The Pharma Innovation Journal 2016; 5(9): 23-30

www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2016; 5(9): 23-30

www.thepharmajournal.com
Received: 07-07-2016
Accepted: 08-08-2016

Pressy P. Prakasia
Department of Botany,
University of Kerala,
Kariavattom campus,
Thiruvananthapuram, Kerala,
India

Ashalatha S. Nair
Department of Botany,
University of Kerala,
Kariavattom campus,
Thiruvananthapuram, Kerala,
India

Correspondence
Pressy P. Prakasia
Department of Botany,
University of Kerala,
Kariavattom campus,
Thiruvananthapuram, 695 581,
Kerala, India.

Pharmacognostic and physicochemical standardization of leaves of *Glycosmis pentaphylla* (Retz.) DC

Pressy P Prakasia and Ashalatha S Nair

Abstract
The present study was undertaken with an objective to investigate the pharmacognosy of the crude drug powder of the leaf of *Glycosmis pentaphylla* an ethno-medicinally important plant belonging to orange family (Rutaceae). Pharmacognostic evaluations were conducted in terms of macroscopic, microscopic, physicochemical and micromeritics analysis according to the WHO and other recommended procedures. The characteristic macroscopic and microscopic features of leaf include presence of oil globules on upper epidermal region, endarch xylem, amphicribral vascular bundles and anomocytic stomata. The Physicochemical analysis reveals values for moisture content, pH, ash values and extractive values which are within the World Health Organisation (WHO) standards for crude drug from medicinal plants. Micromeritic analysis of leaf powder reveals good flow properties of powder which is an important criterion in the manufacture of tablets and capsules. The results of the macroscopic and microscopic evaluations of *Glycosmis pentaphylla* leaf furnish diagnostic features for judging the authenticity, quality, purity and also to differentiate the drug from its closely related species and also to detect adulterants. The present study on pharmacognostic standardisation, physicochemical evaluation of *Glycosmis pentaphylla* leaf might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present state of affairs.

Keywords: *Glycosmis pentaphylla,* macroscopic, microscopic evaluation, physicochemical evaluation

Introduction
The use of natural products with therapeutic properties is as ancient as human civilisation and, for a long time, mineral, plant and animal products were the main sources of drugs [1]. Historically, natural products discovered from medicinal plants and their derivatives have provided numerous clinically useful medicines. The value of natural products in new drug delivery will continue to be significant in the years to come, mainly because of their inherent unmatched chemical structural diversity, "drug-like" properties [2]. With the increasing attractiveness of plant medicines and tremendous range of herbal products now available to the public, regulatory requirements covering therapeutic plants are introduced by World Health Organisation (WHO) and other national health agencies in order to control the quality of these products. According to the WHO, to ensure reproducible quality of medicinal plants (or preparations), physicochemical and phytochemical characterizations are required to be carried out for establishing their identity, purity, and quality standards [3]. Therefore, there is a need for documentation of standardization studies for profiling the quality control parameters of plant-derived crude/herbal drugs.

Glycosmis pentaphylla is commonly called as orange berry belonging to the Rutaceae family, is a shrub or small (1.5–5 m) tree (Figure 1a and 1b), widely distributed, spanning from India, Malaysia and Southern China to the Philippine Islands where it occurs in tropical forests at low altitudes. It is traditionally known to be useful for the treatment of wide panel of diseases like cough, rheumatism, anaemia and jaundice. Methanolic extract of leaves of *Glycosmis pentaphylla* has been reported to possess antinociceptive activity [4].

Glycosmis pentaphylla yields an acridone alkaloid called arborinine which showed significant inhibition of crown gall tumours in a simple potato disc bioassay [5]. Leaf and stem bark extracts of *G. pentaphylla* showed a positive result on hepatoprotective activity on CCl_4 induced hepatic injury in albino rats [6].More than 40 biologically active constituents from various parts of this plant were identified using different solvent systems and reported in literatures. Alkaloids reported from leaves are glycosine, arborinine, glycosminine, glucosamine, glycerine, glycosmicine and γ-fagarine [7, 8].

