# CHARACTERIZATION OF THE ANTIBACTERIAL PROPERTIES OF ESSENTIAL OILS

**Kashish, Ajay Kumar***
*Faculty of Engineering & Technology, Rama University, Mandhana, Kanpur (209217), U.P. India.*

**ABSTRACT**

This study looks at the antibacterial effects of lavender (*Lavandula angustifolia*) and eucalyptus (*Eucalyptus globulus*) essential oils against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Essential oils, which are known for their various biological actions, are gaining popularity as viable alternatives to traditional antibiotics. The investigation begins with the extraction of essential oils from lavender and eucalyptus plants via steam distillation. Gas chromatography-mass spectrometry (GC-MS) analysis is used to determine the chemical contents of the oils. The antibacterial effectiveness of the essential oils is then tested using agar diffusion and microdilution assays. The results show that both lavender and eucalyptus oils had strong inhibitory effects on all tested bacterial strains. Lavender oil is very powerful against *S. aureus*, whilst eucalyptus oil is highly efficient against *E. coli and P. aeruginosa*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values provide further insight into the oils' concentration-dependent antibacterial activity. This work adds to our understanding of essential oils' antimicrobial capabilities, providing insights into their chemical composition and antibacterial mechanisms. The findings demonstrate the viability of adding lavender and eucalyptus oils into antimicrobial formulations or as natural preservatives in a variety of applications, including pharmaceuticals, food, and cosmetics. Furthermore, this study emphasizes the need of investigating natural compounds as potential sources of novel antibacterial agents in the face of rising antibiotic resistance.

**Keywords-** *Lavandula angustifolia, Eucalyptus globulus, Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* etc.

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## 1. INTRODUCTION

Essential oils (EOs) are aromatic liquids extracted from various plant materials, including flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots (Lyczko et al., 2023). There are various extraction procedures, including solvents, pressing, and sublimation (Zhang et al., 2018). Steam distillation is the most frequent approach for obtaining essential oils from these materials (Hanif et al., 2019). Essential oils are widely utilized in medicine, cosmetics, aromatherapy, fragrances, and food preservation. They include chemicals with antioxidant and antibacterial effects, including menthol, eugenol, carvacrol, and thymol (Beya et al., 2021). EOs contain over 50 components at varying concentrations that contribute to antibacterial action (Bhavaniramya et al., 2019). Numerous studies have demonstrated that essential oils have anti-carcinogenic, anti-inflammatory, anti-mutagenic, antibacterial, antifungal, and

antiviral activities (Maddala et al., 2023). In the recent years, due to increasingly negative consumer perceptions of synthetic preservatives, interest in essential oils and their application in food preservation has been amplified. Moreover, the development of resistance to different antimicrobial agents by bacteria, fungi, viruses, parasites, etc. is a great challenge to the medical field for treating the infections caused by them, and hence, there is a pressing need to look for new and novel antimicrobials. EO composition is regulated by genotype, environment, agronomy, and processing technology (Guo et al., 2020). Lavender is a perennial scented plant from the Lamiaceae family that grows as a semi shrub. The plant is endemic to the Mediterranean region and frequently cultivated for essential oil production (Barut et al., 2024). Lavender is one of the most important EO plants. Lavender species essential oils are often utilized in aromatherapy as antibacterial agents (Speranza et al., 2023). Lavender essential oils contain monoterpenoids such as terpinen-4-ol, linalool, linalyl acetate, and 1, 8-cineole, which have been linked to antimicrobial activity, antimutagenicity, and cytotoxicity (Soulaimani et al., 2019). Lavender essential oil has antimicrobial properties against a variety of microorganisms, including gram-positive bacteria like *Staphylococcus aureus*, and gram-negative bacteria like *Pseudomonas aeruginosa, Escherichia coli* (Xylia et al., 2023). Eucalyptus is one of the most important genera in the Myrtaceae family, which contains 140 genera and 3800 species spread across tropical and subtropical regions (Shiferaw et al., 2022). Eucalyptus covers 506,000 ha of land in Ethiopia, primarily in the highlands (1500-3200 m) with favourable moisture and temperature conditions for tree growth (Damtie et al., 2020). Eucalyptus species are aromatic and medicinal plants. Eucalyptus species essential oil has antibacterial and antioxidant qualities that are widely employed in pharmaceutical and cosmetic applications, as well as flavouring and food preservation. Several studies have proved the antibacterial activity of Eucalyptus essential oils against a broad spectrum of microorganisms (Hafsa et al., 2016). The basic constituents of *Eucalyptus globulus* essential oil are gathered during the summer, and one of the most important factors influencing the amount of medicinal plant potency is the timing of plant organ harvesting. Eucalyptus is widely used in traditional medicine, and its essential oil is among the most popular and effective treatments. Many of these symptoms are most likely induced by 1,8-cineole, the active ingredient (Cmikova et al., 2023).



