



In Vitro* Antibacterial Activity of Flower Extract of *Leucas Indica

M. Sowjanya^{1*}, Ch. Venkatrayulu¹, B.V. Sandeep², Vardi Venkateswarlu¹

¹Department of Marine Biology, Vikrama Simhapuri University, Nellore, Andhra Pradesh, India-524324

²Department of Biotechnology, Andhra University, Visakhapatnam, Andhra Pradesh, India-530003

*Corresponding author's E-mail: sowjanyamuthyam86@gmail.com

Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 02 Nov 2023	<i>In this study, the antimicrobial activities of methanol extracts of flowers of <i>Leucas indica</i> was investigated by using agar well diffusion method against selected ten bacterial species (<i>Pseudomonas</i> (MTCC 424), <i>Corynebacterium</i> (MTCC 1349), <i>Spingomonas</i> (MTCC 6362), <i>Bacillus subtilis</i> (MTCC 441), <i>Bacillus licheniformis</i> (MTCC 429), <i>Bacillus coagulans</i> (MTCC 5856), <i>Streptococcus</i> (MTCC 9724), <i>Klebsiella pneumonia</i> (MTCC 452), <i>E. coli</i> (MTCC 443), and <i>Staphylococcus</i> (MTCC 3160). Among the ten bacterial species studied, the flower extract at concentrations of 20µg and 40µg showed antimicrobial activity against <i>Klebsiella pneumonia</i>, <i>E. coli</i>, <i>Streptococcus</i>, <i>Bacillus subtilis</i>, <i>Spingomonas</i>, <i>Staphylococcus</i> and <i>Corynebacterium</i> respectively.</i>
CC License CC-BY-NC-SA 4.0	Keywords: Antimicrobial, Micro Organisms, flower extract, <i>Leucas indica</i> .

1. Introduction

Medicinal plants have a great importance in human being as it shows diverse pharmacological properties and have attained a significant position in health systems all over the world. Even though pharmacological industries have produced a number of new antibiotics in the last decades, resistance to these drugs by microorganisms have led scientists to introduce antimicrobial activity of medicinal plants. An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, protozoa or viruses. The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. Antimicrobial activity of herbs has been known and described for several centuries (Begamboula *et al.*, 2003).

Leucas indica (family: *Lamiaceae*) is an annual herb found throughout India as a weed in cultivated fields, wastelands and roadsides. The flowers are given with honey to treat cough and cold in children. The leaves are applied to the bites of serpents, poisonous insects and scorpion sting. *L. indica* leaves are also used as insecticides and mosquito repellent in rural area. The plant extract with honey is a good remedy for stomach pain and indigestion (1). In the underway effort a comparative crum was conceded out in between, methanolic leave and flower extracts of *Leucas indica* for their antimicrobial activity.

2. Materials And Methods

Collection of Plant material

The aerial parts, leaves and flowers of *Leucas indica* Linn were collected from Marlapudi village, Nellore district, Andhra Pradesh, India, in the month of November – March, 2015. Plant materials were recognized with the help of Gamble, “Flora of the Presidency of Madras” and later verified by comparison with the authentic specimens available in the herbariums of National Botanical Research Institute (NBRI), Lucknow and these plant materials were authenticated by Dr.Padal, faculty of Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. Leaves and flowers of *Leucas indica* were cleaned, shade dried, mechanically grinded. Finally, the coarse powders were separated by sieving and stored in an air tight container for further use.

Preparation of Extracts

Flowers of *Leucas indica* were cleaned, shade dried, mechanically grinded. Finally, the coarse powders were separated by sieving and stored in an air tight container for the study. Accurately weighed 10 gms of *Leucas indica* flower powder were extracted with 250 ml methanol by stirring at 50°C for 3hr. The

extracts were then filtered through Whatman filter paper and the filtrate was concentrated with a vacuum rotary evaporator under low pressure and temperature and stored in desiccator.

Test Organisms

In the present study ten bacterial species were selected to assess susceptibility patterns against the extracts prepared. These species were obtained from National Collection of industrial microorganisms (NCIM), Pune. Out of ten species, four being gram negative organisms (*Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 443), *Sphingomonas* (6362) and *Klebsiella pneumonia* (MTCC 452)) and six belonging to gram positive organisms (*Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441), *Streptococcus* (9724), *Corynebacterium* (MTCC-1349), *Bacillus licheniformis* (MTCC-429) and *Bacillus coagulans* (MTCC-5856)). Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

Culture Media

For antimicrobial activity of *Leucas indica* flowers (Solid and Broth) was used.

