Comparative anatomical study and morphological observation of three medicinal *Vitex* species (Lamiaceae)

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Abstract: : Many species of *Vitex* have medicinal values and despite of being widely utilized, they are sometimes confusing due to high morphological variation. In this study, leaf anatomical characters of three species, *V. pinnata, V. negundo* and *V. trifolia* have been studied comprehensively including the epidermal characters, margin TS, lamina TS, midrib TS and petiole TS. Leaf morphological observation was also conducted. Generally, all three species show similar anatomical characters. However, some anatomical variations can be diagnostic and taxonomically valuable. Morphological characters for the leaf apex and lamina can also be used in delineating these three species**.**

Key words: *Vitex,* Lamiaceae, anatomy, morphology, comparative study

INTRODUCTION

Vitex belongs to the Lamiaceae whose members are very diverse in morphology and also systematically controversial including *Vitex*. *Vitex* is a genus which has palmately compound, three to five leaflets. There are 250 known species worldwide, 25 species occur in Southeast Asia (La Frankie 2010) and 10 species are recorded in Peninsular Malaysia (Turner 1995). Locally, *Vitex* species are known as *Legundi, Leban, Halban, Lemuning, Muning, Demundi* and *Lemuni. Vitex* is commonly used by the locals in traditional medicine preparation to treat various illnesses including during post-partum care. It is used to treat fever and wounds and also used to comfort pain after birth. Many are not aware the existence of variants in *Vitex* which cause the species sometimes difficult to be identified (Azimi *et al*. 2006). Therefore, this comparative study was conducted to investigate the interspecific variation between species especially *V. pinnata, V. negundo* and *V. trifolia*.

METHODOLOGY

Fresh samples of *V. pinnata*, *V. negundo* and *V. trifolia* including leaf and fruit were collected through a series of fieldworks in Peninsular Malaysia and Sarawak. Specimens including replicates were studied morphologically and anatomically. Herbarium vouchers were prepared and deposited in UPSI herbarium and anatomical slides were kept at Plant microtechnique laboratory, UPSI. Herbarium and field samples were studied morphologically and fixed leaf specimens in FAA were then underwent a microtechnique procedure which is a modification of Johansen (1940), Cutler et al. (2008) and Nor Nafizah (2006). Microimages were captured using Nikon Eclipse 2000-U microscope fitted with image analyzer. Anatomical descriptions were made according to Metcalfe and Chalk (1969, 1979). Morphological study including field and herbarium study was done at Universiti Pendidikan Sultan Idris and Forest Research Institute Malaysia herbarium using Leica light and dissecting microscopes.

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RESULT AND DISCUSSION

Morphological and anatomical observations show some variations between the three *Vitex* species studied. Anatomically, all species show high homogeneity and similarity. However, there are some variations shown interspecifically. These anatomical characters are found to be useful and valuable taxonomically especially in delineating the three species, further aid the identification of the species. Details of the anatomical observation were presented in Tables 1, 2 and 3.

Vitex epidermal characters show great similarities. They posess anomocytic stomata where surrounding epidermal cells and subsidiary cells are not differentiated, open areoles and looped marginal venation. Distribution of stomata in *V. pinnata* is denser compared to *V. negundo* and this might be due to high density of tomentose hairs covering the *V. negundo* epidermis and well-segmented macrohairs were also found on the adaxial surface (Musnif *et al.* 2007). On the other hand, *V. trifolia* shows the most dense stomata per area in comparison to the other two species. Significant variations in anatomical characters between them is type of anticlinal wall of the epidermal cells. The anticlinal wall of *V. pinnata* is straight to curved while *V. negundo* and *V. trifolia* they are slightly sinous. Details epidermal characters are presented in Table 1. Microimages of epidermal character of the studied sample are shown in Figure 1.

