Original Research Article

ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF *OCIMUM GRATISSIMUM AND CYMBOPOGON FLEXUOSUS OIL* AGAINST SOME SELECTED PATHOGENIC MICROORGANISM

ABSTRACT

Essential oils are concentrated substances extracted from plant components such as leaves, flowers, fruits, stems, roots, and bark. These oils contain volatile organic compounds that are responsible for their distinctive aroma and therapeutic effects. This study investigated the antimicrobial activity of essential oils from *Ocimum gratissimum L* (Clove basil) and *Cymbopogon flexuosus* (Lemongrass) against two gram-positive bacteria, two gram-negative bacteria, and one fungus, using the agar well diffusion method. Fourier-transform infrared spectroscopy (FTIR) analysis of selected essential oils revealed the presence of functional groups, such as aldehydes, ketones, and aromatic rings. The results showed that both oils exhibited significant antimicrobial activity against all tested microorganisms, with *Cymbopogon flexuosus* (Lemongrass) being more effective, completely inhibiting the growth of *Bacillus paramycoides* and *Bacillus altitudinus* at concentrations up to 40%. The zone of inhibition for *Cymbopogon flexuosus* against *Escherichia coli* and *Pseudomonas spp. RRC15* were 25.5 mm and 8.1 mm at 100% concentration, respectively. *Ocimum gratissimum L* showed lower antimicrobial activity than *Cymbopogon flexuosus*, with the zone of inhibition in the decreasing order of *Bacillus paramycoides, Bacillus altitudinus, Escherichia coli, and Pseudomonas spp. RRC15.* Both essential oils completely inhibited the growth of *Colletotrichum gloeosporioides* at concentrations ranging from 100% to 40%. The antimicrobial activity was attributed to the presence of bioactive compounds, such as citral, eugenol, and other monoterpenes in the essential oils, which disrupt bacterial and fungal cell membranes, leading to cell death. These findings highlight the potential of *Ocimum gratissimum* L. and *Cymbopogon flexuosus* essential oils as natural antimicrobial agents for various applications including plant-based crop protection practices and in food and textile industries.

Key words:

Antimicrobial activity, *Ocimum gratissimum, Cymbopogon flexuosus*, Essential oils, Fourier-transform infrared spectroscopy, Agar well diffusion method, Bioactive compounds, Monoterpenes

1. INTRODUCTION

Plant-derived essential oils are a significant product in agriculture-based industries. They are commonly utilised as flavouring agents in a variety of products including food, beverages, perfumes, pharmaceuticals and cosmetics (1). At least 2000 plant species have been used to produce 3000 different types of essential oils with numerous benefits and out of these 300 oils are important from a commercial perspective (2). Primarily obtained through steam distillation, hydrodistillation, and solvent extraction, these substances are stored in plant oil ducts, resin ducts, glands, or trichomes. They contain 20-100 plant metabolites that are chemically complex mixtures mainly of low-molecular-weight compounds such as terpenoids and phenylpropanoids, along with some aromatic and aliphatic constituents (3). Monoterpenes, sesquiterpenes, and their oxygenated derivatives form the largest chemical groups in essential oils (4,5). These natural compounds exhibit various beneficial properties, including antimicrobial, antiviral, antimutagenic, anticancer, antioxidant, anti-inflammatory, immunomodulatory, and antiprotozoal activities. (6–9).

Owing to their diverse beneficial properties, essential oils also hold potential in agricultural practices, particularly in addressing challenges posed by phytopathogenic microorganisms. Phytopathogenic microorganisms reduce plant yield and compromise fruit quality through postharvest diseases, leading to deterioration of produce resulting in food wastage (10–14). Chemical agents being used in various agriculture practices are harmful and are is detrimental to human health. It also causes environmental contamination, resulting in increase in production costs, and promote pathogen resistance (15,16). In search of safer alternatives, research has turned towards natural solutions like essential oils. The exploration of natural antimicrobials like essential oils from *Ocimum gratissimum* and *Cymbopogon flexuosus* is critical in the era of rising antibiotic resistance. Discovering effective natural compounds could revolutionize the approach to controlling microbial growth in clinical, agricultural, and food preservation sectors.

