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# Chemical composition and antibacterial activity of essential oil of *Cymbopogon citratus* (Lemon grass)

**Neha Sharma, Sanjay Guleria, Aasiya Majeed and KH Salaria**

## Abstract
The present study was carried out to find out the phytochemical constuients and the antibacterial activity of lemongrass against *Escherichia coli, Klebsilla pneumoniae, and Bacillus subtilis*. The essential oil of Lemongrass was analysed by (GC-MS) gas chromatography-mass spectrometry analysis. Twenty-eight compounds were identified, comprising about 96.22 % of the total oil composition. The oil was dominated by citral followed by β-myrcene, cis-geraniol, isoneral, geranyl acetate with some minor components. The essential oil showed strong antibacterial activity against various bacterial strains. Thus, lemongrass is effective against drug resistant organism. It can be suggested that the use of lemongrass oil would be helpful in the treatment of infections caused by the multidrug resistant pathogens.

**Keywords:** Citral, β-myrcene, isoneral, antibacterial, essential oil

## Introduction
In the recent times, developments in the field of antibiotics have raised a global challenge and threat to the human society coined as antimicrobial resistance (AMR) (Adukwu *et al*., 2016) [1]. The global threat of antibiotic resistance has raised the need for various therapeutic alternatives to cope with the growing challenge.Throughout the history, numerous essential oils extracted from plant materials have been used for their taste, odour, antibacterial, preservative and medicinal properties (Burt, 2004) [2]. Lemongrass is one of the essential oil that has been studied for its antibacterial effects. Lemongrass (*Cymbopogon citratus*) and other *Cymbopogon* species are tall, coarse grasses with a strong lemon flavor. It is a tropical and subtropical perennial herb that is divided into two species: East Indian *Cymbopogon flexuosus* stapf and West Indian *Cymbopogon citratus* stapf. *Cymbopogon citratus* stapf has been cultivated over many years for medicinal purposes in different countries throughout the world. The use of lemongrass oil was found in folk remedy for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia, and vascular diseases. It is also reported to have antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine, and sedative effects (McGuffin, 1997) [3].Various literature support the use of lemon grass essential oil against various pathogens including gram positive and gram negative bacteria, fungi and yeast (Naik *et al*., 2010; Shigeharu *et al*., 2001) [4, 5].The essential oil of lemongrass was found to be effective against *Acinetobacter baumanii*, *Aeromonas veronii*, *Enterococcus faecalis, Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica serotype typhimurium*, *Serratia marcesens, Proteus vulgaris, Enterobacter aerogenes, Corynebacterium equii, and Staphylococcus aureus* (Pereira *et al*., 2004) [6]. The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. Hence the present study was carried out to find out the antibacterial activity of lemongrass oil against the selected pathogenic bacteria.

## Material and Methods
### Material
Essential oil of *Cymbopogon citratus* was procured from (CSIR-IIIM, Jammu) Council of scientific & industrial research - Indian institute of integrative medicine, canal road, Jammu. Further, essential oil were evaluated for antimicrobial activity against one gram positive (*Bacillus subtilis* MTCC2389, and two gram negative (*Klebsiella pneumoniae* MTCC7172, *Escherichia coli* MTCC2127) bacterial strains. The microbial strains used in this study were procured from Institute of Microbial Technology - Chandigarh.

**Analysis of essential oil**

GC/MS analysis of essential oil was determined using a Varian HP-5 agilent fused with silica capillary column of dimensions (30 × 0.25mm id, film thickness 0.25 µm). The oven temperature was set at 50°C which was programmed to rise to 300°C with every 3°C rise/min. Helium was used as a carrier gas at a constant flow of one ml per min. Mass spectra were recorded with electron impact voltage and the mass range at 70 eV and 40-500 *m/z* respectively at the speed of one scan per second. The volatile organic compounds were identified by comparing the obtained mass spectra of each component with the mass spectra of the authentic reference compound present in the NIST database or with the literature (Adams, 2007; Mahomoodally *et al*., 2018) [7, 8].

