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Pharmacognostical profile of *Spermacoce ocymoides (Burm. F) DC.* - A Study on a Medicinal Botanical

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ABSTRACT

The aim of the present study was designed to evaluate the pharmacognostical and preliminary phytochemical evaluation of the whole plant Spermacoce ocymoides (Burm F.) DC. The pharmacognostical profiles which includes Organoleptic evaluation, micro morphology of leaves and seeds; microscopic evaluation e.g. like Leaf microscopy, Root microscopy, Stem microscopy, determination of leaf constants e.g. determination of stomatal number and stomatal index, determination of vein-islet and vein termination number; Powder microscopy of whole plants along with determination of average length of trichomes of leaf, stems and whole plants, Determination of length and width of fibres of whole plant; Fluorescence analysis and reagent analysis with powder drugs; Physical properties evaluation of powder materials of the whole plant e.g. Extractive values, Ash values e.g. total ash, Water soluble ash, Acid insoluble ash, Sulphated ash; others e.g. Moisture content, P^H (1% w/v solution), swelling index, foaming index and the powdered plant materials than subjected to successive extraction process with different solvents with increasing order of their polarity using standard extraction processes like reflux condensation process and Preliminary phyto-chemical screening has been done to find out the nature of phyto-constituents present within them for the further research work.

Key words: *Spermacoce ocymoides (Burm F.) DC*, Pharmacognosy, phytochemical.

INTRODUCTION

Nature has a treasure of medicines to treat all kinds of ailments. When our prehistoric ancestors first roamed the earth in search of food, they perhaps learnt from better experience, which plants and herbs edible and which were not. The importance of this information and experience was vital to the health and to the tribe and so was passed down from generation to generation. This formed their medicinal love for thousands of years to come. Out of this fundamental knowledge came corpus of herbal knowledge, which was grown continuously to the present day [1].

We are discussing here about a traditional plant Spermacoce ocymoides (Burm F) DC. belongs to the family *Rubiaceae*, Commonly known as "Purple Leaved Button Weed" (also known as "Basil like button weed") which is a vascular plant without significant woody tissue above or at the ground. Forbs and herbs may be annual, biennial or perennial but always lack significant thickening by secondary woody growth and have buds borne at or below the ground surface. It is found in Odisha and through out of India, also found in Cameron, Congo, Gabon, Uganda etc as a wayside weed.

The plant having several folklore and ethnomrdicinal claims which includes: the plant is active against hookworm, ringworm[2], some bacteria like *Strepto coccus, E.coli, Proteus mirabilis* and so having wound healing properties[3,5], also the whole part uses in diarrhea and dysentery[4], leaves are used to treat eczema and skin

problem[3] etc. The literatures revealed that there is no and lack of scientific reports on pharmacognostical, pharmacological and phytochemical evaluation. So, the aim of the present study was designed to evaluate the pharmacognostical and preliminary phytochemical evaluation of the whole plant Spermacoce ocymoides (Burm. F.) DC for its easy identification and future research work.

Plant Profile:
Botanical Name: *Spermacoce ocymoides Burm. F. DC* Classification:-Domain**:** *Eukaryota*, Kingdom: *Plantae* (Plants), Subkingdom: *Viridaeplantae* (Vascular plants), Super division: *Spermatophyta* (Seed plants), Division: *Magnoliophyta* (Flowering plants), Class: *Magnoliopsida* (Dicotyledons), Subclass: *Asteridae*, Order: *Rubiales*, Family: *Rubiaceae*, Sub-family: *Rubioideae*, Genus: Spermacoce, Species: ocymoides; Synonym: *Borreria ocymoides, Borreia ramisparsa, Bigelovia laevicaulis, Borreria laevicaulis, Spermacoce tenera, Spermacoce prostrata, Spermacoce domingensis, Tardaval ocymoides;* English name: Purple Leaved Button Weed, Odiya name: Ganthia Tulasi;

MATERIALS AND METHODS

Collection and Authentication of Plant:
The plant drug was collected from Nandan kanan area, Bhubaneswar, from the campous of Indira Gandhi Institute of Pharmaceutical Sciences, I.R.C village, Nayapalli, Bhubaneswar, and from the periphery of Balasore district, Odisha, India at morning hour and after noon in the month of February when the plant was in flowering stage.

For authentication of the plant drug, Twig containing leaves and flowers were collected. The specimens were disease free with all parts intact without any injuries and were sent to Regional plant Resource centre (R.P.R.C), Bhubaneswar, Odisha, India, another three sent to two registered Ayurvedic physician of Odisha and a botanist of Balangi college, Baleswar, India respectively for proper authentication and the other one was kept in Pharmacognosy Lab. Of Indira Gandhi inst. Of Pharmaceutical sciences, Bhubaneswar. The sample was identified to be *Spermacoce ocymoides Burm F*. DC (*Rubiaceae*) and the authentication certificate was issued by Dr. P.C Panda, sr. scientist, R.P.R.C, Bhubaneswar, Dr. Rashmi Prava Bahali, AYUSH Medical officer, Balikhand PHC(N), Balasore, Dr.Subhransu sekhar Mishra, Registered Ayurvedic medical practitioner, Mr. M. Rehman, Botanist, Department of Botany, Balangi college, Balasore of Odisha, India. After authentication the collected plant materials were shade dried at room temperature and then they are pulverized in mixer grinder to coarsely powdered drug and passed through sieve 60, stored in a well closed container by keeping away from direct sun light for the further use.

