ORIGINAL ARTICLE

# Anti-bacterial activity of the methanolic extract of *leucas hyssopifolia (Benth.)*

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**Context:** Methanolic extract of *Leucas hyssopifolia* roots was investigated for its anti-bacterial property. **Aim:** Evaluation of anti-bacterial activity of *Leucas* hyssopifolia Benth. **Settings and Design:** Roots of the plant were collected, extracted and finally evaluated for their anti-bacterial activity. **Materials and Methods:** Paper disc diffusion method and microdilution technique were employed for the determination of zone of inhibition and minimal inhibitory concentration, respectively. **Results:** The extract showed anti-bacterial activity against all the tested bacterial strains except *Escherichia coli*. **Conclusions:** Anti-bacterial activity of extract of *Leucas hyssopifolia* roots may be due to the presence of secondary plant metabolites like terpenoids, steroids and flavonoids, which are present in the extract. The extract can be further studied for the isolation of chemical compounds and their biological activity.

**Key words:** Anti-bacterial activity, *Leucas hyssopifolia*, minimal inhibitory concentration, phytochemical screening

## INTRODUCTION

The genus *Leucas* includes about 100 Asiatic and African species. The plant is useful in bronchitis, inflammation, asthma, dyspepsia, paralysis and leucoma. The leaves are useful in fever and urinary discharge.[1] Flowers mixed in honey are used as domestic remedy for cough and cold.[2] It is valuable homoeopathic drug and as such is used for the treatment of chronic malaria and asthma.[3] Dry leaves along with tobacco (1:3) are smoked to treat bleeding as well as itching piles.[4] The plant was evaluated for *in vitro* anti-filarial activity.[5] Flavonoid glycosides were reported from the aerial parts of *Leucas lavandulaefolia*.[6] Alkaloids, steroids and tritepenoids were isolated from *L. aspera*, *L. stricta* and *L. cephalotes*.[7] The aerial parts of *L. neufliseana* were reported to contain diterpenes and flavones.[8] Two aliphatic ketals,[9] phenolic compounds[10] and triterpenoid lactones[11] were also reported from *L. aspera*. *L. cephalotes* showed anti-filarial activity while *L. aspera* exhibited anti-microbial activity.[12]

*L. hyssopifolia* Benth. (Labiatae) is an annual herb found throughout India as a weed in cultivated fields, wastelands and roadsides. To the best of our knowledge, there has not been any work published on this plant and this has been confirmed by detail chemical abstracting.

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## MATERIALS AND METHODS

### Plant Material

The plant material was collected from Nanda Chauri, Chamoli in the month of October. The plant identification was confirmed by the Botanical Survey of India, Dehradun. The voucher specimen (No. 112285) is deposited in the Department of Applied Chemistry, Birla Institute of Applied Sciences, Bhimtal, Nainital, India.

### Extraction

The roots (300 g) of the plant were shade dried, powdered and extracted by using Soxhlet apparatus with methanol (Qualigens) for about 24 h. After extraction, the filtrate was concentrated under reduced pressure using vacuum rotatory evaporator to obtain a residual mass.

### Phytochemical Screening

Phytochemical screening of the methanolic extract of *L. hyssopifolia* roots gave positive tests for terpenoids, steroids,[13,14] flavonoids, reducing sugars and saponins.[15,16]

### Microorganisms

The anti-bacterial activity was conducted against three Gram positive bacteria: *Staphylococcus aureus* (NCIM 2901), *Bacillus mycoides* (MTCC 645) and *Bacillus subtilis* (MTCC 441), four Gram negative bacteria: *Pseudomonas aeruginosa* (NCIM 2046), *Escherichia*

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*coli* (NCIM 2810), *Salmonella typhi* (NCIM 2501) and *Proteus vulgaris* (MTCC 426). Required microorganisms were procured from Institute of Microbial Technology, Chandigarh and National Chemical Laboratory, Pune, India.

