

CHARACTERISTICS AND NUMBER OF TRICHOME OF LEAVES FROM SELECTED CASSIA SPP. IN THAILAND

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Abstract: The genus *Cassia* L. belong to the Caesalpiniaceae family. Most of plants in this genus have been widely used as herbs, foods and ornamental plants for a long time. This study aimed to investigate the trichome number of leaves for identification of selected *Cassia* spp. Sixteen species of *Cassia* found in Thailand were employed in this study. Leaf trichome number was observed under the light microscope (LM) and scanning electron microscopy (SEM). For each species, fresh mature leaves were collected from three different areas. Thirty observation fields were detected and the number of trichome was counted per 1 mm² area. Mean, minimum, maximum and standard deviations were calculated and discussed. The trichome characteristics of investigated *Cassia* spp. were uniseriate, uni- or multicellular non-glandular and multicellular glandular types. However, trichome in some *Cassia* spp. was absent. *Cassia javanica* L. has the highest trichome number in both dorsal (78.94±2.86) and ventral (127.39±2.46) surfaces of the leaf whereas *Cassia surattensis* Burm. f. has the lowest trichome number only on ventral (3.46±0.80) surface. Among 16 *Cassia* spp. can be classified into three major groups based on the presence of trichomes on leaf surfaces. The first group, the trichome was present on both dorsal and ventral surfaces (10 species). The second group, the trichome was present on ventral surface (three species). The last group, the trichome was absent. In conclusion, trichome number is a useful tool for identification of selected Thai *Cassia* spp.

Keywords: *Cassia*, trichome number, characteristics of trichome, Caesalpiniaceae

บทคัดย่อ: พืชสกุล *Cassia* L. จัดอยู่ในวงศ์ Caesalpiniaceae พืชในสกุลนี้ส่วนใหญ่ถูกนำมาใช้เป็นสมุนไพร อาหาร และไม้ประดับมาเป็นเวลานาน วัตถุประสงค์ในการศึกษานี้เพื่อศึกษาลักษณะโครงสร้างของใบเพื่อใช้ในการระบุชนิดของพืชในสกุล *Cassia* บางชนิดในประเทศไทยในแง่ของลักษณะและจำนวนขน พืชสกุล *Cassia* ในประเทศไทยจำนวน 16 ชนิดถูกนำมาทำการศึกษาลักษณะและจำนวนขนภายใต้กล้องจุลทรรศน์และกล้องจุลทรรศน์อิเล็กตรอน โดยแต่ละชนิดจะเก็บเฉพาะใบสดที่โตเต็มที่ ซึ่งเก็บจากสถานที่ต่างๆ ชนิดละ 3 แหล่งทั่วประเทศไทยและทำการนับจำนวน 30 บริเวณต่อพืชตัวอย่าง 1 แหล่ง นับจำนวนขนต่อพื้นที่ 1 ตารางมิลลิเมตร ค่าเฉลี่ย ค่าสุด สูงสุด และค่าส่วนเบี่ยงเบนมาตรฐานของจำนวนขนถูกคำนวณและอภิปราย ลักษณะของขนที่พบในพืชสกุล *Cassia* ได้แก่ ขนแบบเซลล์เดี่ยวและหลายเซลล์ ไม่มีต่อมนขนและขนแบบหลายเซลล์ มีต่อมนขน อย่างไรก็ตามในพืชสกุล *Cassia* บางชนิดพบว่าไม่มีขน ซัยฟฤกซ์ มีจำนวนขนทั้งด้านหลังใบและด้านท้องใบสูงที่สุด (78.94±2.86, 127.39±2.46) ขณะที่ทรงบาดาล มีจำนวนขนด้านท้องใบต่ำที่สุด (3.46±0.80) จากการศึกษาพืชสกุล *Cassia* จำนวน 16 ชนิดสามารถแบ่งพืชออกได้เป็น 3 กลุ่ม ได้แก่ กลุ่มที่ 1 ประกอบด้วย พืชสกุล *Cassia* จำนวน 10 ชนิด โดยสามารถพบขนทั้งด้านหลังใบและด้านท้องใบ กลุ่มที่ 2 ประกอบด้วย พืชสกุล *Cassia* จำนวน 3 ชนิด โดยสามารถพบขนในด้านท้องใบ และกลุ่มที่ 3 ประกอบด้วย พืชสกุล *Cassia* จำนวน 3 ชนิด ไม่พบขนทั้งด้านหลังใบและด้านท้องใบ จากการศึกษาในครั้งนี้พบว่าสามารถใช้จำนวนขนในการระบุชนิดของพืชสกุล *Cassia* บางชนิดในประเทศไทยได้

