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Phytochemical Investigation and Antimicrobial Activity of *Leucas Cephalotes* Roth. Spreng Whole Herb

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ABSTRACT

Leucas cephalotes Roth. Spreng (Lamiaceae) is the well known herb in the Ayurvedic and Modern systems of medicine, to cure various disorders. Commonly known as Dronpushpi or Guma is mainly a rainy season weed found through out India, The plant is authenticated by Taxonomist Dr. Anjula Pandey and specimen (NHCP/NBPGR 2009-9/900) has preserved in NBPGR, Pusa Campus, Delhi. Powdered plant material analyzed for the two major attributes, Pharmacognostic parameters and Phyto-chemical characterization followed by the quantitative analysis of Tannins and Flavonoids. Antimicrobial activity on the Toluene and Methanolic extract was also performed in which Methanolic extract have shown the 59% and 41% zone of inhibition on the bacterial strains and on the fungal strains resp. when compared to the standards.

Key words: Dronpushpi/Guma, Pharmacognosy, Florescence analysis and Antimicrobial activity.

INTRODUCTION

Leucas cephalotes (Roth) Spreng. Syn. *Phlomis cephalotes* Hindi- Guma, Sanskrit- Dronapushpi (family- lamiaceae) is an erect, scabrous or pubescent, stout annual herb, 30-100 cm high and found as a common weed throughout the India.[1] Leaves are yellowish-green; de ovate or ovate lanceolate, subacute, more or less pubescent. Stem is light greenish yellow, surface rough, hairy, quadrangular with four prominent furrows. Roots are cylindrical, zig-zag, smooth, long with

numerous wiry. Inflorescence is sessile, white, crowded in dence, globose, surrounded by numerous foliaceous bracts. Fruits are schizocarpic carcerule, nutlets smooth and brown. Seeds are oblong, trigonous, smooth, dark brown. [2]

Basically Guma is a rainy season weed and commonly found ascending up to 600 - 1,800 m. in Himalayas, mainly in the hilly regions of Nepal, India, Pakistan, Bhutan and Bangladesh. [3] In the East Asia this plant is found in Afghanistan to Western China in open areas at elevation of 1,700 m. [4] The other species of leucas genus are *Leucas aspera* (willd.) spreng., *L. linifolia* spreng., *L. lanata* Benth., *L. stelligera* Wall., *L. urticaefolia* R. Br. *L. Biflora* (Vahl.) Benth., *L. ciliata* Wall ex Benth., *L. indica* (L.) R. Br., *L. Zeylanica* (L.) R. Br. are commonly found in the Asian Countries. [5, 6]

A decoction of the plant is used in the treatment of malarial fever and a domestic medicine for Snake bite. The dried inflorescences are smoked and the smoke exhaled through the nose to treat nose bleeds. [7, 8] Dried leaves along with tobacco (1:3) are smoked to treat bleeding as well as itching piles and fresh leaves eaten as a potent herb. [1, 3, 9] The juice of leaves used topically in psoriasis, skin eruptions, scabies and internally for the treatment of urinary complaints. The flowers are administered in the form of syrup or with honey as a domestic remedy for cough and colds.[6, 10] Whole herb of Dronapushpi was found to be hepatoprotective in CCl₄ induced hepatotoxicity in mice and rats, juice of this plant act as an antibilious in herbal therapy for Jaundice. [11, 12] The whole plant powder in the proportion of 70% in the herbal composition is patented to cure Epileptic convulsions and Cerebral function disorders. [13] This plant has antipyretic action and also considered to be stimulant, expectorant, aperient, diaphoretic, insecticidal and emmenagogue. [6, 14]

Leucas cephalotes (Roth) Spreng whole herb contains new labdane, norlabdane and abietane type diterpenes and protostane type triterpenes, together with common triterpene, five sterols and eight flavones. [15] A rarely found Laballenic acid (octadeca-5, 6-dienoic acid) and others luaric acid, glutaric acid, adipic acid and tridecanoic acid from the seed oil. [16] A major compound β -sitosterol and its glucoside also isolated from this plant. [17]

The volatile compounds of inflorescence and seeds were found caryophyllene oxide 26.56%; delta-fenchene 12.02%; α -cardinal 2.13%; 1-hepten-3-ol 6.53%; menthol 6.30%; deca hydro naphthalene 5.15% and trans-caryophyllene 4.05%. [18]

MATERIALS AND METHODS:

Collection of Plant Material

The plant material was collected from waste lands of Industrial area of Distt. Haridwar, Uttrakhand. The plant was authenticated as *Leucas cephalotes* (Roth) Spreng. F- Lamiaceae by the Dr. Anjula Pandey (Taxonomist), National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi. A voucher specimen (Specimen No: NHCP/NBPGR 2009-9/900) is preserved in Herbarium section of Taxonomic Deptt. of NBPGR, New Delhi.

