, Bodakhe & Ram, 2007, Patil *et al*., 2010, Dhale, 2011, Sharma *et al*., 2011, Pani *et al*., 2011, Prusty *et al*., 2011, Kumar *et al*., 2012, Bodakhe *et al*., 2012, Marasani *et al*., 2013, Manoj *et al*., 2013, Sharma *et al*., 2015, Dej-adisai & Pitakbut, 2015, and Pandey, 2015)

Root – anti-inflammatory activity, and antiobesity activity (root and stem bark) (Yadava & Reddy, 2003, and Balamurugan & Muralidharan, 2010)

Flower – antimicrobial activity (Kulshrestha *et al*., 2011, and Pandey, 2015)

Seed – hemagglutination, and wound healing activity (Lin & NG, 2008, and Neto *et al*., 2011)

Aerial part – anti-inflammatory activity (Rao *et al*., 2008)

Toxicity

Several doses (50 – 2000 mg/kg) of *B. variegata* stem extract revealed nontoxic effect by observing behavioral changes and mortality in normal rats (Bodakhe & Ram, 2007). A new triterpene saponin which is isolated from *B. variegata* leaves was shown nontoxic effect by observing both mortality and morbidity of animals with several doses (25, 50, 100, 200, 400, 1000, and 2000 mg/kg) (Mohamed *et al*., 2009). Root and stem bark methanolic extract with the doses up to 2,000

mg/kg were not indicated any toxic symptoms or mortality in rats (Balamurugan & Muralidharan, 2010).

***Bauhinia winitii* Craib**

Synonym: *Lysiphyllum winitii* (Craib) de Wit

Common name: -

Native distribution: Thailand

Plant description

"A tendrilled climber; branches rusty pubescent, glabrescent. Leaves arranged spirally; stipules minute; petiole about 1 cm long; leaflets 2, free, obliquely ovate, 3-4.5 cm × 1.5-2.5 cm with rounded base and apex. Inflorescence a lateral or terminal, mainly unbranched raceme, 6-15 cm long with a rusty pubescent axis; buds velvety pubescent; bracts linear, 5-8 mm long; pedicel 4-8 mm long; bracteoles slightly shorter than bracts; flowers bisexual; receptacle tubular, 4-6 cm long; calyx with 5

free sepals, 2-3.5 cm long; petals 5, lanceolate, up to 9 cm × 4 cm including the 1.5 cm long claw, posterior petal yellowish and slightly larger; stamens 10, all fertile, about 7 cm long, opening by longitudinal slits; ovary superior, stipitate, glabrous, style about 4 cm long with a peltate stigma. Fruit a tardily dehiscent pod, up to 30 cm × 8 cm, glabrous, 6-10(-more)-seeded. Seed compressed, 10-15 mm in diameter. *B. winitii* is found in open, dry or mixed deciduous forest, rarely above 300 m altitude."

(https://uses.plantnet-project.org/en/Bauhinia_winitii_(PROSEA))

Medicinal uses

Stem and bark – diarrhea, dysentery, cough, relief headache (Ruangrungsi, 2004), and Kaewamatawong, 2008)

Pharmacological activity

Leaf - Phosphodiesterase (PDE) inhibitory activity, and PDE5 inhibitory activity (Temkitthawon *et al*., 2008, and Temkitthawon *et al*., 2011)

Stem and bark - α-glucosidase inhibitory activity, and antioxidant activity (Dejadisai & Pitakbut, 2015)

Plant identification

The preliminary method to identify the medicinal plants is the macroscopic and microscopic evaluations. They can determine the identity and purity of medicinal plant materials. The simplest observation is a visual by eye based on the appearance of morphological characteristics. However, only macroscopic evaluation is possibly insufficient. So, other methods like microscopic evaluation, chemical constituents or molecular identification are necessary coordinate.

Morphological characteristics

Morphological characteristics of medicinal plant materials are categorized according to sensory macroscopic and microscopic evaluations. They are the primary step for the quality and purity control in medicinal plants.

Macroscopic evaluation

Macroscopic evaluation is useful to identify, characterize, and be the reference for the plant materials (Kagithoju *et al*., 2013). The shape, size, color, odor, taste, texture, fracture characteristics of plant materials and appearance of the cut surface are observed by a naked eye, hand lens, and stereomicroscope.

Microscopic evaluation

Microscopic evaluation is used to characterize the structure of cells, and the inclusions in plant cells which represent both plant anatomy and histology (WHO, 2011).

Microscopic leaf measurement

Microscopic leaf measurement is quantitative determination of distinctive cells including stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area, trichome number, etc. These numbers are characteristics and can be used to differentiate plant species.

Trichomes are another characters of epidermal cells. A single epidermal cell is growing external as a long cylinder is called a unicellular trichome. While the epidermal cell is subdivided in several cells is call multicellular trichomes. The study of trichome characteristics in plants is useful. Previous studies reported that some *Bauhinia* species presented unicellular (*B. racemosa*) and multicellular (*B. malabarica*) trichomes by observing on both upper and lower epidermis (Albert & Sharma, 2013). Ruangrungsi, N. introduced trichome index as one of microscopic leaf measurement parameters (Roonyamarai *et al*., 2011, Pitakpawasutthi *et al*., 2018, and Intakhiao *et al*., 2019).

Palisade cells are phytosynthetic cells in the mesophyll of leaf, normally found in the upper epidermal surface. Palisade ratio is one of the parameters which show constant result although the geography is varied, while, other parameters are changed when plants are grown in diverse environments (Mukherjee, 2002 and Khan *et al*., 2014).

Stoma (plural called stomata) is a pair of guard cells which used to exchange gas, mainly CO_2 and O_2 for photosynthesis, and control the water in plants by opening and closing stomatal pore. Stomata are normally found on the leaves, but also on stems and other organs (Daszkowska-Golec & Szarejko, 2013). Because of their function as to control the water, stomata are normally found on the lower epidermis (Martin & Glover, 2007). Subsidiary cells are the epidermal cells which are surrounding the stomata. Stomata types are divided according to the subsidiary cells into four types i. e. a). anomocytic type, b.) anisocytic type, c.) diacytic type, and d.) paracytic type (WHO, 2011).

Table 2 Four types of stomata (WHO, 2011)

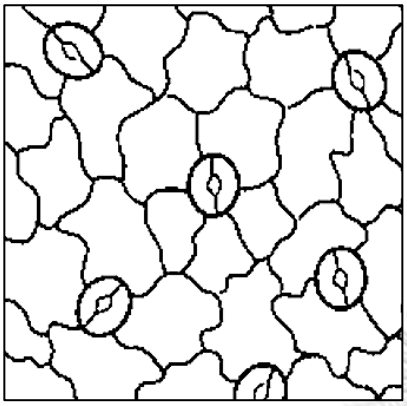
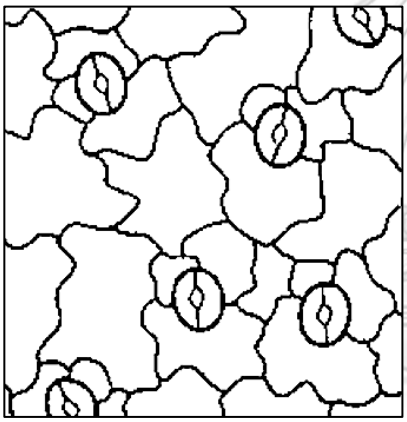
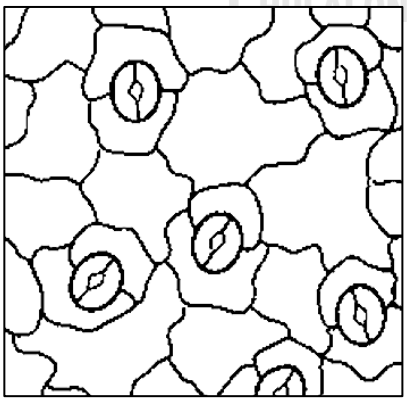
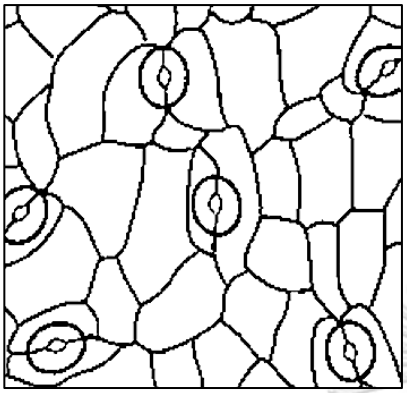
Types of stomata	The arrangement of the surrounding cells
a.) Anomocytic type 	The stoma is surrounded by a varying number of cells, generally not different from those of the epidermal cells.
b.) Anisocytic type 	The stoma is usually surrounded by three or four subsidiary cells, one of which is markedly smaller than the others.
c.) Diacytic type 	The stoma is accompanied by two subsidiary cells, the common wall of which is at right angles to the stoma.

Table 2 (cont.) Four types of stomata (WHO, 2011)

Types of stomata	The arrangement of the surrounding cells
d.) Paracytic type 	The stoma has two subsidiary cells, of which the long axes are parallel to the axis of the stoma.

B. racemosa leaves have been reported that paracytic stomata type was founded on both sides of epidermis (Khan *et al*., 2015). In addition, leaves of *B. ungulatoides* and *B. fotana* have been found paracytic stomata type on the lower epidermal surface (Lin *et al*., 2015).

Modh *et al*. (2011) observed microscopic leaf measurements of *B. variegata* in the parts of palisade ratio, stomatal index, vein-islet number, and vein termination number. They used 5% sodium hydroxide for clearing leaf and then peeled off the epidermis using Jeffrey's maceration fluid (The Pharmacopoeia of India, 1996). *B. purpurea* leaves were quantitated the stomatal number, stomatal index, vein-islet number, vein termination number, and palisade ratio followed Trease and Evans methods (Evans, 2009 and Pahwa *et al*., 2010)

Accordingly, macroscopic and microscopic evaluations are helpful in primary identification of plant materials, and also plays an important role in the standardization of herbal drug (Kagithoju *et al*., 2013).

Physico-chemical analysis

According to "Quality control methods for medicinal plants materials" that established by World Health Organization, the physico-chemical analysis which including loss on drying, total ash, acid insoluble ash, moisture content, and extractive value need to be done for standardization of plant materials (WHO, 2011).

Total ash evaluation determines the remaining plant material after incineration which consists of inorganic compounds or minerals in the form of oxides, sulfates, phosphates or silicates. Acid-insoluble ash evaluation determines the inorganic compounds that cannot formed salts with hydrochloric acid such as silica and aluminium.

Loss on drying and moisture content evaluation are the methods to quantitate the water in plant materials by dry heating and azeotropic distillation respectively. Water content affects microbial growth, or degradation of active ingredients in plant materials, therefore these parameters need to be control.

The evaluation of extractive matters represents the amount of active chemical compounds soluble in specified solvents. Volatile oil content is also a quality parameter determined by water-distillation method (WHO, 2011, and Pradhan *et al*., 2015).

These parameters are helpful for the assessment of quality and could be the reference to check plant materials from the adulteration (Modh *et al*., 2011).

The physico-chemical parameters of some species of *Bauhinia, B. purpurea* stem bark and leaf, *B. tomentosa* flower and leaf, and *B. variegata* leaf have been reported in India (Pahwa *et al*., 2010, Modh *et al*., 2011, Meshram *et al*., 2013, Khare *et al*., 2017, and Saravanapriya & Karpagam, 2018).

Chemical compounds in *Bauhinia* species

The main chemical compounds in plants of *Bauhinia* are usually encountered flavonoids especially kaempferol and quercetin derivatives (Pizzolatti *et al*., 2003). Flavonoids are the essential class of natural products. They are a group of secondary metabolites normally found in several parts of the plant as water soluble glycosides in the vacuoles of the epidermal cells (Harborne & Williams, 2000, Panche *et al*., 2016). They are the key of plant growth, plaques protection (Havsteen, 2002) and most of them are recognized as pigments of flowers in the angiosperm families (Panche *et al*., 2016). They have various pharmacological activities, antioxidant effects against many diseases such as cancer, alzheimer's disease (AD), atherosclerosis, and so on (Burak & Imen, 1999, Duthie & Crozier, 2000, Pietta, 2000, Schroeter *et al*., 2006, Ovando *et al*., 2009, Lee *et al*., 2009), anti-inflammatory, anti-mutagenic, antibacterial, antithrombotic, and anti-carcinogenic activities (Erlund, 2004, Middleton *et al*., 2000, Steinmetz & Potter, 1996, Metodiewa *et al*., 1997, Hayashi *et al*., 1988, Walker *et al*., 2000).

Quercetin and quercitrin

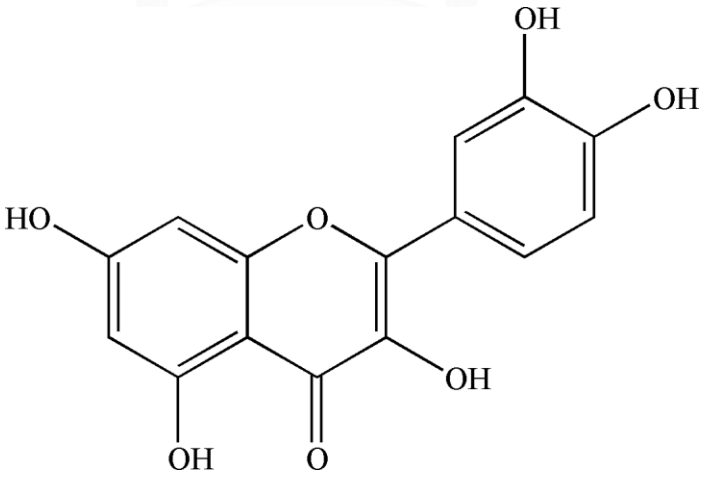


Figure 1 Structure of quercetin

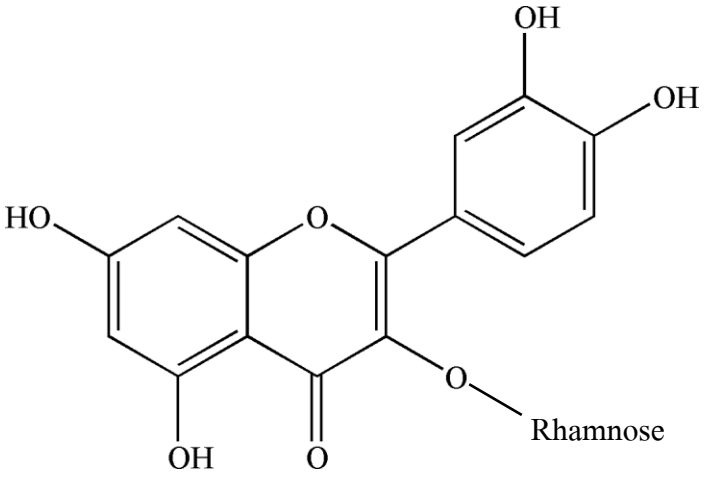


Figure 2 Structure of quercitrin

Quercetin is an aglycone flavonoids, and quercetin which is contain rhamnose is called quercitrin (quercetin-3-*O*-rhamnoside). Quercetin has diverse pharmacological activities, for example improving blood circulation, lowing blood pressure, anti-inflammatory, anti-allergy, antimicrobial, and antitumor activities (Hollman & Katan, 1999, Borska *et al*., 2012, and Seo *et al*., 2015). In addition, quercitrin also has UV protection, antitumor, antimicrobial, anti-aging and anti-allergy activities (Yang *et al*., 2012, Yin *et al*., 2013, and Menaa, 2014). Additionally, both quercetin and quercitrin were reported that they had strongly antioxidant activity in many studies (Yokozawa *et al*., 1997, Seyoum *et al*., 2006, and. Kaewamatawong *et al*., 2008). They are commonly found in fruits, vegetables, and grains like onions, apples, berries, broccoli, green pepper, buckwheat, parsley leaves, leek, cherry, and capers (Scallbert & Williamson, 2000, Baranowski *et al*., 2005, Bhagwat *et al*., 2014, and Seo *et al*., 2015).

Many species of *Bauhinia* consist of flavonoids. The extract of *B. tomentosa* flowers presented the higher yield of rutin content (4.6%) and small amount of quercetin content (Row and Viswanadham, 1954). Kaewamatawong *et al*. (2008), showed seven flavonols which were isolated from *B. malabarica*. Quercetin, guajivarin and quercitrin were isolated from the leaves of *B. ungulata* (Neto *et al*.,

2008). In addition, *B. racemosa* leaves were also found three catechins (epiafzelechin, epicatechin, and catechin) (Sashidhara *et al*., 2012). *B. reticulate, B. variegata, B. purpurea, B. championii, B. splendens* and *B. vahlii* have been reported that their leaves, roots, seeds and pods contained many flavonoids, such as quercitrin, kaempferol-3-galactoside, kaempferol-3-rhamnoside, rutin, apigenin, apigenin-7-*O*-glucoside, methoxylated, methyllenedioxyflavones, naringenin-4'-rhamnoglucoside, bausplendid, butein-4'-arabinosylgalactoside, and agatisflavone (Rabate, 1938, Rahman & Begum, 1966, Spikova & Hubik, 1992, Chen *et al*., 1984, Gupta *et al*., 1980, Laux *et al*., 1985, Bhartiya *et al*., 1979, Bhartiya & Gupta, 1981, and Kumar *et al*., 1990).

Quantitative analysis of flavonoids

Several methods were used for the analysis of flavonoids such as colorimetry, thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), mass spectrometry, and high performance liquid chromatography (HPLC) (Prince *et al*., 1998, Gliszczyriska-Swiglo *et al*., 2006, Roy *et al*., 2009, and Koh *et al*., 2009). Bhandari *et al*., (2007) determined the major flavonoids i. e. apigenin, quercetin, rutin, luteolin, and quercitrin in medicinal plants (*Bauhinia variegata*, *Bacopa monnieri*, *Centella asiatica*, *Ginkgo biloba*, *Lonicera japonica*, *Rosa bourboniana*, *Rosa brunonii*, and *Rosa damascene*) using RP-HPTLC method. *Eugenia uniflora* L. leaves were determined for rutin and quercetin contents using spectrophotometric method (Ramos *et al*., 2017). Flowers and flower buds of three various *Bauhinia* species have been analyzed for caffeic acid, vanillic acid, syringic acid, and kaempferol contents using HPTLC analysis. Kaempferol was found the highest value (1.53%) in both *B. purpurea* and *B. variegata* flower buds (Gupta *et al*., 2015). In addition, the aqueous extract of *B. variegata* leaves was shown quercetin content around 0.20 % W/W using HPTLC method (Kulkami & Garud, 2016).

High performance liquid chromatography (HPLC)

Both qualitative and quantitative analysis of quercetin and quercitrin were performed using HPLC analysis (Jurisic Grubesic *et al*., 2013). This method is sensitive, accurate, precise, rapid, and reproducible (Seal, 2016).

Normal phase and reversed phase HPLC are ordinarily used for qualification and quantification of flavonoids. For the normal phase, mobile phase is non-polar and stationary phase is polar, while, for the reversed phase, mobile phase is polar, and stationary phase is non-polar.

From previous studies, quercetin and quercitrin are usually analyzed by both normal phase and reversed phase HPLC. Two types of elution mode (isocratic and gradient elution) are reported for the mobile phase of HPLC analysis. According to various polarity of flavonoids, reversed phase HPLC is preferable (Baranowski *et al*., 2005). Gradient elution is suitable for finding acceptable separating conditions because of its more effective influence on the selectivity when compared with isocratic elution. Furthermore, the peak width from gradient elution is significantly narrower when compared with isocratic elution. However, gradient elution takes more times for the separation and the column should be clearly washed at least ten column volumes of initial eluent before the next run to eliminate the ghost peak, baseline noise, and other disturbances (Stadalius *et al.*, 1984, Berry & Shansky, 1984, Zhao & Carr, 2000, Neue & Mazzeo, 2001, Dolan, 2002, Boelens *et al*., 2004, and Schellinger & Carr, 2006).

Column temperature is the other part that important and need to be focused because temperature may affect the retention time, peak shape, and selectivity. High temperature will decrease the retention time, while, the separation of peak will not be resolved. The column which is set at suitable temperature will show the stable result, greater reproducibility and robust method (Dolan, 2002). The range of

temperature which is usually used in the research is up to 60 ℃, while only a few studies use high temperature (Wilson, 2000, Smith & Burgess, 1996, and Guillarme *et al*., 2004). However, the conditions of suitably setting of the column need to follow the instructions of each column.

Method validation

For the quantification of chemical constituents, method validation needs to be done for guarantee the precise, robust, and reproducible method. ICH Q2(R1) (2005) is the guideline recoqnized for validation of analytical procedures.

Specificity of the method can be determined *via* the purity tests of chromatographic peaks.

Accuracy, or another term is trueness, presents the closeness of tested amount and the reference value.

Repeatability and intermediate precision are done for the closeness of a series of measurements; repeatability is observed under the same conditions in a short interval of time, and intermediate precision is observed within-laboratories variations such as different days, different analysts, or different instrument, and so on.

Limit of detection and limit of quantification of the methods are the lowest amount of chemical compound that can be detected in the sample and the lowest amount of chemical compound that can be quantitated in the sample.

The range of an analytical method is the interval between the upper and lower concentration of result for demonstration that this concentration range is suitable for precision, accuracy, and linearity.

Robustness of the method represents the ability to resist small, but deliberate variations such as the variation of column temperature, flow rate, or absorbance wavelength. These parameters of method validation are to provide the reliability of the analytical method (ICH, 2005).

HPLC analysis of flavonoid contents such as quercetin, catechin, kaempferol, rutin, and quercitrin in several parts of *Bauhinia* species have been done and the validation for the precise and reliable methods has been reported (Bhandari *et al*., 2007, Xu *et al*., 2012, Peroza *et al*., 2013, Silveira *et al*., 2015, and Beber *et al*., 2018).

Molecular identification

Deoxyribonucleic acid or DNA is a molecule that contains the genetic markers used in the growth, development, functioning and reproduction of all living organisms and viruses. The structures of DNA consist of phosphate group, 5-carbon sugar and nitrogenous base. Two strands of DNA are coiled with each other to form a double helix. Nitrogenous base or nucleotide is a simpler monomer units containing cytosine (C), quinine (G), adenine (A) and thymine (T). Nitrogenous base in DNA has two types, (1) purine is a double-ringed bases and (2) pyrimidine is a single-ringed base. Purine consists of adenine and thymine, which is bonding with two hydrogen bonds and pyrimidine consists of cytosine guanine which is bonding with three hydrogen bonds, are pyrimidine. Double stranded structure or dsDNA is bonded together in a helical fashion by noncovalent bond of the intra-strand base stacking interactions (Figure 3). The two strands of DNA can separated into single-stranded DNA (ssDNA) using a process called melting. The melting temperature (T_m) is depended on the length of DNA molecule and its specific nucleotide sequence (Graur & Li, 2000, Albert *et al*., 2002, Russell, 2002, and Atawodi *et al*., 2010). Melting temperature is the temperature at which one half of DNA duplex will dissociate to

become ssDNA. The melting temperature can calculate from this formula: T_m = 2(A + T) + 4(G + C) (Freier *et al*., 1986).

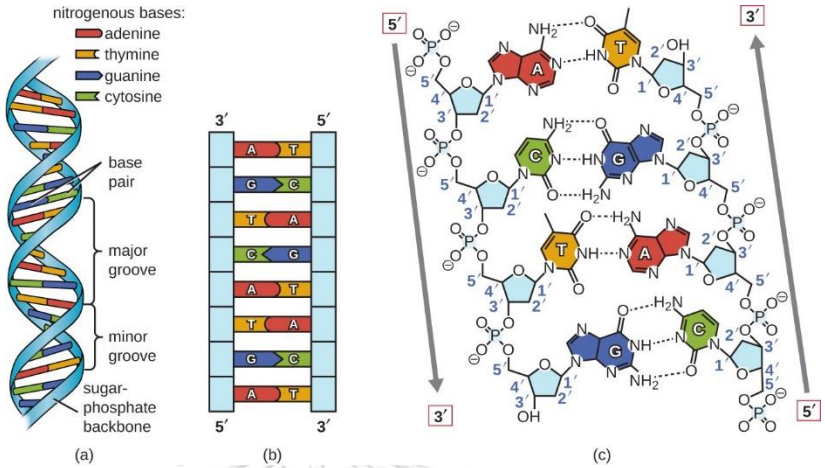


Figure 3 DNA structure

The molecular method or DNA-based techniques have been wildly used for herbal medicine technology and authentication of medicinal plant species. These methods are useful as medicinal plants are frequently substituted or adulterated with other species or their morphological or phytochemically indistinguishable because of their variable sources and chemical complexity. These techniques have been found to be useful and accurate for determination of genetic variation in plants. DNA is an extremely stable macromolecule that is not affected by external factors i. e. growth, environmental factors, storage conditions, harvest processes and can be recovered from fresh, dried, and even processed biological material (Joshi *et al*., 2004, Heubl, 2010).

DNA extraction

There are many alternative protocols for DNA extraction and the choice of protocol depends on the quality and quantity of DNA.

CTAB method

DNA isolation by CTAB method is one of the most popular protocols. The genetic DNA may be isolated by many different methods (Semagn *et al*., 2006). In general, all methods involve disruption and lysis of the start material followed by the removal of proteins and other contaminants and finally recovery of DNA. The small cut pieces of fresh young leaves are frozen rapidly in liquid nitrogen and crushed into fine powder then lysed with the cationic detergent The use of CTAB (cetyl trimethylammonium bromide), a cationic detergent, facilitates the separation of polysaccharides during purification while additives, such as polyvinylpyrrolidone, can aid in removing polyphenols. CTAB based extraction buffers are widely used when purifying DNA from plant tissues.

One option for purifying DNA using CTAB exploits that polysaccharides and DNA have different solubilities in CTAB depending on the concentration of sodium chloride. At higher salt concentrations, polysaccharides are insoluble, while at lower concentrations DNA is insoluble. Consequently, by adjusting salt concentration in lysates containing CTAB, polysaccharides and DNA can be differentially precipitated. The DNA complex is solubilized by raising the salt concentration and precipitated with ethanol or isopropanol.

CTAB-based protocols tend to work very well, but one significant disadvantage is that chloroform extractions are routinely used to separate organic soluble molecules from the DNA (https://opsdiagnostics.com/notes/protocols /ctab_protocol_for_plants.htm).

DNA extraction kit

Another widely DNA isolation method used is the commercial instant DNA extraction kit. The technology makes use of spin columns, which contain a silica-gel-based membrane that binds the DNA. The DNA while bound to the membrane can

be washed and cleaned from contaminants and then eluted from the column (membrane) using water. This method is relatively simple, save time, do not contain harmful chemicals such as phenol or chloroform, involves minimal handling, higher percent yields and the high quality of DNA but this method is expensive.

DNA concentration and purity

DNA quantity and purity is checked by spectrophotometric analysis and agarose gel electrophoresis. According to the absorbance of DNA and protein at 260 nm and 280 nm respectively, the purity of DNA sample is calculated from OD_{260}/OD_{280}, and the accepted ratio ranged from 1.8 – 2.0.

Agarose gel electrophoresis is a method to separate DNA or RNA molecules by size. This is achieved by moving negative charge nucleic acid molecules through agarose matrix with an electric field electrophoresis. Shorter molecules move faster and migrate faster than longer ones. The DNA concentration of a sample can be roughly calculated by comparison of the sample band intensity with that of a molecular weight marker band whose DNA content is known (Weising *et al*., 2005).

Molecular identification

There are two types of DNA-based molecular identification which are used to evaluate DNA polymorphism for authentication of plant taxa (Kaplan *et al*., 2004, Pereira *et al*., 2008, Sucher & Carles, 2008, and Shaw *et al*., 2009).

1. Hybridization-based methods or non-PCR-based methods

2. PCR-based methods

Hybridization-based methods (non-PCR-based methods)

DNA hybridization is a process in which two single stranded DNA fragments anneal into a double-stranded nucleic acid. Restriction Fragment Length Polymorphism (RFLP) implies that a single restriction enzyme produces fragments of

different lengths from the DNA marker of different strains of a species or from different related species. This technique is time-consuming, labor-intensive, and requires a large quantity of high amounts of good quality or un-degraded DNA.

PCR-based methods

Mullis *et al*. (1987) invented the polymerase chain reaction (PCR) method which is played the important role of molecular biology. PCR or *in vitro* enzymetic gene amplification is the technique that increases DNA fragment. It is quick and easy method to characterize, analyze, and generate unlimited copies of any DNA or RNA pieces. The components of PCR reaction consist of DNA template, thermostable DNA polymerase, deoxyribonucleotide triphosphates (dNTPs), oligonucleotide primers, suitable buffers, and magnesium (Mn^{2+}) or manganese ions (Williams *et al*., 1990). The selectivity of the reaction is determined by the choice of the primer(s). Primers are single-stranded pieces of DNA (oligonucleotides) with sequence complementarity to template sequences flanking the targeted region. To allow for exponential amplification, the primers must anneal in opposite directions, so that their 3'-ends face the target amplicon.

The principle of the cycling reaction consists of three steps. In the first step of the first cycle, the original template DNA is made single-stranded by raising the temperature to about 94ºC (denaturing step). In the second step, lowering the temperature to about 35 to 65ºC (depending on primer sequence and experimental strategy) results in primers annealing to their target sequences on the template DNA (annealing step). For the third step, a temperature is chosen at which the activity of the thermostable polymerase is optimal; i. e., usually 65 to 72ºC (elongation step). In the second cycle, the two resulting double-stranded DNAs are again denatured, and both the original strand and the product strand now act as a template. In a typical

PCR assay, three temperature-controlled steps are repeated in a series of 25 to 50 cycles (Figure 4).

The contamination is the problem which researcher needs to aware. The place needs to be cleaned before preparing the PCR product. The contaminations such as dust, skin, hair which are make the many copies of unrelated DNA target and get the error results (Weising *et al*., 2005).

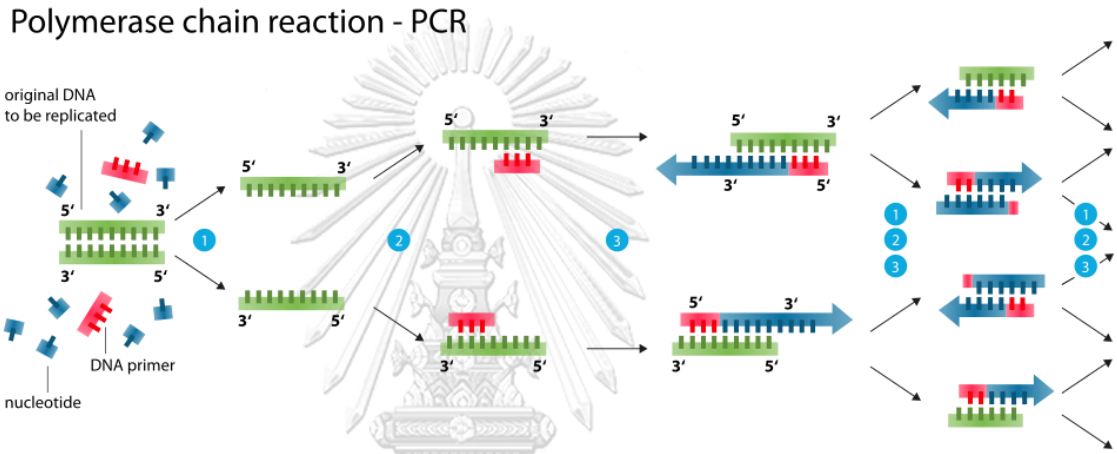


Figure 4 Polymerase Chain Reaction (PCR)

PCR-based markers involve amplification of particular DNA loci, with the help of specific or arbitrary oligonucleotide primers and a thermostable DNA polymerase enzyme. The major advantages of PCR techniques are that mainly only a small amount of DNA required, arbitrary oligonucleotide primers are no need to know the prior sequence information such as Random Amplification of Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR), and many genetic markers can be generated within a short time. These methods can be grouped into 1. Arbitrary or semi-arbitrary primed PCR techniques that need no prior sequence information i. e. Arbitrarily-primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSR) and 2.

Site-targeted PCR techniques that are developed from known DNA sequences i. e. Cleaved Amplified Polymorphic Sequence (CAPS), Simple Sequence Repeats (SSR), Sequence Characterized Amplified Region (SCAR), Sequence-tagged Site (STS) (Heubl *et al*., 2010),

Inter simple sequence repeat (ISSR) markers

Inter simple sequence repeat (ISSR) technique is a PCR based technique reported by Zietkiewicz *et al*. (1994), which involves amplification of DNA segments between two identical microsatellite repeat regions 'oriented in opposite direction using primers designed from microsatellite core regions. The technique uses microsatellite primers, usually 16 – 25 bp long, of di-nucleotide, tri-nucleotide, tetra-nucleotide or penta-nucleotide repeats to target multiple genomic loci. The primers can be either unanchored or more usually anchored at 3' to 5' end with 1 to 4 degenerate bases extend into the flanking sequences.

ISSR markers have many advantages over other marker systems. ISSR technique is simple, quick and less costly like the RAPD technique. ISSR markers have high reproducibility than RAPD primers due to the longer primer length. Development of ISSR markers does not need prior knowledge of the genome to be analyzed; hence, it can be used universally for plant genome analysis.

Phylogenetic tree construction methods

Phylogenetic tree construction

Phylogenetic is one branch of systematic taxonomy and it is a field of biology concerning with identified and understanding the evolutionary relationships among many different kinds of organism. Phylogenies are constructed using all kinds of data such as morphological characteristic data, molecular data and geographical data (Swofford *et al*., 1996). A phylogenetic tree is a tree diagram presenting evolutionary relationships among various biological species that are believed to have common

ancestor. Construction of a phylogenetic tree is controlled by computer operations. Many phylogenetic computer programs such as PHYLIP, NTSYS and Free Tree software are commonly easily to conduct a phylogenetic (Felsenstein, 1981, Rohlf, 1993, and Hampl *et al*., 2001).

Some commonly employed molecular marker methods such as RAPD, ISSR, and AFLP generate a fingerprinting pattern obtained from a particular DNA material. Polymorphisms between the fingerprinting patterns of individuals are scored as presence (1) or absence (0) of particular sized fragments.

Similarity index

As the first step of similarity analysis, multilocus band patterns are applied to various procedures to quantify a pairwise similarity of genotypes represented in the different fingerprinting patterns. Commonly, a similarity index is calculated from band sharing data of each pair of the fingerprints (Weising *et al*., 2005).

There are many similarity coefficients used in molecular marker analysis. The examples of the similarity coefficients are described as follows;

Jaccard's coefficient: J = a/(a+b+c)

Where; a = the number of 1-1 matches

b = the number of 1-0 matches

c = the number of 0-1 matches

(1 = band present, 0 = band absent)

Nei and Li's coefficient: N = 2a/(a+b)(a+c)

Where; a = the number of 1-1 matches

b = the number of 1-0 matches

c = the number of 0-1 matches

(1 = band present, 0 = band absent)

In the formulas of Jaccard's coefficient and Nei and Li's coefficient are derived from comparing the number of bands shared between individuals or population. One of the most commonly used similarity indices is Jaccard's coefficient which avoid including shared abscents band in the calculation of similarity index was chosen to use in this thesis.

Tree construction using distance matrix method

The distance matrix uses evaluation distances in a matrix from between all pairs of species in a data set to construct a phylogenetic tree. One widely used method is the Unweighted Pair Group Method of the Arithmetic Average (UPGMA) method, used distance method for phylogenetic tree construction.

UPGMA was originally developed for constructing taxonomic phenograms which are trees that reflect the phenotypic similarities between operational taxonomic units (OTUs) (Sneath & Sokal, 1973). This method involves clustering of closely species. At each stage of clustering, tree branches are being built and branch lengths are calculated. UPGMA assumes a constant evolutionary rate thus, the two species in cluster are given the same branch length from the node. It is a simple and fast method of tree construction.

Bootstrap analysis

A bootstrap analysis is a simple and effective computer based technique for assessing the accuracy of almost any statistical estimation (Efron & Tibshirani, 1993). It is one of tree evaluation method with provide measure of support for each branch in phylogenetic tree (Felsenstein, 1985). A bootstrap data matrix is created by randomly selecting a column from the original matrix with replacement. Pseudoreplicate

datasets are generated by randomly sampling the original character matrix to create new matrices of the same as the original. The whole process is repeated independently a large number of times (approximately 100-1000 replications). A bootstrap value is count (or percentage) of how often each branch presents in the resampled trees. These bootstrap confidence value can be considered as a reasonable assessment of errors for the estimated tree (Piteekan, 2009).

