



Assessment of Anti-Bacterial Property of Selected Essential Oils, Lemongrass, Lemon, Eucalyptus, Citronella, and Peppermint against *Escherichia coli* DH5 α Strain

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Abstract

Essential oils (EOs) have been used as a common home cure for numerous illnesses, including headaches, anxiety, and insomnia, since ancient times. They are concentrated hydrophobic liquids that are rich in a complex variety of terpenoid hydrocarbons and contain secondary metabolites of plants. The objective of this study was to have a comparative analysis of the antibacterial characteristics of five widely used essential oils: lemongrass, lemon, eucalyptus, citronella, and peppermint. *Escherichia coli* DH5 α strain was chosen because of its quick growth and ease of maintenance. The Agar well diffusion method was used to conduct the microbiological assay. Measure the antibacterial effectiveness of the oils, zones of inhibition were assessed. The current study's findings demonstrate that each of the five essential oils used has discernible antibacterial action, although the most potent impact is found in lemon essential oil. Further studies can be done to find the effect of a mixture of different essential oils in different proportions and the mechanism of its action.

Keywords

Essential oils, Antibacterial Activity, *Escherichia coli*, Lemon

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1. Introduction:

Essential oil is a concentrated hydrophobic liquid made up of volatile chemical components from plants. Volatile oils, ethereal oils, and aetheroleum are other names for essential oils. The fact that the oil captures the essence of the plant in the form of its aroma or flavour gives the chemical its name. A plant portion is pressed or steamed to produce oil, which is then used to make essential oils. A plant's flowers, bark, leaves, or fruit may be used to make essential oils. Essential oils, derived from plant extracts, gained popularity in alternative medicine from the late twelfth century, highlighting antibacterial and antioxidant effects. Essential oils are popular for their potential medicinal benefits, including stress and anxiety relief, demonstrated in studies, and commonly used in massages (Stefflitsch, W. et al, 2015; De Sousa, D. P. et al, 2015)

Peppermint oil has shown promise in reducing tension, headaches, and migraines (Göbel, H. et al, 1996). Research indicates that inhaling essential oils can improve sleep, benefiting both students and postpartum mothers (Afshar, M. K. et al, 2015). While essential oils exhibit antibiotic and antibacterial properties in in-vitro studies, their real-world effectiveness for curing bacterial infections remains unclear. Aromatherapy, a long-practiced therapeutic use of essential oils, involves their use for fragrance in homes, laundry, and natural products. Some oils, particularly citronella, are explored as natural insect repellents, though efficacy varies. Essential oils are also considered for extending the shelf life of foods commercially. While promising, it is important to note that further research is needed to validate and understand the full extent of their potential benefits (Lillehei, A. S. & Halcón, L. L, 2014). Because essential oils are very concentrated plant extracts, they can have negative effects including headaches. They could result in rashes or an allergic reaction. Additionally, essential oils must be diluted before usage.

