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TAXONOMIC VALUE OF LEAF VENATION AND TRICHOME CHARACTERISTICS IN SOME *Byttneria* L. AND *Pterocymbium* R. BR. (MALVACEAE *s.l*)

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ABSTRACT

A study on some leaf anatomical characteristics was undertaken on two genera in Malvaceae *s.l* namely *Byttneria* L. and *Pterocymbium* R. Br. The objective was to determine the taxonomic value of leaf venation characteristics especially in identification and classification of species. In the study, five species were chosen, three *Byttneria* species, namely *B. curtisii, B. jackiana, B. maingayi,* and two *Pterocymbium* species, namely *P. tinctorium* and *P. tubulatum. Byttneria* and *Pterocymbium* have been split into two subfamilies, Byttneroideae and Sterculoideae, of Malvaceae *s.l* respectively. Leaf clearing, staining, mounting and observation under a light microscope were techniques used for the study. Results showed that the similarities and differences in leaf venation could be useful in classification and identification of species. The common characteristics found in all species studied were the presence of entire marginal venation and closed system venation with minimum free ending veinlets in the areolar venation. The variations in the leaf venation can be used to distinguish species, which are complete ultimate marginal venation in *B. curtisii* and *B. maingayi*; curved to looped pattern and incomplete, ultimate marginal venation in *B. jackiana*, *P. tubulatum* and *P. tinctorium*. The presence of simple unicellular trichomes in *B. jackiana* and two types of trichomes (simple unicellular trichomes and multicellular glandular trichomes) and crystals alongside veinlets in *P. tubulatum* can also be used to further identify these two species from other species studied. Therefore, the study showed that leaf anatomical characteristics in *Byttneria* and *Pterocymbium* can be used in species identification and have taxonomic value.

Key words: Malvaceae, *Pterocymbium*, *Byttneria*, leaf venation, marginal and areolar venation, trichomes

INTRODUCTION

It is well known to the systematists that the delimitation of families within the "core" Malvales is problematic (Cronquist, 1981). Identification of species from this order, family, species and genus in the field is difficult especially for specimens or trees without flowers or fruit. According to Kochummen (1973), the families of Malvales are closely related to each other. Sterculiaceae *s.s* is especially closely related to Tiliaceae *s.s* and no clear distinctions exist; the main difference is the tendency for the stamens to be joined as a tube or column in Sterculiaceae *s.s* and free in Tiliaceae *s.s.* He also stated that not all Malayan Sterculiaceae s.s are equally distinct from each other. Several species such as *Pterygota alata* was previously placed in *Sterculia* (*S. alata*). The confusion may result in the wrong identification of sterile material such as in herbarium specimens therefore, anatomical evidence is needed in addition to leaf morphological characteristics for species identification (Hussin & Sani, 1996). Cutler *et al.* (2008) mentioned that lately, leaf sample in most herbarium in China will be analyzed using anatomical methods even though flower and fruit are present in the specimens. Corner (1952) stated even with the presence of flower, this family is a difficult one to define due to the great diversity that is shown. It is closely allied with the *Hibiscus* and jute-families (Malvaceae *s.s*, Tiliaceae *s.s*) and in several aspects, such as the tendency to have unisexual flowers, with the rubber-tree family (Euphorbiaceae).

According to Turner (1995), there are six species of *Byttneria* found at forest fringes of Peninsular Malaysia, and these are *B. beccarii* Warb., *B. curtisii* Oliv., *B. jackiana* Wall., *B. maingayii* Mast., *B. mastersii* Cristóbal, and *B. pilosa* Roxb.. However

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only two species of *Pterocymbium* have been recorded and they are *P. tinctorium* (Blanco) Merr. and *P. tubulatum* (Mast.) Pierre. Kostermans (1950) stated that in the Malesian region there are three species belonging to the genus *Pterocymbium,* with one variety recognized, they are *P. beccarii* K. Schumann, *P. tinctorium* (Blanco) Merrill, with var. *javanicum* (R. Brown) Kostermans and *P. tubulatum* (Masters) Pierre.