From roots carbazoles alkaloids, glycozolicine, 3-formylcarbazaole, glycosinine, glycozoline, glycozolidine, skimmianine, and dictamine have isolated.

Fig 1a: Habit of *Glycosmis pentaphylla*

Fig 1b: Leaves of *Glycosmis pentaphylla*

As aforesaid, *G. pentaphylla* is enriched with ample active constituents and has proved varied pharmacological activities. Hence an investigation to explore its pharmacognostic examination is inevitable. Till date, there is no report on the pharmacognostical evaluation of *Glycosmis pentaphylla* leaf from Kerala. So the aim of the present study is to explore the morphological and microscopical parameters of *Glycosmis pentaphylla* leaves for its proper authentication so that it cannot be easily adulterated.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

Fresh leaves of *Glycosmis pentaphylla* were collected during spring season from district of Thiruvananthapuram (Latitude- 8.54ºN, Longitude- 76.91ºE and Altitude 18.00 m), Kerala, India. The botanical identities were identified by Curator, department of Botany, University of Kerala. The leaves were washed under running tap water, rinsed with distilled water, dried under shade at room temperature for two weeks. The dried leaves were powdered using electric blender, sift through 40# mesh sieve and stored in airtight bottles to free from moisture and humidity until further experimental usage.

2.2 Macro-Morphological Evaluation of Leaf

2.2.1 Organoleptic (Sensory) Parameters

Different organoleptic (sensory) parameters of leaf and leaf powder such as colour, odour, taste and texture of dried leaf powder were evaluated by the sense organs and recorded.

2.2.2 Morphological Parameters

Morphological and related taxonomical observations were made by using compound light microscope and the characters were described in technical terms.

2.3 Microscopic Evaluation of Leaf

2.3.1 Qualitative Microscopy

Transverse section of leaf: The microscopic characters transverse sections of fresh leaf through midrib with small portion of lamina were prepared, stained in Safranin O solution (1.0%) and mounted in glycerin. The sections were observed under Image Analyzer (Olympus-BX51TF, Japan).

2.3.2 Quantitative Microscopy

Quantitative microscopy parameters such as leaf constant studies viz. determination of palisade ratio, stomatal number, stomatal index, vein islet, and vein termination number was carried out using the standard procedures [9].

2.4 Evaluation of Leaf Powder

2.4.1 Powder Microscopy

Coarse powder was used to study the microscopical characters of leaf powder [10].

2.4.2 Behaviour of Leaf Powder with Chemical Reagents

Behaviour of leaf powder with different chemical reagents was performed to detect the occurrence of phytoconstituents along with colour changes under ordinary daylight by standard method [11].

2.4.3 Fluorescence Analysis of Leaf Powder

Fluorescent evaluation of *Glycosmis pentaphylla* dried leaf powder was performed as per reported standard procedures [12].

2.4.4 Physico-Chemical Evaluation of Leaf Powder

Physiochemical parameters such as foreign matter, moisture content, pH, ash constants and soluble extractive values were performed according to the official method prescribed and the WHO guidelines on quality control methods for medical plants material [13, 14].

2.4.5 Micromeritic Evaluation of Dried Leaf Powder

The micromeritic characteristics of leaf powder viz. Bulk density, Tap density, Angle of repose, Hausner's ratio and Carr's index was determined according to the official standard procedures [15-16].

Statistical analysis

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

3. Results

3.1 Macromorphological Evaluation of *Glycosmis pentaphylla* Leaf

3.1.1 Organoleptic Evaluation of Leaf and Leaf Powder

Glycosmis pentaphylla leaf showed dark greenish appearance on upper side and light greenish in colour on lower side. Fresh leaf possesses slight bitter taste with characteristic odour.

Dried leaf powder is greenish brown in appearance (Figure 5). The powder has aromatic odour with slight bitter taste. The results of organoleptic characteristics of fresh leaf and dried leaf powder are compiled in Table 1.

