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Pharmacognostic study and development of quality control parameters in *Glycosmis pentaphylla* (Retz)DC (Rutaceae)

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Abstract
Glycosmis pentaphylla (Retz.) DC. is a medicinal plant that belongs to the family Rutaceae. It is used by various ethnic communities to treat ailments such as asthma, cough, jaundice, fever, dysentery and as a cardiotonic. The plant is considered to be containing much medicinal value and mature fruit, is also eaten raw by local people. The plant has not been explored scientifically for its pharmacognostical details. The present research work focusses on the pharmacognostic characterization of *G. pentaphylla* which includes; macro and microscopic evaluation, physicochemical properties and micrometric evaluation of leaf, stem and root. Anatomical study was conducted by taking transverse section of plant parts. Transverse section of leaf showed the arrangement of the different cellular components i.e. vascular bundles, parenchyma, oil glands, lower epidermis and upper epidermis, respectively. Stem sections showed the presence of vascular bundles, parenchyma, schizogenous cavity etc. On the other hand, root sections exhibited secondary thickenings thereby showing periderm, xylem fibres, cortical cells etc. In fluorescence analysis different colors were observed under ordinary, short and long wavelength ultra violet light. Physicochemical analysis i.e. loss on drying, pH, ash values, foaming index, foreign matter and extractive values were performed. Leaf constants like stomatal characters, vein islet and vein termination number have also been determined. The results of the study showed that, on comparison with stem and root, the leaf possessed maximum values for most of the parameters under study. Better quality control parameters in pharmaceutical industries helps to prevent the adulteration, if any. Hence, this work will help in identification and quality control of *G. pentaphylla* as a medicinal material.

Keywords: *Glycosmis pentaphylla, Pharmacognosy, Physicochemistry, Anatomy*

Introduction

Medicinal plants have been used in health care since ancient times. They are used in treating, curing and preventing various ailments and are considered to play a beneficial role in health. Studies have been carried out globally to verify their safety, efficacy and stability and some of the findings have led to the production of plant-based medicines. The emphasis on the use of medicinal plants had hitherto been placed on the treatment rather than prevention of diseases [1]. On the basis of current knowledge of herbal medicines, it came to know that the extracts of these crude drugs are efficacious. *Glycosmis pentaphylla* (Retz.) DC. also known as a tooth brush tree is a member of the citrus family Rutaceae and is commonly known as orange berry and gin berry. The word *Glycosmis* is derived from Greek where "*Glykys*" means sweet and "*Osme"* means smell; alluding to the sweet scented flowers. The family Rutaceae contains about 100 genera and 800 species of herbs and *G. pentaphylla* is a shrub or a small trees [2]. It is distributed in Bangladesh, India, Malaysia, Southern China to Philippines, and Australia [3]. The plant is used for cough, rheumatism, anaemia and jaundice [4]. Stems and roots of plant are used for treatment of ulcer. Paste of leaves, with a bit of ginger, applied over the navel for worms and other bowel disorders. In parts of Asia the orange berry leaf is boiled down and used to reduce fever, liver complications and various intestinal parasites. The traditional healers in Gazipur district of Bangladesh utilize *G. pentaphylla* for prevention of all forms of cancer [5]. Stem and fruits of *G. pentaphylla* is used by medicinal practitioners in Bangladesh for the treatment of rheumatoid arthritis. Roots were used in India against facial inflammation, rheumatism, jaundice and anaemia [6]. Roots of this species are used for the treatment of inflammation, rheumatism, jaundice, and anaemia [7]. Some of the major classes of compounds reported from *G. pentaphylla* include terpenoids, amides, imides, alkaloids, coumarin, and flavonoids [8]. Previous studies emphasized mostly on identifying different phytochemicals and revealing their medicinal value profile, however, investigation to uncover its various pharmacognostical activities is very scanty. The plant is of great importance yet there isn't any extensive and thorough pharmacognostic data available on its structural anatomy and physicochemical standards, as it is required for the identification and quality standardization of the plant. Therefore, detailed morphological estimation, physicochemical evaluation and micrometric screening is required that will be helpful to avoid any ambiguity [9]. Morphological results are helpful in explanation of an exclusive drug with a major focus on quantitative and qualitative microscopy. Therefore, the current research consist of anatomical, structural, physicochemical and micrometric evaluations of the *G. pentaphylla* along with the assessment of leaf constants.

Materials and Methods

Plant material

The leaf, stem and root of the plant were collected from the hilly areas of Thodupuzha (9.8930°N, 76.7221°E), Kerala, India and was authenticated by Curator, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India. A specimen of plant was deposited at the herbarium of Department of Botany, under voucher No: KUBH 6043. Leaf, stem and roots were dried under shade, were powdered and preserved in amber colored containers at dry place.

Pharmacognostic evaluations

Macroscopic evaluation Macroscopic evaluations were carried out on 5 samples. The taxonomical description was made according to the data given in different books [10].

Microscopic evaluation

In microscopic evaluation of leaf, stem and root, studies were conducted on both grounds qualitatively and quantitatively. Qualitative microscopy involved determination of anatomical features [11, 12] and quantitative microscopy involved determination of foliar epidermal studies like stomatal constants [13], vein islets [14], vein termination [15] and gland dots [14]. The stomatal constants used for the study were listed (Table 1).

