https://doi.org/10.33451/florafauna.v30i2pp267-272
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2024 Vol. 30 No. 2 PP 267-272 ISSN 0971 - 6920 (Print)

Anatomical and morphological characterisation of *Turpinia malabarica*

Nithin Joseph[1], *K. Jayakumar[2] and A. J. Robi[3]

[1] Department of Botany,
St. Thomas College, Palai, Arunapuram
P.O., KOTTAYAM -686574 (KERALA) INDIA
[2]Post Graduate and Research Department of Botany,
SVR NSS College, Vazhoor, Theerthapadapuram
P.O., KOTTAYAM-686505 (KERALA) INDIA
[3] Department Of Botany,
Bishop Abraham Memorial College, Thuruthicad,
PATHANAMTHITTA -689653 (KERALA) INDIA
*Corresponding Author
E-mail: jksvrnss@gmail.com

Received **:** 03.09.2024; ***Revised*** **:** 25.09.2024; ***Accepted*** : 22.10.2024

ABSTRACT

The objective of this study was to investigate the various morphological and anatomical attributes of *Turpinia malabarica*, an angiosperm tree. Field studies were conducted to record the morphological characteristics of the tree and its fruits. Anatomical characterisation was performed using free-hand sections of stem, leaf, and petiole. The microscopic images acquired through these anatomical observations were analysed using Image J, image analysis software. Anatomical leaf characteristics like stomatal index, distribution and stomatal type were studied. Clearing and maceration of leaf samples were also done for further analysis.

Figures : 07 References : 13 Table : 00
KEY WORDS : Extraction, Image J, Maceration, Morphology, Plant anatomy, *Turpinia malabarica*

Introduction

Medicinal plants are a valuable resource for drug discovery, with a significant portion of the population in India relying on traditional medicine for their primary healthcare needs. As a result, there has been a growing interest in exploring ethnobotanical resources around the world for potential drug discovery[10]. Many anti-cancer drugs, such as taxane, vinca alkaloids, podophyllotoxin, and camptothecin, are derived from plants[2]. It is reported that around 75% of plant-derived pharmacological drugs were discovered by screening plants that were medicinally used by ethnic communities[11]. Thus, there is potential to find potent drugs by screening the bioactive compounds of important ethnobotanical medicinal plants.

Interestingly, several plants showing medicinal potential remain scientifically unexplored. One such plant is *Turpinia malabarica* of the family Staphyleaceae. *Turpinia malabarica* is a medium-sized tree primarily found in the wet tropical biome, native to India and Sri Lanka[6]. Various *Turpinia* species are utilized in local Chinese medicines, with a particular focus on their antibacterial and anti-inflammatory effects[12]. Modern studies have identified chemical constituents in *Turpinia* species, such as flavonoids, triterpenoids, megastigans, and phenolic acids[12]. The medicinal properties of numerous species within this genus have been scientifically validated, and bioactive compounds have been identified. For example, *T. formosana* exhibits robust osteogenic potential, with isolated compounds showing the ability to enhance bone formation[4]. *Turpinia insignis* found to contain antibacterial phenolics[7] and polysaccharides from the dried leaves of *Turpinia arguta* demonstrated antioxidative and anti-inflammatory activities, along with inhibitory effects on angiotensin II-induced vascular smooth muscle cell proliferation[13]. Flavonoid glycosides with strong anti-inflammatory activity were identified in the leaves of *Turpinia arguta*[5]. Turformosin A, isolated from *Turpinia formosana*, exhibits cytotoxicity against various cancer cells[8].

