

***Freycinetia* of Mount Nyiut and Palung, West Kalimantan based on Leaf Anatomical Characters
(*Freycinetia* dari Gunung Nyiut dan Gunung Palung, Kalimantan Barat berdasarkan Karakter Anatomi)**

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Memasukkan: September 2014, Diterima: Januari 2015

ABSTRACT

Freycinetia Gaudich. is a climber plant belongs to the family of *Pandanaceae*. Seven species of *Freycinetia* from Mount of Nyiut and Palung located in West Kalimantan have been recognized based on leaf anatomy. Paradermal and transversal leaf sections of those species were observed, and the data was used to construct a dendrogram using Jaccard similarity index and Unweighted Pair Group Method with Arithmetic Average (UPGMA) method. Six main characters have been used to distinguish and evaluate the similarity between its, i.e. number of hypodermal layers, number of palisade layers, shape of sponge tissue, layout of sclerenchyma tissue, stomata size, presence of costal and intercostal cells, and shape of vascular bundle. Cluster analysis based on the anatomical data showed that seven *Freycinetia* species were clustered into two major groups at similarity coefficient 0.38. The first group consisted of *F. angustifolia*, *F. imbricata*, *F. sarawakensis*, and *F. winkleriana*, while the second group consisted of *F. corneri*, *F. sessiliflora* and *F. sumatrana*.

Keywords: Anatomical variation, *Freycinetia*, West Kalimantan

ABSTRAK

Freycinetia Gaudich. adalah tumbuhan pemanjat yang termasuk dalam famili *Pandanaceae*. Tujuh spesies *Freycinetia* dari Gunung Nyiut dan Gunung Palung yang terletak di Kalimantan Barat telah diidentifikasi berdasarkan anatomi daun. Irisan paradermal dan transversal daun dari spesies tersebut telah diamati, dan datanya digunakan untuk membentuk sebuah dendrogram menggunakan indeks kesamaan Jaccard dan metode *Unweighted Pair Group Method with Arithmetic Average* (UPGMA). Enam karakter utama telah digunakan untuk mengetahui dan mengevaluasi kesamaan yaitu jumlah lapisan hipodermal, jumlah lapisan palisade, bentuk jaringan spons, tata letak jaringan sklerenkim, ukuran stomata, kehadiran sel kostal dan interkostal, dan bentuk jaringan pembuluh. Pengelompokan berdasarkan data anatomi menunjukkan bahwa tujuh spesies *Freycinetia* dikelompokkan menjadi dua kelompok besar pada koefisien kesamaan 0.38. Kelompok pertama terdiri dari *F. angustifolia*, *F. imbricata*, *F. sarawakensis* dan *F. winkleriana*, sementara kelompok kedua terdiri dari *F. corneri*, *F. sessiliflora* dan *F. sumatrana*.

Kata Kunci: Variasi anatomi, *Freycinetia*, Kalimantan Barat

INTRODUCTION

Freycinetia is one genus that mostly distributed in the Malesia region particularly in Borneo, Celebes, Papua and Sumatra. The genus comprises approximately 200-300 species all over the world; about 150 species found in Indonesia (Stone 1970). *Freycinetia* was first described by the French botanist Gaudichaud in 1824. Along with *Pandanus*, *Saranga*, *Martellidendron* and

Benstonea, *Freycinetia* were classified into *Pandanaceae* family (Callmander *et al.* 2003, 2012).

Freycinetia is abundantly distributed in Indonesia, particularly New Guinea, Kalimantan, Sulawesi and Sumatra. However, the number of species, ecology, pollination, phenology, ethnobotany, and anatomical data were less informed. Anatomical approach may show correlation between anatomical character and other characters. Therefore, this data

can be used to strengthen taxon boundaries, mainly to support other taxonomic evidences such as uncertain morphological character. Generally, anatomical character is a reliable basis for species differentiation (Stone 1972), but it usually has great usability on infrageneric taxon. These characters are fairly constant, diagnostic and can be used for identification and determination of phylogenetic relationships (Judd et al. 2002).

