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Preliminary phytochemical screening and antimicrobial evaluation of *Leonotis nepetifolia*(L.) R.Br.

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ABSTRACT

The present paper focuses on the phytochemical screening and antimicrobial activity of *Leonotis nepetifolia* (Lamiaceae). In the present study the Petroleum ether, Ethyl acetate and methanol extracts of leaves, stem, inflorescence and root of *Leonotis nepetifolia* were studied for their antibacterial activity by agar disc diffusion against both gram positive, gram negative and one fungal strain. It was observed that alcoholic extracts of leaf showed highest antimicrobial activity against the pathogenic microorganisms followed by inflorescence, root and stem extracts. Phytochemical examination indicated the presence of alkaloids, saponins, tannins and terpenoids.

Key words: *Leonotis nepetifolia*, antibacterial potential, phytochemicals, solvent extracts.

INTRODUCTION

Medicinal plants are known to provide a rich source of raw materials for traditional medicine for the treatment of various ailments since time immemorial. According to World Health Organization¹, 80% people in developing countries particularly those living in villages still depend on traditional plant based medicines principally due to their low price².

Leonotis nepetifolia (Lamiaceae) is an important herbaldrug, which has been used to treat bronchial asthma, diarrhoea, fever, influenza, malaria and is also an analgesic. Whole plant is used for menstrual pain and unspecified female complaints⁸. The plant has been evaluated for its hypotensive potential⁹, anti-inflammatory activity¹⁰ and anti-plasmodial activity¹¹. Methanol extracts of aerial parts has been shown to be significant active in brine shrimp lethality test¹³. This plant exhibited various biological activities such as antifungal and antibacterial activities¹². It has relaxing effect on rat and guinea-pig smooth muscle and rat cardiac muscle¹⁴. Phytochemical studies on this plant revealed presence of iridoid glycosides, phenylethanoid glycosides, labdanoid diterpenoids and coumarins in stem¹⁵, diterpenoids from leaves^{16, 17}, seed oil contains laballic acid, a new allelic acid¹⁸. Very few works regarding the antibacterial activity of *Leonotis nepetifolia* are there^{3,4}. However, detailed investigation of the antibacterial activity of this plant has not been carried out. The present study describes the invitro susceptibility of bacteria isolates to different parts of the plant.

MATERIALS AND METHODS

Plant material:

The specimens *Leonotis nepetifolia* were collected from the chittoor district, Andhra Pradesh, identified with the help of regional and local floras and the voucher specimens were deposited at Sri Krishna Deveraya University Herbarium (SKU), Anantapur. The plant material was washed with water and shade dried, pulverized in mechanical grinder and stored in an airtight container till further successive extractions.

Preparation of extracts:

The samples (leaf, stem, inflorescence and root) collected in bulk quantities for conducting preliminary phytochemical screening and antimicrobial activity. The samples were shade dried, powdered and successively extracted with petroleum ether, ethyl acetate and methanol solvents using soxhlet apparatus for six hours¹⁸. The extracts were filtered and concentrated under reduced pressure to dryness.

Preliminary phytochemical screening

Preliminary phytochemical screening was performed to identify phytochemicals in various solvent extracts of *Leonotis nepetifolia*⁵. The extracts were subjected for phytochemical screening using standard procedures.

Test for antibacterial activity

For the bioassay, eight bacteria and one fungal strain were used. Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumonia*, gram-positive bacteria: *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus* and one fungal strain *Candida albicans*. The microbial strains were obtained from the Microbial Type Culture Collection centre (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. *In vitro* antimicrobial activities of the extracts were evaluated by disc diffusion method. The extracts were diluted in dimethylsulphoxide (DMSO) at the concentrations of 25mg/ml, 50mg/ml and 75mg/ml. The diameters of inhibition zone were measured.

RESULTS AND DISCUSSION

Phytochemical tests indicated the presence of alkaloids, steroids, saponins, tannins, flavanoids, coumarins, volatile oils, glycosides, terpenoids, phenols and glycosides (Table 1). The leaf and inflorescence shows maximum phytochemicals.

