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Characterization of selected *Caesalpinia* species in Thailand based on microscopic leaf anatomy including leaf constant numbers

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Abstract

Caesalpinia L. is a genus of spiny trees, shrubs, and climbers. There are 18 species of *Caesalpinia* reported in Thailand. Besides organoleptic evaluation, microscopic characterization is used for plant authentication. This study aimed to establish the microscopic leaf characteristics of eight *Caesalpinia* species in Thailand. The fresh mature leaves of eight *Caesalpinia* species were collected from three different places throughout Thailand. The midrib transverse sections were investigated under a microscope attached to a digital camera. The laminae were evaluated for stomatal cells, epidermal cells, palisade cells, and trichomes. The results found that there were unicellular nonglandular trichomes in four species. The trichomes appeared on both dorsal and ventral epidermises of *C. digyna* Rottler, *C. minax* Hance, and *C. decapetala* (Roth) Alston, whereas *C. bonduc* (L.) Roxb. had trichomes only at the margin and midrib of both leaf surfaces. *Caesalpinia sappan* L., *C. pulcherrima* (L.) Sw., *C. mimosoides* Lam., and *C. coriaria* (Jacq.) Willd. did not present trichomes on their leaves. Stomatal cells were present only on the ventral epidermis of all species. Trichome characteristics of *C. bonduc* can be used as a key identification for this species. Trichome number and trichome index determined from *C. minax*, *C. digyna*, and *C. decapetala* were found to be overlapped. However, the upper epidermal cell area was capable of identifying *C. decapetala*. Furthermore, the stomatal number could differentiate *C. digyna* from *C. minax*. For non-trichome-containing species, *C. sappan* and *C. pulcherrima* could be identified by stomatal index and upper epidermal cell area. Microscopic leaf constant numbers between *C. mimosoides* and *C. coriaria* were overlapped; however, *C. coriaria* demonstrated its identity due to the isolateral character. Microscopic leaf constant evaluation is proved to be one of the methods capable of plant authentication.

Keywords: *Caesalpinia* L.; Caesalpiniaceae; microscopic leaf anatomy; microscopic leaf constant numbers; plant characterization

1. Introduction

The Caesalpiniaceae family is comprised of spiny trees, shrubs, or incessant herbs. This family contains around 153 genera with 2,175 species [1]. In Thailand, 20 genera with 113 species are found [2]. The genus *Caesalpinia* is a genus of trees, shrubs, and climbers consisting of approximately 150 species distributed throughout the world [3]. Morphological characterization is the first step, which is simple and can be observed macroscopically by the naked eye or magnifying glass and microscopically by microscope. Microscopic characterization of a leaf is recommended as a useful tool for medicinal plant authentication. It is based on the qualitative observation of the cellular structures and quantitative

determination of leaf constant numbers. The observation consists of stomatal type, trichome type, epidermal cell, transverse section of each part of the plant, and leaf constant numbers such as stomatal number, stomatal index, palisade ratio, epidermal cell area, trichome number, and trichome index [4]. The microscopic leaf constant numbers of eight *Caesalpinia* species in Thailand have never been established. The aims of this study were to investigate the microscopic characteristics and constant numbers of the leaves of eight selected *Caesalpinia* species in Thailand.

2. Materials and Methods

Plant collection

The fresh mature leaves of eight *Caesalpinia* species were collected from September 2014 to August 2017. Each species was collected from three different sources throughout Thailand (Table 1). All samples were authenticated by Assoc. Prof. Dr. Nijsiri Ruangrungsi and compared with herbarium specimens at Forest Herbarium Thailand (BKF). Voucher specimens were deposited at the College of Public Health Sciences, Chulalongkorn University, Thailand.

Table 1 List of eight collected *Caesalpinia* species

No.	Scientific name	Place of location (Thailand)	Collecting date (Month, Year)
1	*Caesalpinia sappan* L.	Bangkok	September, 2014
		Phetchabun	September, 2014
		Ratchaburi	November, 2014
2	*Caesalpinia pulcherrima* (L.) Sw.	Bangkok	September, 2014
		Nonthaburi	September, 2014
		Samutsakhon	October, 2014
3	*Caesalpinia bonduc* (L.) Roxb.	Ratchaburi	September, 2014
		Nakhon Ratchasima	October, 2014
		Songkla	December, 2014
4	*Caesalpinia mimosoides* Lam.	Nakhon Ratchasima	November, 2014
		Chiang Mai	December, 2014
		Chiang Rai	February, 2015
5	*Caesalpinia coriaria* (Jacq.) Willd.	Bangkok	June, 2015
		Bangkok	June, 2015
		Prachinburi	July, 2015
6	*Caesalpinia digyna* Rottler	Bangkok	June, 2015
		Songkla	November, 2015
		Songkla	November, 2015
7	*Caesalpinia minax* Hance	Chiang Mai	May, 2015
		Chiang Mai	February, 2016
		Chiang Mai	February, 2016
8	*Caesalpinia decapetala* (Roth) Alston	Chiang Mai	May, 2015
		Chiang Mai	May, 2017
		Chiang Mai	August, 2017

