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Invitro Antimicrobial and Antidiarrhoeal Activity of *Leucas aspera* Plant Extract by Using Agar Well Diffusion Method

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Abstract:

- ***Background:***

Diarrheal diseases remain a major public health concern worldwide due to the increasing prevalence of antimicrobial resistance among enteric pathogens. Medicinal plants are considered promising sources of novel antimicrobial agents. *Leucas aspera* , a medicinal herb widely used in traditional medicine, possesses several pharmacological properties, including antimicrobial activity.

- ***Aim & Objectives:***

The present study was carried out to evaluate the in vitro antimicrobial activity of the ethanolic extract of *Leucas aspera* against selected diarrhea-causing bacterial pathogens using the agar well diffusion method. The objectives were to determine the antibacterial potential of the extract at different concentrations and to compare its efficacy with the standard antibiotic Gentamicin.

- ***Materials & Methods:***

The aerial parts of *Leucas aspera* were shade-dried, powdered, and extracted with 70% ethanol using the maceration extraction method. The antibacterial activity of the extract was evaluated against Escherichia coli and Staphylococcus aureus by the agar well diffusion assay. Different concentrations of the extract (50, 100, 250, and 500 µg/mL) were tested, while Gentamicin served as the positive control. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters.

- ***Results & Discussion:***

The ethanolic extract of *Leucas aspera* exhibited concentration-dependent antibacterial activity against both test organisms. At 500 µg/mL, the extract produced the highest zone of inhibition against E. coli (24.9 ± 0.28 mm) and S. aureus (21.95 ± 0.07 mm), which was comparable to the standard drug Gentamicin. Moderate antibacterial activity was observed at 250 µg/mL, whereas no inhibition was detected at 100 and 50 µg/mL. These findings indicate that the bioactive phytoconstituents present in *Leucas aspera* possess significant antibacterial activity against diarrhea-causing pathogens and support its traditional medicinal use.

- ***Conclusion:***

The present study demonstrated that the ethanolic extract of *Leucas aspera* possesses significant in vitro antibacterial activity against selected diarrhea-causing bacteria. The results suggest that this plant may serve as a promising natural

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source for the development of antimicrobial agents for the management of bacterial diarrhea. Further phytochemical characterization, MIC determination, and in vivo studies are recommended to confirm its therapeutic potential.

Keywords: *Leucas aspera, Agar Well Diffusion, E. coli, S. aereus.*

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I. INTRODUCTION

➢ *Diarrhoea:*

Diarrhoea is a common gastrointestinal disorder characterized by the passage of three or more loose or watery stools within 24 hours. It is a symptom of various infectious and non-infectious diseases rather than a disease itself. Diarrhoea results from an imbalance between intestinal fluid secretion and absorption, leading to excessive loss of water and electrolytes. The major causative pathogens include Escherichia coli, Shigella spp., Salmonella spp., Vibrio cholerae, and Campylobacter jejuni. Non-infectious causes include food intolerance, inflammatory bowel disease, medications, and malabsorption disorders. Diarrhoea is a major public health concern, particularly in developing countries, and is associated with significant morbidity and mortality among children under five years of age.

- *Types*

Acute, Persistent, and Chronic diarrhoea. [1,2]

➢ *Agar Well Diffusion Assay:*

The agar well diffusion assay is an in vitro antimicrobial susceptibility testing method used to evaluate the ability of a test substance to inhibit the growth of microorganisms. In this method, a standardized microbial inoculum is spread uniformly over the surface of agar medium, and wells are punched into the agar and filled with the test extract or antimicrobial agent. During incubation, the substance diffuses into the agar, and its antimicrobial activity is determined by measuring the diameter of the zone of inhibition surrounding each well. This method is widely used for screening the antimicrobial activity of plant extracts and natural products.[3]

II. PLANT PROFILE

Fig 1 *Leucas aspera* - Aerial Parts

➢ *Plant Description:*

Leucas aspera is an annual herb known for its strong, hairy, and sharply four-angled stem growing up to 15 to 60 cm tall. Its narrow and linear leaves are slightly pubescent and have lengths of 8 cm and width of 1.25 cm and the leaf margin is either entire or very slightly serrate. Petioles are short with lengths of 2.5 to 6 mm. *Leucas aspera* has white, small, sessile flowers arranged in dense whorls at the tip or side of branches. The flowers have bracts that are thin with lengths of 6 mm each; the tips of the bracts have bristles accompanied by fringe of fine hairs. Tubular calyxes grow from 8 to 13 mm; the upper part of the calyx has ribs and pubescence. The narrow mouth and the small triangular teeth are characteristic of the calyx, where the upper tooth is larger than the rest. Corolla is about 1 cm long, 5 mm pubescent tube and ringed throat. The short and woolly upper lip is paired with longer lower lip that has short lateral lobes and broad median lobe. Fruit is made up of smooth brown oblong nutlets which are 2.5 mm long, having rounded outer and angular inner face.[4]