Almost all the species of *Byttneria* and *Pterocymbium* are difficult to identify especially in the field because flowers or fruits are usually absent. The morphological characteristics are similar and these factors contribute to difficulties in identification and classification. Therefore, further studies are required in order to enhance the knowledge on these genera. The objective of the study was to quantify significant leaf venation characteristics that could be used for identification of species. Hickey (1973) stated that leaf venation is correlated to plant evolution and has systematic significance in plant identification and classification.

One of the most prominent features of the lamina and one which has long been employed by systematists is trichomes or indumentum. Essentially all the major groups of terrestrial plants appear to have or have had the capacity to develop such structures and trichomes are the basic building blocks from which indumenta are made (Johnson 1975). They have been employed both for classification and identification purposes by many systematists (Dickison, 2000; Batterman & Lammers, 2004; Sonibare *et al.*, 2005). Stace (1969) noted that there is great diversity in the indumentum which consists of unicellular hairs, glandular hairs, peltate trichomes of various types, stalked glands and scales that are of the greatest taxonomic value. Edmonds (1982) stated that the significance of trichomes in plant systematics has long been recognised in the classification of angiosperms. Plant anatomy on the other hand not only enable a deeper understanding of plant physiology, plant growth (with structural correlation) and plant function, but also provides, data which facilitate problem solving in plant taxonomy (Metcalfe & Chalk, 1963; Dickison, 2000).

MATERIALS AND METHODS

Fresh and dried leaf samples were used for the study. Fresh specimens were collected from several forest reserves in Peninsular Malaysia, one being the Pasoh Forest Reserve in Negeri Sembilan. Dried leaf samples were obtained from the FRIM Herbarium in Kepong (KEP) and Universiti Kebangsaan Malaysia Herbarium, in Bangi (UKMB). A list of the species studied is given in Table 1. Voucher specimens are kept at UKMB Herbarium. Fresh leaf specimens collected were fixed in AA (70% Ethanol: 30% Acetic Acid in ratio of 1:3), while dried herbarium samples were boiled. A measurement of 1cm x 1 cm leaf lamina and marginal area were cleared using Basic Fuchsin solution (10% Basic Fuchsin and 10% KOH), and placed in the microwave oven at 60ºC for one to two days depending on the thickness of the leaf specimen. Cleared leaf specimen were then go through dehydration in an alcohol series, clearing in xylene and mounted on slides using Canada Balsam and placed in the oven at 60ºC for nearly two weeks (See Table 2). Slides were then photographed using a digital camera (JVC) mounted on an Olympus microscope. Leaf venation patterns were observed using Cell B Software (soft-imaging system) under 10x, 20x, 40x and 100x magnifications and processed using Adobe Photoshop software. Details of the analysis and descriptions on the leaf venation types followed the classification of Hickey (1973) and the descriptions of trichome types followed those of Metcalfe and Chalk (1979).

RESULTS AND DISCUSSION

The findings of the present study have shown that there are similarities and differences present in the leaf venation (anatomical) characteristics. For both *Byttneria* and *Pterocymbium* the common significant anatomical character found in the study was the entire marginal venation (Figure 1A-E) and as for the areolar venation, closed system venation were observed with minimum free ending veinlets (Figure 2 A-J). The variations in the leaf venation can be used to distinguish species, which are ultimate marginal venation is complete in *B. curtisii* (Figure 1A) and *B. maingayi* (Figure 1C), re-curved to looped patterns and incomplete venation in *B. jackiana* (Figure 1B), *P. tinctorium* (Figure 1D) and *P. tubulatum* (Figure 1E).