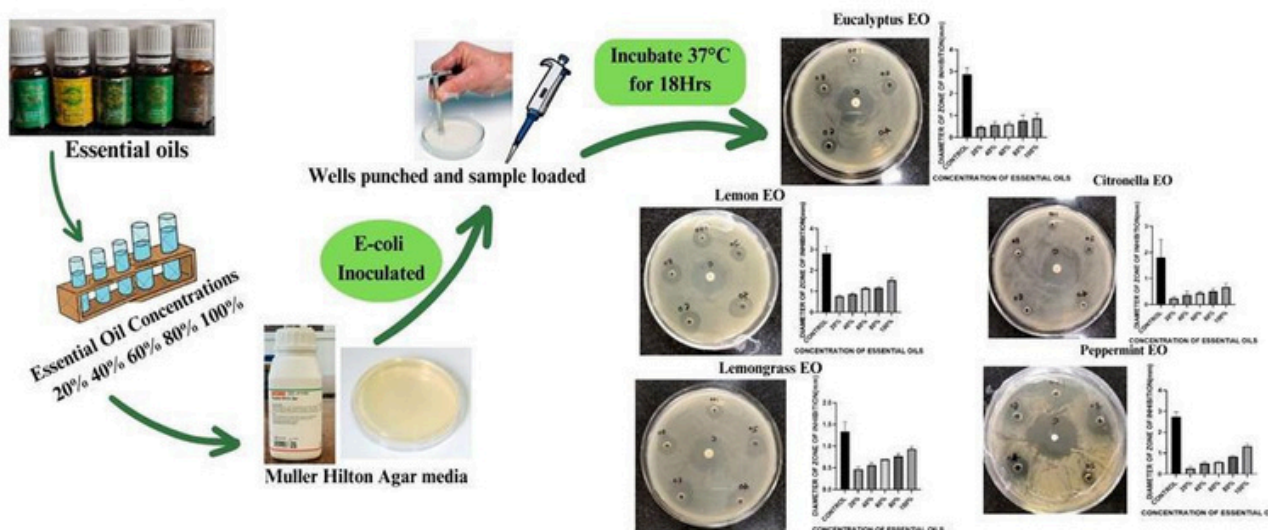


Fig 1: Graphical Abstract

Direct skin contact should be avoided whenever possible. Home remedies for minor diseases frequently employ essential oils have been and still are. Because they can be utilised as scenting components in cosmetics, essential oils also have significant commercial significance and are employed in many cleaning products.

The objective of this project is to conduct a comparative analysis and evaluate the antibacterial efficacy of five widely used essential oils: lemongrass, lemon, eucalyptus, citronella, and peppermint. *Escherichia coli* DH5 strain is the bacteria that has been studied.



Fig 2: Essential Oils used for study: Lemongrass, Lemon, Eucalyptus, Citronella, and Peppermint

Lemongrass Essential Oil

Lemongrass, a tropical grass, serves culinary and herbal purposes, with its oil featuring a citrusy aroma used in various personal care items. While lemongrass is commonly ingested, topically applied, or used in aromatherapy for various ailments, empirical evidence supporting its broad applications is limited. It adds a distinct "lemon" flavour to food and beverages, commonly found in herbal teas. Its use extends to the production of scents, flavours, cosmetics, and pharmaceuticals through. Notably, Citral, a major component, demonstrated anti-inflammatory properties in laboratory rats when combined with the non-steroidal anti-inflammatory drug naproxen.

This suggests the compound's pharmaceutical potential with comparable anti-inflammatory benefits to naproxen alone but with fewer stomach-related adverse effects (Boukhatem, M. N. et al, 2014; Schweitzer, B. et al, 2022).


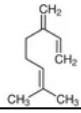

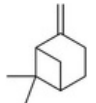
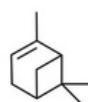
Table 1: Active Compounds of Lemongrass

Name	α -terpineol	Citral
Structure		

Lemon Essential Oil

The lemon tree, *Citrus limon*, introduced in Spain and North Africa between 1000 and 1200 CE, played a historical role in Europe's culinary and medicinal landscapes. Initially considered a variant of citron, it is now recognized as a distinct hybrid species. Lemon's alkaloid-rich composition offers various health benefits, including anticancer, antibacterial, antifungal, antiviral, and antidiabetic properties. Lemon essential oil, composed of d-limonene, -terpinene, -pinene, sesquiterpene hydrocarbons, carbonyl compounds, alcohols, esters, and oxides are obtained through hydro distillation. The terpene constituents, particularly pinene, myrcene, and limonene, contribute to the antibacterial activity by potentially causing membrane rupture. Linalool, a minor monoterpene alcohol, exhibits antibacterial and antifungal properties. Lemon and its essential oil, with proven antimicrobial capabilities, have been integral in Ayurvedic medicine for over 1,000 years. Scientifically validated benefits include uses as a natural teeth whitener, household cleaner, laundry freshener, mood enhancer, and nausea reliever. Comparative research has reinforced lemon's potent antibacterial characteristics, establishing it as a valuable resource in health and wellness (Mahato, N. et al, 2017)

