



Studies on antibacterial, antioxidant, larvicidal, pesticidal activities and phytochemistry of *Leonotis nepetifolia* (Linn) R. Br.

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ABSTRACT

Antibacterial, anti-oxidant, larvicidal, pesticidal activity and phytochemical constituents of the plant *Leonotis nepetifolia* is studied. The plant, *Leonotis nepetifolia* was collected from Villiambakkam Village belonging to Chengalpet District of Tamil Nadu State in India. The acetone, chloroform, ethanol and ethyl acetate extracts of leaves, stem and root of the plant were evaluated for their phytochemical constituents. Antibacterial efficacy was studied through Micro dilution technique on *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 1320), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96) and *Streptococcus epidermidis* (MTCC 435), antioxidant potency through DPPH scavenging activity, larvicidal potency using the nauplii of *Artemia salina* and pesticidal activity using a rice weevil, *Sitophilus oryzae*. The phytochemical studies revealed that Phlobatannins are completely absent in leaf, stem and root of the plant. Steroids are absent in stem and root and Saponin in stem of the plant. Ethyl acetate extract of the stem of the plant showed significant antibacterial activity comparable to Streptomycin as control. The ethanolic extract of the plant, *Leonotis nepetifolia* recorded the EC₅₀ value at 14 mg/ml against DPPH. The root of the plant was found to possess significant larvicidal and pesticidal activity against *Artemia salina* and *Sitophilus oryzae* respectively. The study amply demonstrates that the plant, *Leonotis nepetifolia* can be potentially used as an antibacterial, antioxidant, larvicidal and pesticidal agent.

Keywords: *Leonotis nepetifolia*; Antibacterial; Anti-oxidant; Larvicidal; Pesticidal activity; Phytochemistry

INTRODUCTION

A weed means any plant which is not desired by growers to compete with other plants growing in an area. They are considered as a waste and unwanted plants in human controlled environment. Weeds have been a part of civilization and human battling the weeds are reported. Weeds are also found to resist most of the microbial diseases when compared to the crop which show disease symptom (Udayaprakash et al, 2012) and serve as a good competitive agent for cultivable plants. *Leonotis nepetifolia* is also one among the weed plant which was pantropic in its distribution and considered as a weed of waste land and cultivated areas. Seers of Ayurveda believe that nothing in this world is non-medicinal and this definition suggests that, all plants have potential medicinal value. *Leonotis nepetifolia* is also used as an analgesic, to treat fever, diarrhoea,

bronchial asthma, malaria, influenza etc., (Ganeswari and Venkatraju, 2012). The indigenous system of Medicine in India is mainly practiced based on plants (Pushpan et al, 2012) and India is not remorse in utilizing the plants in its medical system (Udayaprakash et al, 2013). This country is perhaps the largest producer of medicinal herbs and use in its medicinal system of Ayurveda, Siddha and Unani (Dubey et al, 2004). With that in view, in this study, the antibacterial, antioxidant, larvicidal and pesticidal potency along with the phytochemical constituents of the plant, *Leonotis nepetifolia* which was neglected as a weed was conducted.

Leonotis nepetifolia is an erect, loosely branched annual that reaches around 3 meters in height in its single growing season. The stems are strongly angled and the leaves are in pairs opposite each other. The leaves are smooth with serrate margins, triangular in shape and 2-5 inches long. The inflorescence is verticillate and flowers are borne in rounded, spiny clusters that encircle the stem. As the stems elongate, new flower clusters continue to develop above the older ones. The tubular flowers that peek out of the spiny heads are orange, velvety, and long. The plant is found mostly during October to February in parts of Tamil Nadu

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state in India. Common names of the plant are Knod grass, Lion's ear (English), Gathivan (Hindi), Ranabheri (Telugu), Dipmal (Marathi) and Kasi Thumbai or Then Thumbai (Tamil). The present study explores the possibility of the extracts of leaf, stem and root of *Leonotis nepetifolia* as antibacterial, antioxidant, larvicidal and pesticidal agent using different solvent system like Acetone, Chloroform, Ethanol and Ethyl Acetate. Further, the plant extracts were subjected to phytochemical analysis in this present study.