Anatomical observation was intended to serve as a basis for further anatomical study which may elucidate diagnostic characters useful in taxonomy. Several previous authors have reported how useful are the anatomical characters for classifying the *Freycinetia* species, e.g. North & Willis (1970) mentioned that stomata and epidermis have importance to circumscribe the *Freycinetia* in Solomon island (*Freycinetia hombronii* Martelli, *F. inermis* Ridl., *F. cf. laeta* Merr. & L.M.Perry, *F. marantifolia* Hemsl., *F. petiolacea* Merr.& L.M.Perry, *F. solomonensis* B.C. Stone. Lim & Stone (1971) have firstly mentioned about costal and intercostal cell of epidermis and found out the value of epidermal characteristics to recognize six Malayan species (*F. rigidifolia*, *F. sumatrana*, *F. sumatrana* var. *penangiana*, *F. jagorii*, *F. sarawakensis*, *F. imbricata* var. *hispidula*, *F. mariannensis*) and eight non Malayan species of the genus (*F. marantifolia*, *F. rapensis*, *F. javanica*, *F. solomonensis*, *F. kamiana*, *F. corneri*, *F. arborea*, and *F. minahassae*). Huynh (1999, 2002) used anatomical characters to describe unknown species of *Freycinetia* from New Guinea. Pasaribu (2010) adopted the anatomical character used by Lim & Stone (1971) to clarify the fourteen species of Sumatran

Freycinetia. Lastly, Santika & Tihurua (2014) concluded that anatomical characters have diagnostic value to recognize the three genera (*Freycinetia*, *Pandanus* and *Sararanga*) under the *Pandanaceae* family.

The present anatomical study was conducted to understand the similarity among those species and to know the significant contributions of anatomical features to identify the species of *Freycinetia* from Kalimantan.

MATERIAL AND METHODS

Plant material used for this study was consisted of mature leaves of *F. angustifolia*, *F. corneri*, *F. imbricata*, *F. sumatrana*, *F. sarawakensis* and *F. winkleriana*, *F. sessiliflora* collected from Mt. Nyiut and Mt. Palung, West Kalimantan (Table 1).

Paradermal section was prepared using the methodology of Sass (1951). The mature leaves fixed in ethanol 70% were washed using distilled water and then immersed in HNO₃ 50% for 5-10 minutes, then washed using distilled water, and thin-slashed the adaxial and abaxial surfaces using razor blade. The thin section of epidermis were immersed in bleach solution for 3-5 minutes, then washed using distilled water, and colored using 1% safranin. Finally, the colored thin sections were suspended in glycerin 30 % on glass slide and covered using cover glass and then observed under light microscope.

Transverse section was done using paraffin method (Johansen 1940). Leaves were fixed in FAA (10 ml formaldehyde 37%: 5 ml acetic acid:

Table 1. List of the material used for anatomical observation

Species	Collection number	Locality	Voucher
<i>Freycinetia sessiliflora</i>	Fitri 01,02,03	G. Nyiut, Kalbar	BO
<i>Freycinetia sumatrana</i>	Fitri 11, 12, 13, 14	G. Nyiut, G. Palung	BO
<i>Freycinetia winkleriana</i>	Fitri 15, 16, 17	G. Palung, Kalbar	BO
<i>Freycinetia angustifolia</i>	Teysmann 143	Singkawang, Kalbar	BO
<i>Freycinetia corneri</i>	Endert 4453	Kutai Barat , Kaltim	BO
<i>Freycinetia imbricata</i>	Polak 1969	Pontianak, Kalbar	BO
<i>Freycinetia sarawakensis</i>	Endert 4582	Kutai Barat , Kaltim	BO

50 ml ethyl alcohol: 50 ml distilled water) for 24 hours. The leaves were then put in a solution series consisted of 50%, 70%, 95% and 100% of alcohol, alcohol:xylol (3:1), alcohol:xylol (1:1), alcohol:xylol (1:3), absolute xylol I and II for 3 hours of each immersion stage.

Paraffin infiltration was gradually conducted. Subsequently, the formed paraffin blocks were sliced using a rotary microtome with a thickness of 15-20 μm . The paraffin ribbons were arranged on a glass slide then the glass slide was placed on the top of the heater plate $\pm 35^\circ\text{C}$ for 6 hours. Afterwards, paraffin ribbons were double-stained using 1% safranin and 0.5% fast green. First, the paraffin ribbons were added 1% safranin, then were dehydrated in a series of alcohol, 70%, 95% and 100% and second, paraffin ribbons were stained using 0.5% fast green, then were immersed in a solution series of 100% alcohol I and II, xylol I and II, and finally put one entellan on each slide, and covered using cover glass, then dried.

Anatomical characters observed were leaf thickness, shape of epidermal tissue, position of sclerenchyma tissue, number of hypodermic layers, number of mesophyll layers, stomatal type, stomatal position, hypodermic layer, papilla position, papilla shape, size and position of vascular bundle and location of crystal cell.