In-vitro antibacterial activity

The study involves inhibition of microbial growth using disc diffusion technique/cup-plate method on nutrient agar medium. In this method nutrient agar was weighed in a sterile flask and autoclave at 15lbs pressure at 121°C for 15 to 20 min, later the agar media was cooled at room temperature. 10ml nutrient agar media was poured in a sterile Petri dish, 100 µl of test organisms were spread on the surface of media prior to agar solidification. Wells were prepared with help of sterile metal steel borer. Different concentration of plant extract (20µg/ml & 40µg/ml), positive control and negative control (DMSO) at a concentration of 50µg/ml were loaded in the wells. Plates were incubated aerobically at 37°C for 14 hrs. The diameter of zones of inhibition were measured in mm according to Bauer *et al.*, 1966.

3. Results and Discussion

Antimicrobial Activity of Flower Extract of *Leucas indica*

Plant extracts are a potentially useful source of antimicrobial compounds. These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as 'forgotten plants'. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.

The antimicrobial activity of methanol extracts of flowers of *Leucas indica* was investigated by using agar well diffusion method against selected ten bacterial species *Pseudomonas* (MTCC 424), *Corynebacterium* (MTCC 1349), *Spingomonas* (MTCC 6362), *Bacillus subtilis* (MTCC 441), *Bacillus licheniformis* (MTCC 429), *Bacillus coagulans* (MTCC 5856), *Streptococcus* (MTCC 9724), *Klebsiella pneumonia* (MTCC 452), *E. coli* (MTCC 443) and *Staphylococcus* (MTCC 3160). Among the ten bacterial species studied, the flower extract at concentrations of 20µg and 40µg showed antimicrobial activity against *Klebsiella pneumonia*, *E. coli*, *Streptococcus*, *Bacillus subtilis*, *Spingomonas*, *Staphylococcus* and *corynebacteriumas* shown in the Table and Figure. Tetracyclin was taken as positive control and methanol was taken as negative control. Due to the antimicrobial potential of *Leucas indica* it is considered as one of the important traditionally plant which provides a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and wellbeing (Kalyani *et al.*, 2021). The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment. Selvamohan *et al.*, 2012 reported antimicrobial activities with methanol, ethanol and aqueous extracts of seven medicinal plants against medically important bacteria such as *Staphylococcus* sp., *Escherichia coli*, *Klebsiella* sp., *Pseudomonas* sp. by agar well diffusion method. According to Rajesh *et al.*, 2007 antimicrobial potential of seventy-seven extracts from twenty-four plants was screened against eight bacteria and four pathogenic fungi, using microbroth dilution assay, whereas Yaminet *et al.*, 2011 reported similar activities against bacteria from crude extract of *P. integerrima* and *A. indica*.