Table 1: Stomatal constants used for the study

Sl. No	Stomatal constants
1	**Stomatal frequency**
2	**Stomatal index (I)** = $\frac{S}{B+S} X\ 100$
3	**Stomatal area** = $\frac{\pi}{4} X \frac{Stomatal\ length}{Stomatal\ width}$
4	**Stomatal density** = $\frac{No\ of\ Stomata}{Leaf\ area}$
5	**Epidermal cell density** = $\frac{No\ of\ epidermal\ cell}{Leaf\ area}$
6	**Stomatal pore surface area** = $\frac{\pi}{4} X \frac{Pore\ length}{Pore\ width}$
7	**Stomatal intensity %** = $\frac{Stomatal\ density}{Stomatal\ density + Epidermal\ density} X\ 100$
8	**Stomatal shape co-efficient** = $\frac{Stomatal\ width}{Stomatal\ length} X\ 100$

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary and photomicrographs of different magnifications were taken using digital Microscope Eyepiece attachment and Photo Explorer 8.0 SE Basic software. Magnifications of the figures are indicated by the scale bars. Descriptive terms of the anatomical features are as given in the standard anatomy books [16].

Fluorescence analysis

Fluorescent evaluation of *G. pentaphylla* dried plant powder was performed as per reported standard procedures [17]. The colour observed in different radiations was recorded and noted according to the colour chart [18].

Physicochemical analysis

Powdered samples were subjected to physicochemical analysis for their extractive values, along with loss on drying, total ash, water soluble ash and acid insoluble ash.

Ash values

Ash values of the plant material was calculated according to standard procedures [19].

Total ash

The content of total ash (w/w) of air-dried material was calculated with the following formula.

$$\text{Percentage of total ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of acid insoluble ash content

The content of the acid insoluble ash (w/w) of air-dried material was calculated as follows;

$$\text{Percentage of water soluble ash} = \frac{\text{Weight of residue ash}}{\text{Weight of sample}} \times 100$$

Extractive values

Extractive values were calculated according to the procedure described by Arambewela and Arawwawala [20]. About 5.0 g of *G. pentaphylla* leaf, stem and root powder were subjected separately to macerate for 24 h in a closed flask using 100 ml of different solvent *viz*. n-hexane, ethyl acetate, ethanol, acetone and distilled water. The flask was frequently shaken during the first 6 h and then allowed to stand for 18 h. After 24 h, the content in the flask was filtered using Whatman no 42 filter paper. In a flat bottomed shallow dish, 25ml of filtrate was evaporated to dryness, dried at 105 °C and weighed. Percentage of soluble extractive was calculated with reference to the air dried powder.

Loss on drying

Accurately weighed 100 mg powder of leaves was placed in china dish. Then dried it in an oven at temperature of 100 °C for an hour. The powder was weighed again and compared with the original weight of the powder. The loss on drying was calculated by the following expression [21].

$$\text{Loss on drying percentage} = \frac{\text{loss in weight of the sample}}{\text{weight of the sample in gm}} \times 100$$

pH values
A pinch of leaf powder was taken in the test tube and 1 mL water was added. The pH was recorded by dipping pH meter in the aqueous extract.

Determination of Foreign Matter
The foreign matter was determined according to standard protocols [22]. Approximately 100 mg of dried leaf powder was taken and spread it in a thin layer. The foreign matter was detected by inspection with the unaided eye and with a magnifying lens. The foreign matter found was separated and weighed.

Foaming Index
The foaming index was determined according to standard protocols [23]. About 1.0 g of dried plant powder transferred to a 500 ml conical flask containing 100 ml of boiling water. Boiling maintained at moderate temperature for 30 min. The contents cooled and filtered into a 100 ml volumetric flask. The decoction poured into 10 stopper test tubes in successive portions of 1 ml, 2 ml, 3 ml upto 10 ml and adjusted the volume of the each liquid in each tube up to 10 ml. The tubes were shaken in a lengthwise motion for 15 second and allowed to stand for 20 min and the height of the foam measured and the foaming index calculated with the tubes shown to be height of 1.0 cm foam with following formula.

$$\text{Foaming index} = \frac{1000}{A}$$

Where A = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1.0 cm is observed, 1.0 ml of foam indicates to foaming index of 1000.

Swelling Index
About 1.0 g of dried plant powder was taken in a 25 ml glass - stopper measuring cylinder. About 25 ml of water was added and volume occupied by the sample recorded. The mixture was shaken thoroughly every 10 min for an hour. It is kept undisturbed for an overnight. The volume occupied by the material was calculated. Three determinations have carried out by the same process and the mean value of the individual determinations calculated relating to 1.0 g of crude sample.

Statistical analysis
All experiments were repeated at least five times. Results are reported as mean ± standard error of the mean.

Results
Macroscopic evaluation
The leaf and stem were green in colour, aromatic with slight bitter taste. The roots were greyish brown and retained the pungent odour and possessed bitter and pungent taste. Dried leaf powder was greenish brown, whereas the dried stem was willow green in colour and roots showed cream colour. The powdered leaf, stem and roots were also aromatic. Among them roots possessed the highest degree of aroma. All powders were bitter in taste. The results of organoleptic evaluation were summarized in Table 2.