Microscopic leaf anatomical evaluation

The transverse section of the midrib of each *Caesalpinia* species was examined. The leaf samples were cleaned before use. The cross-sectioning of the midrib was done by hand with a razor as thin as possible, transferred onto a slide, two drops of water added, and the anatomical characteristics observed

under a light microscope attached to a digital camera. All pictures were recorded by a digital camera and also illustrated by hand drawing with dimensions of a specific ratio relative to the actual size.

Microscopic leaf constant numbers

The stomatal number, stomatal index, trichome number, trichome index, epidermal cell area, and palisade ratio were evaluated.

The lamina of a fresh mature leaf was soaked in 50% Haiter solution in water (about 3% sodium hypochlorite) for 24 h until it was clear from the chlorophyll, then transferred into a beaker containing chloral hydrate:water (4:1 w v^{-1}) and heated on a water bath for 2 h. The transparent lamina was placed on a slide with 2 – 3 drops of water and the cells were examined under a microscope. The labeled image was recorded using AxioVision software. For the magnification, 40 × was used for examined palisade cells while 20 × power was used for stomatal and epidermal cells and 10 × was used for trichome cells. Every examination investigated both sides of the leaf. Thirty fields of each selected *Caesalpinia* species from three locations were assessed. The average of the 90 fields was calculated.

The stomatal number and stomatal index

The stomatal number and epidermal cells were counted and calculated per one square millimeter (mm^2) of the upper and lower epidermis of the leaf.

The stomatal index was evaluated as the ratio of stomata number (*S*) to all the epidermal cell number (*E*) (including trichome or cicatrix) in the same unit area of the leaf by the following formula [4]:

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

Palisade ratio

The palisade cells beneath four contiguous upper epidermal cells were counted then divided by four to obtain the palisade ratio [4].

Upper epidermal cell area

The epidermal cell area was estimated by dividing one square millimeter by the upper epidermal cell numbers [4].

Trichome number and trichome index

Trichome cells or cicatrices per one square millimeter of epidermis were counted. Trichome index, a percentage proportion of trichome number to all the epidermal cell number in one square millimeter, was calculated using the following formula [4]:

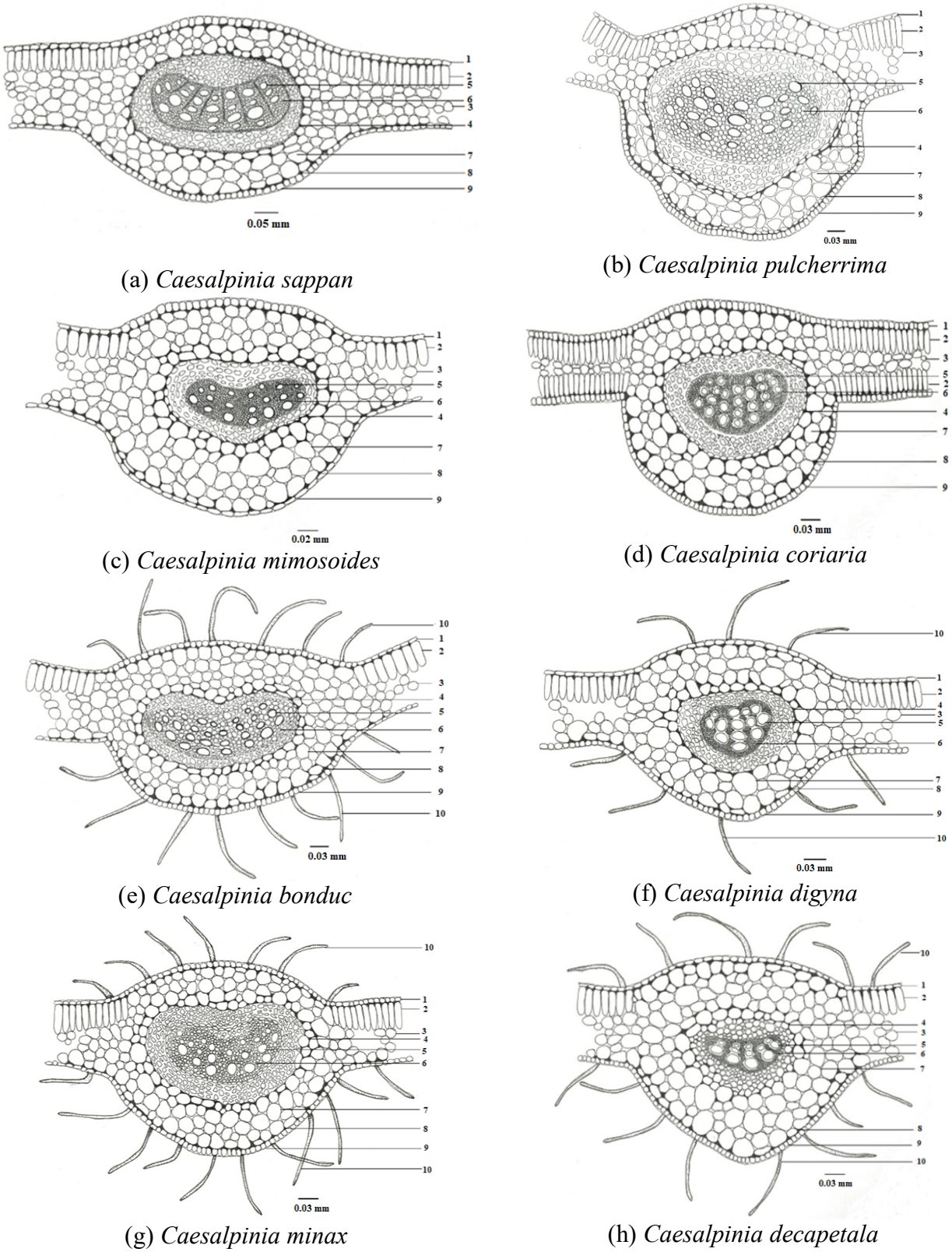
$$Trichome\ index = \frac{T \times 100}{E + S}$$

where T = the number of trichomes including cicatrices per unit area,
E = the number of epidermal cells in the same unit area including trichomes or cicatrices,
S = the number of stomatal cells in the same unit area of leaf.

3. Results and Discussion

Anatomical characteristics

The anatomical characteristics of midrib transverse sections of eight *Caesalpinia* species were obviously distinguished as shown in Fig. 1. *Caesalpinia bonduc*, *C. digyna*, *C. minax*, and *C. decapetala* contained trichomes on both upper and lower epidermis, as shown in Fig. 1 (e) – 1 (h). However, trichomes of *C. bonduc* were found at the margin and midrib of the leaf only. All trichomes were the unicellular nonglandular type. Stomatal cells were present only on the lower epidermis (Fig. 2). Palisade cells beneath epidermal cells are shown in Fig. 3.



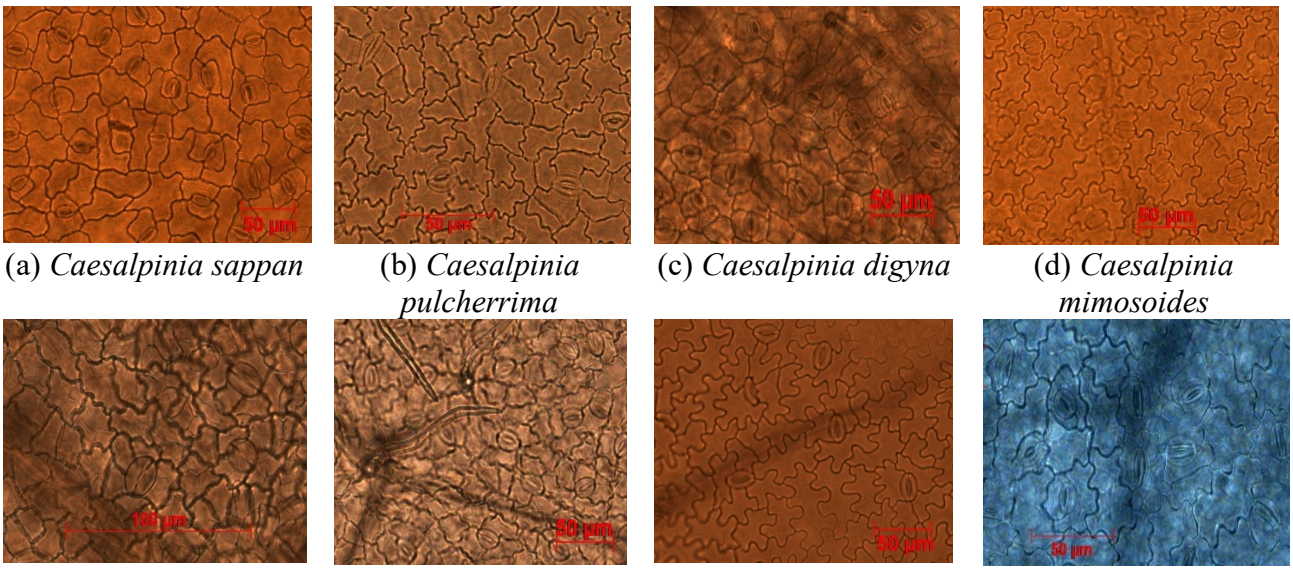
(a) *Caesalpinia sappan* (b) *Caesalpinia pulcherrima*

