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# Antimicrobial Activity of Essential Oils of Four Lemongrass (*Cymbopogon flexuosus* Steud) Varieties

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## ABSTRACT

Antimicrobial activity of the essential oils (EOs) of four lemongrass (*Cymbopogon flexuosus*) varieties 'Krishna', 'Cauveri', 'Nima' and 'Cheerharit' and major EO constituents, viz. citral, geraniol, and geranyl acetate were evaluated. EOs from 30 d-old tillers of these varieties were extracted. Antimicrobial screening of the EO and major constituents was performed by the agar well diffusion method. All the EOs screened displayed strong antibacterial than antifungal activity against the microorganisms used. EOs from 'Krishna' and 'Cauveri' had exceptionally strong inhibitory effects against *Bacillus subtilis*. Among all the bacteria, *Staphylococcus aureus* was highly susceptible to all four EOs. Citral displayed remarkable antimicrobial activity against bacteria and fungi. Geraniol was also effective against fungi *Aspergillus flavus* and *A. fumigatus* while geranyl acetate had reasonable activity against *S. aureus*.

**Keywords:** agar well diffusion, antibacterial, antifungal, *Cymbopogon flexuosus*, zone of inhibition

## INTRODUCTION

*Cymbopogon flexuosus* Steud (family Poaceae), commonly know as East Indian lemongrass, is a perennial herb that yields an essential oil (EO) of immense commercial value in flavour, fragrance, cosmetics and pharmaceuticals (Ganjewala *et al.* 2008). Lemongrass EOs of diverse origin are mainly characterized by the presence of citral (geranial and neral), which accounts for 75-85% of the total EOs (Khanuja *et al.* 2005; Ganjewala *et al.* 2008). Citral is an isomeric mixture of geranial (citral A) and neral (citral B). Besides citral, geraniol and geranyl acetate are present in small amounts in EOs of *Cymbopogon* spp. Citral, due to its characteristic lemon aroma, is of considerable importance in the food and flavour industry; it is also used for the synthesis of vitamin A and ionones (Dawson 1995; Lewinsohn *et al*. 1998). So far, a number of studies have been performed aimed at investigating the EO composition of different *Cymbopogon* spp. and to evaluate their biological activity. The EOs of several *Cymbopogon* are reported to possess antimicrobial, antifungal, antiyeast, insecticidal, antiparasitic, antiviral, and antiprotozoan activities (Pandey *et al.* 1996; Pattanaik *et al.* 1996; Delespaul *et al.* 2000; Saikia *et al.* 2001; Nakahara *et al.* 2003; Pedroso *et al.* 2006; Simic *et al.* 2008). Citral possesses antifungal activity against plant and human pathogens (Rodov *et al.* 1995), and antibacterial (Asthana *et al.* 1992) and insecticidal properties (Rice and Coats 1994). The EOs from lemongrass (*Cymbopogon flexuosus*) posses *in vitro* cytotoxicity against 12 human cancer cell lines (Sharma *et al.* 2009) while the EOs from *C. nardus* and *C. martinii* have strong fungicidal activity (Delespaul *et al.* 2000). *C. nardus* EO has been traditionally used as a mosquito repellent, household fumigant or fragrance agent in food commodities, soaps and cosmetics (Kazuhiko *et al.* 2003). The EO of *C. travancorensis*, composed mainly of citronellol, citronellal, γ-terpinene and β-phellandrene, is reported to have potential antifungal activity (Maridass 2008). Citronellol and citronellal present in the EO are responsible for its antifungal activity (Maridass 2008).

The aim of this study was to evaluate the antimicrobial activity of the EOs extracted from four different lemongrass varieties and to compare with the antimicrobial activity of commercially available major EO constituents, citral, geraniol, and geranyl acetate.

## MATERIALS AND METHODS

### Plant materials

Lemongrass (*Cymbopogon flexuosus* steud) Wat var. 'Krishna', 'Cauveri', 'Nima' and 'Cheerharit' plants were collected from the Central Institute of Medicinal and Aromatic Plant (CIMAP), Resource Station, Hyderabad, India and grown in pots at the Vellore Institute of Technology University, Vellore, Tamil Nadu, India. Lemongrass tillers 30-d of age were harvested and their EOs were extracted by steam distillation in a mini Clevenger apparatus (Clevenger 1928). The EOs were stored in small stoppered tubes in a refrigerator at 4°C.