Table 1: Epidermal characters and venation of *V. pinnata V. negundo* and *V. trifolia.*

Species			Stomata		Venation	Areoles
	Adaxial	Abaxial	Abaxial	Adaxial		
V. pinnata	Straight to curved anticlinal	Straight to curved anticlinal	Anomocytic	-	Looped	Open
V. negundo	Slightly sinous anticlinal	Slightly sinous anticlinal	Anomocytic	-	Looped	Open
V. trifolia	Slightly sinous anticlinal	Slightly sinous anticlinal	Anomocytic	Anomocytic	Looped	Open

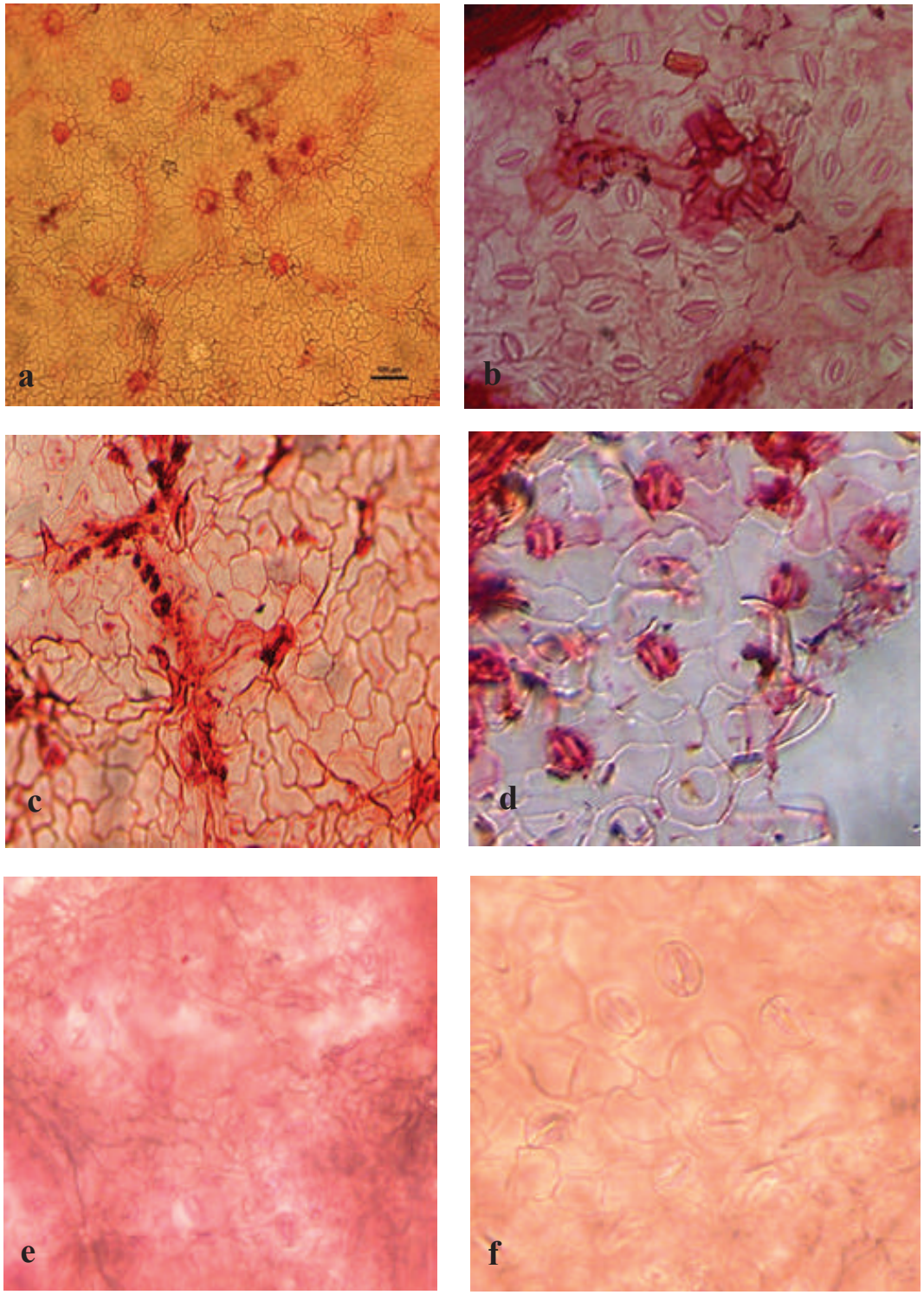

Figure 1. Epidermal characters of *Vitex*. **a.** adaxial epidermis of *V. pinnata*, **b.** abaxial epidermis of *V. negundo* **e.** adaxial epidermis of *V. trifolia*. Scale bar = 100 µm (**a-f t**o the same scale).