In a previous study done by Nurul-Aini *et al*. (2010) on the genera *Grewia* and *Microcos* (Grewioideae) proved that the differences in leaf venation were also found useful to distinguish the two studied genera. The ultimate marginal venation was incomplete and not looped in *Grewia* but complete and looped in all *Microcos* sp. A study by Nurshahidah *et al.* (2011) stated that the leaf venation can be used in identification of species and has systematic significance for the genus *Carallia.* The common anatomical character found in the genus *Carallia* was the incomplete marginal venation. The variations in the leaf venation can be used to distinguish species, which are entire

Table 1. List of specimens and species studied

No.	Species	Locality and Specimen Code
1.	*Byttneria curtisii* Oliv.	Malaysia, Negeri Sembilan, Kuala Pilah, Senaling Inas Forest Reserve, 07.03.1937, Symington, C.F., FMS 43549
2	*B. curtisii* Oliv.	Malaysia, Negeri Sembilan, Kuala Pilah, Senaling Inas Forest Reserve, 21.11.1936, Symington, C.F., FMS 42967
3	*B. curtisii* Oliv.	Malaysia, Perak, Larut Matang, Pondok Tanjung Forest Reserve, 07.08.1934, Symington, C.F., FMS 37374
4	*B. curtisii* Oliv.	Malaysia, Pahang, Bentong, Genting Highlands Rd, 26.03.1973, Kochummen, K.M., FRI 16675
5	*Byttneria jackiana* Wall.	Malaysia, Perak, Kuala Kangsar, Gunung Bubu, Bubu Forest Reseve, 15.02.1967, Whitmore, T.C., FRI 966
6	*B. jackiana* Wall.	Malaysia, Kedah, Padang Terap, Koh Mai Forest Reserve, 01.04.1938, Kiah, M.S., SFN 35118
7	*B. jackiana* Wall.	Malaysia, Kedah, Pendang, Bukit Perak Forest Reserve, 26.11.1969, Everett, B., FRI 13692
8	*Byttneria maingayi* Mast.	Malaysia, Negeri Sembilan, Jelebu, Pasoh Forest Reserve, 01.03.1996, Gardette, E., EG 1665
9	*B.maingayi* Mast.	Malaysia, Negeri Sembilan, Jelebu, Pasoh Forest Reserve, 05.09.1996, Gardette, E., EG 2204
10	*B. maingayi* Mast.	Singapura, Bukit Lalang, 26.02.2009, Leong, T.M., SING 2009-167
11	*B. maingayi* Mast.	Malaysia, Negeri Sembilan, Jelebu, Pasoh Forest Reserve, 24.12.2010, Nurshahidah, M.R., NMR 1 (UKMB)
12	*B. maingayi* Mast.	Malaysia, Negeri Sembilan, Jelebu, Pasoh Forest Reserve, 24.12.2010, Nurshahidah, M.R., NMR 4 (UKMB)
13.	*Pterocymbium tinctorium* (Blanco) Merrill	Malaysia, Kedah, Pulau Langkawi, 23.01.2011, Sani Miran, NBM 13-L (UKMB)
14.	*P. tinctorium* (Blanco) Merrill	Malaysia, Negeri Sembilan, Jelebu, Pasoh Forest Reserve, 23.12.2012, Abu Hussin H. NBM 20 (UKMB)
15.	*P. tinctorium* (Blanco) Merrill	Malaysia, Negeri Sembilan, Jelebu, Pasoh Forest Reserve, 23.12.2012, Abu Hussin H. NBM 21 (UKMB)
16.	*P. tinctorium* (Blanco) Merrill	Malaysia, Negeri Sembilan, Jelebu, Pasoh Forest Reserve, 23.12.2012, Abu Hussin H. NBM 22 (UKMB)
17.	*Pterocymbium tubulatum* (Masters) Pierre	Malaysia, Negeri Sembilan, Jelebu, Pasoh Forest Reserve, 23.12.2012, Abu Hussin H. NBM 01 (UKMB)
18.	*P. tubulatum* (Masters) Pierre	Malaysia, Negeri Sembilan, Jelebu, Pasoh Forest Reserve, 23.12.2012, Abu Hussin H. NBM 02 (UKMB)
19.	*P. tubulatum* (Masters) Pierre	Malaysia, Negeri Sembilan, Jelebu, Pasoh Forest Reserve, 14.02.2001, Wilkie P. PW155