Morphological Evaluation
The morphology of *Spermacoce ocymoides (Burm F.) DC*. investigated by taking minimum 50 different plants and plant parts. Micromorphology of leaves and seeds of the palnt also studied under compound microscope. The morphological and micromorphological descriptions are mentioned in result and discussion briefly.

Micro Morphology of Seed
Determination of length and width of the Seeds: The seeds the plant isolated are observed under microscope and the values for the length and width of 50 seeds were calculated and multiplied them by the factor obtained from calibration of eye piece micrometer with stage micrometer. The average value was calculated and snaps are taken for microscopic view.

Micro morphology of Leaves
The leaf lets are taken from the fresh plant and treated with chloral hydrate solution to isolate the chlorophyll pigments and the treated leaflets are washed with water and observed under microscope from different view by adding glycerin water.

Microscopical Evaluation [6-9, 19-23]
Stem microscopy (T.S and L.S of stem): Fresh plants are collected. A piece of stem transversely holds in thermo coat, and then it firmly holed between first finger and thumb of the left hand. The first finger was kept as erect as possible and the thumb was holed down below the surface of the material. Then the razor blade was placed on the top of the thermo coat at right angles and sections were cuts, then the sections are flooded with water. The thin sections were placed in a watch glass containing chloral hydrate solution. It was boiled to clear the sections. Then chloral hydrate was removed, phloroglucinol, safranin solution and concentrated hydrochloric acid were added to stain the sections. One thin section was taken out and mounted on a clean glass slide. A drop of glycerin was added and a cover-slip was hold between the finger and thumb of the left hand and the edge of the cover-slip rested on the slide at the at the left hand edge of the drop. A dissecting needle was inserted under the right hand edge of the cover slip. The cover-slip was lowered slowly on to the drop such a way that the drop of the liquid exactly filled the space between the

slide and the cover-slip without any air bubbles being trapped inside and observed under compound microscope and microscopic photos are taken. Another piece of stem was longitudinally holds in thermo coat and followed the same process for the L.S of stem and observed under microscope.

Root microscopy: Fresh roots of Spermacoce ocymoides are collected and washed with tap water followed by washing with distilled water. The roots were softened by boiling in water for which water soluble components are removed by soaking in water. T.S of roots was prepared by the same method as mentioned in stem microscopy. The thin sections were placed in a watch glass containing chloral hydrate solution. It was boiled to clear the sections. Then chloral hydrate was removed, phloroglucinol, safranin solution and concentrated hydrochloric acid were added to stain the sections. One thin section was taken out and mounted on a clean glass slide. A drop of glycerin was added and covered with the covered slip as mentioned in stem microscopy. This slide was observed under microscope and microscopic photos are taken.

Leaf microscopy: A part of the leaf passing through the midrib was cut. The cut portion of leaf was holds in the thermo coat. The portions of leaf protruding the surface of the pith are cut. The vertical sides of the pith are tapered off upwards. Sections are taken by moving the blade back and forth and placed in watch glass containing water. Thin sections are selected and placed in chloral hydrate and are cleared by boiling. Cleared sections are stained by using phloroglucinol, and concentrated hydrochloric acid followed by adding of safranin solution.. One thin section was taken out and mounted on a clean glass slide. A drop of glycerin was added and covered with the cover slip. The slide was observed under microscope and microscopic photos are taken.

Determination of leaf constants:

Determination of Stomatal number: Stomatal number is the average number of stomata per square mm of the epidermis of the leaf.

A piece of leaf was cleared by boiling with chloral hydrate solution. The upper and lower epidermis was peeled separately. The peeled epidermis was placed on slide and mounted with glycerin water. Camera lucida and drawing board was arranged for the drawings. With the help of stage micrometer 1 sq. mm was drawn. The prepared slide was placed on the stage, epidermal cells and stomata are traced. The no. of stomata lying in the area of 1 sq. mm are counted including the stomata which having at least half of its area lying within the square are taken as one. Average no. of stomata per sq. mm is calculated by tracing four different fields. Stomatal number is affected by various factors like age of the plant, size of the leaf, environmental conditions etc.

Determination of Stomatal Index: Stomatal Index is the percentage which the numbers of stomata form to the total number of epidermal cells, Stomatal index is not much affected by the factors like factors like age of the plant, size of the leaf, environmental conditions etc. It is relatively constant. Hence it is more significant in the evaluation of a leaf drug. Each stomata being counted as one cell.

A piece of leaf was cleared by boiling with chloral hydrate solution. The upper and lower epidermis was peeled separately. The peeled epidermis was placed on slide and mounted with glycerin water. Camera lucida and drawing board was arranged for the drawings. With the help of stage micrometer 1 sq. mm was drawn. The prepared slide was placed on the stage, epidermal cells and stomata are traced. The no. of stomata lying in the area of 1 sq. mm are counted including the stomata which having at least half of its area lying within the square are taken as one. The no. of stomata and the no. of epidermal cells in each field were counted. Stomatal index was calculated using the formula. Stomatal index (SI) = S X 100 /E+S. Where, S= the number of stomata per unit area and E = the number of epidermal cells in the same unit area of leaf. Values for upper and lower epidermis were determined separately.

Determination of vein-islet Number: A vein islet is the small area of green tissue surrounded by the vein-lets. The vein-islet number is the average number of vein-islets per square mm of a leaf surface midway between midrib and margin. It is determined by taking counting the number of vein-islets in an area of 4 sq. mm of the central part of the leaf between the midrib and the margin.