CHAPTER III

MATERIALS AND METHODOLOGY

Chemicals

2-Mercaptoethanol	Sigma-Aldrich Company Co., St. Louis, MO, U.S.A.
Agarose	Ultrapure TM, Life technologies, U.S.A.
Boric acid	Merck, Daemtadt, Germany
Chloral hydrate	Tokyo Chemical Industry Co., LTD, Tokyo, Japan
Chloroform	Merck, Daemtadt, Germany
Cyltrimethylammonium bromide	Sigma-Aldrich Company Co., St. Louis, MO, U.S.A.
Ethanol (absolute)	Merck, Daemtadt, Germany
Ethanol (AR grade)	RCI Labscan Limited, Bangkok, Thailand
Ethidium bromine	Sigma-Aldrich Company Co., St. Louis, MO, U.S.A.
Ethyl acetate (AR grade)	RCI Labscan Limited, Bangkok, Thailand
Ethylene diamine tetaacetic acid	Merck, Daemtadt, Germany
Formic acid 98-100 %	RCI Labscan Limited, Bangkok, Thailand
Haiter (6% sodium hypochlorite)	Kao Corp., Japan
Hydrochloric acid	RCI Labscan Limited, Bangkok, Thailand

Isoamyl alcohol	Sigma-Aldrich Company Co., St. Louis, MO, U.S.A.
Loading dye	Thermo Fisher Scientific Inc., USA
Methanol (AR grade)	RCI Labscan Limited, Bangkok, Thailand
Methanol (HPLC grade)	RCI Labscan Limited, Bangkok, Thailand
Phosphoric acid	RCI Labscan Limited, Bangkok, Thailand
Polyvinylpyrrolidone	Sigma-Aldrich Company Co., St. Louis, MO, U.S.A.
Toluene	RCI Labscan Limited, Bangkok, Thailand
Tris (hydroxymethyl)-aminomethane	Fluka, Biochemika, Germany

Materials

DNA ladder, 100 bp	Promega, U.S.A.
DNA ladder, 1kb	Thermo Fisher Scientific Inc., USA
DNeasy® plant mini kit	QIAGEN, USA
dNTPs	Thermo Fisher Scientific Inc., USA
Nylon membrane filters (0.45 µm)	RCI Labscan Limited, Bangkok, Thailand
Primer	Eurofins MWG Operon Inc., USA
PTFE membrane syringe filters (0.45 µm)	ANPEL Laboratory Technology (Shanghai), Shanghai, China
Silica gel 60 GF254	Merck, Germany
Taq DNA polymerase	Thermo Fisher Scientific Inc., USA

Instruments

Centrifuge (Model: Sigma 1-14)	Sartorious, Gottingen, Germany
Centrifuge	Labnet international, Inc., U.S.A.
Digital balance (Model: Sl-234)	Denver Instrument, New York, USA
Gel electrophoresis apparatus and power supply	Labnet international, Inc., U.S.A.
Inertsil® ODS-3 5µm C18 column (4.6 x 250 mm)	GL Sciences, Tokyo, Japan
NanoDrop Spectrophotometer ND-1000	Nanodrop Technologies, Inc., Wilmington, DE, U.S.A.
Proflex PCR system	Thermo Fisher Scientific Inc., USA
ReproSil®-Pur ODS-3 C18 guard column	Dr. Maisch GmbH, Ammerbuch, Germany
Rotary evaporator (Model: R-210)	Buchi, Flawil, Switzerland
Sequi-GEN GT Sequencing and power supply cycler	Bio-Rad, U.S.A.
Shimadzu HPLC LC-20A system photo-diode array detector (SPD-M20A) Shimadzu LC Solution software	Shimadzu, Japan
Ultra-pure water purification (Model: NW20VF)	Heal Force, China
Ultraviolet viewing cabinet (Model: CC-80)	CAMAG, Muttenz, Switzerland
Waterbath GFL1083	GFL Gesellschaft Fur Labortechnik mbH, Germany
Zeiss Axio Imager. A2 microscope	Zeiss, Germany

Part I Preliminary quantitative analysis of quercetin and quercitrin in twenty *Bauhinia* species using RP-HPLC method

Sample collection

The mature leaves of selected *Bauhinia* species were collected throughout Thailand and dried at 45ºC in hot air oven. All plants materials were authenticated by expert (N. Ruangrungsi) and herbarium comparison at Forest Herbarium-BKF. Voucher specimens were deposited at college of Public Health Sciences, Chulalongkorn University. Crude drugs were ground into fine powders after removal of any foreign matters.

Twenty *Bauhinia* species were used in this study i. e. *Bauhinia acuminata, Bauhinia aurefolia, Bauhinia bracteata, Bauhinia galpinii, Bauhinia integrifolia, Bauhinia lakhonensis, Bauhinia malabarica, Bauhinia ornate, Bauhinia pottisii, Bauhinia pulla, Bauhinia purpurea, Bauhinia racemose, Bauhinia saccocalyx, Bauhinia siamensis, Bauhinia scandens, Bauhinia sirindhorniae, Bauhinia strychnifolia, Bauhinia tomentosa, Bauhinia variegata,* and *Bauhinia winitii* (Table 3).

Table 3 Twenty *Bauhinia* species in three different collecting locations

No.	Species	Locality		
1	*Bauhinia acuminata*	Bangkok 1	Bangkok 2	Chiang Rai
2	*B. aureifolia*	Bangkok 1	Bangkok 2	Trang
3	*B. bracteata*	Bangkok 1	Bangkok 2	Kanchanaburi
4	*B. galpinii*	Bangkok	Trang	Chiang Rai
5	*B. integrifolia*	Nonthaburi	Bangkok	Chiang Rai
6	*B. lakhonensis*	Bangkok	Chiang Rai	Nong Khai
7	*B. malabarica*	Bangkok	Chonburi	Pathum Thani
8	*B. ornata*	Chiang Rai	Lampang	Kanchanaburi
9	*B. pottsii*	Bangkok	Chiang Rai	Satun

Table 3 (cont.) Twenty *Bauhinia* species in three different collecting locations

No.	Species	Locality		
10	*B. pulla*	Nakhon Sawan	Singburi	Chai Nat
11	*B. purpurea*	Bangkok	Chiang Rai	Lampang
12	*B. racemosa*	Bangkok 1	Bangkok 2	Singburi
13	*B. saccocalyx*	Bangkok	Ratchaburi	Rayong
14	*B. scandens*	Bangkok	Nonthaburi	Kanchanaburi
15	*B. siamensis*	Phitsanulok 1	Phitsanulok 2	Phitsanulok 3
16	*B. sirindhorniae*	Bangkok	Nonthaburi	Kanchanaburi
17	*B. strychnifolia*	Bangkok	Pathum Thani	Chiang Rai
18	*B. tomentosa*	Bangkok 1	Bangkok 2	Pathum Thani
19	*B. variegata*	Lampang 1	Lampang 2	Lampang 3
20	*B. winitii*	Bangkok 1	Bangkok 2	Kanchanaburi

Sample extraction

Five grams of each dried leaf powder of twenty *Bauhinia* species were exhaustively extracted with 95% ethanol using a Soxhlet apparatus. The ethanolic extract was filtered through filter-paper Whatman No. 4 and evaporated to dryness by rotary evaporator. The extract yields were weighed and stored at -20°C to avoid the possibility of degradation of active compounds.

Chromatographic conditions

Solvent A was 0.5% phosphoric acid and solvent B was methanol, filtered through 0.45 µm nylon membrane filters and degasses before analysis. Isocratic mode was set as 50% solvent B for 30 minutes, using flow rate 1.0 ml/min, column temperature was set at 35°C. Each *Bauhinia* ethanolic extract and standards

(quercetin and quercitrin) were prepared in methanol, filtered through 0.45 µm PTFE membrane syringe filter and injected 5 µl. Peak areas were observed under 255 nm and calculated using linear equations from calibration range of quercetin and quercitrin (20, 40, 60, 80 and 100 µg/ml). Each extract was analyzed in triplicate.

Method validation

According to ICH guideline: calibration range, specificity, accuracy, repeatability, intermediate precision, limit of detection, limit of quantitation and robustness were validated for analytical method. *B. malabarica* leaf extract was used as sample matrix in this part.

Calibration range

The calibration range was performed by plotting peak areas that obtained from HPLC analysis *versus* concentrations of standard. The stock solutions of quercetin and quercitrin were dissolved in methanol and prepared 20, 40, 60, 80 and 100 µg/ml for evaluation of the calibration range. The calibration curves of both standards were fitted by linear regression.

Specificity

The specificity was evaluated by peak purity test. The peak purity index of the analyte was processed with Shimadzu LC Solution software. It was determined by comparing the spectra from the upslope, apex, and downslope of the chromatographic peak.

Accuracy

The accuracy of each sample was tested by recovery method. Three different levels of standard solutions (20, 40, 60 µg/ml) were spiked into the extract. The spiked and un-spiked samples were evaluated under the same condition in triplicate. The accuracy was calculated as percent recovery by using following formula:

$$\% \text{ recovery} = \left(\frac{C1}{C2 + C3} \right) \times 100$$

Where: C1 = the amount of compound found in spiked sample

C2 = the amount of compound found in un-spiked sample

C3 = the amount of standard added to sample

Precision

The precision was determined by repeatability (intra-day) and intermediate precision (inter-day) studies. The method was performed by analyzing three level concentrations of sample solution in triplicate on the same day for repeatability and in three different days for intermediate precision. The precision was calculated in term of percent relative standard deviation (% RSD) of compound content by following formula:

$$\% \text{ RSD} = \frac{SD}{Mean} \times 100$$

Where: SD = the standard deviation of each measurement

Limit of detection (LOD)

Limit of detection which is the lowest concentration that can be detected but not accurately quantitated was determined from the calibration curve using following formula:

$$LOD = \frac{3.3 \times SD_{res}}{S}$$

Where: SD_{res} = the residual standard deviation of regression line

S = the slope of regression line

Limit of quantitation (LOQ)

Limit of quantitation which is the lowest concentration that can be accurately quantitated from the calibration curve using following formula:

$$LOQ = \frac{10 \times SD_{res}}{S}$$

Where: SD_{res} = the residual standard deviation of regression line

S = the slope of regression line

Robustness

The robustness was determined for variations in flow rate, column temperature and wavelength. The robustness was calculated in term of % RSD of peak area.

Part II Microscopic leaf characteristics of twenty *Bauhinia* species

Twenty *Bauhinia* spp. fresh mature leaves were macroscopically and microscopically observed. The middle part of fresh mature leaf blade was cut into 0.5 x 0.5 cm, soaked in the solution of Haiter (containing 6 % sodium hypochlorite) : water (1 : 1) until clear, washed and boiled in chloral hydrate solution (4 g in 1 ml of water) until translucent. The upper and lower epidermis were examined under microscope and the stomata, palisade, trichome, and epidermal cell numbers were recorded. Parisade ratio was calculated from the number of palisade cells under four epidermal cells and divided by four. The average of each parameter was calculated from 30 fields of 3 locations.

Trichome index and stomatal index were obtained from following formula:

$$\text{Trichome or Stomata index} = (S \text{ or } T \times 100)/(S+E+T)$$

Whereas S = number of stomata per 1 mm^2

E = number of epidermal cells per 1 mm^2

T= number of trichomes and cicatrices per 1 mm^2

Epidermal cell areas were determined on the upper epidermis by counting the number of epidermal cells, trichomes and cicatrices, and stomata and calculating as follows:

$$\text{Epidermal cell area} = (1/(S+E+T)) \times 10^6$$

Whereas S = stomatal number per 1 mm^2

E = number of epidermal cells per 1 mm^2

T = trichome and cicatrix number per 1 mm^2

The midrib of fresh mature leaf of each *Bauhinia* species was thinly cross-sectioned with blade by hand and observed the anatomy under microscope with the magnification 10X to 40X.

Part III Pharmacognostic specification of *B. malabarica* leaf and quercetin and quercitrin contents by RP-HPLC analysis

Sample collection

Fifteen samples of *B. malabarica* mature leaves were collected throughout Thailand (Bangkok, Chiang Rai, Nakhon Pathom, Trang, Ang Thong, Uthaithani, Nakhon Sawan, Suphanburi, Phuket, and Chainat). All plant materials were authenticated by expert (N. Ruangrungsi) and herbarium comparison at Forest Herbarium-BKF. Voucher

specimens were deposited at college of Public Health Sciences, Chulalongkorn University. The leaves were dried and ground into fine powders after removal of any foreign matters.

Sample extraction for RP-HPLC analysis

Five grams of *B. malabarica* fine powders were exhaustively extracted with 95% ethanol using Soxhlet apparatus, evaporated to dryness and stored in refrigerator for quantification of quercetin and quercitrin using reversed phase high performance liquid chromatography.

Chromatographic conditions

Solvent A was 0.5% phosphoric acid and solvent B was methanol, filtered through 0.45 µm nylon membrane filters and degasses before analysis. Isocratic mode was set as 50% solvent B for 30 minutes, using flow rate 1.0 ml/min, column temperature was set at 35°C. Each *B. malabarica* ethanolic extract and standards (quercetin and quercitrin) were prepared in methanol, filtered through 0.45 µm PTFE membrane syringe filter and injected 5 µl. Peak areas were observed under 255 nm and calculated using linear equations from calibration range of quercetin and quercitrin (20, 40, 60, 80 and 100 µg/ml). Each extract was analyzed in triplicate.

Physico-chemical characteristics

Fine powder of *B. malabarica* dried leaves were determined for the contents of water, loss on drying, ashes, extractive matters and volatile oil according to “Quality control methods for medicinal plants materials” (WHO, 2011). All samples were analyzed in triplicate per location. Grand mean and pooled standard deviation were calculated.

Determination of water content (azeotropic distillation method)

The accurate 50 g of dried powdered *B. malabarica* leaves were transferred to round bottom flask, 200 ml of water-saturated toluene were added and boiled until the water was completely distilled. The water and toluene layer were separated then the volume of water was recorded and calculated in the percentage.

Determination of loss on drying (dry heat method)

The accurate 3 g of dried powdered *B. malabarica* were transferred to a pre-weighed crucible and then dried at 105°C in a hot air oven until constant weight. The crucible was allowed to cool at room temperature in desiccator, weighed and calculated the loss of weight in percentage.

Determination of total ash and acid insoluble ash

The crucible from the aforementioned loss on drying was burnt on a cylinder gas stove until there was no smoke, then incinerated at 500°C in ashing furnaces until white ash was obtained. The crucible was cooled in a desiccator and weighed. The crucible that containing the total ash was added with 25.0 ml of hydrochloric acid (70 g/l), covered with a watch-glass and boiled gently for 5 minutes, the insoluble matter was filtered through an ashless filter-paper Whatman No. 40, the filter-paper was transferred into the same crucible, dried on a hot plate and incinerated at 500°C until ash remaining. The residue was cooled in a desiccator and weighed. The content of total and acid insoluble ashes were calculated in percentage.

Determination of ethanol soluble extractive value

The accurate 5 g of dried powdered *B. malabarica* leaves were macerated with 70 ml of 95% ethanol in closed conical flask for 6 hours under shaking, and standing for 18 hours. After that, the extract was filtered through filter-paper Whatman No. 4, the marc was washed and the filtrate was adjusted to 100 ml with

95% ethanol. Twenty millilitres of the filtrate were transferred to pre-weighed small beaker and evaporated to dryness on a water-bath. Finally, the extract was dried at 105ºC for 6 hours, cooled in a desiccator, weighed and calculated in percentage.

Determination of water soluble extractive value

The accurate 5 g of dried powdered *B. malabarica* leaves were macerated with 70 ml of water in closed conical flask on for 6 hours under shaking, and standing for 18 hours. After that, the extract was filtered through filter-paper Whatman No. 4, the marc was washed and the filtrate was adjusted to 100 ml with water. Twenty millilitres of the filtrate were transferred to pre-weighed small beaker and evaporated to dryness on a water-bath. Finally, the extract was dried at 105ºC for 6 hours, cooled in a desiccator, weighed and calculated in percentage.

Determination of volatile oil content

The accurate 100 g of dried powdered *B. malabarica* leaves were transferred to round bottom flask, 600 ml of water were added and the flask was combined to a Clevenger apparatus for hydro-distillation. After the volatile oil was completely distilled, the volatile oil and water were separated then the volume of volatile oil was recorded and calculated in percentage.

Thin layer chromatographic fingerprint

Three microliters of crude ethanolic extract solution (5 mg/ml) were spotted onto the silica gel GF_{254} TLC plate. TLC plate was developed in saturated TLC chamber with ethyl acetate : formic acid (10 : 1). After development, the plate was observed under day light, UV 254 nm, UV 366 nm and dipped in 1% aluminium chloride and p-anisaldihyde reagents.

Macroscopic and microscopic evaluations of *B. malabarica* leaves

For the macroscopic evaluation of *B. malabarica* fresh and dried mature leaves were observed based on their morphological characters such as shape, size, color, texture, and other characters using naked eye.

For the microscopic evaluations of *B. malabarica*, the midrib of fresh mature leaf was thinly cross-sectioned with blade by hand and observed the anatomical character under microscope with the magnification of 10X to 40X.

Fine powders of *B. malabarica* leaves were mixed with a few drops of water on the slide and observed the histological character under microscope with the magnification of 10X to 40X.

Part IV Molecular identification

DNA extraction and electrophoresis

Genomic DNA was extracted from the fresh young leaf tissues following a CTAB method as described previously by Doyle and Doyle (1987) with a minor modification. One gram of cleaned leaf sample was rapidly ground in liquid nitrogen to a fine powder with mortar and pestle followed by transferred that powder into microcentrifuge tube with 500 µl of CTAB extraction buffer (2% W/V) CTAB, 100mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% (W/V) β-mercaptoethanol). The mixture was incubated at 65ºC for 1 hour then centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was transferred to a new microcentrifuge tube, added 500 µl of chloroform and centrifuged at 10,000 rpm for 10 minutes. The same phase was transferred to a new microcentrifuge tube, added 500 µl of chloroform/isoamyl alcohol (24 : 1) and centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was transferred again to a new microcentrifuge tube, added 1:10 volume of 3M Sodium acetate pH 5.0 followed by added 2 volume of cold absolute ethanol (-20ºC), inverted tube and kept at -20ºC for 1 hour. It was centrifuged at 10,000 rpm

for 10 minutes, the supernatant was gently discarded. DNA pellet was washed using 1 ml of cold 70% ethanol (4ºC) and centrifuged at 10,000 rpm for 10 minutes. The supernatant was smoothly discarded. DNA pellet was dried at room temperature, dissolved in 200 µl of TE buffer and stored at -20ºC. The quantity and quality of genomic DNA were determined by spectrophotometry and 1% agarose gel stained with 2 mg/ml of ethidium bromide, respectively. Fragment size was also estimated using GeneRuler 1 kb and 100 bp DNA ladder.

ISSR amplification

ISSR amplification was performed as stated by Bornet and Branchard (2001); forty-five primers were screened with some *Bauhinia* species. After screening, twenty ISSR primers were revealed the bands and were screened again with twenty two samples (twenty *Bauhinia* species and two out groups) (Table 4). PCR amplifications were performed in 20 µl reaction mixtures; containing a final concentration about 50 ng of DNA, 2.5 mM of $MgCl_2$, 1X of PCR buffer, 0.1 µM of primer, 0.1 µM of each dNTP and 0.5 unit of *Taq* DNA polymerase. ISSR amplifications were performed using a Proflex PCR system thermocycler with an initial denaturation step for 5 minutes at 95ºC, followed by 45 cycles of denaturation step 45 seconds at 95ºC, annealing step 45 seconds at annealing temperature of each primer, extension step 1 minutes at 72ºC and completed with a final extension for 5 minutes at 72ºC. ISSR-PCR product was visualized on 1% agarose gel stained with 2 mg/ml of ethidium bromide. Fragment size was also estimated using GeneRuler 1 kb and 100 bp DNA ladder.

Table 4 Twenty ISSR primers

Primer	Nucleotide sequence (5’ to 3’)	Annealing temperature (°C)
ISSR-01	AGAGAGAGAGAGAGAGT	45
ISSR-03	GAGAGAGAGAGAGAGAT	45
ISSR-05	TCTCTCTCTCTCTCTCC	49

Table 4 (cont.) Twenty ISSR primers

Primer	Nucleotide sequence (5' to 3')	Annealing temperature (°C)
ISSR-07	TGTGTGTGTGTGTGTGA	45
ISSR-08	CTCTCTCTCTCTCTCTC	47
ISSR-09	ACACACACACACACACT	45
ISSR-11	agagagagagagagagYt	43
ISSR-12	agagagagagagagagYc	47
ISSR-13	agagagagagagagagYa	45
ISSR-16	ctctctctctctctctrg	47
ISSR-28	gggtggggtggggtg	47
ISSR-29	hbhagagagagagagagag	37
ISSR-31	AGAGAGAGAGAGAGT	41
ISSR-32	AGAGAGAGAGAGAGC	41
ISSR-33	ATATATATATATATG	47
ISSR-36	CTCTCTCTCTCTCTG	46
ISSR-38	GTGTGTGTGTGTGTC	41
ISSR-41	TTCTTCTTCTTCTTC	35
ISSR-44	GACAGACAGACAGACA	43
ISSR-45	GGAAGGAAGGAAGGAA	43

Data analysis

For the genetic similarity analysis, ISSR fragments were visually scored as present (1) or absent (0) to create a binary data set. The data were entered into a binary data matrix as discrete variables. Jaccard's coefficient of similarity were calculated for all pair-wise comparisons among the *Bauhinia* species as follows: Jaccard = $N_{AB}/(N_{AB}+N_A+N_B)$, where N_{AB} is the number of fragments shared by two

cultivars (A and B), N_A represents amplified fragments in cultivar A and N_B represents fragments in cultivar B (Jaccard, 1908). A dendrogram was constructed using the Unweighted Pair Group Method of the Arithmetic Average (UPGMA), clustering by FreeTree software (Hampl *et al*., 2001). To evaluate the strength of the resulting branching, bootstrap probabilities were calculated using 1,000 bootstrap resampling data by FreeTree software.

CHAPTER IV

RESULTS

Part I Preliminary quantitative analysis of quercetin and quercitrin in twenty *Bauhinia* species using RP-HPLC method

Mature leaves of twenty *Bauhinia* species were collected from three locations throughout Thailand (Table 3). Each species was exhaustively extracted with 95% ethanol using soxhlet method. The yields of ethanolic extracts of 20 species were presented in Table 5. The highest yield was found in *B. lakhonensis* (36.128 g/100 g dried crude drug) and the lowest yield was found in *B. variegata* (16.063 g/100 g dried crude drug). The quantification of quercetin and quercitrin of each species was done in triplicate and presented as mean ± SD (Table 6). The highest contents of quercetin and quercitrin were found in *B. malabarica* as 0.1918 ± 0.0006 and 0.3740 ± 0.0002 g/100 g of dried crude drug. Quercetin was not found in *B. pulla*, *B. racemosa*, *B. saccocalyx*, and *B. tomentosa*. Quercetin was not found in *B. bracteata*, and *B. variegata*.

Table 5 The yields of ethanolic extracts of twenty *Bauhinia* species

No.	Species	Yield of ethanolic extract (g/100 g dried crude drug)
1	*Bauhinia acuminata*	26.127
2	*B. aureifolia*	18.763
3	*B. bracteata*	17.190
4	*B. galpinii*	23.898
5	*B. integrifolia*	24.501
6	*B. lakhonensis*	36.128
7	*B. malabarica*	26.199
8	*B. ornata*	17.527
9	*B. pottsii*	19.159

Table 5 (cont.) The yields of ethanolic extracts of twenty *Bauhinia* species

No.	Species	Yield of ethanolic extract (g/100 g dried crude drug)
10	*B. pulla*	30.038
11	*B. purpurea*	17.150
12	*B. racemosa*	21.553
13	*B. saccocalyx*	23.786
14	*B. scandens*	19.537
15	*B. siamensis*	21.146
16	*B. sirindhorniae*	25.518
17	*B. strychnifolia*	28.379
18	*B. tomentosa*	24.251
19	*B. variegata*	16.063
20	*B. winitii*	30.656

Table 6 Quercetin and quercitrin contents in twenty *Bauhinia* species

No.	Species	Content (mg/g of dried ethanolic extract)*		Content (g/100 g of dried crude drug)*	
		Quercetin	Quercitrin	Quercetin	Quercitrin
1	*Bauhinia acuminata*	0.7912 ± 0.0069	1.4812 ± 0.0009	0.0207 ± 0.0002	0.0387 ± 0.0000
2	*B. aureifolia*	3.4167 ± 0.0031	5.1871 ± 0.0127	0.0641 ± 0.0001	0.0968 ± 0.0002
3	*B. bracteata*	0.6969 ± 0.0012	-	0.0120 ± 0.0000	-
4	*B. galpinii*	1.6757 ± 0.0017	0.4981 ± 0.0018	0.0400 ± 0.0000	0.0119 ± 0.0000
5	*B. integrifolia*	0.3417 ± 0.0032	14.6785 ± 0.0400	0.0084 ± 0.0001	0.3596 ± 0.0010
6	*B. lakhonensis*	3.8483 ± 0.0098	8.9028 ± 0.0008	0.1390 ± 0.0004	0.3216 ± 0.0014
7	*B. malabarica*	7.3211 ± 0.0233	14.2743 ± 0.0092	0.1918 ± 0.0006	0.3740 ± 0.0002
8	*B. ornata*	3.7639 ± 0.0005	2.6335 ± 0.0079	0.0660 ± 0.0000	0.0462 ± 0.0001
9	*B. pottsii*	0.8493 ± 0.0014	10.7628 ± 0.0273	0.0163 ± 0.0000	0.2062 ± 0.0005
10	*B. pulla*	-	3.1372 ± 0.0107	-	0.0942 ± 0.0003
11	*B. purpurea*	0.1550 ± 0.0006	-	0.0027 ± 0.0000	0.0193 ± 0.0005

Table 6 (cont.) Quercetin and quercitrin contents in twenty *Bauhinia* species

No.	Species	Content (mg/g of dried ethanolic extract)*		Content (g/100 g of dried crude drug)*	
		Quercetin	Quercitrin	Quercetin	Quercitrin
12	*B. racemosa*	-	0.7281 ± 0.0046	-	0.0157 ± 0.0001
13	*B. saccocalyx*	-	8.8927 ± 0.0022	-	0.2115 ± 0.0001
14	*B. scandens*	0.2151 ± 0.0004	2.6298 ± 0.0244	0.0042 ± 0.0000	0.0514 ± 0.0005
15	*B. siamensis*	0.2955 ± 0.0010	-	0.0062 ± 0.0000	0.3502 ± 0.0004
16	*B. sirindhorniae*	0.5194 ± 0.0041	5.8135 ± 0.0088	0.0133 ± 0.0000	0.1483 ± 0.0002
17	*B. strychnifolia*	1.0983 ± 0.0008	1.6021 ± 0.0076	0.0312 ± 0.0000	0.0455 ± 0.0002
18	*B. tomentosa*	-	1.0075 ± 0.0031	-	0.0244 ± 0.0001
19	*B. variegata*	0.2948 ± 0.0008	-	0.0047 ± 0.0000	-
20	*B. winitii*	1.3568 ± 0.0023	1.4833 ± 0.0015	0.0416 ± 0.0001	0.0455 ± 0.0000

*mean ± SD from triplicate

Method validation

The absorption spectra of quercetin and quercitrin were performed using photodiode array detector in the range of 200 – 800 nm. The result showed the maximum absorbance of quercetin at 255, 371 nm and the maximum absorbance of quercitrin at 255, 350 nm. So, the optimum absorbance used in this study was 255 nm (Figure 5 and Figure 6).

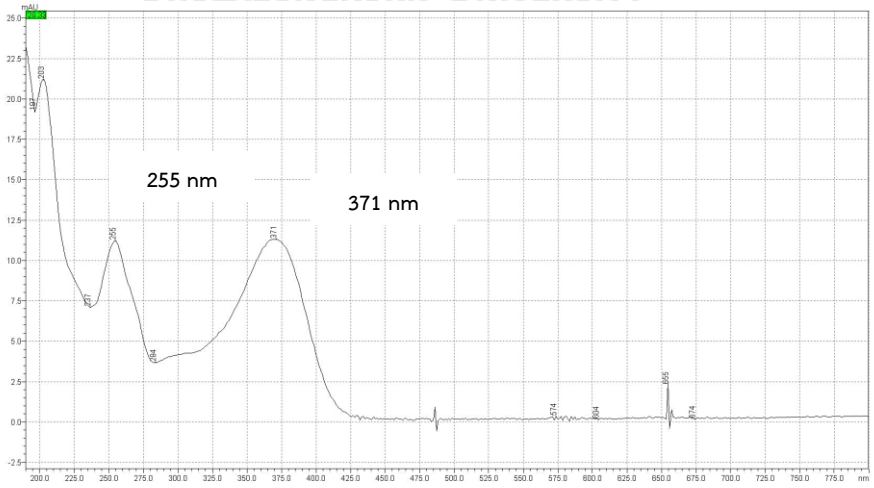


Figure 5 Absorbance spectrum of quercetin

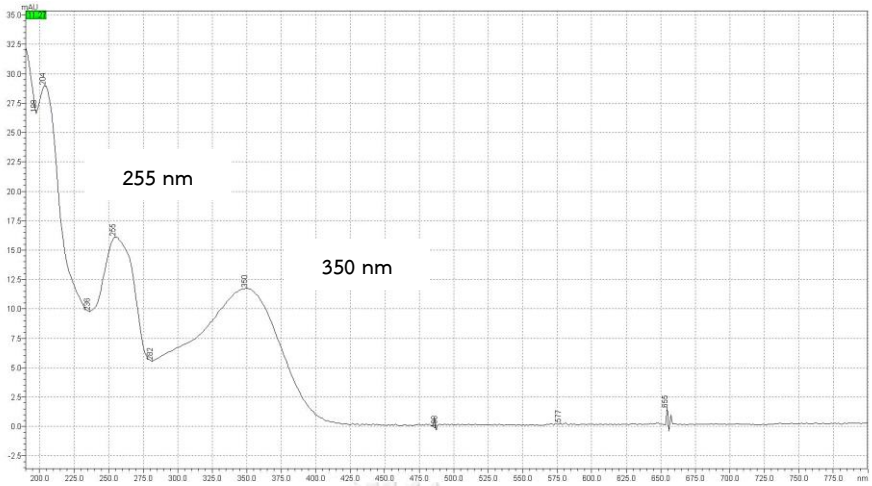


Figure 6 Absorbance spectrum of quercitrin

Linear calibration curves in the range of 20 – 100 µg/ml were created for each compound by plotting the peak area with the concentration. The regression equation of quercetin and quercitrin were y = 18199x – 31136 and y = 14702x - 6863.3 respectively with the coefficient of determination as 0.998 for quercetin and 0.999 for quercitrin (Figure 7 and Figure 8).

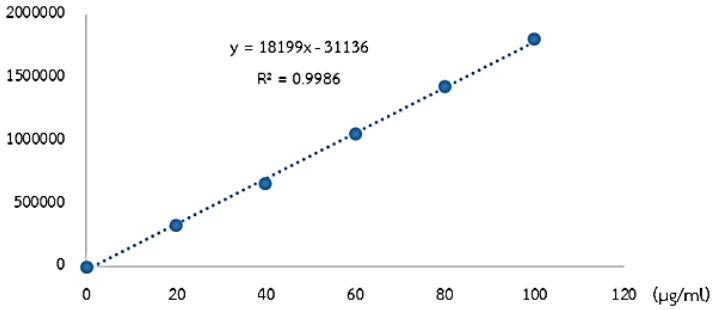


Figure 7 The calibration curve of quercetin

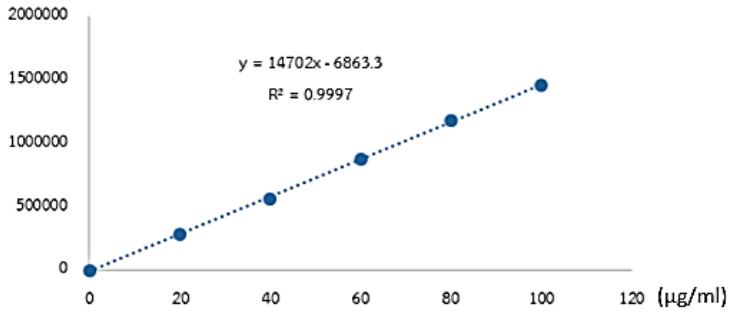


Figure 8 The calibration curve of quercitrin

The peaks of quercetin and quercitrin were well separated at retention times of 20.324 and 11.245, respectively (Figure 9). Peak purity index of quercetin and quercitrin showed 1.000 and 1.000 (Figure 10 and Figure 11).

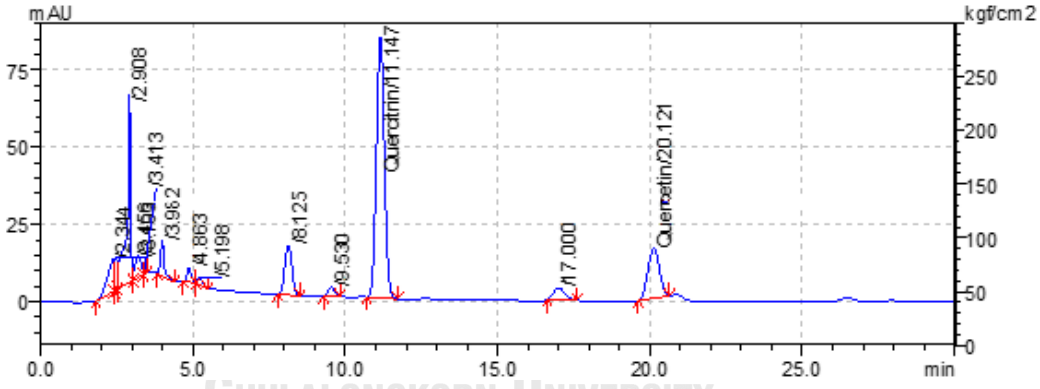


Figure 9 Chromatogram of *B. malabarica* leaf ethanolic extract

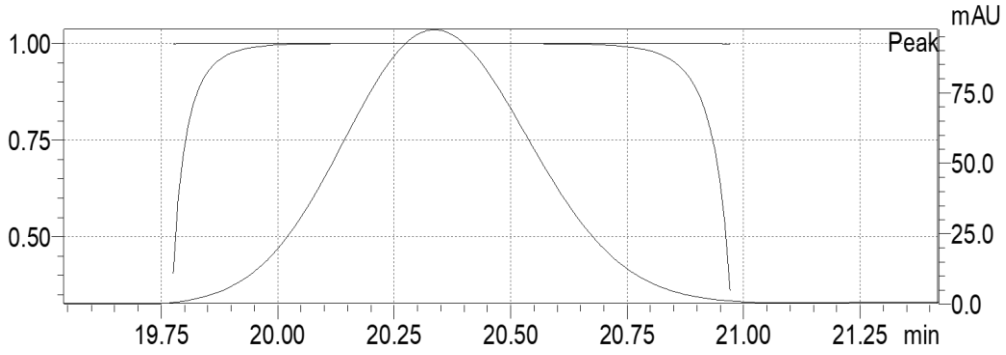


Figure 10 The peak purity of quercetin in *B. malabarica* leaves ethanolic extract

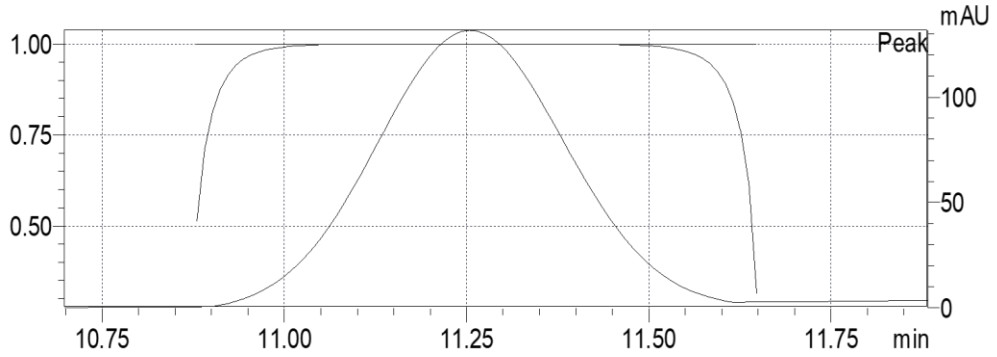


Figure 11 The peak purity of quercitrin in *B. malabarica* leaves ethanolic extract

The accuracy was determined by recovery method which spiked three concentrations (20, 40 and 60 µg/ml) of standard quercetin and quercitrin into *B. malabarica* leaf ethanolic extract. The results ranged from 99.231 – 100.114 and 98.768 – 103.652 % recovery as shown in the Table 7. The precision, both repeatability and intermediate precision were done on spiked sample with three different concentrations in the same day and three different days. The result of repeatability and intermediate precision in quercetin and quercitrin were shown in Table 8 and Table 9. The residual standard deviation of a regression line and the slope of calibration curve in both quercetin and quercitrin were used to calculate LOD and LOQ analysis. LOD and LOQ of quercetin were 4.755 and 14.408 µg/ml. LOD and LOQ of quercitrin were 1.941 and 5.883 µg/ml in quercitrin, respectively. The slightly changes of column temperature, flow rate and wavelength were determined the effect on one sample for robustness of the method. Column temperature was varied from 34 – 36 °C, the flow rate was set in the range of 0.950 – 1.050 ml/min and the detection wavelength was defined at 252 nm, 255 nm and 258 nm. The results were shown in the Table 10 – Table 12.

Table 7 Accuracy of quercetin and quercitrin in *B. malabarica* leaves

Compounds	Spike concentration (µg/ml)	Concentration (µg/ml)	% recovery (n = 3)
Quercetin	0	15.476 ± 0.177	-
	20	35.203 ± 0.405	99.231
	40	54.943 ± 0.822	99.529
	60	75.028 ± 0.123	100.114
Quercitrin	0	25.044 ± 0.408	-
	20	44.985 ± 0.644	99.870
	40	64.243 ± 0.994	98.768
	60	88.149 ± 1.262	103.652

Table 8 Repeatability precition of quercetin and quercitrin in *B. malabarica* leaves

Compound	Concentration (µg/ml)					
	#1	#2	#3	Mean	SD	%RSD
Quercetin	15.668	15.441	15.319	15.476	0.177	1.146
	34.738	35.402	35.470	35.203	0.405	1.150
	54.209	54.789	55.831	54.943	0.822	1.496
	75.045	75.142	74.897	75.028	0.123	0.164
Quercitrin	24.928	24.706	25.497	25.044	0.408	1.629
	44.303	45.068	45.584	44.985	0.644	1.432
	63.746	63.594	65.387	64.243	0.994	1.548
	86.871	88.181	89.395	88.149	1.262	1.432

Table 9 Intermediate precision of quercetin and quercitrin in *B. malabarica* leaves

Compounds	Concentration (µg/ml)					
	Day 1	Day 2	Day 3	Mean	SD	%RSD
Quercetin	16.810	15.668	15.888	16.122	0.606	3.759
	36.114	34.738	34.096	34.983	1.031	2.947
	55.118	54.209	52.444	53.923	1.360	2.522
	75.739	75.045	73.520	74.768	1.135	1.519
Quercitrin	24.927	25.089	25.032	25.016	0.082	0.329
	44.018	43.733	44.445	44.065	0.358	0.813
	62.504	64.102	66.278	64.295	1.895	2.947
	84.634	83.971	85.860	84.822	0.959	1.130

Table 10 Robustness of quercetin and quercitrin in *B. malabarica* leaves (flow rate)

Flow rate (ml/min)		Retention time (min)			Peak area (unit)			Concentration (µg/ml)		
		1	2	3	1	2	3	1	2	3
Quercetin	1.0950	21.275	21.389	21.389	240443	240526	242183	14.571	14.576	14.666
	1.0000	20.233	20.370	20.356	212265	214111	211345	13.040	13.140	12.990
	1.1050	19.447	19.485	19.421	210004	206472	214141	12.917	12.725	13.142
	Mean	20.374			221276.667			13.529		
	SD	0.825			15013.843			0.816		
	%RSD	4.051			6.785			6.031		
Quercitrin	1.0950	11.766	11.826	11.792	348145	351549	352578	24.071	24.299	24.368
	1.0000	11.238	11.264	11.233	306905	309924	306505	21.313	21.515	21.286
	1.1050	10.752	10.772	10.734	303294	299231	310839	21.071	20.799	21.576
	Mean	11.264			320996.667			22.255		
	SD	0.452			22609.047			1.512		
	%RSD	4.011			7.043			6.795		

Table 11 Robustness of quercetin and quercitrin in *B. malabarica* leaves (temperature)

Temperature (°C)		Retention time (min)			Peak area (unit)			Concentration (µg/ml)		
		1	2	3	1	2	3	1	2	3
Quercetin	34	21.075	21.079	21.039	203873	200955	200637	12.584	12.425	12.408
	35	20.233	20.370	20.356	212265	214111	211345	13.040	13.140	12.990
	36	19.645	19.641	19.650	232377	236753	236336	14.133	14.371	14.348
	Mean	20.343			216516.889			13.271		
	SD	0.616			14826.779			0.806		
	%RSD	3.028			6.848			6.072		
Quercitrin	34	11.591	11.595	11.582	294824	292631	292872	20.505	20.358	20.374
	35	11.238	11.264	11.233	306905	309924	306505	21.313	21.515	21.286
	36	10.911	10.913	10.917	336309	341331	342948	23.279	23.615	23.723
	Mean	11.249			313805.444			21.774		
	SD	0.293			20845.247			1.394		
	%RSD	2.602			6.643			6.403		

Table 12 Robustness of quercetin and quercitrin in *B. malabarica* leaves (wavelength)

Wavelength (nm)		Retention time (min)			Peak area (unit)			Concentration (µg/ml)		
		1	2	3	1	2	3	1	2	3
Quercetin	252	20.329	20.370	20.356	207031	208583	205798	12.755	12.840	12.688
	255	20.331	20.370	20.356	212265	214111	211345	13.040	13.140	12.990
	258	20.330	20.371	20.357	200154	203317	199142	12.382	12.553	12.327
	Mean	20.352			206860.667			12.746		
	SD	0.018			5281.132			0.287		
	%RSD	0.087			2.553			2.252		
Quercitrin	252	11.238	11.264	11.233	303611	306814	303438	21.092	21.307	21.081
	255	11.238	11.264	11.233	306905	309924	306505	21.313	21.515	21.286
	258	11.238	11.264	11.233	294969	298049	294313	20.514	20.720	20.471
	Mean	11.245			302725.333			21.033		
	SD	0.014			5638.949			0.377		
	%RSD	0.128			1.863			1.793		

Part II Microscopic leaf characteristics of twenty *Bauhinia* species

Selected twenty *Bauhinia* species revealed the paracytic stomata type on both upper epidermis and lower epidermis (Figure 12 and Figure 13). Nine *Bauhinia* species (*B. aureifolia*, *B. bracteata*, *B. integrifolia*, *B. lakhonensis*, *B. purpurea*, *B. scandens*, *B. strychnifolia*, *B. tomentosa*, *B. variegata*, and *B. winitii*) were found stomata on both sides. Unicellular trichomes and multicellular trichomes were found (Figure 14 and Figure 15). The characteristics of the upper and lower epidermis of twenty *Bauhinia* species was shown in Table 13. *B. pottsii* and *B. saccocalyx* showed abundant trichomes that interfered stomatal number and epidermal cell number determination at lower epidermis. Epidermal cell area and palisade ratio were determined on the upper epidermis and the stomatal number, stomatal index, trichome index, and trichome number were determined on both upper and lower epidermis and the results were presented in the Table 14.

