ISSN-2249-5746

International Journal Of Ayurvedic And Herbal Medicine 2:4 (2012)733:741

Journal Homepage http://interscience.org.uk/index.php/ijahm

Pharmacognostical and Phytochemical investigation of *Geniosporum prostratum* (L) Benth

Gaurav Sharma[1], Dr. Lalit Nagar[2], Dr. Ashashri Shinde[3], Dr. Sudipt Rath[4], Dr. Naresh Khemani[5]

[1]Pharmacologist, Dept. of P.G. Studies in Dravya Guna, National Institute of Ayurveda, Amer road, Joravar Singh gate, Jaipur, Rajasthan, India, 302002

[2]P.G. Scholar, Dept. of P.G. Studies in Dravya Guna, National Institute of Ayurveda, Amer road, Joravar Singh gate, Jaipur, Rajasthan, India,302002

[3]P.G. Scholar, Dept. of P.G. Studies in Dravya Guna, National Institute of Ayurveda, Amer road, Joravar Singh gate, Jaipur, Rajasthan, India, 302002

[4]Lect. Dept. of P.G. Studies in Dravya Guna. National Institute of Ayurveda, Amer road, Joravar Singh gate, Jaipur, Rajasthan, India, 302002

[5]Prof. Dept. of P.G. Studies in Dravya Guna. National Institute of Ayurveda, Amer road, Joravar Singh gate, Jaipur, Rajasthan, India, 302002

Corresponding author: Gaurav Sharma, Pharmacologist, Dept. of P.G. Studies in Dravya Guna, National Institute of Ayurveda, Amer road, Joravar Singh gate, Jaipur, Rajasthan, India, 302002

ABSTRACT

Geniosporum prostratum (L) Benth belongs to family Lamiaceae grow only in Tamil Nadu. Present study deals with Pharmacognostical examination including determination of Loss on drying, ash value and extractive value. The Preliminary Phytochemical screening of whole plant was carried out and it is revealed the presence of various phyto constituents like Alkaloids, Glycosides, Steroids, Triterpenesand, Tannins and Saponins. The result wills useful for the standardization and identification this plant.

INTRODUCTION

The plant *Geniosporum Prostratum* (L) Benth belongs to Lamiaceae family and is found on sandy ground in deccan peninsula, specially near the coast grow only in Tamil Nadu. It is known as nazel-nagai, Bhutulasi in Tamil and is reported to have febrifugal properties (wealth of India). The present paoper deals with the isolation and characterisation of the flavones from the aeial parts of the plant. This plant has three chemical component (1) b- sitoserol (2) ursolic acid (3) 5-0 demethyl nobiletin. Crude extract of whole plant will use for Pharmacognostical and Phytochemical screening.

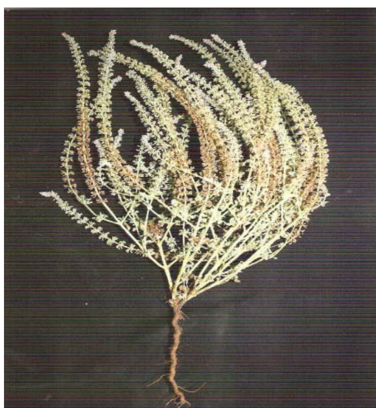

Fig. 1 *Geniosporum prostratum* Entire pant
(Courtesy: Self)

Fig. 2 *Geniosporum prostratum* floral part
(Courtesy: Self)

Plant profile

Kingdom:	Plantae
Subkingdom:	Viridaeplantae
Phylum:	Magnoliophyta
Subphylum:	Euphyllophytina
Infraphylum:	Radiatopses
Class:	Magnoliopsida Dicotyledons
Subclass:	Lamiidae
Superorder:	Lamianae
Order:	Lamiales
Family:	Lamiaceae
Genus:	Geniosporum
Specific epithet:	prostratum - Benth.
Botanical name: -	*Geniosporum prostratum Benth*

MATERIAL AND METHOD

Plant Material

Plant *Geniosporum prostratum* was collected from Cheranmadevi, Tirunelveli district, Tamil Nadu, with the help of a local herbalist and identified by V. Chelladurai Ex. Research Officer- Botany central council for Research in Ayurveda & Siddha Govt. of India .The leaves were air-dried and ground into powder.

Ash Value

- 2.4g of the ground air dried material weighed accurately in a previously ignited crucible.
- Ignite the material by gradually increasing the heat $500^{o}C$-$600^{o}C$ until it is white Cool in desiccators and weigh.