Table 2: Organoleptic parameter of *G. pentaphylla* plant parts

Sl.No	Character	Leaf	Stem	Root
1	Colour	Upper: Dark Green Lower: Light Green	Dark Green	Externally: Brown Internally: Yellow
2	Odour	Aromatic	Aromatic	Aromatic
3	Taste	Bitter	Bitter	Bland
4	Texture	Upper: Smooth Lower: Creased with distinct veins	Smooth	Hard

The leaves were imparipinnately compound, 3-5 foliate (Fig. 1). The plant had sub-opposite leaflets, with the leaves being entire to sub-dentate to sub-crenate. The leaves were attenuate at base and acute to round at apex of the leaf. Venation was found to be reticulate. The length of the leaf was 15.63±0.00 cm and the laminar breadth was 6.44±0.00 cm. The leaves were glandular on both sides and glabrous. Stem was smooth with a length of 55.17±0.20cm and a width of 1.32±0.04cm. It had curved surface and possessed an intermodal length of 27.67±0.29cm. Externally, willow green and internally white and the stem was erect without thorns. (Fig 2). Fresh roots were stout, irregularly hard up to 10-35 cm x 5 cm. Sometimes a number of roots were found in the collar zone, though usually few occur in root nodules. These roots had a dull brownish skin with yellowish colour inside. Procured dried roots in cut pieces were up to 15 cm long and 0.5 to 2 cm in diameter, cylindrical, straight or somewhat curved, surface rough due to longitudinal striations, cracks and scars of lateral rootlets. The roots were externally brownish grey, internally yellowish brown, their fracture is short and smooth (Fig. 3).

Fig 1: Leaf of *G. pentaphylla*

Fig 2: Stem of *G. pentaphylla*

Fig 3: Root of *G. pentaphylla*

Microscopic Evaluation

Morphological descriptions outlined in floras are used as a guideline to propose diagnostic characters. The microscopic features were studied under quantitative and qualitative characters.

Quantitative characters

The results of micro and macromorphological quantitative characters of leaf, stem and root of *G. pentaphylla* were summarized in Table 3 and 4 respectively. The stomata (Fig 4), gland dots, vein islet, vein termination (Fig 5) were also calculated.

Table 3: Micromorphological characters of *G. pentaphylla*

Sl.No	Quantitative characters	Values
1	Number of gland dots	6.23±0.18
2	Stomatal frequency-lower	3.27±0.15
3	Stomatal frequency-upper	3.15±0.04
4	Stomatal Index-upper	25.17±0.15
5	Stomatal Index-lower	17.1±0.12
6	Specific Leaf Area	187.33±1.78
7	Stomatal Area	1.25±0.04
8	Stomatal Density-lower	0.05±0.01
9	Stomatal Density-upper	0.02±0.01
10	Epidermal Cell density-lower	1.4±0.07
11	Epidermal Cell density-upper	2.5±0.14
12	Stomatal Intensity %-lower	3.53±0.11
13	Stomatal Intensity %-upper	1.22±0.16
14	Stomatal Pore Surface Area	80.17±0.20
15	Stomatal Shape Co-efficient	78.33±0.22
16	Vein Termination	7.13±0.11
17	Vein Islet	6.20±0.19

Data are expressed as mean ± standard error

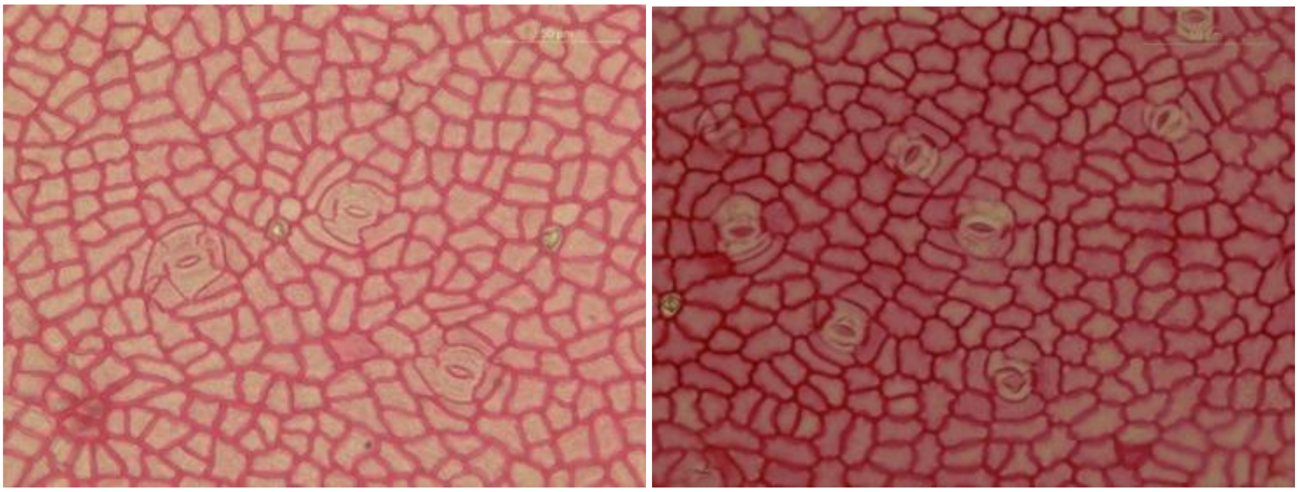

Fig 4 a, b: Distribution of Anomocytic Stomata; a-lower, b-upper Ec: Epidermal Cell; Gc: Guard Cell; Sc: Subsidiary Cell

