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Phytochemical and Antimicrobial Screening of *Spermacoce ocymoides* Burm. f.

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Abstract

Spermacoce ocymoides belongs to the family Rubiaceae. The plant is used in traditional system of medicine for healing various diseases. However, the present study was aimed to evaluate the parameters to determine the quality of the plant. This study comprises morphological, microscopical, phytochemical and pharmacognostic investigations of the plant.

Keywords: *Antimicrobial Screening, Phytochemical screening, Pharmacognosy, Rubiaceae, Spermacoce ocymoides Burm.f*.

Introduction

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of plant of the plant like barks, leaves, flowers, seeds etc. i.e. any part of plant may contain active components. Knowledge of the chemical constituents of plant is desirable because such information will be of value for the synthesis of complex chemical substances. *Spermacoce ocymoides* belongs to the family Rubiaceae. Commonly known as "purple leaved button weed" (also known as "Basil like button weed) which is a vascular plant with significant woody tissue above or at the ground. These herbs may be annual, biennial or perennial but always lack significant thickening by secondary woody growth and have borne at or below the ground surface. It is found in Orissa and throughout the India and also found in Cameron, Congo, Gabon, Uganda etc as wayside weed.

The plant having several folk fore and ethno medicinal claims which includes, the plant is active against hookworm, ringworm and also having wound healing properties. The whole part is used in Diarrhea and Dysentery. The leaves are used to treat eczema and skin problems. The plants are useful in curing gonorrhoea, dysentery and skin disease much study (Kirtikar and Basu 1987). The literature revealed that there is lack information about the phytochemical evaluation of *S. ocymoides* and the results of this investigation are discussed here.

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Materials and Methods

Material

Spermacoce ocymoides Burm.f. belongs to the family Rubiaceae. It is commonly known as "Purple leaved Button Weed". *Spermacoce ocymoides* is an erect or spreading well-branched annual plant with slender stem, it grow up to 25 – 60 cm fall. The plant is harvested from the wild for local medicinal use. The is grown in waste places, disturbed soil, along canals and marshes, in grassy fields and along road; at elevation from sea level up to 650 – 1,400 meters. *Spermacoce ocymoides* leaf is partly fused at base, small, puberulous, margin not hairy. The fresh leaves are green in color, semi-leathery and about out 13 cm long, 0.21 cm wide, linear lanceolate (or) ovate to elliptic.

Collection of plant material

The plant is used in the study was *Spermacoce ocymoides* was collected from the surroundings of Davangere Districts. The plant were identified and authenticated by using Flora of Davangere.

Drying of plant material

The plant materials were dried under shade for about 15-20 days and sliced into small pieces, pulverized using mechanical blender and passed through 40 mesh sieve and stored in an air tight container for further analysis.

Microscopic Analysis

To the prescribed, procedure, the microphotographs were taken by Bright Field Microscope. The microscopy of the plant studied according to method. Traverse section of leaf, stem, root and stomata were prepared and stained with Safrannin as per the procedure (Dwivedi 1990). Powder microscopy is performed according with prescribed procedures (Evans 2002 and Wallis 1985). The microphotographs were

taken by Bright Field Microscope with camera for further detailed studies.

Extraction of powdered plant material

The powdered whole part of *S. ocymoides* were extracted against ~350 ml of water and methanol for 24 hours. The extract obtained was dried at room temperature at room temperature and used for the further study.

Determination of behaviour of plant powder

The powder drugs with different chemical reagents shown different colour when seen on naked eye with respect to the nature of chemicals constituents present in the powdered material. Fluorscence analysis: Many drugs shows fluorescene behaviour when exposed to UV-Radiation according to the nature of phytoconstituents whatever presents in the drugs. It is important to observe all the material on reaction with different chemical reagents under UV-light. The fiuoresence characteristics of powdered drugs were studied under UV- light, the fluorescence is described in result and discussion.

Powdered Microscopy

Dried whole plants were powdered separately and sieved with 40 mesh size sieve to get fine powder. The fine powders of different parts were treated with chloral hydrate solution separately in a beaker and boiled for 10 minutes to clear powder. Then some amount (one portion) from each were transferred to different watch glass for each and stained using phloroglucinol, concentrated hydochloric acid and another portions of each were transferred to another watch glasses and stained using phloroglucinol, safranin. After staining, the powder was taken on a clean slide with the glycerin water solution, then the slides were covered with cover slip and excess solution was wiped with the help of tissue paper. This slide was observed under microscope and the snaps are taken in different microscopic cells.