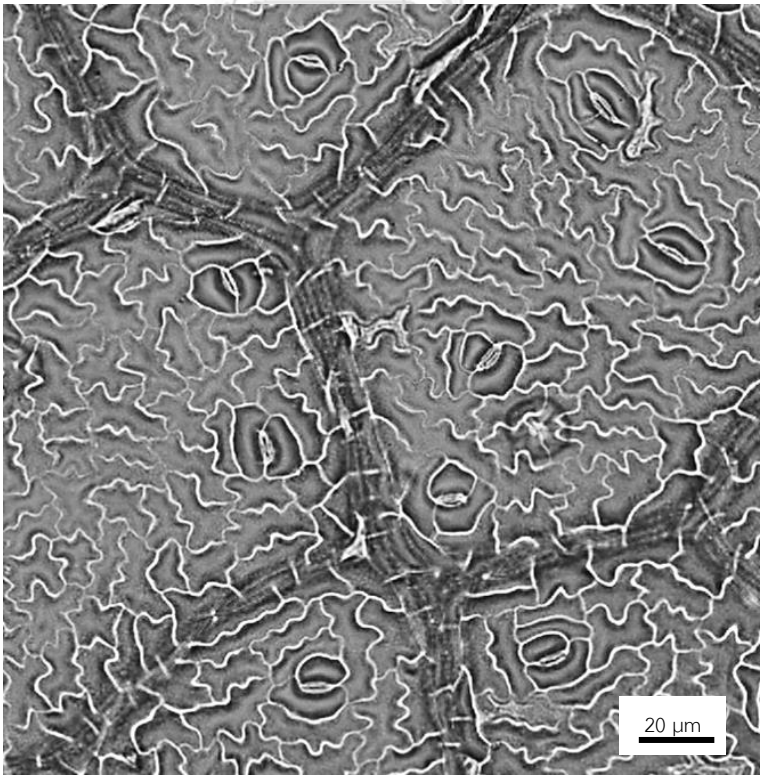


Figure 12 Paracytic stomata type on the upper epidermis of *B. bracteata*

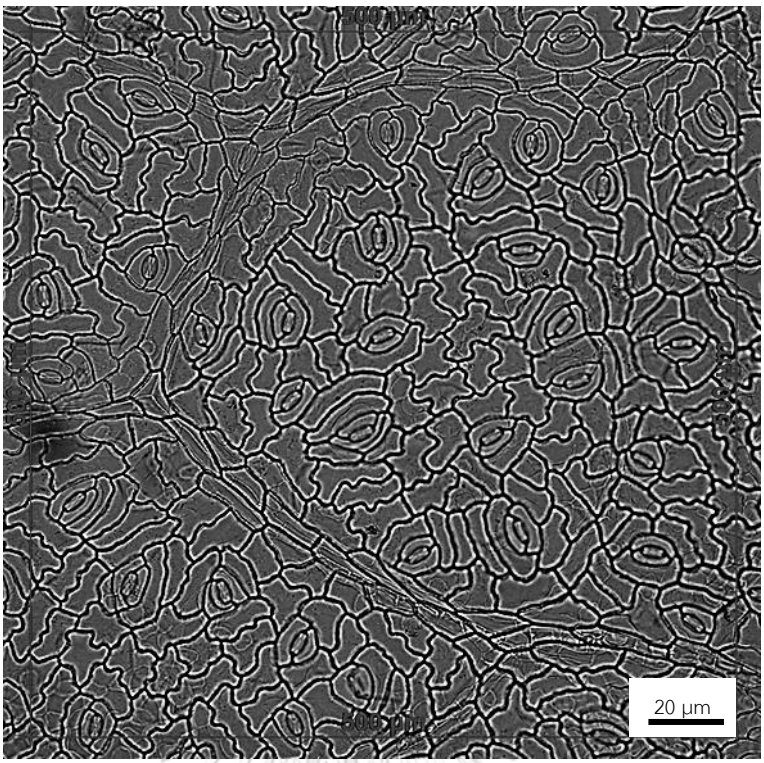


Figure 13 Paracytic stomata type on the lower epidermis of *B. scandens*

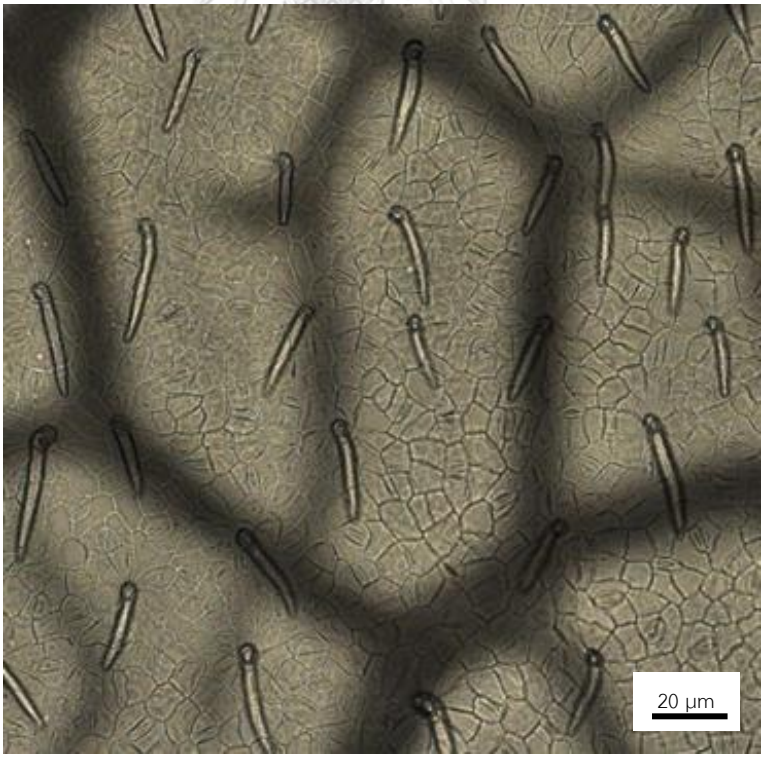


Figure 14 Unicellular trichomes of *B. galpinii*

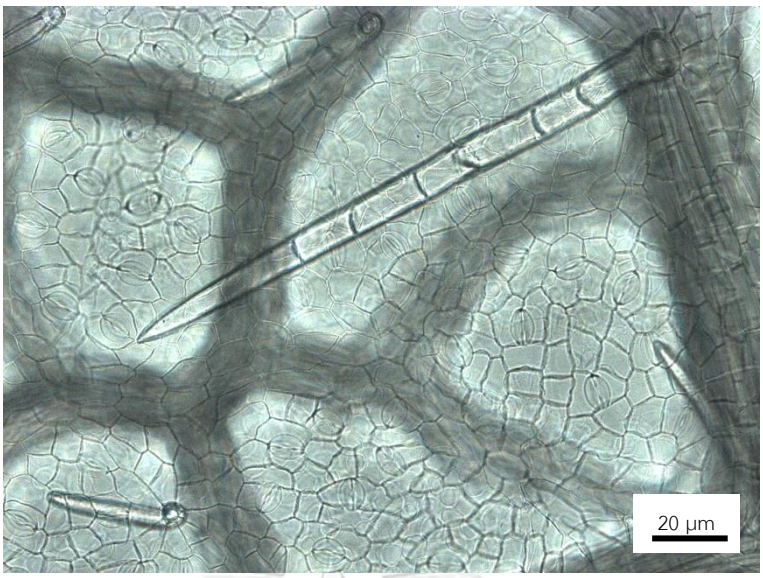


Figure 15 Multicellular trichomes of *B. variegata*

Table 13 The characteristics of upper and lower epidermis of twenty *Bauhinia* species

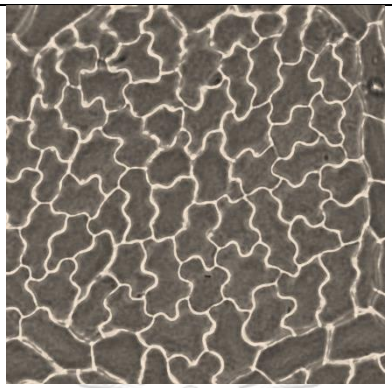
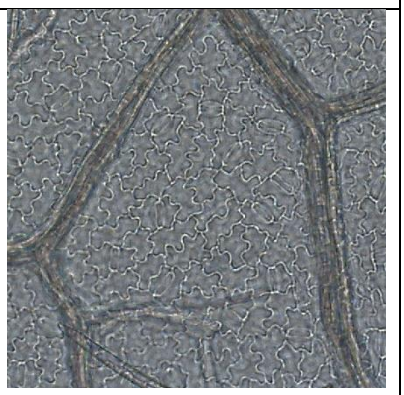
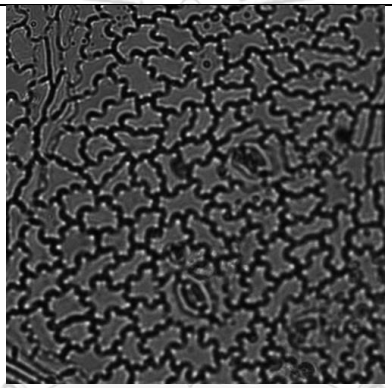
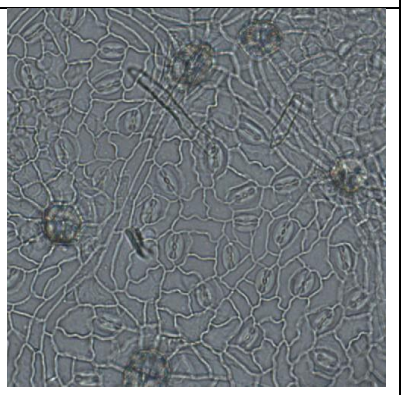
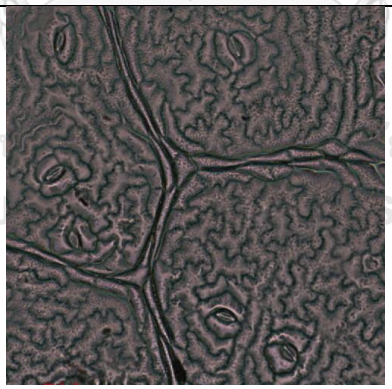
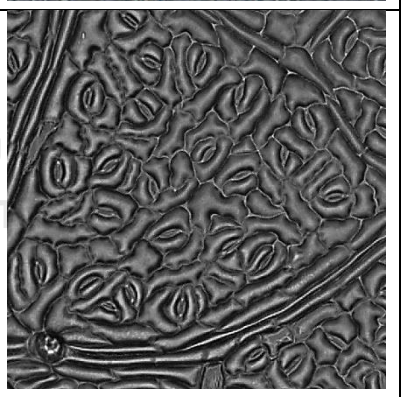
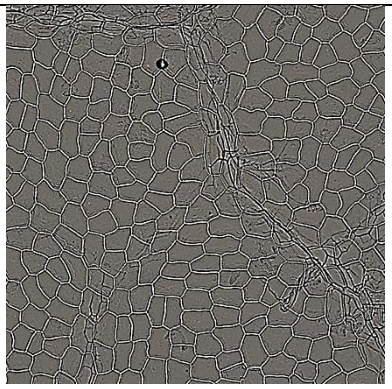
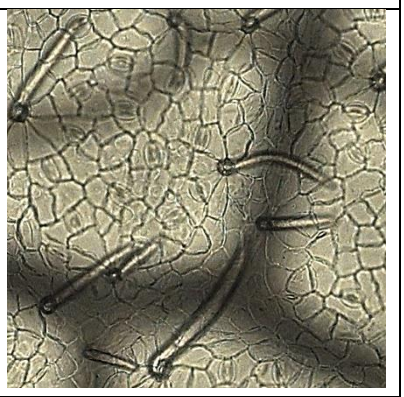
Species	Upper epidermis	Lower epidermis
Bauhinia acuminata		
B. aureifolia		
B. bracteata		
B. galpinii		

Table 13 (cont.) The characteristics of upper and lower epidermis of twenty *Bauhinia* species

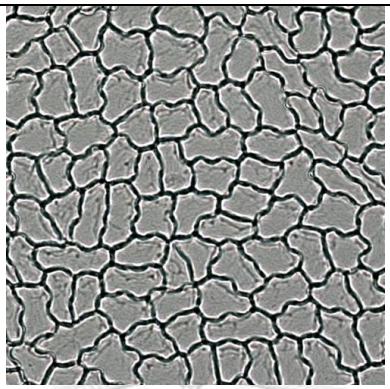
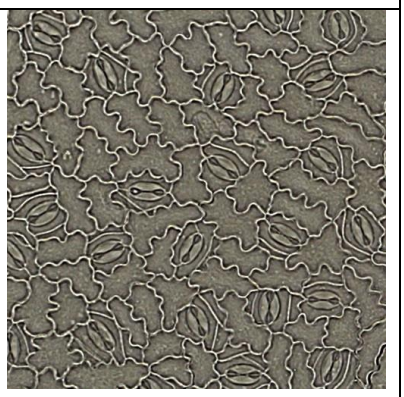
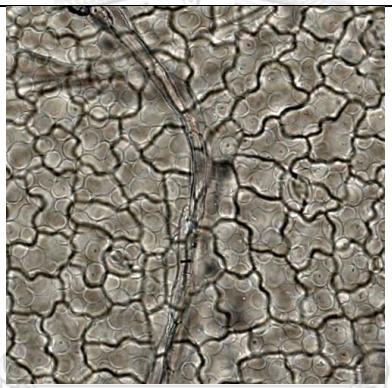
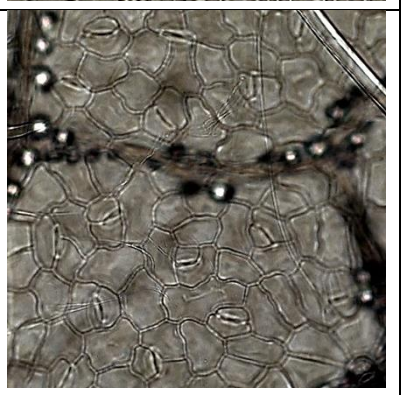
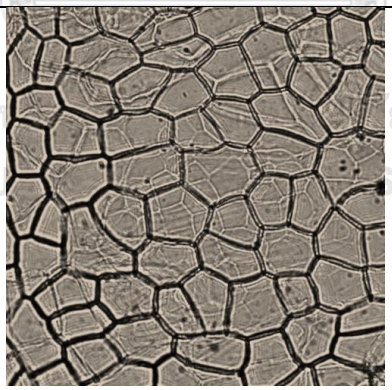
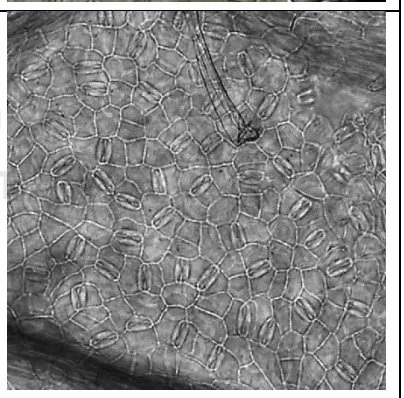
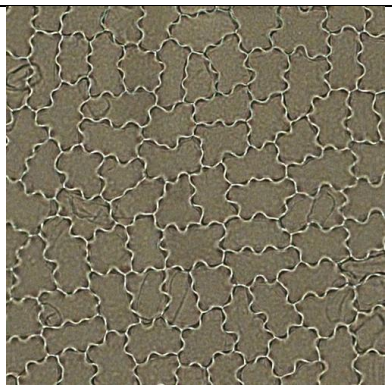
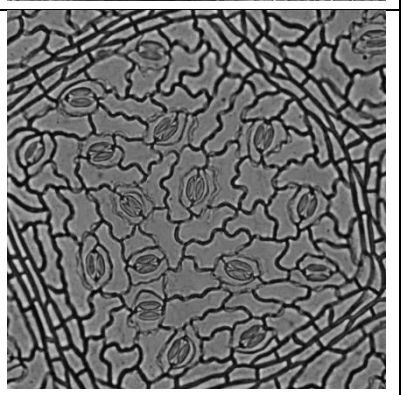
Species	Upper epidermis	Lower epidermis
B. integrifolia		
B. lakhonensis		
B. malabarica		
B. ornata		

Table 13 (cont.) The characteristics of upper and lower epidermis of twenty *Bauhinia* species

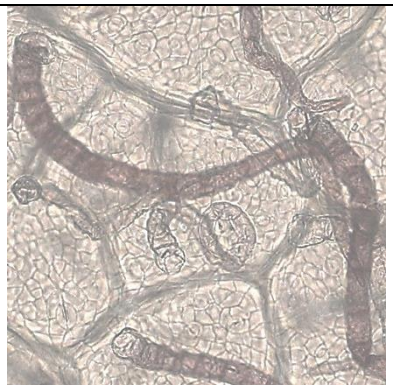
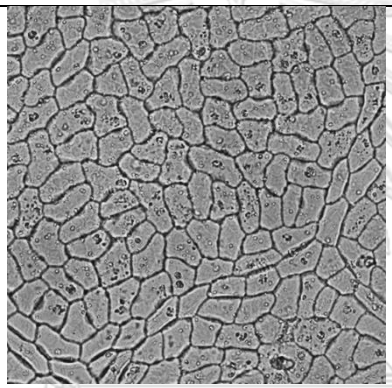
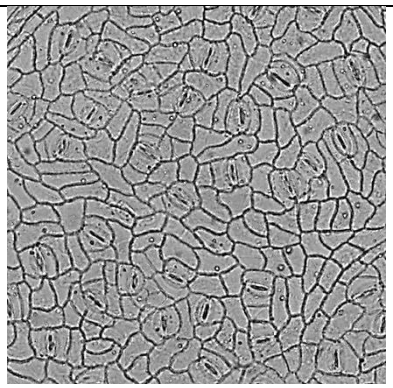
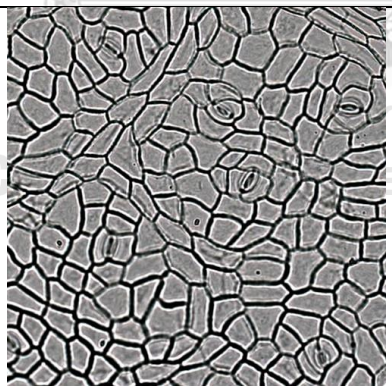
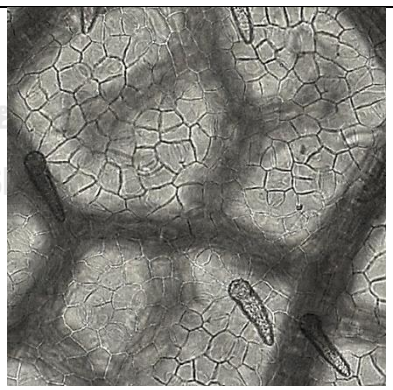
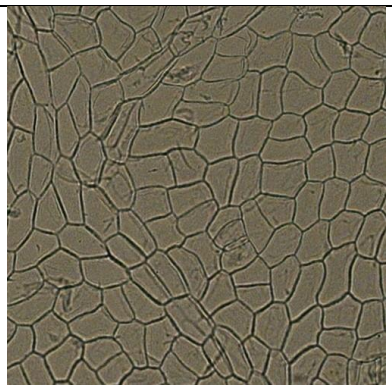
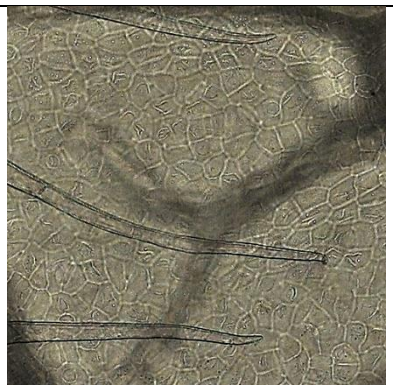
Species	Upper epidermis	Lower epidermis
B. pottsii		
B. pulla		
B. purpurea		
B. racemosa		

Table 13 (cont.) The characteristics of upper and lower epidermis of twenty *Bauhinia* species

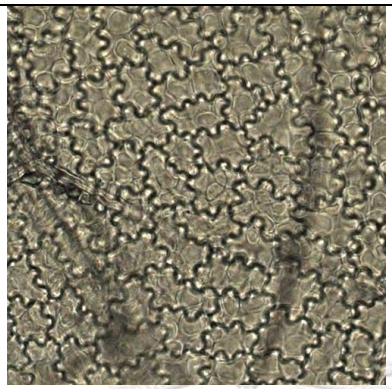
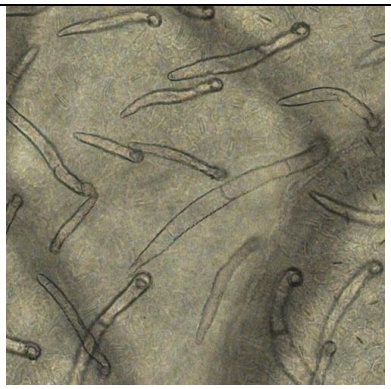
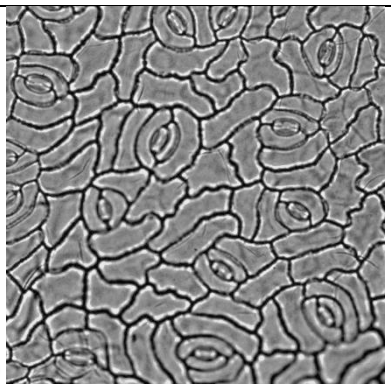
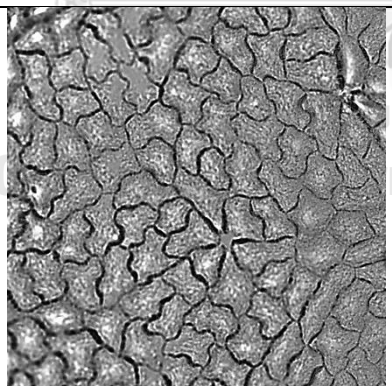
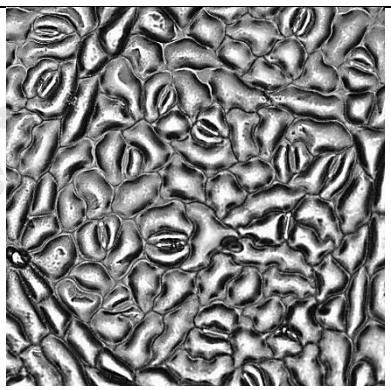
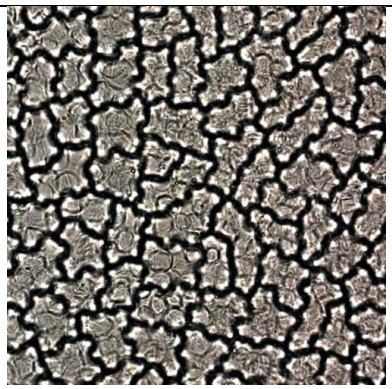
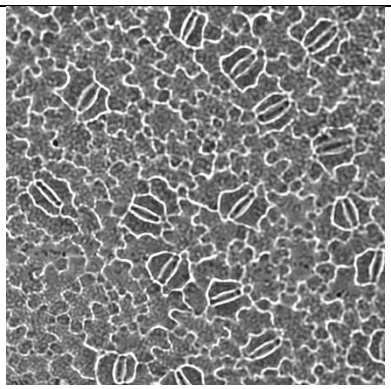
Species	Upper epidermis	Lower epidermis
B. saccocalyx		
B. scandens		
B. siamensis		
B. sirindhorniae		

Table 13 (cont.) The characteristics of upper and lower epidermis of twenty *Bauhinia* species

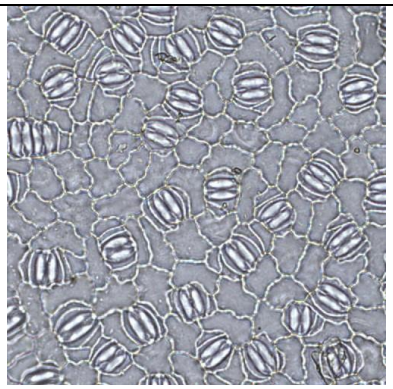
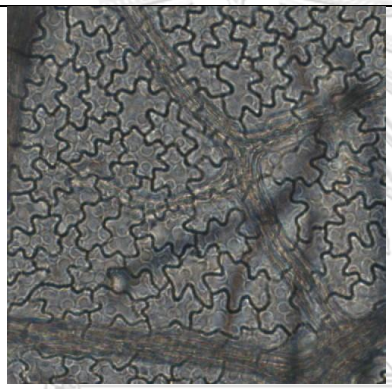
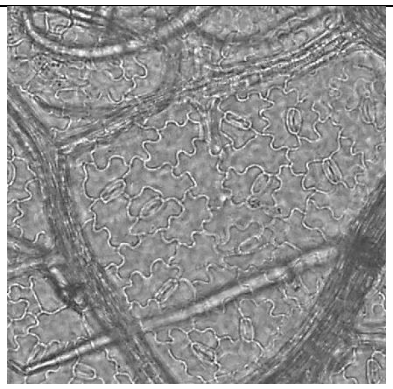
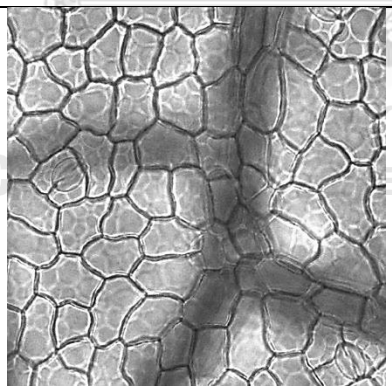
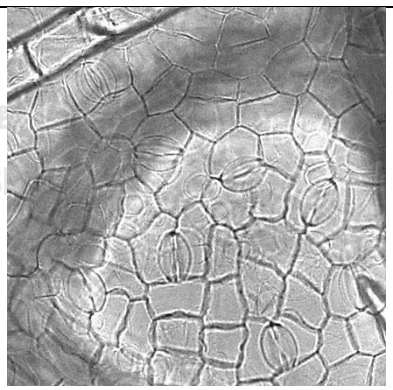
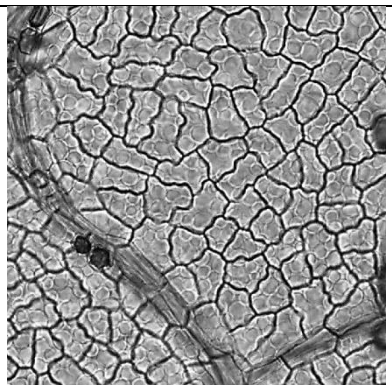
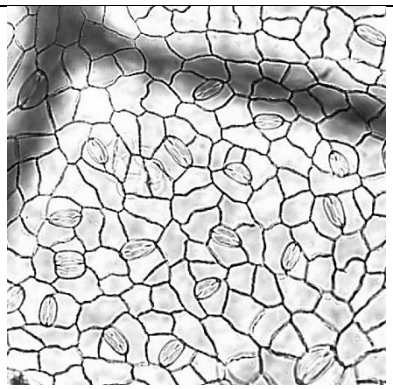
Species	Upper epidermis	Lower epidermis
B. strychnifolia		
B. tomentosa		
B. variegata		
B. winitii		

Table 14 Microscopic leaf measurement of twenty *Bauhinia* species

No.	Species	Epidermal cell area (μm^2)	Palisade ratio	Epidermis	Stomatal number ($/mm^2$)	Stomatal index	Type of trichome	Trichome number ($/mm^2$)	Trichome index
1.	*Bauhinia acuminata*	539.140 ± 10.305 (520. 833 – 554.324)	6.642 ± 0.320 (6.000 – 7.250)	Upper	–	–	–	–	–
				Lower	452.800 ± 13.632 (432.000 – 472.000)	20.607 ± 0.503 (19.459 – 21.377)	Multicellular	9.200 ± 3.951 (4.000 – 16.000)	0.419 ± 0.181 (0.180 – 0.741)
2.	*B. aureifolia*	534.582 ± 7.021 (518.672 – 545.852)	5.008 ± 0.241 (4.750 – 5.500)	Upper	28.800 ± 6.677 (20.000 – 44.00)	1.539 ± 0.356 (1.037 – 2.366)	–	–	–
				Lower	583.600 ± 21.357 (548.000 – 624.000)	16.108 ± 0.498 (15.232 – 16.938)	Unicellular	55.200 ± 10.886 (36.000 – 72.000)	1.523 ± 0.296 (0.991 – 1.991)
3.	*B. bracteata*	352.512 ± 2.642 (347.705 – 357.143)	4.908 ± 0.241 (4.500 – 5.250)	Upper	98.933 ± 7.273 (88.000 – 112.000)	3.487 ± 0.248 (3.086 – 3.972)	Unicellular	7.067 ± 2.716 (4.000 – 12.000)	0.249 ± 0.095 (0.139 – 0.426)
				Lower	468.000 ± 13.736 (448.000 – 488.000)	15.147 ± 0.381 (14.359 – 15.803)	Unicellular	6.800 ± 2.809 (4.000 – 12.000)	0.220 ± 0.090 (0.127 – 0.389)
4.	*B. galpinii*	479.328 ± 4.056 (471.698 – 488.281)	5.808 ± 0.444 (5.000 – 6.250)	Upper	–	–	–	–	–
				Lower	445.867 ± 18.003 (408.000 – 480.000)	10.277 ± 0.372 (9.595 – 11.091)	Multicellular	132.133 ± 11.953 (108.000 – 152.000)	3.045 ± 0.266 (2.495 – 3.493)
5.	*B. integrifolia*	686.829 ± 7.469 (673.854 – 694.444)	5.617 ± 0.424 (5.250 – 6.500)	Upper	6.000 ± 2.924 (4.000 – 12.000)	0.411 ± 0.198 (0.270 – 0.829)	–	–	–
				Lower	238.000 ± 9.381 (212.000 – 256.000)	12.949 ± 0.535 (11.427 – 14.097)	–	–	–
6.	*B. lakhonensis*	708.920 ± 8.664 (694.444 – 724.638)	4.108 ± 0.306 (3.750 – 5.000)	Upper	81.467 ± 7.537 (72.000 – 96.000)	5.772 ± 0.505 (5.000 – 6.761)	Unicellular	6.533 ± 2.460 (4.000 – 12.000)	0.462 ± 0.172 (0.279 – 0.847)
				Lower	204.800 ± 10.314 (192.000 – 228.000)	12.166 ± 0.537 (11.374 – 13.287)	Unicellular	12.667 ± 4.213 (4.000 – 20.000)	0.753 ± 0.252 (0.243 – 1.202)
7.	*B. malabarica*	685.912 ± 8.592 (672.043 – 706.215)	3.767 ± 0.236 (3.500 – 4.250)	Upper	–	–	–	–	–
				Lower	683.867 ± 18.179 (652.000 – 720.000)	18.066 ± 0.431 (17.249 – 19.068)	Multicellular	12.800 ± 3.547 (8.000 – 20.000)	0.338 ± 0.092 (0.206 – 0.521)

Table 14 (cont.) Microscopic leaf measurement of twenty *Bauhinia* species

No.	Species	Epidermal cell area (μm^2)	Palisade ratio	Epidermis	Stomatal number ($/mm^2$)	Stomatal index	Type of trichome	Trichome number ($/mm^2$)	Trichome index
8.	*B. ornata*	874.518 ± 25.304 (841.751 – 919.118)	4.150 ± 0.375 (3.750 – 4.750)	Upper	–	–	–	–	–
				Lower	250.000 ± 13.235 (228.000 – 268.000)	8.957 ± 0.434 (8.297 – 9.558)	–	–	–
9.	*B. pottsii*	473.548 ± 5.544 (460.405 – 483.559)	5.808 ± 0.444 (5.250 – 6.500)	Upper	–	–	Multicellular	27.467 ± 6.538 (16.000 – 36.000)	1.300 ± 0.307 (0.737 – 1.724)
				Lower	*	*	Multicellular	130.533 ± 12.539 (108.000 – 152.000)	*
10.	*B. pulla*	530.124 ± 5.277 (521.921 – 536.481)	6.175 ± 0.384 (5.750 – 7.000)	Upper	–	–	–	–	–
				Lower	451.867 ± 19.125 (428.000 – 484.000)	12.532 ± 0.499 (11.656 – 13.253)	–	–	–
11.	*B. purpurea*	520.423 ± 9.705 (507.099 – 561.798)	5.150 ± 0.423 (4.500 – 6.000)	Upper	64.133 ± 5.303 (56.000 – 72.000)	3.338 ± 0.285 (2.905 – 4.045)	–	–	–
				Lower	1165.200 ± 24.026 (1120.000 – 1208.000)	19.522 ± 0.368 (18.881 – 20.296)	Multicellular	96.667 ± 7.053 (88.000 – 108.000)	1.620 ± 0.119 (1.469 – 1.828)
12.	*B. racemosa*	490.107 ± 4.467 (483.559 – 498.008)	7.292 ± 0.378 (6.750 – 8.000)	Upper	–	–	–	–	–
				Lower	835.067 ± 26.001 (800.000 – 880.000)	23.156 ± 0.598 (22.272 – 24.084)	Multicellular	31.467 ± 4.897 (24.000 – 44.000)	0.873 ± 0.136 (0.657 – 1.221)
13.	*B. saccocalyx*	367.648 ± 3.739 (361.272 – 373.134)	9.217 ± 0.346 (8.750 – 9.750)	Upper	–	–	–	–	–
				Lower	*	*	Unicellular and multicellular	190.533 ± 13.716 (164.000 – 220.000)	*
14.	*B. scandens*	463.130 ± 3.429 (457.875 – 469.043)	9.000 ± 0.410 (8.500 – 9.500)	Upper	10.533 ± 4.133 (4.000 – 20.000)	0.487 ± 0.191 (0.186 – 0.931)	–	–	–
				Lower	154.933 ± 7.926 (144.000 – 168.000)	8.529 ± 0.410 (7.895 – 9.251)	–	–	–

Table 14 (cont.) Microscopic leaf measurement of twenty *Bauhinia* species

No.	Species	Epidermal cell area (μm^2)	Palisade ratio	Epidermis	Stomatal number ($/mm^2$)	Stomatal index	Type of trichome	Trichome number ($/mm^2$)	Trichome index
15.	*B. siamensis*	645.648 ± 8.126 (632.911 – 661.376)	6.633 ± 0.424 (6.000 – 7.250)	Upper	–	–	Unicellular	26.000 ± 3.895 (20.000 – 32.000)	1.678 ± 0.246 (1.272 – 2.094)
				Lower	199.867 ± 9.482 (172.000 – 220.000)	10.208 ± 0.458 (9.034 – 11.294)	Unicellular	24.133 ± 5.406 (16.000 – 32.000)	1.231 ± 0.271 (0.815 – 1.649)
16.	*B. sirindhorniae*	880.405 ± 19.845 (847.458 – 912.409)	4.533 ± 0.370 (4.000 – 5.000)	Upper	–	–	–	–	–
				Lower	245.333 ± 10.148 (232.000 – 256.000)	12.474 ± 0.458 (11.741 – 13.008)	–	–	–
17.	*B. strychnifolia*	498.106 ± 5.790 (486.381 – 507.099)	5.158 ± 0.466 (4.500 – 6.000)	Upper	8.533 ± 3.893 (4.000 – 16.000)	0.425 ± 0.195 (0.198 – 0.806)	–	–	–
				Lower	612.000 ± 14.054 (592.000 – 640.000)	16.414 ± 0.323 (15.846 – 17.131)	–	–	–
18.	*B. tomentosa*	688.769 ± 9.491 (672.043 – 702.247)	5.567 ± 0.227 (5.250 – 6.000)	Upper	–	–	–	–	–
				Lower	395.067 ± 16.532 (368.000 – 428.000)	18.926 ± 0.862 (17.829 – 21.063)	Multicellular	38.533 ± 5.894 (32.000 – 52.000)	1.845 ± 0.278 (1.507 – 2.524)
19.	*B. variegata*	420.411 ± 4.854 (413.223 – 430.293)	5.258 ± 0.325 (4.500 – 5.750)	Upper	47.467 ± 6.867 (40.000 – 60.000)	1.995 ± 0.283 (1.667 – 2.564)	–	–	–
				Lower	620.667 ± 20.906 (576.000 – 656.000)	11.566 ± 0.348 (10.868 – 12.117)	Unicellular and multicellular	48.533 ± 7.026 (36.000 – 60.000)	0.904 ± 0.128 (0.676 – 1.124)
20.	*B. winitii*	566.596 ± 5.155 (556.793 – 577.367)	4.467 ± 0.260 (4.000 – 5.000)	Upper	7.600 ± 3.979 (4.000 – 16.000)	0.430 ± 0.225 (0.225 – 0.909)	–	–	–
				Lower	348.267 ± 15.929 (320.000 – 384.000)	13.422 ± 0.558 (12.422 – 14.701)	–	–	–

* cannot count epidermal cells and stomata numbers due to many numbers of trichomes.

