TAXONOMIC SIGNIFICANCE OF LEAF EPIDERMIS MICROMORPHOLOGICAL CHARACTERISTICS OF *Pentace* L. (MALVACEAE *s.l*) IN MALAYSIA

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Abstract. *Pentace* (Malvaceae *s.l*) is known to have difficulty in indentification at species level due to high similarities in morphological characteristics. A study on the leaf epidermis micromorphological characteristics were done in 18 *Pentace* species from Malaysia under scanning electron microscope to investigatate its' systematic significance. Variations were found in stomata, trichome, waxes and cuticle ornamentation. Six types of stomata observed were anomocytic, parasitic, diacytic, anisocytic, tetrasitic and staurocytic. *P. hirtula* was identified with anomocytic and *P. erectinervia* with tetracytic stomata and *P. excelsa* with wax-coated stomata. Cuticular striae was present only in three species. Film, crustose and granules waxes were found in this study. There were 22 types of trichomes recorded, including simple, non-cushioned armed, stellate, stellate rotate, stellate lepidote, dentate, lepidote, multiradiate clump trichomes, multicellular glandular, stelate rotate, capitate glandular, peltate glandular trichomes, three types of radial, capitate glandular and cushioned stellate. *P. acuta* and *P. eximia* were identified with epicalyx, *P. floribunda* with fan and *P. strychnoidea* with palmatifid radial trichomes. *P. grandiflora* can be identified by the presence of cluster peltate glandular trichomes. In conclusion, the leaf epidermis micromorphological characteristics definitely have taxonomic value in *Pentace* and can be used in differentiation and identification up to the species level.

Keywords: leaf micromorphological, leaf epidermis, *Pentace*, trichomes

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Introduction

Pentace, Colona and *Schoutenia* are important genus in the Malvaceae *s.l* that have the potential to be commercialized as a source of timber due to their high wood resilience. These three genera are from the subfamilies Grewioideae (*Colona*), Brownlowioideae (*Pentace*) and Dombeyoideae (*Schoutenia*) which have been classified by [7] through plastid *atp*B analysis and *rbc*L DNA sequences. *Pentace* known locally as 'melunak' contains approximately 28 species and is widely distributed in Indo-China, southern Thailand, Burma to Java, Borneo and southern Philippines [8]. [9-10] stated that apart from 'melunak', *Pentace* is also known by several other vernacular names depending on the locality of its presence, among them in Myanmar it is known as 'thitka', but 'burma mahogany' is the local name for *P. burmanica*, in Indonesia it is known as 'kayu pinang', in Brunei as 'kedang pinit', in Sabah as 'takalis', while in Sarawak it is known as 'bary baran' and others. A total of 18 species of *Pentace* can be found in Malaysia distributed from lowland to an altitude of 1000 m [8] and according to [17] a total of 12 species Peninsular Malaysia. Most of *Pentace* species formed as large tree canopies [8, 12]. [11] found that the time period for *Pentace* flowering is 3-5 months and some are only once in six years. [6] reported *that P. acuta, P. eximia, P. grandiflora, P. microlepidota, P. perakensis* and *P. strychnoidea* are endemic species in Peninsular Malaysia. Identification problems arise especially for most species that are difficult to distinguish without the presence of flowers and fruits with almost identical external morphological features such as stem and leaf morphological features [7, 11, 17-19]. This study is conducted to used alternative characters as tool to differentiate and identify species in *Pentace*. Study on micromorphology of the leaf epidermis is indispensable in helping to solve problems in the identification of species without the presence of flowers and fruits [20, 19, 22].