Pharmacognostic parameters:**Morphological Characters:**

The macroscopy of a drug includes its visual appearance to the naked eye. Macroscopic identity of a medicinal plant material is based on shape, size, colour, taste, surface characteristic, texture, fracture characteristic and appearance of cut surface. [19]

Microscopical Characters:

The fresh whole herb was boiled with chloral hydrate for several minutes for completely clarified. The size, shape and relative positions of the different cells and tissues, chemical nature of the cell walls and of the cell contents are determined in Microscope. [19]

Foreign Matter Analysis:

The 100 gms sample was weighed then spread in a thin layer and foreign matter was sorted into groups either by visual inspection, using a magnifying lens or with the help of a suitable sieve according to the requirements. The remainder of the sample was sifted through a sieve no. 250; dust regarded as mineral admixture. The portions of sorted foreign matter were weighed. [19]

Crude Fiber Determination:

2 gm of powder drug was taken in a beaker; add 50 ml of 10% v/v HNO₃. Boil with constant stirring. Strain through fine cotton cloth on Buchner funnel and residue washed with boiling water. Transfer the residue from cloth to a beaker and 50 ml of 2.5% v/v NaOH solution was added and heat to boil, maintain at boiling point with constant stirring. Strain and washed the residue with hot water, transfer in cleaned & dried crucible for quantitative determination. Weigh the residue. [20]

Phytochemical Investigation:

The diff. extracts of the whole plant were subjected to preliminary phytochemical screening for the detection of various phytoconstituents:

1. Tests for Alkaloids

a) Dragendroff's test: Dragendroff's Reagent (potassium bismuth iodide) gives reddish brown precipitate.

b) Mayer's test: Mayer's Reagent (Potassium mercuric iodide) gives cream precipitate.

c) Wagner's test: Wagner's Reagent (Iodine in potassium iodide) gives reddish brown precipitate.

d) Hager's test: Hager's Reagent (Saturated picric acid solution) gives yellow precipitate indicated.

2. Tests for Glycosides

a) Keller-killiani Test (for deoxy sugar): 1 ml of glacial acetic acid containing traces of FeCl₃ and 1 ml of conc. H₂SO₄ were added. A reddish-brown colour formed at the junction of two layers and the upper layer turned bluish green.

b) **Legal test (for cardinolides):** Conc. Ethanolic extract was made alkaline with few drops of 10% NaOH and then add freshly prepared Sod. Nitroprusside solution. Blue colouration observed.

c) **Baljet test:** Picric acid is added to the extract and made alkaline, gives a stable orange color.

d) **Borntrager's test:** Dil. HCl is added to powdered drug and heated for 5 mins then filtered and add equal volume of CHCl_3 , shake well and collect the lower org. layer of CHCl_3 , add NH_3 half of its volume, shake well, lower ammonical layer turned rose pink colour.

3. Tests for Saponins

a) **Foam Test:** Powdered drug residue was taken in a test tube and shaken vigorously with a small amount of NaHCO_3 and water. Characteristic honeycomb like froth obtained.

4. Tests for Steroids

a) **Salkowski reaction:** Residue of extract taken in 2 ml of CHCl_3 and 2 ml of conc. H_2SO_4 added from the side of the test-tube and then shaken for few minutes. Red colour developed in the CHCl_3 layer.

5. Tests for Tannins and Phenolic Compounds

a) **Ferric chloride reagent:** FeCl_3 sol. added to test sol. Dark green or deep blue colour is obtained.

b) **Lead acetate test:** A 10% w/v solution of basic lead acetate in distilled water was added to test sol. Precipitation occurred.

c) **Gelatin solution test:** 1% w/v solution of gelatin in water, with 10% sodium chloride and then added to test sol. White precipitate is obtained.