MATERIALS AND METHODS

Plant Source

The stem, leaves and root of the plant *Leonotis nepetifolia* was collected near Villiambakkam village belonging to Chengalpet district of the state of Tamil Nadu, India. The plant parts were chosen as they are healthy and not damaged or diseased. The collected plant parts were cleaned thoroughly in running tap water so as to remove the physical, chemical and biological particulates. The leaves of the plant were shade-dried for 4 days. The stem and root of the plant took nearly 15 days to dry. The dried plant parts were made as a powder using electric blender and stored for further use.

Preparation of plant extracts

The plant extracts were prepared using cold-percolation method. To 15g of each dried pulverized sample 150ml of respective solvent (Acetone, Chloroform, Ethanol and Ethyl acetate) was added and stirred in temperature-controlled shaker at $30 \pm 2^\circ\text{C}$. After 48 hours the extract was filtered and concentrated. These extracts were used for screening antibacterial, antioxidant, larvicidal and pesticidal properties.

Phytochemical analysis

The dried pulverized plant material (15g) extracted with respective solvents were filtered using Whatman No.1 filter paper. Qualitative detection of phytochemicals like, cardiac glycosides, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids were carried out immediately without storage according to standard procedures (Evans, 1994).

Antibacterial assay – Determination of Minimum Inhibitory Concentration (MIC)

The antibacterial potency of all extracts of leaves, stem and root of *Leonotis nepetifolia* were screened against 6 bacterial strains, i.e. *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 1320), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96) and *Streptococcus epidermidis* (MTCC 435) procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

The initial concentration of the plant extract at 100mg/ml was diluted using serial dilution by transferring 5ml of the sterile plant extract into 5ml of sterile

Nutrient broth to obtain 50mg/ml concentration. This was repeated to obtain the dilutions of 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml and finally 1.6 mg/ml (Sule and Agbabiaka, 2008). Each concentration was inoculated with 0.1ml of 24 hours bacterial cell suspension and incubated at 37°C for 24 hours. The growth of the inoculum in the broth is indicated by turbidity or cloudiness of the broth and the lowest concentration of the extract which inhibited the growth of the test organism were taken as the Minimum Inhibitory Concentration (MIC). This was compared with Streptomycin as control.

DPPH free radical scavenging assay

The leaf extracts of *Leonotis nepetifolia* obtained from different solvents were studied for their Free radical scavenging assay using DPPH (2, 2 diphenyl-1-picryl hydrazyl). To 0.5mL of extract of each solvent and the reference compound in various concentrations (15.6, 31.2, 62.5, 125, 250 mg/ml), 0.5mL methanol and 0.5mL of 0.1mM solution of DPPH in methanol was added. After an incubation time of 30 minutes in dark condition at room temperature, absorbance was measured at 517nm using spectrophotometer. The same solution of DPPH in methanol was used as control, whereas Butylated hydroxyanisole (BHA) was used as reference.

Percentage inhibition was calculated using the formula

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

Larvicidal activity

Culture of Larvae

The seeds of *Artemia salina* were procured from Philadelphia, USA. The seeds were incubated in marine water for 48 hours for hatching in a small water tank provided with an aerator pump for aeration. Required light is provided with Philips 40 Watts lamp for 12 hours cycle. After 48 hours, the hatched larvae at nauplii stage were removed and used for the experiment.

Bioassay

Larvae of *Artemia salina* were taken in different test tubes containing extracts of *Leonotis nepetifolia* at five different concentrations (2, 4, 6, 8 and 10 mg/ml). To each test tube containing 10ml of sea water, around 20 larvae were added. The test tubes were maintained in triplicates. At the end of the experimental period the numbers of mobile and dead larvae in each test tube were checked using hand lens. The viability of the larvae was recorded after 24hours. Nauplii were considered dead when they are immobile and stayed at the bottom of the test tubes.

Pesticidal activity

Weevil cultivation

Sitophilus oryzae adults were collected from naturally infested Rice grains from a local market in Chennai, Tamil Nadu. The insects were collected and reared on clean and un-infested rice grains. Nearly 12 jars each containing 400 insects provided with sufficient rice grains capped with muslin cloth to ensure ventilation was reared. After 48 h, the adults were removed and used for the experiment.

