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Research Article

Pharmacognostic Profiling and Total Phenol Quantification of Leaves of *Spatholobus parviflorus* (Roxb. Ex DC.) Kuntze, A Woody Climber

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ABSTRACT

Spatholobus parviflorus is a woody climber which is less evaluated for its medicinal properties. In Kerala, Kani tribes uses the leaves paste to treat conjunctivitis. In this study, micro-macro morphological features, physicochemical constants, phytochemical screening, fluorescence characteristics and element analysis of *Spatholobus parviflorus* (Roxb. ex DC.) Kuntze leaves were studied. Total phenolic content was quantified in fresh leaf sample, dried leaf powder, ethanol, methanol and distilled water extracts by using Bray and Thorpe method. Total phenolic content was maximum in the ethanol extracts (3.78±0.2mg/g). Physicochemical parameters, phytochemical analysis, fluorescent behavior, element analysis of leaf powder and leaf morphology helps in pharmacognostic profiling of the leaves.

Keywords: *Spatholobus parviflorus*, Phenol Quantification, Phytochemical studies, Pharmacognosy, Element analysis, Histochemistry

INTRODUCTION

Plants play an important role in traditional medicine and widely consumed as home remedies. The WHO supports the use of traditional medicine and they are proven to be efficacious and safe. Over three quarters of the world population relies mainly on plants and plant extracts for health care. It is well known that plant produce these chemicals to protect them, but recent research demonstrate that they can also protect the humans against diseases. These drugs are derived either from whole plant or from different plant organs. These phytochemicals or drugs play an important role as antioxidants and also involved in hormonal action, stimulation of enzymes, interference with DNA replication, antibacterial effect etc[1]. The primary benefits of using plant derived medicines are that they are relatively safer than their synthetic alternatives offering profound therapeutic benefits and more affordable treatment[2]. Hence there is a need to validate the ethanomedicinal use of herbal medicine subsequently isolate and characterize the compounds[3]. A knowledge of chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies[4]. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification[5]. All these problems can be solved with pharmacognostic studies of medicinal plants. Pharmacognostic studies ensures plant identity and helps in authentication of the plants and ensures reproducible quality of herbal products which will lead to the safety and efficiency of herbal drugs[34].

Spatholobus parviflorus (Roxb. ex DC.) Kuntze belongs to the family Fabaceae and sub Family: Faboideae in the class Magnoliopsida. This plant is a woody climber which is less evaluated for its medicinal properties. These Woody climbers are widely distributed in a wide geographic range from Nepal, Bhutan and India through south-east Asia to southern China and Indonesia. In northern Thailand the leaves and stems of this species are boiled, with Dicranopteris, and used as a liquid to apply to broken bones as an analgesic[6]. In Kerala, a leaf paste is used to treat conjunctivitis[7]. Gum extracted from the wood, fibre from the bark and oil from the seeds is reputed to have economic use in Bangladesh[8]. As no pharmacognostic work is reported in this plant, the main objective of the present study is the pharmacognostic profiling, phenol quantification of this tribal used less explored medicinal plant *Spatholobus parviflorus*.

MATERIALS AND METHODS

Pharmacognostic Profiling

The fresh leaves were collected from the plant grown (Fig 1) at RET garden, St Mary's College Thrissur. The identification of the plant is carried out on the basis of information collected from MS Swaminathan Research Institute, Ambalavayal, Wayanad with the help of regional and National floras[9,10]. The dust and debris were removed from the plant parts and shade dried. Shade dried leaves were grinded to fine powder by a domestic grinder. Leaves were examined for its morphology studies size, shape etc. Microscopic studies were carried out by taking fresh free hand transverse sections of the leaves and photographed