While the other members within the genus are well-documented, there is a notable absence of data

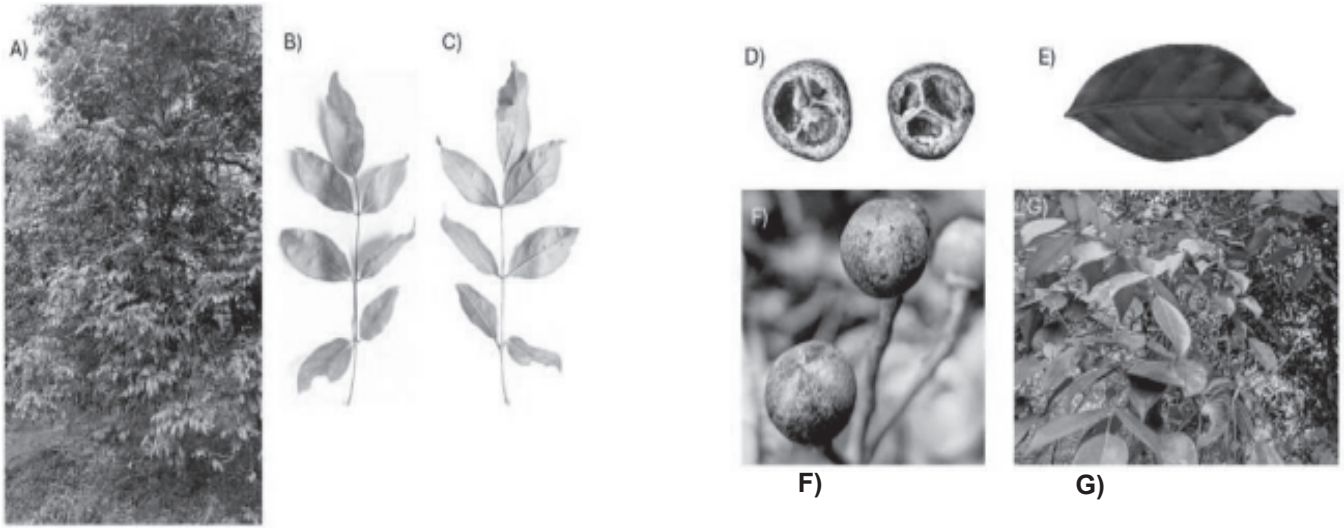

Fig. 1: A) *Turpinia malabarica* Habit, B) Adaxial side of a leaf, C) Abaxial side of a leaf, D) Section of a fruit, E) A single leaflet, F) Fruits with 3-pointed tip, G) A branch with fruits

regarding *Turpinia malabarica*. So, given the large amount of data pointing to the immense medicinal potential of the genus and the lack of scientific validation of the medicinal properties of *Turpinia malabarica* the current study evaluated the morphological and anatomical characteristics of *Turpinia malabarica* which could be a valuable resource for determining adulterants of the plant and may aid in its conservation.

Material and Methods

Plant material

The plant material, *Turpinia malabarica*, used for the study was collected from Nelliyampathy Hills, Palakkad district, Kerala.

Anatomical studies

The free-hand sections of the stem and leaves were dehydrated and stained using safranine. The sections were then mounted in glycerine and observed under a compound microscope to analyse the anatomical characters[1].

Leaf Clearing

In order to study the epidermal and vein characteristics of the leaf, the leaf tissues were cleared using the standard procedure[3]. The clearing process involved cutting the leaves and immersing them in Franklin's solution, which consisted of equal volumes of 35% hydrogen peroxide and concentrated acetic acid for maceration. The setup was kept undisturbed until the leaves became semi-transparent and colourless, with bubbles forming between the two layers of the epidermis. After that, the material was washed in distilled water and stained with safranine, and the epidermal layers were peeled away to reveal the vein structure. The stained veins and epidermal tissues were analysed separately

