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Original Research Article

Pharmacognostic Evaluation and Antioxidant Potential of *Spermacoce hispida*

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Abstract: One significant member of the Rubiaceae family and a frequently used herb in siddha treatment is *Spermacoce hispida* Linn. Phytochemical components found in plant extracts include saponins, tannins, phenolics, steroids, essential oils, flavonoids, and terpenoids. This plant's chemical components have been employed for its antidiabetic, antihypertensive, hepatoprotective, anti-inflammatory, antihyperlipidemic, analgesic, antifungal, anticancer, and antioxidant activities. In the current investigation pharmacognostic evaluation and antioxidant potential of *Spermacoce hispida* was carried out. The outcome of study showed that the methanolic extract of *S.hispida* had strong hydrogen donating ability with an IC50 value of 60.91 µg/ml, respectively and the value was found to be less than the standard vitamin C (IC50 value of 19.77 µg/ml).

Keywords: *S.hispida*, pharmacognostic evaluation and antioxidant potential.

INTRODUCTION

The natural world has a vast storehouse of cures for human problems. The main health care needs of about 80% of the world's population are partially or entirely met by conventional medicine. Since ancient times, physicians have utilised botanicals as the primary treatment in traditional medicine, and its use has greatly contributed to the maintenance of human health [1, 2]. The Rubiaceae family includes the *Spermacoce hispida*. One of the four largest angiosperm families, the Rubiaceae, with 637 genera and 10,700 species [3]. Popular names for S. hispida include "Nattaichuri" in Tamil and "Shaggy button weed" in English [4].

S.hispida

Botanical Description [5, 6]

BIOLOGICAL NAME	*Spermacoce hispida*
FAMILY	Rubiaceae
SYNONYM	*Spermacoce articularlis, Borreria hispida, Borreria articularlis*
VERNACULAR NAMES	**English:** Shaggy button weed, Jointed buttonweed; **Tamil**: Nathaichuri, Cirakkuli, Nathai-choori, Vetuppaccuri; **Hindi:** Madanaghanti, **Sanskrit:** Bukah, Madanaghanti, Madanghanta, Vasukah;
HABIT	A procumbent, scabrid, hirsute or hispid herb.
Ethno-Medicinal Importance	**Leaves:** Leaf juice is used in conjunctivitis. An extract of leaves is given as an astringent in hemorrhoids and gallstones. The leaves are used as haemostatic, in dental carries and tooth ache. **Root:** The root is used for wound healing, dental problems and as an appetizer. **Seed:** The seeds are used as a stimulant, demulscent and for the treatment of diarrhoea and dysentery.
Traditional uses	**Pharmacological uses**
The roots stem, and leaves of S. hispida have been used conventionally for the management of urinary infections, oliguria, venereal diseases, conjunctivitis, hemorrhoids, gallstones, stomach ailments, internal injuries of nerves, and kidney, coughs, malaria, internal heat, dyslipidemia, and for reducing weight [7].	Pharmacological studies revealed a wide spectrum of biological activities such as analgesic, antioxidant, anti-inflammatory, antimicrobial, anticancer, antihyperglycemic, antihypertensive, hepatoprotective, cardioprotective, antihyperlipidemic, anxiolytic properties [7].

MATERIAL AND METHOD

Collection and authentication of plant material

The whole plant of *Spermacoce hispida* was collected from, India, in the month of December, 2016.

Pharmacognostical Standardization [8-9]

Macroscopy

The fresh whole plant of *S. hispida* was used for macroscopical study. The size, shape, color, taste, odour, surface, base, margin and venation were observed.

Microscopy

a) Preparation of histological specimen

The leaf, stem and root of *S. hispida* were cut into required size and fixed in FAA (Formalin 5 ml + Acetic acid 5 ml + 70% Ethanol 90 ml). After 24 hr of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60˚C) until tertiary butyl alcohol solution attained super saturation. The specimens were cased into paraffin blocks.

b) Microtoming

The paraffin embedded specimen was sectioned with the help of rotary microtome. The thickness of the section was 10-12 µm. After dewaxing the sections were stained with toluidine blue. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some phyto-chemical reactions were obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein.

c) Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon photo lab - 2 microscope units. For normal observations bright fields was used. For the study of crystals, starch grains and lignified cells polarized light were employed. The prepared sections were observed through the microscope and distribution of various types of tissues was noted.

Fluorescence Analysis

The powdered material of *S. hispida* as treated with different chemical reagents to detect the phyto - constituents with color changes under ordinary daylight and UV light by the standard method.

Powder Analysis

The coarse powder of the plants (*Spermacoce hispida*) was mounted in glycerine and its anatomical characters were observed.

