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Research Article

PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL STUDIES OF *GNETUM ULA* BRONGN.

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ABSTRACT

Medicinal plants are of great value in the field of treatment and cure of disease. Herbal drug industry would be largely dependent upon the reliable methodologies for the standardization and quality control of traditional systems of medicine which continues to be widely practised in India. *Gnetum ula* (Fam: Gnetaceae) is widely used to cure various ailments like Rheumatism, Bronchitis, piles, inflammation, jaundice, arthritis. The study profile includes organoleptic characters, Microscopic studies, physiochemical characters such as ash value, extractive values, crude fibre content, fluorescence analysis, preliminary phytochemical testing followed by total phenolic and flavonoids assay. Stem of *G.ula* showed the presence of rich fibre content and length of fibres were maximum of 2544µ.Total ash determined was 3.80% and acid insoluble value being 0.98%. .Phytochemical screening showed the presence of Alkaloids, Flavonoids, Phytosterols, Phenols and Tannins and carbohydrates. Total alkaloid and Flavonoid content was found to be more for ethanol extract. However, available literature revealed that no pharmacognostic study has been reported on the stem of *G.ula*.So, data obtained from this study can be used for identification and determination of quality and purity of the plant material in the future.

Keywords: *Gnetum ula*, Gnetaceae, Sclereids. Flavonoids, Fibres

INTRODUCTION

India has been known to be rich repository of medicinal and aromatic plants, which are collected as raw materials for manufacture of drugs and perfumery commodities[1]. World's one fourth population is dependent on traditional medicines to treat various ailments[2] Most of the developed countries deny using these medicines because of lack of documentation and stringent quality control[3].

Gnetaceae, a family of tropical gymnosperms in the order Gnetales composed of one genus, *Gnetum,* with 30 or more species and one of the species selected for the present studies is *Gnetum ula (G.ula).*

G.ula is a large woody climber. It has been found on trees in the hill forests of southwest and south-eastern India (Western Ghats, Nilgiris, hills at Coromandel Coast). It has also been reported from the Andaman and Nicobar Islands[4]. *G. ula* is considered as sacred plant by kodavas of Karnataka, India. *G. ula* is an important plant used in indigenous system of medicine, as antiperiodic[5]. Stem is used to treat jaundice[6-8]. Seed oil and Roasted fruit is used in the treatment of rheumatism[5, 9].

In view of its diverse medicinal application, the main hindrance in its use is the non-availability of an official monograph and incomplete validation of the plant material. With this backdrop, it is of prime importance to make an effort towards standardization of the plant material. The present communication deals with pharmacognostic evaluation of *G.ula* which is achieved by step wise pharmacognostic studies this in turn helps in identifying and authenticating *G. ula*.

MATERIALS AND METHODS

Sample collection, processing and storage

The plant material was collected from Biligirirangana Hills (B.R. Hills) situated in the Western Ghats of Karnataka, and authenticated by Dr. Shiddamallayya.N, at National Ayurveda Dietetics Research Institute, Department of AYUSH, Govt. of India, Bangalore. And the voucher Specimen (No: RRCBI-MUS-0107) was deposited for future references. The stem was shade dried and powdered using mechanical grinder. The powder sample was stored in an air tight container and a portion of it is used for the pharmacognostic studies and phytochemical analysis.

Macro morphology

The stem of *G.ula* was subjected to macroscopic studies which comprised of organoleptic characteristics Viz shape, colour, odour, Fracture. These parameters are considered as quite useful in qualitative control of crude drug and were evaluated as per standard WHO guidelines [10].

Microscopy

Microscopic sections of the stem were cut by free hand sectioning; using razor blade and section were stained with safranine and were mounted on a slide with glycerol and examined microscopically[11].

Powder microscopy

Microscopic examination of the powder drug[12, 13] and determination of fibre length was carried out as per the reported methods[14].

Florescence Analysis

Dried stem powder of the *G.ula* were treated with various chemical reagents and Different solvent extract of *G.ula* were exposed to visible, ultraviolet light to study their fluoresce behaviour[15,16].

Physicochemical Parameters

Physicochemical values such as ash values, extractive values, and crude fibre analysis (Dutch method) were determined according to the well-established protocols [17-19].

Phytochemical Screening

The portion of the powder was taken in a test tube and solvents (petroleum ether, chloroform and ethanol) was added to it such that plant powder soaked in it and shaken well. The solution then filtered with the help of muslin cloth and filtered extract were taken and used for further phytochemical analysis[20].

Estimation of Total Phenol and Flavonoid content

Total phenols of different solvent extract were determined by Folin Ciocalteu reagent Phenolic content was expressed in terms of Gallic acid equivalent[21]. Flavonoids were also estimated by aluminium chloride colorimetric method for all the extracts taking Quercetine as standard [22].