Materials and Methods

Most leaf material investigated were obtained from herbarium specimens available at UKM Herbarium (UKMB). Fully developed leaves were selected from dried herbarium specimens. For each leaf, an area from the middle of the lamina, including midrib and leaf margin, was taken. All material was observed under scanning electron microscope, samples of leaf specimens (adaxial and abaxial surfaces) were directly mounted on stubs and coated with gold and observed under JOEL JSM-6400 scanning electron microscope. There were 18 species chosen namely *Pentace acuta* Ridl., *Pentace adenophora* Kosterm., *Pentace borneensis* Pierre., *Pentace chartacea* Kosterm, *Pentace* curtisii King., *Pentace erectinervia* Kosterm, *Pentace excelsa* Kochummen, *Pentace eximia* King., *Pentace floribunda* King., *Pentace grandiflora* Kochummen, *Pentace hirtula* Ridl., *Pentace laxiflora* Merr, *Pentace macrophylla* King., *Pentace microlepidota* Kosterm., *Pentace polyantha* Hassk, *Pentace rigida* Kosterm., *Pentace strychnoidea* King. and *Pentace triptera* Mast.

Results and Discussion

Variation between species can be observed through several features such as stomata shape and type, epidermal anticlinal wall pattern either on adaxial or abaxial epidermis, wax type, cuticular ornamentation pattern and also trichome type.

Stomatal characteristics. Six types of stomata were found namely anomocytic, parasitic, diacitic, anisocytic, tetrasitic and also staurocytic. Anisocytic stomata were found in 10 species, whilst paracytic were found in five species studied. *Pentace hirtula* can be identified through the presence of anomocytic stomata whilst *P. erectinervia* can be identified by the presence of tetracytic stomata. [5] only recorded the presence of anomocytic stomata in *Pentace* but did not state specific species. Similarly, studies conducted by [14-15] and [24], found only anomocytic type of stomata in Malvaceae *s.l.* The results of this study clearly shown an addition of data to previous studies with another five types of stomata. This may due to the greater number of species studied and the more appropriate methods used for the observation of the presence of stomata. Heterostomatically (more than one type of stomata), were found only in *P. macrophylla, P. borneensis* and *P. chartacea.* There are three types of stomata that can be found in *P. borneensis*, namely anisocytic, tetracytic and staurocytic, two types of stomata can be found in *P. macrophylla*, namely anisocytic and diacytic stomata. Whereas for *P. chartacea*, anomocytic stomata can be seen present together with anisocytic stomata. The shape of guard cells also has taxonomic value either at the genus or species level [13]. The shape of of guard cells in *P. excelsa* is elliptic, differ from other species (round shape), that can be used as diagnostic feature for this species. As for the stomata rims, all species have outstanding rims whether raised or not raised. *P. grandiflora* is the only species with an outstanding and raised stomata frame while other five species (*P. borneensis, P. eximia, P. laxiflora, P. microlepidota* and *P. rigida*) were without raised stomata. Wax -coated stomata only found in *P. erectinervia* and *P. excelsa*. In addition to that, four study species recorded the presence of cuticular striae on the stomata namely *P. acuta, P. eximia, P. rigida* and *P. strychnoidea* (Table 1). Previous study on 32 species of *Microcos* (Malvaceae *s.l*) by [5], recorded the presence of cuticular striae only in *M. tomentosa*, making it a diagnostic feature for that species. Findings by [5] together with the results of this study prove that *Pentace* has non-uniformity in terms of the presence of cuticular striae on stomata. It is proven that the stomata characters have taxonomic value especially in species differentiation.

