PHARMACOGNOSTIC STUDIES AND PRELIMINARY PHYTOCHEMICAL SCREENING ON THE ROOT OF *GLYCOSMIS PENTAPHYLLA* (Retz.,) DC., (RUTACEAE)

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**Abstract**

*Glycosmis pentaphylla* (Retz.,)DC (Rutaceae) is an important traditional medicinal plant being used for treatment of cough, rheumatism, anemia, antioxidant and jaundice. Systematic pharmacognostical evaluation of the root of the plant has been carried out with respect to macroscopy, microscopy, physicochemical parameters and estimation of different standards. TLC profiles were developed for petroleum ether, chloroform, ethyl acetate and ethanol extracts. The preliminary phytochemical investigations indicated presences of alkaloids, terpenoids, flavanoids, tannins, sugar, glycoside and phenolic compounds. The results obtained are useful for standardization of the root established the macro, micro, powder microscopy and physiochemical parameters to characterize the genuine plant drug. This parameter can be utilized for quick identification of the drug and are particularly useful in the case of powdered forms and also contribute towards establishing pharmacopoeial standards for the specified plant. *Glycosmis pentaphylla* possesses medicinally important secondary metabolites; these can be employed in the treatment of various diseases.

**Keywords**

*Glycosmis Pentaphylla*, Root, Macroscopy, Microscopy, Physicochemical, Phytochemical.

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INTRODUCTION

Glycosmis pentaphylla (Retz.,)DC., (Family - Rutaceae) an odorous shrub (or) a small tree found all over India and sometimes grown in gardens for its dark green glossy leaves and white or pinkish berries. In India it is locally known as Ashvashakaota, Ban-nimbu, kirmira, Glougu, Kula, Pannai, Gurodagida and Panal in various region. The plant is used in indigenous medicine for cough, rheumatism, anemia and jaundice. The juice of the leaves which is bitter is used in fever, liver complication and in vermifuge.[1] The paste of the leaves with ginger is applied in eczema and skin affection. A decoction of the root is given for facial inflammations and rheumatism. The twigs are fibrous and astringent, they are used as toothache, wood also used in snake bite. [2] The roots are reported to posses glycozoline, furoquinolone and carbazole alkaloids. Some glycolone, quinazolone alkaloid like glycophymine, glycomide and glycosmine have been isolated from flowers of the plant. The crude extracts of the bark have been studied for hepatoprotective activity and antiabetic activity. [3] The detail pharmacognostical studies of leaf of the plant have been reported by Arora.,et al., since there is no report of systematic pharmacognostical and phytochemical studies on root, in order to fix some standards for its identification, this study was planned to study detailed pharmacognosy and preliminary phytochemistry of the same. [4]

MATERIALS METHODS

Plant Material

The fresh root of Glycosmis pentaphylla (Retz.,)DC., was collected in the mouth of September form Thirumala hills, Thirupathi (Dt), Andra pradesh, India. It was identified and authenticated by Professor Jayaraman, Plant Anatomical Research Center, West Tambaram, Chennai (PARC/2012/1081) and a voucher specimen of the root is deposited in the department for further references. The root samples of different organs were cut and removed from the plant and fixed in FAA ( Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol – 90 ml ). After 24 hrs of fixing , the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the schedule given by Sass, 1940. [5] Infiltration of the specimens was carried by gradual addition of Paraffin wax (Melting point 58-600C) until TBA solution attained super saturation. [6] The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 μm. Dewaxing of the sections was by customary procedure (Johansen,1940). [7] The sections were stained with Toluidine blue as per the method published by O’ Brien et al. (1964). [8] Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytological reaction were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast green and I/KI (For starch) [9] Powdered materials of glycosmis pentaphylla root were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscoic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. [10] Since these structures have birefringent property, under polarized light they upper bright against dark background. [11] Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books (Esau, 1964). [12]

Powder microscopic

For powder microscopy, powdered plant materials were sieved through 60 mesh cleared and preliminary examination, behavior of powder with different chemical reagents and microscopical examination was carried out. [13]

Physicochemical analysis

Percentage of total ash, acid-insoluble ash, water soluble ash, sulphated ash and also loss on drying and crude fiber content were calculated as per the Indian pharmacopeia. [14] The total ash of the powdered root was tested for different inorganic constituents. Various extracts were prepared for the study of extractive values of the root.[15]