Mature leaves of each *Bauhinia* species were collected and cleaned. The midrib was cut thinly using blade by hand. The leaf midrib cross-section of twenty *Bauhinia* species was drawn and shown in Figure 16 – 35. The arrangement of the upper and lower epidermal cells, palisade cells, sponge cells, collenchyma, sclerenchyma cells, xylem, phloem and stomata was presented. *B. winitii* was shown the different characters of palisade cells. This species was presented two layers of palisade cells on the upper epidermis as shown in Figure 35.

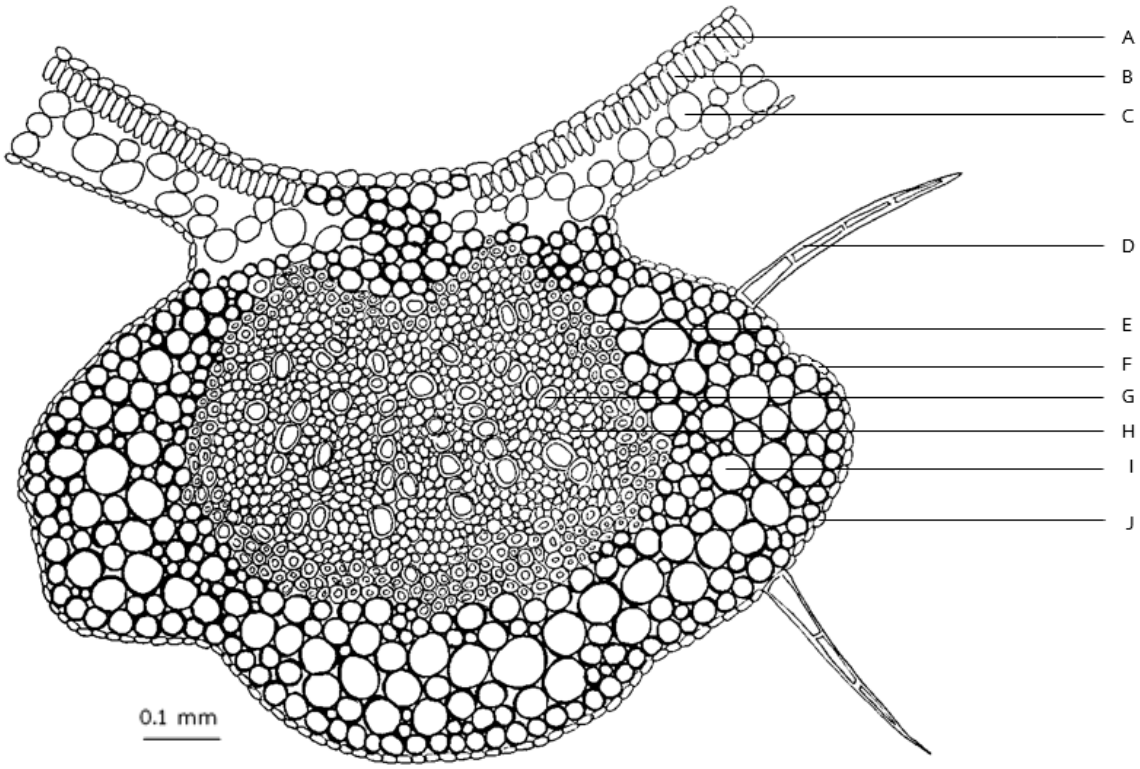


Figure 16 Anatomical characteristics of *B. acuminata* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells,
D = multicellular trichome, E = sclerenchyma, F = stomata, G = xylem, H = phloem,
I = collenchyma, J = lower epidermis

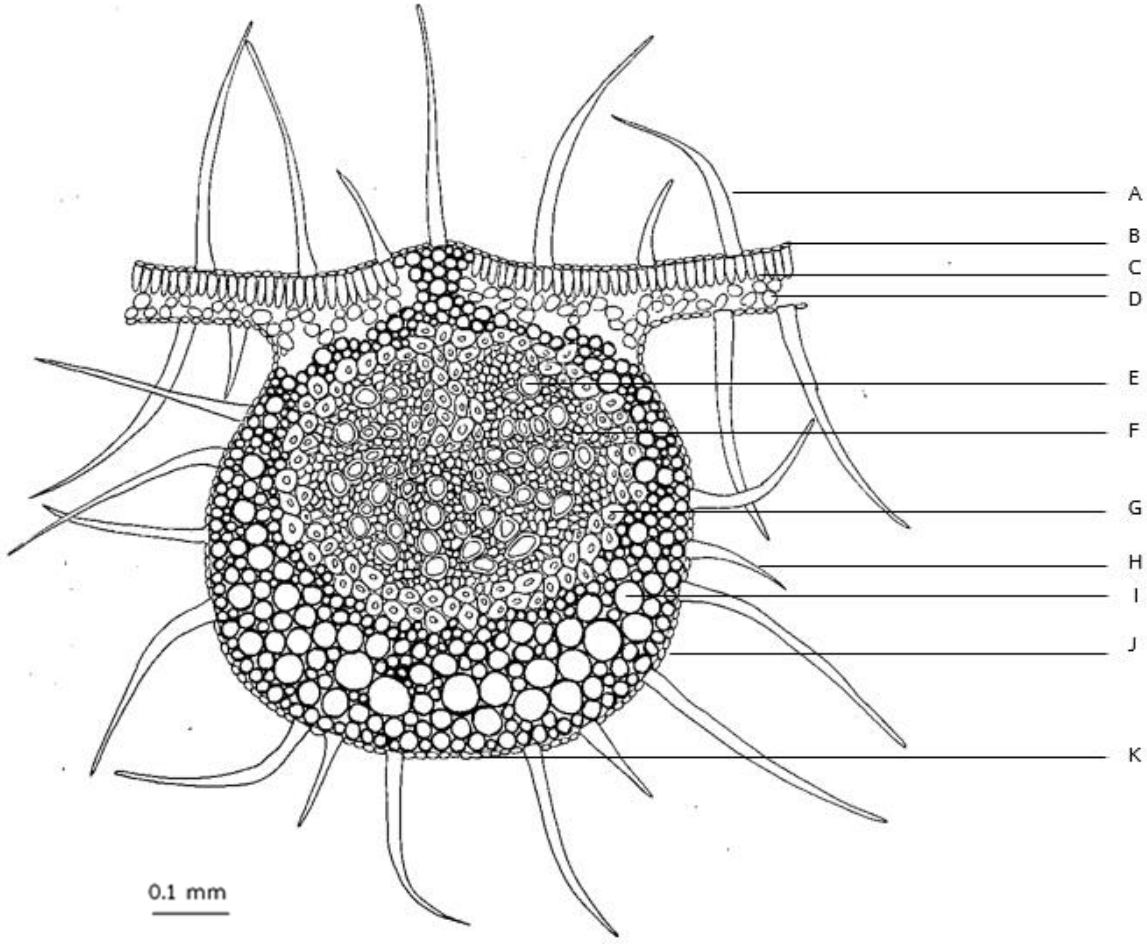


Figure 17 Anatomical characteristics of *B. aureifolia* leaf midrib (cross-section)
A = unicellular trichome (upper epidermis) B = upper epidermis,
C = palisade cells, D = sponge cells, E = xylem, F = phloem, G = sclerenchyma cells,
H = unicellular trichome (lower epidermis), I = collenchyma, J = lower epidermis,
K = stomata

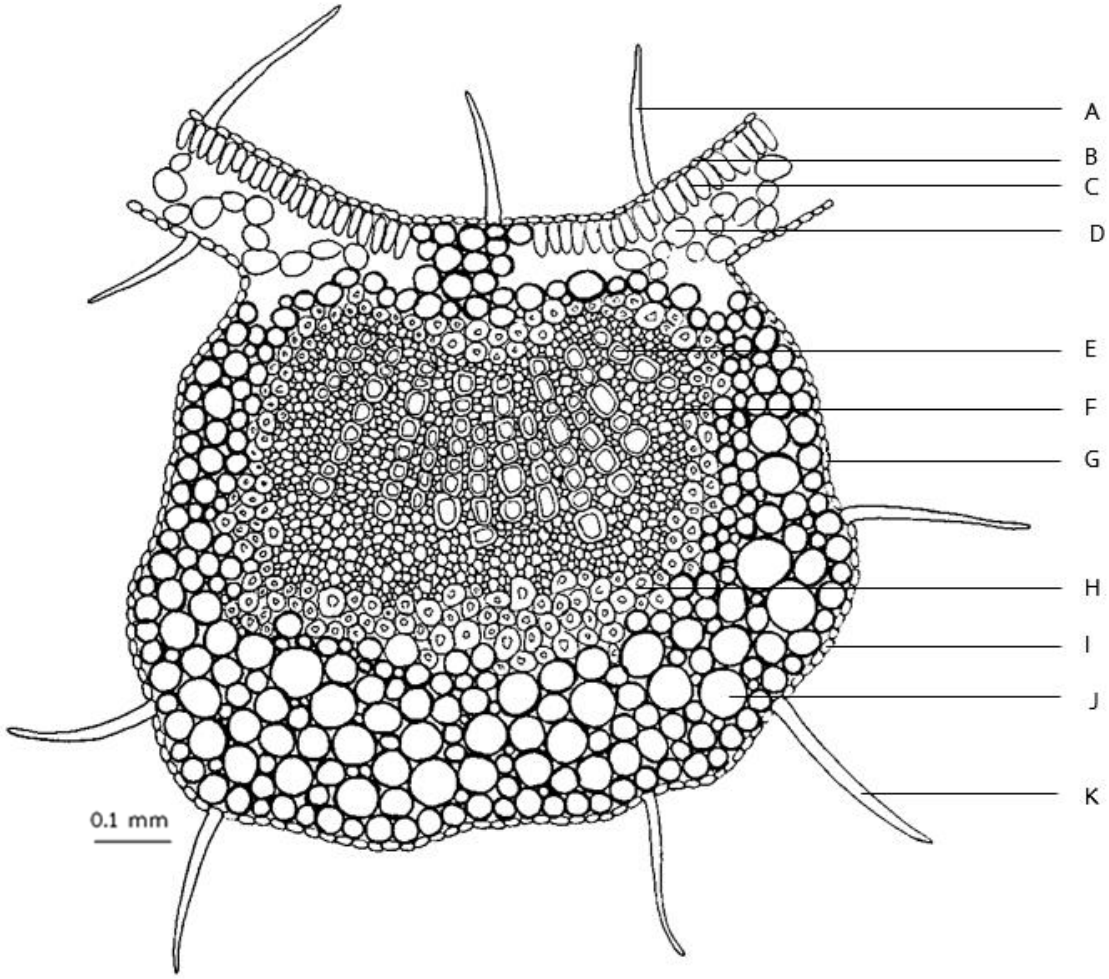


Figure 18 Anatomical characteristics of *B. bracteata* leaf midrib (cross-section)
A = unicellular trichome (upper epidermis) B = upper epidermis,
C = palisade cells, D = sponge cells, E = xylem, F = phloem, G = stomata,
H = sclerenchyma cells, I = lower epidermis, J = collenchyma,
K = unicellular trichome (lower epidermis)

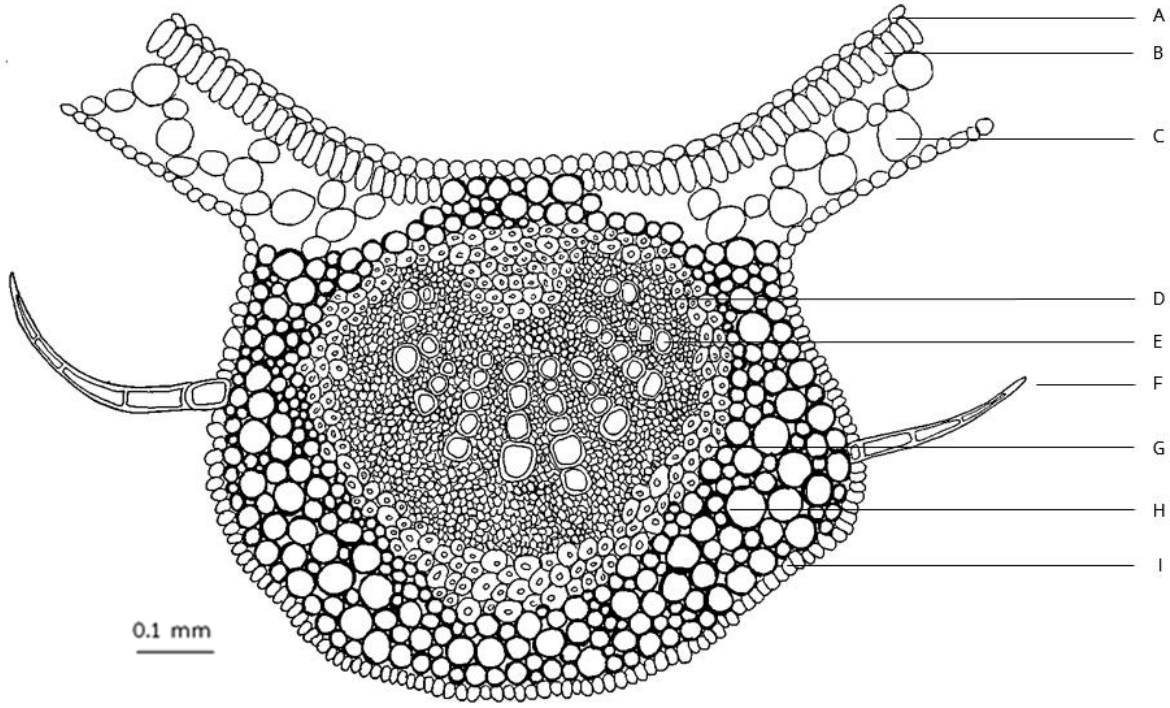


Figure 19 Anatomical characteristics of *B. galpinii* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells, D = phloem,
E = xylem, F = multicellular trichome, G = sclerenchyma cells, H = collenchyma,
I = lower epidermis

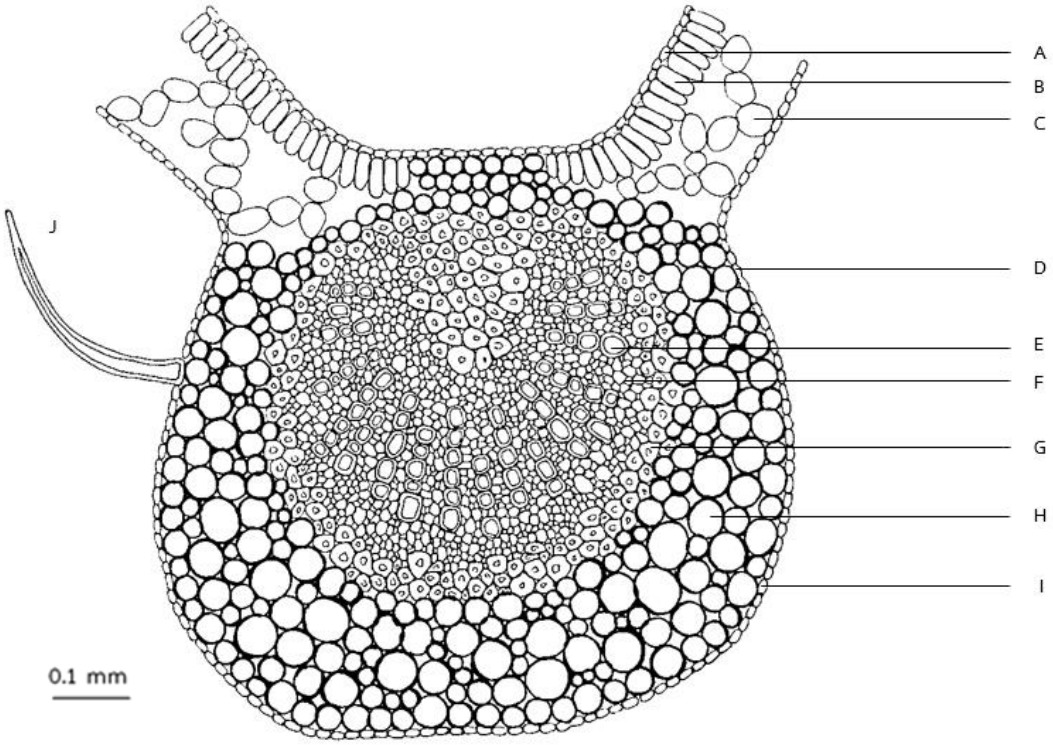


Figure 20 Anatomical characteristics of *B. integrifolia* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells, D = stomata,
E = xylem, F = phloem, G = sclerenchyma cells, H = collenchyma,
I = lower epidermis, J = unicellular trichome

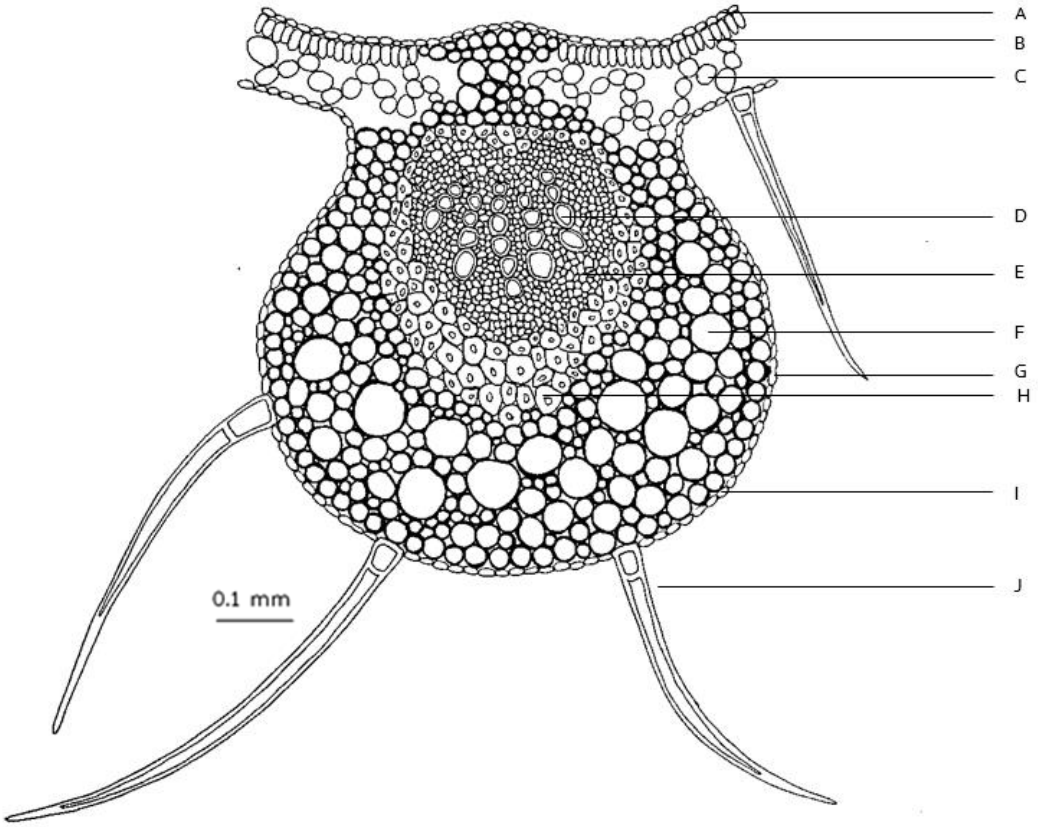


Figure 21 Anatomical characteristics of *B. lakhonensis* leaf midrib (cross-section) A = upper epidermis, B = palisade cells, C = sponge cells, D = xylem, E = phloem, F = collenchyma, G stomata, H = sclerenchyma cells, I = lower pidermis, J = multicellular trichome

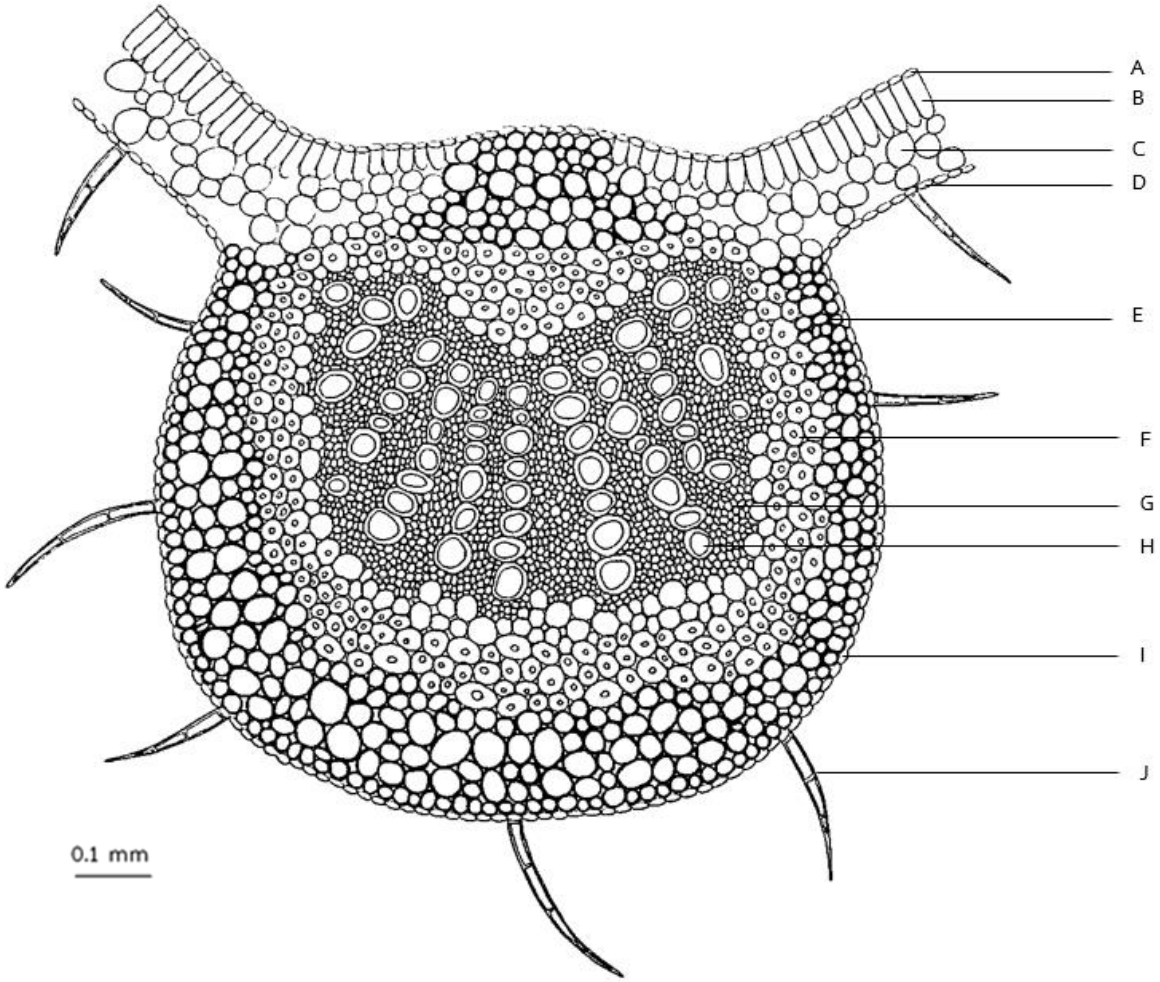


Figure 22 Anatomical characteristics of *B. malabarica* leaf midrib (cross-section) A=upper epidermis, B=palisade cells, C=sponge cells, D=lower epidermis, E=collenchyma, F=scherenchyma cells, G=phloem, H=xylem, I=stomata, J=multicellular trichome

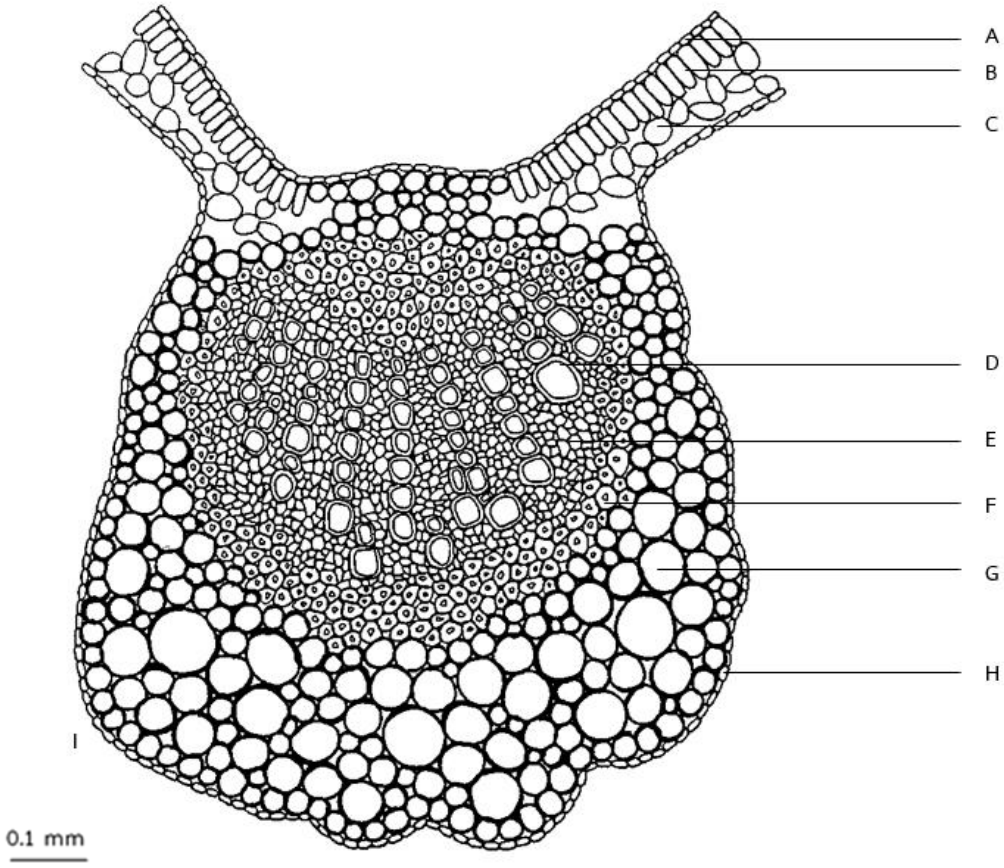


Figure 23 Anatomical characteristics of *B. ornata* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells, D = xylem,
E = phloem, F = sclerenchyma cells, G = collenchyma, H = lower epidermis,
I = stomata

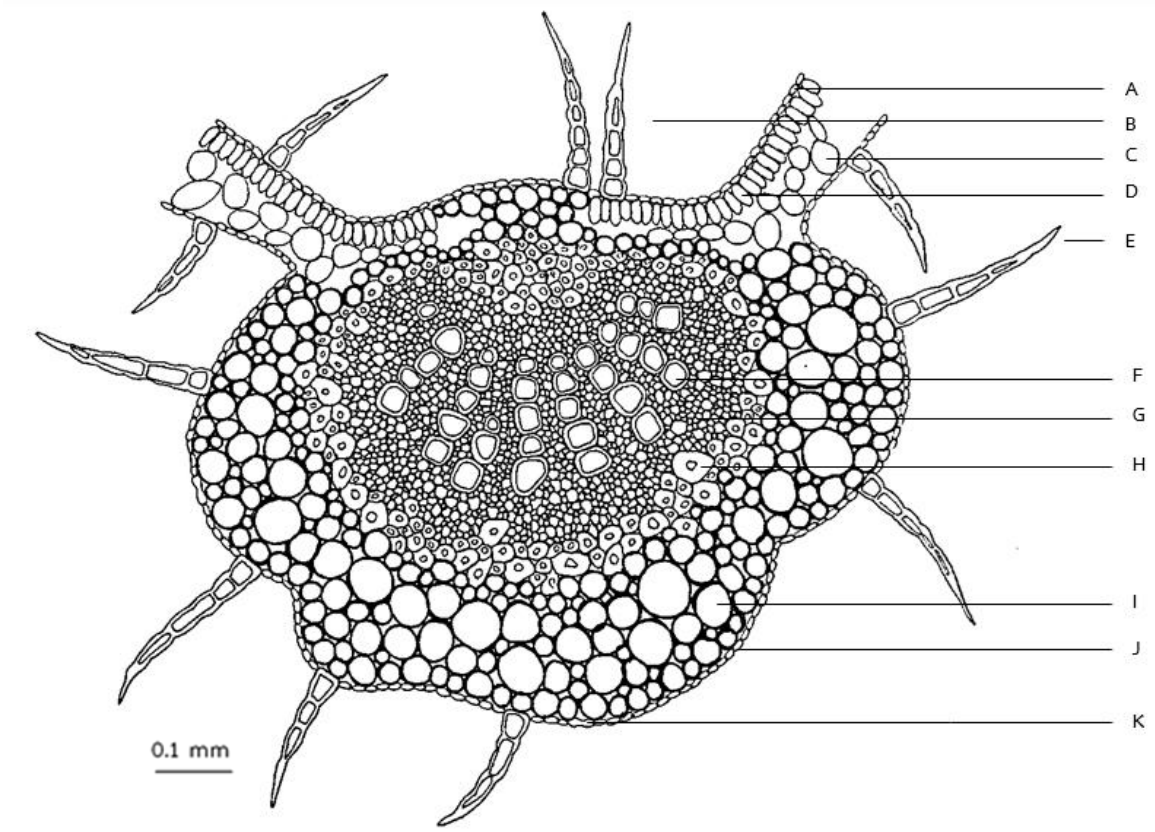


Figure 24 Anatomical characteristics of *B. pottsii* leaf midrib (cross-section)
A = upper epidermis, B = multicellular trichome (upper epidermis),
C = sponge cell, D = palisade cells, E = multicellular trichome (lower epidermis),
F = xylem, G = phloem, H = sclerenchyma cells, I = collenchyma,
J = lower epidermis, K = stomata

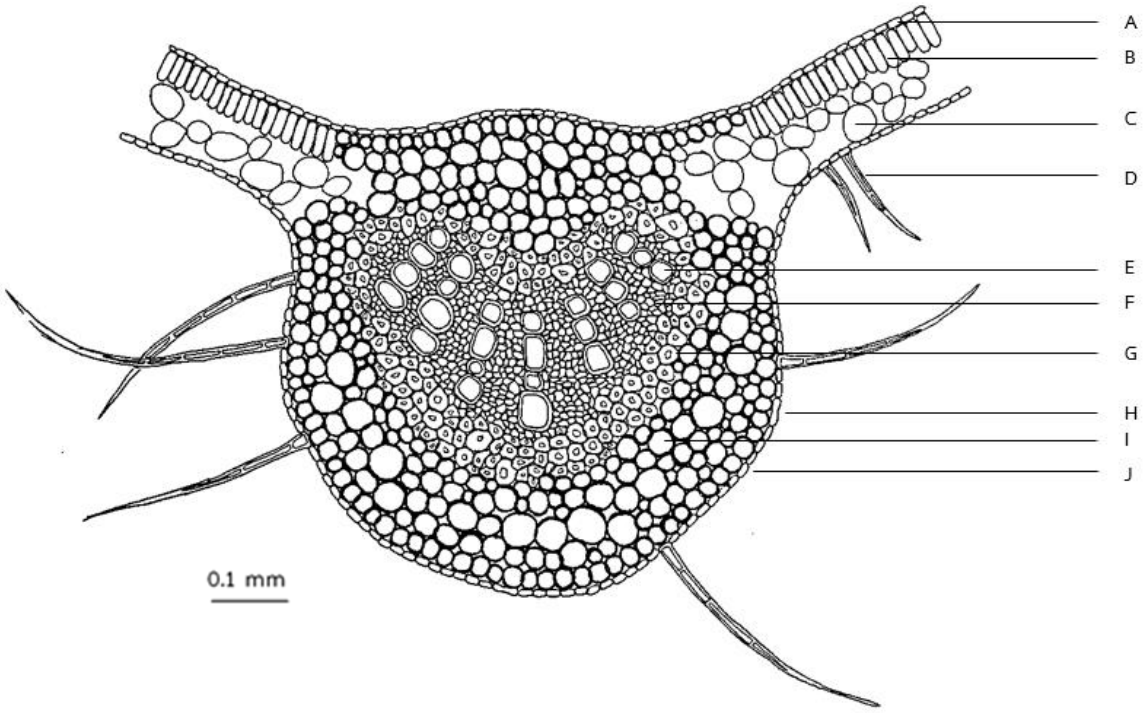


Figure 25 Anatomical characteristics of *B. pulla* leaf midrib (cross-section)
A = lower epidermis, B = palisade cells, C = sponge cells,
D = multicellular trichome, E = xylem, F = phloem, G = sclerenchyma cells,
H = stomata, I = collenchyma, J = lower epidermis

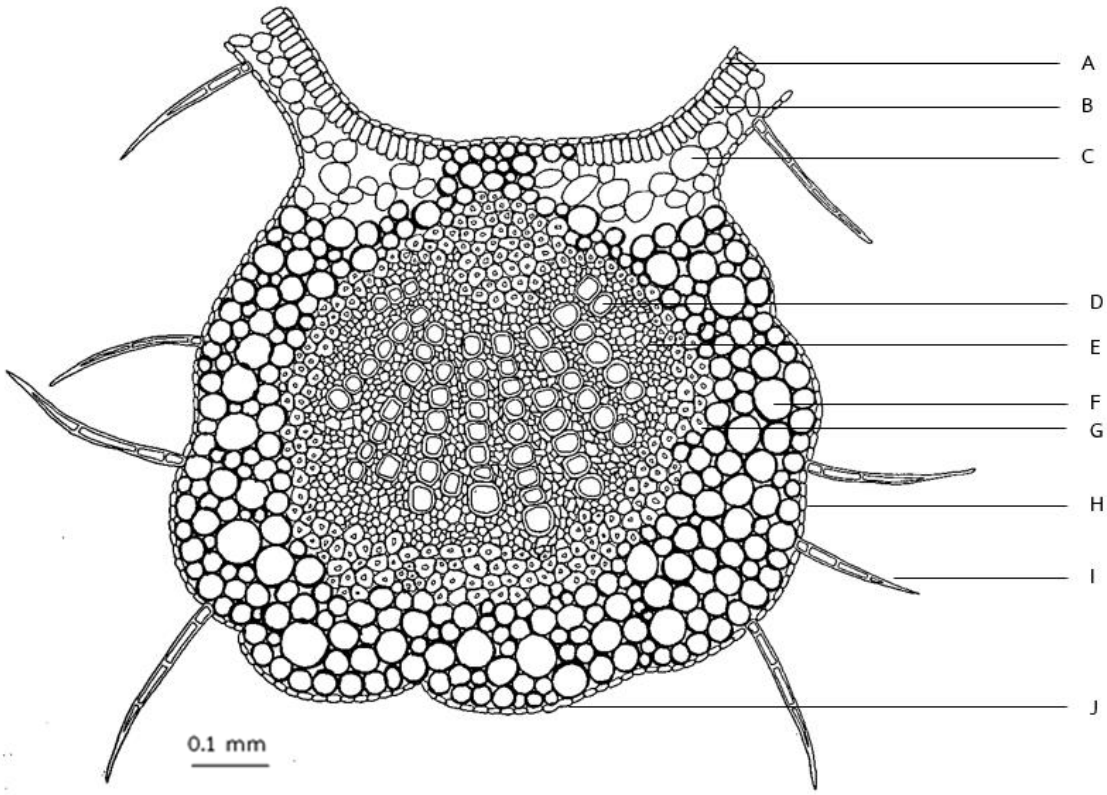


Figure 26 Anatomical characteristics of *B. purpurea* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells, D = xylem,
E = phloem, F = collenchyma, G = sclerenchyma cells, H = lower epidermis,
I = multicellular trichome, J = stomata

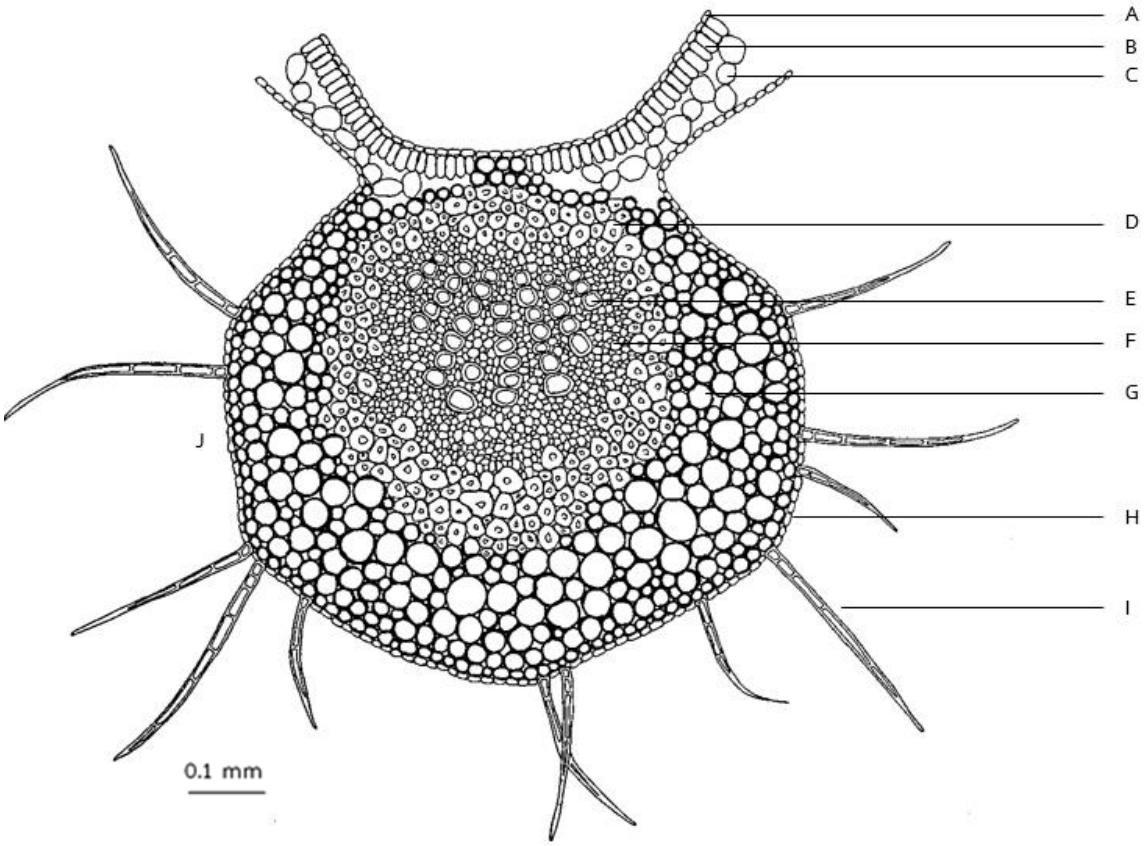


Figure 27 Anatomical characteristics of *B. racemosa* leaf midrib (cross-section) A = upper epidermis, B = palisade cells, C = sponge cells, D = sclerenchyma cells, E = xylem, F = phloem, G = collenchyma, H = lower epidermis, I = multicellular trichome, J = stomata