(c) *Caesalpinia mimosoides* (d) *Caesalpinia coriaria*

(e) *Caesalpinia bonduc* (f) *Caesalpinia digyna*

(g) *Caesalpinia minax* (h) *Caesalpinia decapetala*

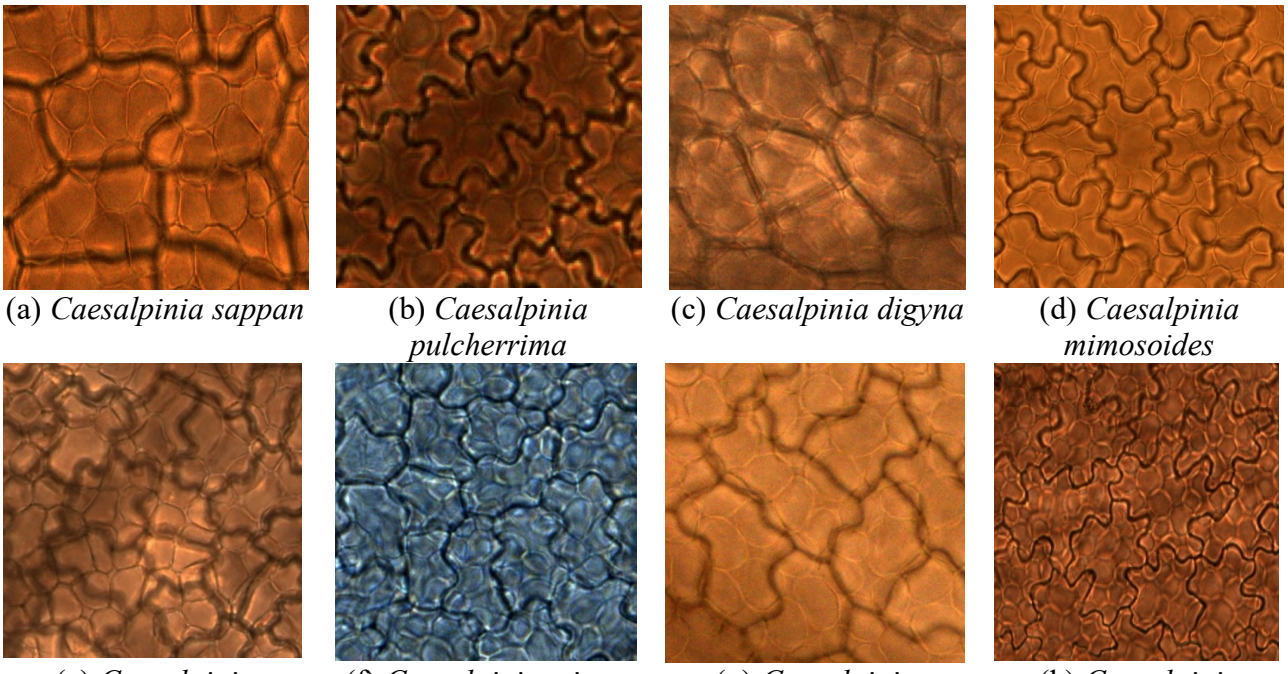
Fig. 1 Midrib transverse section of the leaf of eight *Caesalpinia* species (a) *C. sappan*, (b) *C. pulcherrima*, (c) *C. mimosoides*, (d) *C. coriaria*, (e) *C. bonduc*, (f) *C. digyna*, (g) *C. minax*, (h) *C. decapetala* 1. Upper epidermis, 2. Palisade cell, 3. Spongy cell, 4. Sclerenchyma, 5. Xylem tissue, 6. Phloem tissue, 7. Parenchyma, 8. Collenchyma, 9. Lower epidermis, 10. Unicellular non-glandular trichome