## 2. MATERIAL AND METHODS

### 2.1 Material

#### 2.1.1 Plants and their essential oil

Essential oil was purchased from FFDC Kannauj, Uttar Pradesh, India. Two distinct essential oils were employed as antibacterial agents in varying doses. The chosen essential oils are listed here (table 1).

**Table: 1** Plant name and their essential oil

| S.no. | Name of the plants | Essential oil |
|---|---|---|
| **1.** | *Eucalyptus globulus* | Eucalyptus oil |
| **2.** | *Lavender angustofolia* | Lavender oil |

#### 2.1.2 Microorganism used

The selected bacteria mentioned below:

- *Escherichia coli (E. coli)*
- *Pseudomonas aeruginosa (P. aeruginosa)*
- *Staphylococcus aureus (S. aureus)*

#### 2.1.3 Chemical utilized

The chemicals were used as required for present work limited e.g. Ethanol used as surface sterilization, equipment sterilization. Streptomycin used as positive control which was antibacterial agent (antibiotic) and equipment are used autoclave, incubator, hot air oven,

electronic weighing machine, laminar air flow, refrigerator and freezer, hot plate, magnetic stirrer, petri dishes, beaker, flask, measuring cylinder, micropipettes and tips, scissors, filter paper, parafilm, aluminium wrapper, laboratory gloves, cotton plug, matchbox, burner, antibiotic zone reader.

**2.2 Methods**

**2.2.1 Media composition**

- **Nutrient agar**

Nutrient agar is an ideal medium for demonstration because it allows colonies to survive longer at room temperature without facing the risk of overgrowing, which can happen with more nutritional mediums.

**Table: 2** Composition and amount of NA

| Ingredient | Gm/litre |
|---|---|
| Peptone | 5.00g |
| HM peptone B # | 1.50g |
| Yeast extract | 1.50g |
| Sodium chloride | 5.00g |
| Agar | 15.00g |

- **Preparation of nutrient agar plates**

  Plates of Nutrient Agar (NA) were made by dissolving 14 grams of nutrient agar in 500 ml of distilled water and then autoclaved. Pouring and plating were done in a sterile environment. These plates were stored in the refrigerator for 24 hours.

**2.2.2 Formulation of essential oils against specific bacteria.**

The composition was created by combining just selected essential oils. The compositions were created using the accords of two essential oils at various concentrations. Each formulation has a basic quantity of one essential oil. In this study, lavender oil and eucalyptus oil, are denoted as L and E respectively. Take one crude oil (Lavender) in amount of 2 microlitre is also used in each plate and antibiotic in amount of 2 microlitre also.



**2.2.3 Essential oil formulations**

- **Lavender and Eucalyptus oils (LE) formulation**

The formulations of lavender and eucalyptus oils were prepared at different amount of concentration of both essential oils mentioned here (Table 3

**Table: 3** Formulations of lavender and eucalyptus oils

| Sr. No. | Name of the formulation | Concentration of essential oils (in microlitre) | |
|---|---|---|---|
| | | Lavender | Eucalyptus |
| **1.** | LE 1 | 3µl | 3µl |
| **2.** | LE 2 | 4µl | 2µl |
| **3.** | LE 3 | 2µl | 4µl |

The concentrations of all formulations differ from one another since each essential oil in this study has a unique concentration when combined with another essential oil. In the construction of entire formulations of selected essential oils, specified quantities have been used, such as lavender and eucalyptus formulations and take 2*µ*l in each concentration.

**2.2.4 Inoculum preparation**

*Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* were obtained from the Department of Microbiology, Rama Medical

College. Table 4 lists the ideal parameters for inoculum preparation, including growth medium, growth condition, temperature, and incubation period. All bacterial strains were grown in Nutrient Agar and incubated at 25°Celsius for 24 hours.