Table 2: Active compounds of Lemon

Name	Structure
Linalool	
Myrcene	
Limonene	
2-β-pinene	
α-pinene	

Eucalyptus Essential Oil

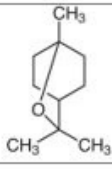
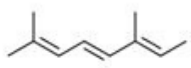
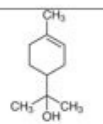
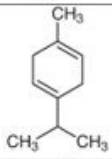
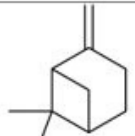
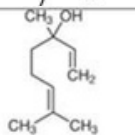
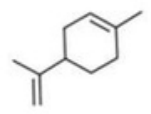
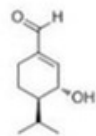
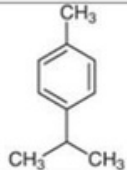
A plentiful amount of biologically active essential oils, including those with bacteriostatic, fungistatic, and anti-inflammatory activities, may be found in the Myrtaceae family. Different Myrtaceae species have strong antibacterial potential, and their volatile oils are used as antimicrobial and antifungal agents in lotions, soaps, and toothpastes. The word eucalyptus, which in Greek means "well-covered," is where the genus name Eucalyptus originates. It alludes to the blooms' cup-like envelope when in bud.

Although it is Australian in origin, ancient medicine systems including Chinese, Indian Ayurvedic, and Greco-European have absorbed and incorporated its therapeutic benefits. By steam distillation and rectification from fresh leaves or terminal branches, its volatile oil is extracted. The active component in eucalyptus oil, eucalyptol (1, 8-cineole), is what gives the oil its many pharmacological effects. The family has been growing and using the Eucalyptus genus extensively for a long time (García-Márquez, J. et al, 2021) (Kumar, A. et al, 2015).

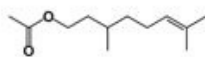
Traditional medicine uses several eucalyptus species as an antiseptic and to treat illnesses of the upper respiratory tract, including colds, the flu, and sinus congestion. In many regions of the world, E. citriodora, E. globulus, and E. teretecorni have been found to have antibacterial, analgesic, and anti-inflammatory properties. About 1.36% of the essential oil found in E. citriodora leaves is citronellal, which makes up the bulk (57%), followed by citronellol (15.89%), citronellyl acetate (15.33%), and other substances. The antibacterial, antifungal, anticandidal, expectorant, and cough-stimulating properties of this essential oil were demonstrated.

The major active compounds in Eucalyptus Essential oil are - Aromadendrene, Alloocimene, 1,8-cineole, Citronellal, Citronellol, citronellyl acetate, p-cymene, Eucamalol, Limonene, Linalool, β- pinene, γ-terpinene and α- terpinol. (Sowndhararajan, K. et al, 2018)

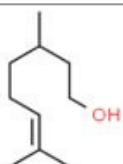
Table 3: Active compounds of Eucalyptus

Name	Structure
1,8-cineole	
Alloocimene	
α- terpinol	
γ-terpinene	
β- pinene	
Linalool	
Limonene	
Eucamalol	
p-cymene	

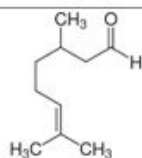
citronellyl acetate



Citronellol



Citronellal



Aromadendrene



Citronella Essential Oil

Citronella essential oil, derived from the leaves and stems of the *Cymbopogon Nardus* plant indigenous to Asia, particularly in countries like Indonesia, Sri Lanka, and India, is well-known for its fresh and lemony fragrance. The plant, traditionally used in Southeast Asian nations as a natural insect repellent and in traditional medicine, made its way to the West in the 19th century and is now cultivated in various tropical locales, including South America, Africa, and the Caribbean.