(a) *Caesalpinia sappan* (b) *Caesalpinia pulcherrima* (c) *Caesalpinia digyna* (d) *Caesalpinia mimosoides*

(e) *Caesalpinia decapetala* (f) *Caesalpinia minax* (g) *Caesalpinia bonduc* (h) *Caesalpinia coriaria*

Fig. 2 Stomatal cells of eight *Caesalpinia* species (a) *C. sappan*, (b) *C. pulcherrima*, (c) *C. digyna*, (d) *C. mimosoides*, (e) *C. decapetala*, (f) *C. minax*, (g) *C. bonduc*, (h) *C. coriaria*



(a) *Caesalpinia sappan* (b) *Caesalpinia pulcherrima* (c) *Caesalpinia digyna* (d) *Caesalpinia mimosoides*

(e) *Caesalpinia decapetala* (f) *Caesalpinia minax* (g) *Caesalpinia bonduc* (h) *Caesalpinia coriaria*

Fig. 3 Palisade cells of eight *Caesalpinia* species (a) *C. sappan*, (b) *C. pulcherrima*, (c) *C. digyna*, (d) *C. mimosoides*, (e) *C. decapetala*, (f) *C. minax*, (g) *C. bonduc*, (h) *C. coriaria*

The microscopic leaf constant numbers of eight *Caesalpinia* species are presented in Table 2 and 3. Trichome characteristics of *C. bonduc* can be used as an identification key for this species. Trichome number and trichome index determined from *C. minax*, *C. digyna*, and *C. decapetala* were found to be

overlapped. However, the upper epidermal cell area was capable of identifying *C. decapetala*. Furthermore, the stomatal number could differentiate *C. digyna* from *C. minax*. For non-trichome-containing species, *C. sappan* and *C. pulcherrima* can be identified by stomatal index and upper epidermal cell area. Microscopic leaf constant numbers between *C. mimosoides* and *C. coriaria* were overlapping, however, *C. coriaria* demonstrated its identity due to the isolateral character (Fig. 1 (d)).

Table 2 Microscopic leaf constant numbers of eight *Caesalpinia* species

***Caesalpinia* species**	**Lower epidermis**			**Upper epidermis**		
	Stomatal number	**Epidermal cell number**	**Stomatal index**	**Epidermis cell area (μm²)**	**Palisade ratio**	**Tri chome**
C. sappan	291.91 ± 23.63	1362.93 ± 63.73	17.62 ± 1.07	1452.50 ± 148.21	6.34 ± 0.63	-
	(230 – 348)	(1214 – 1504)	(15.32 – 19.82)	(1141.55 – 1724.14)	(5.00 – 7.50)	
C. pulcherrima	372.96 ± 57.17	3732.00 ± 454.41	9.07 ± 0.73	457.24 ± 36.19	5.90 ± 0.69	-
	(278 – 484)	(2938 – 4590)	(7.82 – 10.98)	(386.10 – 524.66)	(4.75 – 7.50)	
C. bonduc	115.62 ± 17.34	1498.69 ± 390.42	7.53 ± 1.72	947.79 ± 159.96	3.98 ± 0.84	+
	(90 - 154)	(900 – 2002)	(4.81 – 10.87)	(687.76 – 1225.49)	(3.00 – 5.75)	
C. mimosoides	265.29 ± 73.49	1289.36 ± 251.65	16.80 ± 1.75	886.80 ± 117.48	5.83 ± 0.80	-
	(130 – 386)	(862 – 1600)	(11.93 – 19.84)	(657.03 – 1103.75)	(4.63 – 7.50)	
C. coriaria	342.67 ± 96.53	1500.31 ± 163.30	18.27 ± 2.58	697.47 ± 53.43	9.78 ± 2.22	-
	(226 – 562)	(1216 – 1832)	(14.89 – 24.96)	(565.61 – 771.60)	(6.13 – 13.63)	
C. digyna	320.54 ± 48.54	1746.42 ± 244.21	15.52 ± 1.14	808.68 ± 78.44	6.04 ± 0.58	+
	(248 – 412)	(1372 – 2156)	(13.34 – 18.77)	(666.67 – 957.85)	(5.13 – 7.88)	
C. minax	156.11 ± 15.15	1138.49 ± 56.53	12.06 ± 0.99	905.86 ± 56.36	5.61 ± 0.51	+
	(128 - 192)	(960 – 1276)	(10.19 – 14.89)	(796.17 – 1091.70)	(4.63 – 6.75)	
C. decapetala	254.82 ± 23.14	3472.13 ± 230.94	6.60 ± 0.59	318.50 ± 36.55	4.20 ± 0.40	+
	(208 – 312)	(2754 – 3958)	(5.45 – 8.65)	(279.96 – 397.46)	(3.25 – 5.25)	

