MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Sundrarajoo *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 18 (2022) 473-482

RESEARCH ARTICLE

Morphological and Anatomical Studies on *Glycosmis perakensis V.Naray* (Rutaceae)

Previnaa Sundrarajoo[a,*], Sugumaran Manickam[b], Nur Fatihah Mohd Yusoff[a], Noorjahan Banu Mohammed Alitheen[a], Parameswari Namasivayam[a]

[a] Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia,43400, UPM Serdang, Selangor, Malaysia; [b] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia

Abstract There are a number of underexplored plant species from the family of Rutaceae despite their economic importance, including *Glycosmis perakensis* V.Naray. Therefore, the main purpose of this study is to enhance its taxonomic characterization and identification of this plant species by examining the morphology and anatomy of selected parts. The histological procedure for *G. perakensis* stems and leaves were optimized by increasing the incubation time for the fixation, clearing, and infiltration steps of the standard paraffin embedding method. Different types of stains were also used to identify and differentiate the plant tissues. The anatomy of plant parts such as stem, leaf, and petiole were studied using light microscopy. Pellucid dots and stomata exist on both abaxial and adaxial leaf surfaces. Statistical analysis on stomatal index and stomatal density showed a significant difference between abaxial and adaxial surfaces. Histological analyses showed the presence of sclerenchyma, collenchyma, and parenchyma cells, vascular bundle, prismatic crystals of calcium oxalate, and secretory cavities in the plant leaf and stem transverse sections. This is the first study reporting on the histological morphology of stem, petiole, and leaf of *G. perakensis.* The findings of this study will be a valuable tool for the identification of this plant.

Keywords: light microscopy, prismatic crystal, secretory cavities, histology, stomata.

Introduction

The family Rutaceae placed in the order Sapindales comprises more than 2000 species across 160 genera, most of which are woody shrubs and trees [20]. Several plants from this family are from the genus *Citrus.* In Peninsular Malaysia, there are 19 genera and about 60 species, and they are found in most forest types [4]. However, they are most common in dry regions, and a few species can be found in coastal areas, including mangrove forests, and there are several species that grow on limestone hills. The presence of secretory cavities (schizogenous, lysigenous, schizolysigenouos) in almost all the organs is a notable feature in the Rutaceae family [22, 10,16]. The secretory cavity is where the synthesis and/or accumulation of biological substances takes place, such as the essential oil [29].

Glycosmis, a genus from the Rutaceae family, is of evergreen glabrous shrub, distributed in warm and temperate regions of the world such as the Southeast Asia and Australia [2008]. The genus *Glycosmis* is a rich source of secondary metabolites such as alkaloids, flavonoids, phenolic glycosides, quinones, terpenoids, and glycerides isolated from different parts of the *Glycosmis* plant. A review on phytochemical and pharmacological perspectives of plant extracts of *Glycosmis* genus revealed that roughly 233 phytoconstituents were isolated from the genus [1,6,37]. Plant species from the genus *Glycosmis* have been reported to have several potent bioactivities. For example, *G. pentaphylla* leaf powder has been reported to have properties that may reduce or prevent arsenic toxicity in humans [7].

***For correspondence:**
previnaa1994@gmail.com

Received: 6 Dec 2021
Accepted: 26 August 2022

Also, the methanolic extract from stem and leaves of *G. pentaphylla* has been reported to possess antioxidant activity as well as highly potent cytotoxic activity [12]. Other than that, *G. arborea* leaf extracts showed significant wound healing properties when tested against rats, further supporting the folk medicinal use of this plant [31]. Alkaloids from *G. cochinchinensis* twigs were reported to have antibacterial activity [33]. With some of the species showing good medicinal properties, it is perhaps worthwhile studying their morphological features in detail, which will enhance scientific literacy in this genus.

Glycosmis perakensis V.Naray is native to Malaysia [14]. In this study, the anatomy and morphological features of *G. perakensis* plant parts such as the leaf, petiole, and stem were investigated. With no such studies reported so far on *G. perakensis*, the findings would directly improve the understanding of the cell structure buildup of this species. Such study may also indirectly add conservation value to the species as according to the IUCN red list of threatened species, *G. perakensis* is listed as vulnerable [5]. *G. perakensis* was also included in 'The World List of Threatened Trees' [30]. With more scientific studies reported of such rare species, the location of its existence may be more protected in time to come. The species' current conservation status is unknown and may be worse than "vulnerable", given the extensive destruction of tropical rain forests.

Materials and Methods

Plant material

G. perakensis leaf, stem, and fruit samples were collected from Rimba Ilmu Botanic Garden, University of Malaya, Kuala Lumpur. Identification of the species was made by a taxonomist, and an herbarium voucher material with the number KLU 49657 was deposited in the Kuala Lumpur Herbarium (KLU) located at Rimba Ilmu Botanic Garden, Institute of Biological Sciences, Faculty of Science, University of Malaya.

Plant morphological study

The leaf architecture was classified according to [2]. Scientific photography of the respective plant parts was captured using Canon EOS 700D DSLR camera.

Light microscopy

Leaf imprint method

The epidermal and stomatal cell was analyzed according to [27], by making impressions of the leaf surface according to [9]**.** Slides were viewed under a light microscope coupled with a camera (Dino Capture 2.0). Four replicates were done. The leaf was first divided equally into four sections for each replicate, including apical, middle, distal, and basal. A thin layer of clear nail varnish was applied onto the section. Clear cellophane tape was adhered firmly onto the completely dried nail varnish. The cellophane with leaf imprint was removed slowly from the leaf and was adhered onto the slide (sticky side facing down). Five unit areas (area =46.007 mm2, radius= 3.827mm) were chosen from different portions of the slide. These steps were carried out for each section of the leaf to calculate the stomatal density and stomatal index. The unit area used was the microscopic field of view at x10 magnification.