3.1.2 Macroscopic Features of Leaf

The leaves are imparipinnately compound, 3-5 foliate (Figure 2). Leaflets are sub-opposite. Leaves are entire to sub-dentate to sub-crenate (Figure 3). Attenuate at base and acute to round at apex of the leaf. Venation is reticulate. The size of the leaf is having an average length of 17.27 ± 0.44 cm and width of 5.8 ± 0.14 cm. Upto 12 pairs of lateral nerves are present on the leaf. Rachis 6-10 cm long and petiole is 2.0 mm long. The leaves are glandular on both sides and glabrous (Figure 4).

Fig 2: Imparipinnate compound leaf.

Fig 3: A leaf showing entire, sub dentate margins

Table 1: Organoleptic evaluation of leaf and leaf powder of *Glycosmis pentaphylla*

Parameter	Observation	
	Leaf	Leaf powder
Colour	Abaxial – dark green	Greenish brown
	Adaxial – light green	
Odour	Characteristic	Characteristic
Taste	Slightly bitter	Slightly bitter
Texture	Abaxial - smooth	Coarse
	Adaxial -creased with distinct veins	
Fracture	Fibrous	NA

3.1.3 Microscopic Evaluation of Leaf

Photographs detailing the microscopic features of the leaves are shown in Figure 6a-6f. Morphological descriptions outlined in floras are used as a guideline to propose diagnostic differentiating macro-microscopic characters of *Glycosmis pentaphylla* leaf.

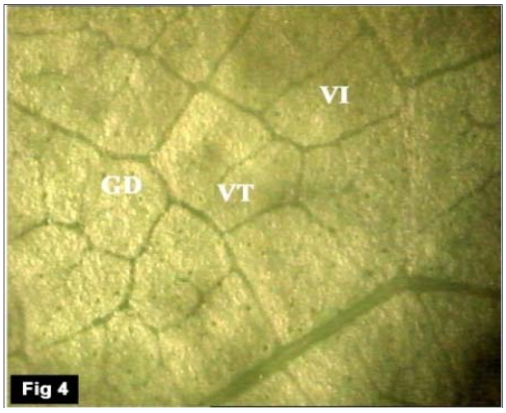

Fig 4: Cleared abaxial surface of leaf showing numerous gland dots, vein islets, vein terminations; GD- gland dots, VI- vein islets, VT- vein terminations.

Fig 5: Greenish brown dry leaf powder of *Glycosmis pentaphylla*

a. Transverse section (T.S) of Leaf

T.S. of *Glycosmis pentaphylla* leaf showed its typical dorsoventral nature. 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 shows central non-lignified phloem, lignified xylem with well-defined xylem fibers, vessels, and parenchyma. In the midrib portion, single layer of epidermis is followed by 2 cell deep compactly arranged palisade mesophyll. Below this comes the spongy mesophyll, which is less chlorophyllous. Along the line where the palisade and spongy mesophyll meet, are placed vascular bundles. Vascular bundles are amphicribral and surrounded by endodermis. Endarch type of xylem formation is seen in the vascular bundle. Trichomes are unicellular. Collenchymas cells are present below and upper layer of epidermis.

b. Lamina

Lamina shows 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.

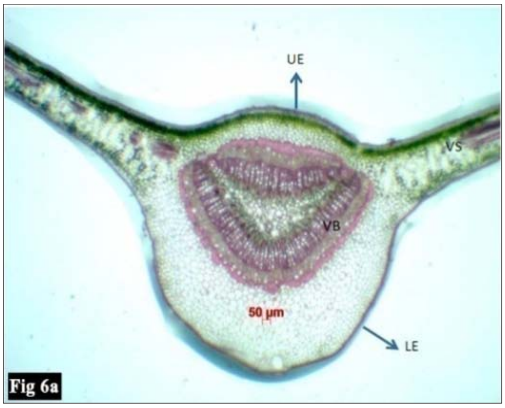

Fig 6a: T.S of leaf through midrib with lamina (4X).