RESULTS

Macro morphology character

G.ula is a lofty dioecious climber (Figure 1) with dichotomous branches, bark thick scaly, young shoots jointed and swollen at the insertion of the leaves. Stem is brown in colour, cylindrical in shape, with a characteristic odour and slightly bitter in taste, fibrous in nature (Figure 2).

Microscopy studies

Transverse section of stem (Figure 3) shows epidermous, composed of thick walled Quadrangular cells, covered by thick cuticle. In cortex group of lignified pericyclic fibres followed by a layer of horse shoe shaped stone cells or sclerides were observed (Figure 5). The inner portion of stem of *G.ula* has 4 -7 layers of medullary ray cells, secondary phloem parenchyma and fibres were found to be present. Secondary xylem characteristics such as xylem vessel, xylem parenchyma and xylem fibres were found (Figure 4). Pith is large with rounded cells, containing abundant starch granules.

Powder study

The microscopic examination of the powder shows Fibres, xylem vessels, calcium oxalate crystals, horse shoe shaped stone cells, starch granules (Figure 6). The length of the fibres in stem was found to be minimum of 1124µ, maximum of 2544µ and average of 1834 µ.

Fluorescent studies

Powder of *G.ula,* when treated with different chemical reagent showed different colour reactions, in accordance to the nature of the constituents present in it. Many plants constituents show fluorescence in visible light and some of the metabolites shows fluorescence only when they are exposed to ultraviolet light. Results are summarised in Table 1 and 2.

Physiochemical characteristics

The Percentage of total ash, acid insoluble ash, water soluble ash, loss on drying content, crude fibre content were shown in table 3. This parameter helps to determine the quality and purity of the drug. Extractive value (Table 4) determines the active constituents present in the drug.

Phytochemical Analysis

The crude drug extracted in different solvents was tested for various phytoconstituents for adulterated drugs and the results were shown in Table 5. Result obtained for total phenolic and flavonoids are in Table 6.

Table 1: Fluorescence characteristics of stem powder of *G.ula*

Treatment	Visible	Short UV (254 nm)	Long UV (365nm)
Drug	Brown	No fluorescence	Dark brown
Drug + water	Brownish	No fluorescence	Green Fluorescence
Drug +1NHCl	Magenta Brown	No fluorescence	Neon Green Fluorescence
Drug +1$NHNO_3$	Magenta Brown	No fluorescence	Neon Green
Drug +1NH_2SO_4	Magenta Brown	No fluorescence	Dark Green
Drug +1NNaOH	Dark brown	No fluorescence	Green
Drug +1N Alc NaOH	Dark brown	No fluorescence	Neon green
Drug +1N KOH	Dark brown	No fluorescence	Dark green
Drug +1N Alc KOH	Dark brown	No fluorescence	Dark green
Drug + Ammonia	Dark brown	No fluorescence	Dark green

Table 2: Fluorescence characteristics of stem powder extracts of *G.ula*

Extract	Visible light	Short UV (254nm)	Long UV(365nm)
Petroleum ether	Pale brown	Dark green	Light magenta
Chloroform	Brownish green	Dark green	Light pink
Methanol	Mud brown	Dark green	Dark magenta

Table 3: Physiochemical parameters

Parameters	Values
Total ash (%w/w)	3.8019%
Acid Insoluble ash(%w/w)	O.981%
Water soluble ash(%w/w)	1.432%
Crude Fibre content	64%
Loss on Drying (%w/w)	1.57%

Table 4: Extractive values of *G.ula*

Extracts	Yield (% w/w)
Petroleum ether	33.45%
Chloroform	21.13%
Ethanol	10.72%

Table 5: Phytochemical Analysis

Sl no	Tests	Petroleum ether extract	Chloroform extract	Methanol extract
1	Test for Alkaloids	+	+	+
2	Test for Flavonoids	-	+	+
3	Test for Phenols	-	+	+
4	Test for Phytosterols	+	+	+
5	Test for Tannins	-	+	+
6	Test for Saponins	-	-	+
7	Test for Resins	+	-	-
8	Test for carbohydrates	+	+	+
9	Test for Glycosides	-	-	-
10	Test for Proteins and Amino acids	-	-	-

+ indicates Present, - -Indicates Not determined

Table 6: Results of Total phenols and Flavonoids

Extract	Total Phenolics (mg of GAE)/ gm	Total flavonoids (mg of Quercetine/gm)
Peteroleum ether	10.06±0.46	17.0±0.32
Chloroform	28.72±0.95	24.77±0.32
Ethanol	63.16±0.69	45.33±0.64

PHOTOMICROGRAPHS

Figure 1

Figure 2

Macro morphology character

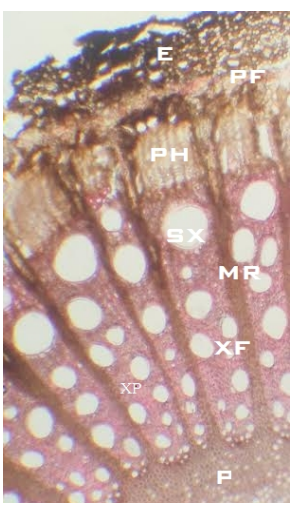


Figure 3: Transverse section of stem 10x
E = Epidermis PF =Pericyclic fibres PH =Phloem SX =secondary xylem MR =medullary ray XP =Xylem parenchyme XF =Xylem fibres P= Pith.