Statistical analysis on the stomatal density and index

The stomatal index and stomatal density were calculated and validated statistically, according to [27]. Stomatal index was determined using the formula, stomatal index (%) = (S/S+E) x 100 where, S and E are the number of stomata and epidermal cells respectively in a microscopic view field. Data was then analyzed using the IBM SPSS Statistics 26 software.

Histology studies

The anatomical features of *G. perakensis* were studied using fresh materials. Plant parts were processed using the paraffin embedding method according to [15] with minor modifications. Briefly, plant parts, including leaf blade (apical, basal, middle, and distal parts), petiole, and stem, were fixed in FAA (37% formaldehyde /glacial acetic acid/ 95% ethanol/ distilled water: 2:1:10:7), two days for leaves, three days for petiole and stem. After rinsing with 50% ethanol, samples were then dehydrated in tertiary butyl alcohol series (50, 70, 85, 95, and 100% (v/v)) followed by immersing the samples in pure tertiary butanol overnight. As for clearing, samples were passed through a graded series of histoclear: tertiary butanol from 1:3,1:1 to 3:1 and then 100% (v/v) histoclear. Leaves were immersed for 1 hour, whereas petiole and stem were immersed for two hours during each step dehydration and clearing. Leaf samples were then infiltrated for four days, petiole, and stem samples were infiltrated for six days in paraffin wax. The

specimens were then embedded in Paraffin Plus and cut to 9-10µm obtain sections using the rotary microtome (LEICA RM2235). Sections were then stained with basic Fuchsin and Astra blue according to [18], Toluidine Blue or Safranin and Fast green FCF according to [15]. Slides were then mounted in DPX medium (Merck) and viewed under the light microscope (Leica Microscope DM1000).

Results and Discussion

Macroscopic evaluation

G. perakensis leaf attachment is alternate, and they are pinnately compound with 7-9 foliate as shown in Figure 1a and 1b. The leaf laminar has a symmetrical elliptic shape. The laminar also has a reticulate venation pattern. The base shape is cunate, and the apex shape is straight. The position of the petiolar attachment is marginal. Leaf margins are entire and unlobed. *Glycosmis perakensis* is very similar and could be regarded as a variety of Glycosmis *gracilis* but differs mainly in its elliptic leaflets [34]. According to [24], *Glycosmis longipetala* has simple, unifoliate, and *Glycosmis cochinchinensis* has simple leaf, unlike *Glycosmis perakensis*. However, another species from the same genus, *Glycosmis pentaphylla* has almost similar features with *Glycosmis perakensis* as it has leaves that are pinnately compound, entire, acute to round at the apex, and attenuate at the base. Their leaves also have a similar venation pattern, reticulate [28].

The *G. perakensis* plant has an ellipsoid fruit with an average length of 1.5 cm and 1.2 cm width (Figure 1c and 1d). It also has dicotyledonous seed, as shown in Figure 1d. Plants from the genus *Glycosmis* have either globose or ellipsoid fruit, and this characteristic can be used to distinguish between its species easily. For instance, a plant from the same genus, *G. erythrocarpa* was reported to have ellipsoid fruits, while *G. parviflora* has globose fruits [34].

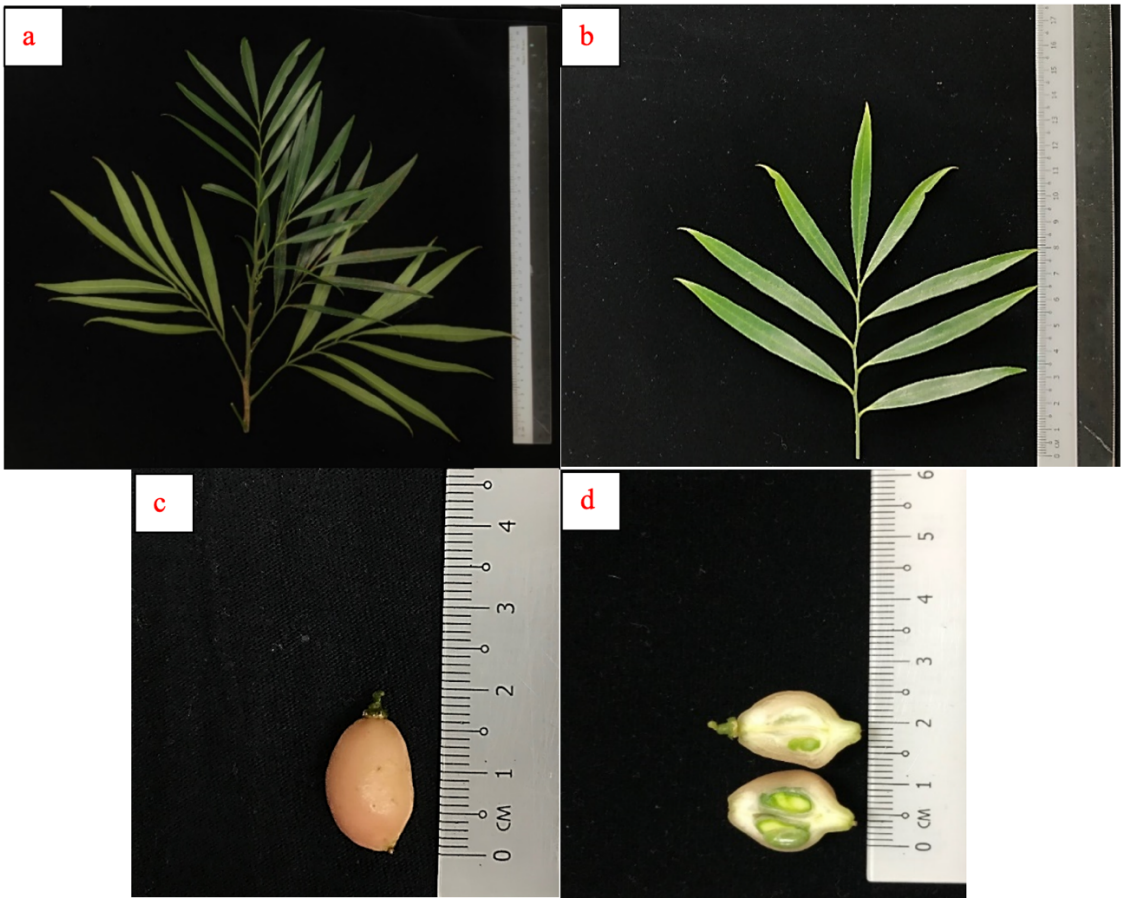

Figure 1. Morphological feature of *Glycosmis perakensis* plant leaf, leaflet and fruit. a: Pinnately compound leaf with leaflets arranged along the stem b: Leaf with 8 leaflets along the rachis. c: Mature ellipsoid fruit. d: Mature fruit with dicotyledonous seed.

