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A COMPARATIVE STUDY OF PHYTOCHEMICALS AND ENDOPHYTIC BACTERIAL EXUDATES OF LEUCAS ASPERA

Microbiology

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ABSTRACT

Leucas aspera is a tropical herb that comes under the family of Lamiaceae. This plant is traditionally used for treating various infections. Endophytic bacteria are symbiotic bacteria that reside within the plant. However, shoots are preferable due to their beneficial properties. Here we are attempting a comparative analysis of phytochemicals and endophytic bacterial exudates from Leucas aspera. Phytochemical screening of leaf and stem extract was done using solvent extraction. Endophytic bacteria were isolated and characterised microscopically and biochemically. Antibacterial activity of the plant extract and exudate from endophytic bacteria were performed against certain clinically isolated bacteria using Kirby-Bauer method. The antibacterial activity of both the stem and endophytic bacteria was carried out against three pathogenic bacteria Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes. From the study it is evident that the endophytic bacterial exudate from Leucas aspera possesses more potential antibacterial activity than the shoot extract and Staphylococcus aureus showed maximum inhibition. The study revealed that further research on this plant may lead to exploring novel bioactive compounds.

KEYWORDS

Endophytic bacteria, Leucas aspera, Phytochemicals, Antibacterial activity

INTRODUCTION

Leucas aspera is commonly known as 'thumba or thumbai' widely distributed throughout India (*Vasudha., 2019*). It also produces numerous phytochemicals such as alkaloids, glycosides, flavonoid, phenols, quinine,saponins,tannins and terpenoids. The plant also contains various pharmacological activities like antifungal, antioxidant, antimicrobial and cytotoxic activities. Traditionally the plant *Leucas aspera* is used for treating coughs, colds, bronchitis, asthma, sinus infections, diarrhoea, constipation, ingestion, stomach ulcers, bronchitis, toothache, skin diseases, eye problems, rashes, sore throat and vaginitis *(Dayamritha et al.,2021; Rai et al.,2005; Chandra et al.,2012).*

Bioactive compounds can be produced by medicinal plants at very low levels. Endophytes are microorganisms known to produce bioactive compounds. Endophytes can be bacteria or fungi. They can colonise various parts of the plant as root, leaves, stem and flowers. Bary (1866) endophyte means any organism that grows within the plant seeds and buds (Dayamritha *et al.,*2021).

Nowadays, endophytic bacteria have received great attention because of their intimate and non – detrimental association with plants. They release an array of bioactive compounds that play an important role in biological control of various phytopathogens. Endophytes are microorganisms that live inside living tissues of plants. The endophytes utilise the nutrients in the host plant and help in the promotion of basic metabolic function. Because endophytes live in a steady environment inside the plant have more antagonistic potentiality than microorganisms isolated from the rhizosphere, plant surface, or soil (Darsha *et al*.,2018; Salem *et al.,*2016).

The bioactive compounds from the endophytic bacteria were found to be active in inhibiting pathogenic microorganisms such as *Staphylococcus aureus, Escherichia coli* and *Streptococcus pyogenes.* In the current research we are analysing the effect of plant extract and the endophytic bacterial extract against few pathogens.

MATERIALS AND METHODS

1. Collection Of Host Plant And Surface Sterilisation

Healthy leaves of *Leucas aspera* collected from the field of Palakkad district (10.7867° N, 76.6548° E). The plant material was cut into small pieces using a sterile blade. It was washed in distilled water and surface sterilised with 0.1% HgCl2 for 5 minutes and washed in sterile distilled water 3-4 times to remove the surface sterilising agent. It was then placed in 70% ethyl alcohol for 5 minutes and again washed in sterile distilled water 3-4 times. Sterilised samples were dried on filter sheets (*Dayamritha et al.,2021).*

2. Isolation Of Bacterial Endophytes

The surface sterilised plant parts were aseptically placed on nutrient agar plates and incubated at 37 degrees Celsius for 24-36 hours. After incubation, the bacterial colony was sub-cultured on nutrient agar and each bacterial colony was checked for purity and sub-cultured again *(Dayamrita et al.,2021).*

3. Morphological, Cultural And Biochemical Characterization Of Endophytic Bacteria

The size, shape, Gram staining and motility was estimated along with various biochemical tests such as indole reduction test, methyl red test, Voges Proskauer's test, citrate utilisation test, nitrate reduction test, urease test, catalase test and oxidase test.

4. Extraction Of Leaf Extract Of *Leucas Aspera*

The stem and leaf of Leucas aspera were collected and properly washed in tap water and rinsed in distilled water and dried in shade for 7 days and pulverised using motor and pistol to obtain powdered form. The acetone extract of the plant was prepared by soaking 10 g samples in 70 ml of acetone and water for 12 hours. The extract was filtered using filter paper and stored in an airtight container for phytochemical screening. (Latha *et al.,*2013).

5. Extraction Of Secondary Metabolite From Endophytic Bacteria

Growth from a mature culture of the strain was inoculated aseptically in 250 ml conical flask containing 100 ml of nutrient broth and incubated at 37 degrees Celsius for 14 days with shaking. After the incubation period, extraction of bioactive compounds was carried out using solvent methods.

6. Antibacterial Activity Of Plant Extract Of *Leucas Aspera* And Bioactive Compound Of Endophytic Bacteria From The Same Plant

The antimicrobial activity of the obtained bioactive compound and plant extract was assessed by Kirby-Bauer method against the test organisms *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes*.