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with Mims Photomicroscope. Analysis of Physicochemical constants of the powder leaf has been done to evaluate the quality and purity of the drug. Various physicochemical parameters like Ash values and Extractive values were calculated as per WHO guidelines[11,12]. The fluorescence behavior of powdered drug with different chemical reagents were studied in Daylight, short (254nm) and long (365nm) UV radiations[13].The phytochemical analysis of crude drug the alcoholic extract (10 gms of dry leaf powder with 100ml of various solvents, ethanol and methanol for 48hours and kept in a magnetic stirrer for 6 hours) were carried out to study the presence and absence of primary and secondary metabolites using standard conventional protocols[14,15]. Analysis of leaf powder for major elements is conducted by digesting with 5ml HNO_3, 3ml $HClO_4$ AND 1ML HF and analyzed with ICP-AES system[16].

Phenol Histochemistry and Quantification

The presence of phenols in the fresh leaf sections were histochemically evaluated using phenol stains. Fresh free hand transverse sections of the leaves were taken and stained with 1) Potassium dichromate[17], 2) Ferric chloride and by fixing fresh samples in a ferrous sulphate solution in formalin for 48 hours[18], Hoepfner-Vorsatz test[19,20] and studied under Mims Photomicroscope. Quantitative analysis of total phenol was estimated in fresh leaf sample, dried powder, ethanol, methanol and distilled water extract using Bray and Thorpe method[21]. Weighed exactly 50mg of fresh leaf, dried powder, ethanol, methanol and distilled water extract and mixed with10ml of 80% ethanol. Colchicine is used as standard. Total phenol values are expressed in terms of colchicines equivalent mg/g of sample. The estimations were repeated two times with triplicates and average values taken and read at 650 nm in systronics spectrophotometer.

Statistical Analysis

All the experiment done is replicated thrice and mean±SD value depicted.

RESULTS

Pharmacognostic Profiling

Macroscopic features of leaves

Leaf stalks are 9-13 cm, stiff. Leaflets are leathery, slightly stiff on the underside, smooth above Fig 2. Lateral veins are 7-9 pairs, usually not branched. Middle leaflet is broadly elliptic, 14-17 cm long, 9.5-12 cm broad, rounded at both ends or slightly narrowed at base. Side leaflets are asymmetric, broadly ovate, 12-16 × 6.5-10 cm, base rounded, apex blunt.

Microscopic features of leaves

The transverse section showed epidermis on both upper and lower surface Fig 3. The outer wall of the epidermis is thick and cutinized. It gives out many unicellular hairs Fig 4. The lamina portion encloses elongated palisade mesophyll tissue just below epidermis on dorsal side and with lower spongy mesophyll with intercellular spaces ventral side. The mesophyll is filled with chlorophyll pigments. In the midrib region, below to epidermis 3-4 layers of polygonal parenchymatous cells found followed by5-6 layers of sclerenchymatous cells limited by an uniseriate endodermis enclosing the xylem and phloem tissue.

Table 1: Ash values and Extractive values of powdered leaves of *Spatholobus parviflorus*

S. No	Parameter	% w/w
	Ash Values	
1	Total Ash	7.98%
2	Water Soluble ash	2.64%
3	Acid insoluble ash	5.42%
	Extractive Values	
4	Alcohol (90%) soluble extractive	11.2 %.
5	Water soluble extractive	16 %

Physiochemical analysis of the powdered leaf namely ash values and extractive values is represented in Table 1.

Fluorescence Studies

The fluorescence characters of the leaf powder observed in day /visible light and UV light when treated with different reagents is summarised in Table 2.

Phytochemical Analysis

The phytochemical analysis revealed the presence of Steroid, Saponin Glycosides, Phenols and Tannins, Fixed oils and Fat and carbohydrates in ethanol and methanol leaf extracts (Table 3).

Elemental Analysis

The results of element analysis of leaf are recorded in Table 4. The element like C >H> N >Ca >Mg >Na >Mn >Fe >Zn> Cu >Ni> Cr> Co decreasing order as mentioned here.

Histochemistry and Total Phenol Quantification

The leaf sections were stained and noted for the presence of phenols (Fig 5,6,7). This is prominent by the brown staining of the leaf epidermis and mesophyll cells.The staining was prominent in the xylem elements.