### Microorganisms

Four bacteria viz. *Escherichia coli* (MTCC901), *Salmonella typhi* (MTCC735), *Staphylococcus aureus* (MTCC96), *Bacillus subtillis* (1429) and two fungi, *Aspergillus flavus* (MTCC2723) and *A. fumigatus* were used for antimicrobial assays. All the microorganisms except for *A. fumigatus* used in the present study were obtained from the Microbial Type Culture Collection (MTTC), Institute of Microbial Technology, Chandigarh and the National Collection of Industrial Microorganisms (NCIM), Pune, India.

### Maintenance of microorganisms

The bacterial and fungal cultures were inoculated in Muller Hinton Agar (MHA) and Sabouraud's Dextrose Agar (SDA) (HiMedia, India), respectively. The isolated colonies were again inoculated into broth for plating and sub-cultured and maintained on agar slants. The agar slants were stored at 4°C.

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Research Note

**Table 1** Antibacterial activity of EOs of four lemongrass varieties Krishna, Cauveri, Nima and Cheerharit. Data in the table are presented as mean ± standard deviation (S.D.) of three independent experiments.

| Microorganisms | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | Krishna | Cauveri | Nima | Cheerharit | Standard |
| **Bacteria** | | | | | **Streptomycin** |
| *Escherichia coli* | 32 (± 1) | 28 (± 1) | 23 (± 1) | 25 (± 1) | 21 |
| *Salmonella typhi* | 26 (± 1) | 28 (± 1) | 30 (± 1) | 27 (± 1) | 22 |
| *Staphylococcus aureus* | 38 (± 1) | 30 (± 1) | 32 (± 1) | 33 (± 1) | 16 |
| *Bacillus subtillis* | 47 (± 1) | 45 (± 2) | 28 (± 1) | 27 (± 1) | 22 |
| **Fungi** | | | | | **Ketaconazole** |
| *Aspergillus flavus* | 26 (± 1) | 32 (± 1.7) | 28 (± 1) | 27 (± 2) | 22 |
| *A. fumigatus* | 25 (± 1) | 32 (± 1.7) | 20 (± 1) | 30 (± 1.7) | -- |

**Table 2** Antibacterial activities of the major EO constituents, citral, geraniol and geranyl acetate. Data in the table are presented as mean ± standard deviation (S.D.) of three independent experiments.

| Microorganisms | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Citral | Geraniol | Geranyl acetate | Standard |
| **Bacteria** | | | | **Streptomycin** |
| *Escherichia coli* | 31 (± 1) | 10 (± 1) | 11 (± 2) | 21 |
| *Salmonella typhi* | 44 (± 2) | 13 (± 1) | 11 (± 1) | 22 |
| *Staphylococcus aureus* | 22 (± 2) | 10 (± 1) | 23 (± 2) | 16 |
| *Bacillus subtillis* | 45 (± 1) | 11 (± 2) | 17 (± 1) | 22 |
| **Fungi** | | | | **Ketaconazole** |
| *Aspergillus flavus* | 52 (± 1) | 27 (± 2) | 10 (± 1) | 22 |
| *A. fumigatus* | 23 (± 1) | 33 (± 1) | 10 (± 2) | -- |

## Antimicrobial screening

Antibacterial activity was determined by using the well diffusion method (Maridass 2008). The medium was sterilized in an autoclave at 121°C at 15 lb for 15 min. The medium was then poured into sterilized Petri dishes and left to solidify in a laminar air flow chamber. The desired strains of bacteria were then swabbed onto the medium. A well was punched at the centre of the plate with the help of a borer (6 mm). EO (10 µL/10 µL of DMSO) was poured into the well with the help of a micropipette. After diffusion, the plates were incubated at 37°C for 24 hrs. After incubation, growth inhibition was measured and recorded.

A similar procedure was followed to determine antifungal activity, except that SDA was used and the incubation period was 48 hrs.

## RESULTS AND DISCUSSION

Results of the antimicrobial assays are summarized in **Table 1**. In general, lemongrass EOs possessed stronger antibacterial than antifungal activity. The EOs from 'Krishna' and 'Cauveri' displayed exceptionally strong activity against *B. subtillis* with a zone of inhibition of 45-47 mm. However, both 'Krishna' and 'Cauveri' EOs also had significant inhibitory effects against three other bacteria *E. coli*, *S. typhi* and *S. aureus*. 'Nima' EO was very effective against *S. aureus* and *S. typhi*. The EO from 'Cheerharit' possessed considerable activity against all bacteria except *S. aureus*. The EO from 'Krishna' was most effective against all bacteria except *S. typhi* most probably due to the abundance of citral (70-75%) in the EO.