**Table: 4** Optimal conditions for inoculum preparation

| Sr. No. | Bacterial strains | Growth medium | Growth condition, temperature | Incubation period |
|---|---|---|---|---|
| **1.** | *Escherichia coli* | Nutrient agar | **Aerobic, 37°C** | 2 days |
| **2.** | *Pseudomonas aeruginosa* | Nutrient agar | **Obligately aerobic, 37°C** | 2 days |
| **3.** | *Staphylococcus aureus* | Nutrient agar | **Facultative aerobic, 30-37°C** | 2 days |



**2.2.5 Methods of analysing antimicrobial activity**

There are several categories of assessment of antimicrobial activity but the broad categories of assessment of antibacterial activities are in vitro disk or well diffusion, agar or broth dilution and micro dilution. Most of the investigators have used these types of methods. These methods are based on investigation and reported for standardized testing of antibiotics. The methods can be used to easily determine the antimicrobial as well as antifungal activity and calculate a minimum inhibitory concentration. Amongst several methods, disk or well diffusion method has additional advantages like low cost, obtainability of results within short duration of time period without specialized laboratory facilities. In the present study disk diffusion method was used for determination of antibacterial activities of different essential oils and formulations against selected bacterial strains.

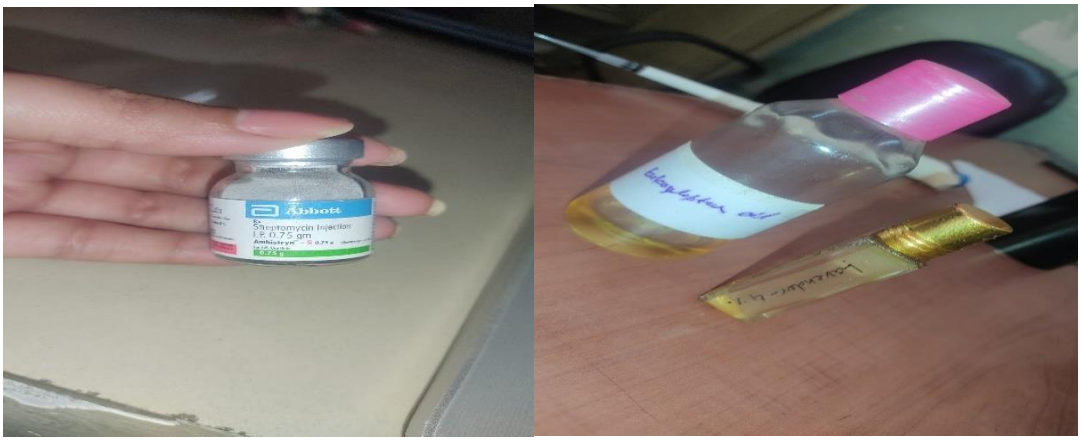
**2.2.6 Disc Diffusion Assay**

The disc diffusion assay was performed in sterilized petri plates of 8 cm diameter as per the method described in Indian Pharmacopoeia. After solidification of Nutrient Agar in petri plates, the suspension culture of bacteria (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*) was spread on respective plates. Autoclaved 5 mm diameter dried discs of Whatman filter paper no. 1 were used as discs. The different amounts of concentration of essential oils and formulations were loaded on discs and an antibacterial drug. Anti-bacterial drug Streptomycin was used as positive control. After that the plates were incubated for the period of 18 to 24 hours on 29˚C. Petri plates were incubated at 29°C for 24 to 48 hours to check zone of inhibition. The values of zone of inhibition are given in result section as mean of tests was performed in doublets (Sharma et al., 2014).

**3 RESULTS AND DISCUSSION**

**3.1 Sample collection**

The main materials used in this in this project are essential oils (lavender and eucalyptus) and antibiotic which are collected from the FFDC and antibiotic is purchased from local pharma shop shown in Fig 1.1(a) and (b)




**(a)** **(b)**

**Figure 1.1 – (a) Streptomycin antibiotic, (b) Lavender oil and Eucalyptus oil sample**

### 3.2 Preparation of media

Media is prepared by adding the 14g of NA media in 500ml of distilled water and kept in autoclave for 30 min then kept for 3-4 min for cooling. Pouring and plating was done under sterile condition in LAF which is shown in Fig 1.2