Table 3 The summary of microscopic leaf constant numbers of four *Caesalpinia* species containing trichome

***Caesalpinia* species**	**Lower epidermis**			**Upper epidermis**		
	Trichome number	**Epidermal cell number**	**Trichome index**	**Trichome number**	**Epidermal cell number**	**Trichome index**
C. bonduc	-*	-	-	-	-	-
C. digyna	15.15 ± 2.16	1694.16 ± 206.93	0.92 ± 0.17	30.93 ± 3.28	2245.33 ± 258.89	1.28 ± 0.23
	(11 – 21)	(1312 – 2122)	(0.66 – 1.41)	(23 – 39)	(1810 – 2800)	(0.89 – 2.00)
C. minax	10.61 ± 1.57	1624.73 ± 321.59	0.67 ± 0.16	18.02 ± 2.43	2090.12 ± 373.33	0.88 ± 0.18
	(8 – 15)	(1196 – 2374)	(0.35 – 1.06)	(14 – 24)	(1616 – 2816)	(0.56 – 1.31)
C. decapetala	18.69 ± 2.30	3098.08 ± 218.51	0.60 ± 0.07	30.72 ± 6.09	3338.83 ± 209.94	0.92 ± 0.18
	(15 – 27)	(2636 – 3510)	(0.47 – 0.81)	(20 – 42)	(2876 – 3812)	(0.57 – 1.36)

*Could not detect but trichomes found at midrib and margin of the leaf

Leaf anatomy, especially at the midrib part, is considered as a stable region regarding the conservation of its structures when submitted to the image acquisition process [5]. In this study, the leaves of eight *Caesalpinia* species were examined to study the midrib transverse section. The structures of upper epidermis, palisade cell, spongy cell, sclerenchyma, xylem tissue, phloem tissue, parenchyma, collenchyma, and lower epidermis were illustrated. Unicellular nonglandular trichomes, found in *C. bonduc*, *C. digyna*, *C. minax*, and *C. decapetala*, were in agreement with the previous study [6]. In

2015, Mehra and team reported that unicellular trichomes were present on the upper and lower epidermis from the transverse section through midrib of *C. bonduc* in India [7]. *Caesalpinia bonduc* in this study also demonstrated trichomes only at both epidermises of the midrib cross section. So, the trichome numbers and trichome index determined from the lamina could not be evaluated. This is an identity of *C. bonduc* that would be beneficial for the species authentication.

To identify the plant at the species level, microscopic leaf constant measurement is one of the most useful parameters [8]. The palisade ratio is counted as a reliable taxonomic character that does not vary with the environment. It is constant in different parts of an individual leaf and shows the same range in the leaves of a single species from a range of habitats and also in leaves of a single species collected over a sequence of years. The palisade ratios among plant species in various genera have been studied [9]. This study demonstrated the palisade ratio of selected *Caesalpinia* species in Thailand. It was found that the palisade ratio among the eight species seemed to be overlapped. The palisade ratio of *C. bonduc* leaves in India was found to be higher than the finding in this study (14 – 18 *vs* 3 – 6) [10]. The value of epidermal cell area is relatively constant within a small range for each species, regardless of the overlapping with closely related species [11]. This study found that the upper epidermal cell area was useful in differentiating *C. sappan* from *C. pulcherrima.*

4. Conclusion

The qualitative and quantitative microscopic characteristics of the leaves of the eight selected *Caesalpinia* species in Thailand were established. The anatomical characteristics of the midrib as well as the constant numbers of the lamina could be used as a tool for authentication of these plants. The microscopic leaf characteristics of other *Caesalpinia* species in Thailand should be further investigated for pharmacognostic applications.

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