The essential oil extracted from citronella leaves and stems is a versatile product widely utilized in home goods such as candles, soaps, and insect repellents. Citronella oil is obtained through steam distillation of the fresh or dried leaves and stems of the citronella grass plant, resulting in a clear or pale-yellow liquid with a potent lemony, sweet, and pleasant scent. The primary chemical components of citronella oil, including Citronellal, Geraniol, Citronellol, Limonene, Camphene, Linalool, -pinene, and -pinene, along with trace levels of other substances like Citral, linalool, and camphene, contribute to its distinctive insect-repellent and anti-fungal properties (Carson, C. et al,2002) (Unalan, I. et al,2019)

Table 5: Active Compounds of Citronella

Name	Limonene	Elemol	Isopulegol	Citronellol	Geraniol	Citronellal
Structure						

2. Materials and Methods:

In this comprehensive study, the antibacterial properties of five essential oils—Lemon, Lemongrass, Citronella, Eucalyptus, and Peppermint—were thoroughly examined against *Escherichia coli* DH5 α strain. The methodological approach involved meticulous preparation steps, including autoclaving of glassware, media, and micropipette tips, followed by a crucial UV treatment for 20 minutes to ensure absolute sterility. The media, comprising of LB Broth and MHA Media, were meticulously formulated, and subjected to sterilization procedures. *Escherichia coli* DH5 α was cultured, underwent staining procedures, and its concentration was diluted using acetone to create standard solutions for the essential oils. The inoculation of bacteria onto agar plates was carried out using a Micropipette, and the bacterial spread was evenly achieved with an L-rod under sterile conditions. The preparation of wells, crucial for testing essential oil concentrations, involved the use of a 4mm gel puncher, leaving space for a control zone. This control included Azithromycin Discs, a well-known antibiotic. Following incubation in a 37°C incubator for 18 hours, the zones of inhibition were measured, providing valuable insights into the antibacterial effectiveness of the tested essential oils.

Peppermint Essential Oil

Peppermint (*Mentha x piperita*), belonging to the Lamiaceae family, is a hybrid of water mint and spearmint, thought to have originated in Europe and the Middle East. Recognized for its revitalizing scent and minty aroma, peppermint has a rich history of medicinal use, addressing ailments like headaches and digestive issues in various civilizations. Today, it finds applications in the food and cosmetics industries, grown easily in cool, moist environments through seeds or cuttings. Extracted from the leaves, peppermint essential oil serves diverse purposes, from aromatherapy and toothpaste to soaps. Its versatility is evident in promoting relaxation, reducing stress, aiding digestion, relieving migraines, addressing chest congestion, and enhancing skincare. The oil's active compounds, including Limonene, Methyl acetate, menthone, and L-menthol, exhibit antimicrobial properties, adding to its holistic benefits (Nostrro, A. et al,2007).

Table 4: Active compounds of Peppermint

Name	Limonene	Methyl acetate	Menthone	L-menthol
Structure				

This meticulous methodology ensures a robust exploration of the potential antibacterial applications of Lemon, Lemongrass, Citronella, Eucalyptus, and Peppermint essential oils.

Table 6: Dilutions of Essential Oils

Concentration	Volume of Essential oil in (µL)	Volume of Acetone in (µL)
20%	20	80
40%	40	60
60%	60	40
80%	80	20
100%	100	0

3. Results and Discussion:

3.1 Observation :

Table 7: Measurement of the Zone of Inhibition

Name of Essential Oil	Plate number	Zone of Inhibition (cm) at Different Concentration of EO					
		Control	20%	40%	60%	80%	100%
Lemongrass	Lemongrass 1	1.2	0.5	0.6	0.7	0.7	1.0
	Lemongrass 2	1.2	0.5	0.5	0.7	0.8	0.9
	Lemongrass 3	1.6	0.4	0.6	0.7	0.8	0.9
Lemon	Lemon 1	2.6	0.8	0.9	1.1	1.1	1.6
	Lemon 2	2.6	0.8	0.9	1.2	1.2	1.6
	Lemon 3	3.2	0.7	0.8	1.1	1.2	1.4
Eucalyptus	Eucalyptus 1	2.8	0.5	0.7	0.7	1.0	1.0
	Eucalyptus 2	2.6	0.4	0.4	0.5	0.5	0.6
	Eucalyptus 3	3.2	0.5	0.6	0.6	0.8	1.0
Citronella	Citronella 1	2.6	0.2	0.2	0.4	0.4	0.5
	Citronella 2	1.4	0.2	0.4	0.4	0.5	0.8
	Citronella 3	1.4	0.3	0.5	0.5	0.6	0.7
Peppermint	Peppermint 1	2.6	0.4	0.5	0.5	0.8	1.4
	Peppermint 2	3.0	0.2	0.6	0.6	0.8	1.4
	Peppermint 3	2.6	0.2	0.4	0.6	0.9	1.2