6. Tests for Flavonoids

a) **Shinoda Test:** Test residue dissolved in 5 ml ethanol (95% v/v) and reacted with few drops of conc. HCl and 0.5 g of Mg metal. The pink, crimson or magenta colour is developed.

b) **Ammonia Test:** Filter paper strips were dipped in the alcoholic extract and ammoniated. Strips turned yellow.

7. Tests for Amino Acids & Proteins

a) **Ninhydrin test:** 0.1% w/v solution of Ninhydrine in n-butanol and then added to test sol. A violet or purple colour is developed.

b) **Cysteine test:** To the 5 ml of test sol., 2 ml of 40% w/v NaOH and 2 drops of 10% w/v lead acetate solution, resulting solution is boiled for few minutes to obtain black precipitate.

c) **Biuret test:** Residue taken in water and add 4% NaOH solution then add few drops of 1% CuSO_4 sol. Violet or pink colour is obtained.

d) **Xanthoprotein test (for tyrosine and tryptophan):** Test sol. added with 1ml of conc. H_2SO_4 , white precipitate is observed, boil and add NH_4OH and precipitate turns yellow.

e) **Millon's test (Mercuric Nitrate solution):** Aq. residue taken with 5 ml of Million's reagent. White precipitate gently warmed to turns pink or red.

8. Tests for Sugars

- a) **Molisch test:** Test solution with few drops of α -naphthol and then conc. H_2SO_4 . Violet colour ring was formed at the junction of two layers.
- b) **Fehling's test:** Fehling A and Fehling B solution were mixed in equal amount and boiled then added with test solution and again heated. Yellow to brick red colour.
- c) **Benedict's test:** Test solution and Benedict's reagent was mixed and heated in water bath for 5-10 minutes. Yellow to red or green colour.
- d) **Tollen's phloroglucinol test for galactose:** 2.5 ml of conc. HCl and 4 ml of 0.5 % phloroglucinol added to test sol. then heated on water bath. Yellow to red colour. [21]

Fluorescence Analysis

Fluorescence analysis of the drug was conducted on the powder to observe diff. types of fluorescent characteristics. Subsequently, powdered sample was treated with 1-N Sodium hydroxide in Methanol, 1-N Sodium hydroxide in water, 50% Sulphuric acid, 50% nitric acid, Different solvents and observed under day light, short wavelength UV and long wavelength UV light. The observations shown in Table no. 03. [22]

Quantitative Determination:

Any estimation method applied to plant tissue should allow determination of a particular compound or of a group of compounds in weight percent.

Total Flavonoids Content:

10 g of the powdered crude sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. [20]

Total Tannins Content:

1 gm of sample/crude powdered drug was extracted with 100 ml of water by shake for one hour and left for overnight, allowed the solid material to settle and filtered the liquid through a Whatman no. 1 filter paper, the first 20 ml of filtrate was discarded and then 10 ml of the filtrate taken in to conical flask. To it 750 ml of water and 25 ml of Indigo sulphuric acid sol. was added and titrated with 0.1 N $KMnO_4$ solution. The flask was shaken vigorously till a golden yellow end point was reached. A blank determination was also performed and percentage calculated. Each ml of $KMnO_4$ solution is equivalent to 0.004157 gm of total tannins. [23]

Antimicrobial activity

The fresh whole plant of *Leucas cephalotes* were collected and shade dried at room temperature for 2 weeks. Dried part then stored at 20°C before using them individually to solvent extraction procedures. The antimicrobial activity was tested against different bacterial and fungal strains which were obtained from Arbro Industrial Testing Lab., Kirti Nagar, New Delhi. The microbial strains with their laboratory registration number were: *Bacillus cereus* (ATCC 11778), *Shigella flexneri* (MTCC 1457), *Candida albicans* (MTCC 227). The Cup Plate method was used to test the antimicrobial activity in present investigation ie adopted from Rose & Miller 1939. The antibacterial activity of plant extract was tested on **Assay Medium no. 11 (No. 11) (Himedia-**