In the present study phenol was qualitatively detected by the presence of blue colouration by the Folin test. The various extract prepared were analysed quantitatively for the phenol. In the present study, the total phenol is estimated in the 50mg each of fresh leaves, dry leaf, ethanol extract, distilled water extract and methanol extract. The results of quantitative estimation of phenols are tabulated in Fig 3. The amount of phenol content varied from 0. 86 mg/gm sample to 3.9 mg/gm sample. High phenolic content observed in ethanol extract.

DISCUSSION

To maintain the standard and reproducibility of herbal preparation the drug used should be pure and not adulterated. This can be ensured by pharmacognostical studies. Thus in recent years emphasis has been given for the standardisation of medicinal plants. Pharmacognostical studies helps in the accurate identification and evaluation of plant drugs[22].

WHO stress that microscopic and macroscopic description of a medicinal plant is the first step towards establishing its purity, identity and should be carried out before any other tests are to be undertaken[23]. Ash value is the residue remaining after incineration of plant material. It gives an idea of the earthy matter or inorganic

Figure 1: *Spatholobus parviflorus* plant.

Figure 2: *Spatholobus parviflorus* leaflets

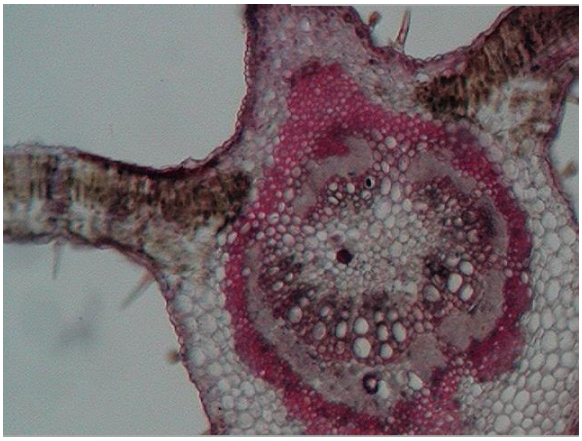

Figure 3: TS of leaf midrib region of *Spatholobus parviflorus*

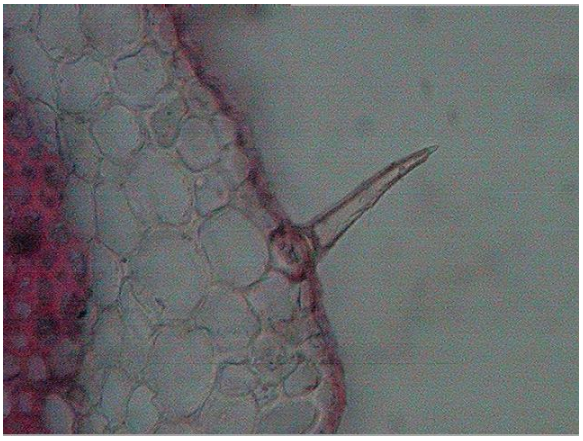

Figure 4: TS of the leaf showing unicellular hairs of *Spatholobus parviflorus*

Figure 5a: Histochemical staining for phenols with $Fecl_3$

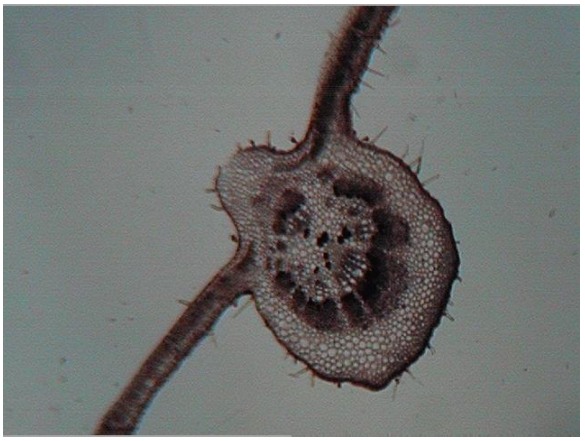

Figure 5b: Histochemical staining for phenols with $FeSO_4$

composition and impurities present along with the drug. In the presents study, extractive value is high with water. Extractive value gives a rough idea of the chemical constituents extracted from a specific amount of air dried material[24]. The compounds extracted with water gives an idea about sugar acids and inorganic compounds whereas polar constituents phenols, steroids, glycosides, flavonoids etc are dissolved in alcohol. Extractive values of the plant parts with various solvents can be used to determine the exhausted and adulterated drugs[25].

Fluorescence analysis is used to characterise the crude leaf powder. It is the phenomenon exhibited by various chemical constituents present in the plant material under UV light. This can be used to characterise the crude drugs. Thus crude drug is often assessed qualitatively and forms an important parameter of pharmacognostical evaluation[25]. Singh *et al*.[26] reported that all secondary metabolites could impart medicinal properties to the plant and has specific healing properties, healthy action and non toxic effects. It is well reported that phytochemicals are found to have a