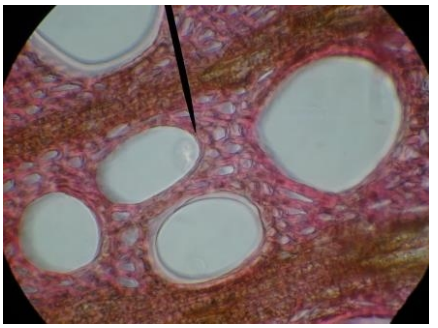


Figure 4: Secondary Xylem

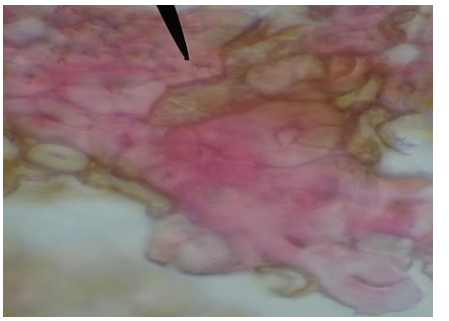
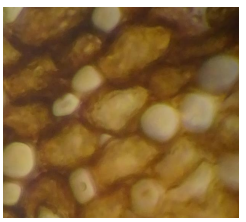
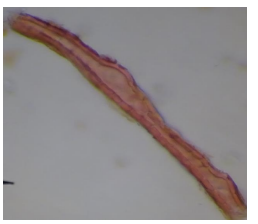


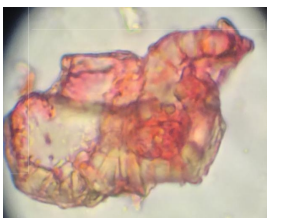
Figure 5: Sclerides at 40x



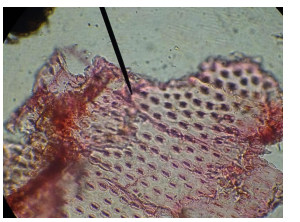
Cork Cells



Fibre



Group of Stone Cells



Xylem

Figure 6: Powder microscopy at 40x

DISCUSSION

Medicinal plants are having utmost interest because they are safe to humankind. The advantage of natural drugs is easily available, economic and less or no side effects. But on the other hand they are the victims of adulteration .Therapeutics efficacy of medicinal plants depends upon the Quality and Quantity of chemical constituents. The misuse of herbal medicine or natural product starts with wrong identification. The present information provides the basis for identification and authentication of the plant *G.ula*. Organoleptic characters of stem of *G.ula* are brown, cylindrical in shape with fibrous fracture, help in identification of the plant material. Microscopic studies of the stem section shows a thick cork layer and shoe shape stone cells or sclerides and powder analysis showed a large number of fibres and its length was calculated using standard protocol. Maximum and minimum length of the fibres gives us the distinguishing character of the plant to identify. Powder material of *G.ula* was examined under day light and UV light to find out the presence of fluorescence compound within them. *G.ula* exhibited clear fluorescence behaviour at different radiations which can be taken as Standard fluorescence pattern for this species. Ash values are used to indicate the quality and purity of the crude drug.it tells about the impurity like carbonate, oxalate and silicate. Total ash of *G.ula* is 3.4%, low implying that the crude drug has less organic matter. Moisture content of the Crude drug is very less, indicates that plant can be stored for a long period of time discouraging the growth of microbes. Crude fibre content determined by standard procedure indicates the stem parts of the plant is rich in fibres and are lengthy too. Preliminary Phytochemical analysis of crude extract showed the presence of carbohydrates, flavonoids, alkaloids, phenols and tannins. Total phenolic and flavonoid assay reveals about the plant potent therapeutic property which must be explored.

CONCLUSION

In the present study process of standardization is achieved by stepwise pharmacognostic studies, which helps us to measure the quality, purity and sample identification of the plant material. The pharmacognostic constants, diagnostic microscopic features and the numerical standard reported in this work could be useful for the preparation of monograph of this plant. Detailed phytochemical studies and pharmacological evaluation of this plant is on-going and will be achieved in near future.

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