M004) and antifungal activity was tested on **Sabouraud Dextrose Agar (SDA) (Himedia-MU063)**. The No. 11 & SDA plates were prepared by pouring a few ml of molten media into sterile Petri plates. The plates were allowed to solidify for 5 min and appropriate amount of inoculum's suspension (0.1 %) was swabbed uniformly on agar medium. A sterile disc was placed on the surface of medium and an appropriate concentration of extracts (5 mg/disc) was loaded on it. The extract was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 18-20 h. Inhibition zones formed around the disc were measured with transparent ruler in millimeter (mm). **Gentamycin** (10 µg/ml), **Amikacin** (10 µg/ml) and **Nystatin** (100 µg/ml) were used as standard for antibacterial and antifungal activity testing resp. [24, 25]

RESULTS AND DISCUSSIONS

Pharmacognostic Parameters:

Macroscopy:

Macroscopic characters are useful in the identification of Organoleptic features such as colour, odour, taste, size, shape, touch and fracture were analyzed on the different plant parts. Stems were usually stout, slightly fibrous and light green, odourless and slightly bitter in taste. Leaves were ovate, serrate, dark green, odourless and having pungent taste. Flowers were white in colour, sessile, globose, surrounded by foliaceous bracts, odourless and tasteless. Roots are tapering, tortuous, fibrous nature, odourless and having characteristic taste. These characters shown in Table No. 01 and Figure No. 1 & 2.

Microscopy:

The TS of stem, leaf and root were observed and different histological parameters determined under microscope.

A: Transverse Section of Stem (Figure. 3)

TS of Stem found to be quadrangular and having outlined ridges covered with thick cuticle and with cortex parenchymal cells forms 3 to 7 rows. Vascular bundles are bicollateral and found with in the ridges. Xylem fully developed, contains trachides and parenchyma. Phloem contains narrow circles surrounded by xylem vessels and fixing towards the pith cells. Pericycle and endodermis is present and outer layer consist diff. types of trichomes such as single cell, double cell, covering and glandular trichomes. Pith cells are collenchymatous and contain prismatic shaped crystals.

B: Transverse Section of Leaf (Figure. 4)

TS of midrib of leaf contain diff. types of trichomes in a big mass ie glandular, non-glandular and multilayered with the single layered palisade cells. Vascular bundles are bicollateral, mesophyll cells are parenchymatous and central part is collenchymatous. Midrib contains deep groove on upper side and wide ridges on lower side.

C: Transverse Section of Root (Figure. 5)

TS of root contain out line of thick walled epidermis cells in 1-3 layers, very few simple trichomes are present, cortex parenchymatous cells with 8-10 layered, tangentially elongated. Endodermis elongated 4-6 layered parenchymatous cells, phloem consist of sieve tubes,

companion cells and parenchyma, secondary xylem is present with xylem vessels, fibres and parenchyma. Centered pith contains xylem parenchyma to distal end of the root.

Phytochemical Analysis:

The diff. extracts subjected to various phytochemical tests, to identify the active constituents, which show in table no. 2. By this analysis, Ethanolic and Methanolic extracts found to have more active constituents compare to other extracts. Phytochemicals such as Carbohydrate, Proteins & amino acids, Glycosides, Flavonoids, Tannins & Phenols and Steroids are commonly found in most of the extracts.

Quantitative Determination:

Qualitative phytochemical investigations show that whole plant of *Leucas cephalotes* contains tannins & flavonoids in excess amount. Therefore the quantitative amount of tannins & flavonoids estimated. The percentage amounts of these are shown in table no. 5. From the quantitative analysis we found presence of Flavonoids & Tannins in whole herb of Guma. These are main Phytoconstituents are responsible for various therapeutic effects of *Leucas cephalotes* whole herb.

Antimicrobial screening

The results of antimicrobial activity experiments are presented in Table 2. In these experiments, the Methanolic extract of whole herb showed highest activity against *Bacillus cereus* and *Shigella flexineri* but found negative against *Candida albicans*. Another Toluene extract of the plant found totally negative against all the strains.