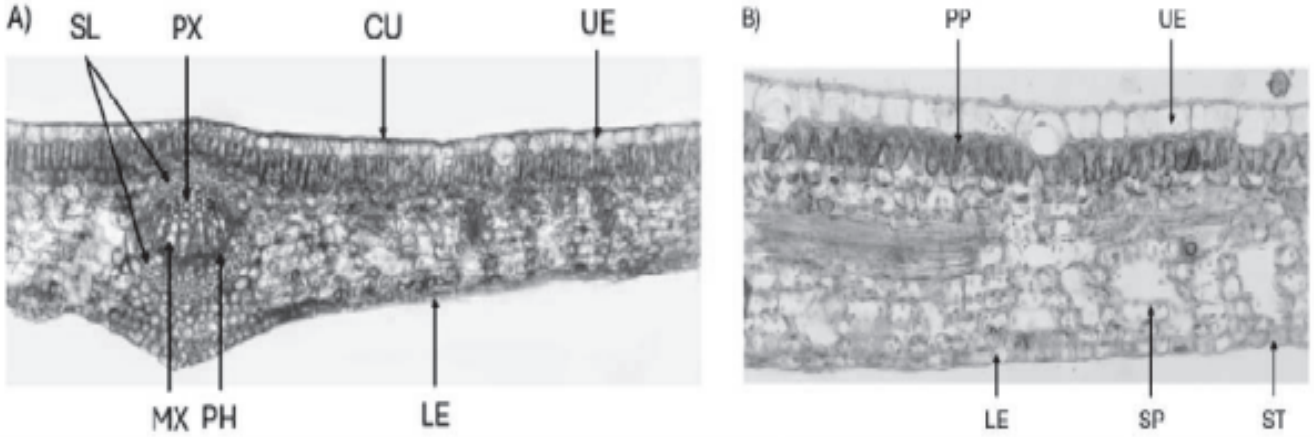

Fig. 2: A) Fresh leaflet cross-section, B) Preserved leaflet cross-section: A portion enlarged. (CU: Cuticle, UE: Upper Epidermis, LE: Lower Epidermis, SL: Sclrenchyma, PX: Protoxylem, MX: Metaxylem, PH: Phloem, PP: Palisade Parenchyma, SP: Spongy Parenchyma, ST: Stomata).

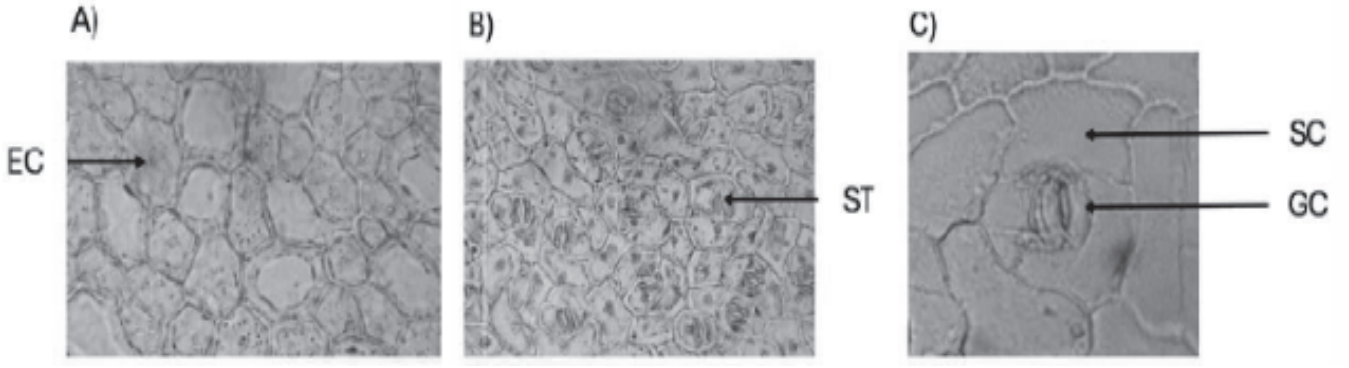

Fig. 3: A) Peel of upper epidermis after leaf clearing, B) Peel of lower epidermis, C) Single anisocytic stomata enlarged (EC: Epidermal cell, ST: Stomata, GC: Guard cell, SC: Subsidiary cell).

under a compound microscope.

Stomatal Analysis

The upper and lower epidermal layers were carefully peeled out, stained in safranine, mounted in glycerine, and observed under a compound microscope for stomatal measurements using ImageJ version 1.54h (https://imagej.net/ij/index.html) and analysis of stomatal index, stomatal type, and stomatal distribution. The total number of stomatal and epidermal cells from 4 microscopic (40x) fields were counted to determine the average stomatal index [9].