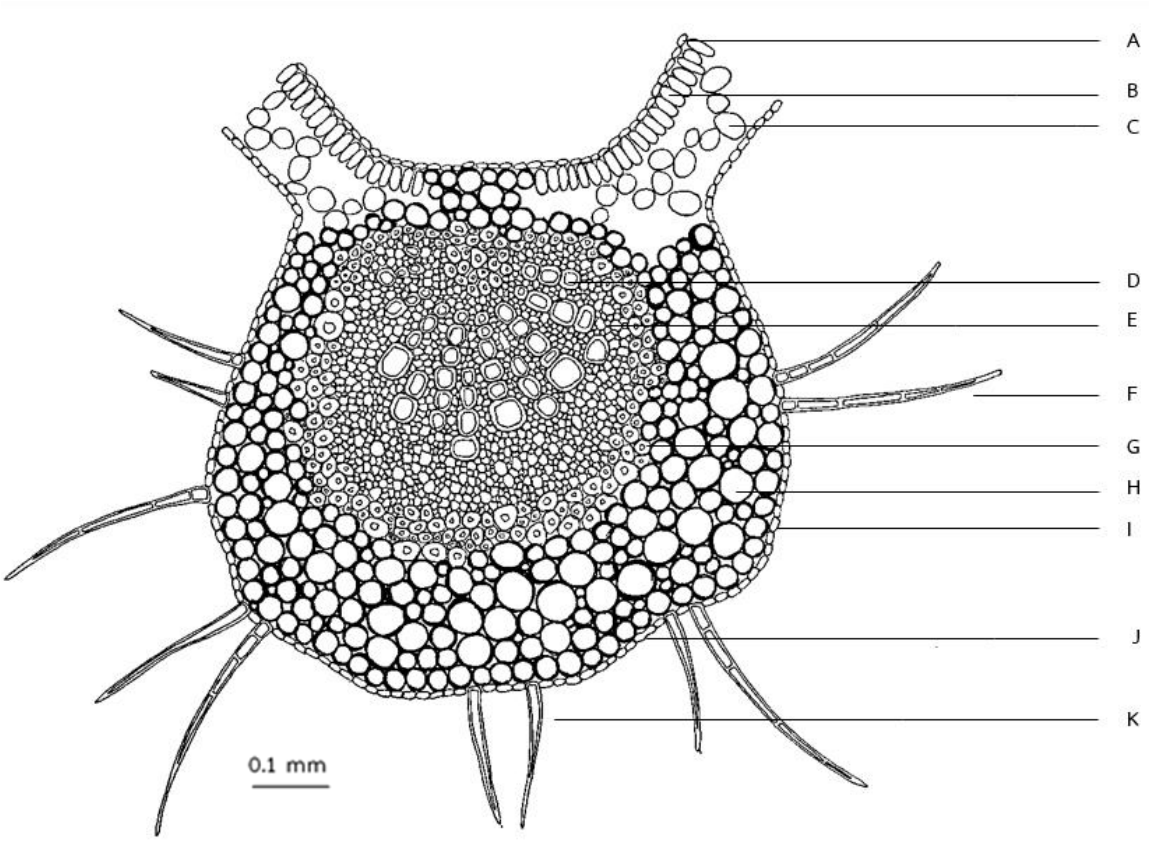


Figure 28 Anatomical characteristics of *B. saccocalyx* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells, D = xylem,
E = phloem, F = multicellular trichome, G = sclerenchyma cells, H = collenchyma,
I = lower epidermis, J = stomata, K = unicellular trichome

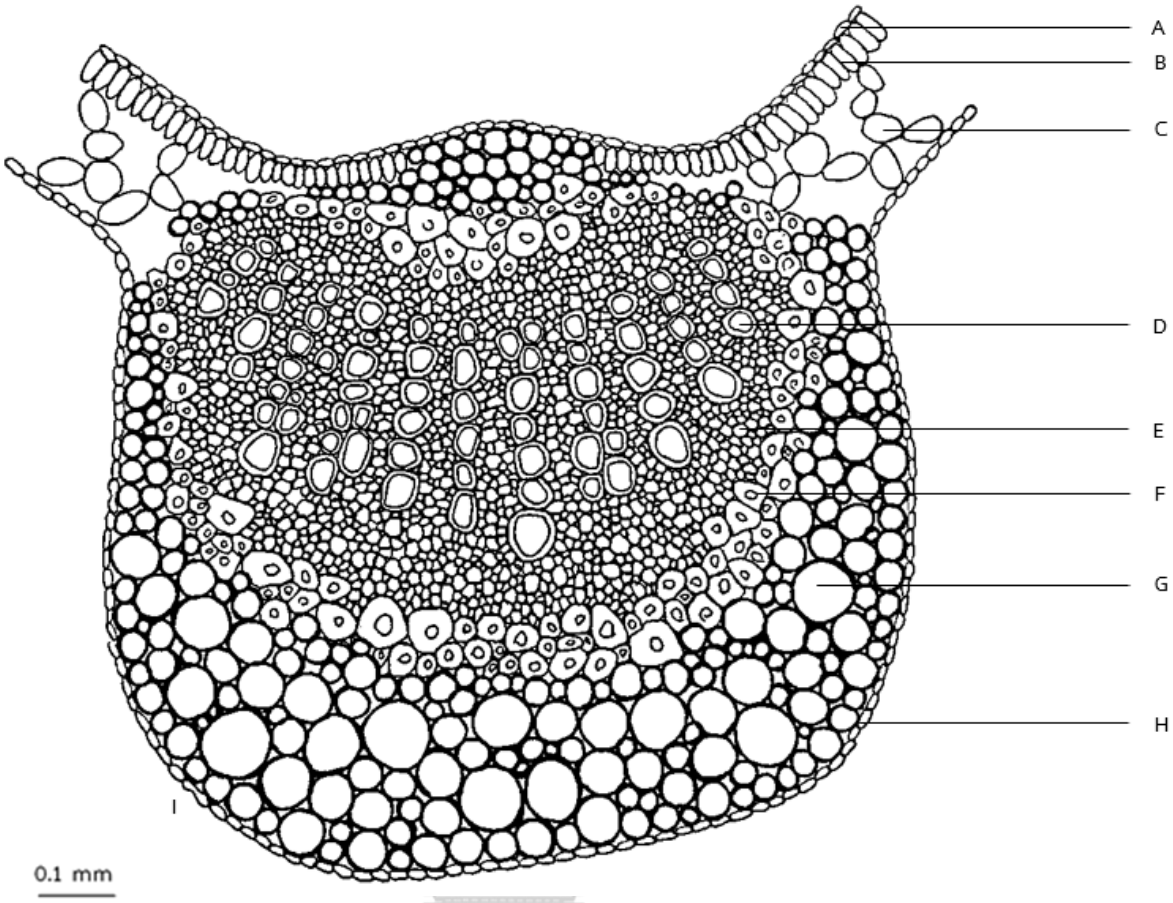


Figure 29 Anatomical characteristics of *B. scandens* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells, D = xylem,
E = phloem, F = sclerenchyma, G = collenchyma, H = lower epidermis, I = stomata

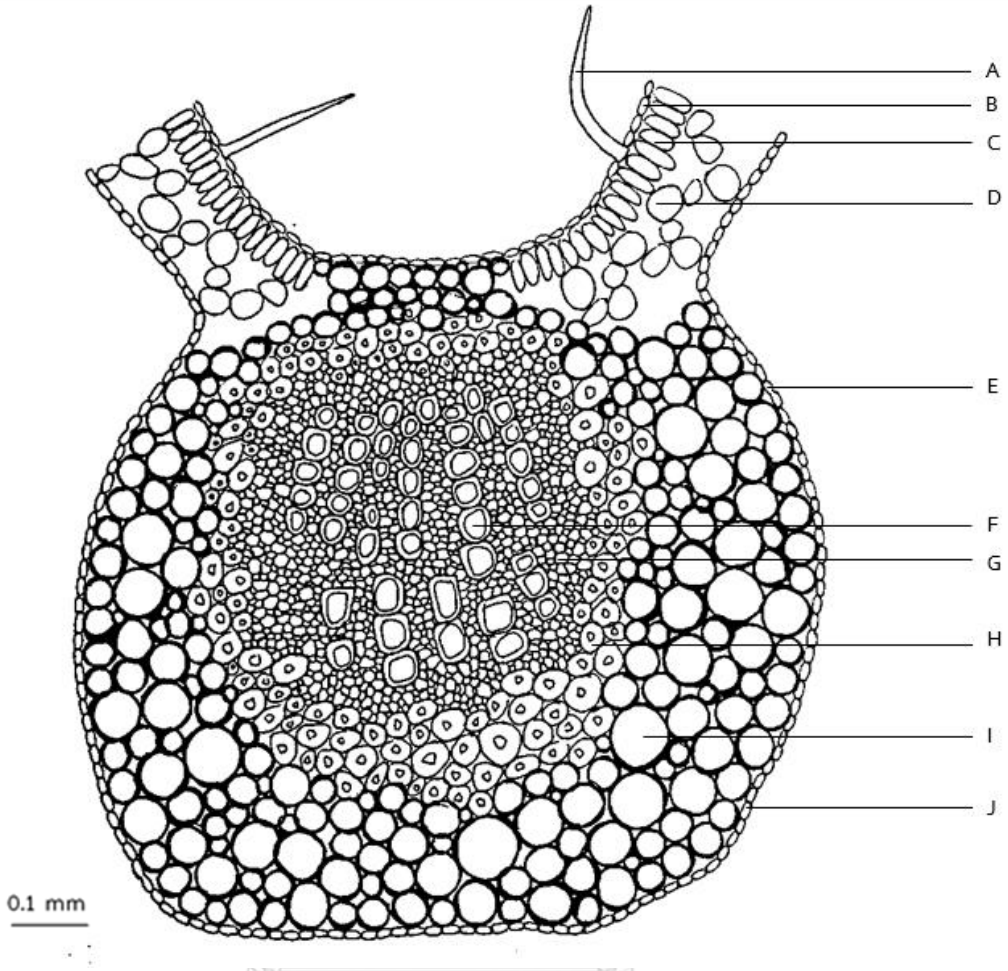


Figure 30 Anatomical characteristics of *B. siamensis* leaf midrib (cross-section)
A = unicellular trichome, B = upper epidermis, C = palisade cells,
D = sponge cells, E = stomata, F = xylem, G = phloem, H = sclerenchyma,
I = collenchyma, J = lower epidermis

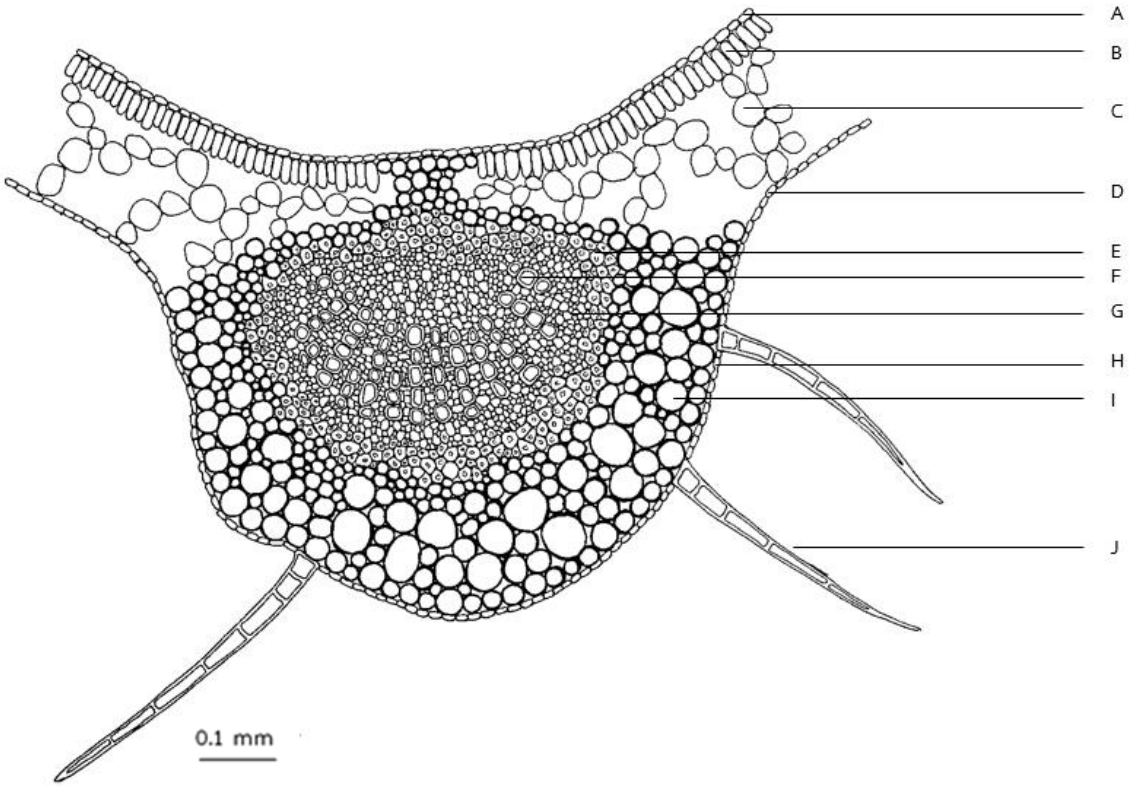


Figure 31 Anatomical characteristics of *B. sirindhorniae* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells,
D = lower epidermis, E = sclerenchyma, F = xylem, G = phloem, H = stomata,
I = collenchyma, J = multicellular trichome

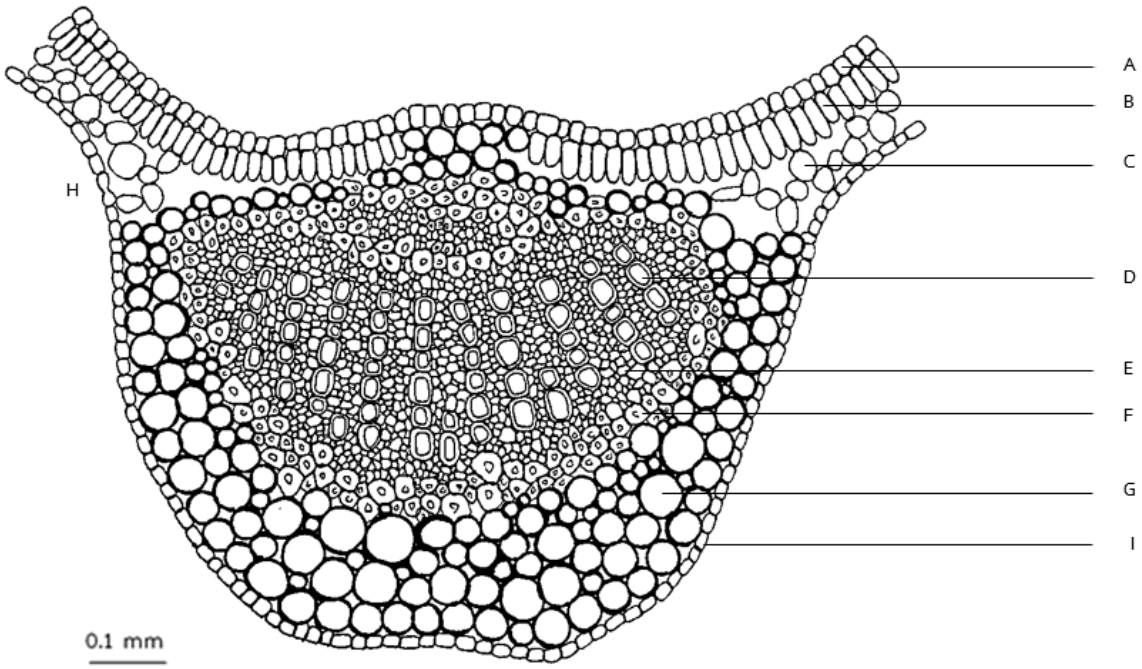


Figure 32 Anatomical characteristics of *B. strychnifolia* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells, D = xylem,
E = phloem, F = sclerenchyma, G = collenchyma, H = stomata, I = lower epidermis

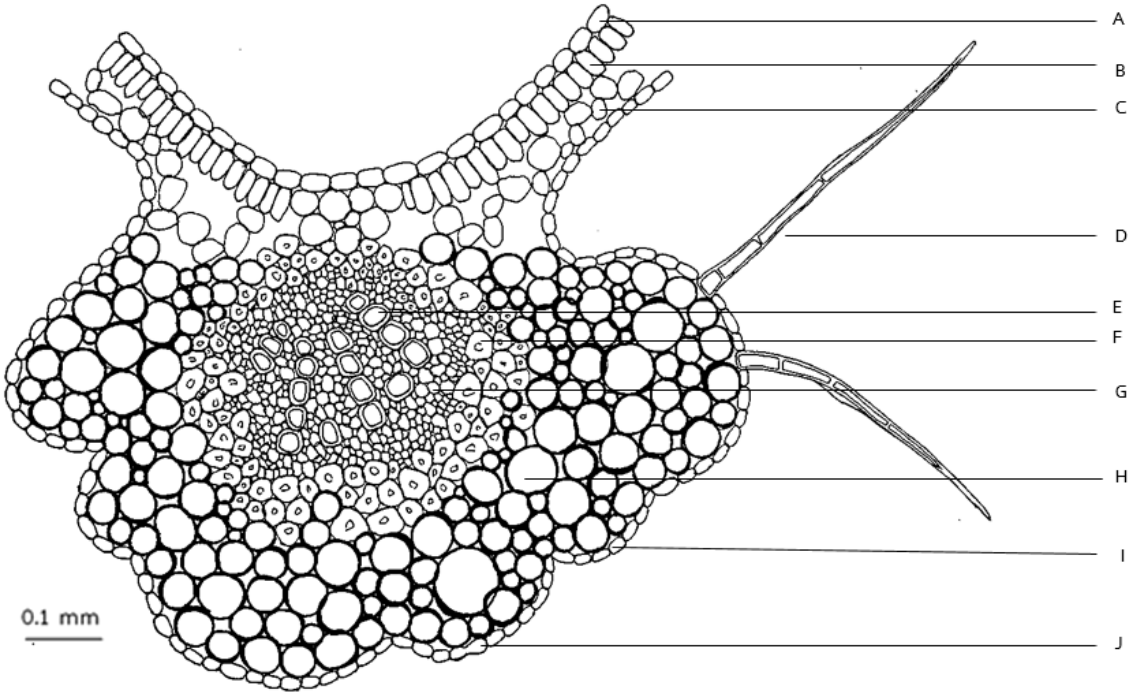


Figure 33 Anatomical characteristics of *B. tomentosa* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells,
D = multicellular trichome, E = xylem, F = sclerenchyma cells, G = phloem,
H = collenchyma, I = lower epidermis, J = stomata

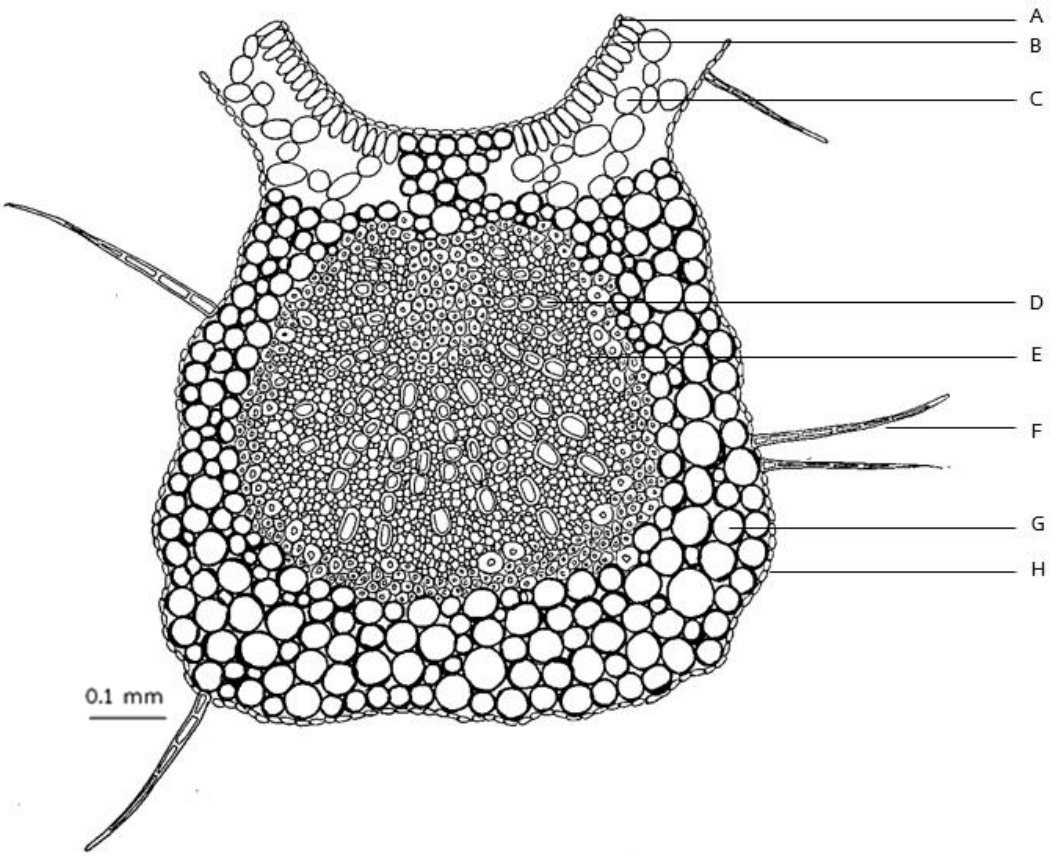


Figure 34 Anatomical characteristics of *B. variegata* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells, D = xylem,
E = phloem, F = multicellular trichome, G = collenchyma, H = lower epidermis

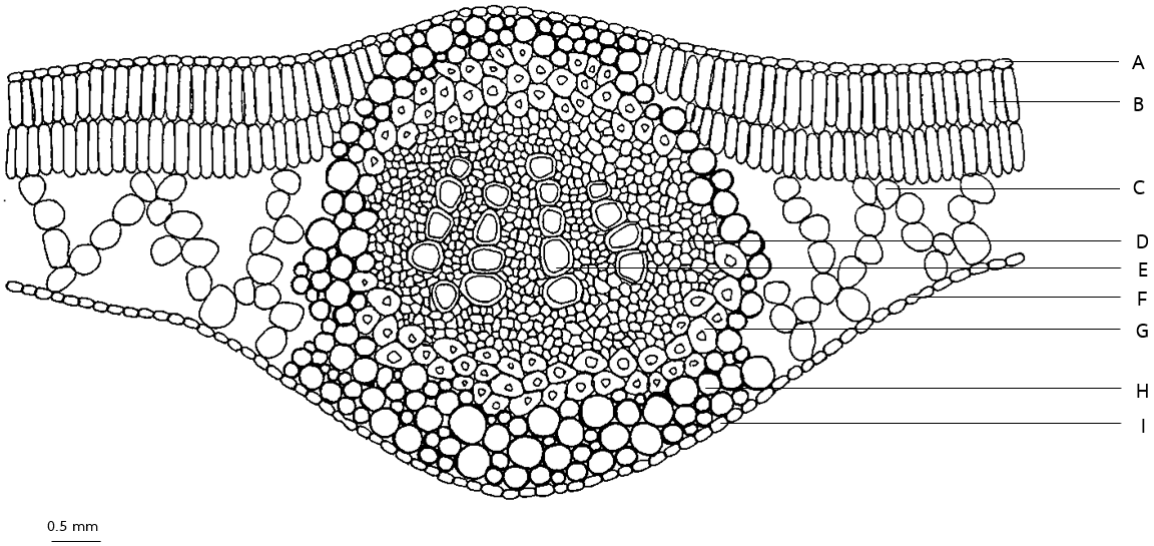


Figure 35 Anatomical characteristics of *B. winitii* leaf midrib (cross-section)
A = upper epidermis, B = palisade cells, C = sponge cells, D = phloem,
E = xylem, F = stomata, G = sclerenchyma, H = collenchyma, I = lower epidermis

Part III Pharmacognostic specification of *B. malabarica* leaf and quercetin and quercitrin contents by RP-HPLC analysis

Fifteen samples of dried *B. malabarica* mature leaves were ground into fine powders and exhaustively extracted with 95% ethanol using soxhlet apparatus. The extract was evaporated to dryness, weighed and calculated the %yield (g/100 g) (Table 15). The highest yield was 30.579 g/100 g, and the lowest yield was 18.471 g/100 g. The quantification of quercetin and quercitrin contents in *B. malabarica* mature leaves were analyzed using RP-HPLC method. The average of quercetin and quercitrin contents in fifteen different locations were 0.1796 ± 0.0678 and 0.3833 ± 0.2138 g/100 g of dried crude drug, respectively (Table 15).

Physico-chemical parameters showed that loss on drying, total ash, acid insoluble ash and water content should not be more than 8.00, 7.08, 1.79 and 8.28 % of dry weight while ethanol and water soluble extractive values should not be less than 13.78 and 16.47 % of dry weight respectively. Volatile oil content could not be detected in *B. malabarica* mature leaves using water distillation method (Table 16).

Fingerprinting of ethanolic extract of *B. malabarica* mature leaves was determined using thin layer chromatographic method. The developed TLC plate was observed under day light, UV 254 nm, UV 366 nm and dipped with p-anisaldehyde and heated with 105 ℃ for 5 minutes and 1% aluminium chloride reagents and observed under UV 366 nm (Figure 36).

Table 15 Yield of *B. malabarica* mature leaves ethanolic extract from 15 different places throughout Thailand

Source	Content (mg/100 g of dried ethanolic extract)*		% yield (g/100g)	Content (g/100 g of dried crude drug)*	
	Quercetin	Quercitrin		Quercetin	Quercitrin
1	6.5111 ± 0.0184	21.9186 ± 0.0442	27.382	0.1783 ± 0.0005	0.6002 ± 0.0012
2	6.1067 ± 0.0120	25.9711 ± 0.0214	26.691	0.1630 ± 0.0003	0.6932 ± 0.0006
3	4.5451 ± 0.0085	9.9210 ± 0.0109	29.267	0.1330 ± 0.0002	0.2904 ± 0.0003
4	4.2478 ± 0.0010	5.3450 ± 0.0088	26.862	0.1141 ± 0.0000	0.1436 ± 0.0002
5	3.2308 ± 0.0086	3.5605 ± 0.0039	30.579	0.0988 ± 0.0003	0.1089 ± 0.0001
6	5.9752 ± 0.0155	9.7721 ± 0.0184	25.115	0.1501 ± 0.0004	0.2454 ± 0.0005
7	3.8733 ± 0.0067	40.6667 ± 0.0406	18.471	0.0715 ± 0.0001	0.7512 ± 0.0007
8	6.5193 ± 0.0110	11.3567 ± 0.0335	23.262	0.1517 ± 0.0003	0.2642 ± 0.0008
9	9.1160 ± 0.0131	10.2160 ± 0.0060	29.887	0.2724 ± 0.0004	0.3053 ± 0.0002
10	7.3213 ± 0.0240	14.2740 ± 0.0087	26.199	0.1918 ± 0.0006	0.3740 ± 0.0002
11	8.3047 ± 0.0162	7.9713 ± 0.0099	26.657	0.2214 ± 0.0004	0.2125 ± 0.0003
12	9.5080 ± 0.0151	9.3187 ± 0.0042	29.908	0.2844 ± 0.0005	0.2787 ± 0.0001
13	6.0373 ± 0.0321	27.7733 ± 0.0092	25.429	0.1535 ± 0.0008	0.7062 ± 0.0002
14	8.6820 ± 0.0314	10.4533 ± 0.0012	24.184	0.2100 ± 0.0008	0.2528 ± 0.0000
15	10.2520 ± 0.0080	17.8587 ± 0.0185	29.302	0.3004 ± 0.0002	0.5233 ± 0.0005

*Mean ± SD from triplicate

Table 16 Physico-chemical parameters of *B. malabarica* dried leaves.

Parameter	Content (% by weight)
Water content	8.280 ± 0.407
Ethanol soluble extractive	13.781 ± 0.197
Water soluble extractive	16.474 ± 0.389
Loss on drying	7.998 ± 0.046
Total ash	7.079 ± 0.047
Acid insoluble ash	1.788 ± 0.184
Volatile oil content	0

*The parameters were shown as grand mean ± pooled standard deviation. Samples were from 15 different places throughout Thailand and each sample was done in triplicate.

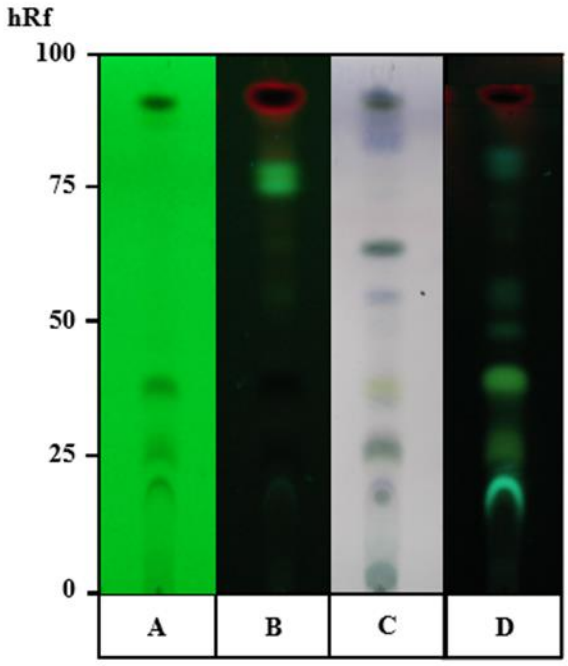


Figure 36 TLC fingerprint of *B. malabarica* leaves ethanolic extract
A=detection under UV 254 nm, B=detection under UV 366 nm,
C=stained with p-anisaldehyde ragent and heated 105 ℃ for 5 minutes and
D=stained with 1% aluminium chloride reagent and detected under UV 366 nm.

Macroscopic and microscopic evaluations of *B. malabarica* leaves

B. malabarica leaves are green color on both sides. The fresh mature leaves shape was bilobed, rough surface, thick leaf, 6 – 10 cm wide, 5 – 7 cm long (Figure 37) and sour taste. Flowers are small white or light green.

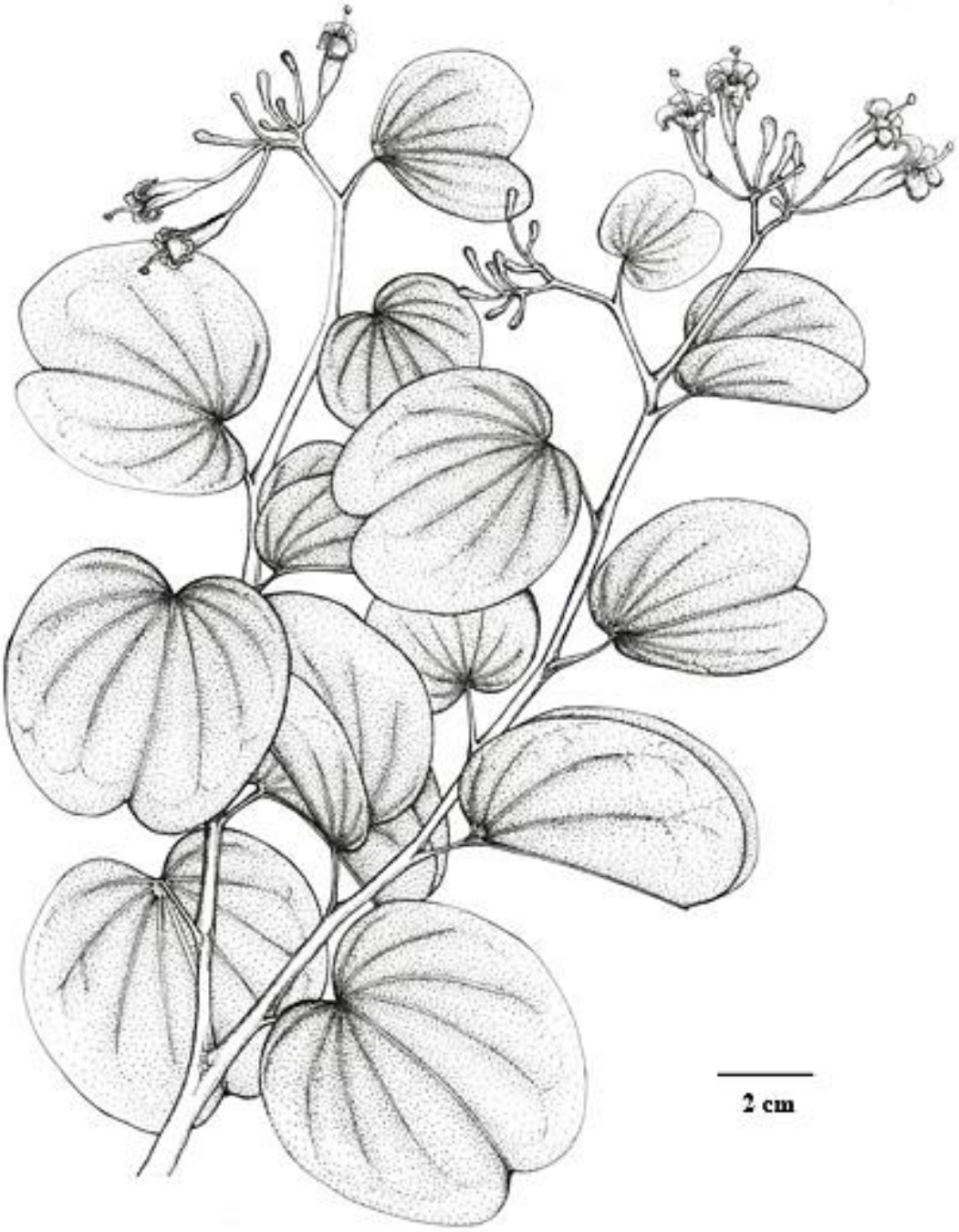


Figure 37 The branches with flowers of *B. malabarica*

Dried *B. malabarica* leaves was greenish brown color (Figure 38). The histological characteristics of *B. malabarica* leaf powder was presented i. e. multicellular trichome, paracytic stomata, epidermis, spiral vessel, prism of calcium oxalate and fibers (Figure 39). Anatomical characteristic of *B. malabarica* leaf midrib (cross-section) was shown in Figure 40.