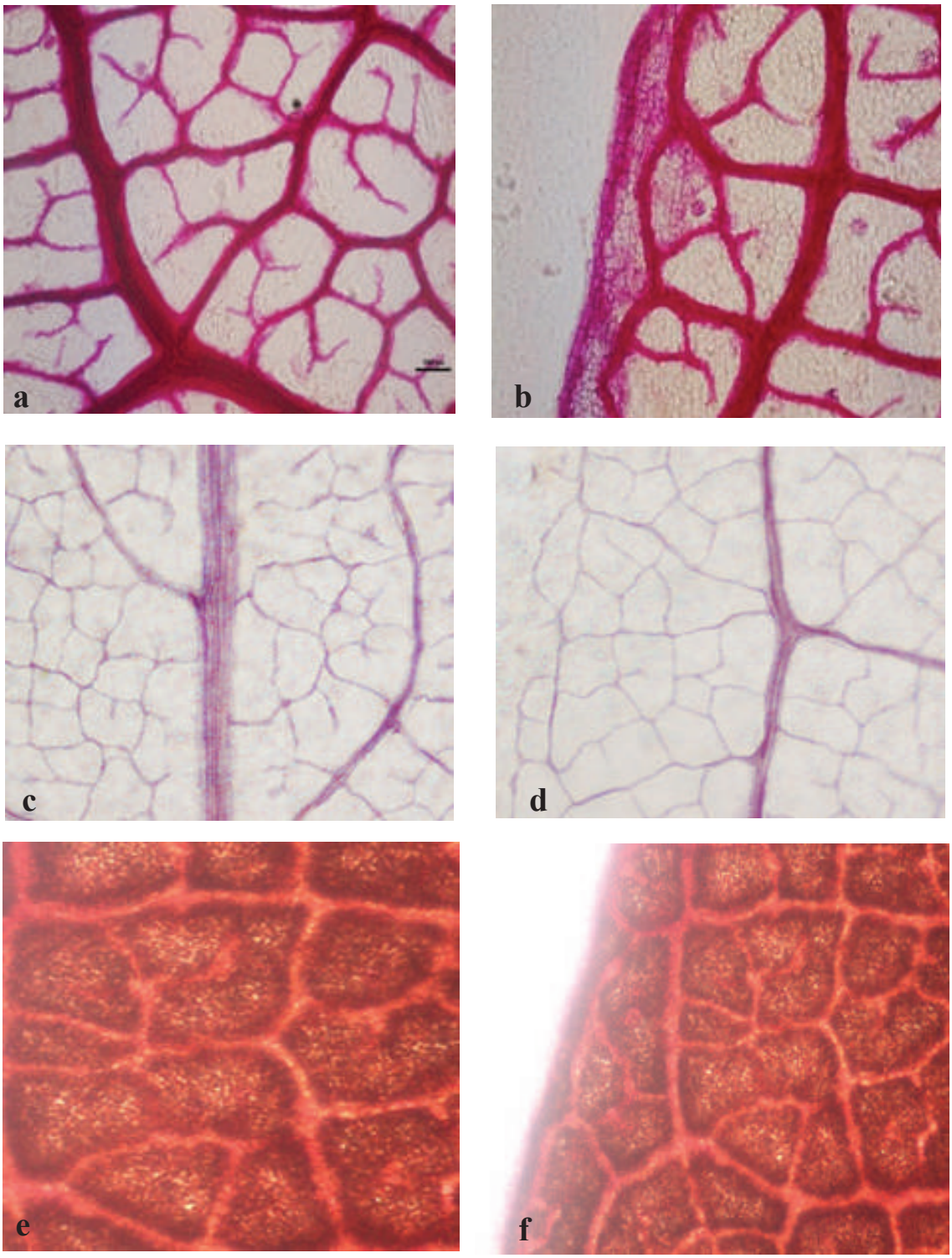

Figure 2. Areole and marginal venation of *Vitex.* **a.** open veinlets in areoles of *V. pinnata.* **b.** Looped marginal venation in *V. pinnata*, **c.** open veinlets in areoles of *V. negundo*, **d.** Looped marginal venation in *V.negundo.* **d.** open veinlets in areoles of *V. trifolia*, **d.** Looped marginal venation in *V. trifolia*. Scale bar in a = 100 µm (**a - f** to the same scale).