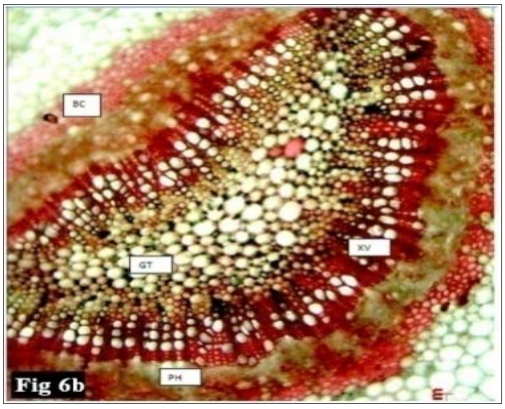

Fig 6b: T.S of midrib- a portion enlarged (10X)

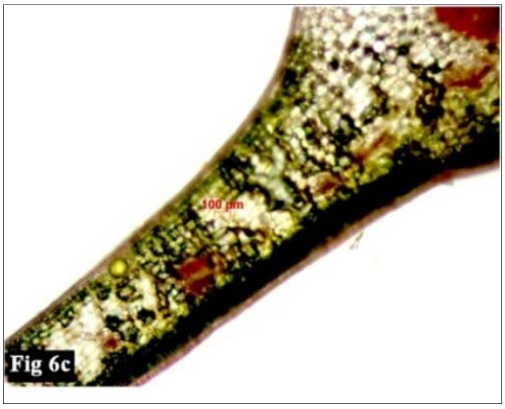

Fig 6c: T.S of lamina – a portion enlarged (10X).

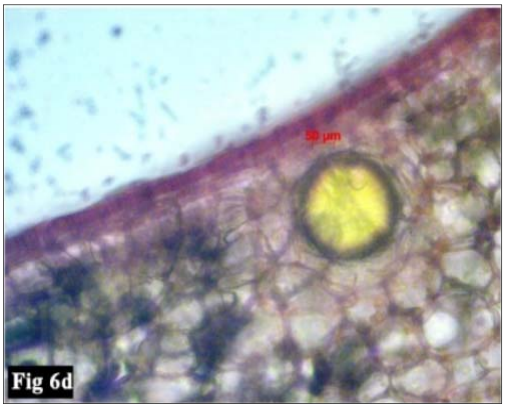

Fig 6d: Upper epidermal region of T.S of leaf showing oil globule (40X)

Fig 6e: Upper epidermal region of T.S of leaf showing secretory cavity (40X).

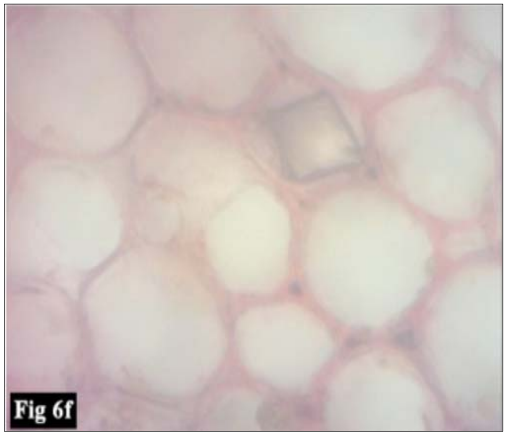

Fig 6f: Section of T.S of leaf showing the presence of calcium oxalate crystal in collechyma cell.

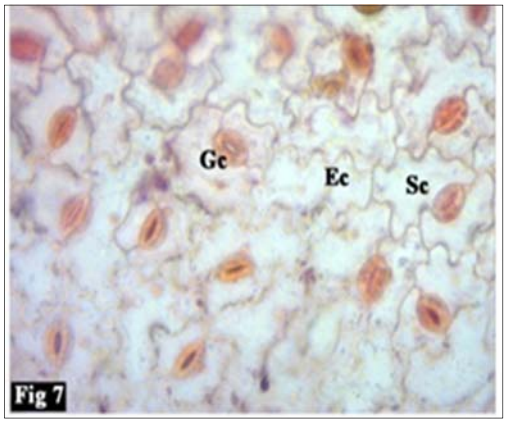

Fig 7: Paradermal section of the abaxial side of leaf showing abundant anomocytic stomata. Ec- epidermal cell, Gc- guard cell, Sc- subsidary cell

3.1.4 Quantitative Microscopy of Leaf

Glycosmis pentaphylla leaf surface shows anomocytic type of stomata which is characteristics of family Rutaceae (Figure 7). Leaf surface also shows the presence of veins, vein islets and vein terminations (Figure 4). Leaf constants such as palisade ratio, stomatal number, stomatal index, veinlet terminations and vein-islet number were measured. The results are shown in Table 2.