Total ash

(Carbone free ash)

- Cool the crucibles.
- Dry on water bath then hot plate and ignite to constant weight.
- Allow the residue in cool in desiccators for 30min.
- Weight without delay.
- Calculate total ash in mg per g of air dried material.

Acid Insoluble Ash

- Add HCl 10% (25ml) to the crucible containing the total ash.
- Cover with watch glass.
- Boil gently for 5 min.
- Rinse the watch glass with 5 ml of hot water and add this liquid to the crucible.
- Collect the insoluble mater on an ash less filter paper.
- Wash with hot water until the filtrate is neutral.
- Transfer the filter paper containing the insoluble matter to the original crucible.
- Dry on hot plate and ignite to constant weight.
- Allow the residue in cool in desiccators for 30min.
- Weight without delay.
- Calculate the content of acid insoluble ash in mg per g of air dried material.

Extractive values

- Determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material.
- Solvents used
    - Water
    - Methanol (different concentration)
    - Chloroform
- Hot extraction with water.

- Place about 4.0g of dried material accurately weighed.
- Added 100 ml solvent.
- Shake well and allow standing for 1hr.
- Attach a reflux condenser and boil gently.
- Cool and weigh.
- Read just to the original weight with the original solvent.
- Shake well and filter rapidly through a dried filter.
- Transfer 25 ml of the filtrate to a tarred flat bottomed dish and evaporate in a water bath to 105°C for 6 hours.
- Cool in desiccators for 30 min and weigh without delay.
- Calculate the matter extractible in mg per g of air dried material.

Loss on drying

Weight out of dried crude powder of drug place in hot air oven for 30 min. after time interval weight out or drug and calculate weight difference.

Preliminary Phytochemical Investigation:

Screening for Alkaloids:

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer’s reagents are added (**Siddiqui et al., 1997**). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer’s reagent .The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence yellow precipitation.

Screening for Glycosides

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer. (**Siddiqui et al., 1997**).

Screening for Flavonoids

Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids. **(Siddiqui et al., 1997)**

Screening for Triterpenes

Four milligrams of extract was treated 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for Triterpenes. **(Siddiqui et al., 1997)**

Screening for Steroids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed green bluish color for steroids. **(Siddiqui et al., 1997)**

Screening for Carbohydrates

5 mg of extract was dissolved in distilled water and filtered. The filtrate was used to test the presence of Carbohydrates. Filtrate was treated with 2 drops of alcoholic alpha naphthol solution (Molish reagent) in a test tube and 2ml of concentrated sulphuric acid and adds carefully along the test tube. Formation of a violet ring at the junction indicates the presence of Carbohydrates.

Screening for Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added, filter it and observed for Blue-black ,blue-green precipitate was observed for gallic tannins and green black for catecholic tannins.**(Iyengar, 1995)**

Screening for Saponins

Took 0.5mg of crud extract was shaken with water in a test tube. Forthing which persist on warning was taken as a preliminary evidence for the presence of Saponins. **(Odebiyi et al., 1978)**

Screening for Proteins

Took two ml on crude extract solution added an equal volume of 10 % sodium hydroxide solution. Added 2 to 3 drops of 1% copper sulphate solution and mixed well. Development of violet or purple colour indicates proteins and rosy pink indicates peptones. **(Patil, 2004)**

Chromatography

Preparation of TLC plate

TLC plates are usually commercially available, with standard particle size ranges to improve reproducibility. They are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like calcium sulfate (gypsum) and water. This mixture is spread as a thick slurry on an glass . The resultant plate is dried and activated by heating in an oven for thirty minutes at 110 °C. The thickness of the adsorbent layer is typically around 0.1 – 0.25 mm for analytical purposes and around 0.5 – 2.0 mm for preparative TLC.

To run a TLC, the following procedure is carried out:

A small spot of solution containing the sample is applied to a plate, about 1.5 centimeters from the bottom edge. The solvent is allowed to completely evaporate off, otherwise a very poor or no separation will be achieved. A small amount of an appropriate solvent (elutant) is poured in to a glass beaker or any other suitable transparent container (separation chamber) to a depth of less than 1 centimeter. A strip of filter paper is put into the chamber, so that its bottom touches the solvent, and the paper lies on the chamber wall and reaches almost to the top of the container. The container is closed with a cover glass or any other lid and is left for a few minutes to let the solvent vapors ascend the filter paper and saturate the air in the chamber. The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the elutant in the chamber, and the lid is closed. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate (elutes the sample). When the solvent front reaches no higher than the top of the filter paper in the chamber, the plate should be removed (continuation of the elution will give a misleading result) and dried.

Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent. By changing the solvent, or perhaps using a mixture, the separation of components can be adjusted.

Mobile phase: A mixture of 95 volumes of chloroform and 5 volume of methanol.

Test Solution: Dissolve about 200 mg of the extract under examination with 10.0 ml methanol, filter.

Reference solution: A 0.02 per cent w/v solution of ursolic acid in the methanol.

Apply to the plate each of solution as bands. Allow the mobile phase to rise up to 8 cm. Dry the plate in air and spray the plate with a anisaldehyde- sulphuric acid reagent solution. Heat at 110° for 10 minutes and examine the plate under 365 nm and under day light.

The chromatogram obtained with the test solution shows a band corresponding to the band obtained using reference solution indicating the presence of Ursolic acid.

RESULT AND DISCUSSION

Study was undertaken for Pharmacognostical and Phytochemical investigation of *Geniosporum prostratum* (l) Benth. Research work establishes the data that enable the identification of the plant material for future investigation & form an important aspect of drug studies. The yield of extracts was found in *Geniosporum prostratum (L)* Benthin table 1 Physico-Chemical Parameters in table 2, Phytochemial constituents in table 3. From the phytochemical screening Alkaloids, Glycosides, Steroids, Triterpenes, Tannins and Saponons was found in the crude extract.

Table 1: Percentage yield of extracts

Plant	% Extractive Value
Aqueous	27.52
75 % Methanol	25.45
50 % Methanol	30.35
Methanol	22.75
chloroform extract	11.68
Petroleum Ether	7.25

Table 2: Physico-Chemical Parameters

S.no.	Parameters	% w/w
1.	Ash values	
	(a) Total Ash	17.5
	(b) Acid Insoluble Ash	7.5
	(c) Water Soluble Ash	12.5
	(d) Sulphated Ash	13.4
2.	Extractive Values	
	(a) Alcohol Soluble Extractive	10.2
	(b) Water soluble Extractive	13.4
3.	Loss on Drying	11.4

Table 3: Phytochemial constituents of crude extract of *Geniosporum prostratum (L) Benth*

Phytochemical constituents	Result
Alkaloids Mayer's test	+ve
Flavonoids	+ve
Glycosides Keller Killiani Test	+ve
Steroids	-ve
Carbohydrates Iodine Test	-ve
Tannins	+ve
Phlobatannins	-ve
Triterpenes	+ve
Proteins Biuret Test	-ve
Saponons Frothing Test	+ve

TLC Observation:

Rf Value of Test Sample :

- Under day light : 0.35, 0.46, 0.54, 0.68, 0.89
- Under 365 nm: 0.29, 0.35, 0.41, 0.46, 0.54, 0.68, 0.74, 0.89
- After Derivatization with anisaldehyde- sulphuric acid: 0.35, 0.41, 0.54, 0.68, 0.74, 0.89

Rf Value of Reference sample (Ursolic acid):

- Under day light : 0.35
- Under 365 nm : 0.35
- After Derivatization with anisaldehyde- sulphuric acid: 0.35

Thin Layer Chromatography plate show that the Reference sample of Ursolic acid have Rf value 0.35. And in this sample Chromatography plate have contained 0.35 Rf value. Ursolic acid is present in the sample. This Study was use full for identification and standardization of this plant.

REFERENCES

1. Pitil CS.; Singh VP. ; Satyanarayan PS.; Jain NK. ; Singh A. and Kulkarni SK. (2003) Pharmacology. 69 (2), 59-67.
2. Sharma Gaurav et al. (2011) ; Anti Ulcer activity of chloroform extract of Geniosporum prostratum (L) Bent, Journal of drug ResearchVol.1, Issue 1,6-13
3. Siddique AA, Alim (1007); Practical pharmaceutical Chemistry, 1 ed., CBS publisher and distributor, New Delhi, 126-131
4. Lyenger MA (1997); Study of crude Drugs, 8th ed., Manipal power press, Manipal, India, 2-4
5. Odebiyi OO.; Preliminary Phytochemical and Antimicrobial Examinations of Leaves of *Securidaca longipedunculata Niger*. J Pharm. 1978; 9(1):29–30.
6. World health Orgianization (1998); Quality Control Methods for Medicinal Plant Material, 8-44