Figure 38 Dried *B. malabarica* leaves

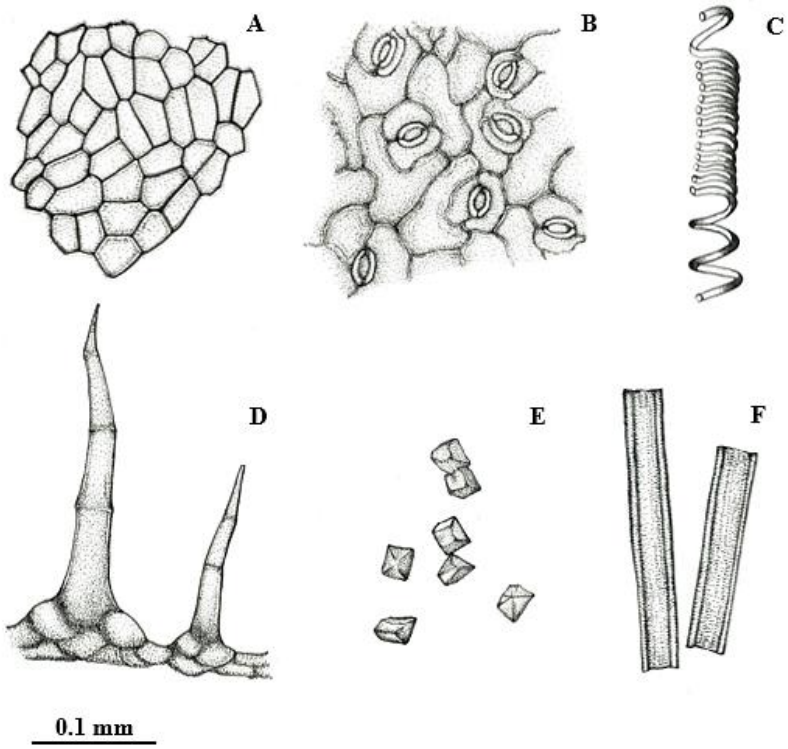


Figure 39 Histological characteristics of *B. malabarica* leaves powder
A=epidermis, B=paracytic stomata, C=spiral vessel,
D=multicellular trichome, E=prism of calcium oxalate, F=fibers

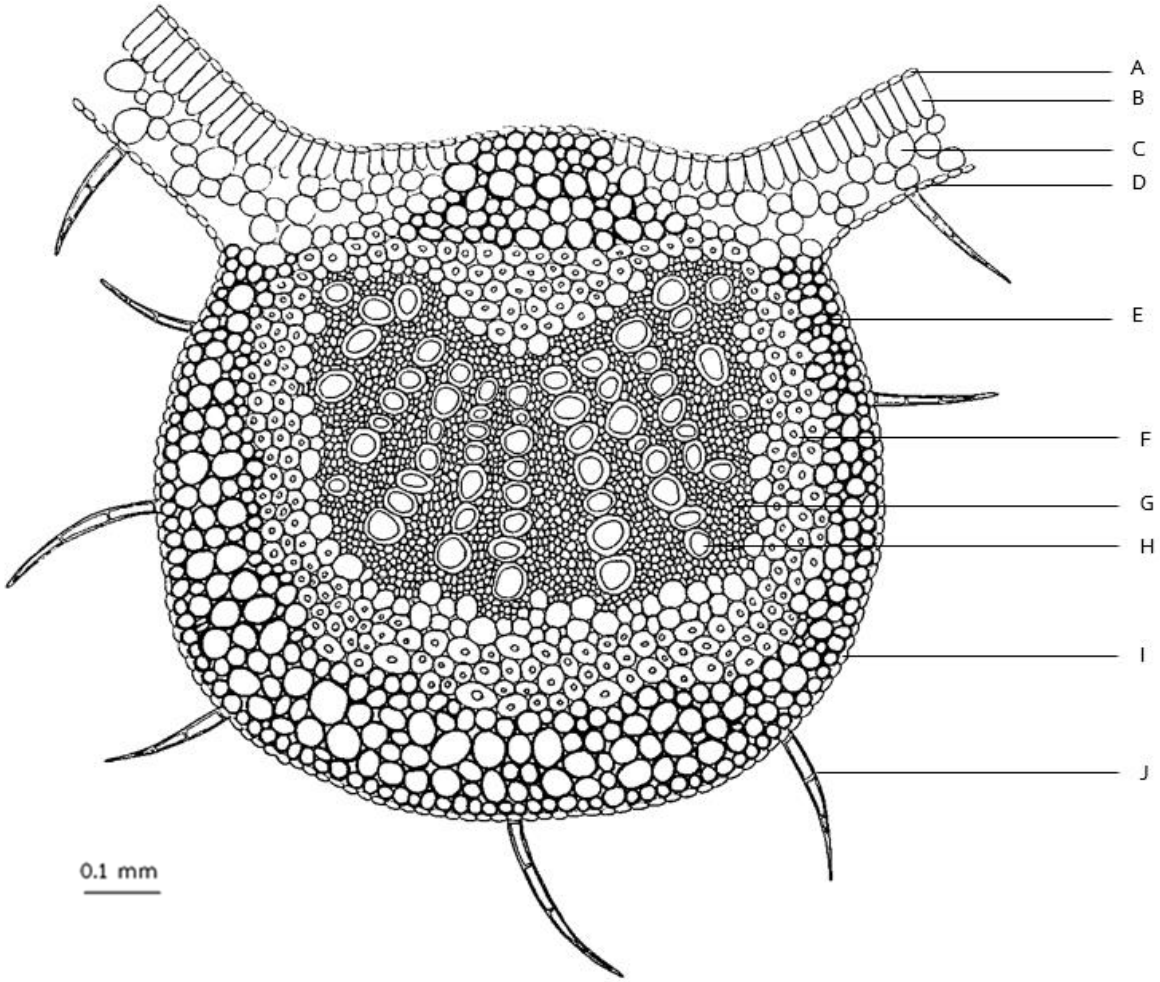


Figure 40 Anatomical characteristics of *B. malabarica* leaf midrib (cross-section) A=upper epidermis, B=palisade cells, C=sponge cells, D=lower epidermis, E=collenchyma, F=scherenchyma cells, G=phloem, H=xylem, I=stomata, J=multicellular trichome

Part IV Molecular identification

The young leaves of twenty *Bauhinia* species, *Millettia utilis*, and *Millingtonia hortensis* were extracted using CTAB method and DNA extraction kit. All samples were checked the purity, and the purity of each sample which is calculated from OD_{260}/OD_{280} was in the range of 1.8 – 2.0 using Nanodrop spectrophotometer. *Millettia utilis*, and *Millingtonia hortensis* were chosen to be outgroup. *Millettia utilis* is in the same family (Leguminosae). *Millingtonia hortensis* is in family (Bignoniaceae). Twenty two samples were screened with twenty ISSR primers. Gel electrophoresis revealed that six ISSR primers were shown clear bands and could use to be the marker for identification of DNA fingerprinting of twenty *Bauhinia* species (ISSR-01, ISSR-05, ISSR-07, ISSR-11, ISSR-12, and ISSR-32) (Figure 41 – 46). ISSR primers produced 841 bands between 187 – 3358 bp. No band was found in negative control amplification. The result was 100% polymorphism. The summary of ISSR primer sequences and the number of ISSR products were presented in Table 17.

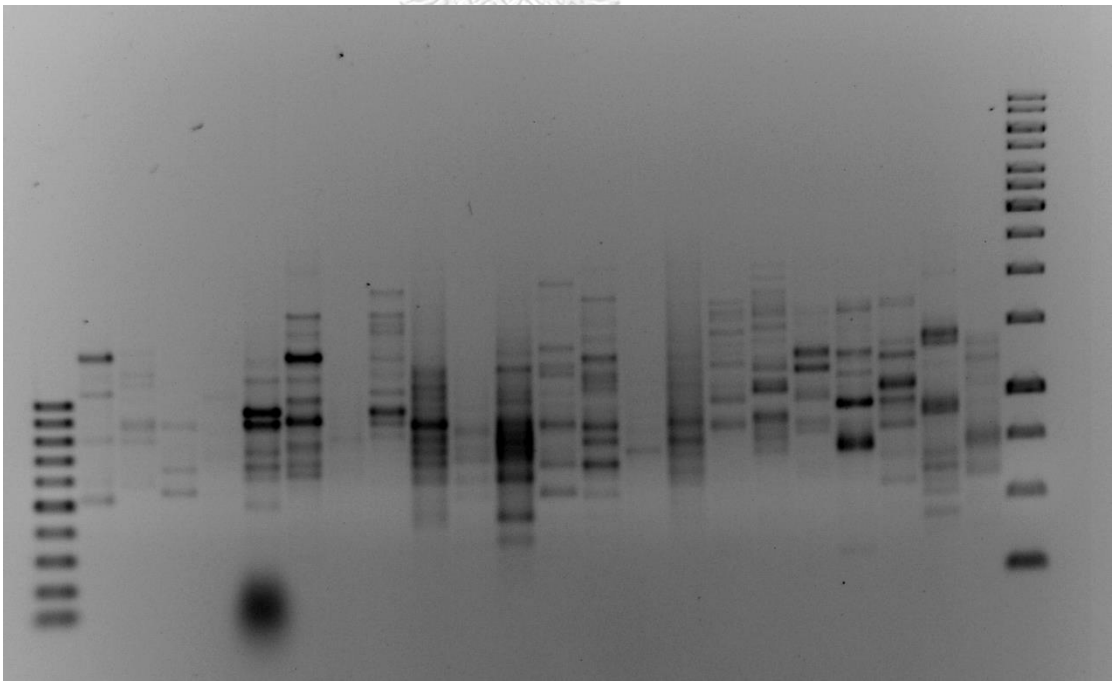


Figure 41 Fingerprint of ISSR-01

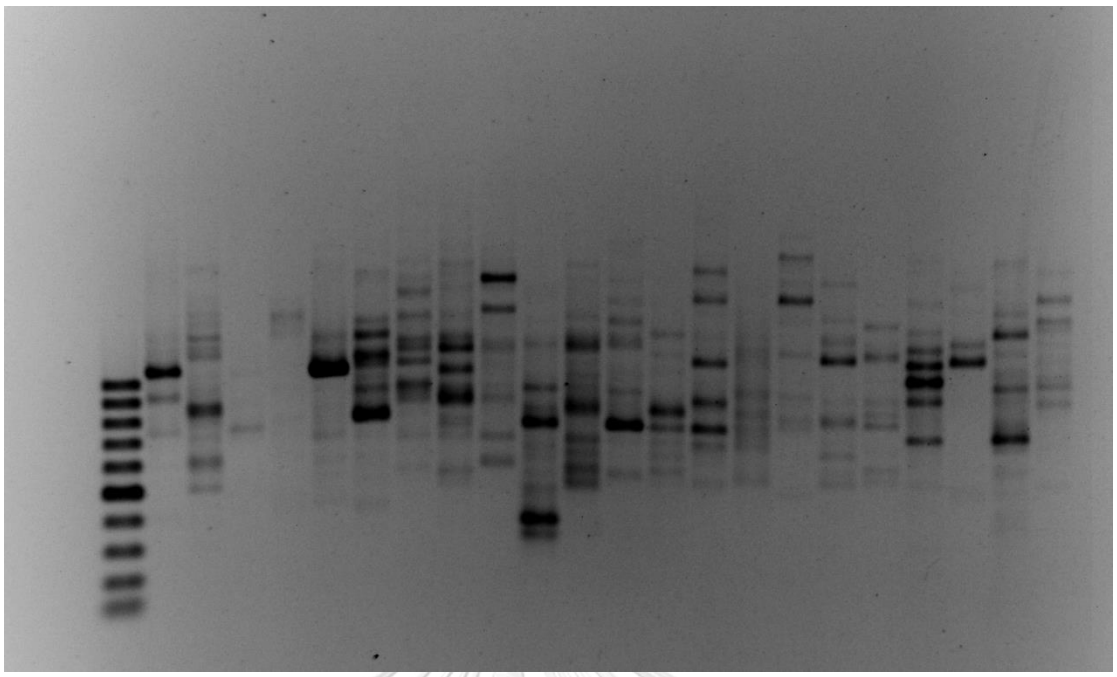


Figure 42 Fingerprint of ISSR-05

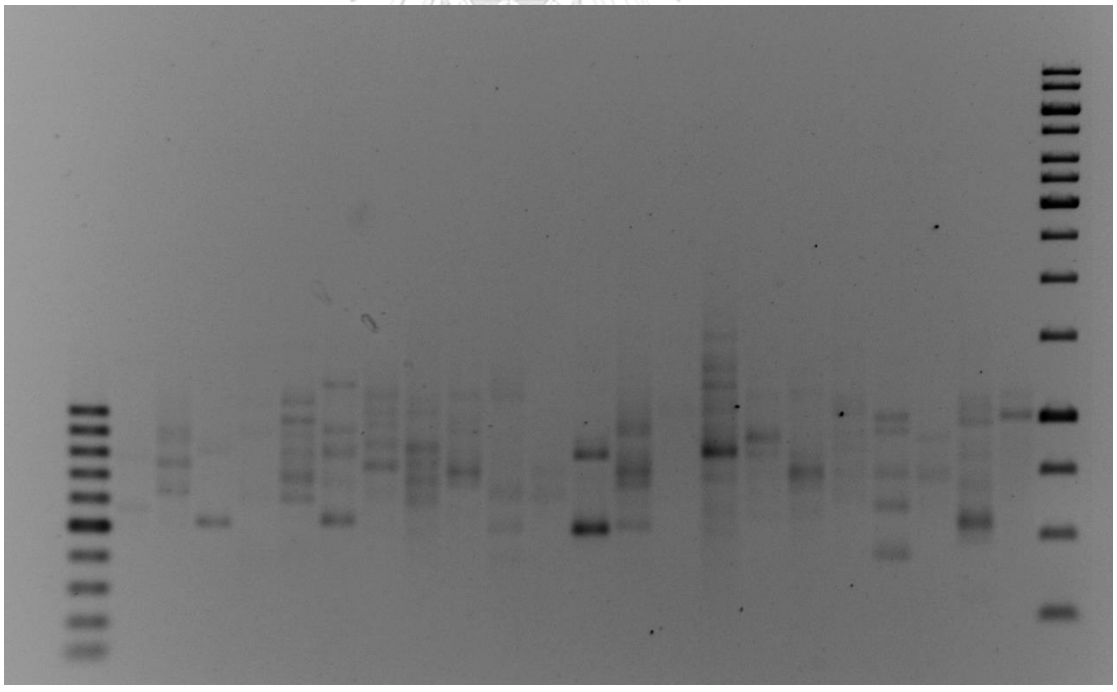


Figure 43 Fingerprint of ISSR-07

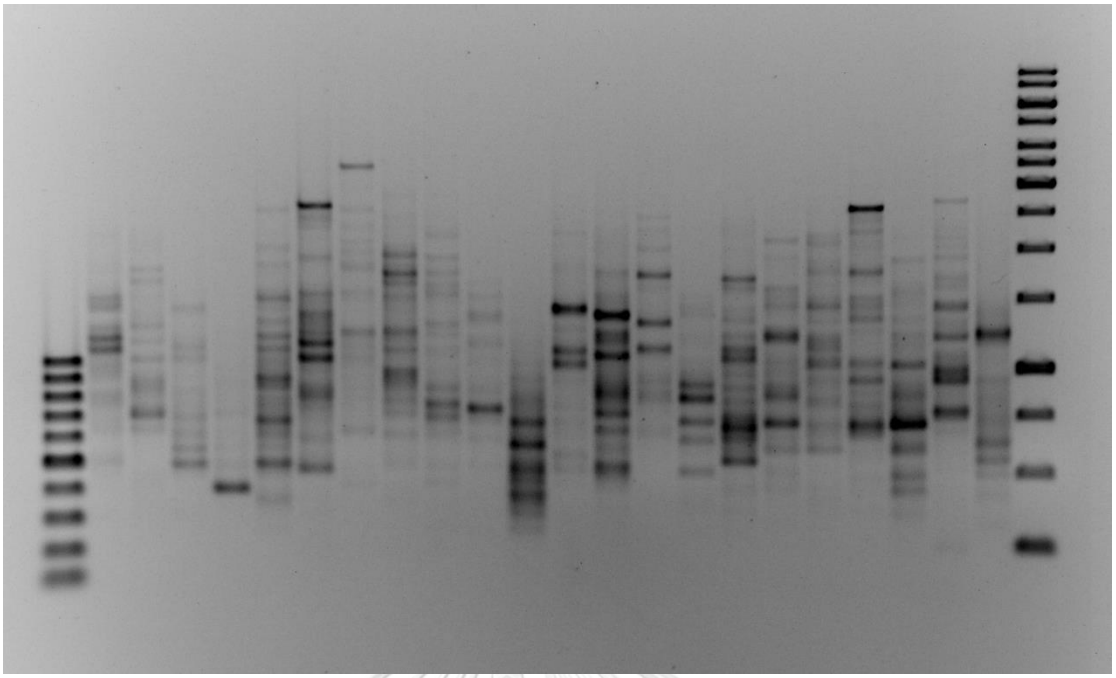


Figure 44 Fingerprint of ISSR-11

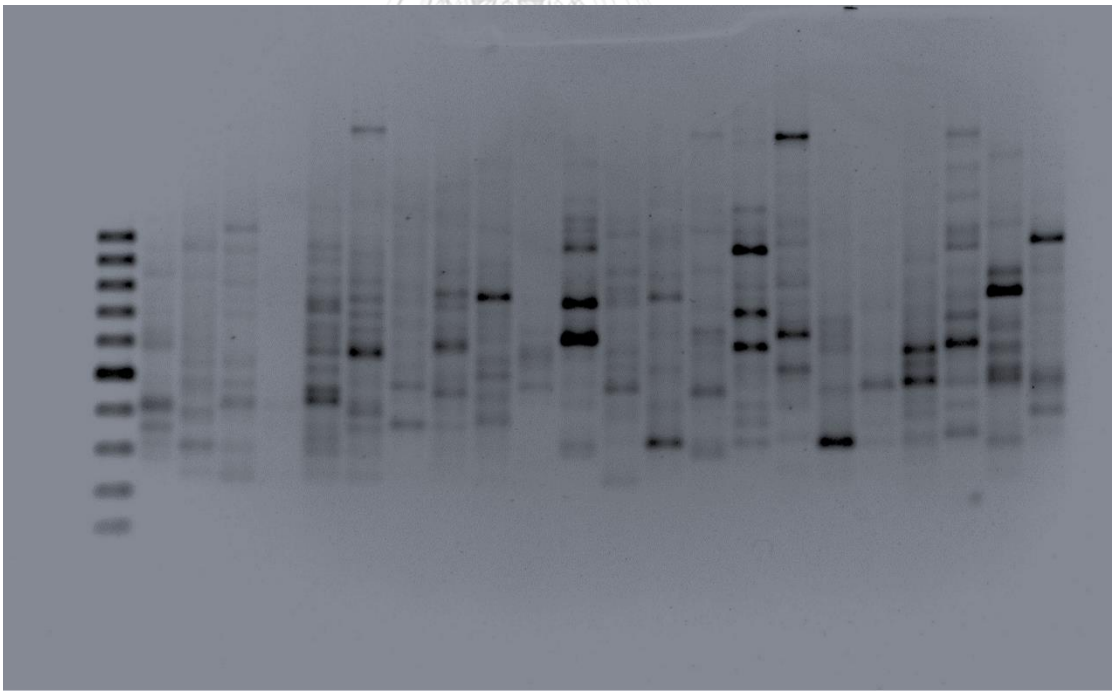


Figure 45 Fingerprint of ISSR-12

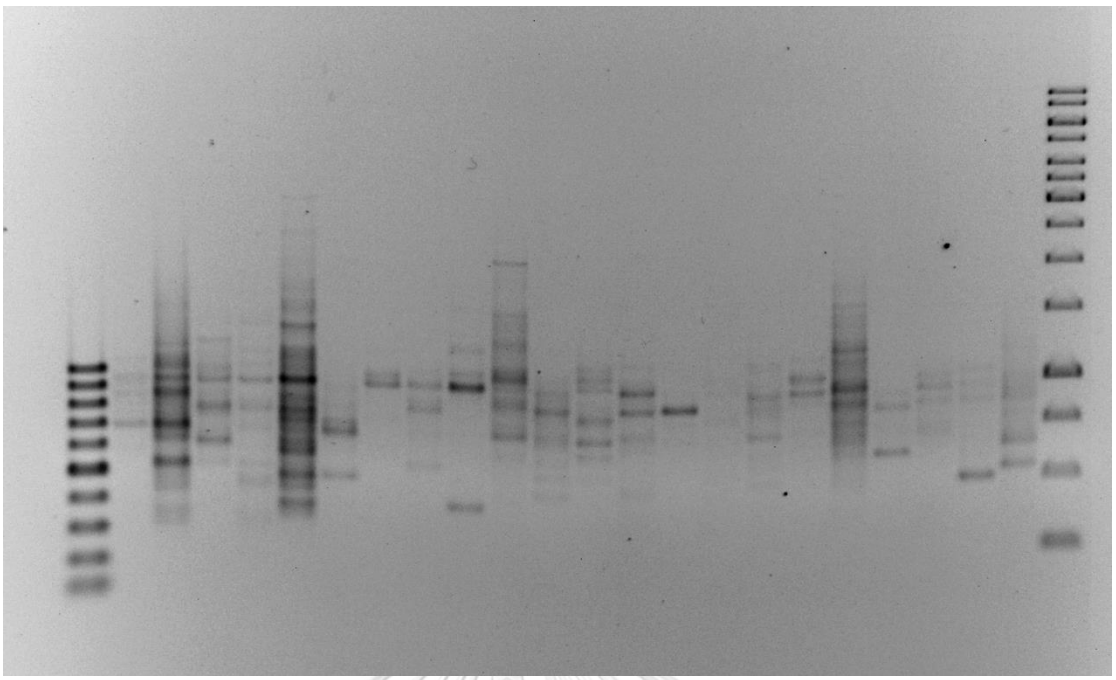


Figure 46 Fingerprint of ISSR-32

For phylogenetic analysis of twenty *Bauhinia*, and two outgroups, phylogenetic trees were constructed by UPGMA method (Figure 47). Dendrogram was constructed based on the Jaccard's similarity coefficient. The ISSR dendrogram indicated 2 different groups. Group 1 could be divided into three subgroups (1a, 1b, and 1c). Group 2 could be divided into two subgroups (2a, and 2b).

Group 1a consisted of *B. lakhonensis*, *B. aureifolia*, *B. sirindhorniae*, *B. strychnifolia*, *B. integrifolia*, and *B. pulla*.

Group 1b consisted of *B. bracteata*, *B. ornata*, *B. racemosa*, *B. tomentosa*, *B. siamensis*, *B. winitii*, and *Millettia utilis*.

Group 1c consisted of *B. galpinii*, and *B. acuminata*.

Group 2a consisted of *B. malabarica*.

Groups 2b consisted of *B. pottsii*, *B. scandens*, *B. variegata*, *B. saccocalyx*, and *B. purpurea*.

Millingtonia hortensis which is different of family and genus was out of both groups.

Table 17 ISSR primer sequences, annealing temperatures, and the number of ISSR products of twenty *Bauhinia* species

Primer	Nucleotide sequence (5' to 3')	No. of bands	Size of bands (bp)	Polymorphism (%)
ISSR-01	AGAGAGAGAGAGAGAGT	120	190 – 1966	100
ISSR-05	TCTCTCTCTCTCTCTCC	182	386 – 2509	100
ISSR-07	TGTGTGTGTGTGTGTGA	70	418 – 1484	100
ISSR-11	agagagagagagagagYt	188	250 – 3358	100
ISSR-12	agagagagagagagagYc	185	187 – 1609	100
ISSR-32	AGAGAGAGAGAGAGC	96	319 – 2379	100
	Total	**841**	**187– 3358**	

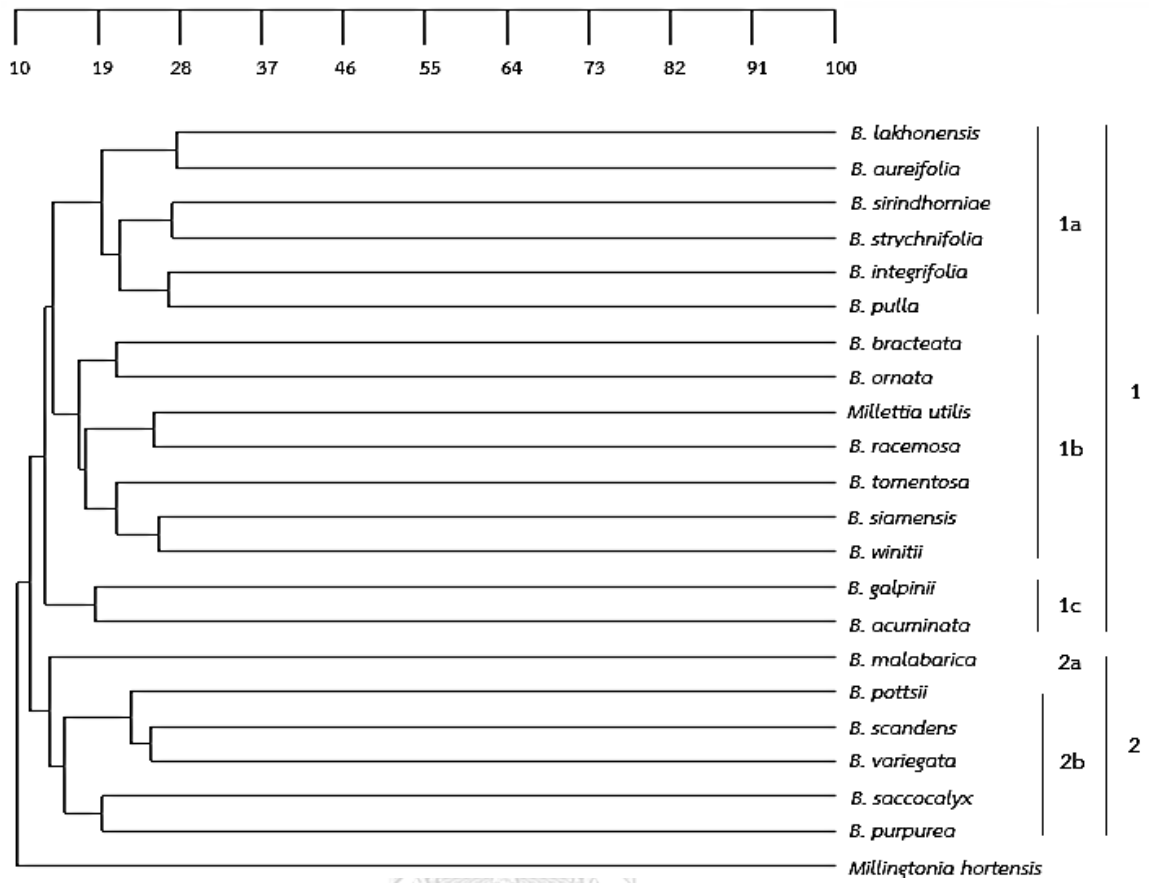


Figure 47 Dendrogram of twenty *Bauhinia* species, and two outgroups (*Millettia utilis*, and *Millingtonia hortensis*) based on ISSR markers (UPGMA)

The Jaccard's similarity matrix was used to analyze the genetic similarity coefficients among twenty *Bauhinia* species in Thailand. The highest similarity value was presented between *B. aureifolia* and *B. lakhonensis* at 0.279. The lowest similarity value was presented between *B. scandens* and *B. galpinii* at 0.036. The similarity index of twenty *Bauhinia* species and two outgroups (*Millettia utilis*, and *Millingtonia hortensis*) was shown in Table 18.

Table 18 Similarity index of selected *Bauhinia* species and outgroup

	B. lakhonensis	*B. aureifolia*	*B. sirindhorniae*	*B. strychnifolia*	*B. integrifolia*	*B. pulla*	*B. bracteata*	*B. ornata*	*Millettia utilis*	*B. racemosa*	*B. tomentosa*	*B. siamensis*	*B. winitii*	*B. galpinii*	*B. acuminata*	*B. malabarica*	*B. pottsii*	*B. scandens*	*B. variegata*	*B. saccocalyx*	*B. purpurea*	*Millingtonia hortensis*
B. lakhonensis	1.000																					
B. aureifolia	0.279	1.000																				
B. sirindhorniae	0.126	0.268	1.000																			
B. strychnifolia	0.174	0.192	0.275	1.000																		
B. integrifolia	0.189	0.191	0.192	0.206	1.000																	
B. pulla	0.267	0.188	0.216	0.256	0.272	1.000																
B. bracteata	0.135	0.230	0.150	0.179	0.142	0.161	1.000															
B. ornata	0.064	0.097	0.163	0.189	0.111	0.156	0.214	1.000														
Millettia utilis	0.130	0.104	0.133	0.198	0.163	0.134	0.166	0.163	1.000													
B. racemosa	0.098	0.082	0.255	0.201	0.165	0.243	0.205	0.151	0.255	1.000												
B. tomentosa	0.120	0.109	0.137	0.157	0.130	0.156	0.169	0.162	0.216	0.186	1.000											
B. siamensis	0.181	0.202	0.165	0.108	0.105	0.130	0.185	0.180	0.213	0.187	0.202	1.000										
B. winitii	0.137	0.178	0.108	0.091	0.115	0.091	0.199	0.163	0.153	0.128	0.228	0.260	1.000									
B. galpinii	0.073	0.112	0.116	0.074	0.149	0.082	0.118	0.189	0.128	0.101	0.124	0.169	0.180	1.000								
B. acuminata	0.155	0.150	0.240	0.122	0.149	0.171	0.162	0.097	0.138	0.122	0.116	0.167	0.145	0.190	1.000							
B. malabarica	0.133	0.051	0.095	0.117	0.156	0.140	0.102	0.113	0.128	0.124	0.105	0.177	0.193	0.125	0.102	1.000						
B. pottsii	0.137	0.097	0.131	0.174	0.161	0.121	0.110	0.103	0.087	0.137	0.113	0.159	0.084	0.045	0.099	0.093	1.000					
B. scandens	0.175	0.075	0.198	0.209	0.117	0.124	0.197	0.169	0.077	0.167	0.154	0.089	0.198	0.036	0.149	0.132	0.209	1.000				
B. variegata	0.193	0.096	0.096	0.229	0.174	0.093	0.153	0.100	0.147	0.126	0.186	0.084	0.162	0.082	0.045	0.206	0.249	0.252	1.000			
B. saccocalyx	0.101	0.065	0.083	0.126	0.137	0.082	0.147	0.146	0.044	0.096	0.107	0.099	0.082	0.116	0.088	0.119	0.148	0.196	0.117	1.000		
B. purpurea	0.088	0.043	0.072	0.132	0.154	0.087	0.121	0.071	0.086	0.104	0.203	0.127	0.153	0.085	0.081	0.149	0.147	0.107	0.223	0.199	1.000	
Millingtonia hortensis	0.044	0.095	0.059	0.098	0.108	0.088	0.184	0.141	0.107	0.131	0.075	0.138	0.079	0.091	0.171	0.132	0.045	0.121	0.065	0.094	0.123	1.000

CHAPTER V

DISCUSSION AND CONCLUSION

RP-HPLC is the popular method which is used for the separation of secondary metabolites in plants. In this study, RP-HPLC exhibits a potential in separating quercetin and quercitrin in all twenty *Bauhinia* species. *B. malabarica* leaves have been used in Thai traditional remedy for a long time. Kaewamatawong *et al*. (2008) reported that seven flavonols including quercetin and quercitrin have been isolated from the methanolic extracts of *B. malabarica* leaves by various chromatographic techniques. In this study, the ethanolic leaf extracts of fourteen *Bauhinia* species contained both quercetin and quercitrin. *B. malabarica* extract was revealed the highest yields of quercetin and quercitrin (0.1918 and 0.3740 g/100 g of dried crude drug respectively). *B. bracteata* and *B. variegata* extracts contained only quercetin. The study in Brazil also reported only quercetin found in 70% ethanolic leaf extracts of *B. variegata* (Silveira *et al*., 2015).

In this study, the optimum wavelength was set at 255 nm which could be absorbed by both quercetin and quercitrin. Peak purity determination based on selected multiple spectral inputs of diode array detector is capable to differentiate coeluted compounds. If the peak is pure, spectra taken at several points during a peak elution should all be identical (Papadoyannis & Gika, 2004). Method validation is done to confirm the reliability of the quantitative analysis. In this study, the quantification of quercetin and quercitrin in *Bauhinia* leaf extracts were developed. The results of method validation of this study were in the acceptable range. The acceptable %recovery is between 80 – 120 % (ICH Guideline, 2005). The result of %RSD determined the error of the method, the acceptable RSD was not more than 15 % (U.S. Department of Health and Human Sevices, 2001). The small variations of column temperature, flow rate and detection wavelength resulted in %RSD < 8, so,

the method was robust. However, RP-HPLC analysis of quercetin and quercitrin in *Bauhinia* species in this study was preliminary because only one sample of each species was used for quantification.

For the macroscopic evaluation of twenty *Bauhinia* species, it could be divided into three groups, i. e. 1) tree group (*B. malabarica*, *B. purpurea*, *B. racemosa*, and *B. variegata*), 2) shrub group (*B. acuminata*, *B. galpinii*, *B. pottsii*, *B. saccocalyx*, and *B. tomentosa*), and 3) climber group (*B. aureifolia*, *B. bracteata*, *B. integrifolia*, *B. lakhonensis*, *B. ornata*, *B. pulla*, *B. scandens*, *B. siamensis*, *B. sirindhorniae*, *B. strychnifolia*, and *B. winitii*). However, the characteristics of the leaf could not absolutely identify because all nineteen *Bauhinia* species had the butterfly-leaf shape except the leaf of *B. strychnifolia*. So, others plant authentication methods such as microscopic evaluation, and DNA fingerprinting of each species could be the important parameters.

Microscopic evaluation of leaves consisted of midrib cross-section, and microscopic leaf measurement were performed. The midrib cross-section of each *Bauhinia* species showed the distinctive arrangement of upper epidermis, palisade cells, spongy cells, sclerenchyma, collenchyma, phloem tissue, xylem tissue, and lower epidermis. Moreover, the presence or absence of unicellular trichomes and multicellular trichomes is one of the important characteristics of each species and could be used for differentiation of twenty *Bauhinia* species. Six species revealed the absence of trichomes (*B. ornata*, *B. pulla*, *B. scandens*, *B. sirindhorniae*, *B. strychnifolia*, and *B. winitii*). *B. bracteata*, *B. lakhonensis*, and *B. siamensis* presented trichomes in both upper and lower epidermis.

Midrib cross-section and microscopic leaf measurement of twenty *Bauhinia* species could be used to identify and differentiate in some species. *B. winitii* was only one of twenty *Bauhinia* species which found two layers of palisade cells. This

character could be used to differentiate from other samples. *B. saccocalyx* was found both unicellular trichome and multicellular trichome on both upper and lower epidermis in midrib and surface of leaves. This species could not be determined the stomatal number and epidermal cell number on the lower epidermis due to a large number of trichomes. The result of trichome number of *B. saccocalyx* was presented the highest value up to 200 per mm^2 of lower epidermis which could be used to differentiate from other species. *B. purpurea* and *B. variegata* had similar midrib anatomy. However, they could be characterized by trichome number. The result of trichome number of *B. purpurea* were found around two times of trichome number of *B. variegata* (Table 13). Furthermore, B. purpurea was shown the highest amount of stomatal number (1120 – 1208 per mm^2) while other species was found around 140 – 800 per mm^2. The midrib cross-section and lamina observation of *B. scandens* showed no trichome on both upper and lower sides and revealed the lowest amount of stomatal number. The stomata type is the other important parameter which is used for authentication of the plant species. In this study, twenty *Bauhinia* species revealed the paracytic stomata type. Stomata are usually present on the lower epidermis of the leaf more than on the upper side because of the protection of water loss. However, eight *Bauhinia* species had found stomata on both upper and lower sides (*B. aureifolia*, *B. integrifolia*, *B. lakhonenesis*, *B. purpurea*, *B. scandens*, *B. strychnifolia*, *B. variegata*, and *B. winitii*).

Kotresha and Seetharam (1995) performed the epidermal studies in dry leaves of some *Bauhinia* species and reported that anomocytic, paracytic, anisocytic, and tetracytic stomata types have been presented. *B. malabarica* and *B. acuminata* were reported in accordance with this research as hypostomatic. *B. acuminata* showed trichomes on lower epidermis only as found in this research. On the contrary, *B. racemosa* was reported to have stomata at both sides of epidermis but

this research found only at lower epidermis. This research observed the mature fresh leaves and found only paracytic stomata type were in twenty *Bauhinia* species.

Other characters of leaves which could be used to characterize twenty *Bauhinia* species were the characteristics of epidermal cells on the upper and lower sides. Twenty *Bauhinia* species were revealed the different characters of upper and lower epidermis (Table 12).

Stomata are easily affected by external environmental conditions such as temperature, moisture, radiation, carbon dioxide in the atmosphere and humidity and nutrient in the soil. The density, size, and shape of stomata from different plants possess different characteristics. In addition, the type of stomata from the same genus or from different germplasm resources of the same species also revealed the various results (Jia *et al*., 2015 and Zhu *et al*., 2016).

Palisade ratio is another parameter for identification and evaluation of plant species. This parameter can be defined as the average number of palisade cells present beneath each upper epidermis cell. This value remains constant within a range for a given plant species and is of diagnostic value in differentiating the species. This value dose not based on geographical variation (Mukherjee, 2002). Modh *et al*. (2011) studied the microscopic leaf measurement of *B. variegata* in India and reported the palisade ratio as 4.8. This study found the palisade ratio as 4.50 – 5.75 of *B. variegata* in Thailand. Furthermore, they showed unicellular and multicellular trichomes in *B. variegata* leaf powder as same as this study. Unicellular and multicellular trichomes of *B. variegata* were presented on the lower epidermis. Trichome characteristics could be an important parameter for identifying plant species.

B. malabarica leaves have been used in Thai remedy for a long time. However, the study of the pharmacognostic specification of this plant was not established. Standaridization of plant materials could be followed from many guidelines. This study was followed WHO guideline (2011). The determination of water content, loss on drying, total ash, acid insoluble ash, water extractive, and ethanol extractive values were done. The determination of volatile oil could not be detected. All parameters could be established for standardization of *B. malabarica* leaves in Thailand. TLC fingerprints of ethanolic extract of *B. malabarica* mature leaves were demonstrated. This method could be the simply and easily method for identification of *B. malabarica* leaves.

The misidentification usage of plants can cause a risk practical application in both agriculture and medicine due to their side-effects and toxicity. Many methods have been employed for medicinal plant authentication such as morphological, anatomical, chemical constituents and DNA markers.

Inter-simple sequence repeat (ISSR) marker was chosen to identify a genetic marker to establish the relationships of twenty *Bauhinia* species. Nowadays, the studies of molecular identification of *Bauhinia* species are not popular while the uses of them are known for a long time. The ISSR marker approach particularly valuable in the study of the genus *Bauhinia*, where extensive genetic characterizations of the nuclear genomes are lacking. This study employed the ISSR technique in the analysis of twenty *Bauhinia* species to determine their genetic relatedness. The results indicated that these species could be easily characterized by ISSR markers. The dendrogram obtained by the UPGMA method using the 841 ISSR markers scored in the twenty *Bauhinia* species clearly shows two defined groups, group 1 and group 2, in Figure 47. *B. malabarica* was separated into one subgroup of group 2. *Millingtonia hortensis* which is outgroup from different family was out of related groups while *Millettia utilis* could not separate from *Bauhinia* species. Each primer could used to

be the fingerprint of these twenty *Bauhinia* species because the result was presented 100 % polymorphic. This ISSR-PCR method from this study revealed the good fingerprint of each *Bauhinia* species and could separate plants in different family.

The identification of plants is the first priority of the studies. Morphological and biochemical markers that are used to identify and differentiate plants are affected by environmental factors, age of plants, collection of plants, and growth places while molecular markers are not affected. However, the molecular identification could not identify the part of plants. So, the authentication of the plant material needs several methods to identify plant species and part used.

In conclusion, twenty *Bauhinia* species were revealed for preliminary contents of quercetin and its glycoside (quercitrin) in the ethanolic leaf extracts. Microscopic anatomical characteristics of their midribs and microscopic leaf measurements of their lamina were demonstrated. DNA fingerprint and dendrogram among twenty *Bauhinia* species were illustrated. Pharmacognostic specification of *B. malabarica* crude drug with special reference to the quercetin and quercitrin contents were established.