คำสำคัญ *Cassia*, จำนวนขน, ลักษณะของขน, Caesalpiniaceae

INTRODUCTION

Cassia L. is a genus belonging to family Caesalpiniaceae (Dave and Ledwani, 2012). There are thirty-three species found in Thailand (Pooma & Suddee, 2014) and there is a great diversity of habit within the genus ranging from trees to prostrate annual herbs. The taxonomy of the plants in this genus is confused with several synonyms at the species and the morphological features of some species are often similar. *Cassia* plants possess valuable traditional and medicinal properties. This genus is known as a natural source of anthraquinone, which has been used for laxative and treatment of skin disease (Thomson, 1971). Some member of the genus are important e.g. *C. alata* and *C. angustifolia* have been notified as a list Herbal Medicinal Products A.D. 2006 of Thailand (National Drug Committee, 2006).

Macroscopic/microscopic evaluation, chemical compound and genetic assessment have been commonly used for identification of medicinal plant. Microscopic evaluation is a conventional method for identification of plant structural feature under microscope due to their simple, rapid and inexpensive method. Trichomes are found in most plants and can comprise either single or various cells and can be secretory glandular or non-glandular. Trichome is plant leaf hair which is useful for mechanical barrier against various external factors, such as herbivores and pathogens, UV light, extreme temperatures and excessive water loss (Werker, 2000; Hülkamp, 2004). Glandular trichomes produce various substances, which are store at the plant surface may provide chemical against several types of herbivore and pathogen (Wagner, 1991).

Recently, trichome number has been used for identification in family Solanaceae and Lamiaceae (Khokhar *et al.*, 2012). Most *Cassia* spp. have commonly covered trichomes on their leaves but the trichome number has never been investigated. Therefore, this study aimed to investigate the trichome characteristics and trichome number of selected Thai *Cassia* spp.

MATERIALS AND METHODS

Plant materials

The fresh mature leaves of 16 *Cassia* spp. were collected from various locations in Thailand. Plant specimens were identified by Associate Professor Dr. Nijsiri Ruangrangsri, College of Public Health Sciences, Chulalongkorn University and Faculty of Pharmacy, Rangsit University, Thailand and compared to the herbarium specimens at The Botanical Garden Organization, Ministry of Natural Resource and Environment and Forest Herbarium Thailand. The voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. Their location and collecting data were shown in Table 1.

Table 1. List of 16 selected *Cassia* spp. and their different collecting localities

No.	Scientific Name	Thai Name	Locality	Collecting date	Voucher ID
1	<i>Cassia bakeriana</i> Craib	Kalapaphruek	Bangkok	August, 2012	CB011
		(กัลปพฤกษ์)	Phitsanulok	August, 2012	CB012
		Chaiyaphruek	Si Sa Ket	September, 2012	CB013
		(ชัยพฤกษ์)			
2	<i>Cassia fistula</i> L.	Daug-kapi (ดอกกะปิ)			
		Ratchaphruek	Bangkok	August, 2012	CF011
		(ราชพฤกษ์)	Phitsanulok	August, 2012	CF012
		Khuun (คูน)	Si Sa Ket	September, 2012	CF013
		Lomlaeng (ลมแล้ง)			
		Ku-phe-ya (กุเพยะ)			
3	<i>Cassia grandis</i> L. f.	Chaiyaphruek			
		(ชัยพฤกษ์)			
		Kanlaphruek	Bangkok	August, 2012	CGR011
		(กัลปพฤกษ์)	Phitsanulok	August, 2012	CGR012
4	<i>Cassia javanica</i> L.	Kalapaphruek	Si Sa Ket	August, 2012	CGR013
		(กัลปพฤกษ์)			
		Chaiyaphruek	Bangkok	August, 2012	CJ011
		(ชัยพฤกษ์)	Phitsanulok	August, 2012	CJ012
		Ratchaphruek	Si Sa Ket	September, 2012	CJ013
		(ราชพฤกษ์)			
5	<i>Cassia alata</i> L.	Kalapaphruek			
		(กัลปพฤกษ์)			
		Lak khoei lak klua			
		(ลักเขยลักเกลือ)			
		Chum het thet	Bangkok	August, 2012	CA011
		(ชุมเห็ดเทศ)	Phitsanulok	August, 2012	CA012
6	<i>Cassia angustifolia</i> Vahl	Chum het yai	Si Sa Ket	September, 2012	CA013
		(ชุมเห็ดใหญ่)			
		Lap muen luang			
		(ลาบมีนหลวง)			
7	<i>Cassia garrettiana</i> Craib	Makhaam khaek	Bangkok	January, 2013	CAN011
		(มะขามแขก)	Phitsanulok	March, 2013	CAN012
		Som khaek (ส้มแขก)	Si Sa Ket	May, 2014	CAN013
7	<i>Cassia garrettiana</i> Craib	Samae san (สามสาร)	Bangkok	May, 2102	CGA011
		Khi lek khok	Phitsanulok	June, 2012	CGA012
		(ขี้เหล็กโขก)	Si Sa Ket	July, 2012	CGA013
		Khi lek phae			
		(ขี้เหล็กแพะ)			
		Ngai-san (ไผ่ชาน)			