A piece of leaf was cleared by boiling in chloral hydrate solution. Camera Lucida and drawing board was arranged for the drawings. With the help of stage micrometer 1 sq. mm was drawn. The cleared leaf was mounted on the slide and a drop of glycerin water was added then covered with cover slip. The above prepared slide was placed on the stage of the microscope. Veins are traced which are included within the square. The outlines of those islets which overlap two adjacent sides of the square are also traced. The no. of vein-islets in the sq. mm is counted. The islets which are intersected by the sides of square are included on two adjacent sides and excluded on other two sides. The average no. of vein islets number was calculated by taking four different fields of same leaf of one sq.mm from midway between midrib and margin.

Determination of Vein-let termination Number: It is defined as the no. of vein let terminated per sq. mm of the leaf surface, midway between midrib of the leaf and its margin. A vein termination is the ultimate free termination of vein-let. The average number of terminated vein-let islets was calculated by taking four different fields of same leaf of one sq.mm from midway between midrib and its margin by same procedure as determination of vein-islet number.

Powder Microscopy

Powder microscopy of leaves, flowers, roots and whole plant: Dried leaves, dried flowers, dried leaves and dried whole plants were powdered separately and sieved with 40 mesh size sieve to get a fine powder. The fine powders of different parts and whole plants were treated with chloral hydrate solution separately in a beaker and boiled for 10 minutes to clear the powder. Then some amount (one portion) from each were transferred to different watch glasses for each and stained using phloroglucinol, concentrated hydrochloric 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.

Quantitative Microscopy

Determination of average length of trichomes of leaves, stems and whole plant: The eyepiece micrometer was calibrated with stage micrometer e.g eye piece micrometer was placed on the circular diaphragm within the ocular after unscrewing the upper lens. The upper lens was then replaced. The stage micrometer was placed on the stage and focused till the two scales are superimposed and the factor was calculated as shown in below. Then the slide was observed under microscope at low power. The lengths of the different trichomes of powdered leaves and whole plants were measured by focusing them on the lines of eyepiece micrometer and noted. The values for 25 trichomes were calculated and multiplied them by the factor obtained from calibration of eye piece micrometer with stage micrometer. The average value was calculated.

Determination of length and width of fibres of whole plant: The procedure is same as determination of trichome. The lengths and width of the different fibres of powdered whole plants were measured by focusing them on the lines of eyepiece micrometer and noted. The values for the length and width of 25 fibres were calculated and multiplied them by the factor obtained from calibration of eye piece micrometer with stage micrometer. The average value was calculated.

Determination of Physical Constant [9, 10-17, 20]

Loss on drying: The glass stoppered shallow weighing bottles were dried and weighed and 5mg of the powdered drugs were transferred to the Petridis. The Petridis then covered and the Petridis along with the contents were weighed. The loaded Petridis then placed in the hot air oven; the cover removed and left it also in the over. The powdered drugs were then dried to constant weight for 30 min. at a temperature of 105^0C. After drying was completed the hot air oven was opened and the Petridis closed promptly and allowed to cool to room temperature in a desiccator before weighing. The processes were continued until three constant readings were observed. The Petridis and the contents were then weighed and LOD were calculated.[10]

Also moisture content of the plant materials determined by using moisture analyzer (CONTECH) at 105^0 c for 10 min.

LOD= (weight of water in sample) × 100 / (total weight of wet sample)

Extractive value

Petroleum Ether, Chloroform, Methanol and Water Soluble Extractive value: 5gm each of air dried drug coarsely powdered (whole plant part) taken separately in 4 different stoppered conical flask and macerated with 100ml of petroleum ether, Chloroform, Methanol and chloroform water (5:95) respectively with shaking frequently during the first 6hrs and allowing to stand for another 18hrs. Thereafter, they are filtered rapidly taking precautions against loss of solvents and then 25ml of the filtrates were evaporated to dryness in a tarred flat bottom shallow dish, dried at 80^0C and weighed. The percentages of different extractives are calculated with reference to the air dried drug.[11,12]

Ash value [13,14]

Determination of ash values: Residue of the crude drugs after incineration contains mostly inorganic salts known as ash. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. sometimes, inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects

'total ash'. Such variables are then removed by treating with acid and then acid insoluble ash value is determined. Types of ash values are Total ash, Acid insoluble ash, water soluble ash and sulphated ash.

Total Ash: carbon and organic matter present in the drug is converted to ash at temperature 450º C. It mostly contains carbonates, phosphates, silicates and silica.

2 gm of air dried drugs were weighed accurately in a silica crucible and incinerated at a temperature not exceeding 450^0C until free from carbon, cooled and weighed. The residues were collected and the percentages of ash with reference to the air dried drug were calculated.

Water Soluble Ash: Water soluble ash is produced by separating the water soluble materials from the total ash. In this case most of the water insoluble salts which may contribute in the total ash are removed to find out water soluble ash.

The ash were boiled for 5 minutes with 25ml of distilled water and the insoluble residues were collected on an ash less filter paper, washed with hot water, and ignited for 30 minutes at a temperature not exceeding 450ºC. The weight of the insoluble matters were subtracted from the weight of the ash, the difference in weight represents the water soluble ash. The percentages of water soluble ash with reference to the air dried drugs were then calculated.

Acid Insoluble Ash: Total ash may be treated with 2N HCl, which removes many inorganic salts to yield silica from the residue of acid insoluble ash.

The ash were boiled for 5 minutes with 25ml of 2M hydrochloric acid and the insoluble matters were collected in ash less filter papers, washed with hot water, ignited, cooled in a desiccator and weighed. The percentage of acid insoluble ash with reference to the air dried drugs was then calculated.