Table 2: Quantitative microscopy of leaf of *Glycosmis pentaphylla*

Parameter	Value
Palisade ratio	3.30 ± 0.03
Stomatal frequency (upper surface)	3.15 ± 0.02
Stomatal frequency (lower surface)	5.25 ± 0.04
Stomatal index(upper surface)	13.15 ± 0.02
Stomatal index (lower surface)	20.59 ± 0.33
Vein islet	5.68 ± 0.4
Vein termination	7.55 ± 1.2

Stomatal number and stomatal index of 10 determinations (each of 1 sq mm); palisade ratio –average of 25 groups, each of 4 epidermal cells; vein islet and vein termination number – average of 10 sets of 2 mm x 2mm area, having 4 squares each of 1 sq mm. Data are expressed as mean ± standard error done in triplicate.

3.2 Evaluation of Leaf Powder

3.2.1 Powder Microscopic Characters

The powder microscopy of *G. pentaphylla* leaf powder (Figure 8) revealed the presence of parenchymatous layer of cells, anomocytic stomata, spirally thickened xylem vessels, prismatic crystals of calcium oxalate, non-glandular trichomes, and small starch grains.

3.2.2 Behaviour of Leaf powder with chemical reagents

The observations (Figure 9) resulted from the leaf powder reaction with chemical reagents are recorded in Table 3.

3.2.3 Fluorescence Analysis of Leaf Powder

The characteristic colour behaviour of dried powdered drug dissolved in organic solvents was observed both under visible and UV light. The reactions of the drugs thus emitted fluorescence light are summarized in Table 4. The powdered drug solutions had exhibited a wide range of fluorescence colours under the UV and visible light.

Fig 8: Powder microscopic characters of *Glycosmis pentaphylla* leaf
a. Conducting strand Xylem vessel with spiral thickening, b. Epidermis with cell walls, c. A single anomocytic stoma, d. Vessel with pits, e. Compound starch grain, f. Trichome, g. Stone cell, h. Fibre, i. Calcium oxalate crystal

Table 3: Chemical analysis of *Glycosmis pentaphylla* leaf powder

Treatment	Observation*	Inference of constituent
Powder (P) as such	Greenish brown	-
P+ Distilled water	Tea green	Saponin absent
P+ Conc. H_2SO_4	Brick red	Carbohydrate
P+ MgHCl	Black colour	Flavonoid present
P+ Mayer's Reagent	Yellow ppt	Alkaloid present
P+ Aqueous sodium nitrite	Red colour	Phytosterol present
P+ Spot test	No change	Oil absent
P+ Ammonia	Indian orange	Cardiac glycoside absent
P+ Picric acid	Maize yellow	Alkaloid present
P+ Dragendorff's reagent	Indian orange	Alkaloid present
P+ Conc HNO_3	Yellow	Presence of tannin
P+ 1 N NaOH	Primrose yellow	Flavonoids present
P+ 40% NaOH + Lead Acetate	Maize yellow	Tannin absent
P+ Conc.HNO_3+ Ammonia	Apricot	Xanthoprotein absent
P+ Iodine	Black colour	Starch present
P+ Ninhydrin reagent	Reddish orange	Amino acid present

*observation according to the Wilson Colour Chart [17]