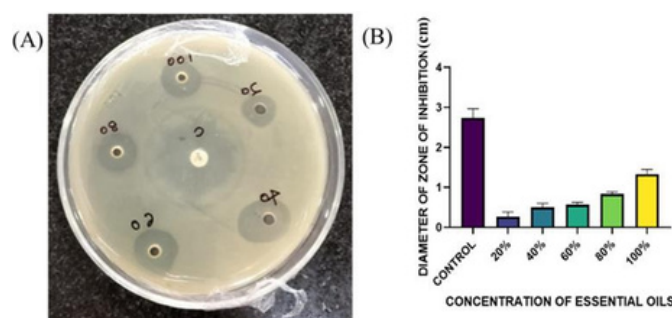


Fig.4: Effect of Lemon EO on Escherichia Coli DH5α (A) Agar Plate Showing Zones of Inhibition at Different Concentrations. (B) Bar Diagram Representing Zones of Inhibition at Different Concentrations

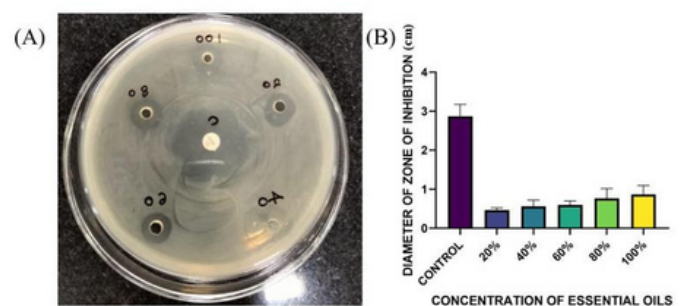


Fig.5: Effect of Eucalyptus EO on Escherichia Coli DH5α (A) Agar Plate Showing Zones of Inhibition at Different Concentrations. (B) Bar Diagram Representing Zones of Inhibition at Different Concentrations

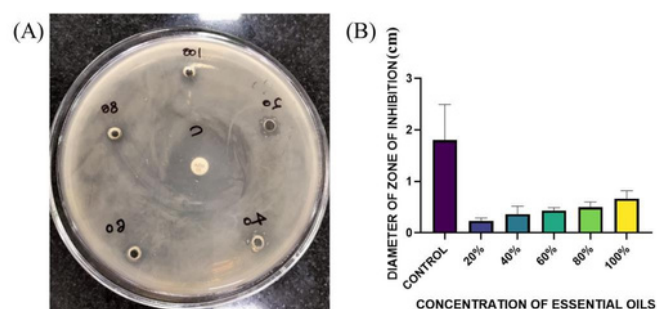


Fig.6: Effect of Citronella EO on Escherichia Coli DH5α (A) Agar Plate Showing Zones of Inhibition at Different Concentrations. (B) Bar Diagram Representing Zones of Inhibition at Different Concentrations

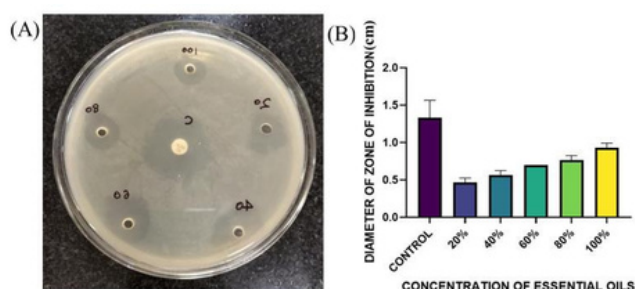


Fig.3: Effect of Lemongrass EO on Escherichia Coli DH5α (A) Agar Plate Showing Zones of Inhibition at Different Concentrations. (B) Bar Diagram Representing Zones of Inhibition at Different Concentrations.