Sulphated Ash: The sulphated ash test is an analytical test for determining the inorganic content of a sample by weight which are typically metal oxides.

Silica crucibles were heated to redness for 10 minutes allowed to cool in a dessicator and weighed. 2gm of the powdered drugs each were then transferred to the crucible and the contents along with the crucible were weighed accurately. At first these were ignited, until the substances were thoroughly charred. The residues were then cooled and moistened with concentrated sulphuric acid, heated gently until the white fumes are no longer involved and ignited at $800^0 \pm 25^0$C until all black particles have been disappeared. The ignitions were conducted in a place protest from air currents. The crucibles were then allowed to cool, few drops of concentrated sulphuric acid were added and heated. Ignitions were done as before, allowed to cool and weighed. The operations were repeated until two successive weighing do not differ by more than 0.5mg.

Determination of swelling index

1 gram of coarsely powdered plant material (whole plant) taken in a 25 ml glass stoppered measuring cylinder and the internal diameter of the cylinder is about 16 mm. 25 ml of water was added and volume occupied by the sample recorded. The mixture is shaken thoroughly every 10 minute For 1 hour. Allowed to stand for 12 hr at room temperature. Then the volume occupied by the plant material was calculated. Three determinations has carried out by the same process and the mean value of the individual determinations calculated relating to 1 gram of plant materials. [15]

Determination of foaming index

1gram of plant material (whole plant) reduced to a coarse powder. Weigh accurately and transferred to a 500ml conical flask containing 100ml of boiling water. Moderate boiling maintained for 30 min. Cooled and filtered into a 100ml volumetric flask. The decoction poured into 10 test tubes in successive portions into 1 ml, 2 ml, 3 ml and adjusted the volume of the each liquid in each tube up to 10 ml. The tubes were shaken in a lengthwise motion for 15 second and allowed to stand for 15 minutes and the height of the foam measured and the foaming index calculated with the tubes shown to be height of 1cm foam with following formula. Foaming index = 1000/A, (Where A = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed, 1ml of foam indicates to foaming index of 1000.).[15]

pH Determination:

pH of 1% Solution: One gram of accurately weighed each drug was treated with 100ml. of distilled water and filtered. pH of the filtrates were checked with a pH meter having (METTLER TOLEDO) standardized glass electrode.[16]

Powder drug analysis:
Powder drug with chemical reagents: The powder drugs with different chemical reagents shown different colour when seen on naked eye with respect to the nature of chemical constituents present in the powdered material. [9]

Fluorescence Analysis: Many drugs show fluorescence behaviour when they exposed to ultraviolet radiation according to the nature of the phyto-constituents whatever presents in the drugs. It is important to observe all materials on reaction with different chemical reagents under U.V. light. The fluorescence characteristics of powdered drugs were studied under U.V. light after treating with different chemical reagents is described in result and discussion.[9]

Qualatative Phytochemical Analysis
Different extracts obtained from the dried whole plant S. ocymoides by successive solvent extraction by reflux condensation process using petroleum ether, chloroform, methanol, water respectively with increasing order of their polarity are analyzed for different Phytoconstituents present in these by the method of qualitative phytochemical screening. The different chemical tests were carried out like Molisch's test, Benedict's test, Fehling's test, Barfoed's test, Dragendroff's test, Mayer's test, Wagner's test, Hager's test, Ninhydrin test, Biuret test, Million's test, Xanthoproteic test, Test with tannic acid, Test with heavy metals, Spot Test, Saponification test, Keller – Killiani test, Legal test, Baljet's test, Borntrager's test, Test for Cyanogenetic glycosides, modified Borntrager's test, Liebermann – Burchard's test, Salkowski's test, Fluorescence test, Shinoda's test, Foam test etc. [18-20]

RESULTS AND DISCUSSION

Morphology
Spermacoce ocymoides is a dicotyledonous weak, erect annual herb, usually well-branched, 4-50 cm tall, seed bearing multi branched vascular herb with fine fibrous roots; stems with clear and prominent hairs on the quadragonal surface and crisped angle, 2.5 to 11 cm long (fig). Leaf-blades (margin) are entire (fig), ovate-lanceolate (fig), 0.5-5 cm long (fig), 0.3-2.8cm wide (midrib to margin 0.2 to 1.4 shown in fig), smooth and concavely narrowed into the petiole at the base, glabrous on both surfaces with hairy upper surface. Including midrib of leaflet its having 10-11 main vein; petiole 0-7 mm long, with scattered hairs; few and small white flowered clusters found at many of the nodes, 2.5-6 mm in diameter in the month of february, Calyx-tube are transversely oblong and 0.4-0.5 mm long; 2-4 lobes and found to be 0.5-0.8 mm long. Corolla found to be whitish about 0.2-0.3mm long tube with triangular lobes of 0.3 mm long, 0.3-0.4 mm wide. Anthers situated just above the lobes of the corolla. Style and stigma are approximately 0.2 mm long and wide respectively; Fruits are oblong and compressed. Seeds are brown (fig. 1), oblong-ellipsoid, strong, not soften in water within 24 hours and about thousand seeds occupies 6ml in a 25 ml measuring cylinder.