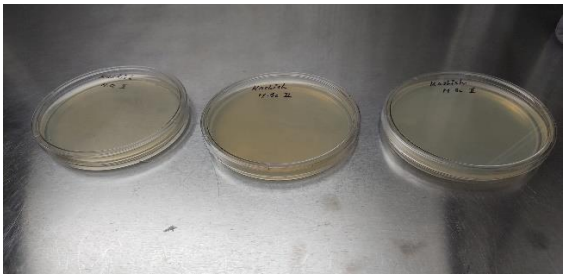
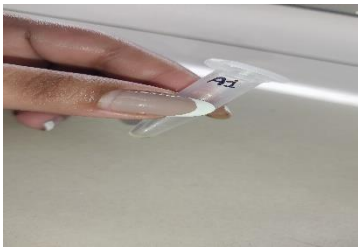


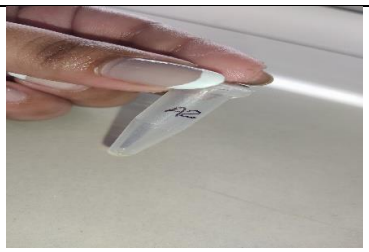
**Figure 1.2 Agar plates**

### 3.3 Essential oil concentration

#### 3.3.1 Lavender and Eucalyptus oils (LE) formulation

The formulations of lavender and eucalyptus oils were created with varying concentrations of both essential oils. The concentrations of all formulations varied because each essential oil in this study has a different concentration when mixed with another essential oil shown below

| Sr. No. | Name of the formulation | Concentration of essential oils (in microlitre) | | Pictures |
|---|---|---|---|---|
| | | Lavender | Eucalyptus | |
| 1. | LE 1 | 3µl | 3µl |  |

| | | | | |
|---|---|---|---|---|
| **2.** | LE 2 | 4µl | 2µl |  |
| **3.** | LE 3 | 2µl | 4µl |  |



**3.4 Inoculum preparation**

All bacterial strains (*Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*) were cultured in NA media for 24 hours in Bacterial laboratory in Microbiology department shown in Fig 1.3 (a), (b), and (c)

**(a) (b) (c)**

**Figure 1.3 – (a)** *Pseudomonas aeruginosa* **(b)** *Escherichia coli* **(c)** *Staphylococcus aureus.*

**3.5 Antibacterial activity of essential oils by zone of inhibition test**

Determination of antibacterial activity of the selected essential oils and formulations against *E. coli, S. aureus, P. aeruginosa* was by the zone of inhibition test. Two microliters of essential oils and formulations of essential oils were pipetted onto sterile paper disks. The petri plates were incubated at 29 C for 24 to 48 hours to check zone of inhibition. Areas of clearing around the disks after incubation were measured and indicate that the oils have some antibacterial activity. Diameters of zones of inhibition were measured in centimeter and recorded. The disk's initial clear zone displays the zone of inhibition of various concentration of oils such as lavender, eucalyptus, and crude oil (lavender) and streptomycin on different bacteria shown in Fig. 1.4 (a), (b) and (c).

(a) (b) (c)

**Figure 1.4- (a)** *Pseudomonas aeruginosa* **(b)** *Escherichia coli* **(c)** *Staphylococcus aureus.*

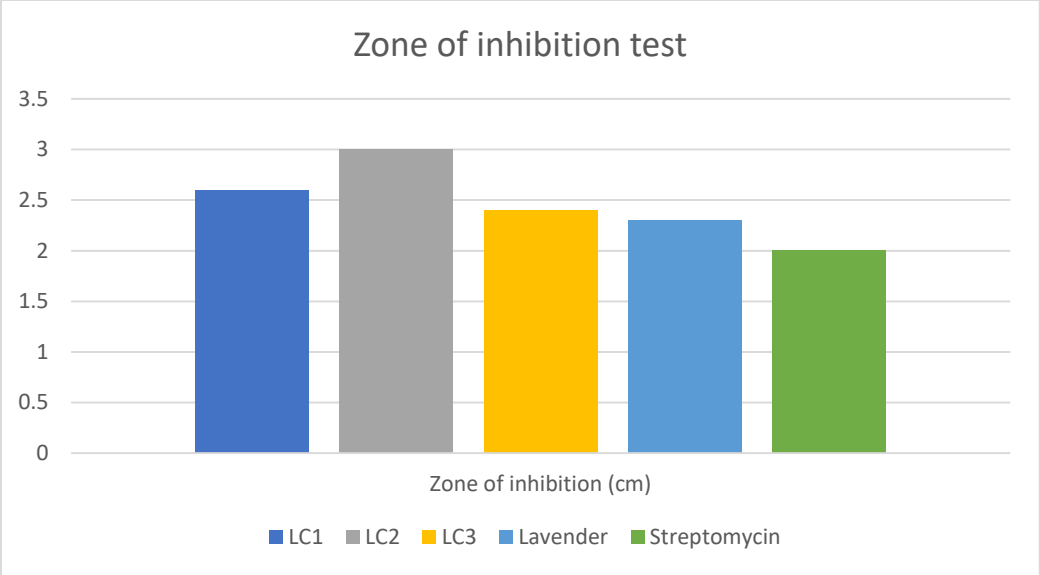
**3.5.1 Antibacterial activity of Lavender and Eucalyptus oil Formulations, Crude oil (Lavender)and Streptomycin against *Escherichia coli***