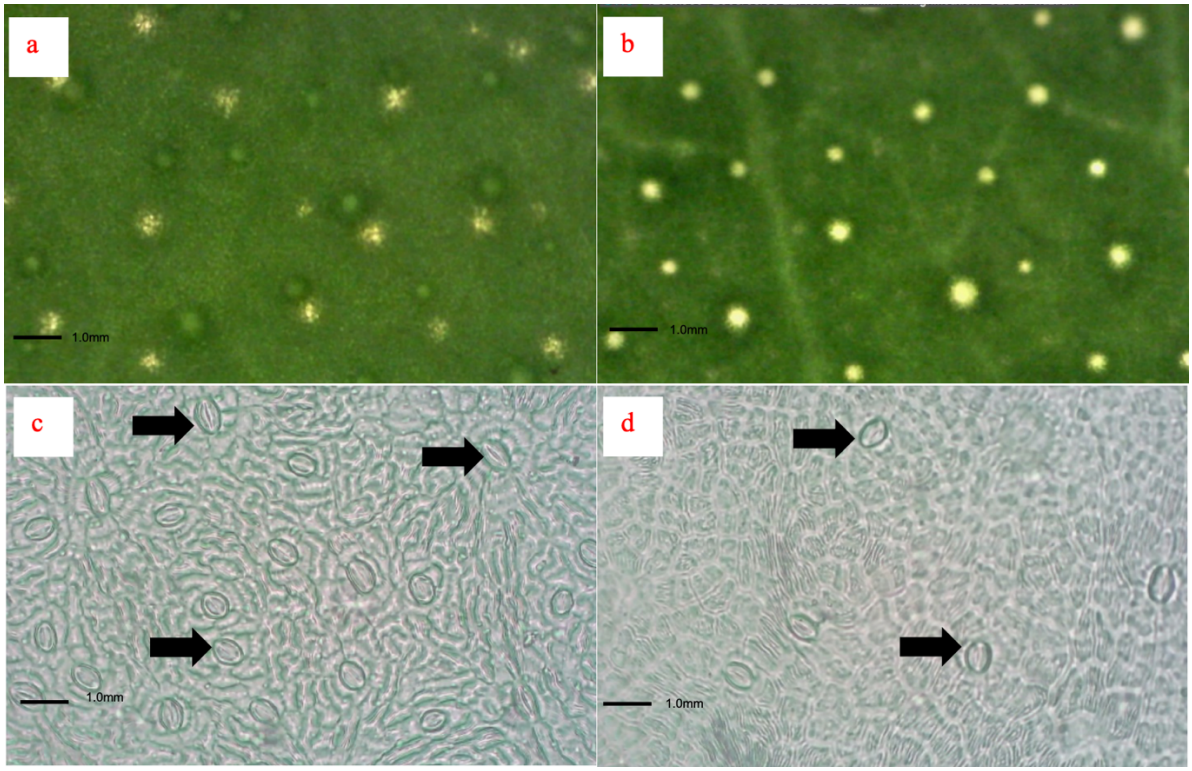

Figure 2. *Glycosmis perakensis* leaf imprint of both abaxial and adaxial surfaces. a, c: Leaf abaxial surface. b, d: Leaf adaxial surface. a, b: Pellucid dots on leaf surfaces. c, d: Leaf epidermal surfaces (*arrow;* stomata). d: Striated cuticle on the adaxial surface. Bars: 1.0mm.

Table 1. Significant difference in stomatal density and stomatal index between abaxial and adaxial surfaces.

Parameters	Values*±SEM
Stomatal density:	
Abaxial	8.090 ± 0.134a
Adaxial	2.220 ± 0.187b
Stomatal index:	
Abaxial	4.608 ± 0.078a
Adaxial	1.885 ± 0.135b

Note. * Values expressed as mean of three reading; SEM: Standard Error Mean
[ab] Values marked with different letters are significantly different between the leaf surfaces (abaxial and adaxial) ($P < 0.005$)

Microscopic evaluation

Leaf abaxial and adaxial surfaces

The microscopic features of the leaf surfaces are shown in Figure 2, where the pellucid dots were visible on both abaxial (Figure 2a) and adaxial (Figure 2b) surfaces which corresponds to the presence of secretory cavities on both surfaces (Figure 3a, d and e). These secretory cavities are potential sites for essential oil production, which contributes to the aroma of this plant. This is a notable feature in the Rutaceae family [22]. Besides that, species from the Rutaceae family mostly have stomata that are confined to the abaxial side [23]. For example, the epidermal peel of *Zanthoxylum macrophylla* (a species in the Rutaceae family) leaf revealed that stomata were present only on the abaxial surface

(hypostomatic) [13]. In contrast, *G. perakensis* leaves are amphystomatic since stomata were observed on both abaxial and adaxial surfaces (Figure 2c and 2d). From the frontal view, the stomata were slightly embedded in the epidermal cells. The epidermal cells were polygonal, rectangular, or isodiametric with straight to curved anticlinal wall pattern on both surfaces. The stomata on both the abaxial and adaxial surface were identified as paracytic or anomocytic. The stomata type on a leaf of a closely related plant species*, G. pentaphylla* showed anomocytic on the abaxial surface, whereas no data was provided for the stomata type on the adaxial surface [28]. The average stomatal index is shown in Table 1. Stomatal density and stomatal index on the abaxial surface were significantly higher ($p<0.005$) than on the adaxial surface of the leaves. In addition, striated cuticle was observed on the adaxial surface of the leaf imprint (Figure 2d). The cuticle is the hydrophobic layer covering the epidermis that acts as a protective barrier [38]. According to [11], wax deposition in plants increases under stress conditions.