Maceration

For maceration, small flakes of tissues were cut out from the leaf midrib. The tissue samples were then boiled in a mixture containing equal volumes of 10% nitric acid and 10% chromic acid[1]. After boiling for a few minutes, the acid was drained off, and the tissue samples were washed in distilled water until the acid residue was removed. The tissue was then stained with safranine and mounted onto a slide. The macerated tissue was spread onto the glass slide by gently tapping the top of the coverslip. The tissue was then observed under a compound microscope.

Image Analysis

Images of the leaf cross-section, vein pattern and epidermis were captured using the Magnus Magcam DC-3 camera under a compound microscope and analysed using ImageJ version 1.54h (https://imagej.net/ij/index.html). The measure tool option in ImageJ was calibrated at 5x and 10x magnification using images of a stage micrometer captured on the same microscope. The calibrated measure tool was then used to obtain anatomical measurements of the epidermis, veins, and leaf sections.

Results and Discussion

Morphology

Turpinia malabarica are small to large trees with bright young leaves (Fig.1-A). The leaflets were opposite and imparipinnate, with seven leaflets per leaf on

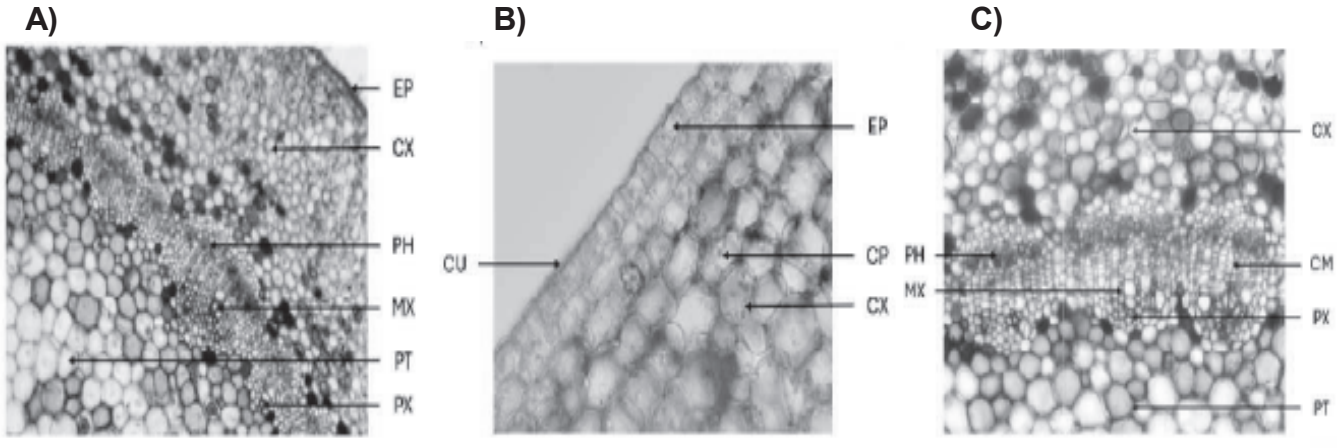

Fig. 4: A) Primary stem cross-section, B) Primary stem CS: Epidermal portion enlarged, C) Primary stem CS: Vascular bundles enlarged (CU: Cuticle, EP: Epidermis, CP: Chloroplast, CX: Cortex, PH: Phloem, CM: Cambium, MX: Metaxylem, PX: Protoxylem, PT: Pith)