Table 1. List of 16 selected *Cassia* spp. and their different collecting localities (Cont.)

No.	Scientific Name	Thai Name	Locality	Collecting date	Voucher ID
8	<i>Cassia hirsuta</i> L.	Dap phit (ดัปพีย)	Bangkok	May, 2013	CH011
		Phong pheng (โพงเฟง)	Phitsanulok	May, 2013	CH012
			Chachoengsao	March, 2012	CH015
9	<i>Cassia occidentalis</i> L.	Chum het lek (ชุมเห็ดเล็ก)	Bangkok	August, 2012	CO011
			Phitsanulok	August, 2012	CO012
		Chum het thet (ชุมเห็ดเทศ)	Si Sa Ket	September, 2012	CO013
		Khi lek phuak (ขี้เหล็กฝือก)			
		Kheelek phee (ขี้เหล็กผี)			
10	<i>Cassia spectabilis</i> DC.	Khee lek	Bangkok	August, 2012	CSP011
		American (ขี้เหล็กอเมริกัน)	Phitsanulok	August, 2012	CSP012
			Si Sa Ket	September, 2012	CSP013
11	<i>Cassia siamea</i> Lam.	Kheelek (ขี้เหล็ก)	Bangkok	August, 2012	CSI011
		Khee lek ban (ขี้เหล็กบ้าน)	Phitsanulok	August, 2012	CSI012
			Si Sa Ket	September, 2012	CSI013
		Khee lek luang (ขี้เหล็กหลวง)			
12	<i>Cassia sophera</i> L.	Phak khet (ผักเค็ด)	Bangkok	August, 2012	CSO011
			Phitsanulok	August, 2012	CSO012
		Phak khlet (ผักเค็ด)	Si Sa Ket	September, 2012	CSO013
		Phak wan ban (ผักหวานบ้าน)			
13	<i>Cassia sulfurea</i> DC. ex Collad.	Trueng badaan (ตริงบาดาล)	Bangkok	August, 2012	CSL011
			Phitsanulok	August, 2012	CSL012
		Sakeng (สะแก)	Si Sa Ket	September, 2012	CSL013
		Sakong (สะแก)			
14	<i>Cassia surattensis</i> Burm. f.	Song badan (ทรงบาดาล)	Bangkok	August, 2012	CSU011
			Phitsanulok	August, 2012	CSU012
		Khee lek wan (ขี้เหล็กหวาน)	Si Sa Ket	September, 2012	CSU013
15	<i>Cassia timoriensis</i> DC.	Kheelek luead (ขี้เหล็กเลือด)	Bangkok	August, 2012	CTI011
			Phitsanulok	August, 2012	CTI012
		Kheelek daeng (ขี้เหล็กแดง)	Si Sa Ket	September, 2012	CTI013
16	<i>Cassia tora</i> L.	Chumhet thai (ชุมเห็ดไทย)	Bangkok	August, 2012	CTO011
			Phitsanulok	August, 2012	CTO012
		Chumhet na (ชุมเห็ดนา)	Si Sa Ket	September, 2012	CTO013
Total number of <i>Cassia</i> species, 48 samples					