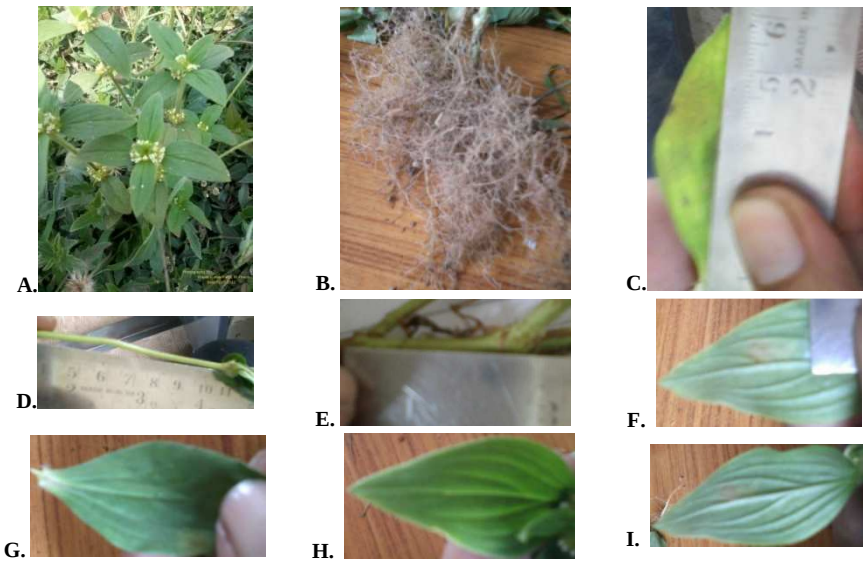


Figure 1. A: Whole plant, B: Roots, C: Max. length of leaflet, D: Max. length of stem, E: Minimum length of stem, F: length from midrib to margin, G: Base of leaf-let, H: Apex of leaf let, I: Veins develops from mid rib.

Micro morphology of the leaves: Leaves of the *S.ocymoides* shown covering trichomes as shown in the fig. . the leaves contain wavy epidermal cells with rubiaceous parallel celled stomata as shown in fig. and also vein islet and terminated veins are shown in fig.2.

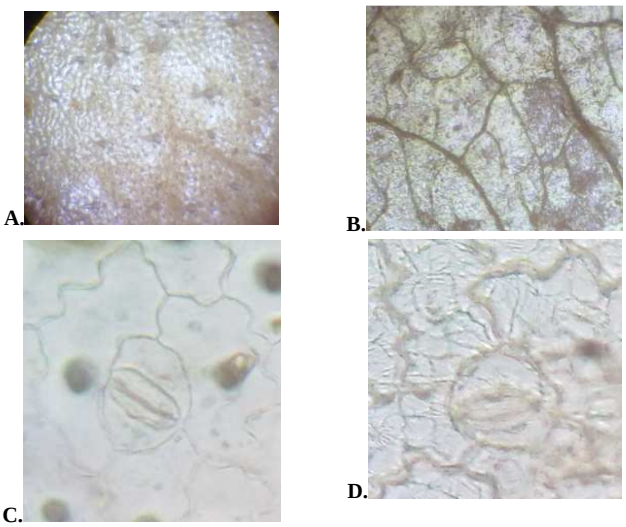
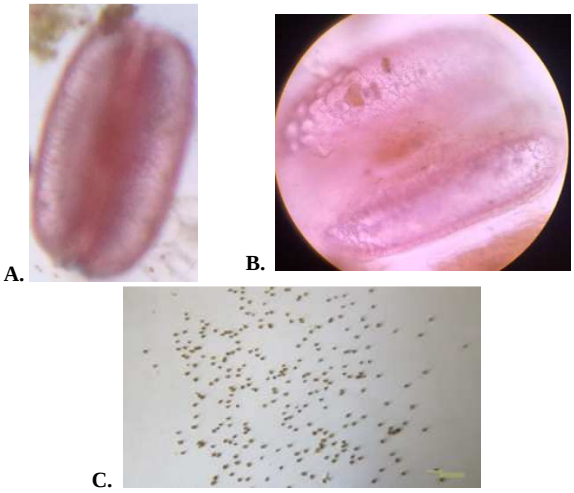


Figure 2. A: stomata on epidermal leaf surface, B: Vein lets and terminated veins, C: stomata with epidermal cells of upper surface, D: stomata with epidermal cells of lower surface.

Micro morphology of the seeds: Seed looks pinkish with safranin solution, Testa appears reticulate at lower magnification but anareolate at higher magnification (fig.3). Meshes areoles polygonal not in regular rows. Wall is thick, margin with meshes. The average length and width are calculated to be431.22 $\pm$ 11.16 µm and 271.46 $\pm$ 6.69 µm respectively. (1 division of eyepiece micrometer = 16.393 µm). Minimum, maximum and average length and width of the seeds are tabulated in table1.



Fifure 3. A: Microscopic view of seed with safranin solution, B: Scanning of seed in 40x, C: seeds in naked eye.