Phytochemical analysis

The preliminary phytochemical analysis of powdered root of Glycosmis pentaphylla was extracted with petroleum ether (40-60 °C), chloroform, ethyl acetate, ethanol and water successively using cold maceration technique. [16] The extract were concentrated under vacuum evaporator, (Superfit, India) dried and weighed. Each extract was tested for presence of different phytoconstituents viz Alkaloids, flavonoids, carbohydrate, tannin, phenolic compound, triterpenoid and glycoside by usual prescribed methods. [17] The TLC pattern of petroleum ether (40-60 °C), chloroform, ethyl acetate, ethanol and aqueous extracts was studied using precoated silica gel G plate (Merck). [18-21]
RESULT AND DISCUSSION

The root morphological studies revealed that roots of the plant were brown coloured, having characteristic odour and bland taste. The microscopical characteristic of the root is 19 mm in diameter. it exhibits well developed secondary growth (Fig.1) 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 (Fig 2 & 3)

Fig.1- Transverse section of Root of *Glycosmis pentaphylla* (4 X & 10 X)

CO – Cortex, SPh – Secondary phloem, SX – Secondary xylem, Pe – Periderm

Fig.2- Transverse section of Root of *Glycosmis pentaphylla* (16 X)

Fi-Fibre, Pe – Peridrem, Co-Cortex, CPh- collapsed phloem, Se – Sclereide, PhF-Phloem fibre, Sph – Secondary phloem, SX – Secondary xylem,

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 (Fig.3) 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 (Fig.1.2) xylem rays are thin, slightly wavy and ray cells are also thick and lignified. The powder microscopical studies revealed the presence of parenchymatous cells and fibres in the root (Fig.4.1-4.5)

Fig.3- Transverse section of Root of Glycosmis pentaphylla (40 X)

CPh - Collapsed Phloem
XF – Xylem Fibre
NCPh – Non collapsed phloem
SX – Secondary xylem
XF – Xylem fibre
XR – Xylem rays
Ve – Vessel

Powder microscopy

For powder microscopy, powdered plant materials were showed presences of phloem fibers, Parenchyma cells, cork cell and xylem vessels.

Fig 4.1-Fibers and Vessels  Fig 4.2 – Cork cells  Fig 4.3-Xylem vessels

The physiochemical parameter values are given in the (Table.1). The qualitative chemical investigation showed that the plant contains alkaloids, steroid, flavonids, carbohydrate, triterpenoid, glycoside, tannin and phenolic compounds and the results are presented in (Table.2). The TLC pattern of extracts of Glycosmis pentaphylla root are presented in (Table .3.)
Table-1. Physicochemical parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% w/w mean ± SEM</td>
</tr>
<tr>
<td>Total ash</td>
<td>6.53 ± 0.25</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>16.1 ± 0.60</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>1.87 ± 0.02</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>1.78 ± 0.04</td>
</tr>
<tr>
<td>Sulphated ash</td>
<td>2.45 ± 0.07</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>8.7 ± 0.40</td>
</tr>
<tr>
<td>Petroleum ether extractive</td>
<td>9.88 ± 0.07</td>
</tr>
<tr>
<td>Chloroform extractive</td>
<td>6.82 ± 0.11</td>
</tr>
<tr>
<td>Ethyl acetate extractive</td>
<td>4.92 ± 0.08</td>
</tr>
<tr>
<td>Ethanol extractive</td>
<td>18.87 ± 0.88</td>
</tr>
<tr>
<td>Aqueous extractive</td>
<td>10.96 ± 0.44</td>
</tr>
</tbody>
</table>

* Mean value of three readings n=3, mean ± SD

Table-2. Phytochemical Analysis.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthroquines</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table-3. Description of TLC pattern of extracts of *Glycosmis pentaphylla* root.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>No. of Spots</th>
<th>Rf value (Iodine chamber)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>3</td>
<td>0.68, 0.84, 0.98</td>
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<tr>
<td>Chloroform</td>
<td>5</td>
<td>0.49, 0.70, 0.79, 0.856, 0.94</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>2</td>
<td>0.67, 0.92</td>
</tr>
<tr>
<td>Ethanol</td>
<td>5</td>
<td>0.49, 0.63, 0.80, 0.86, 0.98</td>
</tr>
<tr>
<td>Aqueous</td>
<td>4</td>
<td>0.39, 0.68, 0.76, 0.90</td>
</tr>
</tbody>
</table>

CONCLUSION

The present work was done to explore the macro, micro and physiochemical parameters of the plant. These parameters reveal the genuinity of the plant source and can be used to identify the drug. TLC profiles were done to identify the chemical constitutes of the plant. The preliminary phytochemical screening of various parts prepared in different solvents of *Glycosmis pentaphylla* indicated that the plant possesses medicinally important phytochemicals for various diseases.

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REFERENCES


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