Apparently as we search for environmentally friendly alternatives, the dual benefits of essential oils become increasingly important. This dual purpose highlights not only their potential as powerful antimicrobials but also highlights the economic importance of essential oils from the *Cymbopogon genus*. Essential oils from the *Cymbopogon genus* are economically significant in fragrance and flavor industries. It includes about 140 species worldwide, with 45 species found in India, each varying in oil content and composition (17–19). Lemongrass (*C. flexuosus*) is widely cultivated in Brazil, Mexico, Dominica, Haiti, Indonesia, and China, and in India it is cultivated in states like Kerala, Assam, Maharashtra, and Uttar Pradesh. The essential oils from *Cymbopogon* species are rich in terpenoids, such as geraniol, citronellol, and citronellal. Citral, another terpenoid, is used in synthesizing ionone and vitamin A (20). *Cymbopogon flexuosus*, a tall perennial grass reaching 1.5m with a lemony scent and dark-green foliage, thrives in tropical and subtropical climates at temperatures between 10°C and 33°C, requiring abundant sunlight for oil production. It is sensitive to cold and cannot withstand frost. Oil is extracted from fresh plant materials, mainly stalks and leaves, through hydrodistillation (21). Studies show lemongrass extract, oil, citral, and citral-derived compounds have antimicrobial, allelopathic, anthelmintic, anti-inflammatory, anticancer, and antioxidant properties, and can repel insects and mosquitoes.

Similarly, the essential oil of *Ocimum gratissimum* also offers a wide array of beneficial properties. The essential oil *Ocimum gratissimum L.* (Lamiaceae), is native to Africa, Asia, and South America, is part of a genus with about 30 species in tropical regions, producing essential oils for perfumery, cosmetics, pharmaceuticals, and food industries (22,23). Also known as clove basil, African basil, or wild basil, *O. gratissimum* can grow up to 3 m tall with a woody stem base, ovate leaves 5–13 cm long and 3–9 cm wide, and petioles 1–6 cm long. Its flowers form in inflorescences 5–30 cm long, either simple or branched (Matasyoh et al., 2007). The plant is used for food flavoring and medicinal purposes due to its anti-inflammatory, analgesic, hepatoprotective, antimutagenic, antihypertensive, and anticarcinogenic properties (22,23). It exhibits antinociceptive, antagonistic, antibacterial, and antifungal effects on intestinal motility (24,25).

Despite the widespread use of essential oils for microbial infections, limited research has comprehensively profiled the antimicrobial properties of *Ocimum gratissimum* and *Cymbopogon flexuosus* oils against selected pathogens. These oils, traditionally used for their presumed therapeutic benefits, have shown significant antimicrobial activities in previous studies. This research intends to fill this void by examining their antimicrobial efficacy against specific bacterial and fungal strains.

This study specifically aims to (1) evaluate the antimicrobial activity of *Ocimum gratissimum* and *Cymbopogon flexuosus* essential oils against two gram-positive bacteria, two gram-negative bacteria, and one fungal strain using the agar well diffusion method, and (2) characterize the active compounds responsible for this activity via Fourier-transform infrared spectroscopy (FTIR)

2. MATERIALS AND METHODS

2.1 Collection of materials

2.1.1 Bacterial Strains:

- *Escherichia coli*
- *Pseudomonas spp*. RRC15
- *Bacillus altitudinis*
- *Bacillus paramycoides*

These strains were obtained from the Department of Microbiology, College of Basic Sciences and Humanities, GBPUA&T, Pantnagar.

2.1.2 Fungal Strain: The fungal strain *Colletotrichum gloeosporioides* was isolated from anthracnose-affected mango fruits in the Department of Plant Pathology, College of Agriculture, GBPUA&T, Pantnagar.

2.1.3 Essential Oils: The essential oils utilized in the antimicrobial assays, *Ocimum gratissimum* and *Cymbopogon flexuosus*, were procured from Aromaaz International, Sahibabad, Ghaziabad, Uttar Pradesh.

2.2 Characterization of essential oil through Fourier Transform Infrared Spectroscopy

FTIR spectroscopy is based on the principle that molecular vibrations absorb infrared (IR) radiation at specific frequencies. Each type of bond within a molecule (e.g., C-H, O-H, C=O) absorbs IR radiation at characteristic frequencies, producing a unique spectrum. The essential oils were analysed utilising a Fourier-transform infrared spectroscopy phase II spectrometer. FTIR spectra were obtained using Bruker software and subsequently analysed to determine the functional groups present in the essential oil (26). Spectra were obtained with Bruker software over the range of 600-3900 cm^{-1}, which corresponds to the mid-infrared region. This region exhibits specific vibrational bands associated with functional groups, allowing the determination of the sample composition based on this mid-IR spectral fingerprint.