Bioassay

Two ml. of the plant extract constituted using 20mg, 40mg, 60mg, 80 mg and 100 mg/ml of the extract of different solvents of *Leonotis nepetifolia* was poured onto a dry and sterile Petridish and was allowed to dry. A plug of cotton was used to wipe the extract from the plate. The cotton plug on which the extract was adsorbed was placed in a Petridish along with adult *Sitophilus oryzae* (20 numbers) and few grams of rice grains for them to feed on. The observations were recorded in the time interval of 24 and 48 hours. The pesticidal activity against the weevils is provided in percent basis depending upon the mortality of the number of insects.

RESULTS

Antibacterial efficacy

Among the extracts of leaves studied for their antibacterial potency, the ethyl acetate extract of the leaves, stem and root of *Leonotis nepetifolia* showed inhibitory concentration at minimum when compared with the other solvents like acetone, chloroform and ethanol. When the same was studied as organism specific resistance, it was ethyl acetate extract of stem against *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Bacillus subtilis*; ethyl acetate extract of leaf against *Escherichia coli*; ethanol extract of leaf against *Staphylococcus aureus*. It was ethyl acetate extract of stem of *Leonotis nepetifolia* which showed significant level of inhibitory concentration. The minimum inhibitory concentration recorded for each solvent against the studied bacteria is presented in Table 1.

Antioxidant activity

Among different extracts of leaves, stem and root of the plant *Leonotis nepetifolia* studied for their free radical scavenging activity using DPPH, the acetone, ethanol and ethyl acetate extracts of leaves showed better anti-oxidant activity when compared with that of control. The EC₅₀ value recorded for these three solvents of leaves recorded lower value of EC₅₀. However, this cannot be compared with that of BHA as they have recorded EC₅₀ value at µg/ml level. The values recorded for the assay against each extracts of different plant parts of *Leonotis nepetifolia* is presented in Table 2.

Larvicidal Activity

Among the leaves, stem and root of *Leonotis nepetifolia* tested for its larvicidal activity against *Artemia salina*, the root of the plant showed larvicidal activity of 100 % even at lower level of concentration when compared to leaf and stem at 24 hours time period. The larvicidal potency recorded for different solvent extracts of the leaves, stem and root is given in Table 3.

Pesticidal Activity

Among the four solvents of leaves, stem and root of *Leonotis nepetifolia* tested for its pesticidal activity against the storage pest, *Sitophilus oryzae*, ethanolic extract of root of the plant showed significant mortality rate at 48 hours of time period. This has recorded 90 % mortality at the lowest concentration of 20mg and recorded 95 % and 100 % concentration at 40 and 60 mg respectively. The chloroform extract of the leaf of the plant is the other significant contributor as a pesticidal agent. The pesticidal potency recorded for different plant parts is given in Table 4.

Phytoconstituents

Test for the presence of Tannins, Flavonoids, Terpenoids, Steroids, Phlobatannins, Saponins and Cardiac glycosides in plant parts of *Leonotis nepetifolia* showed complete absence of phlobatannins, steroids in stem and root, saponins in stem and root and tannin was absent in root of the plant. The presence of terpenoids was detected with all the four solvents from the leaves and similarly, cardiac glycosides were recorded from the stem of the plant. The presence of different phytochemicals from the extracts of different solvent is presented in Table 5.

DISCUSSION

The traditional medicine system like Chinese Traditional medicine, Kampo medicine system in Japan, Korean Chinese Medicine, Jamu in Indonesia, Ayurveda and Siddha system of medicine in India are widely using plants in their system of medicine (Makkar et al, 2009). As products from plants are natural with novel mechanism of action and effective treatment of infections and diseases with their minimal side effects, screening of plants to validate their medical potency is recommended (Shihabudeen et al, 2010). Hence, plants have been widely used as an important source of traditional medicine as they function as the reservoir of chemical agents, it is reasonable to make use of local weeds (Udayaprakash et al, 2012). It is also necessary to study in search of such plants for their ethno medical use and isolation of compounds subsequently to add them under potential list of drugs (James and Friday, 2010). Current study provides an inward knowledge on the phytochemistry and bio-efficacy, i.e. antibacterial potency, anti-oxidant potency, larvicidal potency and pesticidal potency of one such plant, *Leonotis nepetifolia*.