Table 2. Dehydration and mounting process

Steps	Solutions	Time
Dehydration		
1	Alcohol 50%	5 mins
2	Alcohol 70% (few drops of absolute hydrochloric acid – HCl)	2 mins
3	Alcohol 95%	5 mins
4	Alcohol 100%	5 mins
5	Alcohol 100% + Xylene	5 mins
6	Xylene	5 mins
Mounting	Canada Balsam	–
Drying	–	2 weeks

Table 3. Leaf venation characteristics of *Byttneria* and *Pterocymbium* species studied

Species	Marginal venation	Ultimate marginal venation	Areolar venation	Trichome	Crystal
Byttneria curtisii	Entire	Complete	Closed system venation with minimum free ending	–	Absent
B. jackiana	Entire	Curved to looped pattern and incomplete	Closed system venation with minimum free ending	Simple unicellular	Absent
B. maingayi	Entire	Complete	Closed system venation with minimum free ending	–	Absent
Pterocymbium tinctorium	Entire	Curved to looped pattern and incomplete	Closed system venation with minimum free ending	–	Absent
P. tubulatum	Entire	Curved to looped pattern and incomplete	Closed system venation with minimum free ending	Simple unicellular, Multicellular glandular	Present alongside veins

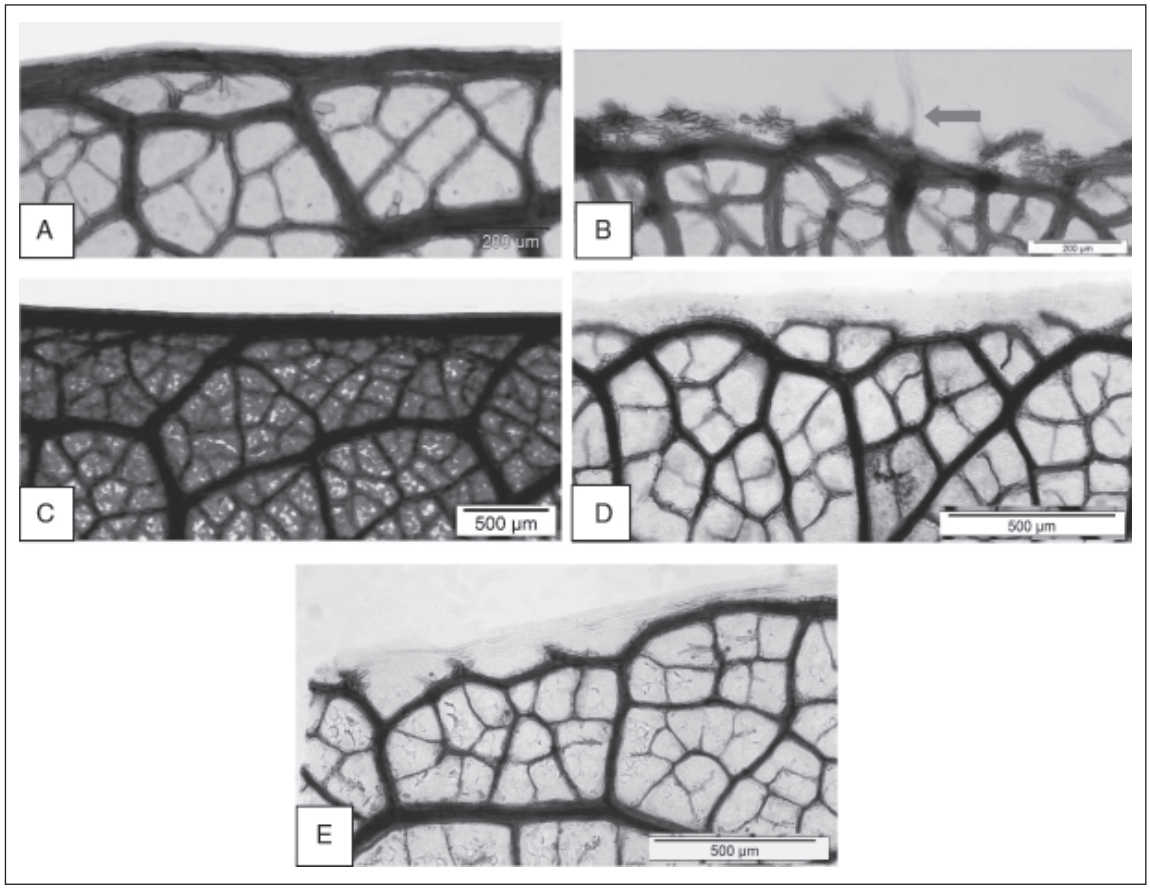

Fig. 1. (A-E). Ultimate marginal venation. A) & C). Complete ultimate marginal venation in *B. curtisii* and *B. maingayi*. B). Re-curved to loops pattern and incomplete with trichomes present (red arrow) in *B. jackiana*. D) & E). Re-curved to loops pattern and incomplete in *P. tinctorium* and *P. tubulatum*. Scale: A) – C) 200 µm, D) & E) 500 µm.