Anti Microbial Analysis

Bacterial test organisms

1. *Escherihia coli*

Escherihia coli are usually found in the gastro-intestinal tracts of warm blooded organisms. The most common causes of urinary tract infection in humans in *Escherihia coli* causing atleast five types of gastro-intestinal diseases in humans. Pathogenicity is generally due to the present of one (or) more virulence factors, including in vasiveness factors, heat-liable and heat stable enterotoxins Pathogenic strains are usually identified by detection of specific virulence factors of a serotype associated with a virulence factor

2. *Bacillus subtilis*

It is a food poisoning, Gram positive, facultative aerobic, sporulating bacteria normally found in soil *Bacillus subtilis* is normally considered as being non-pathogenic, but it has been linked to food-borne illnesses, causing diarrhea, it is a food poisoning, Gram positive, Nausea, vomiting. *Bacillus subtilis* produces subtitism, which an extra cellular enzyme that catalyzes the breakdown of proteins into polypeptides, resembles trysin in its action, and has shown to be a potent occupation allergen.

Preparation of Plant Extract

About 20 mg of solvent extract of plant part *Spermacoce ocymiodes* will be dissolved in 1ml of Dimethyle Sulfoxide (DMOS). This considered as concentrations screened; 31.25, 62.5, 125,250, 500 and 1000mg. the inoculum of the plant extract will be prepared at the time of loading.

Description

Media: Peptone 10g, NaCl 10g , yeast extract 5g, Agar 20g in 1000 ml of distilled water.

Initially, the stock cultures of bacterial, we revived by inoculating in broth media and grown at 37^0 C for 18 hr, the agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hrs old cultures (100 l) and spread evenly on the plant .after 20 min, the wells were filled with of compound and antibiotic at different concentrations. All the plates were incubated at 37^0 C for 24 hr and the diameter in inhibition zone were noted.

Results and Discussion

Pharmacognostic Investigation

The detail and systematic Pharmacognistic evaluation would give valuable information for the further studies. The detailed morphology of *Spermacoce ocymoides* was carried out to support proper identification of drug (Plate 2) (Kokate 1953 and Khandelwal 2007).

Stomata

Spermacoce ocymoides showed various kind of stomato are predominately present and in *Spermacoce ocymoides*, Rubiaceae parallel or parasitic celled stomata (Plate 1. D).The type of stomata reported in *Borreria sp*. predominantly diacytic (Wong *et.al*, 2014) and *Spilanths acmella* (Kavya and Pramod 2015) diacytic and anomocytic stomata were present.

Microscopy

Microscopy of Root (T.S)

A transverse section of root consist of Cork cell, Cortex, Pholem, Vessels and Xylem fibers. Xylem consists of vessels and are arranged in a row formed a waxy ray (rays of xylem). The vessel are combined together centrally which leads to a large central vessel. Phloem found in the Periphery of cortex (Plate 1. A)

Microscopy of Stem (T.S)

A transverse section of Stem shows lignified tissue, wide pith with thin walled non lignified spongy Parenchymatous cell consist of oil cells and Calcium Oxalate crystals. Covering trichomes are appeared from

the epidermis; also it consists of Cortex, cortical collenchymatous fibers which separates the Cortex and secondary fibers; secondary xylem consist of medium to large multiple vessel; phloem are appeared in group with thick and straight medullary rays towards the pith (Plate 1. B).

Microscopy of Leaf (T.S)

A transvers section of leaf shows that the cells are epidermis are irregular in size with square shaped and curved angle. Unicellular covering trichomes are frequently developed from the upper epidermis and lower epidermis along with from the surface of the midrib. Palisade cells are slightly cylindrical and irregular in size but closely arranged, cell walls are slightly thin. Vascular bundle is located at nearly middle of the midrib which consists of the xylem and Pholem. Midrib is uniseriate and cells are pentagonal to hexagonal shaped with curved angle and of various size (Plate 1. C).

Phytochemical Analysis

Preliminary analysis of crude solvents extracts

In the present study, two solvent (methanol and water) used to extract the phytochemicals from the selected plant *Spermacoce ocymoides*.

Therefore preliminary phytochemicals screening of *Spermacoce ocymoides* plants powder is done by following standard method (Table 1). **(Raaman 2016).**

Behaviour of whole plant powder with different chemical reagents

Behaviour of *Spermacoce ocymoides* with different chemical reagents is to detect the color changes under ordinary day light by standard method. (Table 2).

Anti-Bacterial Analysis

The following tables contains the zone of inhibition in mm.

Antibacterial activity of solvent extracts of *Spermacoce ocymoides* against *Bacillus subtilis* (Table 3), (Plate 3. A & B).