Determination of characteristics and number of trichome

The light microscope (LM) attached a digital camera and scanning electron microscope (SEM) were used in this study. The central lamina of cleaned fresh mature leaf were cut, soaked in bleaching agent such as water : Haiter bleaching solution (1:1) to remove the chlorophyll until it was clear, rinsed with water 2-3 times then was investigated the trichomes on both surfaces by wet mounting in glycerin and examining under the LM. The images were record using AxioVision Release 4.8.2 program. The studied area was avoided from the veinlet, margin, or unclear field. The trichome characteristics were investigated and the trichomes in 1 mm² area were counted. Thirty fields of each species from three different sources were examined. Mean, minimum, maximum, and standard deviations of trichome number on both surfaces were calculated and discussed (WHO, 1998).

RESULTS AND DISCUSSION

Forty eight samples of *Cassia* spp. were examined. Trichome numbers and trichome characteristics were examined as shown in Table 2. Micrographs of *Cassia* spp. taken from SEM were demonstrated in Figure 1-3.

Table 2. The trichome number and trichome characteristics of *Cassia* spp.*

No.	<i>Cassia</i> spp.	Trichome number		Trichome characteristics	
		Mean $\pm$ SD (Min-Max)		Dorsal surface	Ventral surface
		Dorsal surface	Ventral surface		
1	<i>C. bakeriana</i>	42.21 $\pm$ 1.31 (39-44)	71.48 $\pm$ 2.64 (66-77)	multicellular non-glandular	multicellular non-glandular
2	<i>C. fistula</i>	32.00 $\pm$ 1.56 (29-34)	94.47 $\pm$ 2.21 (90-98)	unicellular non-glandular	unicellular non-glandular
3	<i>C. grandis</i>	22.04 $\pm$ 2.13 (19-25)	46.36 $\pm$ 2.95 (42-52)	multicellular non-glandular	multicellular non-glandular
4	<i>C. javanica</i>	78.94 $\pm$ 2.86 (72-88)	127.39 $\pm$ 2.46 (124-135)	multicellular non-glandular	multicellular non-glandular
5	<i>C. alata</i>	8.59 $\pm$ 1.47 (6-12)	7.29 $\pm$ 1.08 (5-10)	unicellular non-glandular	unicellular non-glandular
6	<i>C. angustifolia</i>	12.42 $\pm$ 2.28 (9-18)	49.43 $\pm$ 3.64 (42-58)	unicellular non-glandular	unicellular non-glandular
7	<i>C. siamea</i>	31.39 $\pm$ 2.45 (26-35)	57.44 $\pm$ 2.60 (52-65)	unicellular non-glandular	unicellular non-glandular
8	<i>C. spectabilis</i>	20.37 $\pm$ 2.30 (16-26)	55.67 $\pm$ 2.48 (52-62)	multicellular non-glandular	multicellular non-glandular
9	<i>C. timoriensis</i>	11.33 $\pm$ 1.73 (8-14)	22.31 $\pm$ 1.65 (19-25)	multicellular non-glandular	multicellular non-glandular
10	<i>C. hirsuta</i>	5.00 $\pm$ 0.50 (4-6) n.c.	8.21 $\pm$ 0.95 (6-10) n.c.	multicellular non-glandular multicellular glandular	multicellular non-glandular multicellular glandular
11	<i>C. sulfurea</i>	-	10.20 $\pm$ 1.79 (6-15)	-	multicellular non-glandular

Table 2. The trichome number and trichome characteristics of *Cassia* spp.* (Cont.)

No.	<i>Cassia</i> spp.	Trichome number		Trichome characteristics	
		Mean $\pm$ SD (Min-Max)		Dorsal surface	Ventral surface
		Dorsal surface	Ventral surface		
12	<i>C. surattensis</i>	-	3.46 $\pm$ 0.80 (2-5)	-	multicellular non-glandular
13	<i>C. tora</i>	-	63.49 $\pm$ 2.34 (58-69)	-	multicellular non-glandular
14	<i>C. garrettiana</i>	-	-	-	-
15	<i>C. occidentalis</i>	-	-	-	-
16	<i>C. sophera</i>	-	-	-	-