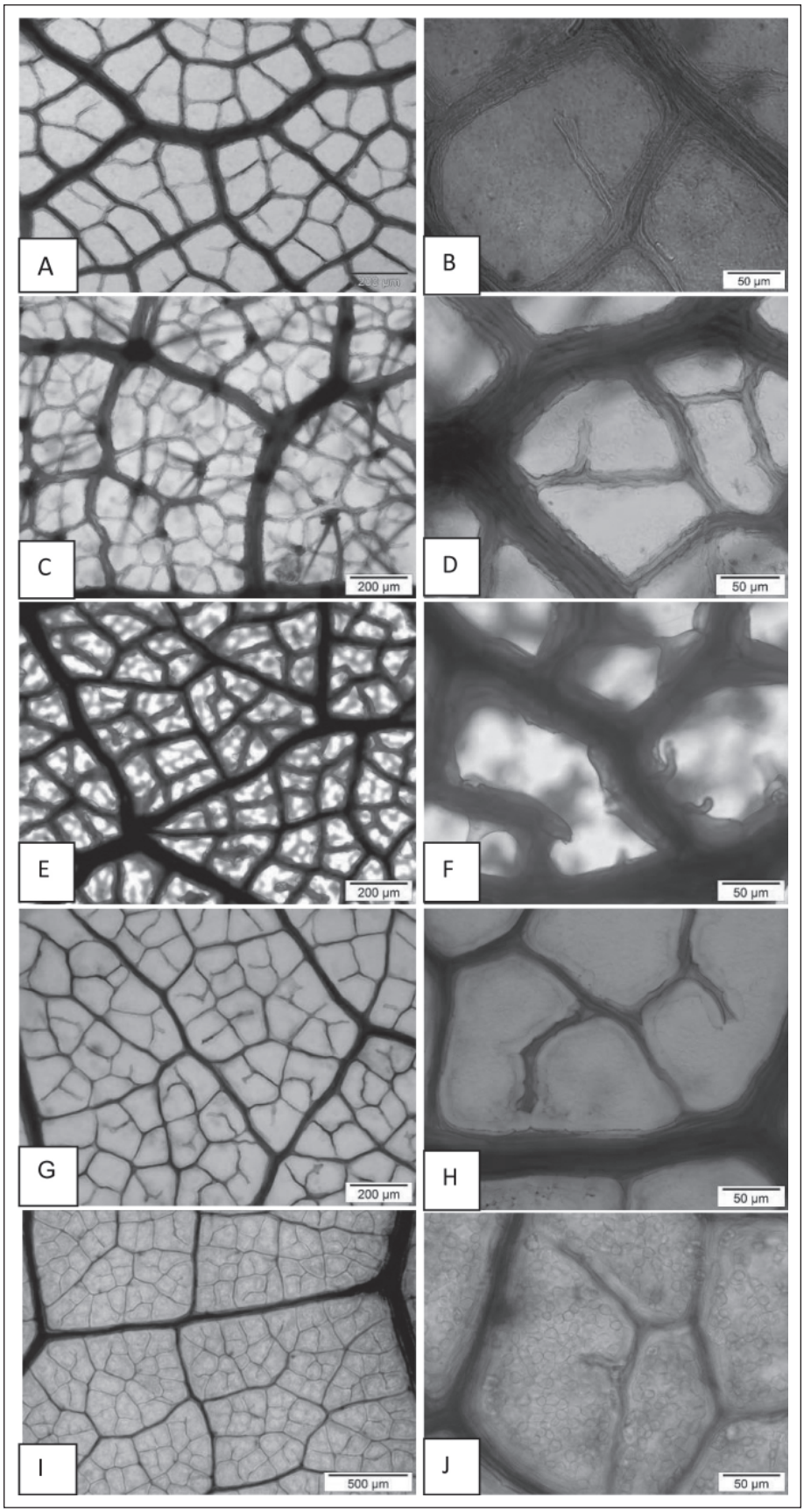

Fig. 2. (A-J). Areolar venation. Major closed venation and minor incomplete venation in all species studied. A-B) *Byttneria curtisii*. C-D) *Byttneria jackiana*. E-F) *Byttneria maingayi*. G-H) *Pterocymbium tinctorium*. I-J) *Pterocymbium tubulatum.* Scale: A, C, E, G & I) 200 µm, B, D, F, H & J) 50 µm.