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APPENDIX

Table 19 Microscopic leaf measurement of *B. acuminata* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1908	524.109	-	-	-	-	25	6.250
2	1916	521.921	-	-	-	-	29	7.250
3	1908	524.109	-	-	-	-	26	6.500
4	1864	536.481	-	-	-	-	24	6.000
5	1840	543.478	-	-	-	-	27	6.750
6	1840	543.478	-	-	-	-	26	6.500
7	1816	550.661	-	-	-	-	26	6.500
8	1840	543.478	-	-	-	-	26	6.500
9	1832	545.852	-	-	-	-	26	6.500
10	1840	543.478	-	-	-	-	27	6.750
11	1876	533.049	-	-	-	-	26	6.500
12	1864	536.481	-	-	-	-	25	6.250
13	1804	554.324	-	-	-	-	27	6.750
14	1848	541.126	-	-	-	-	28	7.000
15	1824	548.246	-	-	-	-	29	7.250
16	1920	520.833	-	-	-	-	25	6.250
17	1916	521.921	-	-	-	-	28	7.000
18	1860	537.634	-	-	-	-	26	6.500
19	1900	526.316	-	-	-	-	26	6.500
20	1840	543.478	-	-	-	-	26	6.500
21	1876	533.049	-	-	-	-	27	6.750
22	1820	549.451	-	-	-	-	25	6.250
23	1816	550.661	-	-	-	-	28	7.000
24	1840	543.478	-	-	-	-	27	6.750
25	1836	544.662	-	-	-	-	26	6.500
26	1908	524.109	-	-	-	-	27	6.750
27	1852	539.957	-	-	-	-	28	7.000
28	1824	548.246	-	-	-	-	26	6.500
29	1808	553.097	-	-	-	-	29	7.250
30	1828	547.046	-	-	-	-	26	6.500

Table 20 Microscopic leaf measurement of *B. acuminata* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	1728	452	20.620	12	0.547
2	1720	432	20.037	4	0.186
3	1748	468	21.043	8	0.360
4	1728	472	21.377	8	0.362
5	1752	456	20.615	4	0.181
6	1736	444	20.255	12	0.547
7	1724	472	21.377	12	0.543
8	1712	452	20.810	8	0.368
9	1728	452	20.658	8	0.366
10	1752	452	20.397	12	0.542
11	1760	456	20.541	4	0.180
12	1736	468	21.157	8	0.362
13	1748	472	21.185	8	0.359
14	1720	436	20.111	12	0.554
15	1728	436	20.111	4	0.185
16	1748	448	20.327	8	0.363
17	1752	448	20.327	4	0.181
18	1728	436	20.074	8	0.368
19	1728	472	21.377	8	0.362
20	1728	472	21.300	16	0.722
21	1776	452	20.215	8	0.358
22	1748	460	20.796	4	0.181
23	1728	460	20.947	8	0.364
24	1720	452	20.772	4	0.184
25	1724	468	21.234	12	0.544
26	1724	448	20.513	12	0.549
27	1712	432	20.000	16	0.741
28	1712	432	20.000	16	0.741
29	1776	432	19.459	12	0.541
30	1728	452	20.583	16	0.729

Table 21 Microscopic leaf measurement of *B. aureifolia* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1816	537.634	44	2.366	-	-	19	4.750
2	1812	541.126	36	1.948	-	-	19	4.750
3	1844	535.332	24	1.285	-	-	21	5.250
4	1840	534.188	32	1.709	-	-	21	5.250
5	1908	518.672	20	1.037	-	-	22	5.500
6	1812	543.478	28	1.522	-	-	21	5.250
7	1876	521.921	40	2.088	-	-	19	4.750
8	1844	534.188	28	1.496	-	-	21	5.250
9	1808	545.852	24	1.310	-	-	20	5.000
10	1848	531.915	32	1.702	-	-	22	5.500
11	1832	537.634	28	1.505	-	-	21	5.250
12	1824	541.126	24	1.299	-	-	20	5.000
13	1828	535.332	40	2.141	-	-	20	5.000
14	1812	544.662	24	1.307	-	-	19	4.750
15	1840	536.481	24	1.288	-	-	20	5.000
16	1876	525.210	28	1.471	-	-	19	4.750
17	1884	524.109	24	1.258	-	-	20	5.000
18	1828	538.793	28	1.509	-	-	20	5.000
19	1848	530.786	36	1.911	-	-	19	4.750
20	1820	542.299	24	1.302	-	-	20	5.000
21	1848	534.188	24	1.282	-	-	20	5.000
22	1876	525.210	28	1.471	-	-	20	5.000
23	1852	528.541	40	2.114	-	-	20	5.000
24	1820	542.299	24	1.302	-	-	19	4.750
25	1840	536.481	24	1.288	-	-	19	4.750
26	1852	533.049	24	1.279	-	-	20	5.000
27	1864	529.661	24	1.271	-	-	19	4.750
28	1840	537.634	20	1.075	-	-	20	5.000
29	1864	528.541	28	1.480	-	-	22	5.500
30	1808	541.126	40	2.165	-	-	19	4.750

Table 22 Microscopic leaf measurement of *B. aureifolia* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	2964	584	16.276	40	1.115
2	3000	584	16.079	48	1.322
3	2948	552	15.576	44	1.242
4	3008	556	15.393	48	1.329
5	2988	548	15.307	44	1.229
6	2988	608	16.740	36	0.991
7	2988	588	16.279	36	0.997
8	3008	596	16.302	52	1.422
9	3000	552	15.249	68	1.878
10	3000	552	15.333	48	1.333
11	2948	584	16.276	56	1.561
12	2956	584	16.258	52	1.448
13	2956	612	16.850	64	1.762
14	3008	624	16.938	52	1.412
15	3000	612	16.703	52	1.419
16	2968	556	15.531	56	1.564
17	2976	588	16.172	72	1.980
18	2976	588	16.225	60	1.656
19	3008	584	15.939	72	1.965
20	3000	600	16.376	64	1.747
21	2992	596	16.392	48	1.320
22	2992	596	16.410	44	1.211
23	3004	552	15.232	68	1.876
24	2952	556	15.583	60	1.682
25	2952	588	16.352	56	1.557
26	3008	584	16.026	52	1.427
27	3008	584	15.956	68	1.858
28	2956	588	16.261	72	1.991
29	2972	608	16.648	72	1.972
30	2988	604	16.575	52	1.427

Table 23 Microscopic leaf measurement of *B. bracteata* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	2704	355.114	100	3.551	12	0.4261	21	5.250
2	2736	352.609	92	3.244	8	0.2821	18	4.500
3	2732	352.113	100	3.521	8	0.2817	21	5.250
4	2752	348.675	108	3.766	8	0.2789	19	4.750
5	2760	347.705	104	3.616	12	0.4172	20	5.000
6	2760	350.631	88	3.086	4	0.1403	21	5.250
7	2712	355.619	96	3.414	4	0.1422	19	4.750
8	2728	354.108	92	3.258	4	0.1416	20	5.000
9	2724	352.113	108	3.803	8	0.2817	19	4.750
10	2724	353.107	100	3.531	8	0.2825	20	5.000
11	2764	349.650	92	3.217	4	0.1399	20	5.000
12	2712	354.610	100	3.546	8	0.2837	19	4.750
13	2704	354.108	112	3.966	8	0.2833	21	5.250
14	2704	357.143	88	3.143	8	0.2857	20	5.000
15	2724	353.607	92	3.253	12	0.4243	19	4.750
16	2736	352.113	100	3.521	4	0.1408	18	4.500
17	2736	351.617	100	3.516	8	0.2813	19	4.750
18	2736	352.609	92	3.244	8	0.2821	21	5.250
19	2752	348.675	108	3.766	8	0.2789	20	5.000
20	2764	348.189	104	3.621	4	0.1393	19	4.750
21	2712	356.633	88	3.138	4	0.1427	20	5.000
22	2712	355.619	96	3.414	4	0.1422	21	5.250
23	2728	353.107	96	3.390	8	0.2825	20	5.000
24	2728	351.617	108	3.797	8	0.2813	20	5.000
25	2752	350.140	100	3.501	4	0.1401	19	4.750
26	2752	350.140	92	3.221	12	0.4202	18	4.500
27	2736	351.617	100	3.516	8	0.2813	18	4.500
28	2704	357.143	92	3.286	4	0.1429	19	4.750
29	2704	354.610	112	3.972	4	0.1418	20	5.000
30	2736	350.631	108	3.787	8	0.2805	20	5.000

Table 24 Microscopic leaf measurement of B. *bracteata* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	2592	456	14.941	4	0.131
2	2640	488	15.561	8	0.255
3	2624	480	15.424	8	0.257
4	2592	464	15.163	4	0.131
5	2664	448	14.359	8	0.256
6	2592	456	14.921	8	0.262
7	2616	448	14.602	4	0.130
8	2640	456	14.710	4	0.129
9	2640	480	15.345	8	0.256
10	2624	472	15.187	12	0.386
11	2624	480	15.404	12	0.385
12	2592	456	14.941	4	0.131
13	2592	456	14.941	4	0.131
14	2640	464	14.910	8	0.257
15	2592	480	15.584	8	0.260
16	2592	488	15.803	8	0.259
17	2648	488	15.541	4	0.127
18	2640	464	14.929	4	0.129
19	2624	464	14.987	8	0.258
20	2592	448	14.698	8	0.262
21	2600	456	14.902	4	0.131
22	2600	488	15.762	8	0.258
23	2592	480	15.564	12	0.389
24	2592	472	15.365	8	0.260
25	2640	472	15.148	4	0.128
26	2640	456	14.672	12	0.386
27	2592	480	15.605	4	0.130
28	2600	456	14.902	4	0.131
29	2600	456	14.902	4	0.131
30	2624	488	15.641	8	0.256

Table 25 Microscopic leaf measurement of *B. galpinii* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	2096	477.099	-	-	-	-	21	5.250
2	2072	482.625	-	-	-	-	26	6.500
3	2112	473.485	-	-	-	-	24	6.000
4	2088	478.927	-	-	-	-	22	5.500
5	2088	478.927	-	-	-	-	23	5.750
6	2112	473.485	-	-	-	-	24	6.000
7	2080	480.769	-	-	-	-	23	5.750
8	2072	482.625	-	-	-	-	25	6.250
9	2072	482.625	-	-	-	-	21	5.250
10	2088	478.927	-	-	-	-	24	6.000
11	2088	478.927	-	-	-	-	26	6.500
12	2112	473.485	-	-	-	-	21	5.250
13	2120	471.698	-	-	-	-	22	5.500
14	2048	488.281	-	-	-	-	26	6.500
15	2072	482.625	-	-	-	-	24	6.000
16	2096	477.099	-	-	-	-	24	6.000
17	2112	473.485	-	-	-	-	23	5.750
18	2048	488.281	-	-	-	-	23	5.750
19	2088	478.927	-	-	-	-	21	5.250
20	2088	478.927	-	-	-	-	21	5.250
21	2096	477.099	-	-	-	-	22	5.500
22	2080	480.769	-	-	-	-	24	6.000
23	2072	482.625	-	-	-	-	26	6.500
24	2088	478.927	-	-	-	-	21	5.250
25	2096	477.099	-	-	-	-	24	6.000
26	2072	482.625	-	-	-	-	21	5.250
27	2096	477.099	-	-	-	-	21	5.250
28	2072	482.625	-	-	-	-	24	6.000
29	2072	482.625	-	-	-	-	26	6.500
30	2096	477.099	-	-	-	-	24	6.000

Table 26 Microscopic leaf measurement of *B. galpinii* (lowerepidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	3796	436	9.954	148	3.379
2	3752	444	10.259	132	3.050
3	3740	460	10.570	152	3.493
4	3788	448	10.266	128	2.933
5	3760	420	9.722	140	3.241
6	3780	436	10.028	132	3.036
7	3752	432	10.009	132	3.058
8	3768	444	10.221	132	3.039
9	3748	448	10.332	140	3.229
10	3768	480	10.919	148	3.367
11	3772	476	10.887	124	2.836
12	3780	436	10.009	140	3.214
13	3768	420	9.704	140	3.235
14	3752	432	10.028	124	2.878
15	3740	444	10.268	140	3.238
16	3740	448	10.419	112	2.605
17	3760	436	10.037	148	3.407
18	3752	432	10.065	108	2.516
19	3752	444	10.287	120	2.780
20	3788	460	10.502	132	3.014
21	3740	480	11.091	108	2.495
22	3732	476	10.938	144	3.309
23	3740	436	10.083	148	3.423
24	3752	444	10.268	128	2.960
25	3788	444	10.156	140	3.202
26	3720	408	9.595	124	2.916
27	3748	436	10.121	124	2.878
28	3780	472	10.786	124	2.834
29	3788	460	10.502	132	3.014
30	3760	444	10.268	120	2.775

Table 27 Microscopic leaf measurement of *B. integrifolia* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1468	679.348	4	0.272	-	-	23	5.750
2	1436	694.444	4	0.278	-	-	24	6.000
3	1476	673.854	8	0.539	-	-	24	6.000
4	1452	686.813	4	0.275	-	-	21	5.250
5	1440	690.608	8	0.552	-	-	24	6.000
6	1436	690.608	12	0.829	-	-	25	6.250
7	1440	692.521	4	0.277	-	-	23	5.750
8	1460	683.060	4	0.273	-	-	25	6.250
9	1440	690.608	8	0.552	-	-	20	5.000
10	1468	675.676	12	0.811	-	-	24	6.000
11	1436	694.444	4	0.278	-	-	20	5.000
12	1436	694.444	4	0.278	-	-	20	5.000
13	1440	692.521	4	0.277	-	-	20	5.000
14	1440	692.521	4	0.277	-	-	22	5.500
15	1440	692.521	4	0.277	-	-	21	5.250
16	1436	694.444	4	0.278	-	-	21	5.250
17	1436	694.444	4	0.278	-	-	24	6.000
18	1468	677.507	8	0.542	-	-	23	5.750
19	1468	675.676	12	0.811	-	-	20	5.000
20	1468	679.348	4	0.272	-	-	23	5.750
21	1436	694.444	4	0.278	-	-	21	5.250
22	1436	692.521	8	0.554	-	-	23	5.750
23	1440	692.521	4	0.277	-	-	23	5.750
24	1440	692.521	4	0.277	-	-	24	6.000
25	1452	683.060	12	0.820	-	-	22	5.500
26	1436	692.521	8	0.554	-	-	22	5.500
27	1468	679.348	4	0.272	-	-	25	6.250
28	1468	679.348	4	0.272	-	-	20	5.000
29	1476	675.676	4	0.270	-	-	23	5.750
30	1468	677.507	8	0.542	-	-	24	6.000

Table 28 Microscopic leaf measurement of *B. integrifolia* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	1572	232	12.860	-	-
2	1636	240	12.793	-	-
3	1584	240	13.158	-	-
4	1616	240	12.931	-	-
5	1572	236	13.053	-	-
6	1632	212	11.497	-	-
7	1624	236	12.688	-	-
8	1576	240	13.216	-	-
9	1604	232	12.636	-	-
10	1628	224	12.095	-	-
11	1616	232	12.554	-	-
12	1612	224	12.200	-	-
13	1624	232	12.500	-	-
14	1560	256	14.097	-	-
15	1592	248	13.478	-	-
16	1604	240	13.015	-	-
17	1604	232	12.636	-	-
18	1636	232	12.420	-	-
19	1572	248	13.626	-	-
20	1560	240	13.333	-	-
21	1584	248	13.537	-	-
22	1560	240	13.333	-	-
23	1624	240	12.876	-	-
24	1572	232	12.860	-	-
25	1600	232	12.664	-	-
26	1604	256	13.763	-	-
27	1604	248	13.391	-	-
28	1616	240	12.931	-	-
29	1604	248	13.391	-	-
30	1616	240	12.931	-	-

Table 29 Microscopic leaf measurement of *B. lakhonensis* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1344	696.379	84	5.850	8	0.557	16	4.000
2	1304	724.638	72	5.217	4	0.290	18	4.500
3	1324	710.227	80	5.682	4	0.284	17	4.250
4	1316	704.225	96	6.761	8	0.563	15	3.750
5	1336	698.324	88	6.145	8	0.559	17	4.250
6	1308	714.286	84	6.000	8	0.571	16	4.000
7	1324	710.227	76	5.398	8	0.568	15	3.750
8	1328	702.247	88	6.180	8	0.562	17	4.250
9	1312	718.391	72	5.172	8	0.575	19	4.750
10	1328	708.215	80	5.666	4	0.283	17	4.250
11	1344	698.324	80	5.587	8	0.559	17	4.250
12	1304	718.391	84	6.034	4	0.287	16	4.000
13	1324	714.286	72	5.143	4	0.286	20	5.000
14	1320	712.251	80	5.698	4	0.285	16	4.000
15	1328	700.280	96	6.723	4	0.280	18	4.500
16	1316	708.215	88	6.232	8	0.567	16	4.000
17	1304	718.391	84	6.034	4	0.287	17	4.250
18	1304	724.638	72	5.217	4	0.290	15	3.750
19	1324	710.227	80	5.682	4	0.284	16	4.000
20	1336	696.379	96	6.685	4	0.279	15	3.750
21	1308	712.251	88	6.268	8	0.570	16	4.000
22	1324	706.215	84	5.932	8	0.565	15	3.750
23	1324	706.215	80	5.650	12	0.847	17	4.250
24	1344	696.379	80	5.571	12	0.836	15	3.750
25	1344	704.225	72	5.070	4	0.282	17	4.250
26	1316	716.332	72	5.158	8	0.573	16	4.000
27	1360	694.444	72	5.000	8	0.556	16	4.000
28	1316	710.227	88	6.250	4	0.284	15	3.750
29	1316	710.227	84	5.966	8	0.568	17	4.250
30	1304	722.543	72	5.202	8	0.578	16	4.000

Table 30 Microscopic leaf measurement of *B. lakhonensis* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	1452	212	12.589	20	1.188
2	1492	208	12.121	16	0.932
3	1468	192	11.456	16	0.955
4	1460	204	12.172	12	0.716
5	1488	228	13.194	12	0.694
6	1468	216	12.736	12	0.708
7	1448	204	12.201	20	1.196
8	1496	196	11.475	16	0.937
9	1464	200	11.905	16	0.952
10	1452	192	11.538	20	1.202
11	1444	208	12.500	12	0.721
12	1452	212	12.619	16	0.952
13	1468	208	12.352	8	0.475
14	1480	192	11.401	12	0.713
15	1468	204	12.143	8	0.476
16	1460	212	12.589	12	0.713
17	1480	228	13.287	8	0.466
18	1488	216	12.587	12	0.699
19	1480	196	11.639	8	0.475
20	1452	200	12.019	12	0.721
21	1468	192	11.456	16	0.955
22	1452	200	11.990	16	0.959
23	1452	216	12.888	8	0.477
24	1468	204	12.143	8	0.476
25	1452	192	11.650	4	0.243
26	1480	192	11.374	16	0.948
27	1480	208	12.264	8	0.472
28	1468	212	12.530	12	0.709
29	1444	208	12.530	8	0.482
30	1444	192	11.622	16	0.969

Table 31 Microscopic leaf measurement of *B. malabarica* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1436	696.379	-	-	-	-	15	3.750
2	1452	688.705	-	-	-	-	15	3.750
3	1448	690.608	-	-	-	-	16	4.000
4	1488	672.043	-	-	-	-	15	3.750
5	1456	686.813	-	-	-	-	14	3.500
6	1484	673.854	-	-	-	-	15	3.750
7	1448	690.608	-	-	-	-	14	3.500
8	1452	688.705	-	-	-	-	16	4.000
9	1468	681.199	-	-	-	-	16	4.000
10	1436	696.379	-	-	-	-	15	3.750
11	1476	677.507	-	-	-	-	14	3.500
12	1476	677.507	-	-	-	-	14	3.500
13	1472	679.348	-	-	-	-	14	3.500
14	1432	698.324	-	-	-	-	15	3.750
15	1428	700.280	-	-	-	-	14	3.500
16	1472	679.348	-	-	-	-	14	3.500
17	1468	681.199	-	-	-	-	16	4.000
18	1460	684.932	-	-	-	-	14	3.500
19	1416	706.215	-	-	-	-	16	4.000
20	1472	679.348	-	-	-	-	14	3.500
21	1468	681.199	-	-	-	-	15	3.750
22	1440	694.444	-	-	-	-	16	4.000
23	1480	675.676	-	-	-	-	15	3.750
24	1464	683.060	-	-	-	-	14	3.500
25	1436	696.379	-	-	-	-	15	3.750
26	1472	679.348	-	-	-	-	16	4.000
27	1472	679.348	-	-	-	-	15	3.750
28	1460	684.932	-	-	-	-	17	4.250
29	1448	690.608	-	-	-	-	16	4.000
30	1464	683.060	-	-	-	-	17	4.250

Table 32 Microscopic leaf measurement of *B. malabarica* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	3040	700	18.637	16	0.426
2	3052	684	18.269	8	0.214
3	3140	680	17.708	20	0.521
4	3160	676	17.549	16	0.415
5	3112	692	18.115	16	0.419
6	3120	680	17.820	16	0.419
7	3156	720	18.538	8	0.206
8	3112	676	17.771	16	0.421
9	3084	668	17.747	12	0.319
10	3036	688	18.435	8	0.214
11	3052	652	17.565	8	0.216
12	3040	660	17.799	8	0.216
13	3056	668	17.880	12	0.321
14	3076	672	17.872	12	0.319
15	3108	692	18.134	16	0.419
16	3052	680	18.162	12	0.321
17	3040	692	18.503	8	0.214
18	3084	708	18.592	16	0.420
19	3040	676	18.133	12	0.322
20	3040	720	19.068	16	0.424
21	3112	676	17.808	8	0.211
22	3156	688	17.842	12	0.311
23	3112	652	17.249	16	0.423
24	3084	660	17.572	12	0.319
25	3080	680	18.008	16	0.424
26	3092	708	18.592	8	0.210
27	3120	700	18.267	12	0.313
28	3072	684	18.134	16	0.424
29	3076	712	18.717	16	0.421
30	3160	672	17.482	12	0.312

Table 33 Microscopic leaf measurement of *B. ornata* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1180	847.458	-	-	-	-	15	3.750
2	1152	868.056	-	-	-	-	16	4.000
3	1184	844.595	-	-	-	-	15	3.750
4	1100	909.091	-	-	-	-	15	3.750
5	1148	871.080	-	-	-	-	18	4.500
6	1168	856.164	-	-	-	-	19	4.750
7	1160	862.069	-	-	-	-	18	4.500
8	1188	841.751	-	-	-	-	17	4.250
9	1152	868.056	-	-	-	-	16	4.000
10	1088	919.118	-	-	-	-	19	4.750
11	1100	909.091	-	-	-	-	17	4.250
12	1152	868.056	-	-	-	-	15	3.750
13	1120	892.857	-	-	-	-	16	4.000
14	1144	874.126	-	-	-	-	15	3.750
15	1168	856.164	-	-	-	-	15	3.750
16	1180	847.458	-	-	-	-	19	4.750
17	1152	868.056	-	-	-	-	18	4.500
18	1100	909.091	-	-	-	-	18	4.500
19	1148	871.080	-	-	-	-	19	4.750
20	1148	871.080	-	-	-	-	17	4.250
21	1168	856.164	-	-	-	-	15	3.750
22	1180	847.458	-	-	-	-	15	3.750
23	1152	868.056	-	-	-	-	16	4.000
24	1100	909.091	-	-	-	-	16	4.000
25	1088	919.118	-	-	-	-	15	3.750
26	1152	868.056	-	-	-	-	17	4.250
27	1180	847.458	-	-	-	-	19	4.750
28	1100	909.091	-	-	-	-	17	4.250
29	1100	909.091	-	-	-	-	15	3.750
30	1180	847.458	-	-	-	-	16	4.000

Table 34 Microscopic leaf measurement of *B. ornata* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	2552	240	8.596	-	-
2	2492	256	9.316	-	-
3	2600	252	8.836	-	-
4	2568	240	8.547	-	-
5	2608	260	9.066	-	-
6	2516	244	8.841	-	-
7	2536	260	9.299	-	-
8	2516	264	9.496	-	-
9	2536	268	9.558	-	-
10	2508	228	8.333	-	-
11	2560	236	8.441	-	-
12	2520	264	9.483	-	-
13	2548	236	8.477	-	-
14	2568	256	9.065	-	-
15	2516	240	8.708	-	-
16	2492	252	9.184	-	-
17	2516	232	8.443	-	-
18	2568	240	8.547	-	-
19	2588	260	9.129	-	-
20	2552	268	9.504	-	-
21	2516	228	8.309	-	-
22	2520	228	8.297	-	-
23	2568	240	8.547	-	-
24	2548	248	8.870	-	-
25	2512	264	9.510	-	-
26	2560	268	9.477	-	-
27	2520	264	9.483	-	-
28	2548	244	8.739	-	-
29	2560	260	9.220	-	-
30	2508	260	9.393	-	-

Table 35 Microscopic leaf measurement of *B. pottsii* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	2096	469.043	-	-	36	1.689	21	5.250
2	2072	478.011	-	-	20	0.956	26	6.500
3	2112	468.165	-	-	24	1.124	24	6.000
4	2088	475.285	-	-	16	0.760	22	5.500
5	2088	471.698	-	-	32	1.509	23	5.750
6	2104	469.043	-	-	28	1.313	24	6.000
7	2056	478.011	-	-	36	1.721	23	5.750
8	2080	474.383	-	-	28	1.328	25	6.250
9	2064	478.927	-	-	24	1.149	21	5.250
10	2072	478.927	-	-	16	0.766	24	6.000
11	2088	474.383	-	-	20	0.949	26	6.500
12	2088	471.698	-	-	32	1.509	21	5.250
13	2108	466.418	-	-	36	1.679	22	5.500
14	2120	465.549	-	-	28	1.304	26	6.500
15	2048	480.769	-	-	32	1.538	24	6.000
16	2076	476.190	-	-	24	1.143	24	6.000
17	2096	469.043	-	-	36	1.689	23	5.750
18	2156	460.405	-	-	16	0.737	23	5.750
19	2048	482.625	-	-	24	1.158	21	5.250
20	2088	472.590	-	-	28	1.323	21	5.250
21	2064	477.099	-	-	32	1.527	22	5.500
22	2052	478.927	-	-	36	1.724	24	6.000
23	2120	466.418	-	-	24	1.119	26	6.500
24	2072	475.285	-	-	32	1.521	21	5.250
25	2104	467.290	-	-	36	1.682	24	6.000
26	2096	469.925	-	-	32	1.504	21	5.250
27	2080	476.190	-	-	20	0.952	21	5.250
28	2048	483.559	-	-	20	0.967	24	6.000
29	2072	476.190	-	-	28	1.333	26	6.500
30	2080	474.383	-	-	28	1.328	24	6.000

Table 36 Microscopic leaf measurement of *B. pottsii* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	-	-	-	148	-
2	-	-	-	132	-
3	-	-	-	152	-
4	-	-	-	128	-
5	-	-	-	140	-
6	-	-	-	132	-
7	-	-	-	132	-
8	-	-	-	132	-
9	-	-	-	140	-
10	-	-	-	148	-
11	-	-	-	124	-
12	-	-	-	140	-
13	-	-	-	140	-
14	-	-	-	124	-
15	-	-	-	112	-
16	-	-	-	112	-
17	-	-	-	148	-
18	-	-	-	108	-
19	-	-	-	120	-
20	-	-	-	132	-
21	-	-	-	108	-
22	-	-	-	144	-
23	-	-	-	128	-
24	-	-	-	140	-
25	-	-	-	124	-
26	-	-	-	124	-
27	-	-	-	132	-
28	-	-	-	120	-
29	-	-	-	112	-
30	-	-	-	140	-

Table 37 Microscopic leaf measurement of *B. pulla* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1908	524.109	-	-	-	-	24	6.000
2	1888	529.661	-	-	-	-	23	5.750
3	1864	536.481	-	-	-	-	28	7.000
4	1908	524.109	-	-	-	-	24	6.000
5	1916	521.921	-	-	-	-	23	5.750
6	1872	534.188	-	-	-	-	23	5.750
7	1880	531.915	-	-	-	-	24	6.000
8	1908	524.109	-	-	-	-	25	6.250
9	1888	529.661	-	-	-	-	25	6.250
10	1864	536.481	-	-	-	-	24	6.000
11	1880	531.915	-	-	-	-	26	6.500
12	1916	521.921	-	-	-	-	25	6.250
13	1908	524.109	-	-	-	-	23	5.750
14	1864	536.481	-	-	-	-	27	6.750
15	1872	534.188	-	-	-	-	24	6.000
16	1880	531.915	-	-	-	-	23	5.750
17	1908	524.109	-	-	-	-	25	6.250
18	1888	529.661	-	-	-	-	28	7.000
19	1864	536.481	-	-	-	-	23	5.750
20	1916	521.921	-	-	-	-	28	7.000
21	1872	534.188	-	-	-	-	26	6.500
22	1880	531.915	-	-	-	-	25	6.250
23	1908	524.109	-	-	-	-	23	5.750
24	1868	535.332	-	-	-	-	24	6.000
25	1864	536.481	-	-	-	-	24	6.000
26	1888	529.661	-	-	-	-	25	6.250
27	1880	531.915	-	-	-	-	25	6.250
28	1864	536.481	-	-	-	-	24	6.000
29	1872	534.188	-	-	-	-	26	6.500
30	1908	524.109	-	-	-	-	24	6.000

Table 38 Microscopic leaf measurement of *B. pulla* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	3184	436	12.044	-	-
2	3248	432	11.739	-	-
3	3264	432	11.688	-	-
4	3168	456	12.583	-	-
5	3244	456	12.324	-	-
6	3200	448	12.281	-	-
7	3192	428	11.823	-	-
8	3168	484	13.253	-	-
9	3184	480	13.100	-	-
10	3204	428	11.784	-	-
11	3244	436	11.848	-	-
12	3264	460	12.352	-	-
13	3192	448	12.308	-	-
14	3168	468	12.871	-	-
15	3160	476	13.091	-	-
16	3244	440	11.944	-	-
17	3264	452	12.164	-	-
18	3200	472	12.854	-	-
19	3176	484	13.224	-	-
20	3176	460	12.651	-	-
21	3176	448	12.362	-	-
22	3244	428	11.656	-	-
23	3248	440	11.931	-	-
24	3244	480	12.889	-	-
25	3200	476	12.949	-	-
26	3168	428	11.902	-	-
27	3168	432	12.000	-	-
28	3192	432	11.921	-	-
29	3160	448	12.417	-	-
30	3244	468	12.608	-	-

Table 39 Microscopic leaf measurement of *B. purpurea* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1840	525.210	64	3.361	-	-	23	5.750
2	1888	513.347	60	3.080	-	-	20	5.000
3	1856	519.751	68	3.534	-	-	21	5.250
4	1880	512.295	72	3.689	-	-	24	6.000
5	1864	517.598	68	3.520	-	-	20	5.000
6	1912	507.099	60	3.043	-	-	21	5.250
7	1848	525.210	56	2.941	-	-	22	5.500
8	1888	510.204	72	3.673	-	-	20	5.000
9	1840	526.316	60	3.158	-	-	20	5.000
10	1848	524.109	60	3.145	-	-	18	4.500
11	1888	512.295	64	3.279	-	-	22	5.500
12	1880	512.295	72	3.689	-	-	20	5.000
13	1872	517.598	60	3.106	-	-	21	5.250
14	1840	526.316	60	3.158	-	-	20	5.000
15	1840	525.210	64	3.361	-	-	21	5.250
16	1848	520.833	72	3.750	-	-	19	4.750
17	1888	511.247	68	3.476	-	-	21	5.250
18	1880	513.347	68	3.491	-	-	21	5.250
19	1880	515.464	60	3.093	-	-	22	5.500
20	1864	519.751	60	3.119	-	-	22	5.500
21	1872	517.598	60	3.106	-	-	24	6.000
22	1848	523.013	64	3.347	-	-	21	5.250
23	1840	523.013	72	3.766	-	-	18	4.500
24	1848	523.013	64	3.347	-	-	19	4.750
25	1840	525.210	64	3.361	-	-	21	5.250
26	1880	513.347	68	3.491	-	-	18	4.500
27	1708	561.798	72	4.045	-	-	18	4.500
28	1840	526.316	60	3.158	-	-	19	4.750
29	1848	525.210	56	2.941	-	-	23	5.750
30	1872	518.672	56	2.905	-	-	19	4.750

Table 40 Microscopic leaf measurement of *B. purpurea* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	4720	1184	19.733	96	1.600
2	4656	1152	19.539	88	1.493
3	4704	1200	20.000	96	1.600
4	4688	1208	20.187	88	1.471
5	4760	1168	19.402	92	1.528
6	4700	1136	19.112	108	1.817
7	4640	1160	19.634	108	1.828
8	4656	1176	19.865	88	1.486
9	4736	1168	19.493	88	1.469
10	4728	1136	19.060	96	1.611
11	4680	1160	19.502	108	1.816
12	4656	1152	19.499	100	1.693
13	4768	1168	19.376	92	1.526
14	4768	1188	19.643	92	1.521
15	4752	1152	19.174	104	1.731
16	4700	1200	20.000	100	1.667
17	4712	1168	19.532	100	1.672
18	4720	1136	19.060	104	1.745
19	4720	1160	19.398	100	1.672
20	4768	1136	18.933	96	1.600
21	4688	1136	19.163	104	1.754
22	4672	1152	19.433	104	1.754
23	4656	1208	20.296	88	1.478
24	4656	1168	19.743	92	1.555
25	4688	1184	19.852	92	1.543
26	4720	1184	19.760	88	1.469
27	4720	1200	19.907	108	1.792
28	4700	1152	19.394	88	1.481
29	4720	1120	18.881	92	1.551
30	4752	1144	19.079	100	1.668

Table 41 Microscopic leaf measurement of *B. racemosa* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	2060	485.437	-	-	-	-	31	7.750
2	2048	488.281	-	-	-	-	29	7.250
3	2020	495.050	-	-	-	-	32	8.000
4	2020	495.050	-	-	-	-	27	6.750
5	2036	491.159	-	-	-	-	29	7.250
6	2068	483.559	-	-	-	-	28	7.000
7	2036	491.159	-	-	-	-	29	7.250
8	2040	490.196	-	-	-	-	31	7.750
9	2060	485.437	-	-	-	-	27	6.750
10	2060	485.437	-	-	-	-	28	7.000
11	2048	488.281	-	-	-	-	32	8.000
12	2036	491.159	-	-	-	-	29	7.250
13	2020	495.050	-	-	-	-	27	6.750
14	2008	498.008	-	-	-	-	30	7.500
15	2008	498.008	-	-	-	-	28	7.000
16	2060	485.437	-	-	-	-	30	7.500
17	2020	495.050	-	-	-	-	28	7.000
18	2020	495.050	-	-	-	-	28	7.000
19	2068	483.559	-	-	-	-	31	7.750
20	2068	483.559	-	-	-	-	30	7.500
21	2020	495.050	-	-	-	-	28	7.000
22	2048	488.281	-	-	-	-	27	6.750
23	2036	491.159	-	-	-	-	28	7.000
24	2036	491.159	-	-	-	-	30	7.500
25	2060	485.437	-	-	-	-	31	7.750
26	2048	488.281	-	-	-	-	29	7.250
27	2036	491.159	-	-	-	-	29	7.250
28	2020	495.050	-	-	-	-	28	7.000
29	2048	488.281	-	-	-	-	30	7.500
30	2060	485.437	-	-	-	-	31	7.750

Table 42 Microscopic leaf measurement of *B. racemosa* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	2752	800	22.297	36	1.003
2	2720	848	23.529	36	0.999
3	2712	868	24.084	24	0.666
4	2704	828	23.258	28	0.787
5	2740	832	23.085	32	0.888
6	2768	804	22.284	36	0.998
7	2720	840	23.411	28	0.780
8	2720	848	23.503	40	1.109
9	2712	868	24.058	28	0.776
10	2760	868	23.716	32	0.874
11	2728	808	22.620	36	1.008
12	2748	880	24.044	32	0.874
13	2740	800	22.422	28	0.785
14	2760	848	23.297	32	0.879
15	2768	820	22.677	28	0.774
16	2712	816	22.947	28	0.787
17	2720	820	22.982	28	0.785
18	2712	800	22.573	32	0.903
19	2720	868	24.031	24	0.664
20	2752	828	22.898	36	0.996
21	2760	820	22.752	24	0.666
22	2752	840	23.153	36	0.992
23	2720	840	23.385	32	0.891
24	2768	848	23.220	36	0.986
25	2760	868	23.768	24	0.657
26	2752	808	22.420	44	1.221
27	2752	880	24.044	28	0.765
28	2720	848	23.556	32	0.889
29	2768	808	22.395	32	0.887
30	2760	800	22.272	32	0.891

Table 43 Microscopic leaf measurement of *B. saccocalyx* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	2760	362.319	-	-	-	-	35	8.750
2	2712	368.732	-	-	-	-	38	9.500
3	2688	372.024	-	-	-	-	37	9.250
4	2728	366.569	-	-	-	-	39	9.750
5	2752	363.372	-	-	-	-	38	9.500
6	2720	367.647	-	-	-	-	37	9.250
7	2704	369.822	-	-	-	-	37	9.250
8	2760	362.319	-	-	-	-	35	8.750
9	2752	363.372	-	-	-	-	36	9.000
10	2720	367.647	-	-	-	-	38	9.500
11	2688	372.024	-	-	-	-	36	9.000
12	2688	372.024	-	-	-	-	38	9.500
13	2680	373.134	-	-	-	-	35	8.750
14	2696	370.920	-	-	-	-	39	9.750
15	2752	363.372	-	-	-	-	38	9.500
16	2768	361.272	-	-	-	-	36	9.000
17	2760	362.319	-	-	-	-	38	9.500
18	2752	363.372	-	-	-	-	39	9.750
19	2720	367.647	-	-	-	-	38	9.500
20	2688	372.024	-	-	-	-	39	9.750
21	2720	367.647	-	-	-	-	36	9.000
22	2688	372.024	-	-	-	-	37	9.250
23	2704	369.822	-	-	-	-	37	9.250
24	2704	369.822	-	-	-	-	36	9.000
25	2760	362.319	-	-	-	-	37	9.250
26	2712	368.732	-	-	-	-	35	8.750
27	2688	372.024	-	-	-	-	37	9.250
28	2720	367.647	-	-	-	-	35	8.750
29	2720	367.647	-	-	-	-	35	8.750
30	2704	369.822	-	-	-	-	35	8.750

Table 44 Microscopic leaf measurement of *B. saccocalyx* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	-	-	-	204	-
2	-	-	-	168	-
3	-	-	-	200	-
4	-	-	-	204	-
5	-	-	-	192	-
6	-	-	-	184	-
7	-	-	-	200	-
8	-	-	-	196	-
9	-	-	-	180	-
10	-	-	-	192	-
11	-	-	-	180	-
12	-	-	-	188	-
13	-	-	-	204	-
14	-	-	-	180	-
15	-	-	-	192	-
16	-	-	-	200	-
17	-	-	-	184	-
18	-	-	-	180	-
19	-	-	-	212	-
20	-	-	-	204	-
21	-	-	-	192	-
22	-	-	-	164	-
23	-	-	-	184	-
24	-	-	-	204	-
25	-	-	-	200	-
26	-	-	-	192	-
27	-	-	-	220	-
28	-	-	-	172	-
29	-	-	-	172	-
30	-	-	-	172	-