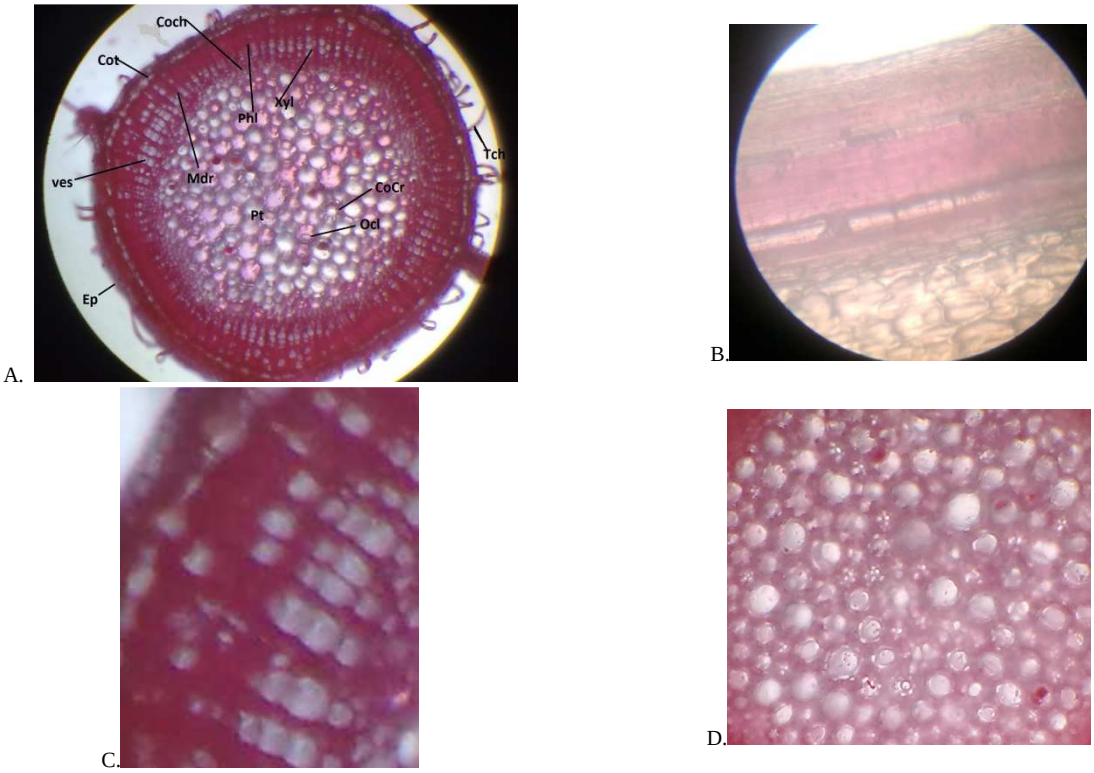
Table: 1

Measurement Of seeds	Minimum (in µm)	Average (in µm)	Maximum (in µm)
Length	295.07	431.22 $\pm$ 11.16	508.18
Width	196.71	271.46 $\pm$ 6.69	311.46

Microscopical evaluation

Stem microscopy (T.S and L.S of stem): T.S of the stem shown (fig.4) lignified tissue, wide pith with thin walled non lignified spongy parenchymatous cell consists of oil cells and calcium oxalate crystals. Covering trichomes are

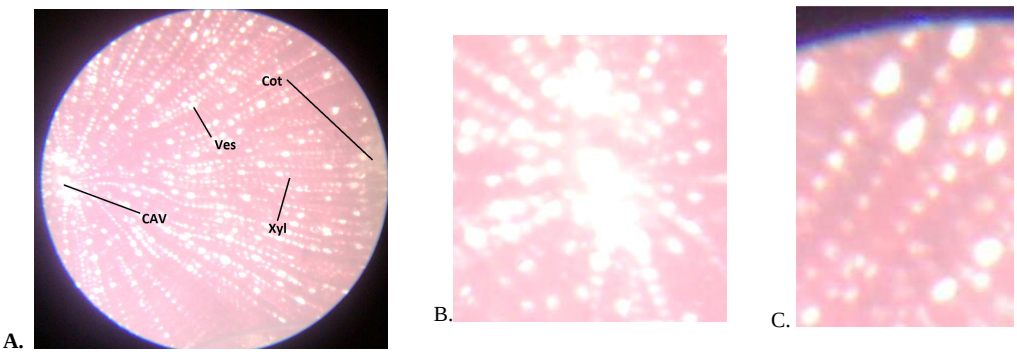
appeared from the epidermis; also it consists of cortex, cortical collenchymatous fibers which separates the cortex and secondary fibres; secondary xylem consists of medium to large multiple vessels; phloems are appeared in group with thick and straight medullary rays towards the pith; the L.S of the stem shown in fig.4



A: T.S of stem; B: L.S of stem; C: xylems with vessels & medullar rays in T.S; D: Pith in T.S

Figure 4. A: Cot: cortex; Coch: Cortical collenchyma; Ves: Vessels; Xyl: Xylem; Phl: Phloem; Tch: Trichomes; Ep: Epidermis; Pt-Pith; Mdr: Medullar rays; CoCr: Calcium oxalate crystal; Ocl: Oil cell.