Table 4: Fluorescence analysis of powdered leaf of *Glycosmis* table

Treatment	Observation*	
	Ordinary light	Short UV (254 nm)
Powder (P) as such	Coriander brown	Fern green
P+ Distilled water	Coriander brown	Fern green
P+ Acetone	Coriander brown	Spinach green
P+ Ethanol	Fluorescent green	Willow green
P+ Benzene	Light brown	Uranium green
P+ Chloroform	Pea green	Pod green
P+ Diethyl ether	Agathia green	Scheeles green
P+ Methanol	Agathia green	Lettuce green
P+ Petroleum ether	Pea green	Scheeles green
P+ Glacial acetic acid	Scheeles green	Salomon yellow
P+ 1.0 M Sulphuric acid	Parsley green	Nasturtrium orange
P+ Nitric acid	Parsley green	Light nalpes yellow
P+ 1.0 M Hydrochloric acid	Parsley green	Maize yellow
P+ 5.0% $FeCl_3$	Straw green	Chrysanthmemum crimson
P+ 1N NaOH + Methanol	Parsley green	Fern green
P+ 10% Iodine	Saffron yellow	Lettuce green
P+ ethyl acetate	Cyprus green	Willow green
P+ 5.0% KOH	Pea green	Scheeles green
P+ Ammonia solution	Lemon yellow	Lettuce green
P+ Toluene / Potassium dichromate	Parsley green	Willow green

*observation according to the Wilson Colour Chart

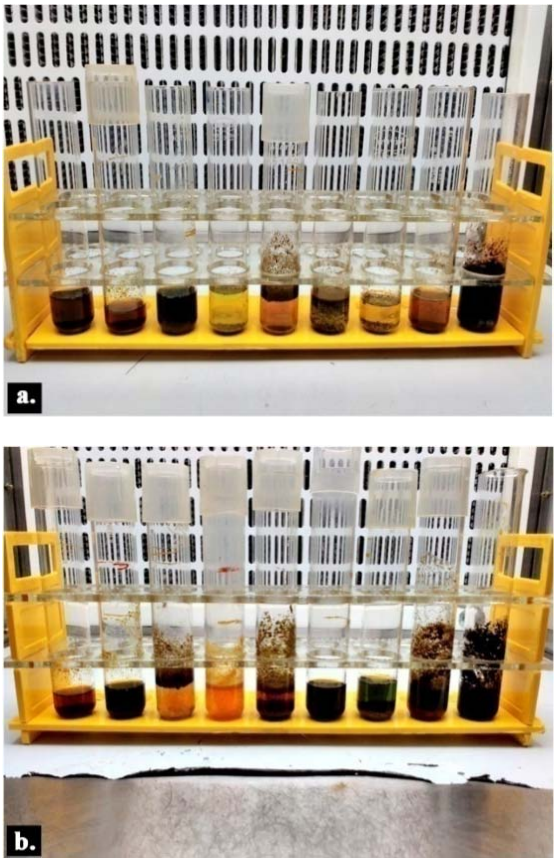

Fig 9: Behaviour of dry leaf powder with chemical reagents

3.2.3 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 5. The leaf of *Glycosmis 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 5. The leaf powder showed moisture content of 11.57 ± 0.004%. Moisture content of the drug was not too high, thus it could not encourage bacterial or fungal growth. The pH value of 1% and 10% leaf powder solution was measured, having pH of 6.6 and 6.4 respectively. The pH of 6.6 and 6.4 indicates that the drug is slightly acidic in nature. The foaming index of the powdered leaves was found to be less than 100 units. The result indicates less saponin content in the powdered drug. Swelling index is 5.25 ± 0.18 ml. The recommended procedures were followed for calculating ash constants. The analytical result of total ash was found to be 12.37 ± 0.04 w/w %. The ash was amorphous and greyish white in colour. The water soluble ash (7.82 ± 0.02%) was analyzed to be lesser than alcohol soluble ash (9.13 ± 0.03 w/w %) and acid insoluble ash (8.46 ± 0.07 w/w %). On the same hand, water soluble extractive value was found to be lesser than chloroform and ethyl acetate extractive value. Among the different extractive values analyzed, alcohol soluble extractive value was the highest (14.59 ± 0.29%) followed by ethyl acetate extractive value (9.32 ± 0.11%).