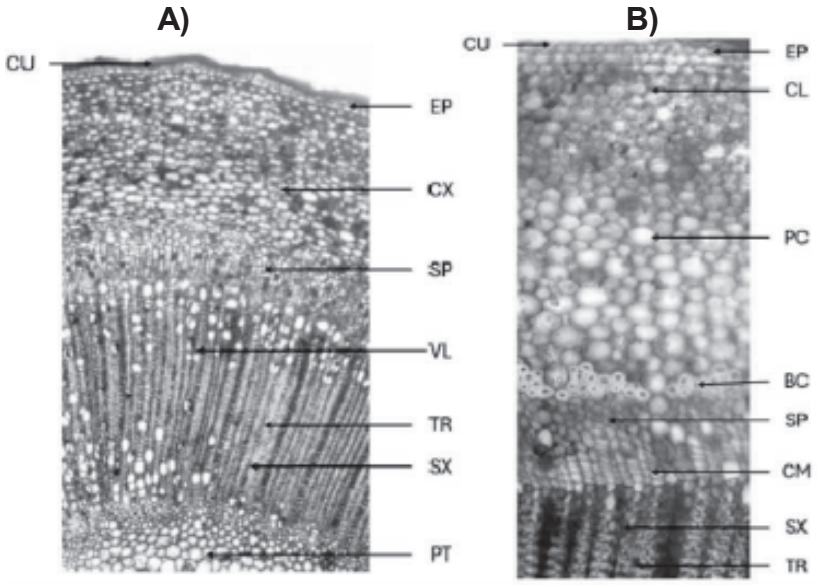

Fig. 5: A) Secondary Stem cross-section, B) Secondary Stem CS: A Portion Enlarged (CU: Cuticle, EP: Epidermis, CL: Chlorenchyma, PC: Parenchyma, CX: Cortex, BC: Bundle Cap SP: Secondary Phloem, CM: Cambium, SX: Secondary Xylem, VL: Vessels, TR: Tracheids, PT: Pith)

average (Fig.1-B&C). The leaflets were elliptic in shape, and the margin of the leaflet was serrated (Fig.1-E). Though usually unpaired, some leaves had paired even number of leaflets. The flowers were small and regular, arranged in a panicle. The fruit was a 3-celled globose drupe the size of a cherry with a 1–3-pointed tip (Fig.1-F).

Anatomy

Leaf Anatomy

The leaves lacked any conspicuous hairs. The single-layered upper epidermis was covered by a waxy cuticle, followed by two layers of palisade parenchyma (Fig.2-A). The palisade layer consisted of elongated chlorenchyma cells followed by 6-7 layers of spongy parenchyma with intercellular spaces and a lower epidermis with interspersed stomata. The stomata were of anisocytic type (Fig.3-C). The upper and lower portions of the vascular bundles were covered with sclerenchyma cells, with the protoxylem lying towards the upper epidermis and the metaxylem lying towards the lower epidermis (Fig.2-A). The upper epidermis lacked any stomata (Fig.3-A).

The average thickness of the leaf and upper epidermis was measured as 251.03 μm and 31.32 μm,