Figure 1: *Leucas Chephalotes* whole herb

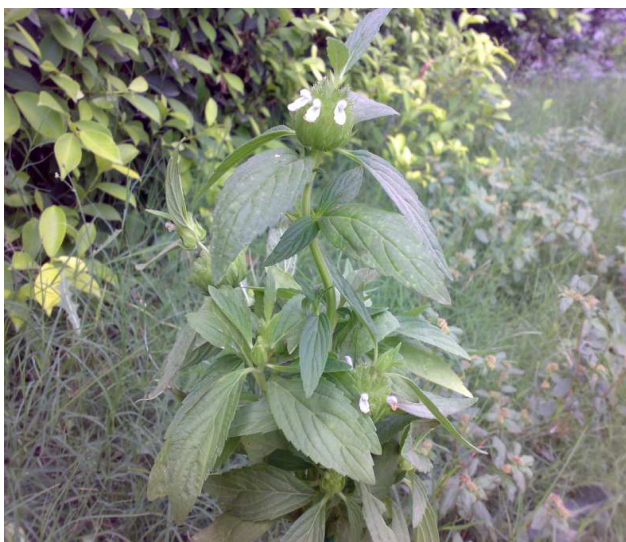
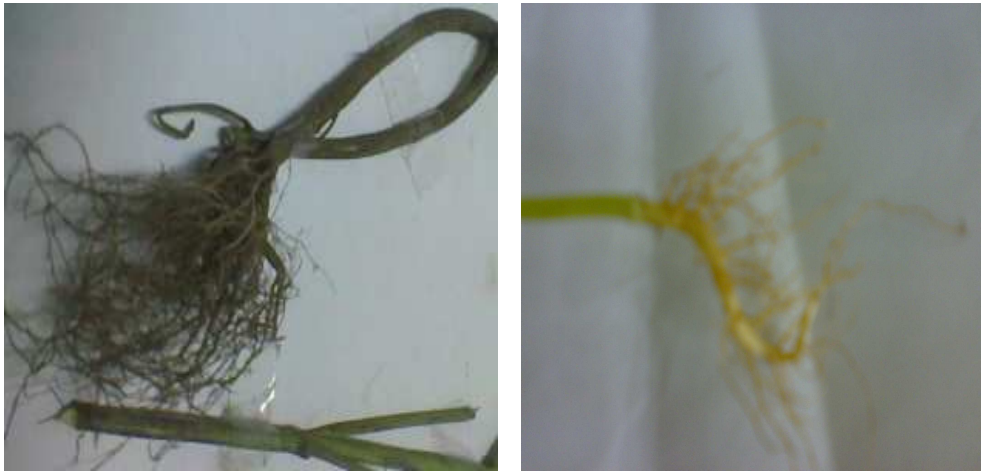
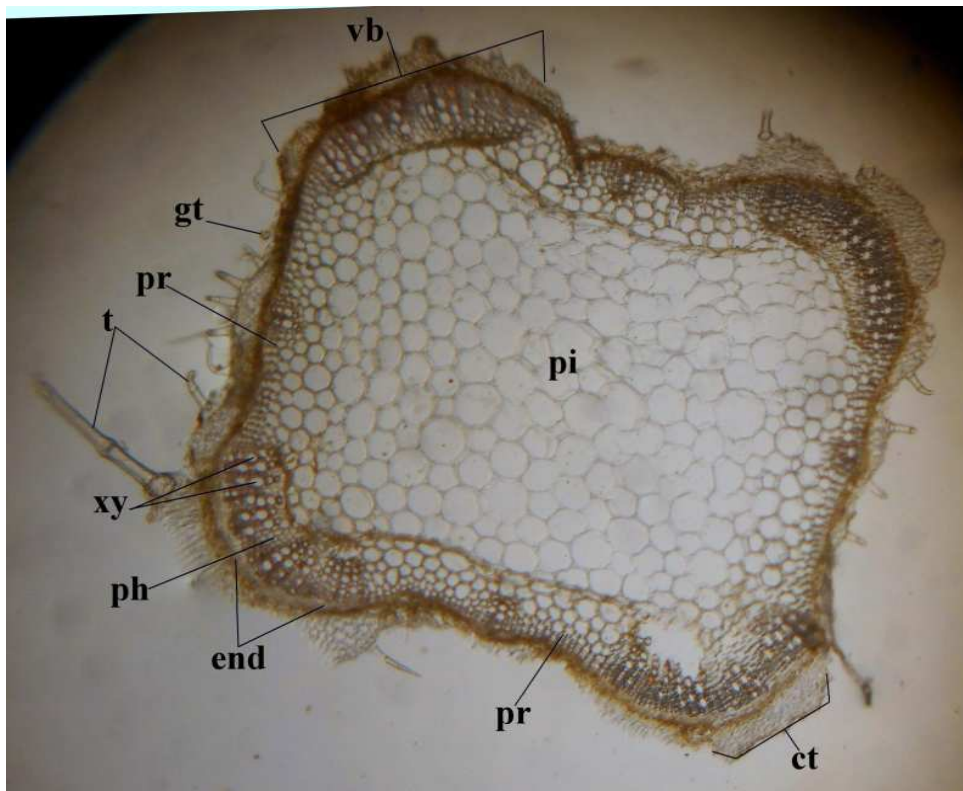