Table 1. Stomata characteristics in *Pentace* species studied

Species	Type of stomata	Stomatal rim	Stomatal frame	Stomata covered by wax
P. acuta	Paracytic	Outstanding and raised	Obscure	Absence
P. adenophora	Paracytic	Obscure	Absence	Absence
P. borneensis	Anisocytic, Tetracytic Staurocytic	Outstanding and raised	Outstanding but not raised	Absence
P. chartacea	Anomocytic, Anisocytic	Outstanding but not raised	Obscure	Absence
P. curtisii	Anisocytic	Outstanding but not raised	Absence	Absence
P. erectinervia	Anisocytic	Outstanding and raised	Obscure	Present
P. excels	Tetracytic	Outstanding but not raised	Obscure	Present
P. eximia	Paracytic	Outstanding but not raised	Outstanding but not raised	Absence
P. floribunda	Anisocytic	Obscure	Absence	Absence
P. grandiflora	Paracytic	Outstanding and raised	Outstanding and raised	Absence
P. hirtula	Anomocytic	Outstanding but not raised	Obscure	Absence
P. laxiflora	Anisocytic	Outstanding but not raised	Outstanding but not raised	Absence
P. macrophylla	Anisocytic, Diacytic	Outstanding but not raised	Obscure	Absence
P. microlepidota	Anisocytic	Outstanding but not raised	Outstanding but not raised	Absence
P. polyantha	Absence	Obscure	Absence	Absence
P. rigida	Anisocytic	Outstanding but not raised	Outstanding but not raised	Absence
P. strychnoidea	Anisocytic	Outstanding and raised	Obscure	Absence
P. triptera	Paracytic	Outstanding and raised	Obscure	Absence

Cuticular ornamentation and wax characteristics. Seven species namely *P. acuta, P. borneensis, P. floribunda, P. grandifora, P. macrophylla, P. polyantha* and *P. rigida* showed the presence of crustaceous wax-type and granules on the surface of the adaxial epidermis. For the other seven species namely *P. curtisii, P. erectinervia, P. excelsa, P. eximia, P. hitrula, P. laxiflora* and also *P. microlepidota* only granules wax was present. Whereas for *P. chartacea* only crustose wax present. The presence of crustose wax could be observed in two species studied namely *P. strychnoidea* and *P. triptera.* On the abaxial epidermis surface, the presence of crustose wax and granules could also be seen in five study species namely *P. borneensis, P. floribunda, P. macrophylla, P. rigida* and *P. strychnoidea*. Three other species namely *P. chartacea, P. excelsa* and *P. hirtula* showed only crustose wax present while for *P. erectinetvia, P. grandiflora* and *P. laxiflora* a thin film layer of wax was present. *P. eximia* and *P. microlepidota* had granular wax only, whilst *P. acuta* showed the presence of granular wax and crustose. Granular wax and crust were first discovered by De Bary (1871), which was the first study done on the presence of wax on the cuticle [3]. *Pentace triptera* was observed to have thin film layer and also crustose wax. *P. adonophora* is the only species with crustose wax present on the abaxial epidermis (Table 2 and 3). As for cuticular ornamentation, for easy reference the cuticular ornamentation was classified into five patterns as shown in Table 2.

Table 2. **Patterns of cuticular ornamentation found in this study**

Pattern	Description
1	Clear cuticular ornamentation Raised anticlinal wall and sunken periclinal wall
2	Clear cuticular ornamentation Sunken anticlinal wall and raised periclinal wall
3	Slightly obscure cuticular ornamentation Raised anticlinal wall and sunken periclinal wall
4	Slightly obscure cuticular ornamentation Obscure anticlinal and periclinal wall
5	Obscure cuticular ornamentation High density of trichomes

Findings have shown eight species with Pattern 1 (*P. acuta*), *P. erectinervia, P. floribunda, P. hirtula, P. laxiflora, P. microlepidota, P. polyantha, P. strychnoidea*), two species with Pattern 2 (*P. chartacea*), *P. curtisii*), six species with Pattern 3 (*P. borneensis, P. excelsa, P. grandiflora, P. macrophylla, P. rigida, P. triptera*) while for Pattern 4 owned by *P. eximia*. The adaxial cuticle ornamentation pattern of *P. adenophora* could not be observed due to lack of samples. As for abaxial cuticle ornamentation, two species have Pattern 1 (*P. microlepidota, P. strychnoidea*) and two species have Pattern 2 (*P. acuta, P. grandiflora*), *P. microlepidota* has Pattern 3 while the rest with Pattern 5 (*P. adenophora, P. chartacea, P. curtisii, P. erectinervia, P. excelsa, P. eximia, P. floribunda, P. hirtula, P. laxiflora, P. macrophylla, P. polyantha, P. rigida, P. triptera*). Table 3 shows the types of waxes possessed by the *Pentace* species studied. The results of the study clearly indicate that cuticle ornamentation features can be used to distinguish species in the genus *Pentace.*