Anatomy of leaf blade

The transversal sections of the leaves of *Glycosmis perakensis* revealed unilayered epidermis on both abaxial (lower) and adaxial(upper) surfaces covered externally by a layer of lignified cuticle (Figure 3a-c). The stomata are located at the same level as the epidermal cells and were more evident on the abaxial surface of the leaf transverse section (Figure 3a). This is due to the significant abundance of stomatal density on the abaxial leaf surface compared to the adaxial side, as demonstrated in Table 1. The mesophyll cells were dorsiventrally formed as a layer of compactly arranged palisade parenchyma, and up to nine layers of spongy parenchyma were arranged with minimal airspaces (Figure 3h and i). Schizogenous secretory cavities were identified along the epidermis on both surfaces of the leaf transverse section (Figure 3a, d, and e). Secretory cavities can be formed by the disintegration of cells (lysigenous spaces), separation of cells (schizogenous spaces), or a combination of both processes (schizo-lysigenous spaces) [17]. The presence of secretory structures is one of the characteristics of Myrtaceae and Rutaceae [23] as they are commonly found in these families. These structures are also the primary sites for the synthesis and collection of essential oils [8].

Plants from the Rutaceae family were generally described to have abundant crystals of calcium oxalate that may be solitary or clustered or a mixture of both [23,32]. Prismatic crystals of calcium oxalate were found in the mesophyll, mainly around the vascular bundles of the *G. perakensis* leaf transverse section (Figure 3k). The type of crystals present in the tissues can be used as a diagnostic feature at the genus level. For example, different types of crystals were found present in different species. A study on 14 neotropical Rutaceae species by [25] revealed that in all species of the genus *Pilocarpus* (from the Rutaceae family)*,* calcium oxalate druses were observed, whereas rod-shaped crystals were observed in other genera. Prismatic crystals around the secretory cavity were found on the *Citrus medica* leaf transverse section [26].

In the description of the anatomy of numerous plant families, including Rutaceae, by [23], reported that plants in the Rutaceae family possesses both glandular and non-glandular trichomes. However, a study done on the *Zanthoxylum armatum* (a species in the Rutaceae) by [3] on the leaf, stem bark, and fruit anatomy reported that neither trichomes nor any other appendages were present. On the other hand, according to a study on plant species from the Rutaceae family by [25], there were two types of glandular trichomes present, capitate and peltate trichome. Unicellular trichomes were also reported to be present on *Glycosmis pentaphylla* leaves (observed with transverse sections) [28]. Current anatomical study on transverse sections of *G. perakensis* leaves did not indicate the presence of trichomes on either leaf surface. Therefore, it is evident that trichomes are not necessarily present in all species of the Rutaceae family, as reported by [23].

The cross-section of the midrib showed a convex contour (Figure 3g). The epidermis was unilayered and was externally covered by a thin layer of cuticle. The midrib was mostly made up of ground tissue, mainly parenchyma with clusters of collenchyma along the epidermis on both the upper and lower sides of the midrib. Collateral vascular bundle in the midrib (Figure 3g and j) was lined by lignified pericyclic fibre. The bundle sheath or endodermis were more visible in the smaller veins compared to the lateral vein and midrib (Figure 3f). The occasional presence of secretory cavity at the midrib was observed on the leaf transverse section (Figure 3g).