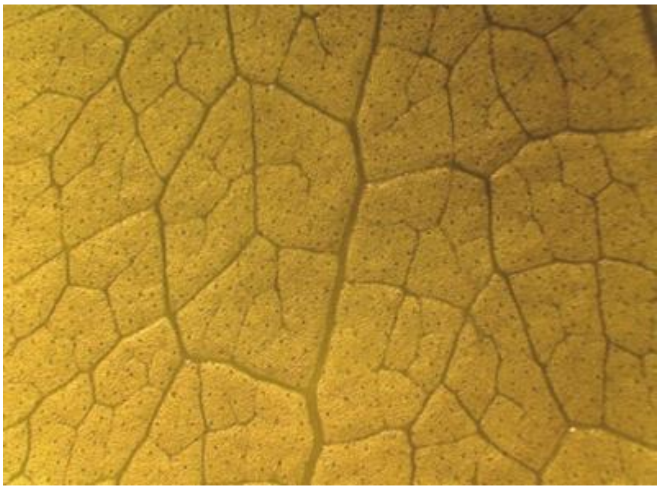

Fig 5: Cleared abaxial surface of leaf showing numerous gland dots, vein islets, vein terminations; GD- gland dots, VI- vein islets, VT- vein terminations

Table 4: Macromorphological characters of *G. pentaphylla*

Sl.No	Quantitative Characters	Values
1	Laminar length	14.43±0.08
2	Laminar breadth	6.17±0.11
3	Petiole length	0.20±0.01
4	Petiole breadth	0.32±0.01
5	Leaf area	59.52±0.16
6	Base angle	71.15±0.11
7	Apex angle	82.30±0.21
8	Length/Breadth ratio of Leaf	2.34±0.18
9	Length/Breadth ratio of Petiole	0.87±0.02
10	Apex Angle/Base Angle	1.03±0.04
11	Stem length	55.17±0.20
12	Stem Diameter	1.32±0.04
13	Stem Length/Stem Diameter	36.23±0.23
14	Internode Length	27.67±0.29
15	Root Length	34.17±0.20
16	Root Diameter	5.27±0.22
17	Root length/Root diameter	6.23±0.18
18	Number of roots per plant	4.23±0.29

Qualitative Microscopy

Transverse section (T.S) of Leaf

T.S. of the leaves of *G. pentaphylla* leaf showed its typical dorsoventral nature (Fig. 6). Upper and lower epidermis, lamina, mesophyll, and midrib region were observed as important diagnostic characters. Palisade tissue appeared in double layer just below upper epidermis in lamina region. Midrib showed central non-lignified phloem, lignified xylem with well-defined xylem fibers, vessels, and parenchyma. In the midrib portion, 2 cell followed single layer of epidermis deep compactly arranged palisade mesophyll. Below this less chlorophyllous spongy mesophyll was observed. Vascular bundles appeared along the line where the palisade and spongy mesophyll meet. Vascular bundles were amphicribral and surrounded by endodermis. Endarch type of xylem formation was observed in the vascular bundle. Trichomes were unicellular. Collenchyma cells were present below and upper layer of epidermis**.** Lamina showed a row of narrow and compactly arranged palisade cells embedded with oval to spherical oil cells followed by few rows of spongy parenchyma and small vascular bundles sheathed dorsiventrally.

Fig 6: T.S of leaf through midrib with lamina (4X) UE: Upper Epidermis; OG: Oil Globule; VB: Vascular Bundle; LE: Lower Epidermis

Transverse Section (T.S) of Stem T.S. of the stem of *G. pentaphylla* leaf showed its typical dorsoventral nature (Fig. 7a and 7b). Upper and lower epidermis, xylem vessels, oil globules were observed as important diagnostic characters. The central cortical region showed parenchyma cells. Non-lignified phloem, lignified xylem with well-defined xylem fibers, vessels, and parenchyma were also seen. Vascular bundles were amphicribral and surrounded by endodermis. Endarchtype of xylem formation was observed in the vascular bundle. Lower epidermal cells were embedded with oval to spherical oil cells and small vascular bundles sheathed dorsiventrally.