Table 1: Antibacterial property of *Leonotis nepetifolia* (MIC recorded – mg/ml; Control µg/ml)

Plant part	Solvent	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>Staph. Aureus</i>	<i>Strept. epidermidis</i>
Leaves	Acetone	25	50	25	12.5	12.5	50
	Chloroform	100	50	100	50	50	50
	Ethanol	50	100	100	12.5	1.56	50
	Ethyl Acetate	1.56	25	25	25	6.25	50
Stem	Acetone	25	50	50	50	12.5	12.5
	Chloroform	100	100	100	100	100	100
	Ethanol	25	25	25	25	25	25
	Ethyl Acetate	25	12.5	12.5	12.5	12.5	25
Root	Acetone	12.5	25	100	100	100	50
	Chloroform	100	100	50	100	50	100
	Ethanol	50	50	50	50	50	50
	Ethyl Acetate	25	25	25	25	25	50
Control	Streptomycin	6.25	25	50	12.5	100	25

Table 2: Inhibition percentage of the plant extracts and BHA to DPPH and their respective percent inhibition values recorded

Plant part	Solvent	15.6 mg/ml	31.2 mg/ml	62.5 mg/ml	125 mg/ml	250 mg/ml	EC ₅₀ mg/ml
Leaves	Acetone	47.22	66.67	75.56	88.33	95	18.18
	Chloroform	43.33	51.11	66.67	75.56	91.67	28.8
	Ethanol	52.22	66.67	75.56	93.33	95	14.0
	Ethyl Acetate	47.22	66.67	75.56	85.56	95	18.18
Stem	Acetone	-	-	47.22	66.67	75.56	65.7
	Chloroform	-	0.56	26.11	47.22	66.67	145.3
	Ethanol	-	36.11	43.33	66.67	79.33	81.1
	Ethyl Acetate	-	26.11	36.11	52.22	78.78	118.5
Root	Acetone	-	-	36.11	52.22	61.67	118.5
	Chloroform	-	-	26.11	36.11	43.33	ND
	Ethanol	-	-	43.11	47.22	66.67	143.1
	Ethyl Acetate	-	-	36.11	47.22	66.67	143.1
Control (In µg/ml)	BHA	36.11	66.67	85.56	88.23	93.33	25.78

ND = Not Detected

Udayaprakash et al, (2012) studied the antimicrobial property of the methanolic extract of leaves of the plant *Leonotis nepetifolia* and recommended further studies on this line as the plant showed wide spectrum antimicrobial potency. With continuation of this study, the current study revealed, the antibacterial property of the plant with different solvent system like acetone, chloroform, ethyl acetate and chloroform and with the plant parts of leaf, root and stem. This study added further data as ethyl acetate extract of stem of the plant showed higher antibacterial potency.

The free radical scavenging test conducted on different plant parts of *Leonotis nepetifolia*, shows that the leaves of the plant serve as a better source when compared with other plant parts. Among the solvent system, the ethanolic extract showed better activity followed by acetone and ethyl acetate. The antioxidant activity of methanolic (Usharani et al, 2013) and ethanolic extract (Gurunagarajan and Brinda, 2010) of the leaves of *Leonotis nepetifolia* are reported. The assay

on larvicidal activity using *Artemia salina* is valuable for establishing toxicity and cytotoxicity (Udayaprakash et al, 2013). The root of the plant showed better activity when compared to other plant parts. David et al, (2007) reported the lethality of brine shrimp towards the extracts of *Leonotis nepetifolia*. From this study, it is evident that only the ethanolic extract of the root of *Leonotis nepetifolia* resulted in significant activity against the pest, *Sitophilus oryzae*. Moreira et al, (2007) studied the pesticidal activity of the plant against *Sitophilus zeamais* and reported that there was no activity. This study provides the data that root are better plant part for larvicidal and pesticidal activity when compared to leaves and stem of the plant, *Leonotis nepetifolia*.