The antibacterial properties of lavender and eucalyptus oil Formulations, Crude oil (Lavender)and Streptomycin were demonstrated in the zone of inhibition ranging from 2cm to 3cm in diameter, against *Escherichia coli*. The zone of inhibition values is shown in table 5 and in graphical form (graph 1.1) for the purpose of comparison.



**Table: 5 Antibacterial activity of Lavender and Eucalyptus oil Formulations, Crude oil (Lavender)and Streptomycin against *Escherichia coli***

| Sr. No. | Antibacterial agent | Zone of inhibition (cm) |
|---|---|---|
| **1.** | LC1 | 2.6 |
| **2.** | LC2 | 3.0 |
| **3.** | LC3 | 2.4 |
| **4.** | Lavender | 2.3 |
| **5.** | Streptomycin | 2.0 |




**Graph 1.1- Zone of inhibition test result in *E. coli***

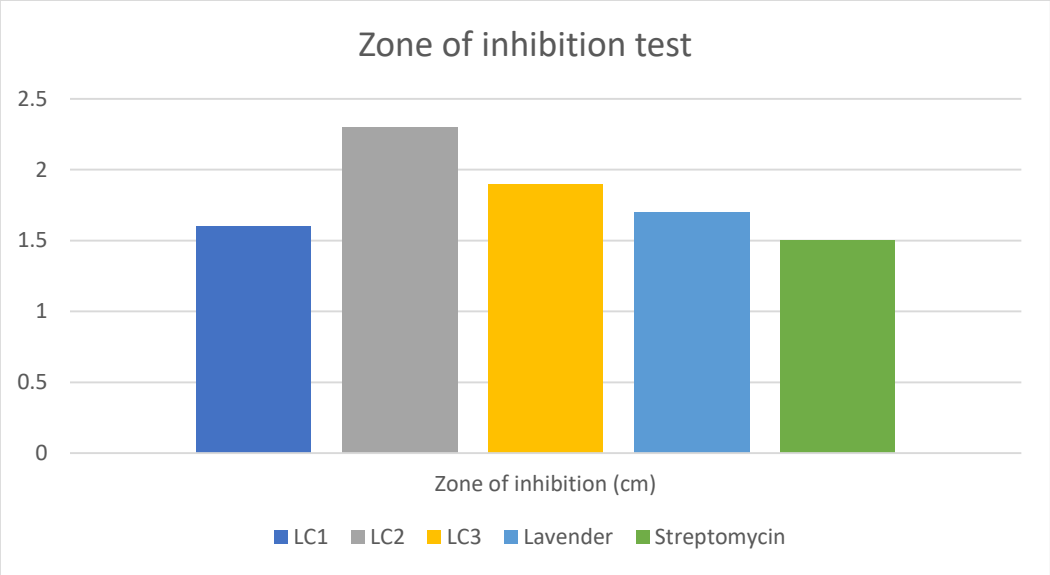


**3.5.2 Antibacterial activity of Lavender and Eucalyptus oil Formulations, Crude oil (Lavender)and Streptomycin against *Staphylococcus aureus***

The antibacterial properties of lavender and eucalyptus oil Formulations, Crude oil (Lavender)and Streptomycin were demonstrated in the zone of inhibition ranging from 1.5cm to 1.7cm in diameter, against *Staphylococcus aureus*. The zone of inhibition values is shown in table 6 and in graphical form (graph 1.2) for the purpose of comparison.