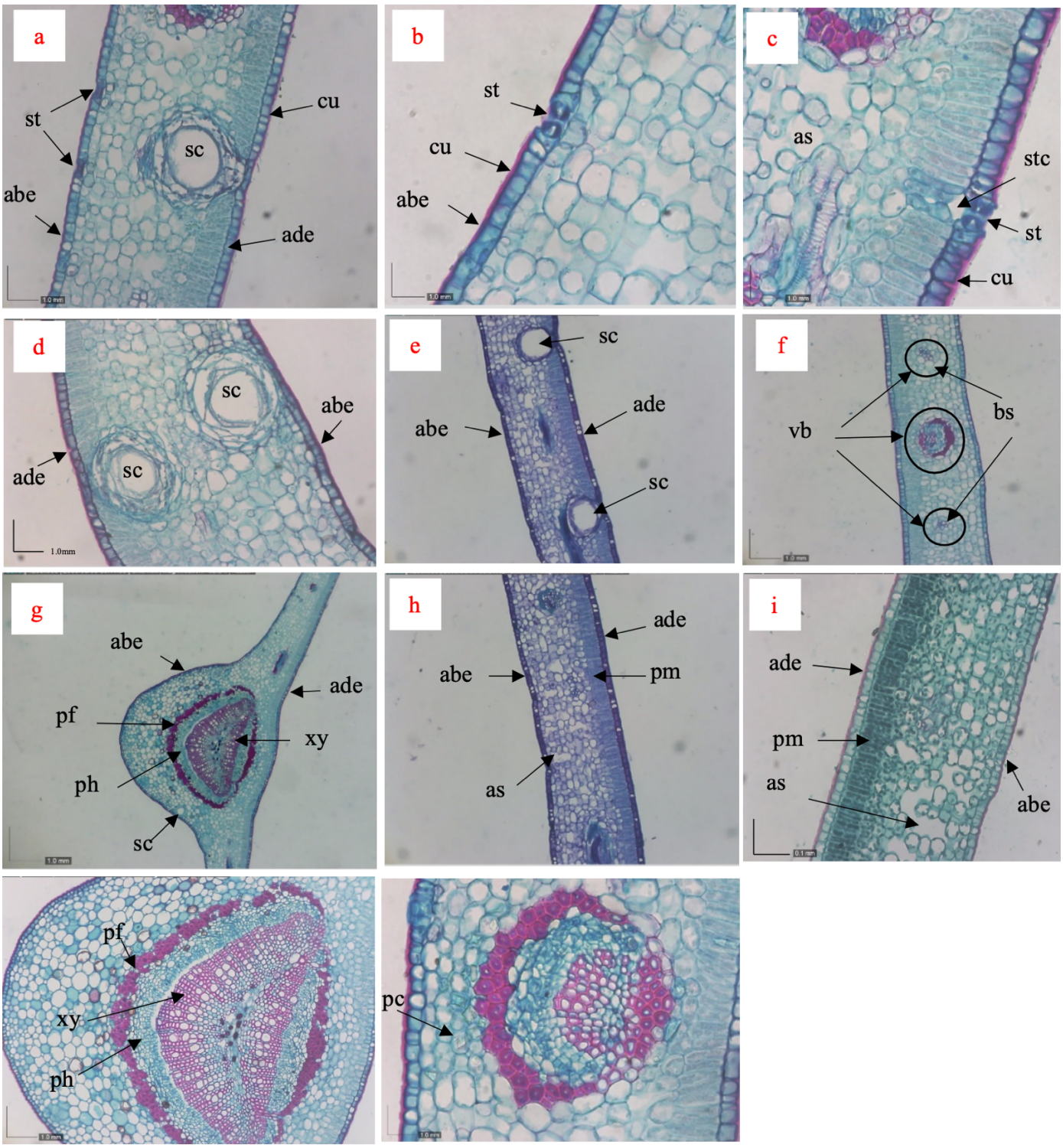

Figure 3. Leaf anatomy of *Glycosmis perakensis* [a, b, c, d, f, g, i, j, k: stained in astra blue/basic fuchsin; e, h: stained in toluidine blue; i: stained in Safranin/Fast green fast FCF]. a: thin lignified cuticle stained red by basic fuchsin on both abaxial and adaxial surface; a: stomata more evident on the abaxial surface; b: stomata on the abaxial surface; c: stomata on the adaxial surface; d: schizogenous secretory cavity; e: schizogenous secretory cavity along the epidermis of adaxial surface; f: bundle sheath is present in smaller veins and is absent in the lateral vein; g: transverse section of the blade; h, i: leaf blade showing palisade mesophyll on the adaxial surface; j: Leaf midrib; k: leaf blade cross-section showing prismatic crystal around the vascular bundle. Abbreviations: stomata(st), abaxial epidermis (abe), adaxial epidermis (ade), secretory cavity (sc), cuticle (cu), air space (as), stomata chamber (stc), vascular bundle (vs), bundle sheath (bs), xylem (xy), phloem (ph), pericyclic fibre (pf), palisade mesophyll (pm), prismatic crystal (pc). Bars: a, b, c, d, e, f, g, h, j=1.0mm; i=0.1mm.

Anatomy of petiole

The petiole outline in the transverse section was circular (Figure 4a). The epidermis had a quadrangular to rectangular aspect (Figure 4b and d). Internal to the epidermis, secretory cavity of both schizogenous and lysigenous was observed on both the abaxial and adaxial surfaces of the petiole transverse section (Figure 4a). The secretory cavities were present mainly in the large cortex region (Figure 4b-d). According to [23], species from Rutaceae generally has secretory cavity that is either schizogenous or lysigenous almost always present in the mesophyll. However, [36] reported that exposure to common fixative solutions causes destructive swelling in the epithelial cells that gives the false impression that the glands are developed lysigenously and could be responsible for the lysigeny reports in the literature, conversely [19] consider that the gland is formed schizolysigenously.

The vascular system was organized with open free vascular bundle (Figure 4a). Pericyclic fibres were observed on the cross-sections (Figure 4a). Diamond-shaped solitary crystals were present in the cortex region (Figure 4b). No idioblasts were observed. It was reported that crystals are commonly present in the mesophyll cells of the Rutaceae plants [23].

Since there are no reported studies on the petiolar features of other species in the genus *Glycosmis*, *G. perakensis* petiole transverse sections were compared within the species' at the familial level, i.e., Rutaceae. *Clausena excavata,* a plant species in the Rutaceae, has almost similar petiolar features except for the petiole outline that had irregular shape of the adaxial surface and circular abaxial surface. It also has trichomes that were unicellular and multiseptate around the petiole, especially on the abaxial epidermis. Moreover, it has large secretory cavity present in the ground tissue near the epidermis [39]. Petiolar features can be used to differentiate plant species from the Rutaceae family as different plant species have different features. This is evident in the genus *Pilocarpus* from where species displayed variation in the organisation of the vascular system in petioles. They were organised in closed cylinder, open with a single cylinder, or with free vascular bundles [25,21].