Table 5: Physicochemical constants of leaf powder of *Glycosmis pentaphylla*

Parameter	Value
Foreign matter	0
Loss on drying	11.57 ±0.004%
pH	
1.0%	6.6 ± 0.0
10%	6.4 ± 0.0
Foaming index	<100 units
Swelling index	5.25 ± 0.18 ml
Ash constants (% w/w)	
Total ash value	12.37 ± 0.04
Acid insoluble ash	8.46 ± 0.07
Water soluble ash	7.82±0.02
Alcohol soluble ash	9.13±0.03
Sulphated ash	3.84±0.05
Extractive value	
Ethanol	14.59 ± 0.29
Chloroform	5.33 ± 0.21
Ethyl acetate	9.32 ± 0.11
Water	7.52 ± 0.28

Data are expressed as mean ± standard error done in triplicate.

3.2.4 Micromeritic Evaluation of Leaf Powder

The Micromeritic properties also showed good flow properties for the leaf powder (Table 6). The leaf powder of *Glycosmis pentaphylla* has Hausner's ratio of 2.10 ± 0.02 and Carr's compressibility index of 9.38 ± 1.02%. The powder showed a good flow rate of 2.56 g s^{-1} with 23.3° angle of repose.

Table 6: Micrometric parameters of leaf powder of *Glycosmis pentaphylla*

Parameter	Value
Bulk density	0.22 ± 0.003 ml/g
Tap density	0.28 ± 0.007 ml/g
Hausner's ratio	2.05 ± 0.02
Carr's compressibility index	9.38 ± 1.02%
Flow rate	2.56 g s^{-1}
Angle of repose	23.3°

Data are expressed as mean ± standard error done in triplicate.

4. Discussion

Ethnomedically the roots, stem and leaves of *Glycosmis pentaphylla* are used by local people in the treatment of various conditions. 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 [18]. So in the present study important diagnostic characters determining the authenticity and purity of the medicinally important leaf part are 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 leaf and leaf powder showed some important characteristics of the drugs i.e. the aromatic odour and bitter tongue sensation aiding in screening the preliminary phytochemical constituents present. In macroscopic study of leaf, the observed morphological features of the leaf of *Glycosmis 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 *Glycosmis pentaphylla* leaf that could be used to distinguish among other genus.

Other important histological aspect is the quantitative microscopy of leaves. 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 [19]. 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. In fluorescence analysis the powdered drug treated with different reagents were observed under visible and short UV light. The colour change for the leaf powder was distinctive and reproducible revealing the solvent properties to the phytoconstituents.

The moisture content of *Glycosmis pentaphylla* leaf powder (11.57%), which is within the recommended range of 8-14% for vegetable drug 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 its adulteration/contamination with inorganic matter. Total ash content which is the total amount of material remaining after ignition is not sufficient to reflect the quality of leaves, since the plant materials often contain calcium oxalate crystals in particular. Acid insoluble ash gives more consistent values than the total ash [20]. Water soluble ash represents the water soluble portion of the total ash. Ethanol extractive value showed the highest value, 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 *Glycosmis pentaphylla.* The ethanol permeates the cells of the leaf powder and thus, a better extractant for *Glycosmis pentaphylla* leaf. The Micromeritic properties like bulk density, tap density, angle of repose, Hausner's ratio and Carr's index indicates the flow properties as well as interparticulate resistance between powders. This information predicts the stability and solubility of crude drug. Increase in bulk density reduces paste thickness which is important in preparation of drugs. The ratio of tapped density to the bulk density of the powder is called Hausner's ratio. This ratio is a useful measure of cohesion reflecting particle friction. With a Hausner's ratio higher than 1.4, the powder is considered a cohesive difficult to fluidize powder. Ratios lower than 1.25 characterizes as free-flowing powder. Carr's compressibility index is good if the value ranges between 5% - 15%. If the angle of repose is more than 50 °, the powder will not flow satisfactorily and if it is near 25 °, the powder will flow easily. The micrometric properties help to characterize and standardize the pre-formulation properties of the herbal drug powder, in order to determine its suitability for formulation into solid dosage forms.

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* plant.