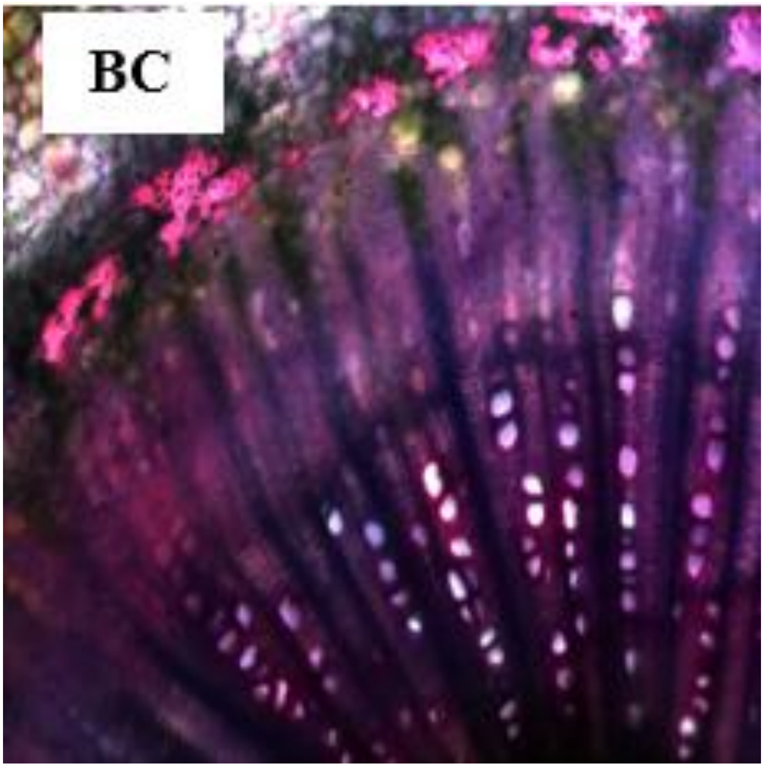

Fig 7a: T.S of Stem (10X) BC-Bundle Cap, XV-Xylem Vessel

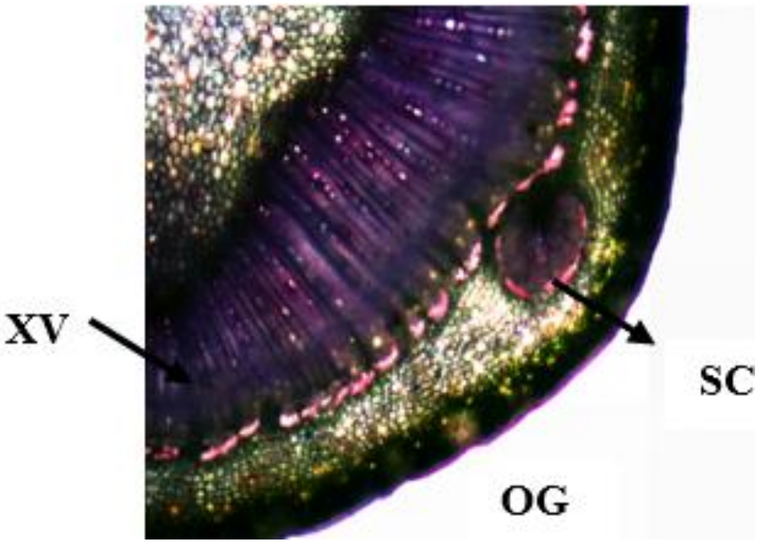

Fig 7b: T.S of Stem (10X) OG- Oil Globule; SC- Schizogenous Cavity

Transverse Section (T. S) of Root

The microscopical characteristic of the root exhibited well developed secondary growth. The epidermis of the root was broken at several places exposing the inner tissues. Inner to the epidermis, a thick cylinder of cortex was present. The vascular cylinder consisted of a wide, dense central cylinder of secondary xylem and outer fairly wide secondary phloem. The secondary phloem exhibited outer zone of collapsed phloem and inner narrow cylinder of non-collapsed phloem. The collapsed phloem comprised of crushed phloem elements which were seen in dark thick tangential bands. The non-collapsed phloem was very narrow and exhibited small, sieve elements which occurred in compact radical rows. Discontinuous fairly thick phloem fibres were seen both in the collapsed and non-collapsed phloem zones. Secondary xylem was thick and solid measuring 1.2 mm in diameter. The secondary xylem included vessels, xylem fibres, xylem rays and xylem parenchyma. The vessels were narrow, thick walled mostly solitary and diffuse in distribution. The vessels were 10 – 30 µm in diameter. The xylem fibers were highly thick walled and lignified (Figure 8b). The cell lumen had narrow xylem parenchyma in thin, concentric successive layers all along the thickness of the xylem. The parenchyma was apotrachial. Most of the parenchyma cylinders were one cell in thickness (Fig. 8b). The xylem rays were thin, slightly wavy and ray cells were also thick and lignified. The microscopical studies revealed the presence of parenchymatous cells and fibres in the root.