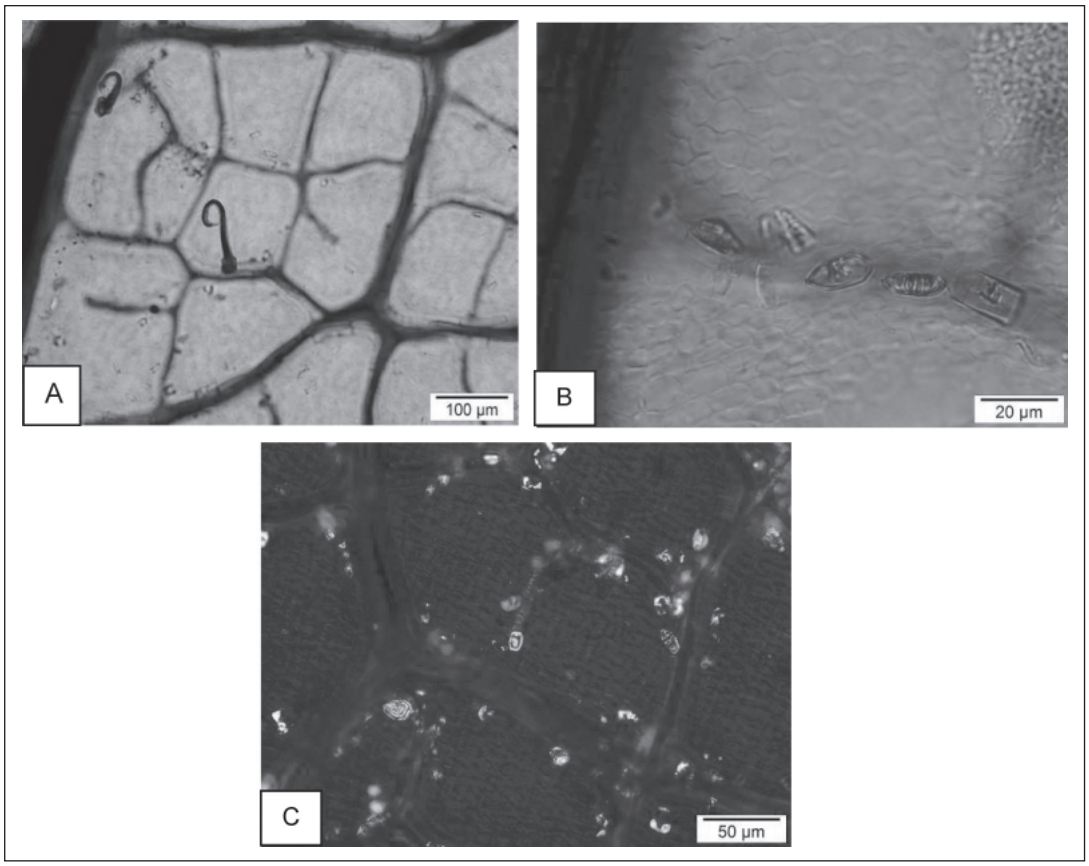

Fig. 3. Areolar venation of *P. tubulatum*. A) Simple unicellular trichomes. B) Multicellular glandular trichomes. C) Crystals alongside the veinlets. Scale: A) 100 µm, B) 20 µm, C) 50 µm.

marginal venation in *C. brachiata, C. corrifolia,* and *C. suffruticosa* and dentate and sub-dentate for *C. borneensis* and *C. eugenioidea,* respectively. The areolar venation was incomplete, with branch-endings and swollen veinlets, except in the case of *C. borneensis* where closed areolar venation was observed. Cork warts present at the leaf margin of *C. euginioidea* can be used to distinguish this species from the others.

The findings of the study also showed that the presence of simple unicellular trichomes at the leaf margin and lamina of *B. jackiana* (Figure 1B) and two types of trichomes, simple unicellular trichomes and multicellular glandular trichomes (Figure 3A & B) and crystals (Figure 3C) alongside veinlets in *P. tubulatum* are diagnostic characters for these species and can be used to distinguish them from the other species studied. A few previous studies stated that the presence of crystals can be used as supportive data for plant identification at the family, genera and species levels (Franceschi & Horner, 1980; Horner & Wagner, 1980; Leelavathi *et al.,* 1984; Metcalfe & Chalk, 1950; Prabhakar *et al*., 1988; Prabhakar & Ramayya, 1980; Solereder, 1908). Solitary crystals are frequently found throughout the family (Sterculiaceae *s.s*) and may be useful as a diagnostic feature (Chattaway, 1937). According to Hussin & Sani (1998), druses occurred in all species of *Sterculia.* Druses also present in *Pterygota alata*, *Kleinhovia hospita* and *Commersonia bartramia* with addition of solitary crystals in *Kleinhovia hospita* (Hussin & Sani, 1997). Presence of prismatic, rectangular crystals and stegmata were also found in *Grewia* and not observed in *Microcos* showing that leaf venation can be used in identification of species and was significant in classification (Nurul-Aini *et al*., 2010).