5. Conclusion

Pharmacological standardisation of *Glycosmis pentaphylla* leaves provide information about its identity, quality and purity to be used as crude drugs. The results collectively might be useful to supplement information for future studies on *Glycosmis pentaphylla* leaves.

6. References

1. Pasquale De. Pharmacognosy: The oldest modern science. J. Ethnopharmacol. 1984; 11:1-16.
2. Sarker SD, Nahar L. Natural products Isolation, Methods and Isolation. In: An introduction to natural products isolation. Humana Press, New York, 2012, 4.
3. Annan K, Dickson RA, Amponsah IK, Jato J, Nooni IK. Pharmacognostic evaluation and physicochemical analysis of *Paullinia pinnata* L. (Sapindaceae). J Pharmacogn Phytochem. 2013; 2(2):203-208.
4. Shams Ud Doha KM, MehnajAkter, Zobaer Al Mahumud, Apurba Sarker Apu, Md Amran Howlader. Antinociceptive activity of the methanol extracts of leaves of *Eugenia fruticosa* (Roxb.) and *Glycosmis pentaphylla* (Retz.) in Swiss albino mice. Journal of Applied Pharmaceutical Science. 2012; 02(04):99-102.
5. Quader MA, Nutan MTH, Rashid MA. Antitumor alkaloid from *Glycosmis pentaphylla*. Fitoterapia. 1999; 70:305-07.
6. Borah RC, Mohan P, Choudhary BH, Barua IC. Hepatprotective activity of *Phyllanthus fratermus* L and *Glycosmis pentaphylla* (Retz) Correa used against jaundice in NE India in Borah. Bioprospecting of commercially important plants: Proceedings of the national symposium on biochemical approaches for utilization and exploitation of commercially important plants, Jorhat, India. 2004, 12-14.

7. Chakraborty DP. Glycozoline, a carbazole derivative from *Glycosmis pentaphylla.* Phytochem. 1969; 08: 769-72.
8. Govindachari TR, Pai BR, Subramaniam PS. Alkaloids of *Glycosmis pentaphylla* (Retz.) Correa. Tetrahedron.1966; 22:3245-52.
9. Khandelwal KR. Practical Pharmacognosy, Techniques and Experiments. In. Leaf Constants. Nirali Prakashan, Pune, 2008, 146.
10. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. Ninth edition. Nirali Prakashan Publishers, Pune, 2003.
11. Singh VK, Govil Gurdip Singh. Recent progress in medicinal plants. Vol. I. SCI Tech publishing, LIC Texas, 2005, 325.
12. Gupta MK, Sharma PK, Ansari SH, Lagarkha R. Pharmacognostical evaluation of *Grewia asiatica* fruits. Int J Plant Sci. 2006; 1(2):249-51.
13. Indian Pharmacopoeia. Government of India Ministry of Health & Family Welfare. Published by The Indian Pharmacopoeia Commission. Ghaziabad, 2010, 3.
14. Kokate CK. Practical Pharmacognosy. Fourth Edition. Vallabh Prakashan, New Delhi, 2003, 122-26.
15. Abdullah EC, Geldart D. The use of bulk density measurements as flowability indicators. Powder Technol1999; 102:151-65.
16. United States Pharmacopoeia. Bulk density and tapped density. Asian ed. 2003, 2125.
17. Wilson FR. Horticultural Colour Chart. Henry Stone and Son Ltd, Banbury, 1938, 1-200.
18. Mukesh S. Sikarwar, Boey Jia Hui, Kumutha Subramaniam, Bavani Devi Valeisamy, Ling KarYean, Kaveti Balaji. Pharmacognostical, Phytochemical and Total Phenolic Content of *Artocarpus altilis* (Parkinson) Fosberg Leaves. Journal of Applied Pharmaceutical Science. 2015; 5(05):094-100.
19. Kumar S, Kumar V, Prakash O. Microscopic evaluation and physiochemical analysis of *Dillenia indica* leaf. Asian Pac J Trop Biomed. 2011; 1:337-340.
20. Joshi Saroja, Vidhu Aeri. Practical Pharmacognosy. Frank Brothers (Pub) Pvt Ltd, New Delhi, 291-292.