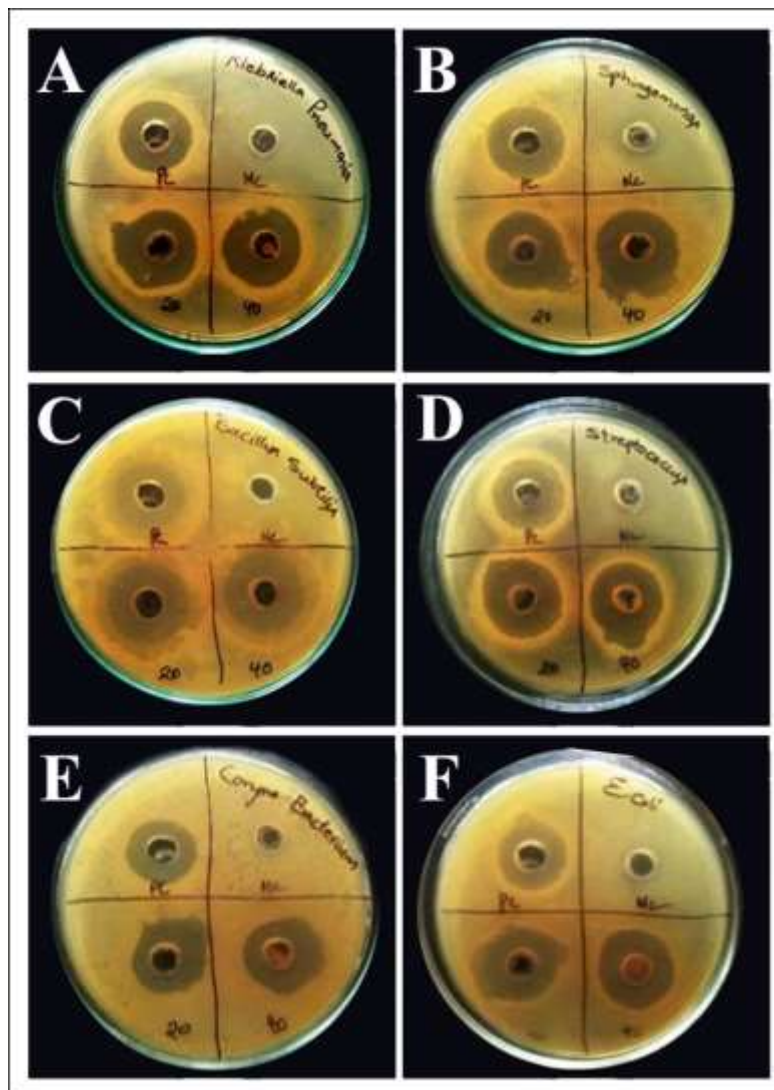


Fig:1 Antimicrobial activity of *Leucas indica* flowers extracts against *Klebsiella Pneumonia* (A), *Sphingomonas* (B), *Bacillus subtilis* (C), *Streptococcus* (D), *Coryne Bacterium* (E), *E.coli* (F).

Table: 1. Antimicrobial activity of *Leucas indica* flowers

Name of the organism	Zone of Diameter (mM)		Positive Control (Tetracycline)	Negative Control (Methanol)
	20µg	40 µg		
<i>Klebsiella pneumonia</i>	15	16	14	-
<i>Sphingomonas</i>	16	18	14	-
<i>Bacillus subtilis</i>	18	20	14	-
<i>Streptococcus</i>	29	19	14	-
<i>Escherichia coli</i>	18	19	14	-
<i>Corynea bacterium</i>	15	17	14	-
<i>Staphylococcus aureus</i>	15	15	14	-

4. Conclusion

It The *in-vitro* antimicrobial evaluation confirms that the *Leucas indica* flowers contain biologically active compounds which exhibit significant antimicrobial activity against some pathogenic bacteria and fungi. *Leucas indica* have some measurable antimicrobial response on both bacteria and fungi tested. However, the methanolic extract of *Leucas indica* showed significant antimicrobial activity against several pathogenic bacteria as well as fungi than the other parts tested.

Future Scope

The future scope of in vitro antibacterial activity of *Leucas indica* flower extract holds promise in developing novel antibiotics, herbal remedies, and eco-friendly agricultural solutions. Research into its phytochemical composition and clinical applications is essential to harness its potential as an antimicrobial agent in an era of increasing antibiotic resistance.

Conflict of Interest: None

Acknowledgments

We, the authors are thankful to the Department of Biotechnology Andhra University, and Department of Marine Biology, Vikrama Simhapuri University, Kakatur, Nellore, Andhra Pradesh for providing the necessary amenities to carry out the present research work.

References:

- Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turck. (1966), Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 36:493-496.
- Cheng, Huan Chen, Yu, Chenn W and Chang (2009), Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. *OBio resource Technology* 100, 452-456.
- Dinesh Reddy G and Kalyani P (2023), Green synthesis of silver nanoparticles from *Aspergillus flavus* and their antibacterial performance. *Chemical Product and Process Modeling*. <https://doi.org/10.1515/cppm-2022-0054>.
- Iwu MM, Duncan AR, Okunji CO (1999), New antimicrobials of plant origin. In: Janick J, editor. *Perspectives on new crops and new uses*. Alexandria, VA: ASHS Press; 1999. pp. 457-62.
- Kalyani P, Botsa SM, Kumari KS (2021), Purification, structural elucidation and anticancer activity of a compound extracted from *Aspergillus fumigatus* strain MF-1. *Archives of Microbiology*. 203:4635–4640.
- Kalyani P, Geetha S, Hemalatha KPJ (2016), Optimization of cultural conditions for improved production and bioactive metabolites by *Aspergillus niger* (MTCC-961). *Ejpmr* 3(10):255-260.
- Madhava Chetty K, Sivaji K, Tulasirao K (2008), Flowering plants of Chittor Distric, Andhra Pradesh. Turupathi; India Students offset printers; 277.
- Rajesh Dabur, Amita Gupta, T K Mandal, Desh Deepak Singh, Vivek Bajpai , A M Gurav, G S Lavekar, ANTIMICROBIAL ACTIVITY OF SOME INDIAN MEDICINAL PLANTS, *Afr. J. Trad. CAM* (2007) 4 (3): 313 – 318, ISSN 0189-6016©2007.
- Selvamohan T, Ramadas V, Shibila Selva Kishore S (2012), Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria .3 (5):3374-3381.
- Yamin Bibi, Sobia Nisa, Fayyaz M Chaudhary and Muhammad Zia (2011), Antibacterial activity of some selected medicinal plants of Pakistan. Bibi et al., *BMC Complementary and Alternative Medicine* 2011, 11:52.