Solereder (1908), Metcalfe and Chalk (1950), Munsif *et al*. (2007) and Nurhanim (2011) also emphasized the taxonomic significance of trichomes. Metcalfe and Chalk (1950) recorded 10 types of glandular and eglandular types of trichomes in the Malvales, five types of trichomes occurred in Sterculiaceae *s.s* which were predominantly stellate, accompanied by simple unicellular, uniseriate, glandular and peltate types. Nurhanim (2011) stated that the type of trichomes has taxonomic significance up to the level of genera. Nurhanim (2011) also mentioned that peltate multicellular and coil-armed trichomes are the diagnostic charactersistics of *Schoutenia* and can be used to distinguish it from *Colona* and *Pentace*. Munsif *et*

al. (2007) also used trichome types to differentiate three genera in the family Verbenaceae namely *Lantana*, *Verbena* dan *Vitex*. Hussin and Sani (1996) mentioned that the presence of trichomes is a very useful character for differentiating the three species studied (*Pterygota alata, Kleinhovia hospita* and *Commersonia bartramia*).

The results of the present study have proved that leaf venation characteristics, presence and types of trichomes and the presence of crystals alongside veinlets can be used in identification of species and have taxonomic value for both genera (*Byttneria* and *Pterocymbium*). However these leaf anatomical characteristics are not sufficient to divide all the studied species into the two different genera, and two different subfamilies, as various other anatomical characteristics should be incorporated and more taxa included.

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