Leaf transverse sections (T.S) show some significant variations as clearly summarized in **Tables 2** and **3**. *V. pinnata* margin tips are acute and incurved whilst, margin tip of *V. negundo* and *V. trifolia* are obtuse and incurved. Stomata are not observed on margin T.S of all of the studied species of *Vitex*. Chlorenchyma tissues which composed of palisade and spongy mesophyll cells show similarity between them especially the layer numbers of palisade. Palisade of all the studied species are arranged in double layers and followed by two to three layers of spongy tissues. Vascular bundles are closed type and ensheathed by sclerenchymatous tissues. Stomata are not seen in all the studied *Vitex* leaf lamina sections.

The vascular bundle present in *V. pinnata, V. negundo* and *V. trifolia* were closed vascular bundle and sclerenchyma sheath present in all *Vitex* spesies. Midrib T.S show high similarity except the slight different in the outline, *V. pinnata* is ridged adaxially and half rounded abaxially, *V. negundo* is flat adaxially and 'U' shaped to rounded abaxially whereas, midrib outline of *V. trifolia* is flat adaxially and rounded abaxially. (**Figure 3g, 3h & 3i**) Details of leaf TS is summarized in **Table 2**.

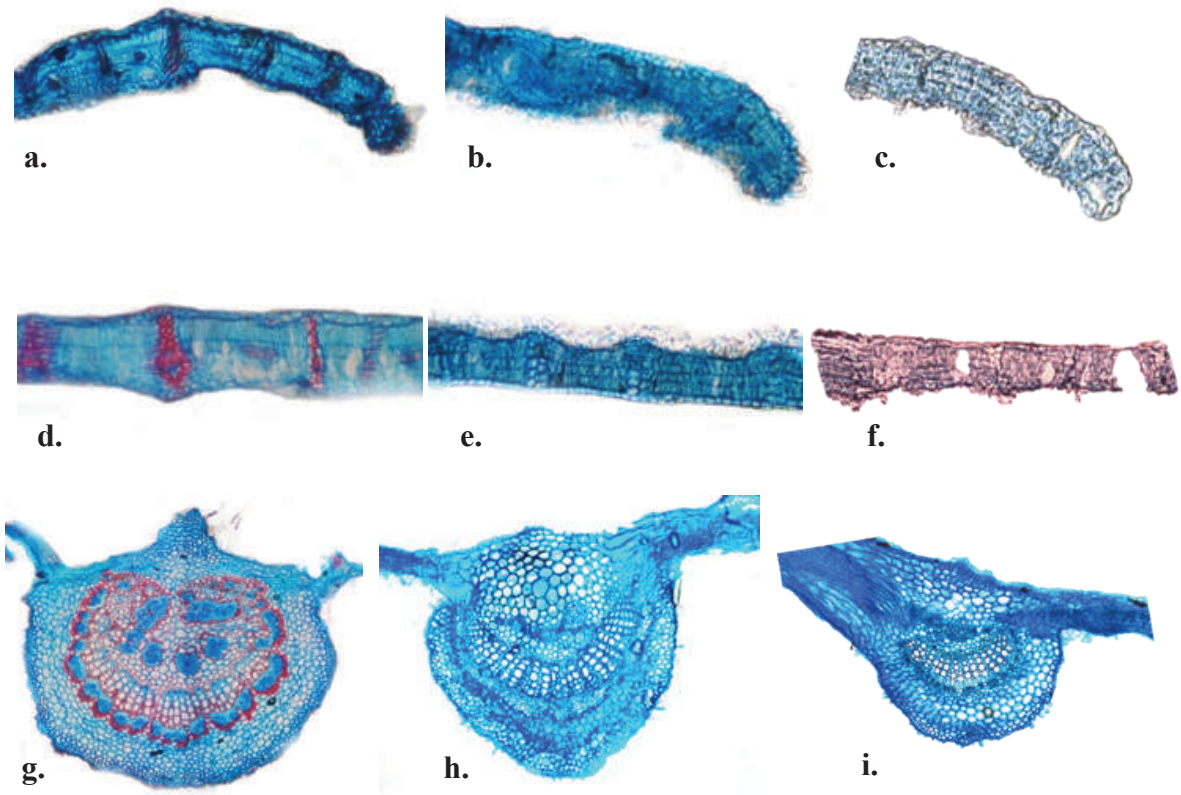