Table 2 : Fluorescence characteristics of the powdered leaves of *Spatholobus parviflorus*

S. No	Treatment	Day Light	UV 254 nm (short)	UV 365nm (Long)
1	Powder + Water	Brown	Fluorescent Green	Brown
2	Powder+Alcohol	Pale Green	Fluorescent Green	Green
3	Powder +10% NaOH	Red	Green	Brown
4	Powder +50%HNO_3	Orange	Fluorescent Green	Brown
5	Powder+50%H_2SO_4	Brown	Dark Green	Dark Brown
6	Powder +acetic acid	Green	Dark Green	Pale Green
7	Powder + dil HNO_3	Orange	Green	Brown
8	Powder+5% Iodine	ReddishBrown	Dark Green	Brownish Green
9	Powder +5%$Fecl_3$	Brown	Dark Green	Green
10	Powder +Methanol	Green	Fluorescent Green	Light Green
11	Powder+Dil NH_3	Brown	Dark Green	Dark Brown

Table 3 :Preliminary Phytochemical Screening of the leaf extracts of *Spatholobus parviflorus*

S. No	Plant Constituents	Test /Reagent	Ethanol/ Methanol	Observations
1	Carbohydrates	Fehling Test	++	Red ppt
2	Starch	Iodine Test	++	Blue colouration
3	Sugar	Anthrone Reagent Test	+++	Blue Black Colouration
4	Proteins	Biuret Test:	++	Violet or pink colour appears.
5	Amino Acids	Ninhydrin Test:	--	No violet or purple colour
6	Fixed oils and Fat	Filter Paper Test	++	oil stains on filter paper
7	Alkaloid	Ammoniacal Test	--	No Creamish ppt
8	Steroid	Salkowski reaction	++	Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
8		Liebermann Burchard Test	+++	Blue Green Ring
9	Cardiac Glycosides	Legal Test	--	No Pink to red colour appears
10	AnthraquinoneGlycosides	Borntrager'stest	--	Ammoniacal layer do not turns pink or red.
11	Saponin Glycosides	Foam test:	++	Froth appearence
12	Phenols and Tannins	Folin Test	+++	Blue Colouration
13	Flavonoids	Ethylacetate Test	++	No Yellow colouration

Table 4: Elements analysis of leaf ppm of *Spatholobus parviflorus*

S. No	Elements	mg/gm	Properties
1	Calcium	10.45	Bone strength,heart attack,premenstrual syndrome
2	Cobalt	BDL	Cancer treatment
3	Chromium	0.00084	Balancing sugar levels,improves heart functioning
4	Copper	0.00919	Chest wounds prevent inflammation in arthritis
5	Iron	0.07598	Prevent anemia and Cough
6	Magnesium	2.710	Insulin sensitivity ,reduce blood pressure
7	Manganese	0.09174	Metabolising protein ,diabetics
8	Sodium	0.15267	Production of energy transport of amino acids and glucose
9	Nickel	0.00186	Healthy skin,bone structure
10	Zinc	0.02464	Growth ,control hair loss wound healing
11	Carbon	431.500	Energy metabolism
12	Nitrogen	23.400	Protein metabolism
13	Hydrogen	54.500	Energy metabolism
14	Sulphur	ND	Protein metabolism