Physico-Chemical Constants [10]

Various physiochemical parameter like LOD, Ash value (Total ash, acid insoluble ash, water soluble ash) and extractive value were determined as per standards procedure.

Preparation of Extract

The fresh leaves of *S. hispida* was dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No 40 and stored in an airtight container for further

Extraction procedure

The coarse selected plant materials were extracted with 1-1.5 liters of methanol continuous hot percolation using soxhlet apparatus. After completion of extraction, extract was filtered and the solvent was removed by under reduced pressure. The dried extract was stored in desiccators.

Qualitative Phytochemical Analysis [11]

The crude methanolic extract of *S. hispida* (MESH) was analyzed for the presence of various phytoconstituents by following standard phytochemical protocols.

***In-vitro* Free radical scavenging activity**

The scavenging activity for DPPH free radicals was measured according to the procedure described by Soni *et al*., 2022. An aliquot of 3 ml of 0.004% DPPH solution in ethanol and 0.1 ml of plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition activity was calculated as [(A0-A1)/A0] ×100, where A0 was the absorbance of the control, and A1 was the absorbance of the plant extract/ ascorbic acid [12].

RESULT AND DISCUSSION

It is seen from the literature that *S. hispida* is a very significant plant for its large number of medicinal properties. The seeds of *S. hispida* are used to make a leg yam that is taken twice daily to treat bloody diarrhoea and help people lose weight. For situations like urinary infections, oliguria, etc., the roots are dried and powdered and given with cow's milk twice daily. The roots' choornam is also taken twice daily to treat problems like internal heat, sexual diseases, etc. Samoolam decoction is effective at treating headaches, and it has recently been discovered that this herb contains abundant amounts of calcium and phosphorus. As a result, administering this medication in the form of chooranam or kudineer (decoction) is advised in conditions like bone diseases, fractures, etc. The macroscopic and microscopic were tabulated in table 1&2.The result of Fluorescence analysis of powder drug was tabulated in table 3. The standardization parameters were loss on drying at 100-105^{0}C (9.19%w/w), total ash value (1.8%, w/w), acid insoluble ash value (1.1 %, w/w), and water soluble ash value (1.9% w/w). Water soluble extractive value (11.2%, w/w) and alcohol soluble extractive value were found to be (6.4%, w/w) respectively (Table 4). The phytochemical analysis showed that the methanolic extract of *S. hispida* contained Phenolic compounds/Tannins, Flavonoids, Steroids, Saponins, Triterpenoids, Proteins Carbohydrates and Glycosides(table.6) .The antioxidant potential was determined by DPPH method and the result was tabulated in table 7. In the present study, the IC50 values of methanolic *S. hispida* extract was found to be 60.91µg/ml.

Table 1: Macroscopic characteristic of *S. hispida*

Leaves	Flower	Fruit
The leaves are opposite, decussate, elliptic- oblong to linear lanceolate measuring 3.5 – 5mm x 0.5 – 1.5mm in size.	The flowers occur in auxiliary or terminal sessile heads. Flowers are tetramerous; petals and sepals are valvate; petals pinkish, ovary inferior, bicarpellary, many ovules: style two.	The fruit is a capsule.

Table 2: Microscopic characteristic of *S. hispida*

Leaves	Stem	Root
Dorsiventral with fairly thick lamina and prominent midrib. Midrib exhibits shallow adaxial groove and thin wide abaxial convex part. The lower part of the midrib consists of fairly thick epidermis with squarish thin walled cells.The ground tissue includes 5 or 6 layers of	• In a slightly old stem, measuring about 2.5mm thick, the cortical cells are crushed and compressed. The pith cells are disintegrated. • The vascular cylinder exhibits secondary growth having wide secondary phloem. In this stem which is further old, there is a narrow periderm, distinct cortex,	• The root studied in 4mm thick. It has undergone advanced stage of secondary growth. It consists of a thin, less prominent superficial periderm, which is just initiated. • A wide cortical zone comprising 8 – 10 layers of

Leaves	Stem	Root
compact, wide thin walled parenchyma cells. On the upper side also, there is a thin pillar of parenchymna cells; the palisade cells extend up to either side of the parenchyma pillar. The vascular bundle is broadly triangular, collateral and located at the upper end of the midrib. It consists of 6 or 7 thin, parallel rows of narrow xylem elements and small clusters of sieve elements. The vascular bundle is 120 x 160μm in size.	wide secondary phloem and dense thick secondary xylem. • Secondary xylem includes diffusely distributed solitary vessels and xylem fibers. • The vessels are circular to angular and thin walled, the vessels are both wide and narrow; the diameter of the vessels range from 15 – 40 μm. • Calcium oxalate crystals are common in the cortical cells. They are druses.The druses are 30 – 60 μm thick.	tangentially oblong phloem. Druses are abundant in the cortical cells. Secondary phloem is a wide and radially seriated. Phloem elements are narrow and thin walled. • Secondary xylem is thick solid cylinder and consists of narrow, angular, thin walled and solitary vessels which are diffuse in distribution. • Xylem fibers are thin walled and lignified. The vessels are up 40μm wide.