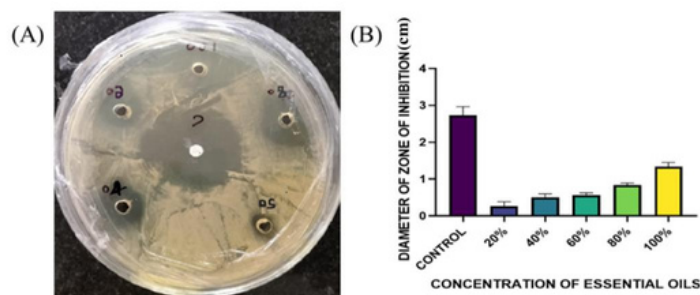


Fig.7: Effect of Peppermint EO on Escherichia Coli DH5α (A) Agar Plate Showing Zones of Inhibition at Different Concentrations. (B) Bar Diagram Representing Zones of Inhibition at Different Concentrations

Table 8: Mean and Error values of Zones of Inhibition

	Control	20%	40%	60%	80%	100%
Lemongrass EO	1.33 ± 0.13	0.46 ± 0.03	0.56 ± 0.03	0.7 ± 0	0.76 ± 0.03	0.93 ± 0.03
Lemon EO	2.8 ± 0.2	0.76 ± 0.03	0.86 ± 0.03	1.13 ± 0.03	1.16 ± 0.03	1.53 ± 0.06
Eucalyptus EO	2.86 ± 0.17	0.46 ± 0.03	0.56 ± 0.08	0.6 ± 0.05	0.76 ± 0.14	0.86 ± 0.13
Citronella EO	1.8 ± 0.4	0.23 ± 0.03	0.36 ± 0.08	0.43 ± 0.03	0.5 ± 0.05	0.66 ± 0.08
Peppermint EO	2.73 ± 0.13	0.26 ± 0.06	0.5 ± 0.05	0.56 ± 0.03	0.83 ± 0.03	• ± 0.06

3.2. Discussion:

The results of the antibacterial assay are given in [Table 8](#). Clear zones of inhibition were observed for all five oils.

Lemongrass Essential Oil - It has been found to have the least error value, emphasizing its antibacterial efficacy against *Escherichia coli*. The findings align with significant antibacterial activity reported in numerous studies, such as those by [Prabuseenivasan et al. \(2006\)](#), [Young et al. \(2000\)](#), and [Lei et al. \(2017\)](#). ([Prabuseenivasan, S. et al. \(2006\)](#)). The zones of inhibition, as depicted in [Fig.5](#), underscore the notable bactericidal effects of Lemongrass Essential Oil against *Escherichia coli*. The results from [Table 8](#) further support these observations, revealing concentration-dependent trends in zones of inhibition ranging from 0.4 cm to 1.6 cm across three plates.

Lemon Essential Oil - The study highlights its effectiveness against *Escherichia coli*, as the most impactful among the five essential oils. The zones of inhibition, displayed in [Fig.4](#), further illustrate Lemon Essential Oil's potent antibacterial action. Citations from [Chao et al. \(2008\)](#), [Burt et al. \(2007\)](#), and other relevant studies substantiate its antimicrobial efficacy ([Chao, S. et al. \(2000\)](#)).

The active compounds contributing to its activity, detailed in [Table.2](#), contribute to understanding the mechanisms underlying Lemon Essential Oil's antibacterial effects. The observed results from [Table 8](#) reinforce these findings, displaying zones of inhibition ranging from 0.7 cm to 3.2 cm at different concentrations.