Table 3. Type of waxes and pattern of leaf epidermis cuticular ornamentation

Species	Type of waxes		Pattern of cuticular ornamentation	
	Adaxial	**Abaxial**	**Adaxial**	**Abaxial**
P. acuta	Crustose, Granule	Crust, Granule	1	2
P. adenophora	No data	Crustose	No data	5
P. borneensis	Crustose, Granule	Crustose, Granule	3	3
P. chartacea	Crustose	Crustose	2	5
P. curtisii	Granule	No data	2	5
P. erectinervia	Granule	Film layer	1	5
P. excelsa	Granule	Crustose	3	5
P. eximia	Granule	Granule	4	5
P. floribunda	Crustose, Granule	Crustose, Granule	1	5
P. grandiflora	Crustose, Granule	Film layer	3	2
P. hirtula	Granule	Crustose	1	5
P. laxiflora	Granule	Film layer	1	5
P. macrophylla	Crustose, Granule	Crustose, Granule	3	5
P. microlepidota	Granule	Granule	1	1
P. polyantha	Crustose, Granule	No data	1	5
P. rigida	Crustose, Granule	Crustose, Granule	3	5
P. strychnoidea	Crustose	Crustose, Granule	1	1
P. triptera	Crustose	Crustose, Film layer	3	5

Trichomes on the adaxial and abaxial epidermis of leaf. There are various types of trichomes observed in 18 *Pentace* species studied (Figure 1(a-l)), for which the presence of trichomes on the adaxial and abaxial epidermis of the leaf lamina is seen to have taxonomic value in *Pentace*. Among the types of trichomes that can be used as a diagnostic feature is the radial trichome and this radial trichome is the first trichome found in higher plants. The results of this study have successfully classified radial trichomes into three types, namely epicalyx, fan and palmatifid radial trichomes, based on the structure and shape of the radial trichomes. The results showed that trichomes found in *P. acuta* and *P. eximia* were identified as epicalyx radial trichomes (Figure 1(a)), fan radial trichomes were found in *P. floribunda* (Figure 1(c)), whilst *P. strychnoidea* had palmatifid radial trichomes (Figure 1(c)). *P. grandiflora* can be identified by the presence of clustered peltate glandular trichomes and other several types of trichomes have been identified such as stellate, fasciculate, multiradiate and rosulate, dendritic, lepidote (stellate rotate, stellate lepidote, dentate, lepidote), papillae and glandular trichomes [23]. The terminology for the trichomes adopted by [23] according to the suitability of the percentage of radial adhesion of the trichomes to its site. Stellate rotate with 15 - 30 % radial attachment, stellate lepidote with 30 - 50 % radial attachmen, dentate is 50 - 80 % radial attachment while for lepidote with 80 - 100 % radial attachment at its base. Several types of trichomes have been found in several species of *Pentac*e studied namely as stellate lepidote (Figure 1(f)) and lepidote trichomes (Figure 1(g)).

Apart from the above types of trichomes, there are several other types of trichomes identified in this study and also can be used as diagnostic feature of certain species such as cushioned stellate (short and thick wall arms) in *P. floribunda* (Figure 1(d)), cushioned stellate (elongate and thin wall arms) in *P. laxiflora* (Fig. 1e), capitate glandular in *P. excels* (Figure 1(h)), simple trichome in *P. laxiflora* (Figure 1(i)), 2-armed trichome in *P. floribunda* (Figure 1(j)), 3-armed trichome in *P. laxiflora* (Figure 1(k)) and 4-armed trichome in *P. floribunda* (Figure 1(k)). [5, 8] in his study also stated that the presence of

trichomes alone is sometimes sufficient to distinguish species. Table 4 shows the presence of trichome types in *Pentace* species studied.