➢ *Botanical Classification:*

- Kingdom: Plantae (Plant).
- Subkingdom: Tracheobionta (Vascular plants).
- Superdivision: Spermatophyta (Seed plants).
- Division: Angiospermae.
- Class: Dicotyledonae.
- Subclass: Gamopetalae.
- Series: Bicarpellatae.
- Family: Labiatae or Lamiaceae.
- Genus: Leucas.
- Species: aspera.[5]

III. METHODOLOGY

➢ *Collection and Authentication:*

The aerial parts of *Leucas aspera* were collected from the local area of Anthiyur in the month of December 2026, Erode District, Tamil Nadu, India. The plant material was authenticated by Dr. P. RADHA; Research officer (Botany) Sci II, I/C; Siddha Medicinal Plants Garden/ Mettur Dam, Tamil Nadu- 636401 .and a voucher specimen {C0205250021} was submitted at the SSM College of Pharmacy, Erode (638312) Tamil Nadu, India.

➢ *Extraction of Plant Material by Maceration:*

The ethanolic extract of *Leucas aspera* was prepared by maceration method.

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Maceration is a simple, traditional solid-liquid extraction method used to isolate bioactive compounds from plant materials using an organic solvent.

➢ *Principle:*

Maceration is an extraction technique based on the principle that when plant material is soaked in a suitable solvent, the solvent penetrates the plant cells and dissolves the soluble phytoconstituents. These dissolved constituents then diffuse from the plant material into the surrounding solvent until equilibrium is reached. The extract is subsequently separated by filtration.

➢ *Apparatus Required for Maceration:*

- Plant material / Crude drug powder
- Solvent (ethanol)
- Maceration container (Glass bottle or conical flask)
- Measuring cylinder
- Filter paper
- Funnel
- Storage container

➢ *Procedure:*

First, the plant material was collected, washed, and dried at a temperature not exceeding 40°C. After drying, the material was ground into a coarse powder using a mechanical grinder. A known quantity of the powdered drug was placed in a clean, closed container and soaked with a suitable solvent (ethanol, water, or hydroalcoholic solvent) in an appropriate ratio (1:10 w/v), until the material is completely immersed. The container is tightly closed or seal and kept at room temperature for about 3-7 days, with occasional shaking or stirring to improve the dissolution of active constituents into the solvent. After the maceration period, the mixture is filtered using filter paper or muslin cloth to separate the liquid extract from the plant residue. The filtrate is obtained is then concentrated by evaporating the solvent using a water bath or rotary evaporator if required. Finally, the concentrated extract is collected and stored in an airtight container for further study or use. [6,7]

➢ *Agar Well Diffusion Method:*

- *Principle:*

Antimicrobials from the sample were permitted to diffuse from the sample into the medium and react on the plate which has been inoculated with the test microorganisms. All the zones of inhibition obtained would be circular zones since there would be a confluent growth on the plate. Size of the zone of inhibition could be measured in mm.

- *Materials Required:*

Both the bacteria species i.e. (S. aureus – 902 and E. coli – 443) were procured from MTCC Chandigarh, India. Nutrient Agar medium, Nutrient broth, Gentamicin solution were procured from Himedia, India. Samples for testing, petri plates, test tubes, beakers, conical flasks were from Borosil, India.

- *Nutrient Agar Medium:*

To prepare the medium, 2.8 grams of Nutrient Agar Medium from HiMedia was used. This was added to 100 ml of distilled water. The resulting mixture was then placed inside the autoclave and sterilized under high pressure, 15 pounds per square inch, at 121 degree centigrade for 15 minutes. Afterwards, we mixed thoroughly before pouring it inside petri plates that have a diameter of 100 mm. We poured 25 to 30 ml of the medium in each petri dish.

We also prepared the broth medium. In doing this, we used 2.8 grams of nutrient medium from HiMedia. It was mixed with 100 ml of distilled water and heated until fully dissolved. The nutrient broth medium and the Nutrient Agar Medium from HiMedia were both prepared properly and carefully.

- *Procedure:*

Plates containing nutrient agar medium in the quantity of 20 ml were utilized. These petri plates were exposed to bacterial strains that were 24 hours old. Bacterial strains were standardized to have the same turbidity of 0.5 as measured using OD according to McFarland Standard. Some openings were done to these plates and the test compound LA was introduced in the concentrations of 500, 250, 100 and 50 µg/ml. These petri plates, containing test compound LA, were incubated at 37 degrees centigrade for 24 hours. Inhibition of the growth of the bacteria by the test compound LA was quantified by the size of the zones in which bacteria failed to grow. Gentamicin was used for comparative purpose to observe the inhibitory effect of the test compound LA. Data were analyzed using GraphPad Prism 6.0 software from the United States of America.). [8,9]

IV. OBSERVATION AND RESULTS

➢ *Extraction:*

Calculation of percentage yield of *Leucas aspera :*

$$Percentage\ yield\ (\%) = \left(\frac{Weight\ of\ extract\ obtained}{Weight\ of\ plant\ material\ used}\right) \times 100$$

$$= \frac{9.03}{100} \times 100$$

$$= 9.03\ \%w/w$$

The percentage yield of the extraction of *Leucas aspera* was found to be 9.03%w/w respectfully.