**Determination of antibacterial activity**

**Agar disc diffusion method**

The agar disc diffusion assay were used to test the antibacterial activity of essential oil (Andrews, 2001) [9]. 20 ml of the autoclaved nutrient agar (NA) medium was poured into the petriplates and allowed to solidify. Then 100 µl of microbial suspension ($10^8$ CFU/ml) was uniformly spread over the NA plates which were then kept to dry for 5 minutes, filter paper disc were impregnated with EO diluted with DMSO in the ratio of 1:1 and also with positive control Chloramphenicol of concentration 3µg. The discs were then placed on the inoculated nutrient agar surface and the plates were left at room temperature for about 30 min in order to allow the diffusion of oils into the agar. After that the plates were incubated at $37^0$C for 24 h. After incubation, the inhibition zone diameters were measured.

**Results**

**Chemical composition of essential oil**

The volatile constuients present in the EO of Lemongrass showed a wide range of phytochemical constuients. Twenty-eight components were identified, comprising about 96.22 % of the total EO composition (Table 1). Major bioactive components present is citral (37.83%) followed by β-myrcene (10.54%), cis-geraniol (6.09%), isoneral (5.37%), geranyl acetate (4.51%).The minor constituents identified in the EO were linalool (3.59%), 3-thujene (3.42%), 2-allylphenol (2.97%), α-terpinolene (2.97%), d-limonene (2.79%), 2,3,5-trimethylanizole (2.75%), citronellol (2.46%), sabinene hydrate (2.23%), 2-Carene (1.33%), camphene (1.28%), caryophyllene (1.28%), cis-verbenol (0.83%), α-Pinene (0.80%), γ-muurolene (0.73%), cis-carveol (0.50%), eucalyptol (0.38%), caryophyllene oxide (0.38%), piperitone (0.25%), (-)-trans-isopiperitenol (0.24%), epoxynerol (0.22%), (-)-cis-isopiperitenol (0.16%) and tricyclene (0.14%).The percent terpene composition of the essential oil was depicted in Fig.1. In another study, citral is reported to be rich component in *Cymbopogon citratus* essential oil (Hadjilouka *et al*., 2017) [10]. The volatile organic constituent is known to be influenced by several extrinsic and intrinsic factors like soil, climate, season, geographical location, extraction methods, genetic makeup and developmental stage of the plant (Rizzo *et al*., 2020) [11].The biological activities of essential oil depend on the bioactive components, the proportion in which they are present and the interaction between different components of oil (Burt, 2004) [2].

**Antimicrobial activity of essential oil**

Antimicrobial activity of plant essential oil was tested against 3 bacterial strains by disc diffusion assay is presented in Table 2 & Plate 1. Two gram –ve and one gram +ve bacteria were used for testing their susceptibility by loading 2.33 mg of essential oil on their respective discs. Results showed that EO displayed antibacterial activity against all strains but at varying level. Regarding, *K. pneumoniae*, inhibition zone diameters (IZD) revealed that it was most susceptible to the EO of *Cymbopogon citratus* (19.01±0.48mm) followed by the *B. subtilis*, inhibition zone diameters (IZD) (17.2mm ±1.0 mm), *E.coli*, inhibition zone diameters (IZD) (15.5±1.0mm) in comparison to chloramphenicol which showed highest inhibition zone at 3µg. Plant EOs have been shown to be agents that kill or limit the development of harmful bacteria by virtue of their antibacterial capabilities. In addition, lemongrass has been shown to have antibacterial effects against a variety of bacteria types (Ilango *et al*., 2019) [12]. It has been reported that citral showed strong antibacterial action against both gram+ve and gram-ve bacteria (Onawunmi, 2008) [13]. The phytochemical constuients like alkaloids, flavonoids, and tannins of plants are responsible for the antimicrobial activity (Jayashree *et al*., 2013) [14]. The antibacterial effect of essential oils against bacteria may be due to their hydrophobic nature which facilitates their interaction with bacterial cell membrane and thereby result in toxic effects on membrane structure and function (Nazzaro *et al*., 2013) [15].