Eucalyptus Essential Oil- The study depicts its potent antibacterial action against *Escherichia coli*, as evident in [Fig.3](#). The antimicrobial properties of Eucalyptus Essential Oil have been previously demonstrated in studies by [Balakrishnan et al. \(2019\)](#) and [Astani et al. \(2010\)](#). These studies underline its effectiveness against various dangerous bacteria, including *Escherichia coli* and *Staphylococcus aureus*. ([Prabuseenivasan, S. et al. \(2006\)](#)) The versatile applications of Eucalyptus Essential Oil in preventing bacterial development in water and microbial growth in food products are highlighted based on the works of [Meireles et al. \(2017\)](#) and [Nazzaro et al. \(2013\)](#). The observed results from [Table 8](#) further support the observed robust antibacterial capabilities, revealing concentration-dependent zones of inhibition ranging from 0.4 cm to 3.2 cm.

Citronella Essential Oil -Citronella Essential Oil's antibacterial prowess is discussed based on relevant studies, including the findings of [Chalermglin et al. \(2019\)](#), [Mahmoudvand et al. \(2019\)](#), and [Ogbolu et al. \(2007\)](#) (20).

Although [Fig.6](#) illustrates its antibacterial activity, it is noted that Citronella Essential Oil shows comparatively less potency, potentially due to its high volatile nature. Citronella Oil is a promising natural alternative to synthetic antimicrobial medicines. ([Kalemba, D., & Kunicka, A. et al. \(2003\)](#)) Which needs further research to optimize its usage. The observed results from [Table 8](#) align with these observations, indicating zones of inhibition ranging from 0.2 cm to 0.7 cm.

Peppermint Essential Oil - Peppermint Essential Oil, supported by studies such as [Kalemba and Kunicka \(2003\)](#), [Silva et al. \(2016\)](#), [Sienkiewicz et al. \(2013\)](#), and [Adukwu et al. \(2021\)](#) ([Verma, R. S. et al. \(2000\)](#))

The potent antibacterial action of Peppermint Essential Oil is evident in [Fig.7](#), positioning it as a compelling natural substitute, second only to Lemon Essential Oil. The collective studies underline its suitability as a powerful antibacterial agent, especially against multidrug-resistant strains, it has potential as a valuable alternative to traditional antibiotics. The observed results from [Table 8](#) further support these findings, illustrating concentration-dependent zones of inhibition ranging from 0.2 cm to 1.4 cm.

4. Conclusion:

Essential oils (EOs) are concentrated hydrophobic liquids that are rich in a complex variety of terpenic hydrocarbons and contain secondary metabolites of plants. They have been used as a common home cure for numerous illnesses, including headaches, anxiety, and insomnia, since ancient times. The present study examined the antibacterial properties of five widely used essential oils: lemongrass, lemon, eucalyptus, citronella, and peppermint. *Escherichia coli* DH5α strain was chosen because of its quick growth and ease of maintenance. The Agar well diffusion method was used to conduct the microbiological assay. Measurement of the zones of inhibition demonstrated the antibacterial effectiveness of EOs. The experimental results presented underscore the concentration-dependent antibacterial efficacy of Lemongrass, Lemon, Eucalyptus, Citronella, and Peppermint essential oils. Lemongrass essential oil consistently exhibited significant bactericidal properties across all three plates, emphasizing its potential as a potent antimicrobial agent.

Lemon essential oil stood out as highly effective, with significantly larger zones of inhibition correlating with increasing concentrations, reinforcing its traditional uses and potential as a natural food preservative. Eucalyptus essential oil demonstrated potent antibacterial action, particularly at higher concentrations, suggesting its versatile applications in preventing bacterial growth in water and food products. Citronella essential oil displayed moderate inhibitory effects, and while its potency varied at different concentrations, further research may optimize its usage. Peppermint essential oil highlighted robust antibacterial properties, with a clear concentration-dependent trend, highlighting its potential as a natural alternative to traditional antibiotics.

Lemongrass, Lemon, Eucalyptus, Citronella and Peppermint essential oils can be used as a prospective candidate for future studies to check for antifungal, antiviral and antiprotozoal activity. The active compounds showing significant antibacterial properties could be extracted and formulated for medicinal or therapeutic purposes.

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