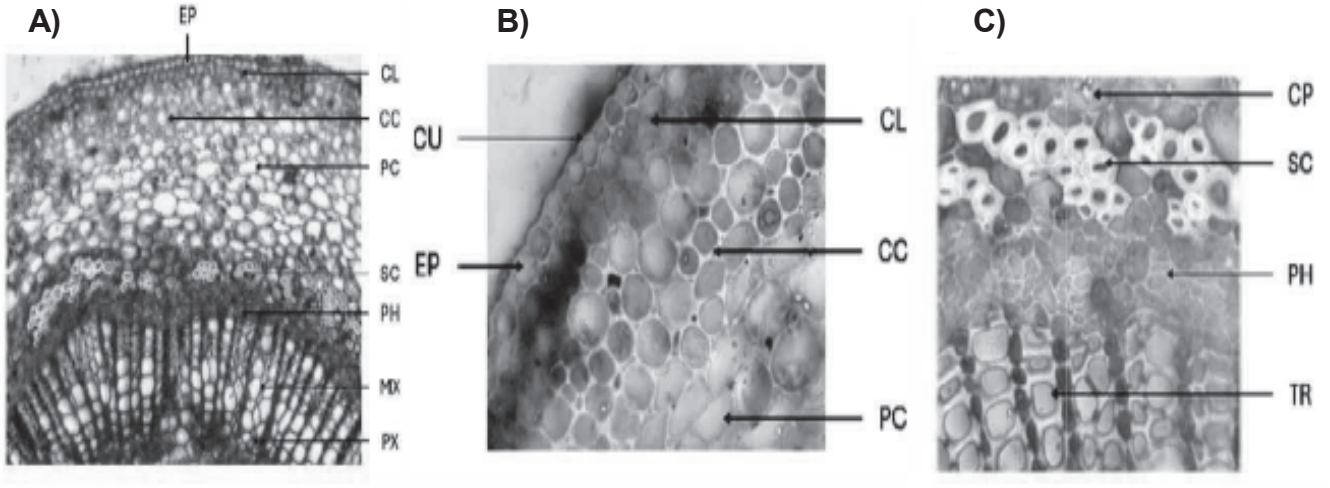

Fig. 6: A) Petiole Cross section: A portion Enlarged, B) Petiole CS: Epidermal Region Enlarged, C) Petiole CS: Vascular Region enlarged (CU: Cuticle, EP: Epidermis, CP: Chloroplast, CL: Chlorenchyma, CC: Collenchyma, PC: Parenchyma, SC: Sclerenchyma, CX: Cortex, PH: Phloem, MX: Metaxylem, PX: Protoxylem, TR: Tracheid).

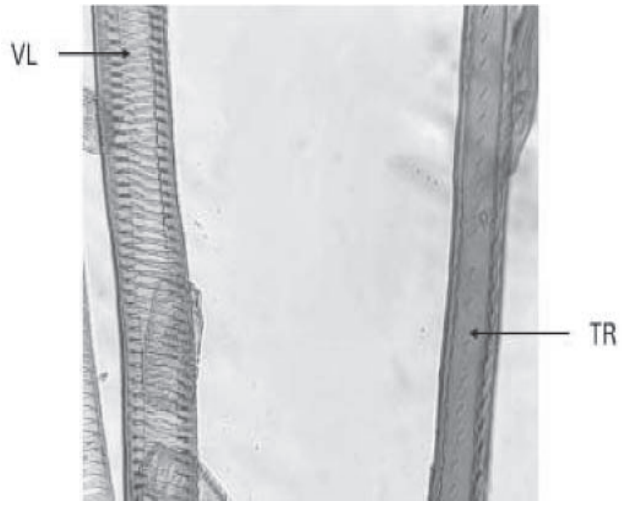

Fig. 7: Vessels and tracheids observed in macerated leaf tissue

respectively. The palisade layer had an average thickness of 66.74 μm, whereas the spongy layer had 139.89 μm thickness. The first layer of palisade cells had an average height of 45.09 μm. The lower epidermis was 17.67 μm in thickness.

Primary Stem Anatomy

The stem was circular in outline with the absence of any visible hairs. The single-layered upper epidermis was covered by a waxy cuticle followed by a parenchymatous cortex (Fig.4-B). Some cells of the cortex were chlorenchymatous in nature and were photosynthetically active. The multi-layered cortex was followed by a parenchymatous pericycle and a broken ring of numerous vascular bundles (Fig.4-A). The vascular bundles were conjoint, collateral and open (Fig.4-C). The xylem was endarch, with the protoxylem lying towards the pith and the metaxylem towards the epidermis. The xylem contained both tracheids and vessels. A multi-layered cambium could be seen between the xylem and phloem (Fig.4-C). A prominent bundle cap was absent.

Anatomy of stem secondary growth

The cross-section was circular in outline. A thick cuticle was followed by a uniseriate epidermis and multilayered chlorenchymatous and parenchymatous cortex. A multilayered cambial ring was present between the xylem and phloem. The bundles had a prominent sclerenchymatous bundle cap followed by primary and secondary phloem, respectively (Fig.5-B). The cambial ring was followed by the secondary xylem. The cambial ring produced more secondary xylem towards the pith and less secondary phloem towards the periphery. The secondary growth was thus found normal. The xylem was followed by a parenchymatous pith (Fig.5-A).

Petiole Anatomy

The Uniseriate epidermis was covered by a thick waxy cuticle. The multi-layered cortex was divided into three zones; the upper cortex or hypodermis was chlorenchymatous in nature, while the middle layer was made of collenchyma and the lower layer was composed of parenchyma cells (Fig.6-B). The cortex was followed by a heterogeneous pericycle made of sclerenchyma and parenchyma (Fig.6-C). The pericycle was followed by the phloem. The bundles were conjoint, collateral and closed. The xylem was endarch in nature. A parenchymatous pith followed the xylem. Some cells of the inner cortex were also chlorenchymatous in nature with the presence of chloroplasts (Fig.6-A).