Figure 3. Leaf T.S of *Vitex.* **a.** Margin T.S of *V. pinnata*. **b**. Margin T.S of *V. negundo*. **c.** Margin T.S of *V. trifolia*. **d.** Lamina T.S of *V. pinnata*. **e.** Lamina T.S of *V. negundo*. **f.** Lamina T.S of *V. trifolia*. **g.** Midrib T.S of *V. pinnata*. **h.** Midrib T.S of *V. negundo* and **i.** Midrib T.S of *V. trifolia*.

Petiole anatomical characters are also of great similarities except the outline which can be useful to differentiate between the three *Vitex* species. All *V. pinnata, V. negundo* and *V. trifolia* have lobed like projection at both flanges, flattern adaxially in both *V. trifolia* and *V. negundo* but ridged adaxially in *V. pinnata* (**Figure 4a, 4b & 4c**). Details of petiole are presented in **Table 4.**

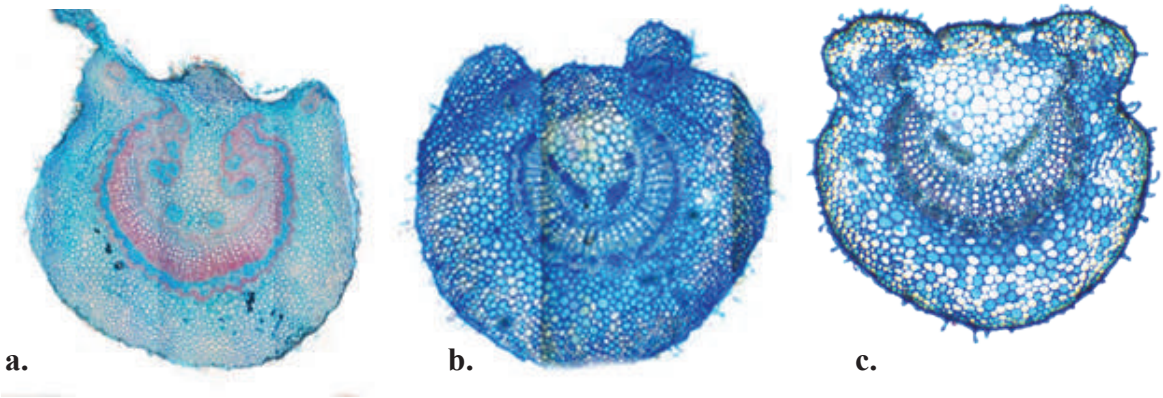

Figure 4. Petiole T.S of *Vitex*. **a.** *V. pinnata.* **b.** *V. negundo* **c.** *V. trifolia.*

Table 2. Anatomical characters on leaf lamina, midrib and margin TS

Spesies	Midrib Outline	Margin Outline	Epidermal cell		Stomata	Chlorenchyma		Sclerenchyma sheath	Crystal Type	Trichome	Vascular Bundles
			Adaxial	Abaxial		Palisade	Spongy				
V. pinnata	Convex adaxially, rounded abaxially	Obtuse to slightly acute; slightly incurved	1:½-1½:1	1:1-1:½	Not seen	2 lyrs	2-3 lyrs	Present	Druses	Non-glandular	Closed
V. negundo	Flattern adaxially, ‘U’shaped abaxially	Obtuse; slightly incurved	1:1½-1:1	1:1-1:½	Not seen	2 lyrs	2-3 lyrs	Present	Druses	Non-glandular	Closed
V. trifolia	Flattex adaxially, rounded abaxially	Obtuse; slightly incurved	1:½ - 1:½	1:1-1:1	Not seen	2 lyrs	2-3 lyrs	Present	-	Non-glandular	Closed