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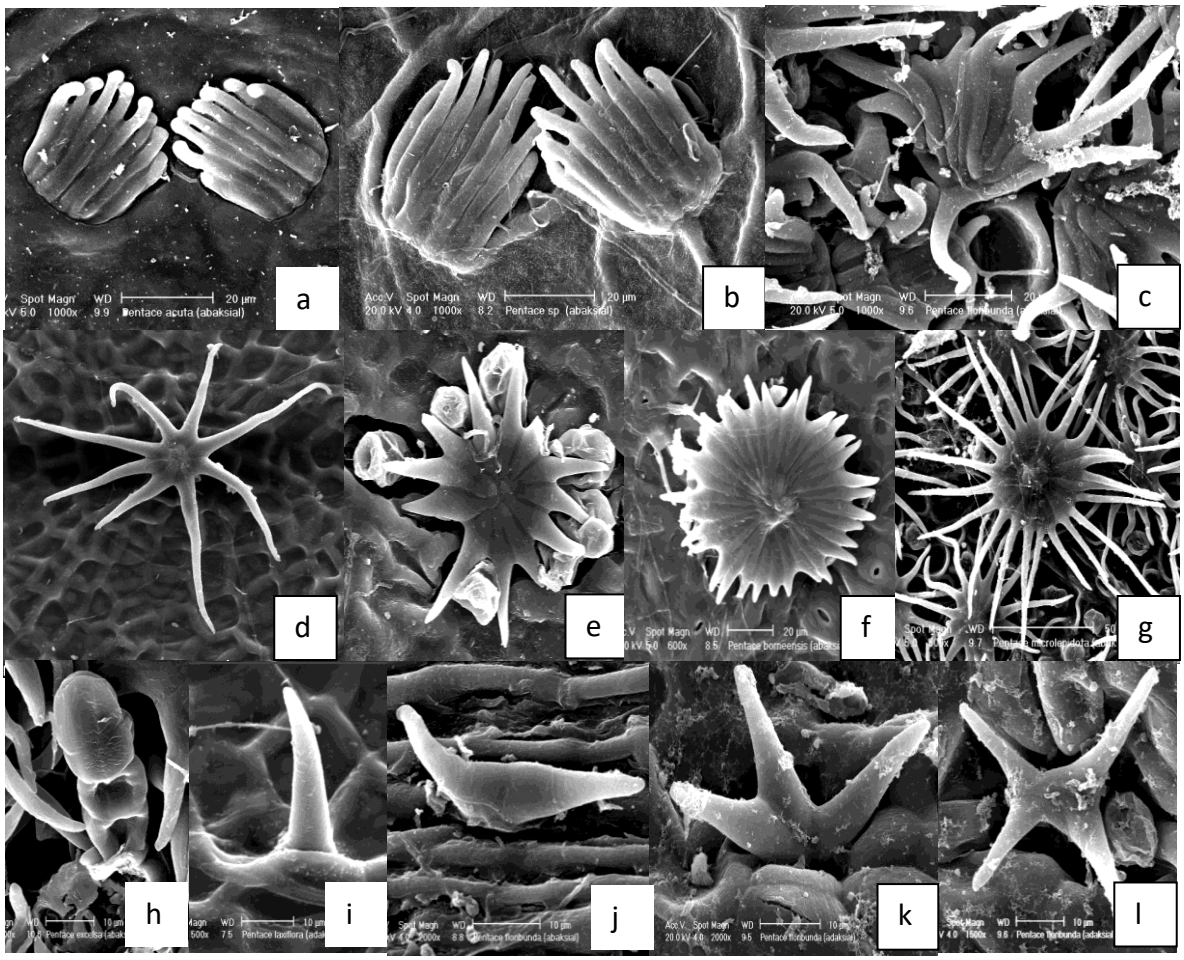