* n = 3, each sample were done in triplicate

- = absence trichome

n.c. = not counted

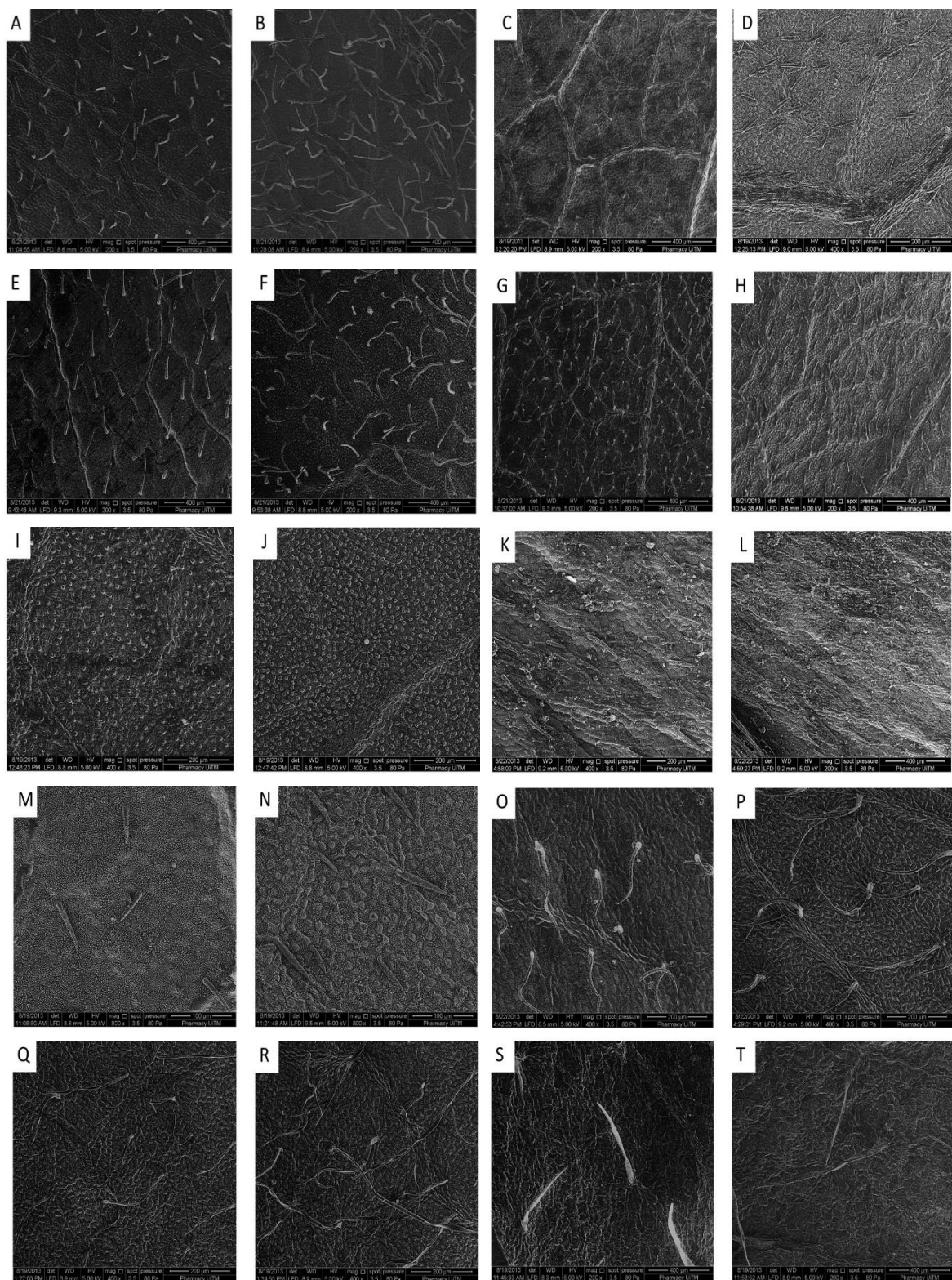


Figure 1. SEM micrographs of 10 *Cassia* spp. Trichome was present on both dorsal and ventral surfaces: (A),(B) *C. bakeriana*, (C),(D) *C. fistula*, (E),(F) *C. grandis*, (G),(H) *C. javanica*, (I),(J) *C. alata*, (K),(L) *C. angustifolia*, (M),(N) *C. siamea*, (O),(P) *C. spectabilis*, (Q),(R) *C. timoriensis*, (S),(T) *C. hirsuta*