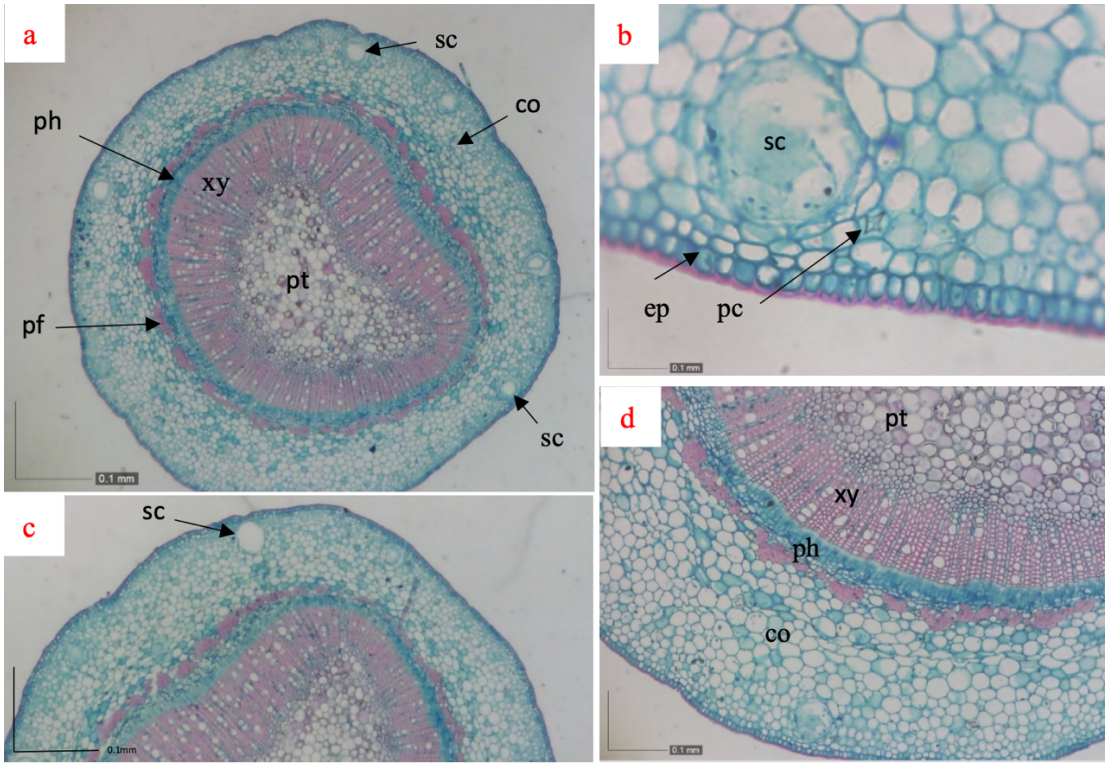

Figure 4. Transverse section of the *Glycosmis perakensis* petiole [a, b, c, d: stained by astra blue/basic fuchsin]. a: Overview of the petiole showing both schizogenous and lysigenous secretory cavity at the cortex region; b: prismatic crystal at cortex and lysigenous cavity; c: schizogenous cavity d: cross section showing large cortex region. Abbreviations: phloem (ph), xylem (xy), cortex (co), pericyclic fibre (pf), pith (pt), secretory cavity (sc), prismatic crystal (pc), epidermal cell (ep). Bars: 0.1mm.

Anatomy of stem

Transverse section of the stem is shown in (Figure 5). The epidermis had a quadrangular to rectangular aspect with a thin cuticle layer on the outer layer of the cell wall (Figure 5b). Just beneath the uniseriate layer of the epidermis, the cortex region consists of 15-24 cell layers, with a few layers (2 to 3) of collenchyma cells and 11-22 layers of parenchymal cells (Figure 5a and b). Cortex cells are hexagonal and orbicular shaped. Stomata were inserted on the same level of the epidermal cells (Figure 5c). Secretory cavities (schizogenous gland) were present at the cortex region below the epidermis layer (Figure 5b). Prismatic crystals were present at the cortex region, mainly in the parenchyma cell layers. The vascular bundle was arranged in an amphicribal arrangement where the xylem was surrounded by a ring of phloem (Figure 5a). Isolated patches of pericyclic fibre were present, with 2-6 cell layers between the inner cortex region and the phloem. The stem cross-section has phloem that consists of 5-7 layers of cell and distinct cambium with 2-3 layers of cell. The large central portion of the stem was occupied by pith, where it comprises hexagonal or orbicular parenchymatous cells, which also contain prismatic crystals (Figure 5a).

There are no reported studies on the stem anatomy for the genus *Glycosmis*. Most plant species from Rutaceae generally have all the features that are present in the cross-section of the stem of *G. perakensis,* such as pericycle that contains isolated strands or bands of sclerenchyma, phloem, and xylem constituting closed cylinder traversed by narrow rays, large secretory cavities that are commonly present in the parenchymatous tissue and they also have abundant crystals in the cortex or phloem [23]. The features that could be used to distinguish between genus are the number of cortex layer and sclerenchyma cell layers on phloem, which was used in a study to differentiate between three different species from the genus *Haplophyllum [*35].