Table 3: Fluorescence Analysis of *S.hispida* with various reagents

S. No	Reagents	Under Ordinary light	Under UV light	
			Long wavelength	Short wavelength
1.	Powder as such	Greenish brown	light brown	Greenish yellow
2.	Dry powder was placed on glass slide affixed with nitrocellulose	Light brown	bright brown	Bright green
3.	Powder treated with 1M NaOH in Methanol	Greenish brown	light brown	Dark green
4.	Powder treated with 1N NaOH in Methanol, dried and then mounted in Nitrocellulose in Amylacetate	Bright yellowish brown	light Yellow	Bright green
5.	Powder treated with 1M HCl	Yellowish brown	dark brown	green
6.	Powder treated with 1M HCl, dried and then mounted in nitrocellulose in amylacetate	Light brown	Dark yellow	Pale green
7.	Powder treated with 1M NaOH in water	Greenish brown	yellowish green	Bright green
8.	Powder treated with 1M NaOH in water, dried and then mounted in nitrocellulose in amylacetate	Yellowish brown	greenish brown	Bluish green
9.	Powder treated with 50% HNO_3	Brown	Darkness	Bright green
10.	Powder treated with 50% H_2SO_4	yellowish brown	yellowish green	Bright green

Table 4: Physiochemical Values of *S.hispida*

S. No	PARAMETERS	RESULTS
1.	**ORGANOLEPTIC CHARACTERISTICS**	
	Appearance	Powder
	Colour	Dark brown
	Odour	No characteristics odour
	Taste	Bitter
2.	Loss on drying	9.19 %w/w
3.	**ASH VALUES (%)**	
	Total ash	1.8
	Water soluble ash	1.9
	Acid insoluble ash	1.1
4.	Alcohol soluble matter (%)	6.4
5.	Water soluble matter (%)	11.2
6.	**EXTRACTIVES (%)**	
	Methanol extract	4.2

Table 5: Estimation of Inorganic Constituents in *S.hispida*

S. No	Elements	Quantity of elements (mg/g) in dried powder
1	Zn	1.660
2	Mn	3.876
3	Cu	0.687
4	Cr	0.123
5	Pb	0.31
6	As	<0.001
7	Co	26
8	Na	<0.01
9	K	<0.01

Table 6: Qualitative phytochemical analysis of methanolic extract of *S.hispida*

Type of Constituent	Methanol extract
Alkaloids	-
Phenolic compounds/Tannins	+ + +
Flavonoids	+
Saponins	+
Steroids	+ + +
Triterpenoids	+ + +
Proteins	+ +
Carbohydrates	+
Glycosides	+ +

Table 7: *In-vitro* antioxidant activity of MESH by DPPH inhibition Assay

Conc (µg/ml)	Percentage Inhibition	
	Vitamin C	MESH
10	34.8 ±0.98	11.14±0.84
20	55.9±0.92	19.6± 1.2
40	63.4±0.85	32.8+1.10
60	76.7±0.92	49.6+1.18
80	83.2±1.02	64.9+1.02
IC_{50} (µg/ml)	19.77	60.91

Data are presented as the mean ± SEM (n = 3)

CONCLUSION

The present investigation documented the pharmacognostical study of *S.hispida* which comprises of microscopically, macroscopically, powder microscopy and determination of physico-chemical constants like ash values, extractive values, loss on drying and estimation of inorganic constituents. These parameters serves as standards providing an imperative basis of information in ascertaining the uniqueness and genuinity to decide on the quality and clarity of the plant material, further serve as an identity tool in differentiating *S.hispida* among its other species. The qualitative phytochemical screening of the extracts were analyzed for the presence of alkaloids, carbohydrates, glycosides and anthraquinones, flavanoids, tannins and phenolic compounds, proteins and amino acids, saponins, sterols and or triterpenes. In this study, methanolic extract of *S.hispida* have showed the presence of numerous phytoconstituents. In the present study, methanolic extract of *S.hispida* displayed significant decrease in absorbance of DPPH radical, indicating the reaction between plant extracts and radical progresses, which results in the scavenging of the radical by hydrogen.

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