Cluster analysis was conducted based on the similarity of anatomical characters using simple matching coefficient and Unweighted Pair Group Method with Arithmetic Average (UPGMA) method. All analysis were performed using NTSYS version 2.2 (Rohlf 2002).

RESULTS

The observation of the transverse section of leaves showed that all species of *Freycinetia* observed have similar in the basic anatomical structure, but character variation was found in three characters, i.e. epidermis cells, the cells of sponge tissue and the number of hypodermis

layers (Table 2). To differentiate among *Freycinetia* species, an identification key based on anatomical characters is also provided.

Two main groups (A dan B) could be recognized. Group A consisted of 2 subgroups, 1 & 2. Subgroup 1 contained of two species, *F. imbricata*, and *F. angustifolia*, while subgroup 2 contained of *F. winkleriana*, and *F. sarawakensis*. Group B consisted of three species, *F. sessiliflora*, *F. corneri* and *F. sumatrana*.

The main dendrogram showed that among seven *Freycinetia* species, *F. sarawakensis* and *F. winkleriana* has the highest similarity, 0.72. They were merged into one group because they shared four similar characters, the presence of costal and intercostals cells, presence of calcium oxalic crystal, number of palisade and hypodermic layers. While, *F. imbricata*, and *F. angustifolia* were similar in six characters, i.e. the presence of costal and intercostals cells, shape and size of epidermis cell, attendance of papilose in epidermis layer, sclerenchyma position, and number of the hypodermic coat.

Group B consisted of three species, namely *F. sessiliflora*, *F. corneri* and *F. sumatrana*. Those species shared three similar characters which are attendance of costal and intercostals cells, attendance of papilose in epidermis layer, presence granula in epidermis layer. *Freycinetia sumatrana* differed from *F. sessiliflora* and *F. corneri* in four characters, i.e. shape of epidermis cell, costal cell, hypodermic cell of abaxial surface, palisade tissue, and vascular bundle.

DISCUSSION

Seven *Freycinetia* species have similar anatomical structures, but several characters are varied including epidermal cells, cells of sponges, hypodermic layer and the size of the stomata (Figure 2).

Epidermis

The epidermal cell structure of abaxial surface

is more variable than adaxial epidermis throughout the species. Epidermal cells are hexagonal, rectangular and notched at the edges on paradermal section, whereas epidermal cells on transversal section are rectangular. The hexagonal

epidermal cells are found in *F. angustifolia* and *F. imbricata*, whereas *F. sarawakensis* has rectangular epidermal cells with papillae. *Freycinetia sumatrana* and *F. winkleriana* possess hexagonal and rectangular epidermal cells, while rectangular

Table 2. Anatomical Characters of *Freycinetia*

Characters	Taxa						
	<i>F. Angustifolia</i>	<i>F. corneri</i>	<i>F. imbricata</i>	<i>F. sessiliflora</i>	<i>F. sarawakensis</i>	<i>F. Sumatrana</i>	<i>F. Winkleriana</i>
-Shape of epidermis cell	Hexagonal	Quadrangular	Hexagonal	Quadrangular	Quadrangular	Quadrangular and hexagonal	Quadrangular and hexagonal
	30,0-40,0	60,0-100,0	30,0-60,0	60,0-90,0	60,0-72,0	60,0-100,0	30,0-72,0
Shape of costal cell	absent	Quadrangular	Rectangular	Quadrangular	Absent	Quadrangular	Absent
-intercostals cell	absent	Flat	Quadrangular	Round	Absent	Quadrangular	Absent
Present of							
-costal intercostals cell	absent	Present	absent	Present	Absent	Present	Absent
-crystal calcium oksalat.	absent	absent	Present	Present	Present	Present	Present
Shape of stomata	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Tetracytic
Size of stomata (µm ²)	266,64-330,41	216,74-530,51	286,45-554,06	319,3-490,30	252,04-430,11	215,92-406,73	250,70-328,48
-Pappilose of epidermis	Absent	Absent	Absent	Absent	Present	Absent	Absent
-Granula of epidermis	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Position of Sclerenchym	Hypodermic & palisade tissue	Hypodermic & palisade tissue	Hypodermic & palisade tissue	Hypodermic & palisade tissue	Hypodermic & palisade tissue	Sponge tissue & palisade tissue	Hypodermic & spongy tissue
No. Hypodermic layers	2	2	2	4	3	4	3
-palisade layers	2	2	3	3-4	3	2-4	4
Shape of Sponge cell	Isodia	Branching	Branching	Isodia	Branching	Isodia	Branching
Shape of hypodermic cell abaxial	metric Round	Flat	Round	metric Flat	Flat	metric Round	Flat
Shape of hypodermic cell adaxial	Round	Flat	Round	Round	Round		Round
Shape of palisade tissue	Flat	Curve	Flat	Curve	Curve	Flat	Curve
Vascular bundle	Round	Oval	Oval	Oval	Round	Round	Round