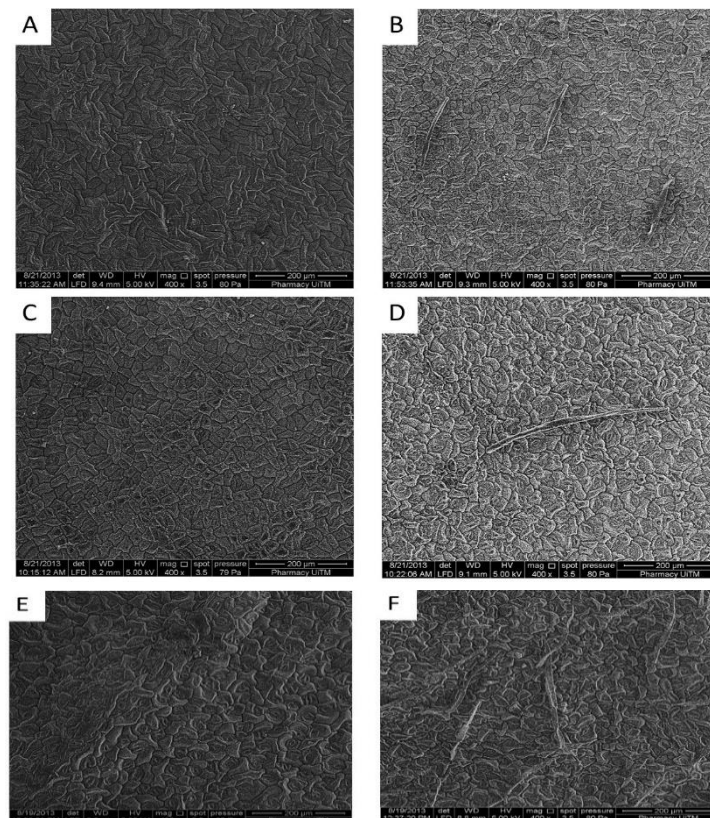


Figure 2. SEM micrographs of three *Cassia* spp. Trichome was present only on ventral surfaces: (A),(B) *C. sulfurea* (C),(D) *C. surattensis*, (E),(F) *C. tora*

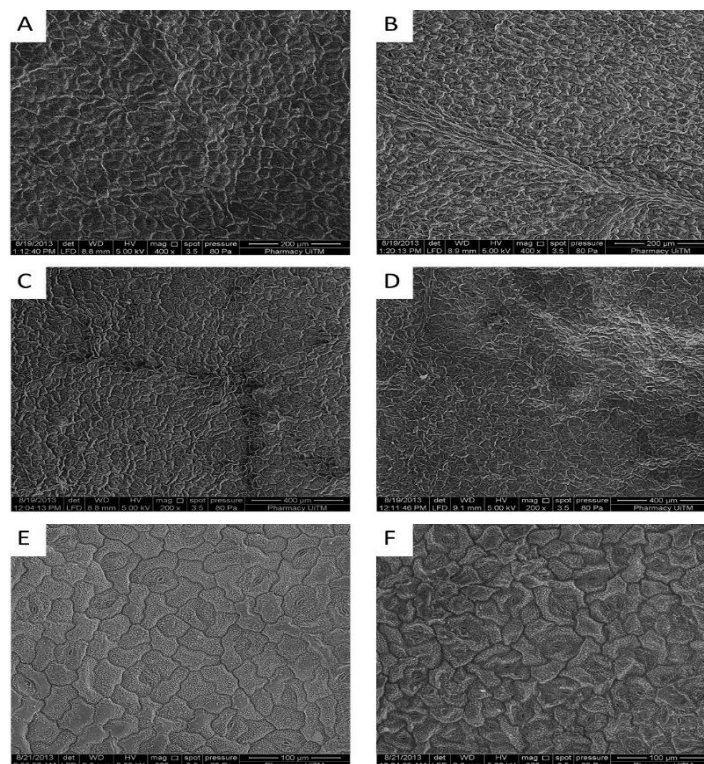


Figure 3. SEM micrographs of three *Cassia* spp. Trichome was absent on both surfaces: (A),(B) *C. garrettiana*, (C),(D) *C. occidentalis*, (E),(F) *C. sophora*