Table 3. Result of anatomical observation on petiole TS

Spesies	Outline	Epidermal cell		Ground tissue (layers)		Vascular tissue	Trichome	Sclerenchyma cells	Crystal Type	Secretory cells	Crystal (type)
		Adaxial	Abaxial	Collenchyma	Parenchyma						
V. pinnata	Rounded , projection on both flanges and ridged adaxially	1½:1-1:½	1½:1-1:½	4-14	8-17	Close	Non-glandular	Incomplete	Druses	Absent	Druses
V. negundo	Rounded with projection on both flanges, flattern adaxially	1:½- 1:1	1½:1-1:½	5-8	8-11	Close	Non-glandular	Incomplete	Druses	Absent	Druses
V. trifolia	Rounded with projection on both flanges, obtuse adaxially	1:1-1:1	1 ½ :1 – 1 ½ :1	2-4	4-9	Close	Non-glandular	Incomplete	-	Absent	Druses

Morphological differences between both species are conspicuously seen within the leaf shape (**Table 4**). *V. pinnata* leaf apex is cuspidate while *V. negundo* is acuminate and *V. trifolia* is attenuate. The leaf blade of *V. pinnata* is ovate while both of the leaf blade of *V. negundo* and *V. trifolia* is lanceolate. Both of the species have entire margin. *V. trifolia* has attenuate leaf base while *V. negundo* cuneate leaf base and lastly, *V. pinnata* has acute leaf base. All of the studied species has compound type with three to five leaflets (**Table 4**, **Figure 4, 5 & 6**). These morphological characters observed agreed with Chantaranothai (2011) morphological description of Thailand species.