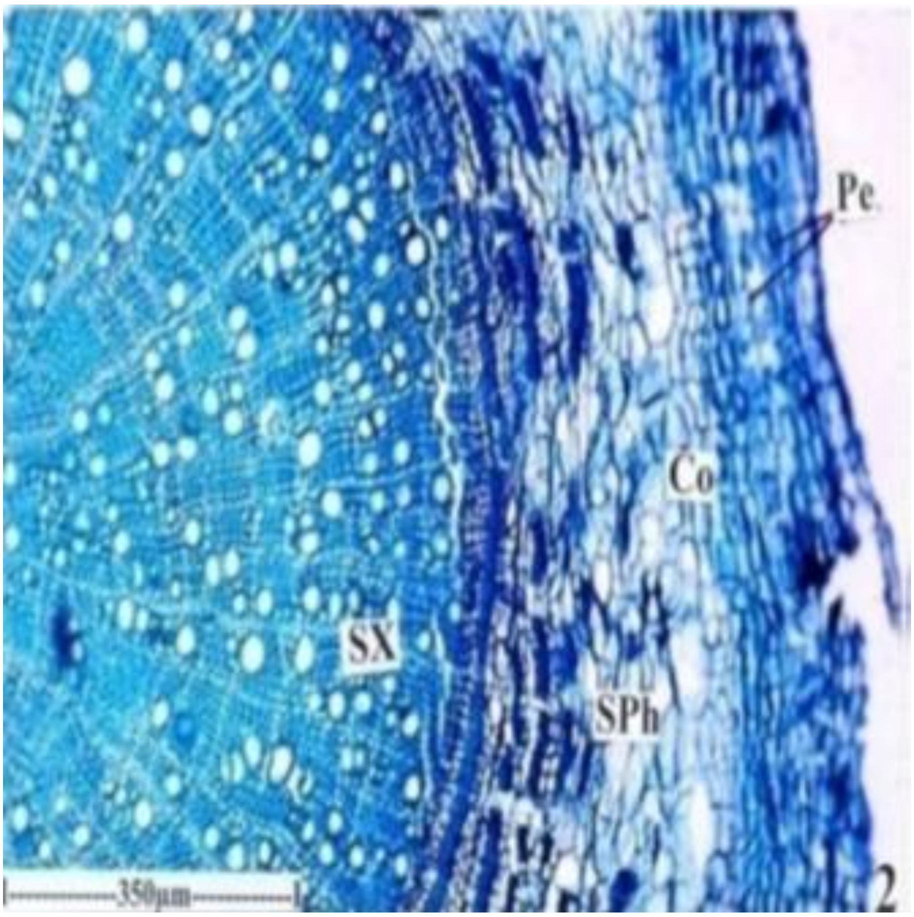

Fig 8a: T.S of Root of *G. pentaphylla* (4X) Co – Cortex SPh – Secondary Phloem SX – Secondary xylem Pe – Periderm

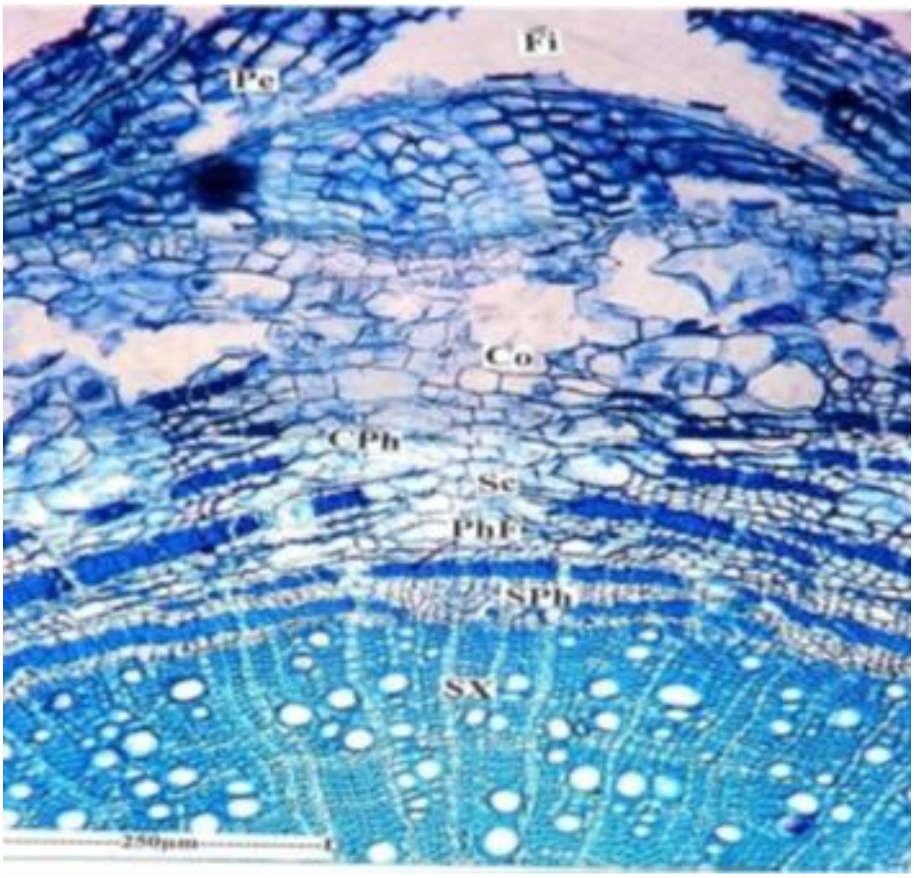

Fig 8b: T.S of root of *G. pentaphylla* (16 X) Fi-Fibre, Pe –Peridrem, Co-Cortex, CPh-collapsed phloem, Se – Sclereide, PhF-Phloem fibre, Sph – Secondary phloem, SX-Secondary xylem

Fluorescence analysis

The characteristic colour behavior of dried powdered drug dissolved in organic solvents like hexane, ethyl acetate, acetone, ethanol and distilled water was observed both under Visible and UV light. The colour reactions of these drug solutions thus emitted fluorescence light were summarized in Table 5. The powdered drug solutions exhibited a wide range of fluorescence colours under the UV and visible light.