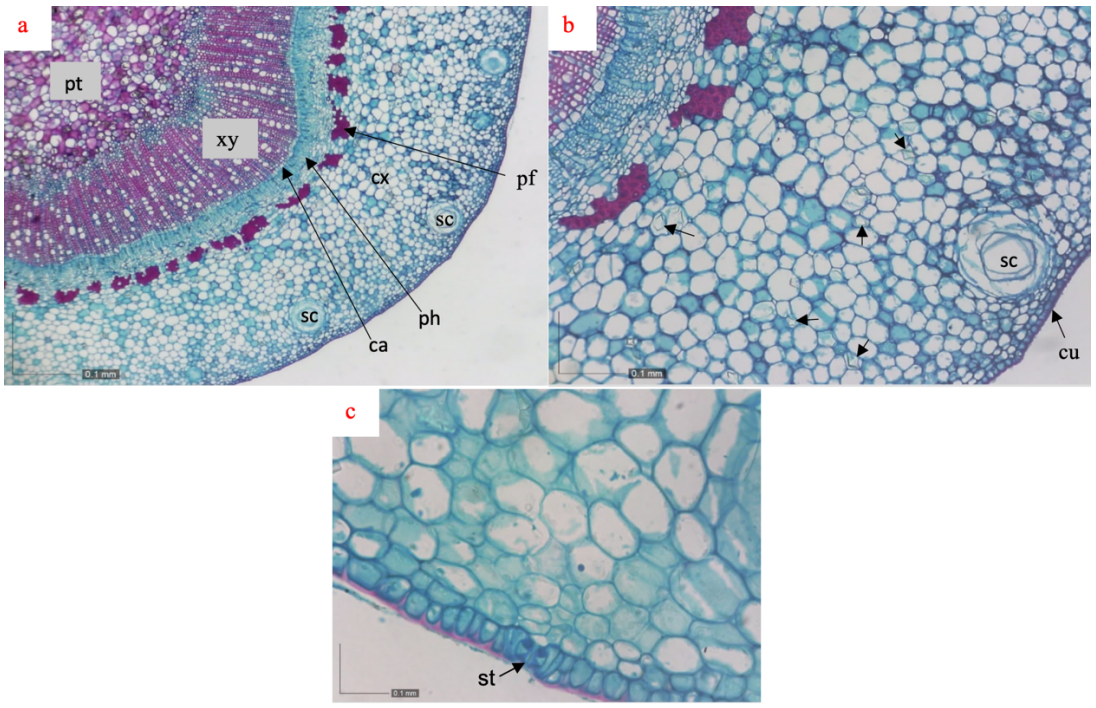

Figure 5. Cross section of *Glycosmis perakensis* stem. [a, b, c: stained by astra blue/basic fuchsin]. a: shows a large cortex region, pericyclic fibres, phloem, cambium, xylem and pith; b: shows a thin layer of cuticle, secretory cavity on the cortex region, uniseriate epidermal layer and calcium oxalate crystals (arrow); c: Stomata in between epidermal cells. Abbreviation: xylem (xy), phloem (ph), cambium (ca), pericyclic fibre (pf), pith (pt), secretory cavity (sc), epidermis (ep), cuticle (cu), stomata (st). Bars: 0.1mm.

Conclusions

In conclusion, this is the first study on the anatomy and morphology on *Glycosmis perakensis* leaf, stem, and fruit. There are very few reported studies on the species from the genus *Glycosmis.* The findings of this study were compared to the findings of other *Glycosmis* species in the literature records, and the differences and similarities between them were stated in detaill. The leaf morphology, presence of calcium oxalate, secretory cavity, stomatal density and the absence of trichomes could be an important diagnostic character at the genus level*. G. perakenesis* stems, leaves, and petioles contain secretory cavities, which are notable features in the Rutaceae family that could contain essential oil. Essential oil could be useful economically and medicinally. Therefore, further studies on its bioactivities should be carried out. The results from this study can be used for future identification and standardization purposes.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

This work was supported by the High Impact Putra Grant (GPB/2017/9559900) of UPM. Ms Previnaa Sundrarajoo is supported by Universiti Putra Malaysia's Graduate Research Fellowship (GRF).

References

[1] Ahmed I, Islam R, Sikder M. A, Haque M. R, Al Mansur M. A, Rashid M. A. (2015). Alkaloid, Sterol and Triterpenoids from *Glycosmis pentaphylla* (Retz.) DC. *Dhaka University Journal of Pharmaceutical Sciences* 13(2): 115-118. doi: https://doi.org/10.3329/dujps.v13i2.21887

[2] Ash A. (1999). *Manual of leaf architecture: morphological description and categorization of dicotyledonous and net-veined monocotyledonous angiosperms*. Smithsonian Institution.

[3] Barkatullah Mi, Jelani Gh, Ahmad Im. (2014). Leaf, stem bark and fruit anatomy of *Zanthoxylum armatum* DC. (Rutaceae). 2014. Pak. J. Bot 46(4):1343-9.

[4] Chooi O. H. (1994). The ethnobotany of Citrus and their relatives. *Korean Journal of Plant Taxonomy (Korea Republic)*.

[5] Chua L.S.L. (1998). *Glycosmis perakensis*. The IUCN Red List of Threatened Species 1998: e. T31782A9653718. https://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T31782A9653718.en. (accessed 06 March 2020)

[6] Cuong N. M, Taylor W. C, Van Sung T. (1999). Glypetelotine, a sulphur-containing indole alkaloid from *Glycosmis petelotii*. *Phytochemistry 52*(8): 1711-1714. doi: https://doi.org/10.1016/S0031-9422(99)00402-1

[7] De A, Nath S, Das A. K, Bandyopadhyay S. K, Banerjee M. C, Mandal T. K. (2017). Effects of *Glycosmis pentaphylla* leaf power against chronic arsenicosis in rats. *International Journal of Pharmaceutical Sciences and Research* 8(5): 2287-2297. doi: 10.13040/IJPSR.0975-8232.8(5).2287-98

[8] Esau K. (1965). *Plant Anatomy. 2nd Edition*. John Wiley, New York.

[9] Grant B. W, Vatnick I. (2004). *Environmental correlates of leaf stomata density. Teaching issues and Experiments in Ecology*. Ecological Society of America 1(1): 1-24.

[10] Groppo M, Pirani J. R, Salatino M. L, Blanco S. R, Kallunki J. A. (2008). *Phylogeny of Rutaceae based on twononcoding regions from cpDNA.* American Journal of Botany 95(8): 985-1005. doi: :10.3732/ajb.2007313

[11] Hollenbach, B., Schreiber, L., Hartung, W., Dietz, K. J. (1997). Cadmium leads to stimulated expression of the lipid transfer protein genes in barley: implications for the involvement of lipid transfer proteins in wax assembly. *Planta* 203(1): 9-19. doi: 10.1007/s004250050159

[12] Howlader M. A, Rizwan F, Sultana S, Rahman M. R, Shams-Ud-Doha K. M, Mowla R, Apu A. S. (2011). Antimicrobial, antioxidant and cytotoxic effects of methanolic extracts of leaves and stems of *Glycosmis pentaphylla* (Retz.). *Journal of Applied Pharmaceutical Science.* 1(8): 137.

[13] Igboabuchi N. A, Ilodibia C. V. (2017). A study on the anatomy of *Zanthoxylum macrophylla* (L.) Sarg. (Rutaceae). *Asian Journal of Biology.* 1-6. doi: 10.9734/AJOB/2017/36104

[14] IPNI. (2020). International Plant Names Index. Published on the Internet http://www.ipni.org, The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Botanic Gardens. [Retrieved 10 December 2020].

[15] Johansen D.A, (1940). *Plant microtechnique.* McGraw-Hill Publ. Co., Ltd, New York, London.

[16] Judd, W. S., Campbell, C. S., Stevens, P. F., Donoghue, M. J. (2008). *Plant Systematics: A Phylogenetic Approach.* Sinauer Associates, Sunderland, MA.