Figure 1. Trichomes: (a) Epicalyx radial trichome in *P. eximia* and *P.acuta*, (b) Palmatid radial trichome in *P. strychnoidea*, (c) Fan radial trichome in *P. floribunda*, (d) *P. floribunda* – cushioned stellate (short and thick wall arms), (e) *P. laxiflora* – cushioned stellate (elongate and thin wall arms), (f) *P. macrophylla*- stellate lepidote, (g) *P. borneensis*-lepidote, (h) *P. excels* - capitate glandular, (i) *P. laxiflora* – Simple, (j) *P. floribunda* – 2-armed, (k) 3-armed trichomes in *P. Laxiflora* and (l) 4-armed trichomes in *P. floribunda*. Scale: 50 µm.

Table 4. Type of trichomes present on the leaf epidermis of *Pentace*

Type of trichomes	Species
Simple	*P. adenophora, P. floribunda, P. hirtula, P. laxiflora*
Non cushioned armed (2 – 4 arms)	*P. erectinervia, P. floribunda, P. laxiflora*
Stellate	*P. adenpohora, P. triptera*
Cushioned stellate (short arms, thick wall)	*P. chartacea, P. floribunda, P. hirtula*
Cushioned stellate (long arms, thick wall)	*P. erectinervia, P. rigida*
Cushioned stellate (long arms, thin wall)	*P. erectinervia, P. laxiflora, P. rigida*
Stellate rotate	*P. hirtula*
Stellate lepidote	*P. chartacea, P. curtisii, P excelsa, P. eximia, P. floribunda, P. laxiflora, P. macrophylla, P. microlepidota, P. rychnoidea P. triptera*
Dentate	*P. acuta, P. borneensis, P. curtisii,P. excelsa, P. floribunda, P. laxiflora, P. macrophylla, P. polyantha, P. strychnoidea, P. triptera*
Lepidote	*P . acuta, P. borneensis, P. chartacea, P. eximia, P floribunda, P. hirtula, P. polyantha*
Tufted multiangulate	*P. eximia*
Tufted multiradiate	*P. adenophora, P. erectinervia*
Multicelullar glandular	*P. curtisii, P. floribunda, P. macrophylla*
Capitate glandular	*P. floribunda, P. triptera*
Capitate glandular (terminal multicellular)	*P. adenophora, P. chartacea*
Capitate glandular (neck multicellular)	*P. curtisii, P. erectinervia, P. excelsa, P. eximia, P. hirtula, P. laxiflora, P. microlepidota, P. polyantha, P. rigida, P. strychnoidea, P. triptera*
Peltate glandular	*P. acuta, P. borneensis, P. erectinervia, P. floribunda, P. hirtula, P. rigida*
Clustered peltate glandular	*P. grandiflora, P. macrophylla*
Radial epicalyx	*P. acuta, P. eximia*
Radial palmatifid	*P. strychnoidea*
Radial fan	*P. floribunda*

Conclusion

Study has proven that leaf micromorphological characteristics can be used as additionnal data to identify some species in *Pentace*. *P. hirtula* can be identified with anomocytic stomata and *P. erectinervia* with tetracytic stomata, *P. excelsa* with observation of elliptic guard cells, *P. grandiflora* with clear and embossed stomata frames, *P. grandiflora* with clustered peltate glandular trichomes, *P. floribunda* with fan radial and *P. strychnoidea* with palmatifid radial trichomes. There are new findings in this study, that the types of stomata, waxes and trichomes especially in the presence of radial trichomes contribute more information to the existing taxonomic and systematic database on the species studied and may be used in the species differentiation in the absence of its flowers and fruit. As a conclusion the micromorphological characteristics of the leaf epidermis in *Pentace* definitely have taxonomic value and can be used in differentiation and identification up to species level if combine with other anatomical characteristics. However, more studies need to be conducted to provide more information on the species studied.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare

Compliance with Ethical Standards

The work is compliant with ethical standards

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