The results showed that there were differences in trichome numbers between 13 species. According to the presence and absence of trichome, three main groups were classified. The first group, the trichome of 10 *Cassia* spp. (*C. bakeriana*, *C. fistula*, *C. grandis*, *C. javanica*, *C. alata*, *C. angustifolia*, *C. siamea*, *C. spectabilis*, *C. timoriensis* and *C. hirsuta*) was shown trichome on both dorsal and ventral surfaces (Figure 1). The second group, three *Cassia* spp. (*C. sulfurea*, *C. surattensis* and *C. tora*) had shown trichome on ventral surface (Figure 2). The last group, three *Cassia* spp. (*C. garrettiana*, *C. occidentalis* and *C. sophera*) had no trichome (Figure 3). The highest value was found on both dorsal and ventral surface of *C. javanica* (78.94 ± 2.86 and 127.39 ± 2.46 , respectively). The lowest trichome number was found on only ventral surface of *C. surattensis* (3.46 ± 0.80).

Trichome number can be used to identify *C. sulfurea* and *C. surattensis* which have similar morphology, i.e. 5-10 m. tall, pinnately compound leaves, even pinnate, inflorescent were racemes with 5 petals bright yellow. However, *C. sulfurea* had the trichome number in ventral surface (10.20 ± 1.79) higher than *C. surattensis* (3.46 ± 0.80). As well as the result of this study can be used to differentiate *C. hirsuta* from *C. occidentalis*. *C. hirsuta* and *C. occidentalis* had similar morphology as they were shrubs, 1.5-2 m. tall, pinnately compound leaves, leaflets arranged oppositely in 4-5 pairs, it is broadly lanceolate to ovate in shape, inflorescent is racemes, petal is brilliant yellow. *C. hirsuta* had covering trichome on both dorsal and ventral surfaces (5.00 ± 0.50 and 8.21 ± 0.95 , respectively) whereas *C. occidentalis* had no covering trichome. Moreover, *C. hirsuta* can easily be distinguished from the other species of *Cassia* by presence of multicellular glandular trichomes with a globular head which agrees with the previous reported (Saheed and Illoh, 2010; Begum et al, 2014). However, the exact density of multicellular glandular trichome of *C. hirsuta* was not calculated because of their uneven distribution on a leaves. Trichome number has been previously used for identification of *Morinda* spp. and *Solanum* spp. from their closely related species (Roonyamarai et al., 2011; Kanjanakantorn et al., 2015).

The type of trichome is usually consistent in many species (Okpon, 1969). Many researchers have found the presence or absence and types of trichomes on the epidermis as classification characters (Adedeji et al., 2007).

Classification of trichome characteristics were based on the number of cell and the presence or absence of glandular cell of trichome. The trichome characteristics of investigated *Cassia* spp. were uniseriate, uni- or multicellular non-glandular and multicellular glandular types (Figure 4). Most trichomes are appressed to the epidermis. The trichome characteristics of *C. fistula* were uniseriate, unicellular non-glandular types which is corroborated by the finding of Saheed and Illoh (2010), Pandya et al. (2012) and Rani and Satish (2014). *C. alata* had short unicellular non-glandular trichome, conical trichomes with bulbous base in the leaflet are important diagnostic features (Figure 5). In *C. angustifolia*, trichomes are frequent on both surfaces. There are unicellular non-glandular trichome, thick walled and conical in shape with cuticular warts (Figure 5). The trichome characteristics are important character for identification of *C. angustifolia*. Moreover, it can be distinguished them from the other species of *Cassia*. The trichome characteristics in this recent study was in agreement with Kidyue et al. (2003) reported that *C. bakeriana*, *C. grandis*, *C. javanica*, *C. spectabilis*, *C. timoriensis*, *C. sulfurea*, *C. surattensis* and *C. tora* had uniseriate, multicellular non-glandular types whereas *C. hirsuta* had multicellular non-glandular and multicellular glandular types. Saheed and Illoh (2010) had reported that the presence or absence of trichomes as well as their types can be useful in characterizing some species in *Cassiinae*.