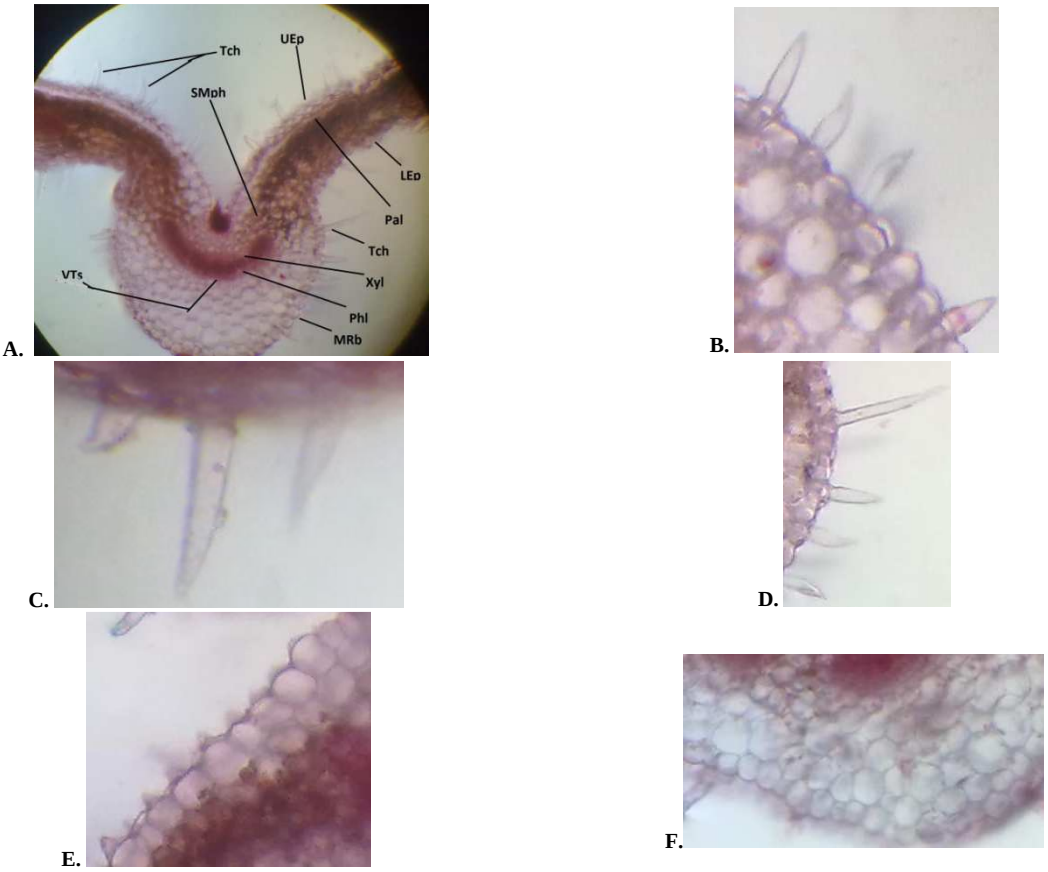
Root microscopy: T.s of the root consists of cork cell, cortex, phloem, vessels, and xylem fibres (fig.5). Xylem consists of vessels and are arranged in a row formed a wavy ray (rays of xylem). The vessels are combined together centrally which leads to a large central vessel. Phloem found in the periphery of cortex.



A: T.S of Root; B: Central aggregated multiple Vessels; C: Xylems with vessels.

Figure.5. A: CAV: Centrally aggregated multiple vessels, Ves: Vessels; Cot: Cortex; Xyl: Xylem

Leaf microscopy: The cells of the 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 phloem. Midrib is uniseriate and cells are pentagonal to hexagonal shape with curved angle and of various size.



A: T.S of leaf; B: Trichomes in upper epidermis; C: Trichomes in lower epidermis; D: Trchomes in midrib; E: Palisade cells in upper epidermis; F: Arrangement of cells in midrib.

Figure 6. A: Tch: Trichomes; SMeh: spongy mesophyles; UEp: Upper epidermis; LEp: Lower epidermis; Pal: Palisade; Xyl: Xylem; Phl: Phloem; MRb: Midrib; VTs: Vascular tissue

Leaf constants

The different leaf constants like stomatal index, stomatal number, vein-islet number and vein let termination number are determined and the data tabulated in table no.2

Table.2

Parameters	Upper Epidermis	Lower epidermis
stomatal number	1130.5 ± 113.93	1666 ± 97.163
stomatal index	7.81 ± 0.443	10.9± 0.344
vein-islet number	410 ± 35.82	-
Vein-termination number	265 ± 10.4	-

Powder microscopy of whole plant: Cork cells, irregular fragment of crystals, rubiaceous parallel celled stomata with wavy epidermal cells, fibres with spiral vessels, fibers with no spiral vessels, covering trichomes, prismatic calcium oxalate crystals, cluster crystals, palisade cells are observed from powder microscopy of the whole plant with occasional finding of free spiral vessels. Crushed seeds are randomly observed with a specific shape and all are shown in fig.

Quantitative microscopy

The average length of trichomes of the dried powdered leaves (149 ± 10.04 µm), stems (228.541 ± 18.203 µm) and whole plant (228.54 ± 18.20 µm) are determined as per the previously mentioned standard methods and the data are tabulated in table.3 **(**1 division of eyepiece micrometer = 12.82 µm**)**

Table.3

Measurement	Plant parts		
	Whole plant	leaves	stems
Average length of trichomes in µm.	228.54 ± 18.20	149 ± 10.04	228.541 ± 18.203

The average length and width of fibres of the powder materials of the plant as whole are determined as per the previously mentioned standard methods and the data are tabulated in table.4 **(**1 division of eyepiece micrometer = 16.393 µm**)**