2.3 Preparation of Essential Oil Concentrations

Various concentrations of essential oils, ranging from 10% to 100%, were prepared using DMSO as a solvent. The selection of concentrations was based on preliminary studies on other bacterial strains showing effective antimicrobial activity within this range.

2.4 Antimicrobial Assay

2.4.1 Antibacterial Assay

An agar well diffusion assay was employed to determine the antimicrobial activity of the essential oils (27).

- **Preparation of Bacterial Culture**: A 24-hour-old bacterial culture was prepared in nutrient broth.
- **Preparation of Agar Plates**: Nutrient agar was prepared with agar of 2% and it is aseptically poured into Petri plates further allowing it to solidify.
- **Inoculation**: Wells of 5 mm diameter were created using a sterile borer. The bacterial culture (100 µL) was spread onto the agar plate using a sterile L spreader.
- **Application of Essential Oils**: 20 µL of essential oil was transferred into each well using a sterile micropipette tip.
- **Incubation**: The plates were incubated at 37°C for 24 hours. Streptomycin and tetracycline were used as positive controls. **Negative controls** consisted of wells filled with DMSO alone for validity and reliability of antimicrobial activity of EOs.
- **Measurement of Inhibition Zones**: The inhibition zones diameter was measured using a ruler and recorded in millimeters.

2.4.2 Antifungal Assay

The antifungal activity was assessed using the following steps:

- **Isolation and Culturing**: Fungal spores from anthracnose-affected mango were purified through repeated culturing in PDA media. One-week-old fungal mycelium was used for the study.
- **Preparation of Agar Plates**: Potato Dextrose Agar media prepared in sterile conditions and was aseptically poured into Petri plates, and 5 mm wells were created using a sterile borer.
- **Inoculation**: Mycelium from the fully grown plate was excised and transferred to the center of another plate containing a bored well.
- **Application of Essential Oils**: 20 µL of essential oil was transferred into each well using a sterile micropipette.
- **Incubation**: The plates were incubated for 7 days at 28^0 C, and Carbendazim (0.2% concentration) served as a positive control. **Negative controls** consisted of wells filled with DMSO alone.
- **Measurement of Inhibition Zones**: The inhibition zones diameter were measured using a ruler and recorded in millimeters.

All experiments were performed in triplicate and mean values are presented in the table (table 1).

3. RESULTS AND DISCUSSION

3.1 FTIR analysis of essential oils

Figure 1 illustrates the FTIR spectra of the essential oil of *Cymbopogon flexuosus*. Examining from higher wavelengths to lower wavelengths, the spectral graph exhibits peaks in the regions of 1743.61 and 1682.32, indicating the presence of C=O (aldehyde/ketone group). Additionally, the presence of peaks in the regions of 348.76, 1561.45, and 1513.88 suggests the presence of aromatic C=C stretching, while a peak at 1461.09 represents C-H asymmetrical bend and C-H bend methylene. The peak in the region of 1322.16 indicates the presence of an OH bond. The region from 1322.16 to 728.32 represents the skeletal vibration C-C. The peak at 728.32 demonstrates 'methylene rocking'. The essential oil is known to comprise the main components, Geranial and Neral, collectively referred to as citral (Fig. 2). It possesses the primary functional groups, aldehyde and methylene, with an aromatic ring. The FTIR results correlate with the functional groups present in the compound.