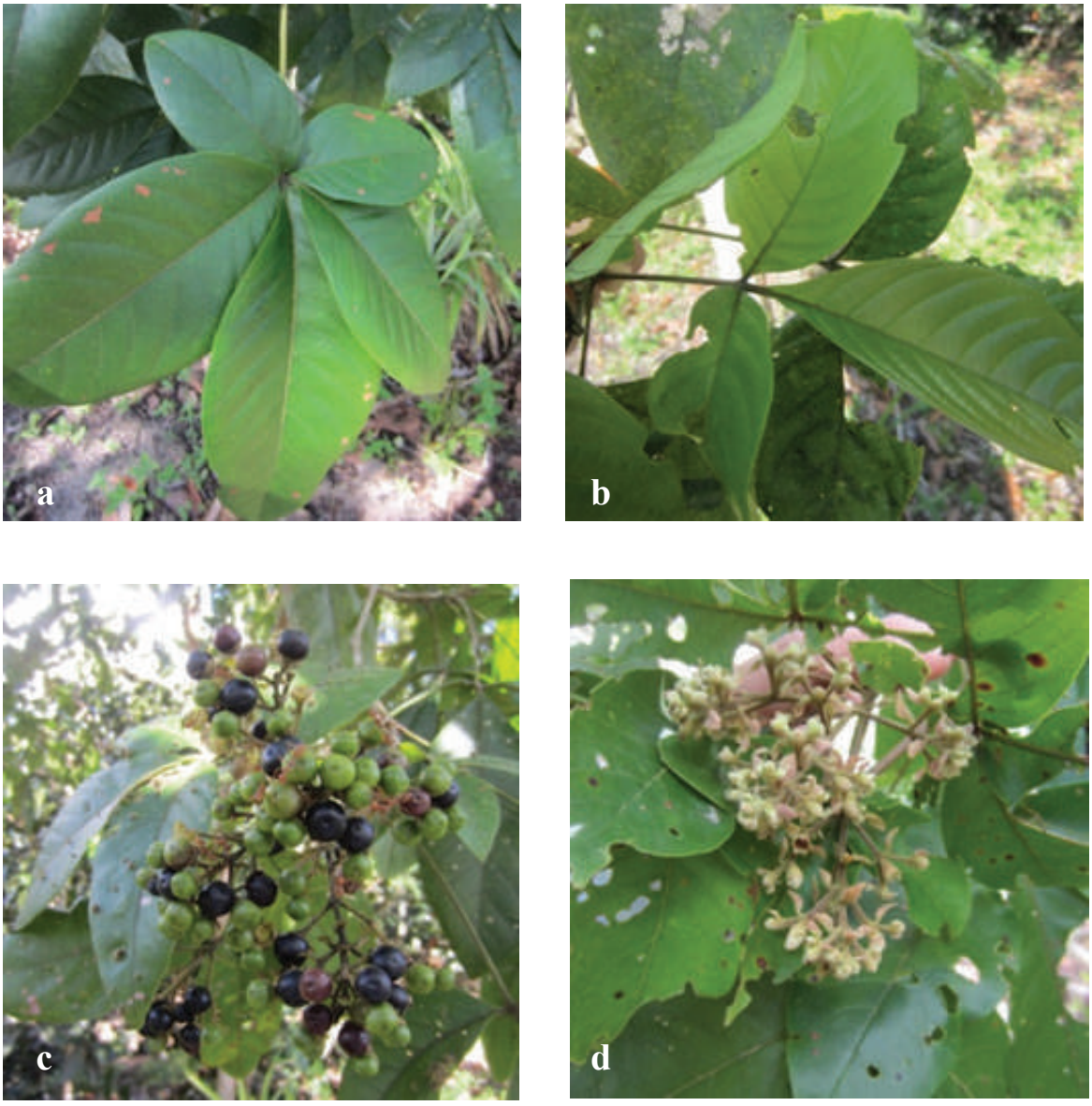

Figure 4. *V. pinnata.* **a.** five leaflets, **b**, three leaflets, **c.** fruits, **d.** flowers.

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Figure 5. *V. negundo*. a. three to five leaflets. b. flower and fruit

Figure 6. *V. trifolia* **a.** three leaflet, **b.** flowers

Table 4. Leaf morphological characters of *V. pinnata, V. negundo* and *V. trifolia*

	Type of tree	Type of leaves	Number of leaflet	Leaves arrangement	Whole leaf lamina shape	Leaf blade	Leaf apex	Leaf base	Leaf margin	Leaf texture
V. pinnata	Small tree	Compound	5	Opposite	Symmetry	Ovate	Cuspidate	Acute	Entire	Coriaceous
V. negundo	Shrub	Compound	5	Opposite	Symmetry	Lanceolate	Accuminate	Cuneate	Entire – undulate	Coriaceous
V. trifolia	Small tree	Compound	3	Opposite	Symmetry	Lanceolate	Attenuate	Attenuate	Entire	Coriaceous

Table 5. Leaf morphological characters of *V. pinnata, V. negundo* and *V. trifolia continue*.

	Leaf venation	Tertiary venation	Trichome	Petiole length	Petiolule (cm)	Leaf length (cm)	Leaf width (cm)	Abax trichome	Adax trichome	Secondary vein distance (cm)
V. pinnata	Pinnate	Scalariform	Absent	5.5-9.0 cm	Almost sessile	9.5-13.0	4.5-5.5	Absent	Absent	0.5-1.5
V. negundo	Pinnate	Scalariform	Present	7.5-9.0 cm	1.5-2.5	11.0-13.0	1.5-2.5	Absent	Absent	0.5
V. trifolia	Pinnate	Scalariform	Present	3.0-6.0 cm	1.0-1.5	7.5-9.0	1.7-2.0	Absent	Absent	0.7-0.9

CONCLUSION

Anatomically, the studied species have high similarity of having the same non-glandular type of trichomes, anamocytic stomatal type and epidermal characters. Communal and high similarity in anatomical characters of *V. pinnata, V. trifolia* and *V. negundo* suggest and support them belongs to the same genus. However, some limited characters are found to be useful in delineating these *Vitex* spesies. Petiole outline of the species for example can be vey helpful to differentiate the three studied spesies. Rounded petiole outline with flanges seen in *V. pinnata, V. negundo* and, *V. trifolia*. However, *V. pinnata* petiole is ridged adaxially inbetween the two flanges and flatten inbetween flanges observed in *V. negundo* and *V. trifolia*. Obtuse outline abaxially differentiate *V. trifolia* from *V. negundo*. Midrib outline are also valuable characters for species identification. Midrib outline of *V. pinnata* is also ridged adaxially which is opposed to *V. negundo* and *V. trifolia* of flattern adaxially.