➢ *Phytochemical Screening:*

Table 1 Phytochemical Inference Data

Phytoconstituents	Inference
Alkaloids	+
Saponins	+

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Phenol and Tannins	+
Carbohydrate and sugars	-
Proteins and amino acids	+
Triterpenoids	+
Steroids	+
Flavonoids	+

➢ *Agar Well Diffusion Method*

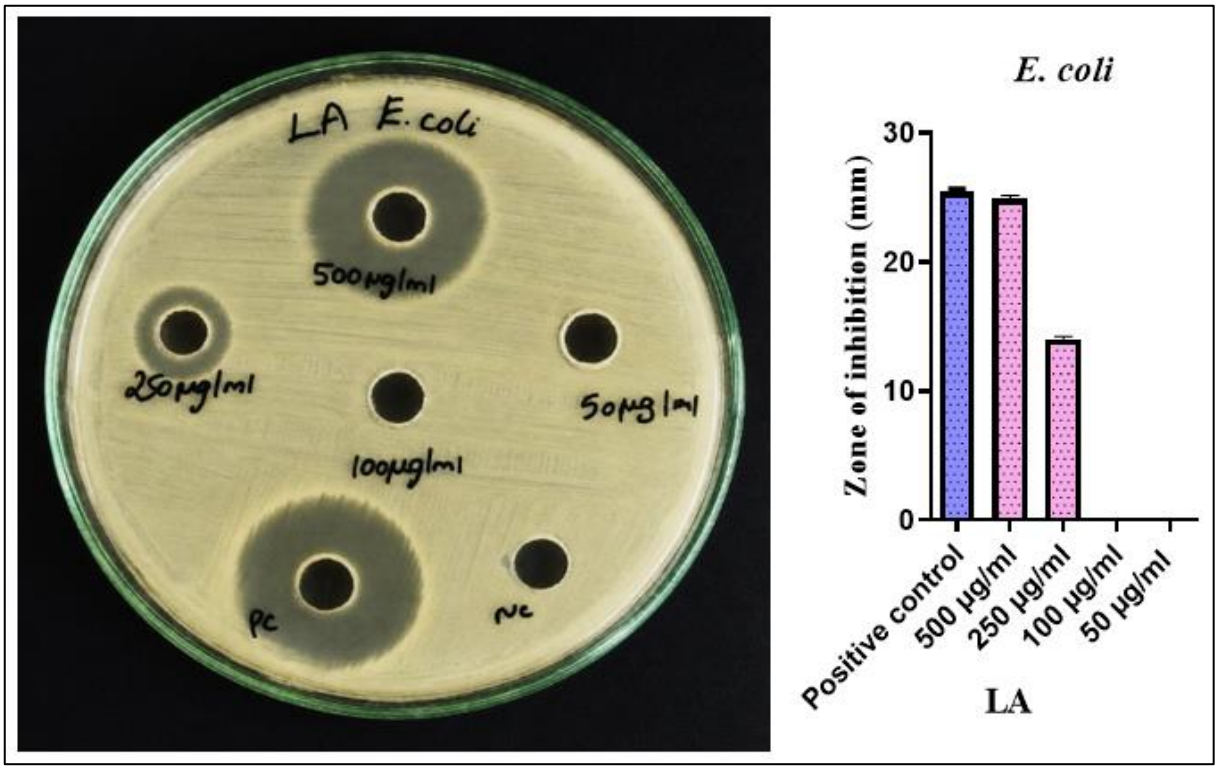

Fig 2 Effect of Sample LA against *E. coli.*

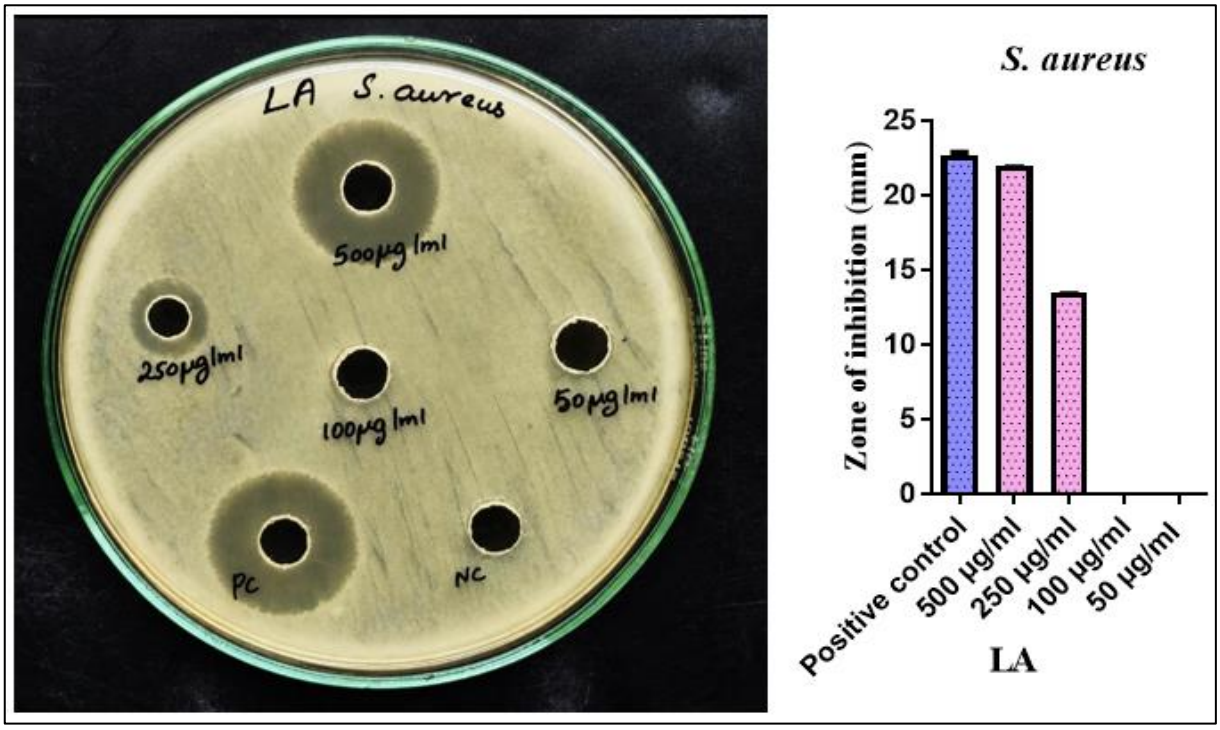

Fig 3 Effect of Sample LA against *S. aureus.*

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Table 2 Zone of Inhibition Obtained by Sample LA against *S. aureus* and *E. coli.*

S.NO	Name of the test organism	Name of the test sample	Zone of inhibition (mm) Mean ± SD				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*E. coli*	LA	24.9±0.28	14.05±0.21	0	0	25.45±0.21
2.	*S. aureus*		21.95±0.07	13.45±0.07	0	0	22.7±0.14

SD – Standard Deviation, *Significance - $p< 0.05$

V. DISCUSSION

The present study demonstrated that the ethanolic extract of *Leucas aspera* possesses significant antibacterial activity against the selected diarrhea-causing bacterial pathogens, Escherichia coli and Staphylococcus aureus. The antibacterial activity increased with increasing concentration of the extract, indicating a concentration-dependent effect. The highest zone of inhibition was observed at 500 µg/mL, producing inhibition zones of 24.9 ± 0.28 mm against E. coli and 21.95 ± 0.07 mm against S. aureus, which were comparable to the standard antibiotic Gentamicin. Moderate antibacterial activity was observed at 250 µg/mL, whereas no inhibition was detected at 100 and 50 µg/mL. These findings suggest that an adequate concentration of the extract is essential for effective antibacterial action.

The observed antibacterial activity may be attributed to the presence of bioactive phytoconstituents such as alkaloids, saponins, phenols, and tannins identified during phytochemical screening. These compounds are known to interfere with bacterial cell wall integrity, membrane permeability, and essential metabolic processes, thereby inhibiting bacterial growth. Overall, the findings support the traditional medicinal use of *Leucas aspera* in the treatment of gastrointestinal infections and indicate its potential as a natural antimicrobial agent.

VI. CONCLUSION

The present study concludes that the ethanolic extract of *Leucas aspera* exhibits significant in vitro antibacterial activity against diarrhea-causing pathogens, particularly Escherichia coli and Staphylococcus aureus, as demonstrated by the agar well diffusion assay. The extract showed maximum activity at 500 µg/mL, with antibacterial efficacy comparable to Gentamicin, indicating its strong therapeutic potential. The presence of phytoconstituents and bioactive compounds may be responsible for the observed antimicrobial activity. These findings suggest that *Leucas aspera* could serve as a promising natural source for the development of antimicrobial agents for the management of bacterial diarrhea. However, further studies including minimum inhibitory concentration (MIC), toxicity evaluation, isolation of active compounds, and in vivo investigations are required to confirm its safety and clinical efficacy.

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