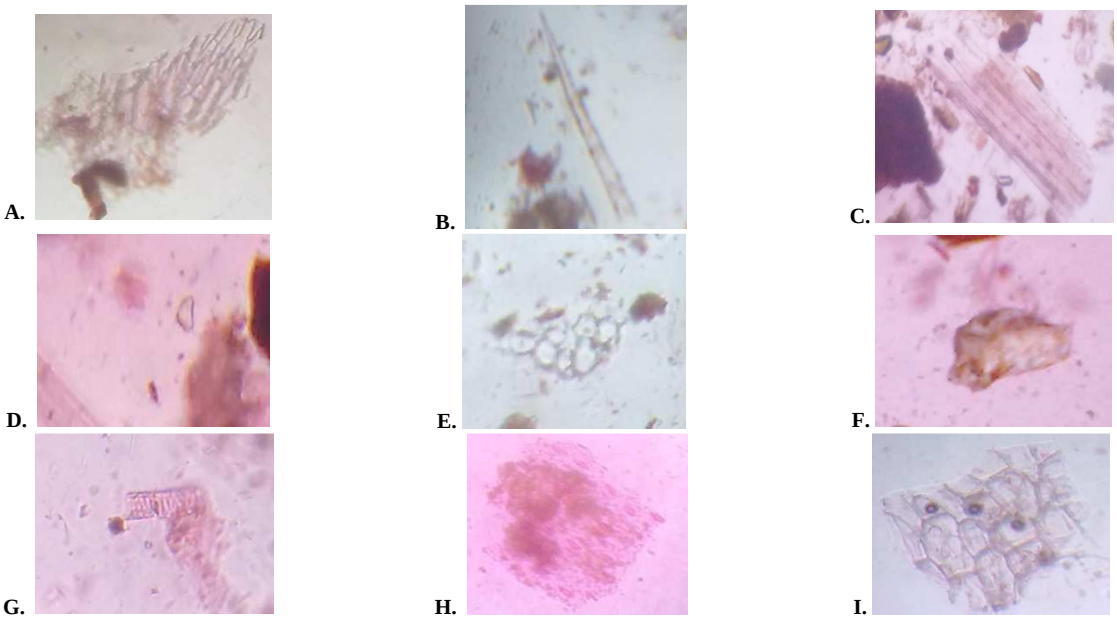


Figure 7. A: Irregular fragment of crystal, B: Covering trichome, C. Fiber with spiral vessel, C: prismatic calcium oxalate crystal, D: Cork cell, E: Cluster crystal, F: Spiral vessel, G: Parallel celled Stomata with wavy epidermal cell, H: palisade cells.

Table.4

Plant part	Measurement	Minimum (in µm)	Average (in µm)	Maximum (in µm)
Whole plant	Length	147.537	320 ± 17.96	606.541
	Width	49.179	120.08 ± 5.73	229.502

Physical constants

Physical evaluation of different parameters has been done by previously mentioned standard method. The colour, odour, taste of the dried whole plant powder are found to be light green, characteristic and tasteless to slightly bitter respectively; Moisture content in leaves, stems, roots and whole plant are found to be 12.15 w/w, 11.4% w/w, 9.2% w/w and 10.2% w/w respectively and data are tabulated in table no.; Extractive values, swelling index, pH (1% and 10% w/v) of dried whole plant are determined and data are tabulated in table no.; The total ash, water soluble ash, acid insoluble ash and sulphated ash of dried plant as whole and leaves powder are determined and data are tabulated in table no.5; Foaming index of different parts of the plants and in whole plant are determined and tabulated in table no.5

Table.5

Sl.no	**Parameters**	**Parts of the plant**			
		Whole plant	**Leaves**	**Stems**	**Roots**
1	LOD (hot air oven) (Moisture analyzer)	10.2% w/w 12.2% w/w	12.1%w/w 14.2%w/w	11.4%w/w 13%w/w	9.2%w/w 10.4%w/w
2	Foaming index	200	200	100	200
3	P^H (1% solution)	5.62	5.3	5.65	5.2

Sl.no	**Parameters**	**Parts of the plant**	
		Whole plant	**Leaves**
4	**Ash Values (w/w)**		
	Total Ash	13.86%	9.06%
	Water Soluble ash	4.68%	3.06%
	Acid Insoluble ash	6.36%	4.15%
	Sulphated ash	9.21%	6.02%

Parts of the plant	Sl.no	Parameters	Values
Whole plant	5	Extractive Values	(w/w)
		Ether soluble	1.66%
		Chloroform soluble	2.66%
		Methanol soluble	10.33%
		Water soluble	15.4%
	6	Swelling index	6.46

Powder drug analysis

The powder drug analysis of plant as whole are analyzed with different reagents in naked eye and under uv light (254 nm). The different colour observation are mentioned in table no.6 and 7 respectively.

Table.6

Reagents	Colour observed
Powder as such	Light Green
Powder + 10% NaOH	Reddish brown
Powder + 50% HCl	Yellowish
Powder + Concentrated HCl	Pale yellow
Powder + Concentrated H_2SO_4	Dark brown
Powder + 50% H_2SO_4	Light amber colour
Powder + 50% HNO_3 solution	Amber colour
Powder + Concentrated HNO_3	Yellowish orange
Powder +5% Ferric chloride solution	Reddish brown

Table.7

Reagents	Fluorescence Observed (nm)
Powder as such	Light Green
Powder + IN NaOH in methanol	Grayish white
Powder + IN NaOH in water	Grayish to yellowish green
Powder + 50% HCl	Dark green
Powder + 50% sulphuric acid	Blackish green
Powder + 50% nitric acid	Darkish green
Powder + petroleum ether	Yellowish orange
Powder + Chloroform	Reddish
Powder + Picric acid	Yellowish green
Powder + 5% Ferric chloride solution	Darkish brown
Powder + 5% Iodine solution	Dark green
Powder + Methanol	Wine like
Powder + (HNO_3 + NH_3)	Very light green

Qualitative phytochemical analysis

From the qualitative phytochemical analysis we got know the plant Spermacoce ocymoides contain reducing sugar, cardiac glycosides, alkaloids, saponin, flavonoids, protein, tannins, triterpinoides etc which are may be responsible for therapeutical activity of the plant as per folklore and ethnomedicinal claim.

CONCLUSION

The phramacognostic profiling of Spermacoce ocymoides (Burm. F) DC is a systematic investigation which will provide sufficient data for the conformation of its identity along with quality and purity of this medicinal herb in future and for the further investigation regarding its chief active constituents.

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