Table 1: Preliminary phytochemical screening

Compounds	Leonotis nepetifolia											
	Leaf			Stem			Inflorescence			Root		
	Pe	Ea	Me	Pe	Ea	Me	Pe	Ea	Me	Pe	Ea	Me
Alkaloids	-	-	+	-	-	+	-	+	+	-	-	+
Anthocyanins	-	-	-	-	-	-	-	-	-	-	-	-
Anthocyanidins	-	-	-	-	-	-	-	-	-	-	-	-
Anthracenoglycosides	-	-	-	-	-	-	-	-	-	-	-	-
Antraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Aucubins	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates	-	-	+	-	-	-	-	+	+	-	-	+
Carotenoids	-	-	-	-	-	-	-	-	-	-	-	-
Catacholic compounds	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	-	+	+	-	-	-	-	+	+	-	-	+
Dihydrochalcones	-	-	-	-	-	-	-	-	-	+	-	-
Emodins	-	-	-	-	-	-	-	-	-	-	-	-
Flavanoids	-	+	+	-	-	+	-	+	+	-	+	+
Flavonols	-	-	-	-	-	-	-	-	-	+	-	-
Flavonones	-	-	-	-	-	-	-	-	-	-	-	-
Flavones	-	-	+	-	+	-	-	-	+	-	-	-
Fattyacid	-	-	-	-	-	-	-	-	-	-	-	-
Gallic tannins	-	-	-	-	-	-	-	-	-	-	-	-
Iridoids	-	+	-	-	+	-	-	+	-	-	-	-
Lignins	-	-	+	-	-	-	-	-	+	-	-	+
Phenols	-	+	+	-	-	+	-	+	+	-	+	+
Proteins	+	+	+	-	+	-	+	-	+	-	+	+
Reducing compounds	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	+	+	-	-	+	-	-	+	-	-	+
Steroids	+	+	+	+	+	+	+	+	+	-	+	+
Tannins	-	+	+	-	-	+	-	-	+	-	-	+
Triterpenoids	+	-	+	-	-	+	-	-	+	-	-	-
Volatile oils	+	-	-	-	-	-	+	-	-	+	-	-
Glycosides	-	-	-	+	+	+	+	+	+	+	-	-
Polyoses	-	-	-	-	-	-	-	-	-	-	+	-

Pe: petroleum ether, Ea: ethyl acetate, Me: methanol

The antibacterial activity of the crude solvent extracts of the *Leonotis nepetifolia* was determined against 8 bacterial strains and one fungal strain, which are reported in Table 2-5.

From the above results it can be concluded that leaf extracts followed by inflorescence, root and stem have great potential with antibacterial compounds against pathogenic microorganisms and that they can be used in the treatment of infectious diseases caused by such microorganisms. *Leonotis nepetifolia* showed maximum antibacterial activity, hence can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals

Table 2: Antibacterial activity of the crude Leaf extracts

Organism	Inhibition Zone (mm ⁻¹)								
	Petroleum ether			Ethyl acetate			Methanol		
	Mg/ml								
	25	50	75	25	50	75	25	50	75
<i>P.aeruginosa</i>	-	-	-	-	-	-	6	7	8
<i>M.luteus</i>	-	-	-	-	-	-	8	9	10
<i>B.subtilis</i>	-	-	-	8	8	9	11	12	13
<i>K.pneumonia</i>	6	6	7	7	10	11	12	13	15
<i>S.typhi</i>	6	7	8	6	9	10	10	11	11
<i>S.aureus</i>	-	-	-	6	9	9	13	15	18
<i>B.cereus</i>	6	7	8	7	8	8	10	11	12
<i>E.coli</i>	6	8	9	7	9	10	15	16	17
<i>C.albicans</i>	8	9	10	6	7	8	10	11	12

P.aeruginosa: *pseudomonas aeruginosa*, *M.luteus*: *Micrococcus luteus*, *B.Subtilis*: *Bacillus subtilis*, *K.pneumonia*: *Klebsiella pneumonia*, *S.typhi*: *Salmonella typhi*, *S.aureus*: *Streptococcus aureus*, *B.cereus*: *Bacillus cereus*, *E.coli*: *Escherichia coli*, *C.albicans*: *Candida albicans*

Table 3: Antibacterial activity of the crude Stem extracts

Organism	Inhibition Zone (mm ⁻¹)								
	Petroleum ether			Ethyl acetate			Methanol		
	Mg/ml								
	25	50	75	25	50	75	25	50	75
<i>P.aeruginosa</i>	-	-	-	-	-	-	-	-	-
<i>M.luteus</i>	-	-	-	-	-	-	-	-	-
<i>B.subtilis</i>	-	-	-	-	-	-	6	7	8
<i>K.pneumonia</i>	-	-	-	9	9	10	7	8	9
<i>S.typhi</i>	6	7	8	-	-	-	6	7	8
<i>S.aureus</i>	-	-	-	-	-	-	9	10	11
<i>B.cereus</i>	-	-	-	8	9	11	8	9	10
<i>E.coli</i>	6	7	8	-	-	-	7	8	9
<i>C.albicans</i>	-	-	-	7	8	9	-	-	-

P.aeruginosa: *pseudomonas aeruginosa*, *M.luteus*: *Micrococcus luteus*, *B.Subtilis*: *Bacillus subtilis*, *K.pneumonia*: *Klebsiella pneumonia*, *S.typhi*: *Salmonella typhi*, *S.aureus*: *Streptococcus aureus*, *B.cereus*: *Bacillus cereus*, *E.coli*: *Escherichia coli*, *C.albicans*: *Candida albicans*.