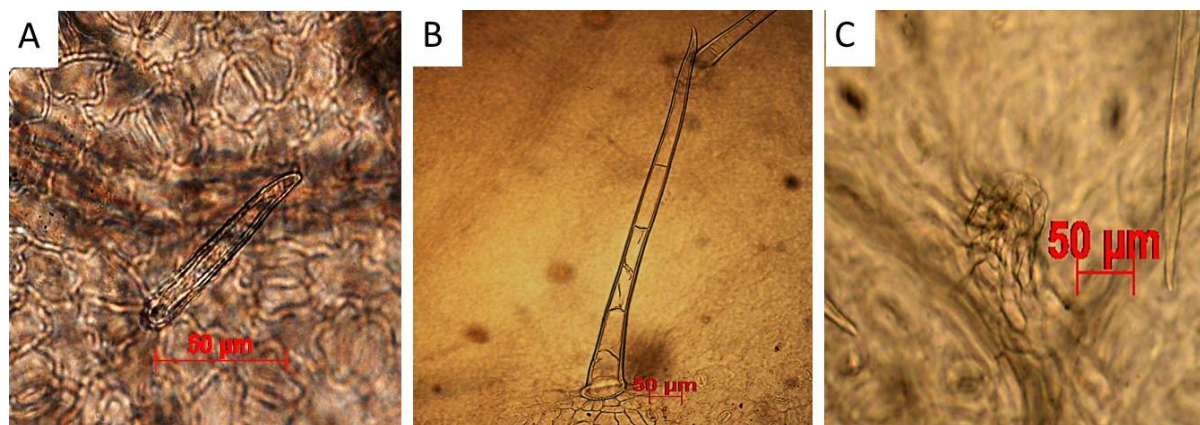


Figure 4. LM micrographs of the trichome characteristic: (A) unicellular non-glandular types, (B) multicellular non-glandular types, (C) globose glandular types

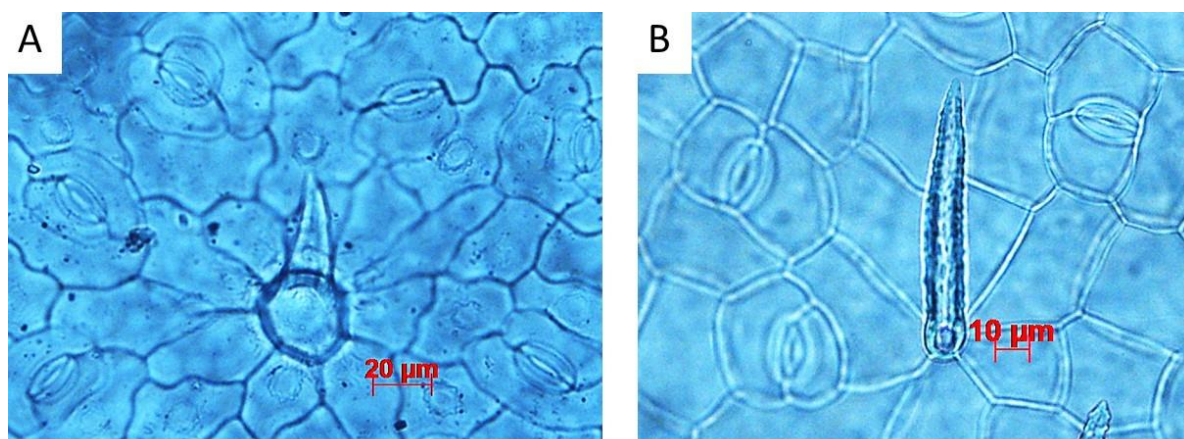


Figure 5. LM micrographs of *C. alata* (A) and *C. angustifolia* (B)

Besides other morphological characters the greatest significance of trichomes is in the identification of angiospermic plants. The taxonomic value of the trichome and their significance in systematic and phylogenetic relationship is well known in Lamiaceae, Verbenaceae and Scrophulariaceae (Navarro and Oualadi, 2000; Khokhar, 2009). The individual species of family Restionaceae and Centrolepidaceae can be distinguished by their unique characteristics of trichomes. Moreover, the T-shaped trichomes of Malpighiaceae and Ericaceae family have been classified on the basis of leaf hair, as an aid to identification of species (Cutler, 1985). Although trichome characteristic and trichome number can provides supporting evidence for plant identification in many families other analytical tools should be applied for more effectiveness in medicinal plants.

CONCLUSION

Identification of medicinal plants is important to develop for more reliability of quality control. The trichome characteristic and trichome number of leaves in this study will provide an important tool for identification at species level of selected Thai *Cassia* spp.

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