Leaf Vein vascular elements and stomatal index

The leaf vein had tracheids with simple pits and vessels with spiral thickenings (Fig. 7). The average stomatal index of the leaf was calculated to be 16.75 (Fig. 8).

Conclusion

The findings from the current research on the morphological and anatomical characteristics of *Turpinia malabarica* plants serve as valuable tools for distinguishing genuine medicinal materials from any substitutes commonly employed in traditional medicine preparations and for identification of adulterants in powders or formulations made from the plant. The data could also be valuable for the preparation of diagnostic keys for medicinal research.

References

1. Anitha R, Kanimozhi S. Pharmacognostic Evaluation of *Ocimum basilicum* L. *Journal of Pharmaceutical Research International*. 2021; **33** (42A): 256-261.

2. Aumeeruddy MZ, Mahomoodally MF. Global documentation of traditionally used medicinal plants in cancer management: A systematic review. *South African Journal of Botany*. 2021;**138**: 424–494.

3. García Gutiérrez E, Ortega Escalona F, Angeles G. A novel, rapid technique for clearing leaf tissues. *Applications in plant sciences*. 2020; **8** (9): 11391. doi: 10.1002/aps3.11391.

4. Imtiyaz Z, Wang YF, Lin YT, Liu HK, Lee MH. Isolated Compounds from *Turpinia formosana* Nakai Induce Ossification. *International Journal of Molecular Sciences*. 2019; **20** (13): 3119. doi:10.3390/ijms20133119.

5. Ma SG, Yuan SP, Hou Q, Li Y, Chen X, Yu SS. Flavonoid glycosides from leaves of *Turpinia arguta* and their anti-inflammatory activity. *Zhongguo Zhong Yao Za Zhi*. 2013; **38** (11): 1747–1750.

6. POWO. "Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet. 2024; http://www.plantsoftheworldonline.org/ Retrieved 18 February 2024.

7. Ramírez Reyes TI, Aguilar Colorado ÁS, Murrieta León DL, Licona Velázquez LS, Bonilla Landa I, Durán Espinosa C, Avendaño Reyes S, Monribot Villanueva JL, Guerrero Analco JA. Identification of Antibacterial Phenolics in Selected Plant Species from Mexican Cloud Forest by Mass Spectrometry Dereplication. *Chemistry & Biodiversity*. 2019; **16**(4): 1800603. doi: 10.1002/cbdv.201800603.

8. Roleira FM, Varela CL, Costa SC, Tavares-da-Silva EJ. Phenolic derivatives from medicinal herbs and plant extracts: Anticancer effects and synthetic approaches to modulate biological activity. In: Atta-ur-Rahman (ed.) Studies in Natural Products Chemistry, Elsevier, 2018. pp. 115-156.

9. Salisbury EJ. On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. Philosophical Transactions of the Royal Society of London. Series B, Containing Papers of a Biological Character. 1928; **216** (439): 1-65.

10. Sreebala G, Eapen J, Deepa MS. Pharmacognostic studies of an ethnomedicinal plant: *Cissus latifolia* Lam. *Journal of Pharmacognosy and Phytochemistry*. 2021;**10** (6): 206–209.

11. Süntar I. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. *Phytochemistry Reviews*. 2020; **19** (5): 1199-1209.

12. Xiao CR, Tu LF, Zhang RZ, Liu DP, Luo YM. Research progress on chemical constituents and biological activities from *Turpinia* species. *Zhongguo Zhong Yao Za Zhi*. 2019; **44** (7): 1295–1304.

13. Yan J, Zhao LJ, Li YM, Zhang ZM, Lin LM, Xia BH. Preparation and Characterization of Polysaccharides from *Turpiniae Folium* and Its Antioxidative, Anti Inflammatory Activities and Antiproliferative Effect on VSMCs. *Chemistry & Biodiversity*. 2022; **19** (11) : 202200459. doi: 10.1002/cbdv.202200459.