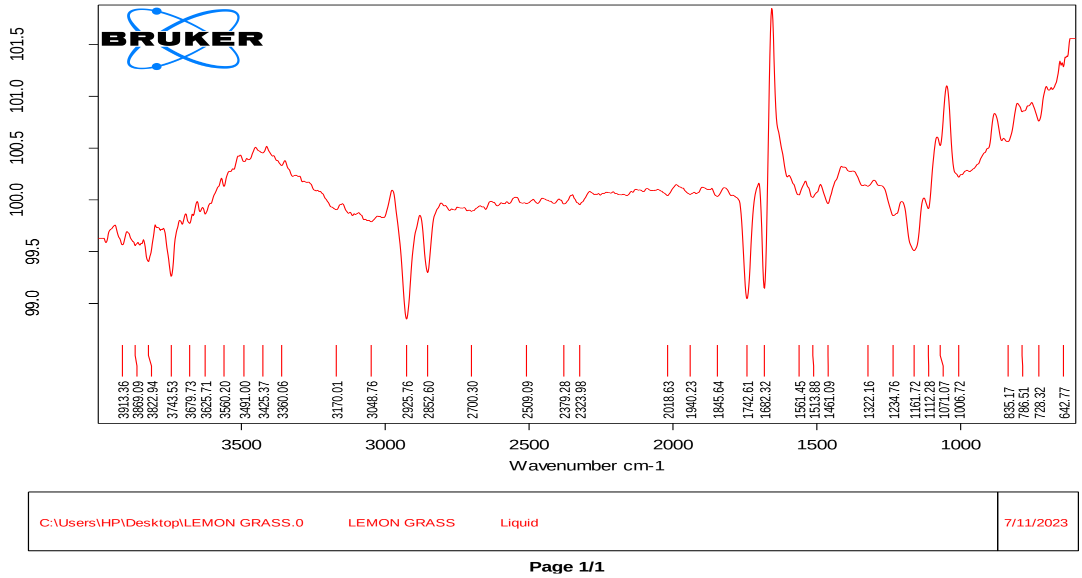
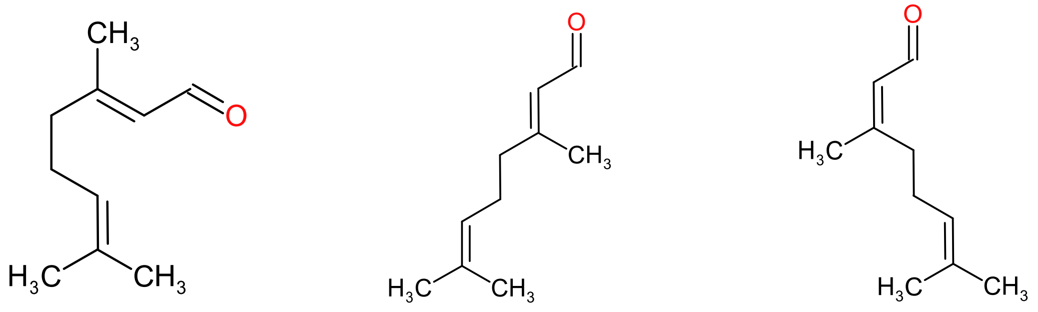


Fig. 1 FTIR of ***Cymbopogon flexuosus***

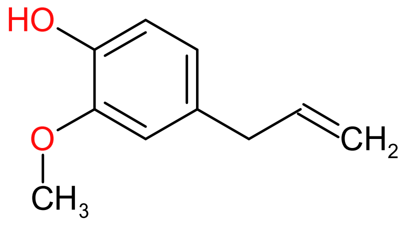


Citral Geranial Neral

Fig 2 Chemical structure of citral, Geranial and Neral

A spectral graph of *Ocimum gratissimum* is presented in Fig. 3. The graph exhibits a broader peak in the region at 3363.10, which indicates the presence of a hydroxyl group. Furthermore, a peak is observed in the region of 1746.15, suggesting the presence of a ketone/ether functional group. The peaks in the regions 1086.49 and 1045.82 indicate cyclic ether/cyclohexane ring vibrations.

Additionally, a peak in wavelength is observed in the regions of 1643.77 and 1575.91, indicating the presence of the C=C conjugate. Eugenol constitutes the major component present in the essential oil of *Ocimum gratissimum*. The chemical structure reveals that it is a cyclohexane with hydroxyl and ether functional groups.