Table 5: Fluorescence analysis of plant powder

Treatment	Observations					
	Leaf		Stem		Root	
	Short UV	Ordinary light	Short UV	Ordinary light	Short UV	Ordinary light
Powder (P) as such	Fern Green	Coriander Brown	Leek Green	Brown	Pale Yellow	Coriander Brown
P + Hexane	Pea Green	Mimosa Yellow	Cyprus Green	Mimosa Yellow	Agathia Green	Mimosa Yellow
P + Ethyl acetate	Cyprus Green	Primrose Yellow	Veronese Green	Uranium Green	Veronese Green	Dresden Yellow
P + Acetone	Veronese Green	Canary Yellow	Veronese Green	Chartreuse Green	Chartreuse Green	Canary Yellow
P + Ethanol	Agathia Green	Dresden Yellow	Veronese Green	Straw Yellow	Pea Green	Primrose Yellow
P + Distilled water	Fern Green	Primrose Yellow	Sap Green	Pea Green	Agathia Green	Sap Green

*Observations made according to Wilson color chart

Physicochemical Evaluation

Air-dried powdered material was used for quantitative determination of different physicochemical evaluation. The results pertaining to these investigations are summarized in Table 6. The leaf, stem and root of *G. pentaphylla* were freshly collected hence there was no adherent inorganic matter. Thus the percentage of foreign matter was found to be nil. The moisture content of the dried powdered material was determined by loss on drying method and is presented in Table. The leaf powder showed moisture content of 11.57 ± 0.004%. On the other hand, the moisture content of stem and root powder were found to be 13.37±0.20% and 14.17 ± 0.45% respectively. Moisture content of the drug was not too high, thus it could not encourage bacterial or fungal growth. The pH value of leaf, stem and root powder solution was measured, having pH of 6.9, 6.0 and 6.8 respectively. These pH values indicates that the drug is slightly acidic in nature. The foaming index of the powdered material was found to be less than 100 units. The result indicates less saponin content in the powdered drug. Swelling index of leaf, stem and root were calculated and it was found that the root powder possessed maximum swelling index. The recommended procedures were followed for calculating ash constants. The analytical result of total ash was found to be highest for leaf (12.37 ± 0.04 w/w %). The ash was amorphous and greyish white in colour. The water soluble ash was analyzed to be lesser than alcohol soluble ash and acid insoluble ash for leaf, stem and root.

Table 6: Physicochemical evaluation

Parameter	Leaf	Stem	Root
Foreign matter	0	0	0
Loss on Drying (LOD)	11.57±0.04%	13.37±0.20%	14.17±0.45
pH	6.9	6.0	6.8
Foaming Index	<100	<100	<100
Swelling Index	5.25±0.18ml	5.25±0.68ml	6.67±0.27ml
Ash constants (%w/w)			
Total Ash	12.37±0.04	10.45±0.17	10.75±1.16
Acid Insoluble	8.46±0.07	7.5±0.2	6.125±0.48
Water Soluble	7.82±0.02	8.07±0.41	7.15±0.13
Extractive value			
Ethanol	14.59±0.29	11.27±0.14	8.87±0.88
Chloroform	5.33±0.21	4.57±0.29	6.82±0.11
Ethyl acetate	9.32±0.11	6.15±0.11	4.92±0.08
Water	7.52±0.28	7.3±0.16	8.65±0.17

Data are expressed as mean ± standard error done in triplicate

Discussion

Owing to the multifarious medicinal properties of *Glycosmis pentaphylla* plant, many researches are encouraged among the scientists in exploring more information on this medicinal plant. Adulteration by the illegal addition of pharmaceutical substances or their analogs and misidentification of crude drugs can cause serious health problems and that an effective control by regulatory authorities is needed to safeguard the consumers. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. Pharmacological studies are more reliable, accurate and inexpensive means to evaluate the plant drugs [24]. So, in the present study important diagnostic characters determining the authenticity and purity of the medicinally important plant parts were observed and recorded. Organoleptic evaluations was done by means of organs of sense thereby define some specific characteristics of the plant material which can be considered as a first step towards establishment of identity and degree of purity. The odour and taste of crude drugs were extremely sensitive criteria based on individual’s perception. Therefore, the description of this feature may sometimes cause some differences of opinion. The organoleptic study of the plant showed some important characteristics of the drugs i.e. the aromatic odour and bitter tongue sensation of leaf and stem, whereas bland tongue sensation of root aiding in the screening of the preliminary phytochemical constituents present. In macroscopic study of leaf, stem and root
the observed morphological features of the leaf of *G. pentaphylla* were in agreement with reported literature. In transverse section of leaf, the occurrence of unicellular trichomes on upper layer of epidermal cells, endarch xylem, amphicribral vascular bundle, oil globule cells on upper epidermal layer of palisade and mid rib region are the important diagnostic features of *G. pentaphylla* leaf that could be used to distinguish among other genus. The stem morphological studies of *G. pentaphylla* revealed that the stem were willow green in colour having odour and bitter taste. In the transverse section of stem, there were occurrence epidermis at the upper layer, xylem vessels, amphicribal vascular bundle, oil globule on upper epidermal region and a central parenchymatic cortical region. The root morphological studies of *G. pentaphylla* revealed that roots