### Anti-Bacterial Activity

Anti-bacterial activity of methanolic extract of roots from *Leucas hyssopifolia* was tested by the paper disc diffusion method according to the slightly modified National Committee for Clinical Laboratory Standards Guidelines.[17] Mueller Hinton agar/broth for bacterial strains was used for the study. A suspension ($10^8$ CFU/ml, determined by using colony counter) of bacterial strains was used. The filter paper discs (6 mm in diameter, Whatman filter paper 1) were individually impregnated with 10 µl of the methanolic extract aliquots (400 mg/ml), which were subsequently placed on the surface of the inoculated petridishes. The petridishes were kept at 4°C for 2 h and then incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters including the diameter of the disc. Controls were set up with equivalent quantities of DMSO (Qualigens), which was used as solvent (15%) for the preparation of the solution of extract and standards. Ciprofloxacin, ampicillin and levofloxacin procured from the microbiology laboratory of department of pharmaceutical sciences Bhimtal, Kumaun University, were used as standards. All the experiments were performed in triplicate and the results (mm of zone of inhibition) were expressed as mean values.

### Determination of Minimal Inhibitory Concentration

Broth microdilution technique was used to determine the minimal inhibitory concentration (MIC).[18] All the experiments were performed in Mueller Hinton broth. Serial doubling dilutions of the extract was prepared in a 96-well microtiter plate ranged from 0.78 to 400.00 mg/ml. Prepared microtiter plates containing microorganism and extract were incubated at 37°C for 24 h. The growth of microorganisms was indicated by the turbidity through visual examination. All the experiments were performed in triplicate. Ciprofloxacin, ampicillin and levofloxacin were used as standards.

## RESULTS

The results obtained for zones of inhibition (mm) and MICs (mg/ml) in Mueller Hinton agar and Mueller Hinton broth, respectively, are summarised in Table 1.

The results showed inhibition zones against all bacterial strains except *E. coli*. The data obtained from disc diffusion method indicated that the methanolic extract of *L. hyssopifolia* roots was most active against *B. subtilis* with the largest inhibition zone (20 mm) while extract was found to be least active against *P. aeruginosa* with the smallest inhibition zone 12 mm. Significantly the extract was more active against Gram positive bacterial strains when compared with Gram negative bacteria, which was further confirmed by the extract's inactivity against *E. coli*.

**Table 1: Anti-bacterial activity of methanolic extract from *Leucas hyssopifolia* roots**

| Microorganisms | Methanolic extract | | MIC[c] | | |
|---|---|---|---|---|---|
| | IZ[a] | MIC[b] | CF | AM | LF |
| *Staphylococcus aureus* (NCIM 2901) | 16 | 6.25 | 0.24 | NT | NT |
| *Bacillus subtilis* (MTCC 441) | 20 | 3.12 | NT | 0.8 | NT |
| *Bacillus mycoides* (MTCC 645) | 16 | 12.50 | 0.24 | NT | NT |
| *Pseudomonas aeruginosa* (NCIM 2046) | 12 | 12.50 | 0.48 | NT | NT |
| *Escherichia coli* (NCIM 2810) | NA | NA | NT | NT | NT |
| *Salmonella typhi* (NCIM 2501) | 14 | 12.25 | NT | NT | 1.20 |
| *Proteus vulgaris* (MTCC 426) | 16 | 6.25 | NT | NT | 0.5 |

IZ – Diameter of zone of inhibition (mm) including disc diameter of 6 mm; NA – Not active; NT – Not tested; CF – Ciprofloxacin; AM – Ampicillin; LF – Levofloxacin; [a]Tested at a concentration of 4 mg/disc; [b]Values given in mg/ml; [c]Values given in µg/ml; MIC – minimal inhibitory concentration

The results of the MICs showed the lowest MIC for *B. subtilis* (3.12 mg/ml), which can be correlated to the highest inhibition zone for *B. subtilis* while the highest MIC was 12.50 mg/ml for *B. mycoides*, *P. aeruginosa* and *S. typhe*.

## DISCUSSION

From the above results, it can be concluded that, extract of *L. hyssopifolia* roots showed anti-bacterial property, which may be due to the presence of terpenoids, saponins, steroids and flavonoids. There are several reports on the anti-microbial activity of plant extracts, which gave positive test for terpenoids,[19] saponins,[20] steroids and flavonoids.[21] Methanolic extract of *L. aspera* also showed anti-microbial activity.[12] Leaf extract of *L. cephalotes* exhibited anti-helmintic activity.[22]

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