Pic 1: Eugenol

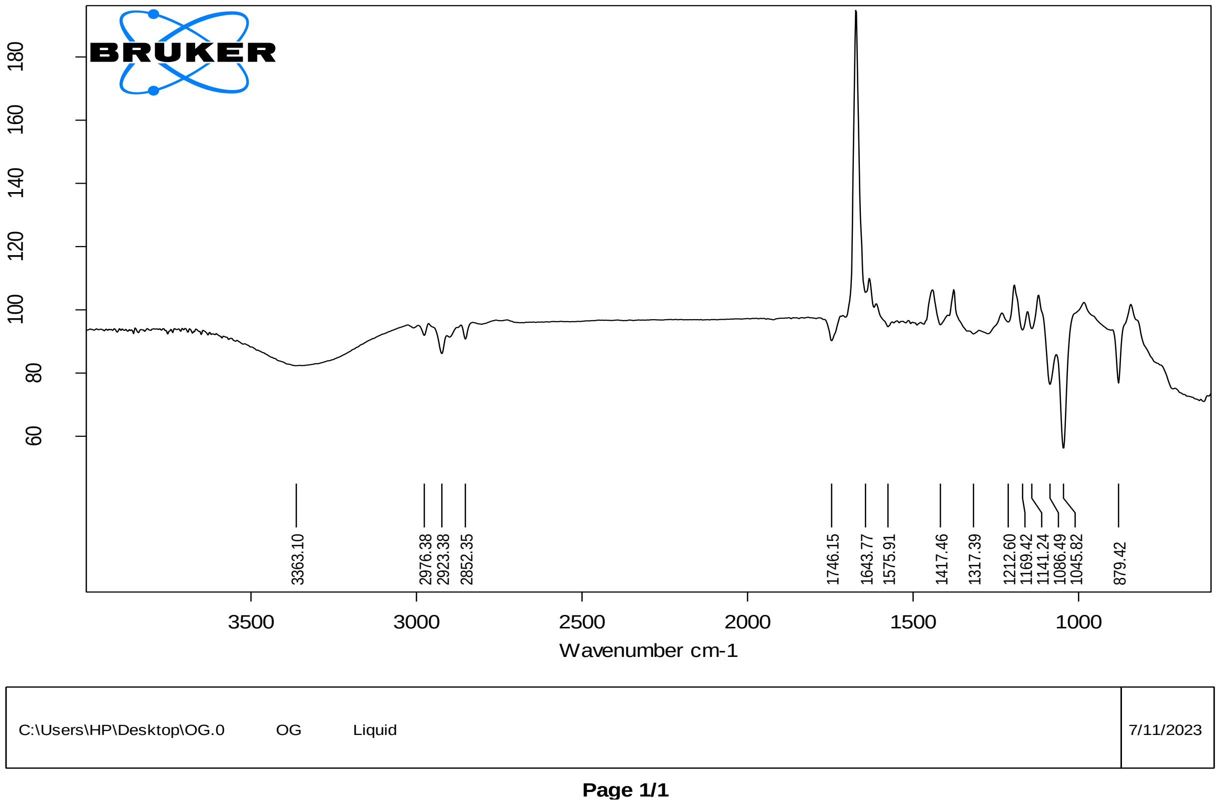


Fig 3 FTIR spectra of *Ocimum gratissimum*

3.2 Antimicrobial activity of essential oils

3.2.1 Antibacterial activity

Table 1 demonstrates the effects of varying concentrations of essential oils on their antimicrobial activity. The results indicated that for all selected bacterial and fungal species, the concentration of the essential oil was directly proportional to the zone of inhibition. At concentrations up to 40%, *Cymbopogon flexuosus* essential oil completely inhibited the growth of *Bacillus altitudinus*, and at concentrations up to 20%, it inhibited *Bacillus paramycoides*. The zone of inhibition against *Pseudomonas spp* was observed to be 8.1 mm for 100 percent concentration of oil and 5.25 for 40 percent concentration. Similarly, against *Escherichia coli*, the zone of inhibition was observed to be 25.5 mm at 100 percent EO concentration and 8.5 mm at 40 percent concentration.

3.2.1.1 EO *Cymbopogon flexuosus* and its antimicrobial activity

From the table 1 it can be observed that EO *C. flexuosus*, (commonly known as lemongrass) is effective against gram positive bacteria when compared to gram negative bacteria. It contains an active component, citral, geranial, neral, and other monoterpenes which are responsible for its antibacterial activity. In a study it was highlighted that citral and other constituents of lemongrass oil possess significant antibacterial properties, particularly against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* (28). A researcher emphasised lemongrass oil's health benefits, particularly its antimicrobial properties due to its high citral content (29). These results suggest that the EO of *C. flexuosus* is more effective against gram-positive organisms due to their simpler cell wall structure (30).

3.1.1.2 Mode of action of Citral a compound present in *Cymbopogon flexuosus* against Bacteria

The antibacterial action of citral against gram-negative bacteria disrupts their lipopolysaccharide rich outer membrane, increasing permeability and leading to cell lysis, altered ATP levels, membrane hyperpolarisation, and reduced cytoplasmic pH, indicating compromised integrity (31–33). This results in leakage of essential components and cell death. Citral induces oxidative stress, generating ROS that damages DNA, proteins, and lipids, thereby enhancing antimicrobial efficacy (33,34). It disrupts metabolic pathways, inhibits cell wall synthesis, impairs homeostasis, and increases the susceptibility to other antimicrobials (35). Citral also inhibits quorum sensing in bacteria, such as *'Pseudomonas aeruginosa*' and *'Cronobacter sakazakii'*, reducing pathogenicity and enhancing immune response vulnerability (33). In Gram-positive bacteria, citral disrupts the cytoplasmic membrane lipid bilayer, increasing permeability and causing intracellular leakage and cell death (34,36,37). It induces morphological changes such as membrane deformation and porosity (38,39). Citral inhibits key metabolic processes, including mitochondrial complex IV, generates ROS, and causes oxidative stress that damages DNA and proteins (40,41). It also downregulates ergosterol biosynthesis, contributing to its antifungal activity (31,42). Furthermore, citral enhances the efficacy of other antimicrobial agents, particularly when combined with nanoparticles, thereby offering effective treatments against resistant bacterial strains (43). This synergistic effect highlights the potential of citral as a natural preservative and therapeutic agent against bacterial infections.