RESULTS

Healthy parts of plant, especially leaf and stem were collected from the paddy field and were washed thoroughly in tap water prior to surface sterilisation to remove dirt and contaminants. The stem and leaves were dried under shade at room temperature and powdered using pestle and mortar. The extraction was done using acetone as the solvent for phytochemical screening. The phytochemical screening shows that Tannin, Terpenoid, Saponin, Steroid, Flavonoid, Alkaloid and Phenol positive.

Table 1: Phytochemical screening of plant extract and exudates of endophytes of *Leucas aspera*

Sl. No:	Test	Plant extract	Endophyte exudate
1	Alkaloids	+	_
2	Glycosides	+	+
3	Flavonoids	+	_
4	Phenols	+	_
5	Quinin's	+	+
6	Saponins	+	_
7	Tannins	+	+
8	Terpenoids	+	+

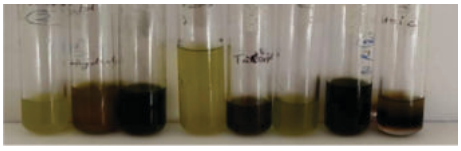

Fig:1. Phytochemical analysis of leaf extract of *Leucas aspera*

The endophytic bacterial strain was isolated from healthy parts of the plant. It ensured that the isolates were endophytes and these were streaked on nutrient agar plates. Four isolates were observed and were named as L1, L2, L3 and L4 respectively. All the isolated endophytic bacteria were Gram positive motile rods. L1, L2, L3 were indole negative and MR positive and able to utilise citrate as carbon source. L4 showed negative results for indole and VP tests but showed a red ring in MR medium indicating test positivity. Based on the microscopy and biochemical test results, L4 might be gram positive motile rod.

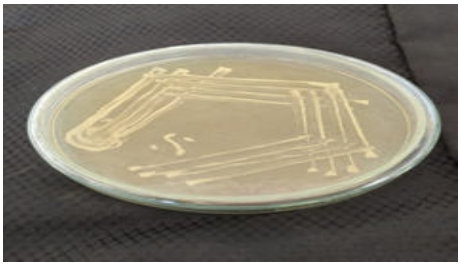

Fig:2. Endophytic bacteria L4

Table 2: Biochemical tests of Endophytic Bacteria

Sl.NO	Biochemical test of Endophytic bacteria	L1	L2	L3	L4
1	Gram staining	Gram Positive, short rod	Gram positive, short rod	Gram positive, short rod	Gram positive, long rod
2	Indole	-	-	-	-
3	MR	+	+	+	+
4	VP	-	-	-	+
5	Citrate	+	+	+	+
6	Motility	motile	motile	motile	motile
7	Oxidase	+	+	+	+

+:Positive; -:Negative

The exudates were extracted by using acetone. From the four endophytes, bacteria producing maximum exudate was selected for antimicrobial activity. Both the endophytic exudate and plant extract were compared with all the four pathogenic test microorganisms such as *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes.* By analysing the zone of inhibition *Escherichia coli,* the zone of bacterial exudate was measured by 11mm. In the case of *Staphylococcus aureus, the zone* of bacterial exudate was found to be wider than plant extract by 12 mm. While testing the antibacterial activity against pathogenic bacteria larger inhibition was against the test organism *Staphylococcus aureus.*

Table 3: Antibacterial activity of bacterial exudates and plant extract from endophytic bacteria against pathogenic bacteria

Sl No:	Human pathogenic bacteria	Plant extract	Bacterial extract
		Zone of Inhibition in millimetres	
1.	Escherichia coli	9mm	10mm
2.	Staphylococcus aureus	11mm	12mm
3.	Streptococcus pyogenes	10mm	11mm

DISCUSSION

Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects (Latha *et al.,2013*). *Leucas aspera* is an annual herbaceous medicinal weed. (*Sasidharan et al.,2012*). In the present attempt *Leucas aspera*, herb with high medicinal importance was used for the isolation of endophytic bacteria. This was mainly done by using the healthy parts from *Leucas aspera.* The control plates showed the effectiveness of surface sterilisation by confirming no microbial growth (*Dayamritha et al.,2021*). It was confirmed that the isolates were endophytes. The isolates were characterised microscopically and biochemically. On comparing the four isolates, endophytic bacteria L4 was selected for further studies based on the quantity of extract. The phytochemical analysis of plant extract shows that the leaves of *Leucas aspera* contain significant phytoconstituents such as tannin, phenol, terpenoid, saponin, steroid, carbohydrates. These phytoconstituents might be responsible for various pharmacological activities *(Lallianwala etal.,2013)*. Theexudates of the endophytes were extracted using acetone. While testing the antibacterial activity against pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pneumonia,* endophytic exudate showed greater inhibitory zone than the shoot extract and was against the test organism *Staphylococcus aureus.* The secondary metabolites of bacterial exudates show more antibacterial activity than the plant extract of *Leucas aspera.*

CONCLUSION

Traditional Indian medicinal herbs have been used in the treatment of different diseases in different countries. The secondary metabolites from plant endophytes are very much helpful in innovative research. *Leucas aspera* is such a medicinally important plant. This study was to report that the exudates of endophytic bacteria from *Leucas aspera* showed an effective antibacterial activity against the tested pathogenic bacteria than that of the plant extract of *Leucas aspera.* The results revealed that the exudates of endophytic bacteria from Leucas aspera are the potential source for the development of new antibacterial compounds.

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