Key Identification of *Freycinetia* in Kalimantan

- 1.a. The intercoastal cells and coastal cells can be differentiated2
 - b. The intercoastal cells and coastal cells have the same shape3
- 2.a. Calcium oxalate crystals scattered on epidermal cells4
 - b. Calcium oxalate crystals on epidermal cells absent.....*F. corneri*
3. a. Granules in the cells of the epidermis present*F. angustifolia*
 - b. Granules in the cells of the epidermis absent5
4. a. Sclerenchyma cells found in the hypodermis and palisade tissue
 -*F. sessiliflora*
 - b. Sclerenchyma cells found in palisade tissue and spongy tissue
 -*F. sumatrana*
5. a. Hypodermis consists of 2 layers *F. imbricata*
 - b. Hypodermis consists of 3 layers 6
6. a. Epidermal cells are rectangular.....*F. sarawakensis*
 - b. Epidermal cells are hexagonal*F. winkleriana*

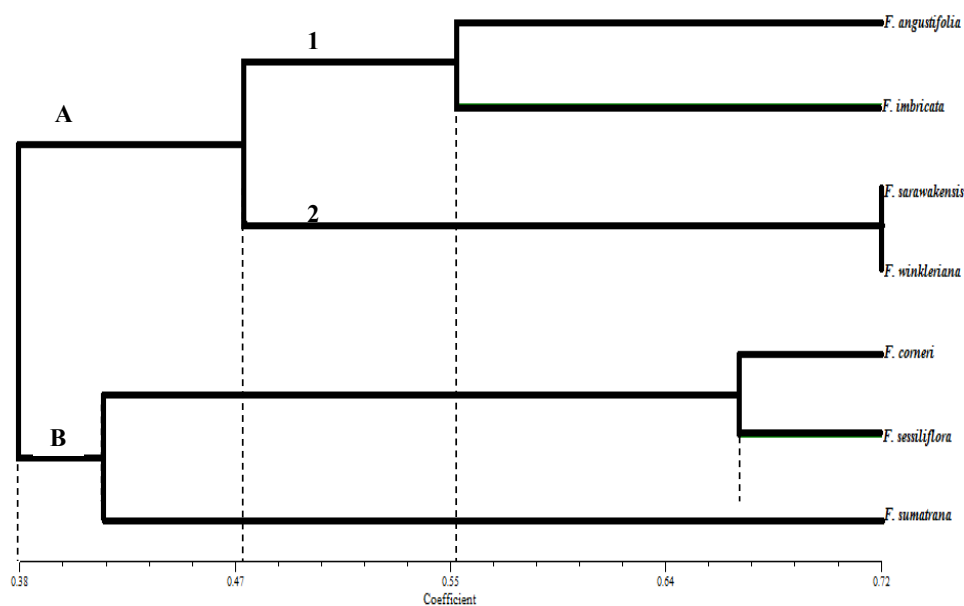


Figure 1. Dendrogram of *Frecynetia* from Borneo based on anatomical characters



Figure 2. Transverse section of leaf, (A) cuticle, (B) sclerenchyma, (C) hypodermis, (D) phloem, (E) palisade, (F) xylem, (G) sponge, (H) epidermis

epidermal cells can be found in *F. corneri* and *F. sessiliflora*.

The abaxial epidermis of paradermal section can be differentiated into costal and intercostal zones in some species. Intercostal cells are varied, short, rectangular, and pentagonal cells. Costal cells are elongated and large; cell walls are moderately thick and straight. The shape of the costal and intercostal cells can be differentiated on

F. sessiliflora, *F. corneri* and *F. sumatrana*, and there are many calcium oxalic crystals in their costal and intercostal zones. The epidermis of adaxial surface is relatively uniform and it is not differentiated into costal and intercostal zones, but most cells have rectangular and square shapes. In abaxial surface of *F. sumatrana*, costal and intercostal zones can be differentiated. Its epidermal cells are rectangular and thick with lignified cell walls of costal zone. Costal cells in the epidermis are few, and have little papillae; whereas intercostal cells in the epidermis are numerous, columnar cells with papillae; simple and large papillae in adjacent cells to stomata. Stomata are sunken with little silica. They are solitary or in pairs in both costal and intercostal zones. *Frecynetia corneri* have the costal and intercostal zones in both epidermal layers, rectangular epidermal cells, no papillae, and no silica.