3.2.1.3 EO *Ocimum gratissimum* and its antimicrobial activity

The EO *Ocimum gratissimum* exhibited a zone of inhibition against these bacterial species in decreasing order as *Bacillus paramycoides*, *Bacillus altitudinus*, *E. coli*, and *Pseudomonas spp. RRC15*. *Ocimum gratissimum* is rich in various phytochemicals, including eugenol, thymol, and other phenolic compounds (44–46) found that essential oils possess notable antimicrobial properties, particularly against gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. This is consistent with the findings of a study (47) who noted that *Ocimum gratissimum* exhibited strong antibacterial activity against common oral pathogens, highlighting its potential for dental applications. The effectiveness of *Ocimum gratissimum* essential oil against gram-negative bacteria is low. This can be attributed to the structural differences between Gram-positive and Gram-negative bacteria, particularly the presence of an outer membrane in Gram-negative bacteria that acts as a barrier to the penetration of hydrophobic compounds. For example, a study by (45) indicated that while eugenol and methyl eugenol showed some antimicrobial activity, their effectiveness against gram-negative bacteria was comparatively weaker owing to their chemical structure.

3.2.1.4 Mode of action of eugenol a compound present in *Ocimum gratissimum* against Bacteria

Ocimum gratissimum essential oil disrupts bacterial cell membranes, resulting in the leakage of intracellular components and subsequent cell death, as demonstrated by (48). The phytochemical composition of the oil, which includes flavonoids and tannins, enhances its antibacterial activity by targeting multiple bacterial pathways (44,49). Eugenol, a phenolic compound present in oil, disrupts gram-positive bacterial cell membranes, interferes with metabolism, and modulates biofilm formation. It integrates into lipid bilayers, increases membrane fluidity, and induces lysis, leading to cellular leakage in *Staphylococcus aureus* and *Streptococcus pneumoniae* (50). High concentrations of eugenol significantly inhibit gram-positive bacteria (51). Eugenol forms hydrogen bonds with proteins and inhibits bacterial metabolic enzymes (52), which is crucial for the treatment of antibiotic-resistant infections (53). It disrupts biofilm formation and increases the bacterial susceptibility to antimicrobials (53). In gram-negative bacteria, eugenol penetrates lipid bilayers and lipopolysaccharides of *Escherichia coli* and *Pseudomonas aeruginosa*, causing cell lysis and leakage at high concentrations (54–56). Eugenol inhibits membrane-bound ATPases and reduces ATP levels and viability of gram-negative bacteria (54). The hydroxyl group disrupts metabolic pathways by forming hydrogen bonds with proteins (52). Eugenol disrupts biofilm formation and enhances the susceptibility to antimicrobial agents (45,57). When combined with other antimicrobials, eugenol exhibits synergistic effects, enhancing activity against resistant strains and reducing antibiotic dosages (56,58).

Compared to *Cymbopagon flexuosus*, *Ocimum gratissimum* exhibits a lower zone of inhibition for bacterial species. This can be attributed to the difference in chemical composition, and the high concentration of active compounds, such as citral, present in *Cymbopogon flexuous* oil, which is highly effective in disrupting the bacterial cell structure.

3.2.2 Antifungal activity

Table 1 depicts the antifungal activity of the essential oils *Cymbopogon flexuosus and Ocimum gratissimum* against *Colletotrichum gloeosporioides.* Essential oils at concentrations of 100%, 80%, 60%, and 40 percent had completely inhibited the growth of fungus and exhibited a total zone of inhibition without any mycelium growth. *Cymbopogon flexuosus essential* oil is rich in bioactive compounds, particularly citral, which is known for its significant antifungal activity against various fungal pathogens. A researcher (59) demonstrated that the essential oil of *Cymbopogon flexuosus* possesses potent antifungal properties against *Aspergillus* species, which are common postharvest pathogens. In these studies (60,61) it is reported that lemongrass essential oil effectively inhibits the growth of key postharvest pathogens, including *Penicillium digitatum* and *Aspergillus niger.* Researchers findings suggested that antifungal activity is concentration dependent, with higher concentrations leading to greater inhibition of fungal spore germination and growth (60,61).