Figure 2: *Leucas Chepalotes* roots



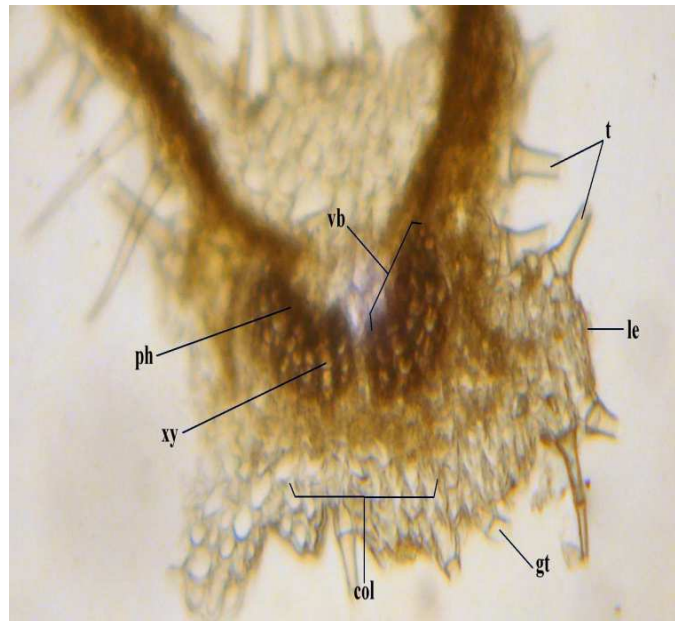
Microscopic characterization

Figure 3. T. S. of stem



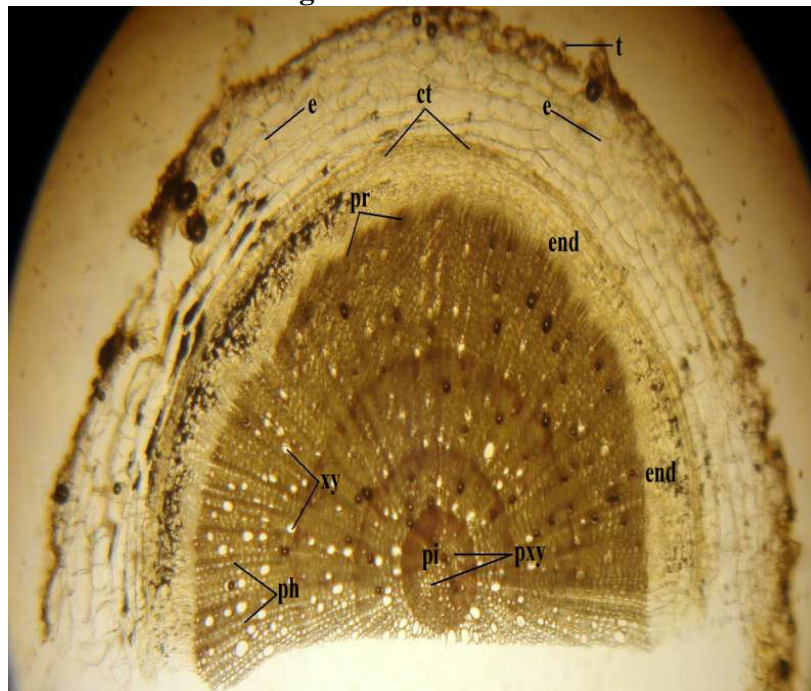
(ct, cortex; end, endodermis; gt, glandular trichome; ph, phloem; pi, pith; pr, pericycle; t, trichome; xy, xylem; vb, vascular bundles)