Table 4: Antibacterial activity of the crude Inflorescence extracts

Organism	Inhibition Zone (mm ⁻¹)								
	Petroleum ether			Ethyl acetate			Methanol		
	Mg/ml								
	25	50	75	25	50	75	25	50	75
<i>P.aeruginosa</i>	-	-	-	-	-	-	10	12	14
<i>M.luteus</i>	-	-	-	-	-	-	12	13	15
<i>B.subtilis</i>	5	6	7	6	7	8	6	7	8
<i>K.pneumonia</i>	6	9	10	8	9	10	9	10	15
<i>S.typhi</i>	6	7	8	6	7	8	7	9	10
<i>S.aureus</i>	6	6	7	7	7	9	14	15	17
<i>B.cereus</i>	-	-	-	9	11	12	10	11	12
<i>E.coli</i>	-	-	-	6	7	8	9	10	12
<i>C.albicans</i>	6	7	8	7	8	9	7	8	9

P.aeruginosa: *pseudomonas aeruginosa*, *M.luteus*: *Micrococcus luteus*, *B.Subtilis*: *Bacillus subtilis*, *K.pneumonia*: *Klebsiella pneumonia*, *S.typhi*: *Salmonella typhi*, *S.aureus*: *Streptococcus aureus*, *B.cereus*: *Bacillus cereus*, *E.coli*: *Escherichia coli*, *C.albicans*: *Candida albicans*.

From this investigation, the results obtained confirmed the therapeutic potency of *Leonotis nepetifolia* used in traditional medicine. Moreover, these results establish a good basis for selection of the plant for further phytochemical and pharmacological investigation. The results of the present study support of the folklore usage of the studied plant and recommends that the plant extract contain certain constituents with antibacterial properties that

can be utilized as antibacterial agents in new drugs for the therapy of infectious disease caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antibacterial and carry out further pharmacological evaluation.

The results showed that the test principle contained phytochemicals of medicinal importance. It might be expected that these constituents were responsible for the antibacterial activity exhibited and it supports the use of the plant in the treatment of various bacterial infections. More research is required for clinical trials, toxicity tests and isolation of active component for possible use in the treatment of various diseases.

Table 5: Antibacterial activity of the crude Root extracts

Organism	Inhibition Zone (mm ³)								
	Petroleum ether			Ethyl acetate			Methanol		
	Mg/ml								
	25	50	75	25	50	75	25	50	75
<i>P.aeruginosa</i>	-	-	-	-	-	-	-	-	-
<i>M.luteus</i>	-	-	-	-	-	-	8	9	10
<i>B.subtilus</i>	-	-	-	8	8	9	11	12	13
<i>K.pneumonia</i>	6	6	7	7	10	11	10	11	12
<i>S.typhi</i>	6	7	8	7	9	10	6	7	8
<i>S.aureus</i>	-	-	-	6	-	-	7	8	9
<i>B.cereus</i>	6	6	7	7	8	9	10	11	12
<i>E.coli</i>	8	9	10	7	9	10	9	10	11
<i>C.albicans</i>	6	7	8	6	7	8	10	11	12

P.aeruginosa: *pseudomonas aeruginosa*, *M.luteus*: *Micrococcus luteus*, *B.Subtilus*: *Bacillus subtilus*, *K.pneumonia*: *Klebsiella pneumonia*, *S.typhi*: *Salmonella typhi*, *S.aureus*: *Streptococcus aureus*, *B.cereus*: *Bacillus cereus*, *E.coli*: *Escherichia coli*, *C.albicans*: *Candida albicans*.

CONCLUSION

Although, a number of antibiotics are widely used in medicine, the search for antimicrobial substances from plants will continue as better and safer drugs to combat with multi-drug resistant microbial infections.

Present study of *Leonotis nepetifolia* with various extracts demonstrated pronounced activity against deadly pathogenic bacteria (Gram + and – negative strains) and on fungal strain.

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