**Table 1:** Chemical composition of the essential oil obtained from the fresh leaves of *Cymbopogon citratus*

| S. No. | Component | Class of component | KI | Percent | Mode of identification |
|---|---|---|---|---|---|
| 1. | Tricyclene | Bicyclic Monoterpene | 980 | 0.14 | GC-MS |
| 2. | 3-thujene | Bicyclic Monoterpene | 982 | 3.42 | GC-MS |
| 3. | α-pinene | Bicyclic Monoterpene | 985 | 0.80 | GC-MS |
| 4. | Camphene | Bicyclic Monoterpene | 993 | 1.28 | GC-MS |
| 5. | β-myrcene | Monoterpene | 1010 | 10.54 | GC-MS |
| 6. | 2-allylphenol | Phenols | 1015 | 2.97 | GC-MS |
| 7. | 2-carene | Bicyclic Monoterpene | 1021 | 1.33 | GC-MS |
| 8. | d-limonene | Monoterpene | 1026 | 2.79 | GC-MS |
| 9. | Eucalyptol | Monoterpene | 1027 | 0.38 | GC-MS |
| 10. | 3-Cyclohexen-1-ol, 5-methylene-6-(1-methylethenyl)-, | Monoterpene | 1046 | 0.17 | GC-MS |
| 11. | α-terpinolene | Monoterpene | 1047 | 2.97 | GC-MS |
| 12. | Linalool | Monoterpene | 1051 | 3.59 | GC-MS |
| 13. | Isoneral | Monoterpene | 1071 | 5.37 | GC-MS |
| 14. | Citral | Monoterpene | 1077 | 37.83 | GC-MS |
| 15. | (E)-isopiperitenol | Monoterpene | 1085 | 0.24 | GC-MS |
| 16. | cis-Carveol | Monoterpene | 1087 | 0.50 | GC-MS |
| 17. | (Z)-isopiperitenol | Monoterpene | 1090 | 0.16 | GC-MS |

| | | | | | |
|---|---|---|---|---|---|
| 18. | Citronellol | Monoterpene | 1091 | 2.46 | GC-MS |
| 19. | (Z)-geraniol | Monoterpene | 1098 | 6.09 | GC-MS |
| 20. | Piperitone | Monoterpene | 1101 | 0.25 | GC-MS |
| 21. | (Z)-verbenol | Monoterpene | 1104 | 0.83 | GC-MS |
| 22. | Epoxynerol | Monoterpene | 1108 | 0.22 | GC-MS |
| 23. | 2,3,5-Trimethylanizole | Monoterpene | 1113 | 2.76 | GC-MS |
| 24. | Sabinene hydrate | Bicyclic Monoterpene | 1120 | 2.23 | GC-MS |
| 25. | Geranyl acetate | Monoterpene | 1231 | 4.51 | GC-MS |
| 26. | Caryophyllene | Sesquiterpene | 1404 | 1.28 | GC-MS |
| 27. | γ-Muurolene | Sesquiterpene | 1453 | 0.73 | GC-MS |
| 28. | Caryophyllene oxide | Sesquiterpene | 1490 | 0.38 | GC-MS |
| Total 96.22% | | | | | |

KI, Kovatis indices
Monoterpene = 81.66 %
Bicyclic Monoterpene = 9.2%
Phenol = 2.97 %
Sesquiterpene = 2.74%
Total identified = 96.22%