Figure 2. T. S. of leaf midrib



(col, collenchymas; le, lower epidermis; gt, glandular trichome; ph, phloem; pr, pericycle; t, trichome; vb, vascular bundles xy, xylem)

Figure 3. T. S. of root



(ct, cortex; end, endodermis; e, epidermis; ph, phloem; pi, pith; pr, pericycle; t, trichome; xy, xylem; pxy, primary xylem)

Table 1. Observations of morphological characters

S. No.	Parameters	Whole plant Parts			
		Stem	Leaf	Root	Flowers
1.	Colour	Externally- Light green Internally- Cream	Dark Green	Externally- Light Brown Internally- Cream	White
2.	Odour	No	No	No	No
3.	Taste	Slightly Bitter	Pungent	Characteristi c	Tasteless
4.	Size	0.3-0.5 in diameter	3.5-6.6 long; 1.2- 2.4 wide	7.5-14.2 cm long ; 0.3- 0.6 cm thick	Small
5.	Shape	Stout	Ovate; Serrate	Tapering; Tortuous	Sessile; Globose
6.	Touch	Plain	Smooth	Smooth Plain	Smooth
7.	Fracture	Slightly Fibrous	–	Fibrous	–

Table 2. Quantitative Observations

Parameters	Result
Foreign Matter	0.336%
Crude Fiber	26.91%
Total Flavonoids	8.07%
Total Tannins	0.1%

Table 3. Observations of Fluorescence Analysis

S.No.	Materials / Treatments	Observations Under UV Cabinet		
		Day Light	At Short Wavelength	At Long Wavelength
1.	Drug Powder	Brownish green	Green	Dark Green
2.	Drug powder rubbed on filter paper	Light Brown	Light green	Green
3.	Powder treated with 1 N NaOH in Methanol	Yellowish green	Light green	Yellow
4.	Powder treated with 1 N NaOH in Water	Brownish green	Florescent green	Green
5.	Powder treated with 1 N HCl Solution	Colourless	Colourless	Light green
6.	Powder treated with 50% HNO ₃	Yellow	Light florescent green	Dark green
7.	Powder treated with 50% H ₂ SO ₄	Light brownish green	Light florescent green	Light green
8.	Methanolic Extract	Yellowish green	Florescent green	Yellow
9.	Water Extract	Brown	Florescent green	Light flor. green
10.	Chloroform Extract	Green	Yellowish Brown	Green
11.	Pet. Ether Extract	Yellow	Florescent green	Orange

Table 4. Phytochemical Analysis

Phytochemicals	Extracts					
	PE	T	CH	Et.	Mt	W
Alkaloids	-	-	-	-	-	-
Glycosides	-	-	-	+	+	+
Saponins	-	-	-	-	-	-
Steroids	+	+	+	+	+	-
Tannins & Phenols	-	-	-	+	+	+
Flavonoids	-	-	-	+	+	+
Amino acid & Proteins	-	-	-	+	+	-
Carbohydrates	-	-	+	+	+	+

Legends: + Positive, - Negative, PE Pet ether, T Toluene, CH Chloroform, Et Ethanol, Mt Methanol, W Water.

Table 5. Observations of Antimicrobial Activity

S.No	Micro Organism	Standards	ZONE OF INHIBITION (mm) ^a		
			Methanol Extract (In DMSO)		Toluene extract (In DMSO)
			Std	Test	Test
1	<i>Bacillus cereus</i> (ATCC 11778)	Amikacin	17.0	10.0	-
2	<i>Shigella flexineri</i> (MTCC 1457)	Gentamycin	28.0	11.5	-
3	<i>Candida albicans</i> (MTCC 227)	Nystatin	23.0	-	-

^a an average of triplicate, - Negative.

CONCLUSION

This plant has a great importance for the Ayurvedic and Modern systems of formulations in which some have been patented for their better acceptability and therapeutic profile. Present study of the Phytochemical & pharmacognostical investigation may serve as new standards to the region specific sample of the plant and key structure for validation of crude plant material and regarding to its future work on Standardization. The positive Antimicrobial activity shows that whole herb extract can be useful for the formulation development against the specific microbial infections.

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