The studies on phytochemistry of the plant showed the absence of Phlobatannin and Saponins in leaves, absence of Phlobatannin, Saponin and Steroids in stem and absence of Tannin, Phlobatannin and Steroid in root. As phlobatannins are termed as a condensed

Table 3: Larvicidal activity of *Leonotis nepetifolia* against the larvae of *Artemia salina*

Plant part	Solvent	2mg/ml	4mg/ml	6mg/ml	8mg/ml	10mg/ml
Leaves	Acetone	45	55	75	95	100
	Chloroform	100	100	100	100	100
	Ethanol	10	100	100	100	100
	Ethyl Acetate	75	100	100	100	100
Stem	Acetone	85	90	90	95	100
	Chloroform	95	100	100	100	100
	Ethanol	75	85	85	85	100
	Ethyl Acetate	80	80	85	90	100
Root	Acetone	100	100	100	100	100
	Chloroform	100	100	100	100	100
	Ethanol	100	100	100	100	100
	Ethyl Acetate	95	100	100	100	100

Table 4: Pesticidal activity of *Leonotis nepetifolia* against *Sitophilus oryzae*. (Mortality rate in % - 48 hours)

Plant part	Solvent	20mg	40mg	60mg	80mg	100mg
Leaves	Acetone	10	10	15	20	20
	Chloroform	30	45	75	80	95
	Ethanol	10	15	15	20	35
	Ethyl Acetate	10	15	15	20	20
Stem	Acetone	0	10	15	15	20
	Chloroform	5	5	10	15	15
	Ethanol	10	15	25	35	45
	Ethyl Acetate	5	10	10	15	20
Root	Acetone	5	5	15	15	20
	Chloroform	5	10	10	15	15
	Ethanol	90	95	100	100	100
	Ethyl Acetate	0	5	5	10	10

Table 5: Presence of phytochemicals in *Leonotis nepetifolia*

Plant part	Solvent	Tan nins	Phloba tan nins	Saponins	Flavonoids	Terpenoids	Cardiac Glycosides	Steroids
Leaves	Acetone	-	-	-	+	+	+	+
	Chloroform	-	-	-	-	+	-	+
	Ethanol	+	-	-	+	+	+	-
	Ethyl Ace-tate	-	-	-	-	+	-	+
Stem	Acetone	+	-	-	+	+	+	-
	Chloroform	-	-	-	-	-	+	-
	Ethanol	+	-	-	+	+	+	-
	Ethyl Ace-tate	-	-	-	-	-	+	-
Root	Acetone	-	-	+	-	+	+	-
	Chloroform	-	-	-	-	-	-	-
	Ethanol	-	-	+	+	-	+	-
	Ethyl Ace-tate	-	-	+	-	-	-	-

+ Positive, - Negative

form of tannins which may form either due to enzymatic action in dead cells or aging of tissues (Chung et al, 1998). Further, acetone and ethanol has served as a good solvent system for isolating maximum compounds in this study. However, the aqueous (Udayaprakash et al, (2013); Moses et al, (2013)) and methanolic extract (Ashish et al, (2011); Gnaneswari and

Venkatraju, (2012)) of the plant resulted in isolation of more compounds. The biological importance of phytochemicals (Udayaprakash et al, 2013) and its ethno-medical claims (Pushpan et al, 2012) of the plant were reviewed. Nair et al, (2005), recommends that the organic extracts of the plants provide more activity when compared to those extracted in water. The extracts of

different plant parts using different solvent of *Leonotis nepetifolia* showed significant difference in their phytochemical constituents, their antibacterial potency, antioxidant, larvicidal and pesticidal properties. This study recommends using different solvent system to know the biological efficacy of the plants.

CONCLUSION

The study proves that the plant, *Leonotis nepetifolia* possess excellent biological property. The ethyl acetate extract of the stem of the plant is superior in its antibacterial efficacy, leaves of the plant possess high antioxidant property when compared with other plant parts, and the root of the plant possesses best larvicidal and pesticidal activity. Hence, it is recommended that the plant, *Leonotis nepetifolia* can be easily utilized for their bio-efficacy and to use suitable solvent system and plant part according to the need of importance.

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