[17] Kalachanis D, Psaras G. K. (2005). Structure and development of the secretory cavities of *Myrtus communis* leaves. *Biologia plantarum.* 49(1): 105-110.
[18] Kraus J. E, De Sousa H. C, Rezende M. H, Castro N. M, Vecchi C, Luque R. (1998). Astra blue and basic fuchsin double staining of plant materials. *Biotechnic & histochemistry.* 73(5): 235-243. doi: https://doi.org/10.3109/10520299809141117
[19] Liang S. J, Wu H, Lun X, Lu D. W. (2006). Secretory cavity development and its relationship with the accumulation of essential oil in fruits of *Citrus medica* L. var. *sarcodactylis* (Noot.) Swingle. *Journal of Integrative Plant Biology*. 48(5): 573-583.
[20] List of plants in the family Rutaceae. (2016). https://www.britannica.com/topic/list-of-plants-in-the-family-Rutaceae-2038278 (accessed 7 May 2019)
[21] Marquete O. (1981). Anatomia e vascularização foliar e floral de *Pilocarpus organensis* Occhioni and Rizzini (Rutaceae). *Arq Jard Bot Rio J*. 25:117–159
[22] Metcalfe C, Chalk L. (1950) Anatomy of the dicotyledons, vol. II. Clarendon Press, Oxford
[23] Metcalfe C. R, Chalk L. (1979). *Anatomy of the Dicotyledons: leaves, stem, and wood, in relation to taxonomy, with notes on economic uses.* Clarendon Press, Oxford
[24] Mou F. J, Zhang D. X. (2009). *Glycosmis longipetala* FJ Mou, DX Zhang, a new species of Rutaceae from China. *Journal of Systematics and Evolution.* 47(2): 162-167. doi: 10.1111/j.1759-6831.2009.00015.x
[25] Muntoreanu T. G, Da Silva Cruz R, Melo-De-Pinna, G. F. (2011). Comparative leaf anatomy and morphology of some neotropical Rutaceae: *Pilocarpus Vahl* and related genera. *Plant systematics and evolution*, *296*(1), 87-99. doi: 10.1007/s00606-011-0478-3
[26] Panara K, Nishteswar K, Harisha C. R, Shukla V. J. (2014). Pharmacognostical evaluation of bijapura (*Citrus medica* Linn.) leaves. *Ayurpharm Int J Ayur Alli Sci.* 3: 112-120.
[27] Paul V, Sharma L, Pandey R, Meena R. C. (2017). Measurements of stomatal density and stomatal index on leaf/plant surfaces. *Manual of ICAR Sponsored Training Programme for Technical Staff of ICAR Institutes on—Physiological Techniques to Analyze the Impact of Climate Change on Crop Plants. 27*.
[28] Prakasia P. P, Nair A. S. (2016). Pharmacognostic and physicochemical standardization of leaves of *Glycosmis pentaphylla* (Retz.) DC. *The Pharma Innovation.* 5(9, Part A): 23.
[29] Rehman R, Hanif M. A, Mushtaq Z, Mochona B, Qi X. (2016). Biosynthetic factories of essential oils: the aromatic plants. *Natural Products Chemistry & Research*. doi: http://dx.doi.org/10.4172/2329-6836.1000227
[30] Sara Oldfield, Charlotte Lusty, Amy M.K, (1998). *The World List of Threatened Trees, The World List of Threatened Trees*. World Conservation Press, Cambridge, UK.
[31] Silambujanaki P, Chandra C. B. T, Kumar K. A, Chitra V. (2011). Wound healing activity of *Glycosmis arborea* leaf extract in rats. *Journal of ethnopharmacology.* 134(1): 198-201. doi: https://doi.org/10.1016/j.jep.2010.11.046
[32] Solereder H. (1908). *Systematic anatomy of the dicotyledons: a handbook for laboratories of pure and applied botany* (Vol. 2). Clarendon Press, Oxford.
[33] Sripisut T, Phakhodee W, Ritthiwigrom T, Cheenpracha S, Prawat U, Deachathai S, Machan T, Laphookhieo S. (2013). Alkaloids from *Glycosmis cochinchinensis* twigs. *Phytochemistry Letters.* 6(3): 337-339. doi: https://doi.org/10.1016/j.phytol.2013.03.018
[34] Stone B. C. (1985). A conspectus of the genus *Glycosmis* Correa: studies in Malesian Rutaceae, III. *Proceedings of the Academy of Natural Sciences of Philadelphia.* 137: 1-27.
[35] Tekin M, Eruygur N. (2016). The structural studies on the medicinal plant *Haplophyllum telephioides*. *Revista Brasileira de Farmacognosia.* 26(5): 544-552. doi: https://doi.org/10.1016/j.bjp.2016.05.007
[36] Turner G. W, Berry A. M, Gifford E. M. (1998). Schizogenous secretory cavities of *Citrus limon* (L.) Burm. F. and a reevaluation of the lysigenous gland concept. *International Journal of Plant Sciences.* 159(1): 75-88. doi: 10.1086/297523
[37] Yasir M, Tripathi M. K, Singh P, Shrivastava R. (2019). The Genus *Glycosmis* [Rutaceae]: A Comprehensive Review on its Phytochemical and Pharmacological Perspectives. *The Natural Products Journal.* 9(2): 98-124. doi: 10.2174/2210315508666180622121212
[38] Yeats T. H, Rose J. K. (2013). The formation and function of plant cuticles. *Plant physiology.* 163(1): 5-20. doi: 10.1104/pp.113.222737
[39] Zaman F. Q, Al-Hakimi A. S, Khamis S, Ruhaizin F. F, Zaidi S. M. (2018). Anatomy and Histochemistry of Structures Producing Aroma in Leaves of *Sygyzium aromaticum* (L.) Merr. and *Clausena excavata* Burm. f. *Pertanika Journal of Tropical Agricultural Science.* 41(1).
[40] Zhang Dianxiang, Thomas G. Hartley. (2008). *GLYCOSMIS* Corrêa. Flora of China. 11: 80–83