This study found that each *Frecynetia* species has specific stomata in epidermal cells, such as stomata in *F. angustifolia* was granular, stomata in *F. sarawakensis* was with papillae, stomata in *F.*

imbricata was arranged in rows in costal and intercostal zones with little calcium oxalic crystals and stomata in *F. sumatrana* was arranged in one line in the costal and intercostal zones with a lot of calcium oxalic crystals.

Stomata

Stomata type of all observed *Freycinetia* species are tetracytic. Tetracytic stomata have guard cells surrounded by four subsidiary cells, two lateral and polar ones (Figure 3, Metcalfe 1961). Stomata are present on the adaxial and abaxial surfaces. The stomatal number of adaxial surface varies, but there are always fewer stomata than that of abaxial surface. The most common stomata position on epidermis cells of *Freycinetia* is flat to other epidermis cells, whereas sunken stomata were only observed in *F. sessiliflora*. In conclusion, stomata are regularly arranged in rows within the epidermis in between costal and intercostals zones, found in *F. imbricata* and *F. sumatrana*. In other species, the stomata are randomly scattered in epidermis layer found in *F. angustifolia*.

Since the stomata of each species are unique, anatomical observations may support the

morphological data of *Freycinetia* from West Kalimantan. *Freycinetia* species found in open areas with high light intensity, *F. sumatrana* and *F. winkleriana*, have more calcium oxalic crystals in epidermis cells, whereas *F. imbricata* found in areas with low light intensity has little calcium oxalic crystals. Kam (1971) stated that calcium oxalic crystals are produced under light conditions, when plant receives large light intensity, then the plant metabolism will be faster, so more calcium oxalic crystals will be produced.

Sclerenchym

Sclerenchym cells can be formed individually or in groups of 2 or 3 cells. Hexagonal, rectangular or triangular cell are arranged in the abaxial and adaxial epidermis layers (Figure 3). However, these cells are also sometimes found in the hypodermis and palisade tissues (*F. sessiliflora*, *F. angustifolia*, *F. corneri*, *F. imbricata* and *F. sarawakensis*); or on a tissue in a palisade and sponge tissues, as in *F. sumatrana*; and spongy tissue and hypodermis on *F. winkleriana*. Each cell is characterized by a narrow concentric layer, circular and oval-shaped, and some cells also have a cone-shaped silica bodies.

Hypodermis

Hypodermis is a prominent cell on the abaxial and adaxial of leaves surfaces. It presents in all observed *Freycinetia* species, and it is usually multiseriate and at least consisted of four layers (Figure 4). Hypodermal cells of adaxial leaf surface is usually somewhat thicker than that of the abaxial leaf surface. The number of hypodermal layers is varied among species, four layers of hypodermal cells were found in *F. sessiliflora* and *F. sumatrana*, three hypodermal layers were found in *F. sarawakensis*, and *F. winkleriana*, while in *F. angustifolia*, *F. corneri*, *F. imbricata* only consisted of two layers. Hypodermal shape on abaxial surface is not uniform compared to that of adaxial hypodermal.

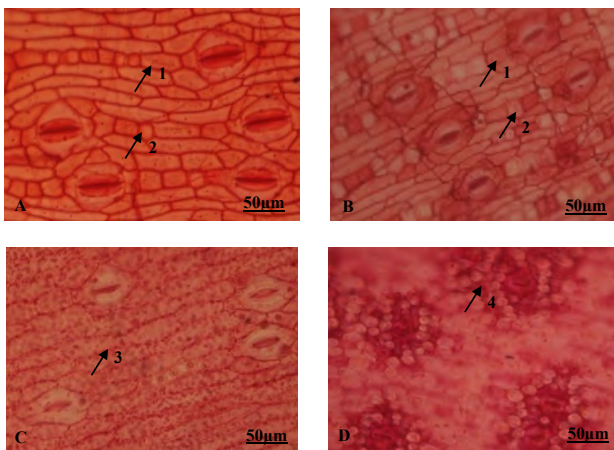


Figure 3. Typical stomata within the cell of epidermis (A) *F. sumatrana*, (B) *F. sessiliflora*, (C) *F. angustifolia*, (D) *F. sarawakensis*, 1. Costal cells, 2. Intercostals cells, 3. Granules, 4. Papillae