**Table: 6 Antibacterial activity of Lavender and Eucalyptus oil Formulations, Crude oil (Lavender) and Streptomycin against *Staphylococcus aureus***

| Sr. No. | Antibacterial agent | Zone of inhibition (cm) |
|---|---|---|
| **1.** | LC1 | 1.6 |
| **2.** | LC2 | 2.3 |
| **3.** | LC3 | 1.9 |
| **4.** | Lavender | 1.7 |
| **5.** | Streptomycin | 1.5 |




**Graph 1.2- Zone of inhibition test result in *S. aureus***

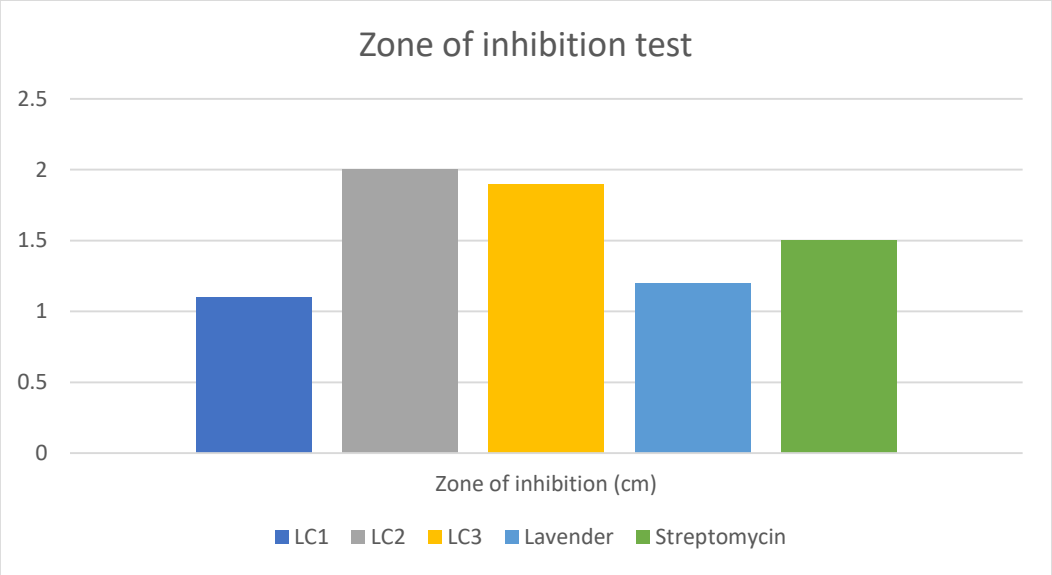


**3.5.3 Antibacterial activity of Lavender and Eucalyptus oil Formulations, Crude oil (Lavender) and Streptomycin against Pseudomonas aeruginosa**

The antibacterial properties of lavender and eucalyptus oil Formulations, Crude oil (Lavender)and Streptomycin were demonstrated in the zone of inhibition ranging from 1.1cm to 1.9cm in diameter, against *Pseudomonas aeruginosa*. The zone of inhibition values is shown in table 7 and in graphical form (graph 1.3) for the purpose of comparison.

**Table:7 Antibacterial activity of Lavender and Eucalyptus oil Formulations, Crude oil (Lavender) and Streptomycin against Pseudomonas aeruginosa**

| Sr. No. | Antibacterial agent | Zone of inhibition (cm) |
|---|---|---|
| **1.** | LC1 | 1.1 |
| **2.** | LC2 | 2.0 |
| **3.** | LC3 | 1.9 |
| **4.** | Lavender | 1.2 |
| **5.** | Streptomycin | 1.5 |




**Graph 1.3 – Zone of inhibition test result in *P. aeruginosa***



**CONCLUSIONS**

The identification of antibacterial activity in essential oils marks a big step forward in using natural substances for medicinal reasons. Researchers have discovered a variety of ways by which essential oils exert their antimicrobial properties, ranging from membrane disruption to interfering with critical bacterial cell functions. This thorough understanding highlights the potency and versatility of essential oils as antimicrobial agents against a wide range of bacterial infections. Furthermore, the variation in antibacterial activity across different essential oils demonstrates the breadth of nature's pharmacopeia and provides prospects for personalized therapeutic approaches. However, obstacles such as extraction process standardization, active component identification, and formulation optimization must be overcome in order to fully realize essential oils' medicinal potential. Nonetheless, the findings of this study open the way for the creation of new antibacterial drugs derived from natural sources, which show promise in reducing antibiotic resistance and addressing public health concerns. We can unleash the full therapeutic potential of these natural substances by continuing to uncover the complexities of essential oil-bacteria interactions and researching new delivery mechanisms. This will provide long-term treatments for infectious disorders.

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