Table 45 Microscopic leaf measurement of *B. scandens* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	2144	462.963	16	0.741	-	-	38	9.500
2	2168	458.716	12	0.550	-	-	38	9.500
3	2168	457.875	16	0.733	-	-	37	9.250
4	2152	462.963	8	0.370	-	-	38	9.500
5	2128	468.165	8	0.375	-	-	36	9.000
6	2128	465.549	20	0.931	-	-	36	9.000
7	2144	464.684	8	0.372	-	-	34	8.500
8	2152	462.107	12	0.555	-	-	34	8.500
9	2168	459.559	8	0.368	-	-	34	8.500
10	2144	463.822	12	0.557	-	-	36	9.000
11	2168	459.559	8	0.368	-	-	38	9.500
12	2152	462.107	12	0.555	-	-	38	9.500
13	2128	467.290	12	0.561	-	-	38	9.500
14	2144	465.549	4	0.186	-	-	37	9.250
15	2128	469.043	4	0.188	-	-	36	9.000
16	2168	458.716	12	0.550	-	-	36	9.000
17	2168	458.716	12	0.550	-	-	34	8.500
18	2144	463.822	12	0.557	-	-	34	8.500
19	2128	469.043	4	0.188	-	-	36	9.000
20	2128	468.165	8	0.375	-	-	36	9.000
21	2144	462.963	16	0.741	-	-	38	9.500
22	2152	461.255	16	0.738	-	-	38	9.500
23	2152	462.963	8	0.370	-	-	34	8.500
24	2168	458.716	12	0.550	-	-	34	8.500
25	2144	464.684	8	0.372	-	-	36	9.000
26	2144	465.549	4	0.186	-	-	34	8.500
27	2128	467.290	12	0.561	-	-	36	9.000
28	2144	462.963	16	0.741	-	-	38	9.500
29	2168	459.559	8	0.368	-	-	34	8.500
30	2168	459.559	8	0.368	-	-	34	8.500

Table 46 Microscopic leaf measurement of *B. scandens* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	1652	168	9.231	-	-
2	1672	148	8.132	-	-
3	1664	152	8.370	-	-
4	1660	160	8.791	-	-
5	1656	148	8.204	-	-
6	1648	152	8.444	-	-
7	1644	152	8.463	-	-
8	1680	144	7.895	-	-
9	1656	156	8.609	-	-
10	1648	160	8.850	-	-
11	1672	160	8.734	-	-
12	1664	160	8.772	-	-
13	1660	168	9.190	-	-
14	1656	144	8.000	-	-
15	1644	152	8.463	-	-
16	1652	144	8.018	-	-
17	1672	152	8.333	-	-
18	1672	148	8.132	-	-
19	1660	160	8.791	-	-
20	1648	168	9.251	-	-
21	1644	152	8.463	-	-
22	1680	152	8.297	-	-
23	1680	144	7.895	-	-
24	1672	156	8.534	-	-
25	1664	156	8.571	-	-
26	1664	160	8.772	-	-
27	1680	168	9.091	-	-
28	1656	168	9.211	-	-
29	1664	144	7.965	-	-
30	1656	152	8.407	-	-

Table 47 Microscopic leaf measurement of *B. siamensis* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1532	642.674	-	-	24	1.542	27	6.750
2	1544	636.132	-	-	28	1.781	29	7.250
3	1512	651.042	-	-	24	1.563	27	6.750
4	1500	657.895	-	-	20	1.316	25	6.250
5	1544	637.755	-	-	24	1.531	26	6.500
6	1552	636.132	-	-	20	1.272	27	6.750
7	1536	642.674	-	-	20	1.285	25	6.250
8	1532	641.026	-	-	28	1.795	28	7.000
9	1528	645.995	-	-	20	1.292	25	6.250
10	1524	644.330	-	-	28	1.804	27	6.750
11	1540	637.755	-	-	28	1.786	28	7.000
12	1508	651.042	-	-	28	1.823	27	6.750
13	1536	639.386	-	-	28	1.790	29	7.250
14	1536	642.674	-	-	20	1.285	24	6.000
15	1504	652.742	-	-	28	1.828	25	6.250
16	1512	649.351	-	-	28	1.818	29	7.250
17	1508	652.742	-	-	24	1.567	27	6.750
18	1536	637.755	-	-	32	2.041	25	6.250
19	1512	649.351	-	-	28	1.818	26	6.500
20	1540	636.132	-	-	32	2.036	27	6.750
21	1528	642.674	-	-	28	1.799	25	6.250
22	1492	661.376	-	-	20	1.323	28	7.000
23	1504	654.450	-	-	24	1.571	24	6.000
24	1528	642.674	-	-	28	1.799	25	6.250
25	1552	634.518	-	-	24	1.523	27	6.750
26	1496	654.450	-	-	32	2.094	28	7.000
27	1488	661.376	-	-	24	1.587	29	7.250
28	1520	645.995	-	-	28	1.809	29	7.250
29	1548	632.911	-	-	32	2.025	24	6.000
30	1500	654.450	-	-	28	1.832	24	6.000

Table 48 Microscopic leaf measurement of *B. siamensis* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	1752	208	10.484	24	1.210
2	1712	204	10.472	32	1.643
3	1720	192	9.877	32	1.646
4	1744	196	9.980	24	1.222
5	1752	196	9.939	24	1.217
6	1720	196	10.145	16	0.828
7	1712	208	10.700	24	1.235
8	1720	200	10.309	20	1.031
9	1752	192	9.736	28	1.420
10	1720	192	9.917	24	1.240
11	1744	192	9.756	32	1.626
12	1752	196	9.980	16	0.815
13	1720	200	10.309	20	1.031
14	1776	208	10.338	28	1.392
15	1712	220	11.294	16	0.821
16	1712	208	10.700	24	1.235
17	1720	220	11.156	32	1.623
18	1712	172	9.034	20	1.050
19	1744	192	9.796	24	1.224
20	1752	196	9.939	24	1.217
21	1720	196	10.124	20	1.033
22	1712	196	10.103	32	1.649
23	1720	208	10.678	20	1.027
24	1712	204	10.559	16	0.828
25	1776	204	10.180	24	1.198
26	1728	208	10.591	28	1.426
27	1720	192	9.959	16	0.830
28	1752	196	9.899	32	1.616
29	1776	196	9.800	28	1.400
30	1752	208	10.484	24	1.210

Table 49 Microscopic leaf measurement of *B. sirindhorniae* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1168	856.164	-	-	-	-	16	4.000
2	1136	880.282	-	-	-	-	18	4.500
3	1104	905.797	-	-	-	-	18	4.500
4	1168	856.164	-	-	-	-	20	5.000
5	1120	892.857	-	-	-	-	16	4.000
6	1152	868.056	-	-	-	-	18	4.500
7	1136	880.282	-	-	-	-	18	4.500
8	1160	862.069	-	-	-	-	18	4.500
9	1112	899.281	-	-	-	-	16	4.000
10	1120	892.857	-	-	-	-	20	5.000
11	1112	899.281	-	-	-	-	18	4.500
12	1168	856.164	-	-	-	-	20	5.000
13	1136	880.282	-	-	-	-	18	4.500
14	1104	905.797	-	-	-	-	18	4.500
15	1168	856.164	-	-	-	-	20	5.000
16	1104	905.797	-	-	-	-	16	4.000
17	1120	892.857	-	-	-	-	16	4.000
18	1152	868.056	-	-	-	-	18	4.500
19	1136	880.282	-	-	-	-	18	4.500
20	1168	856.164	-	-	-	-	18	4.500
21	1120	892.857	-	-	-	-	16	4.000
22	1168	856.164	-	-	-	-	20	5.000
23	1136	880.282	-	-	-	-	20	5.000
24	1104	905.797	-	-	-	-	20	5.000
25	1096	912.409	-	-	-	-	18	4.500
26	1136	880.282	-	-	-	-	18	4.500
27	1180	847.458	-	-	-	-	20	5.000
28	1104	905.797	-	-	-	-	16	4.000
29	1168	856.164	-	-	-	-	18	4.500
30	1136	880.282	-	-	-	-	20	5.000

Table 50 Microscopic leaf measurement of *B. sirindhorniae* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	1728	256	12.903	-	-
2	1712	248	12.653	-	-
3	1728	232	11.837	-	-
4	1720	248	12.602	-	-
5	1728	248	12.551	-	-
6	1744	256	12.800	-	-
7	1704	248	12.705	-	-
8	1712	256	13.008	-	-
9	1728	256	12.903	-	-
10	1712	232	11.934	-	-
11	1728	232	11.837	-	-
12	1720	232	11.885	-	-
13	1720	232	11.885	-	-
14	1728	256	12.903	-	-
15	1712	256	13.008	-	-
16	1712	248	12.653	-	-
17	1712	248	12.653	-	-
18	1728	232	11.837	-	-
19	1720	248	12.602	-	-
20	1728	248	12.551	-	-
21	1744	232	11.741	-	-
22	1712	232	11.934	-	-
23	1704	248	12.705	-	-
24	1712	248	12.653	-	-
25	1720	256	12.955	-	-
26	1728	256	12.903	-	-
27	1728	232	11.837	-	-
28	1712	232	11.934	-	-
29	1720	256	12.955	-	-
30	1728	256	12.903	-	-

Table 51 Microscopic leaf measurement of *B. strychnifolia* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	2008	497.018	4	0.199	-	-	20	5.000
2	1968	506.073	8	0.405	-	-	22	5.500
3	2044	486.381	12	0.584	-	-	22	5.500
4	1968	504.032	16	0.806	-	-	20	5.000
5	2000	498.008	8	0.398	-	-	24	6.000
6	2016	494.071	8	0.395	-	-	20	5.000
7	2016	494.071	8	0.395	-	-	20	5.000
8	2000	498.008	8	0.398	-	-	21	5.250
9	2016	495.050	4	0.198	-	-	20	5.000
10	1984	502.008	8	0.402	-	-	24	6.000
11	2000	499.002	4	0.200	-	-	18	4.500
12	2008	497.018	4	0.199	-	-	21	5.250
13	1976	505.051	4	0.202	-	-	20	5.000
14	1968	504.032	16	0.806	-	-	24	6.000
15	2044	486.381	12	0.584	-	-	21	5.250
16	2008	497.018	4	0.199	-	-	18	4.500
17	1968	507.099	4	0.203	-	-	20	5.000
18	2000	498.008	8	0.398	-	-	20	5.000
19	2016	493.097	12	0.592	-	-	24	6.000
20	2016	494.071	8	0.395	-	-	20	5.000
21	2000	499.002	4	0.200	-	-	21	5.250
22	1968	506.073	8	0.405	-	-	20	5.000
23	1976	502.008	16	0.803	-	-	24	6.000
24	2000	499.002	4	0.200	-	-	18	4.500
25	1968	505.051	12	0.606	-	-	21	5.250
26	2044	487.329	8	0.390	-	-	20	5.000
27	2008	495.050	12	0.594	-	-	20	5.000
28	2008	496.032	8	0.397	-	-	18	4.500
29	2016	493.097	12	0.592	-	-	18	4.500
30	1968	505.051	12	0.606	-	-	20	5.000

Table 52 Microscopic leaf measurement of *B. strychnifolia* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	3120	608	16.309	-	-
2	3104	608	16.379	-	-
3	3088	616	16.631	-	-
4	3120	624	16.667	-	-
5	3152	640	16.878	-	-
6	3096	592	16.052	-	-
7	3136	624	16.596	-	-
8	3104	592	16.017	-	-
9	3120	608	16.309	-	-
10	3144	592	15.846	-	-
11	3120	608	16.309	-	-
12	3104	616	16.559	-	-
13	3152	608	16.170	-	-
14	3104	608	16.379	-	-
15	3088	616	16.631	-	-
16	3112	624	16.702	-	-
17	3120	624	16.667	-	-
18	3128	592	15.914	-	-
19	3120	592	15.948	-	-
20	3104	640	17.094	-	-
21	3096	640	17.131	-	-
22	3136	624	16.596	-	-
23	3120	608	16.309	-	-
24	3120	592	15.948	-	-
25	3136	616	16.418	-	-
26	3096	608	16.415	-	-
27	3104	608	16.379	-	-
28	3104	616	16.559	-	-
29	3120	608	16.309	-	-
30	3120	608	16.309	-	-

Table 53 Microscopic leaf measurement of *B. tomentosa* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1480	675.676	-	-	-	-	22	5.500
2	1468	681.199	-	-	-	-	22	5.500
3	1476	677.507	-	-	-	-	24	6.000
4	1448	690.608	-	-	-	-	22	5.500
5	1476	677.507	-	-	-	-	21	5.250
6	1460	684.932	-	-	-	-	23	5.750
7	1432	698.324	-	-	-	-	22	5.500
8	1440	694.444	-	-	-	-	23	5.750
9	1452	688.705	-	-	-	-	21	5.250
10	1424	702.247	-	-	-	-	23	5.750
11	1448	690.608	-	-	-	-	21	5.250
12	1480	675.676	-	-	-	-	22	5.500
13	1480	675.676	-	-	-	-	22	5.500
14	1472	679.348	-	-	-	-	23	5.750
15	1468	681.199	-	-	-	-	22	5.500
16	1448	690.608	-	-	-	-	21	5.250
17	1460	684.932	-	-	-	-	22	5.500
18	1436	696.379	-	-	-	-	23	5.750
19	1428	700.280	-	-	-	-	22	5.500
20	1432	698.324	-	-	-	-	22	5.500
21	1436	696.379	-	-	-	-	24	6.000
22	1488	672.043	-	-	-	-	21	5.250
23	1444	692.521	-	-	-	-	23	5.750
24	1432	698.324	-	-	-	-	22	5.500
25	1436	696.379	-	-	-	-	23	5.750
26	1424	702.247	-	-	-	-	21	5.250
27	1444	692.521	-	-	-	-	22	5.500
28	1444	692.521	-	-	-	-	22	5.500
29	1480	675.676	-	-	-	-	24	6.000
30	1428	700.280	-	-	-	-	23	5.750

Table 54 Microscopic leaf measurement of *B. tomentosa* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	1656	368	17.829	40	1.938
2	1704	392	18.386	36	1.689
3	1672	396	18.750	44	2.083
4	1704	388	18.267	32	1.507
5	1688	400	18.797	40	1.880
6	1712	400	18.587	40	1.859
7	1652	376	18.147	44	2.124
8	1656	404	19.275	36	1.718
9	1672	400	18.975	36	1.708
10	1648	380	18.447	32	1.553
11	1572	428	21.063	32	1.575
12	1648	372	18.093	36	1.751
13	1596	392	19.406	32	1.584
14	1648	404	19.275	44	2.099
15	1680	408	19.245	32	1.509
16	1680	380	18.130	36	1.718
17	1656	368	17.829	40	1.938
18	1668	396	18.893	32	1.527
19	1652	396	19.002	36	1.727
20	1644	404	19.423	32	1.538
21	1592	404	19.882	36	1.772
22	1600	424	20.583	36	1.748
23	1660	416	19.586	48	2.260
24	1680	388	18.476	32	1.524
25	1692	392	18.421	44	2.068
26	1588	420	20.388	52	2.524
27	1612	420	20.309	36	1.741
28	1664	376	18.008	48	2.299
29	1656	380	18.269	44	2.115
30	1680	380	18.027	48	2.277

Table 55 Microscopic leaf measurement of *B. variegata* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	2300	425.894	48	2.044	-	-	22	5.500
2	2356	415.282	52	2.159	-	-	23	5.750
3	2372	413.907	44	1.821	-	-	22	5.500
4	2320	421.585	52	2.192	-	-	21	5.250
5	2328	422.297	40	1.689	-	-	18	4.500
6	2280	427.350	60	2.564	-	-	22	5.500
7	2308	425.894	40	1.704	-	-	20	5.000
8	2352	415.282	56	2.326	-	-	21	5.250
9	2336	420.875	40	1.684	-	-	20	5.000
10	2328	420.168	52	2.185	-	-	21	5.250
11	2280	430.293	44	1.893	-	-	23	5.750
12	2360	413.907	56	2.318	-	-	19	4.750
13	2372	413.223	48	1.983	-	-	22	5.500
14	2352	418.060	40	1.672	-	-	23	5.750
15	2320	423.729	40	1.695	-	-	20	5.000
16	2300	427.350	40	1.709	-	-	22	5.500
17	2300	426.621	44	1.877	-	-	20	5.000
18	2352	418.060	40	1.672	-	-	21	5.250
19	2320	421.585	52	2.192	-	-	22	5.500
20	2360	415.973	44	1.830	-	-	22	5.500
21	2360	414.594	52	2.156	-	-	22	5.500
22	2312	424.448	44	1.868	-	-	21	5.250
23	2320	420.168	60	2.521	-	-	20	5.000
24	2328	418.760	60	2.513	-	-	21	5.250
25	2328	420.875	48	2.020	-	-	22	5.500
26	2360	415.282	48	1.993	-	-	20	5.000
27	2308	425.170	44	1.871	-	-	19	4.750
28	2320	423.729	40	1.695	-	-	21	5.250
29	2360	416.667	40	1.667	-	-	22	5.500
30	2352	415.282	56	2.326	-	-	19	4.750

Table 56 Microscopic leaf measurement of *B. variegata* (lower epidermis)

No	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	4656	624	11.721	44	0.826
2	4752	592	10.963	56	1.037
3	4696	608	11.377	40	0.749
4	4680	576	10.868	44	0.830
5	4744	656	12.032	52	0.954
6	4664	592	11.161	48	0.905
7	4656	632	11.835	52	0.974
8	4720	624	11.556	56	1.037
9	4680	608	11.420	36	0.676
10	4752	640	11.773	44	0.809
11	4640	596	11.296	40	0.758
12	4656	624	11.685	60	1.124
13	4672	632	11.818	44	0.823
14	4640	632	11.889	44	0.828
15	4752	648	11.877	56	1.026
16	4736	624	11.513	60	1.107
17	4736	616	11.382	60	1.109
18	4680	616	11.510	56	1.046
19	4744	624	11.521	48	0.886
20	4664	640	11.949	52	0.971
21	4672	648	12.072	48	0.894
22	4720	592	11.053	44	0.822
23	4680	640	11.914	52	0.968
24	4752	592	10.987	44	0.817
25	4640	648	12.117	60	1.122
26	4672	592	11.145	48	0.904
27	4656	616	11.588	44	0.828
28	4720	616	11.458	40	0.744
29	4720	632	11.721	40	0.742
30	4752	640	11.773	44	0.809

Table 57 Microscopic leaf measurement of *B. winitii* (upper epidermis)

No.	Epidermal cell number (/ mm^2)	Epidermal cell area (μm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index	Palisade cell number	Palisade ratio
1	1776	560.538	8	0.448	-	-	18	4.500
2	1744	569.476	12	0.683	-	-	18	4.500
3	1760	566.893	4	0.227	-	-	16	4.000
4	1768	563.063	8	0.450	-	-	18	4.500
5	1728	574.713	12	0.690	-	-	19	4.750
6	1752	568.182	8	0.455	-	-	19	4.750
7	1776	560.538	8	0.448	-	-	20	5.000
8	1768	563.063	8	0.450	-	-	18	4.500
9	1728	577.367	4	0.231	-	-	17	4.250
10	1776	561.798	4	0.225	-	-	19	4.750
11	1744	568.182	16	0.909	-	-	18	4.500
12	1776	560.538	8	0.448	-	-	17	4.250
13	1768	564.334	4	0.226	-	-	18	4.500
14	1752	569.476	4	0.228	-	-	17	4.250
15	1728	576.037	8	0.461	-	-	17	4.250
16	1752	569.476	4	0.228	-	-	17	4.250
17	1744	569.476	12	0.683	-	-	18	4.500
18	1776	560.538	8	0.448	-	-	20	5.000
19	1760	566.893	4	0.227	-	-	16	4.000
20	1752	568.182	8	0.455	-	-	17	4.250
21	1760	564.334	12	0.677	-	-	18	4.500
22	1768	560.538	16	0.897	-	-	17	4.250
23	1788	556.793	8	0.445	-	-	17	4.250
24	1776	561.798	4	0.225	-	-	18	4.500
25	1744	572.082	4	0.229	-	-	19	4.750
26	1744	568.182	16	0.909	-	-	19	4.750
27	1760	566.893	4	0.227	-	-	17	4.250
28	1768	564.334	4	0.226	-	-	17	4.250
29	1744	572.082	4	0.229	-	-	19	4.750
30	1744	572.082	4	0.229	-	-	18	4.500

Table 58 Microscopic leaf measurement of *B. winitii* (lower epidermis)

No.	Epidermal cell number (/ mm^2)	Stomatal number (/ mm^2)	Stomatal index	Trichome number (/ mm^2)	Trichome index
1	2240	348	13.447	-	-
2	2264	332	12.789	-	-
3	2256	336	12.963	-	-
4	2256	368	14.024	-	-
5	2224	352	13.665	-	-
6	2240	368	14.110	-	-
7	2228	384	14.701	-	-
8	2240	348	13.447	-	-
9	2232	332	12.949	-	-
10	2256	320	12.422	-	-
11	2256	336	12.963	-	-
12	2256	368	14.024	-	-
13	2240	368	14.110	-	-
14	2240	328	12.773	-	-
15	2240	348	13.447	-	-
16	2264	348	13.323	-	-
17	2264	332	12.789	-	-
18	2256	336	12.963	-	-
19	2256	336	12.963	-	-
20	2224	368	14.198	-	-
21	2240	352	13.580	-	-
22	2228	368	14.176	-	-
23	2240	352	13.580	-	-
24	2232	332	12.949	-	-
25	2256	348	13.364	-	-
26	2256	352	13.497	-	-
27	2240	352	13.580	-	-
28	2240	332	12.908	-	-
29	2264	336	12.923	-	-
30	2256	368	14.024	-	-

Table 59 The percent yield of *B. malabarica* leaf crude drug

No.	Weight of crude drug (g)	Weight of extractable matter (g)	% yield
1	5.0417	1.3805	27.3816
2	5.0482	1.3474	26.6907
3	5.0681	1.3614	26.8621
4	5.0316	1.5386	30.5787
5	5.0535	1.2692	25.1153
6	5.0377	0.9305	18.4707
7	5.0502	1.1748	23.2624
8	5.0624	1.5130	29.8870
9	5.0162	1.4840	29.5841
10	5.0280	1.3142	26.1376
11	5.0422	1.3403	26.5817
12	5.0121	1.5080	30.0872
13	5.0674	1.2745	25.1510
14	5.0779	1.2255	24.1340
15	5.0705	1.4879	29.3442
Mean			26.6179
SD			3.2042

Table 60 Pharmacognostic specification of *B. malabarica* leaf

Source	No.	Moisture (g%)	Loss on drying (g%)	Ash value (g%)		Extractive value (g%)	
				Total ash	Acid insoluble ash	water	ethanol
1	1	8.800	8.803	8.9144	1.8765	13.8621	12.4648
	2	9.200	8.850	8.7974	1.7549	13.4387	12.1709
	3	9.400	8.736	8.8884	1.7269	14.4793	12.5890
2	1	9.400	8.942	5.5849	1.4143	19.8156	14.5054
	2	8.800	8.983	5.5762	1.0702	19.7037	14.2662
	3	8.800	8.922	5.4940	1.1119	19.6671	14.7896
3	1	7.600	7.939	4.7306	0.7199	23.4490	13.2758
	2	7.800	7.916	4.7489	0.6170	24.8856	13.1913
	3	7.400	7.960	4.7285	0.4402	24.4312	13.0710
4	1	7.600	8.552	7.0180	2.3184	17.7413	15.5830
	2	7.200	8.492	7.0480	1.9819	18.2514	15.6558
	3	7.400	8.498	7.0508	1.6903	18.2168	15.8096
5	1	7.200	7.929	6.4112	2.0420	18.0505	13.0587
	2	7.000	7.938	6.5036	2.1220	18.1925	12.7497
	3	7.400	7.843	6.4218	2.0255	17.3152	12.9364
6	1	6.400	7.692	6.1036	1.5137	17.3574	15.6514
	2	6.800	7.752	6.0821	1.3980	17.7164	15.6322
	3	7.600	7.808	6.0934	1.1982	18.0312	15.8682
7	1	7.200	7.357	7.3204	2.1350	18.8031	17.5458
	2	6.800	7.391	7.3343	1.9173	18.2822	17.6778
	3	7.200	7.476	7.2967	1.5297	18.6922	17.9746
8	1	6.800	8.033	7.6536	1.8522	17.2792	14.5389
	2	7.200	7.986	7.7175	1.7565	17.8322	14.3111
	3	8.000	8.014	7.6538	2.0369	17.7653	14.3117
9	1	8.600	8.695	6.7460	1.8897	9.3806	8.7306
	2	9.800	8.738	6.8415	1.8306	8.5168	8.8646
	3	9.400	8.609	6.6594	1.8240	8.3842	8.9833

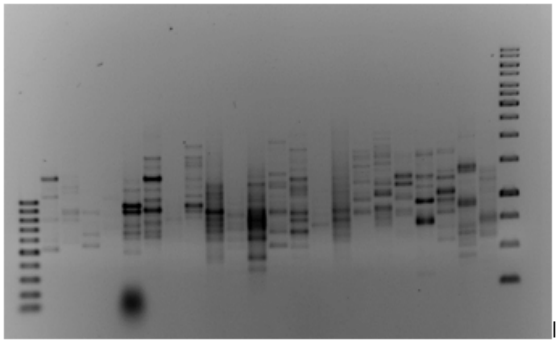
Table 60 (cont.) Pharmacognostic specification of *B. malabarica* leaf

Source	No.	Moisture (g%)	Loss on drying (g%)	Ash value (g%)		Extractive value (g%)	
				Total ash	Acid insoluble ash	water	ethanol
10	1	7.800	7.463	7.0763	1.8674	16.3228	10.2522
	2	8.200	7.515	7.1201	2.2760	16.4801	10.3315
	3	7.800	7.563	7.1994	1.9927	16.8494	9.7588
11	1	8.200	7.695	6.5866	2.1073	16.1488	14.0778
	2	8.200	7.609	6.5351	1.9529	16.3815	14.7120
	3	8.200	7.649	6.6684	2.0715	16.3423	14.4833
12	1	8.600	7.531	8.4072	2.9727	15.1960	17.7016
	2	9.000	7.451	8.4900	2.3137	15.8983	17.8731
	3	8.800	7.399	8.4827	2.3791	16.1701	17.5219
13	1	9.400	9.066	8.6820	1.8663	12.5766	13.7548
	2	10.600	9.079	8.7288	1.9009	12.3561	13.5154
	3	9.600	9.142	8.7555	1.9409	12.1677	13.5354
14	1	8.800	7.546	7.6446	1.8164	15.1030	11.7689
	2	9.200	7.599	7.5986	1.7838	14.7672	11.5644
	3	9.400	7.596	7.6027	1.7912	14.8574	11.5213
15	1	8.800	6.700	7.1769	1.9392	14.3555	13.9635
	2	9.200	6.730	7.1633	1.9648	15.1539	14.0106
	3	10.000	6.724	7.2068	1.7218	14.6525	13.5841

Formulas:

$$\text{Grand mean} = \frac{\bar{x}_1 n_1 + \bar{x}_2 n_2 + \ldots + \bar{x}_k n_k}{n_1 + n_2 + \ldots + n_k}$$

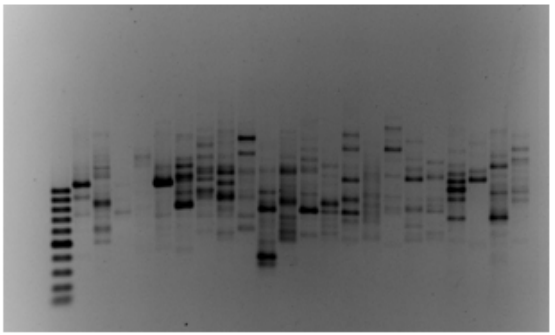
$$\text{Pooled SD} = \sqrt{\frac{\left((n_1 - 1) \times SD_1^2\right) + \left((n_2 - 1) \times SD_2^2\right) + \ldots + \left((n_k - 1) \times SD_k^2\right)}{(n_1 + n_2 + \ldots + n_k) - k}}$$



Number of bands	Track 1	Track 2	Track 3	Track 4	Track 5	Track 6	Track 7	Track 8	Track 9	Track 10	Track 11	Track 12	Track 13	Track 14	Track 15	Track 16	Track 17	Track 18	Track 19	Track 20	Track 21	Track 22
1	1693	1210	876	1381	1299	1715	794	1960	1082	821	1317	1978	1792	690	1046	1702	1966	1254	1607	1645	1385	8096
2	1340	888	649	1051	1156	1539		1712	1023	731	1193	1342	1251		830	1609	1587	1139	1245	1223	902	1207
3	1073	802	549	950	954	1328		1543	859	685	982	1149	1093		734	1422	1462	963	1102	1036	656	727
4	800			896	885	1159		1307	784	585	752	1016	815			1181	1347	806	921	938	591	580
5	523			843	740	1023		1065	722	574	584	837	742			952	1149		699	797	404	
6	190			743	661	895		938	664	525	427	636	629			804	1016			536		
7				721	494	765		877	598		343	515	504				851					
8						667			506													
9									498													
10									444													

Figure 48 Fingerprint and molecular weight plots of ISSR – 01

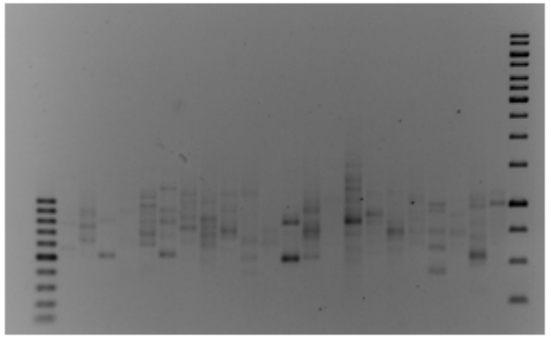
fragment sizes range from 190 to 8096 bps, 100% polymorphic



Number of bands	Track 1	Track 2	Track 3	Track 4	Track 5	Track 6	Track 7	Track 8	Track 9	Track 10	Track 11	Track 12	Track 13	Track 14	Track 15	Track 16	Track 17	Track 18	Track 19	Track 20	Track 21	Track 22
1	1318	1974	1096	1516	2269	1931	2509	2294	1859	1383	2069	1952	1505	2042	1354	2152	1944	1649	1803	1950	2169	2072
2	1253	1726	756	1463	1996	1519	2084	2069	1745	1078	1593	1736	1362	1924	1142	2055	1675	1530	1618	1850	1998	1821
3	1056	1537		1231	1329	1372	1917	1952	1457	1047	1360	1583	1256	1625	1131	1941	1532	1256	1495	1529	1746	1798
4	1030	1445		1093	1116	1338	1733	1558	1155	873	1343	1559	987	1302	1035	1645	1408	1162	1397	1423	1573	1629
5	761	1344		936	786	1098	1680	1435	1012	838	1254	1113	980	1032	893	1402	1283	1033	1381		1557	1547
6		1310		884	719	815	1488	1300	800	667	1186	916	881	1014	773	1254	1029	829	1168		1439	1511
7		1180			566	761	1295	1282	788	655	921	713	754	933		1087	839	759	1061		1265	1367
8		1146			458	733	1186	1148		527	890		737	745		1017	735		784		1193	1271
9		1047					1149	963		430	836		614	720							1036	1250
10		776					975	828		386	706										862	791
11		732					814	777			625											
12		705					638	618														
13		497					618	525														

Figure 49 Fingerprint and molecular weight plots of ISSR – 05

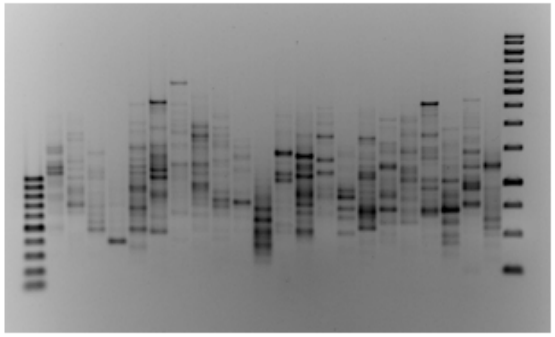
fragment sizes range from 368 to 2509 bps, 100% polymorphic



Number of bands	Track 1	Track 2	Track 3	Track 4	Track 5	Track 6	Track 7	Track 8	Track 9	Track 10	Track 11	Track 12	Track 13	Track 14	Track 15	Track 16	Track 17	Track 18	Track 19	Track 20	Track 21	Track 22
1	785	885	513	1060	1043	1148	1085	993	1099	633	721	794	932	1034	1484	890	735	914	995	884	969	1003
2	569	747		1039	946	900	991	821	948	508	620	695	723	618	1279	812		835	921	726	810	
3	555	625		885	685	796	939	674	898			497	675		1153			739	725		679	
4				616	599	675	840	602	715				516		814				592		535	
5				591		519	737								710				418			

Figure 50 Fingerprint and molecular weight plots of ISSR – 07

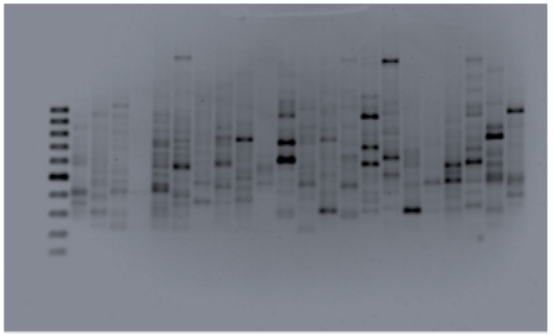
fragment sizes range from 418 to 1484 bps, 100% polymorphic



Number of bands	Track 1	Track 2	Track 3	Track 4	Track 5	Track 6	Track 7	Track 8	Track 9	Track 10	Track 11	Track 12	Track 13	Track 14	Track 15	Track 16	Track 17	Track 18	Track 19	Track 20	Track 21	Track 22
1	1399	1768	1394	714	2560	2620	3358	1928	2182	1499	917	2216	1745	2428	1429	1685	2117	2093	2558	1878	2687	1229
2	1151	1642	1091	558	2019	2433	2559	1731	1907	1332	800	1406	1352	2233	1382	1593	1583	1750	2232	1562	2046	931
3	1077	1249	1025	482	1811	2359	2313	1507	1739	1136	689	1093	1200	2014	1362	1400	1433	1434	1751	1025	1927	727
4	790	1133	691	402	1485	1905	2278	1206	1532	751	585	1011	1065	1720	1202	1325	1202	1136	1440	844	1694	622
5	503	1016	549		1281	1521	2099	1083	1275	617	485	545	845	1296	1167	1200	844	1026	1339	705	1627	553
6		849	491		1176	1339	1988	939	1086	497	388	492	741	1113	898	1056	703	920	1035	592	1457	408
7		710			1084	1196	1965	618	858				658	913	822	876	582	822	932	490	1436	301
8		641			899	1125	1798	498	773				503	828	707	680	491	690	697	427	1247	250
9					681	1029	1534		713					670	619	540		589		338	1207	
10					491	808	1208		486					658	497	414					980	
11					358	594	1035		431												938	
12						477	642														768	
13																					250	

Figure 51 Fingerprint and molecular weight plots of ISSR – 11

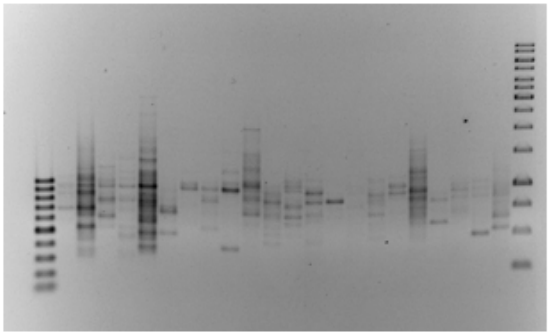
fragment sizes range from 250 to 3358 bps, 100% polymorphic



Number of bands	Track 1	Track 2	Track 3	Track 4	Track 5	Track 6	Track 7	Track 8	Track 9	Track 10	Track 11	Track 12	Track 13	Track 14	Track 15	Track 16	Track 17	Track 18	Track 19	Track 20	Track 21	Track 22
1	845	955	1033	821	1128	1609	1119	1236	1022	595	1348	967	1133	1524	1465	1504	939	995	828	1511	1365	916
2	695	829	940	760	1063	1148	946	1086	724	514	1146	803	937	1140	1071	1257	582	858	708	1445	991	783
3	592	579	803	730	953	914	908	832	581	420	989	732	784	1072	962	1206	506	844	698	1288	768	702
4	411	533	691	403	897	877	777	744	509		911	513	697	970	875	995	362	758	614	1134	692	384
5	351	470	608		814	803	640	698	462		689	460	566	798	722	904	236	637	598	951	563	298
6	311	392	533		720	735	574	563	406		561	396	446	674	627	728		493	491	879	498	
7		309	469		633	681	505	416	342		422	349	336	566	515	549		371	436	772	385	
8			414		560	623	446	335			263	263	254	497	438	428		232	380	673	233	
9			299		505	552	341					187		374	325	249			291	596		
10			235		439	464								233	286				239	511		
11					415	380									244					382		
12					339	333														309		
13					318	223														247		
14					290																	

Figure 52 Fingerprint and molecular weight plots of ISSR – 12

fragment sizes range from 187 to 1609 bps, 100% polymorphic



Number of bands	Track 1	Track 2	Track 3	Track 4	Track 5	Track 6	Track 7	Track 8	Track 9	Track 10	Track 11	Track 12	Track 13	Track 14	Track 15	Track 16	Track 17	Track 18	Track 19	Track 20	Track 21	Track 22
1	949	1176	1210	1345	2379	662	965	905	1132	1957	850	944	1027	765	954	1046	957	1505	784	909	1034	5260
2	854	1029	942	1038	1700	478	915	771	977	1404	756	877	856		866	848	864	1317	571	835	850	3100
3	700	949	783	938	1476			513	891	1171	621	706	754		716	635		1144			484	890
4	600	861	662	777	1313				355	947	473	607	622		632			1077				878
5		703	620	452	1095					790	392	547						892				767
6		533	528		935					637		449						809				633
7		358			767													701				530
8		319			579																	
9					481																	
10					373																	

Figure 53 Fingerprint and molecular weight plots of ISSR – 32

fragment sizes range from 319 to 5260 bps, 100% polymorphic

REFERENCES

VITA

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AWARD RECEIVED	Paphitchaya Thetsana, Chayanon Chaowuttikul, Chanida Palanuvej, and Nijsiri Ruangrungsi. “Discovery of quercetin and quercitrin in Bauhinia spp. Distributed in Thailand” at Rangsit University on “The 5th International Conference on Advanced Pharmaceutical Research (ICAPH 2018)” February 20 – 21, 2018, Rangsit University, Pathum Thani.