were brown coloured, having characteristic odour and bland taste. The root exhibits well developed secondary growth. The epidermis of the root is broken at several places exposing the inner tissues. Inner to the epidermis, a thick cylinder of cortex is present. The vascular cylinder consists of a wide, dense central cylinder of secondary xylem and outer fairly wide secondary phloem. The secondary phloem exhibits outer zone of collapsed phloem and inner narrow cylinder of non-collapsed phloem. The collapsed phloem comprises crushed phloem elements which are seen in dark thick tangential bands. The non- collapsed phloem is very narrow cylinder and exhibits small, sieve elements which occur in compact radical rows. Discontinuous fairly thick phloem fibres are seen both in the collapsed and non-collapsed phloem zones. Secondary xylem is thick and solid measuring 1.2 mm in diameter. The secondary xylem includes vessels, xylem fibres, xylem rays and xylem parenchyma. The vessels are narrow, thick walled mostly solitary and diffuse in distribution. Both narrow and wide vessels are inter mixed. The vessels are 10 – 30 µm in diameter. The xylem fibers are highly thick walled and lignified the cell lumen is narrow xylem parenchyma is thin, concentric successive layers all along the thickness of the xylem. The parenchyma is apotracheial. Most of the parenchyma cylinders are one cell in thickness xylem rays are thin, slightly wavy and ray cells are also thick and lignified. Other important histological aspect is the quantitative microscopy of plant. The various parameters studied here are palisade ratio, stomatal number, stomatal index, vein islet, and vein termination number. The values of these parameters are useful for detecting adulterants [25]. Palisade cells bear a definite relationship to the epidermal cells, a diagnostic feature in the identification of leaves. Stomatal Index is relatively constant and is not much affected by factors *viz*. age of the plant, size of the leaf, environmental conditions etc. It is more significant in the evaluation of leaf drug. The plants of Rutaceae family have a wide range of stomatal index i.e. between 11% and 24%. Like the palisade and stomatal

index, vein islet is also a useful diagnostic feature of leaf. Every plant possesses characteristic tissue features which can be identified by microscopy of leaf powder analysis. When properly mounted in stains and reagents, characteristic tissue features will be observed, which could be used in the identification as well as in the detection of adulterants. The treatment of powdered drug with chemical reagents reveals the presence of different chemical constituents present in the crude drug. The microscopic study also showed the presence of anomocytic stomata which is commonly found in Rutaceae family. Fluorescence property of a material is attributed to its chemical composition. It may tend to change with different wavelengths of light. The same material emit different fluorescence in day light, short ultra violet and long ultra violet. Application of different solvents causes decomposition of the chemicals and the decomposition products or their derivatives emit fluorescence. This fluorescence study is used as a fingerprint for crude drug identification [26]. The fluorescence analysis in the powdered drug treated with different reagents can be used as a future reference to detect adulteration of the material. The colour change for the plant powder was distinctive and reproducible revealing the solvent properties to the phytoconstituents.

Moisture content of the drug was not too high, thus it could not encourage bacterial or fungal growth. The moisture content of *G. pentaphylla* leaf powder (11.57 %), was lower than that of stem and root. At the same time the plant powder possessed moisture content within the recommended range of 8-14% for vegetable drug. This is an indication that the plant can be stored for a long period of time with less probability of microbial attack. Ash values are useful indicators of the purity of any drug and give information relative to adulteration/contamination with inorganic matter. Ash values were considerably high for the leaf. Total ash content which is the total amount of material remaining after ignition is not sufficient to reflect the quality of plant parts, since the plant materials often contain calcium oxalate crystals in particular. Acid insoluble ash gives more consistent values than the total ash. Water soluble ash represents the water soluble portion of the total ash. Ethanol extractive value of the plant parts showed the highest value, especially the leaf ethanol extractive which was found to be 14.59% compared to other extractives of the present study. This may be due to the presence of high amount of alcohol soluble compounds in the leaves of *G. pentaphylla*. The ethanol permeates the cells of the plant powder and thus, a better extractant for *G. pentaphylla*. The process of standardization can be achieved by stepwise pharmacognostic studies as stated above. Therefore, the result generated from this study would be useful in identification and standardization of the plant material towards quality assurance and also for preparation of a monograph on *Glycosmis pentaphylla*.

Conclusion

The current investigation describes the pharmacognostic standardization of *G. pentaphylla*. The macro and microscopic findings laid down the standards that will be helpful in identification and authentication of this plant material, to prevent adulteration or admixture. The pharmacognostic studies serve as an ideal tool in determining the quality of raw drug and the morphological and anatomical characters together could help in distinguishing the original plant from their adulterants. It was found that leaf possessed better pharmacognostic properties on comparison with stem and root. Ash values added more strength to crude drug standardization with prominent results indicating involvement of extraneous matter. Such study on the macro and microscopic anatomy, physicochemical and micrometric parameters are important information which may be useful in confirmation and quality control of this medicinal plant.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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