BDL:BelowDetectablelimit
ND : Not Detected

broad range of activities which may help in protection against chronic diseases. The preliminary phytochemical screening of the alcoholic extracts has confirmed the presence of Steroid, Glycosides, Phenols and Tannins, Fixed oils and Fat and Carbohydrates. Suriyavathana *et*

Table 5 : Total phenolic content in *Spatholobus parviflorus* in different solvents

S. No	Extract	Avg. Concentration in mg/gm
1	Fresh Leaf	0.9±0.1
2	Dry Leaf	0.86±0.2
3	Water	1.96±0.1
4	Methanol	2.98±0.5
5	Ethanol	3.78±0.2

All the results were given as the triplicate of mean ± SD

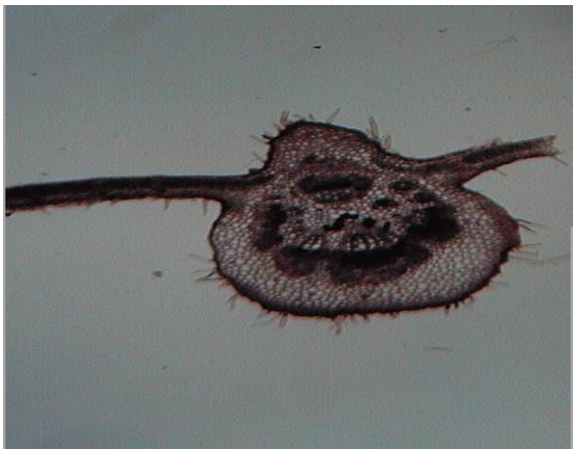

Figure 6: Histochemical staining for phenols with Hoepfner-Vorstaz test

al.[27] in their studies on selected medicinal plants has stated that phytochemical is a natural bioactive
compound found in plants such as vegetables fruits, medicinal plants, flowers, that work to act as defense system aganist diseases.

The elements play a major role in the formation of secondary metabolites which are responsible for pharmacological action of medicinal plants[28]. The trace elements play both curative and preventive role in combating diseases. In the leaf powder presence of trace elements like Ca, Cr,Cu,Fe,Mg,Mn,Na,Ni,Zn has been detected.

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues[29] Johnson *et al.*,[30] in their study has stated that the application of histochemical characters in taxonomic problems is now a common practice for the identification and characterization of taxon. The present study confirmed the presence of phenols in the leaves of *Spatholobus parviflorus*. Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties. The phenolic compounds are heterogeneous groups of substances that are present in almost all plants, inside the vacuole, cytoplasm or consisting the cell wall [31]. Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. The total phenol content play a very important role in the protection of the plants against the deleterious effects of UV rays and also against certain phytopathogenic microorganisms[32]. It is also well known that phenolic compounds contribute to the quality and nutritional value and also provide health beneficial effects[33]. Phenols are found to be useful in the preparation of antimicrobial compounds[26]. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction[27]. This is confirmed by the presence of blue colour in the extracts in comparison to the catechol which is taken as the standard. The phenol possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities[33]. They are also found in many medicinal plants, and herbal medicines containing these compounds have often been used in pharmacy.

The present study on physiochemical parameters, fluorescence analysis, preliminary phytochemical analysis and elemental analysis provide a better understanding of this less explored medicinal plant. These data forms important information in the identification and authentication of the plant material. There is no doubt that this plant is a reservoir of potentially useful chemical compounds which serve as drugs, provide newer leads and clues for modern drug design.

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