Antibacterial activity of solvent extracts of *Spermacoce ocymoides* against *Escherichia coli* (Table 4), (Plate 3. C & D).

Table 1

Preliminary phytochemical screening of Spermacoce ocymoides

Tests	**Dist.Water**	**Methanol**
Carbohydrates	+	+
Reducing sugar	+	+
Glycosides	+	+
Polysaccharides	-	-
Tests for Proteins Free amino acids	-	-
Bradford test	+	+
Tests for alkaloids Dragendroff's test	+	+
Mayer's test	+	-
Tests for steroids Libermann-Burchard test	-	+
Salkowski's test	+	+
Triterpenoids	+	+
Tests for flavonoids Test 1	-	-
Shinoda test	-	-
With sodium hydroxide	+	+

Tests for Tannins		
$FeCl_3$ test	+	+
Dilute HNO_3 test	-	-
Test For Lipid	+	+
Test for Oils	+	+
Test for Saponins	+	+

Table 2
Behaviour of whole plant powder with different chemical reagents

Treatment	**Colour of powder**
Powder + H_2O	Light green
Powder + HCl	Black
Powder + HNO_3	Orange
Powder + $FeCl_3$	Dark brown
Powder + NaOH	Brown
Powder + H_2SO_4	Dark black
Powder +NH_4Cl	Light green
Powder+Glacial acetic acid	Dark green

Table 3
Antibacterial activity of solvent extract of Spermacoce ocymoides against Bacillus subtilis

Sample	**31.25 µg**	**62.50µg**	**100 µg**	**250 µg**	**500 µg**	**1000 µg**	**MIC µg**
01-W	0	0	0	0	0	5	1000
01-M	0	0	0	0	0	5	1000

Table 4
Antibacterial activity of solvent extract of Spermococe ocymoides against Escherichia coli.

Sample	**31.25 µg**	**62.5 µg**	**125 µg**	**250 µg**	**500 µg**	**1000 µg**	**MIC µg**
01-W	0	0	0	0	0	0	NF
01-M	0	0	0	0	0	0	NF

The same type of antibacterial activity reported in *Spilanthes acmella* (Kavya and Pramod 2016).

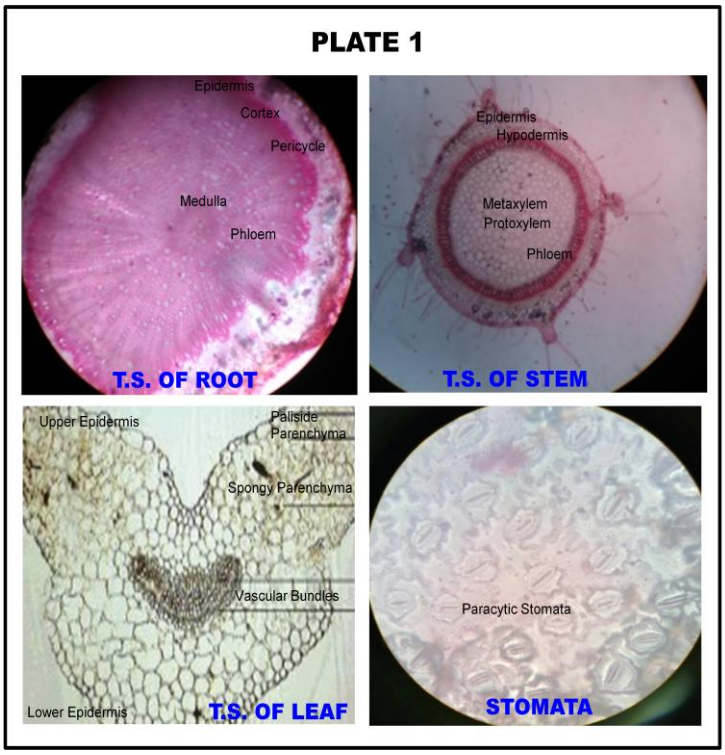


PLATE 1
Epidermis
Cortex
Pericycle
Medulla
Phloem
T.S. OF ROOT
Epidermis
Hypodermis
Metaxylem
Protoxylem
Phloem
T.S. OF STEM
Upper Epidermis
Paliside Parenchyma
Spongy Parenchyma
Vascular Bundles
Lower Epidermis
T.S. OF LEAF
Paracytic Stomata
STOMATA