In a study by researchers (62,63) it is found that lemongrass oil exhibits significant antifungal activity against various *Candida* species, including *Candida albicans*. This study indicated that the antifungal effects were closely associated with the presence of citral in the essential oil, which demonstrated a strong capacity to inhibit fungal growth (63). This aligns with the findings of (64) who reported that lemongrass oil in both powder and oil forms effectively inhibits *Candida albicans*, further emphasising its potential as a natural antifungal agent.

3.2.2.1 Mode of action of Citral a compound present in *Cymbopogon flexuosus* against fungus

Citral exerts antifungal effects by disrupting the fungal cell membrane, increasing its permeability, and causing intracellular leakage. This disruption results in morphological changes such as thinner filaments and fragile hyphae, leading to cell death (65,66). The ability of citral to form charge transfer complexes with membrane components such as tryptophan also contributes to its efficacy (65). Additionally, citral inhibits ergosterol biosynthesis, a key component of fungal cell membranes, thereby compromising membrane integrity and function (31,67). This inhibition enhances the susceptibility of fungi to other antifungal agents, particularly azole-resistant strains (31). Citral also inhibits spore germination and proliferation by interfering with essential metabolic pathways and induces oxidative stress through reactive oxygen species (ROS) generation, damaging cellular components and leading to cell death (65,66,68). Furthermore, citral can enhance the efficacy of other antifungal agents by working synergistically with conventional drugs to combat resistant strains (69).

In a study (70) it is demonstrated that the essential oil of *Ocimum gratissimum* is highly effective against *Colletotrichum gloeosporioides*, a pathogen responsible for postharvest anthracnose in mangoes. This study reported that the oil exhibited fungicidal effects on both mycelial growth and spore germination. (71) reported that hydrodistilled volatile oils from the leaves of *Ocimum gratissimum* demonstrated significant antifungal activity against *Candida albicans*, a common pathogenic fungus. This was further supported by (47), who noted that *Ocimum gratissimum* showed antifungal activity against various *Candida* species, reinforcing its therapeutic relevance.

3.2.2.2 Mode of action of eugenol a compound present in *Ocimum gratissimum* against fungus

The antifungal mechanism of *O. gratissimum* likely involves disrupting the fungal cell membrane, leading to leakage of intracellular components and cell death. This is attributed to eugenol, a major component with strong antifungal properties (72)

The compounds present in *O. gratissimum* disrupts membrane integrity and metabolic processes (73,74). Its lipophilic nature allows it to penetrate fungal lipid bilayers, increase permeability, and cause cell lysis, effectively inhibiting fungi, such as *Candida albicans*, *Trichophyton rubrum*, and *Microsporum canis* (72,75). *O. gratissimum* essential oil, with eugenol as a significant constituent, demonstrates potent antifungal activity with an MICs of 31.25 μg/mL against various fungi (76). Eugenol reduces fungal viability by inhibiting key enzymes that are essential for growth and reproduction (73). It forms hydrogen bonds with proteins and enzymes, disrupting critical biochemical processes within the fungal cells (47). Furthermore, eugenol modulates biofilm formation, rendering biofilm-associated fungi more susceptible to antifungal agents (77,78), which is crucial in clinical settings for challenging biofilm-associated infections. *O. gratissimum* essential oil also exhibits synergistic effects with other antifungal agents, enhancing activity, reducing the required dosages, and minimising potential side effects and resistance development (79).

Table 1 Zone of inhibition against *Escherichia coli*, *Pseudomonas spp*. RRC15, *Bacillus altitudinus*, *Bacillus paramycoides*, and *Colletotrichum gloeosporoides*