Mesophyll

The mesophyll of the *Freycinetia* species differs remarkably among species. It usually consists of 2-5 layers of palisade tissue and 2-5 layers of spongy tissue. *Freycinetia sessiliflora* has the thinnest layer of spongy tissue and has round palisade cell, and consists of 2-4 layers (Figure 4). In this present study, it is found that sponge tissue of a species that grows in open areas or exposed to the sunlight generally has isodiametric of sponge cells, and three layers of palisade tissues, such as *F. sessiliflora*, *F. angustifolia* and *F. sumatrana*. In contrast to that character, *Freycinetia* species grows in moist areas including *F. imbricata*, has a branch-shaped spongy cells, and one to two layers of palisade tissues. In many previous studies, it has been reported that the leaves of plants grown

in moist habitat decrease in the amount of palisade tissue (McDougall 1927; Wilmer 1983).

CONCLUSION

Anatomical structures of *Freycinetia* leaves tend to be similar among species, but variation is found in epidermal cells, cells of sponges, the number of hypodermic layer and the size of the stomata. There are six characters that can be used to distinguish among *Freycinetia* species, i.e. the number of hypodermic layer, the number of palisade layer, sclerencycm tissue, shape of spongy tissue, presence of costal and intercostal cells and shape of vascular bundle. Phenetic analysis based on morphological characters revealed that there are two groups of *Freycinetia* formed by the similarity coefficient 0.38. Cluster analysis based on anatomical data showed that seven *Freycinetia* species were clustered into two major groups at similarity coefficient 0.38. The first group consisted of *F. angustifolia*, *F. imbricata*, *F. sarawakensis*, and *F. winkleriana*, while the second group consisted of *F. corneri*, *F. sessiliflora* and *F. sumatrana*.

ACKNOWLEDGEMENTS

This study was supported by Directorate General of Higher Education (DIKTI), Kemendikbud RI, Government of Indonesia. The author would like to thank the Bogor Agricultural University (IPB), thanks also to Herbarium Bogoriense and Rijksherbarium, Leiden for the opportunity in using the specimens and Anatomy Laboratory of LIPI. Thank also to Prof. Mien A. Rifai and two anonymous reviewers for their opinion in the scientific writing and comments to the manuscript.

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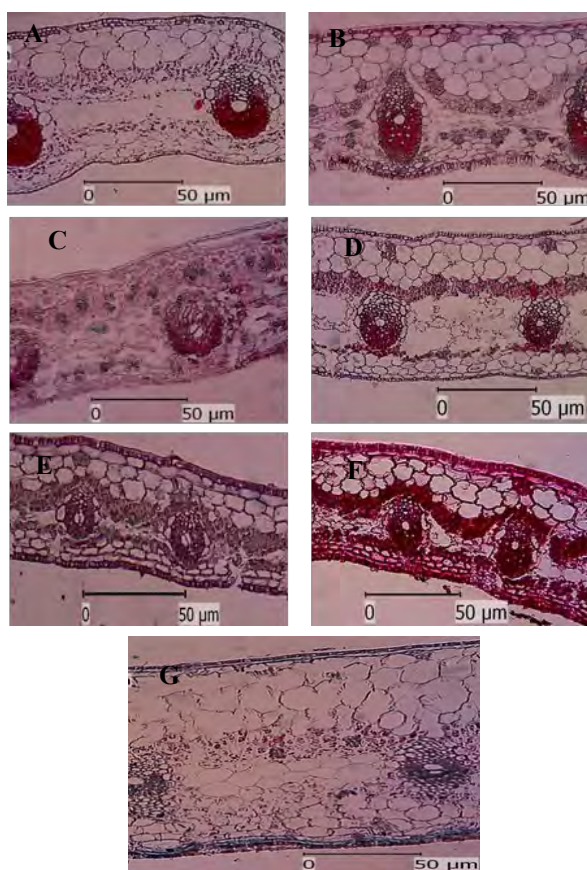


Figure 4. The transverse section of leaves (A). *F. angustifolia*, (B). *F. corneri*, (C). *F. imbricata*, (D). *F. sessiliflora*, (E). *F. sarawakensis*, (F). *F. winkleriana* and (G). *F. sumatrana*

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