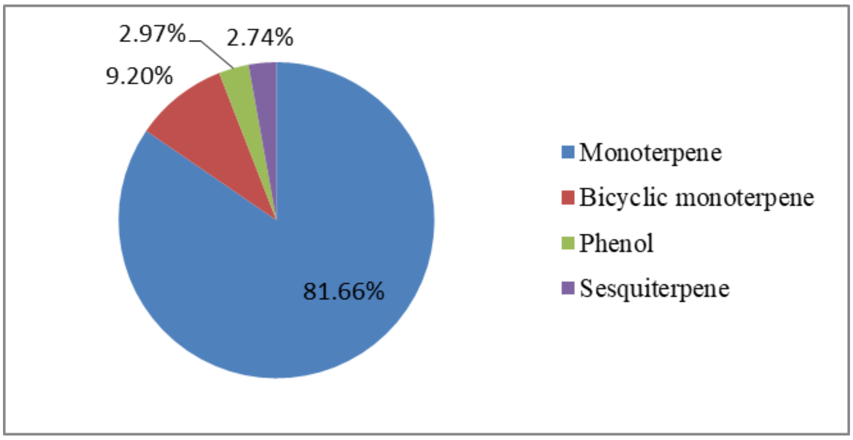


**Fig 1:** Percent terpene composition of essential oil extracted from fresh leaves of *Cymbopogon citratus*

**Table 2:** Antibacterial activity of essential oil (Disc diffusion assay)

| Bacterial strains | Inhibition zone diameter (IZD) in mm |
|---|---|
| *Escherichia coli* | 15.5±0.73 |
| *Bacillus subtilis* | 17.1±0.60 |
| *Klebsilla pneumoniae* | 19±0.89 |

*Data presented is ± s.d. of three replicates.

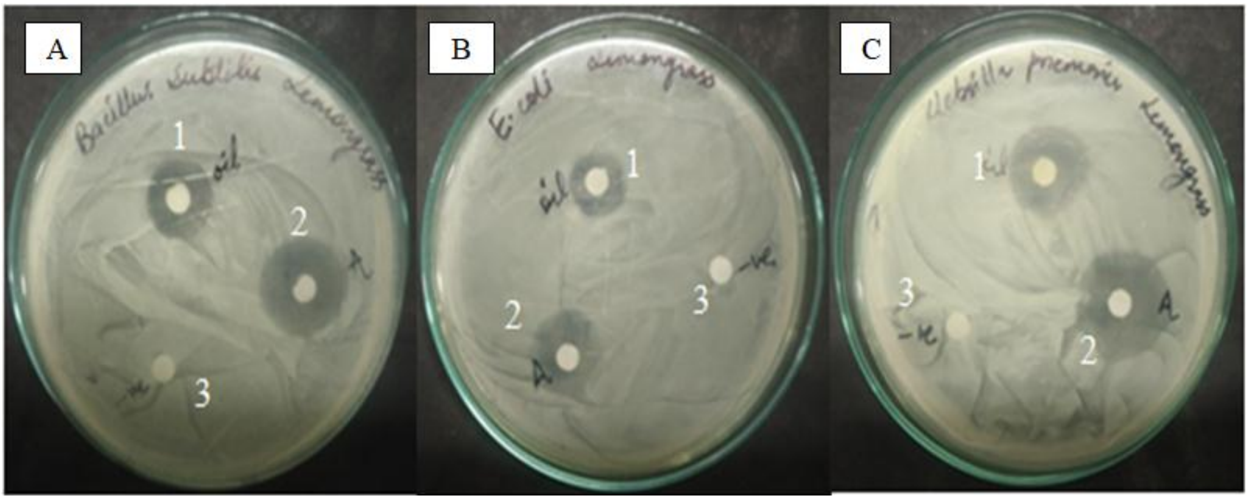


**Plate 1:** Antibacterial activity of *Cymbopogon citratus* essential oil against selected bacteria using agar disc diffusion assay: (A) *B. subtilis* (B) *E. coli* (C) *K. pneumoniae*; (1) Essential oil (2) Chloramphenicol (3) DMSO

## Conclusion

This study indicates that the essential oil isolated from aerial parts of lemongrass was rich in cyclic monoterpene citral and possesses promising *in vitro* antibacterial potential and can be explored for innovative therapeutic or preventive strategies against infections that can be for great interest to both pharmaceutical and food industries.

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