V. pinnata has compound three to five leaflets and share many characters with *V. negundo* and *V. trifolia* morphologically. Compound leaf with three to five leafleats are one of major characters belongs to the genus *Vitex*. Morphologically, these three spesies are able to be differentiated with close observation on the leaf. The shape of leaf blade, apex and based can be used to differentiate them. Cuspidate leaf apex seen in *V. pinnata* whereas acuminate leaf apex in *V. negundo* while, attenuate leaf apex in *V. trifolia*. The leaf blade of *V. pinnata* is ovate while the leaf blade of *V. negundo* and *V. trifolia* is lanceolate. Hopefully, these fundamental findings more or less are able to contribute some information in resolving the taxonomic problem that arose in Lamiaceae, especially species identification of some confusing *Vitex* especially those medicinal. The genus *Vitex* is being one of the most prioritizes to berevised within the family (Azimi et al. 2006). The genus was also claimed has never been fully revised (De Kok, 2008) and suggested to have a comprehensive taxonomic treatment in further research.

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REFERENCES

Azimi, R., Jamzad, Z., Sefidkon, F. and Bakhshi-Khaniki, Gh. (2006). potential value of phytochemical and micromorphological characters in taxonomic treatment of genus *Vitex* L. (Lamiaceae). *Iran. Journ. Bot.* 12 (1) : 15-35.

Chantaranothai, P. (2011). A Revision of the Genus Vitex (Lamiaceae) in Thailand. *Tropical Natural History* 11(2) : 91-118

de Kok, R. (2008). The genus Vitex (Labiatae) in the flora malesian region, excluding Papua New Guinea. *Kew Bulletin* 63 : 17-40.

Johansen, D. A. (1940). *Plant Microtechnique.* Bombay: Tata Mc Graw-Hill Book Company.

LaFrankie, J. (2010). *Trees of Tropical Asia: An Illustrated Guide to Diversity.* Philippines: Black Tree Publications, Inc.

Munsif, S., Mir Ajab Khan, Mushtaq Ahmad, Muhammad Zafar, Ghulam Mujtaba Shah and Nighat Shaheen. (2007.) Leaf Epidermal Anatomy as an Aid to the Identification of Genera *Lantana, Verbena* and *Vitex* of Family Verbenaceae from Pakistan. Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan. View on 20 January 2013 from http://www.fspublishers.org/jass/past-issues/JASSVOL_3_No_2/2.pdf

Nor Nafizah, M.N. (2006). *Comparative Leaf Anatomy, Phytochemistry and Palynology of Nine Genera of Dipterocarpoideae* (Dipterocarpaceae). Ph.D thesis The University of Reading, United Kingdom. (unpublished).

Phongoudome, C. (2000). A Systematic Study on *Vitex* Tourn. (Verbenaceae) in Sabah and Sarawak, Malaysia. MSc Thesis. Faculty of Forestry. Universiti Putra Malaysia. (Unpublished).

Turner, L. M. (1995). A Catalogue of the Vascular Plant of Malaya. *Gards' Bull. Sing*. 47(2) : 347-655.

Slik, J.W.K (2009). *Plant of Southeast Asia.* View on 17 June 2013 from http://www.asianplant.net/ Lamiaceae/Vitex_pinnata.htm

Vishwanathan, A.S. and Basavaraju, R. (2010). A review on *Vitex negundo* L.- A medicinal Important plant. India: Department of Biosciences, Sri Sathya Sai University, Prasanthi Nilayam, Puttaparthi, Andhra Pradesh, India.