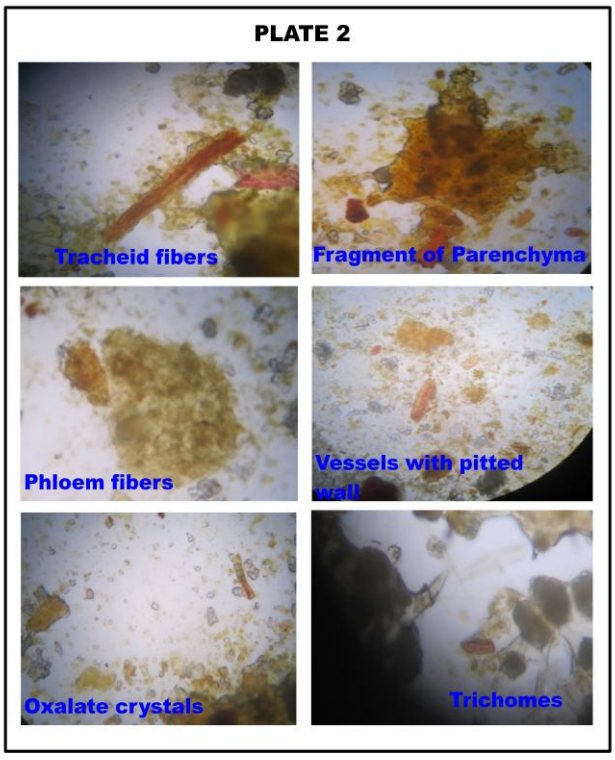
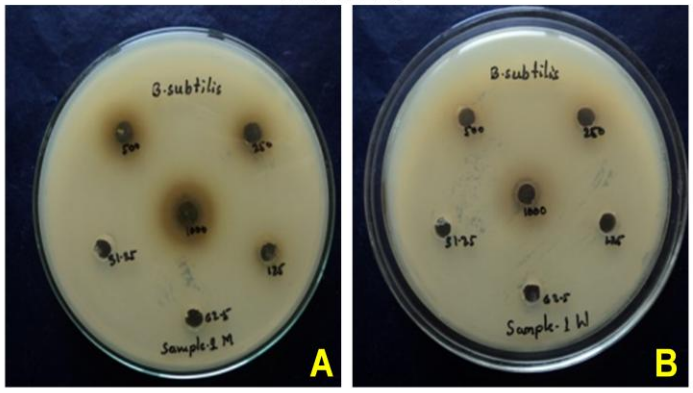
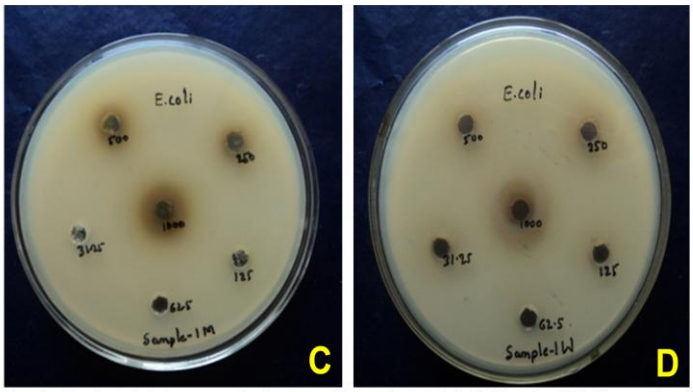


PLATE 2
Tracheid fibers
Fragment of Parenchyma
Phloem fibers
Vessels with pitted wall
Oxalate crystals
Trichomes

PLATE 3



A. Antimicrobial property (zone of bacterial inhibition) of methanol extract against *B.subtilis*
B. Antimicrobial property (zone of bacterial inhibition) of water extract against *B.subtilis*



C . Antimicrobial property (zone of bacterial inhibition) of Methanol extract against *E.coli*
D. Antimicrobial property (zone of bacterial inhibition) of water extract against *E.coli*

Conclusion

In present investigation various standardized such as macroscopic, microscopy, pharmacognostic, phytochemical screening and antibacterial properties was carried out and which could be helpful in authentication of *Spermacoce ocymoides*. The results of present study will also serve as reference material in preparation of monograph. It is present need to conserve the plant for medicinal usage. Tissue culture techniques may be more useful in the conservation point of view and to make the drug available throughout the year. The plants holds great promise as a commonly available medicinal plant and it is indeed no surprise that the plant is referred to in the Indian-traditional circles. From the available literature on various aspects of the plant-traditional to the ethnobotanical and antimicrobial and pharmacognostic and phytochemical screening however there many gaps which need to be filled by concurrent researches in different disciplines. One must make the best use of the naturally available resources which provide valuable raw material for advanced research.

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