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Research Article

ANTIBACTERIAL POTENTIAL OF CRUDE METHANOLIC EXTRACT OF *Leonotis nepetifolia* (L) R. Br

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ABSTRACT
The methanolic extract of leaf, flower and stem of *Leonotis nepetifolia* was analyzed for antibacterial activity against eight pathogenic bacterial isolates viz., *Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Shigella flexneri, Vibrio cholerae, Bacillus subtilis, Micrococcus luteus* and *Saphylococcus aureus*. The extract was also subjected to qualitative tests for alkaloids, tannins, terpenoids, steroids, saponins and flavonoids. The methanolic extract exhibited antibacterial activity against all the bacteria tested indicating the presence of broad spectrum antibacterial compounds in the plant parts. Gram positive bacteria were found to be more susceptible than Gram negative bacteria. The leaf and flower extract of the plant showed higher activity than the stem. Phytochemical examination indicated the presence of alkaloids, saponins, tannins and terpenoids.
Key words: *Leonotis nepetifolia*, antibacterial potential, phytochemicals, methanolic extract

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 Organization[1], 80% people in developing countries particularly those living in villages still depend on traditional plant-based medicines principally due to their low price [2].

Leonotis nepetifolia (Lamiaceae) is an important herbal drug which has been used to treat bronchial asthma, diarrhoea, fever, influenza and malaria and is also an analgestic. This plant exhibited various biological activities such as antifungal and antibacterial activities. Phytochemical examination of this plant parts indicated the presence of different diterpenoids of labdone type, besides the presence of other bioactive compounds. 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 *in vitro* susceptibility of bacterial isolates to different parts of the plant.

MATERIALS AND METHODS

Plant material

Leonotis nepetifolia (Lamiaceae) plants were collected from Bhandara Road, Maharashtra State in December, 2010. The plant was identified by Prof. G. G. Maity, Department of Botany, University of Kalyani (voucher specimen number SNSLN 2). The dried leaf, stem and flowers were homogenized to fine powder and further subjected to extraction.

Preparation of extract

The crude methanol extract was obtained by extracting 10 g of different parts of dried plant powder in 100 ml methanol and kept on a rotary shaker for 24 h. The extract was filtered, centrifuged at 5000 g for 15 min and was dried under reduced pressure. The extract was stored at 4^0C in air tight container.

Phytochemical studies

The samples were subjected to phytochemical screening for the detection of alkaloids, tannins, terpenoids, steroids, saponins, flavonoids etc using the method of Harborne[5].

Test for antibacterial activity

For the bioassay, eight bacteria were used. Gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Shigella flexneri*, *Vibrio cholerae* and Gram positive bacteria: *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*. The bacterial strains were procured from ID and BG Hospital, Kolkata. *In vitro* antibacterial activity of crude methanol extract was studied by agar well diffusion method using Mueller Hinton Agar media (Hi media, India). The extracts were diluted in 100% dimethylsulphoxide (DMSO) at the concentrations of 5 mg ml^{-1} and 2.5 mg ml^{-1}. The diameters of inhibition zone were compared with those produced by commercial control antibiotics Ciprofloxacin (5 mg ml^{-1}).

RESULTS

The antibacterial activity of the crude methanol extract of the *Leonotis nepetifolia* was determined against 8 bacterial strains which are reported in Table 1-3.

The antibacterial activity was noted to be in dose-dependent manner i.e. 5 mg/ml showed higher activity than 2.5 mg/ml against all tested bacteria. Gram positive bacteria were found to be more sensitive. The methanol extract of *Leonotis nepetifolia* was most active against *Bacillus subtilis* and *Micrococcus luteus* in comparison to all the bacteria tested. The study indicated that *Leonotis nepetifolia* leaf and floral extracts showed more activity than the stem extract.

Phytochemical tests indicated the presence alkaloids, steroids saponins, tannins and terpenoids. No flavonoids was detected in crude extract (Table 4).

DISCUSSION

In the present study Gram positive bacteria were found to be more susceptible to the plant extract than Gram negative bacteria which corroborated the previous reports that plant extract are more active against Gram positive bacteria than Gram negative one [6,7], which is due to the differences in their cell wall structure. Gram negative bacteria are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances including antibiotics [8]. The results can be compared with the standard antibiotics (Table 1-3). More or less activities of *L.*

nepetifolia were also recorded against Gram positive bacteria[9].

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 the folklore usage of the studied plant and recommends that the plant extract possess 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 antibacterials and carry out further pharmacological evaluation.

The results showed that the test plant 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.

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Table 1 Antibacterial Activity of Crude Methanol Extract of *Leonotis nepetifolia* Leaf

Bacteria	Diameter of inhibition zone (mm)		
	L. nepetifolia		Ciprofloxacin
	5 mg/mL	2.5 mg/mL	2.5 mg/mL
Bacillus subtilis	22	18	32
Staphylococcus aureus	20	16	24
Micrococcus luteus	24	18	15
Escherichia coli	18	14	30
Vibrio cholerae	14	11	22
Shigella dysenteriae	15	10	24
Shigella flexneri	16	11	28
Pseudomonas aeruginosa	15	12	30

Table 2 Antibacterial Activity of Crude Methanol Extract of *Leonotis nepetifolia* Flower

Bacteria	Diameter of inhibition zone (mm)		
	L. nepetifolia		Ciprofloxacin
	5 mg/mL	2.5 mg/mL	2.5 mg/mL
Bacillus subtilis	24	18	32
Staphylococcus aureus	23	16	24
Micrococcus luteus	24	16	15
Escherichia coli	20	15	30
Vibrio cholerae	18	14	22
Shigella dysenteriae	16	12	24
Shigella flexneri	16	11	28
Pseudomonas aeruginosa	16	12	30

Table 3 Antibacterial Activity of Crude Methanol Extract of *Leonotis nepetifolia* Stem

Bacteria	Diameter of inhibition zone (mm)		
	L. nepetifolia		Ciprofloxacin
	5 mg/mL	2.5 mg/mL	2.5 mg/mL
Bacillus subtilis	20	17	32
Staphylococcus aureus	18	14	24
Micrococcus luteus	19	17	15
Escherichia coli	19	15	30
Vibrio cholerae	16	10	22
Shigella dysenteriae	14	10	24
Shigella flexneri	12	10	28
Pseudomonas aeruginosa	14	11	30

Table 4 Qualitative Determinations of Active Ingredients in Crude Extract of Different Parts of *Leonotis nepetifolia*

Plant extract	Phytoconstituents					
	alkaloids	tannins	terpenoids	steroids	saponins	flavonoids
Leaf	++	++	++	+	++	-
Flower	++	++	++	+	++	-
Stem	+	+	+	+	+	-

+ indicates presence, ++ indicates presence in higher amounts, - indicates absence.

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