Essential oil	**Conc.**	***Escherichia coli***	***Pseudomonas spp.* RRC15**	***Bacillus altitudinus***	***Bacillus paramycoides***	**Colletotrichum gloeosporoides**
Cymbopogon flexuosus	100	25.5	8.1	100.0 (CI)	100.0 (CI)	100.0 (CI)
	80	35.0	5.25	100.0 (CI)	100.0 (CI)	100.0 (CI)
	60	19.5	6.75	100.0 (CI)	100.0 (CI)	100.0 (CI)
	40	8.5	5.25	100.0 (CI)	100.0 (CI)	100.0 (CI)
	30	-	-	37.5	100.0 (CI)	100.0 (CI)
	20	-	-	23.5	100.0 (CI)	100.0 (CI)
	10	-	-	20.5	24.0	100.0 (CI)
Ocimum gratissimum	100	6.5	4.25	17.5	37.5	100.0 (CI)
	80	5.25	2.25	11.5	14.5	100.0 (CI)
	60	4.25	1.25	9.5	16.0	100.0 (CI)
	40	3.75	1.0	9.0	7.5	100.0 (CI)
DMSO*	NA	0	0	0	0	0
Tetracyclin (disc)	10mcg	21.0	4.0	34.5	22.75	NA
Streptomycin	0.25mg/ml	1.0	1.0	18.0	17.0	NA
Carbendazim	0.2%	NA	NA	NA	NA	24.2

*DMSO - Dimethyl sulfoxide, NA-Not applicable

4. CONCLUSION AND SUMMARY

This study investigated the antimicrobial activity of essential oils from *Ocimum gratissimum* and *Cymbopogon flexuosus* against two gram-positive bacteria, two gram-negative bacteria, and one fungus, using the agar well diffusion method. FTIR analysis revealed the presence of functional groups, such as aldehydes, ketones, and aromatic rings, in the essential oils. Both oils exhibited significant antimicrobial activity against all tested microorganisms, with *Cymbopogon flexuosus* being more effective, completely inhibiting the growth of *Bacillus paramycoides* and *Bacillus altitudinus* at concentrations up to 40%. *Ocimum gratissimum* showed lower antimicrobial activity than *Cymbopogon flexuosus*, with the zone of inhibition decreasing in the order of *Bacillus paramycoides*, *Bacillus altitudinus*, *Escherichia coli*, and *Pseudomonas spp*. Both essential oils completely inhibited the growth of *Colletotrichum gloeosporioides* at concentrations ranging from 40% to 100%. The antimicrobial activity was attributed to the presence of bioactive compounds, such as citral, eugenol, and other monoterpenes in the essential oils, which disrupt bacterial and fungal cell membranes, leading to cell death.

Limitations of the study

Present study investigates the antimicrobial effects of *Ocimum gratissimum* and *Cymbopogon flexuosus* essential oils against a limited range of pathogenic microorganisms, including two gram-positive bacteria, two gram-negative bacteria, and one fungus. To gain a more comprehensive understanding of the oils' antimicrobial potential, future research should include a broader spectrum of bacterial and fungal species, especially multi-drug-resistant strains. Although the study provides data on antimicrobial activity at various concentrations, it does not determine the minimum inhibitory concentration (MIC) or minimum bactericidal/fungicidal concentration (MBC/MFC), which would offer detailed insights into the oils' efficacy and potency. While the study attributes antimicrobial activity to compounds like citral and eugenol, it does not explore their mechanisms of action on microbial cell wall synthesis, protein synthesis, and nucleic acid integrity. The in vitro nature of the study limits its ability to predict the in vivo efficacy and safety of these oils, necessitating future research involving animal models or clinical trials. Furthermore, the study does not assess the stability of essential oils under various storage conditions or explore potential synergistic or antagonistic interactions with other antimicrobial agents, which could inform more effective antimicrobial strategies.

As researchers explore and discover its antimicrobial properties, the potential applications of this antimicrobial agent can extend across various industries and offer promising solutions to address microbial challenges. In the food industry, it can serve as an effective natural preservative, contributing to the extension of shelf life for perishable products and the reduction of food waste. This application aligns with increasing consumer demand for clean-label products and natural food additives. In agriculture, the antimicrobial properties of compounds could contribute to sustainable crop management strategies, potentially reducing reliance on synthetic pesticides and promoting environmentally friendly farming practices. Furthermore, the pharmaceutical and textile industries stand to benefit significantly from this antimicrobial agent. In pharmaceuticals, it can be incorporated into new drug formulations or utilised to develop novel treatments for bacterial infections, addressing the growing concern of antibiotic resistance. The textile industry can employ this compound to create insect repellent and antimicrobial fabrics, which are increasingly in demand for medical textiles, sportswear, and everyday clothing. Such applications could enhance hygiene standards, reduce odour-causing bacteria, and potentially extend the lifespan of textile products. Future research should focus on optimising the efficacy of the compound, exploring its mechanisms of action, and conducting comprehensive safety assessments to ensure its suitability for diverse applications.

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