Results of antimicrobial screening with EO constituents revealed that citral possessed highest activity against all bacteria except *S. aureus* whereas the other two constituents, geraniol and geranyl acetate, had only a small effect against the same set of bacteria except for some inhibitory activity showed by geranyl acetate against *S. aureus* (**Table 2**).

It is clear that the EOs of the four lemongrass varieties studied and their EO constituents, citral and geraniol, possess significant antimicrobial activities; in particular, citral exhibits strong antimicrobial activity. Geranyl acetate however, was only reasonably effective against the test organisms (**Fig. 1A, 1B**). A number of studies have documented many useful biological activities *viz.*, antibacterial, antifungal, pesticidal, insecticidal and anticancer of lemongrass EOs and EO constituents (Pattanaik *et al.* 1996; Pandey *et al.* 1996; Delespaul *et al.* 2000; Saikia *et al.* 2001; Nakahara *et al.* 2003; Pedroso *et al.* 2006; Simic *et al.* 2008). Thus, the antimicrobial activity of the lemongrass EOs and constituents reported here (**Tables 1** and **2**) are expected and similar to that of previously published reports on *Cymbopogon* spp. EOs and their bioactivities (Inouye *et al.* 2001; Maizura *et al.* 2008; Rusenova and Parvanov 2009). From **Table 1** it is clear that the EOs studied showed variable antimicrobial activity, these variations are though mainly t be due to the differences in chemical composition of the EOs. Earlier studies have described variable bioactivity of EOs due to variation in their chemical composition (Nakahara *et al.* 2003; Pedroso *et al.* 2006; Maizura *et al.* 2008). Thus, there is a direct relation between antimicrobial acti-

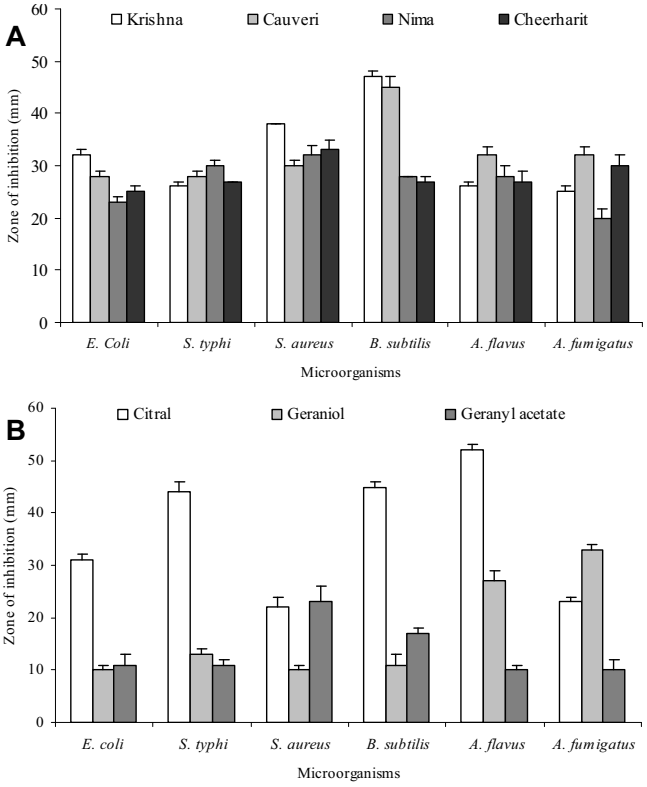


**Fig. 1** Antimicrobial activities of (**A**) EOs of four lemongrass varieties and (**B**) major oil constituents.

vity of the EOs and chemical composition.

In the antifungal screening, the EO from 'Cauveri' demonstrated highest antifungal activity against the test fungi *A. flavus* and *A. fumigatus* (**Table 1**). The EOs from three other lemongrass varieties showed less inhibitory activity against the test fungi. Among the EO constituents, citral showed strong antifungal activity against *A. flavus*, while geraniol significantly inhibited the growth of *A. fumigatus*. Geranyl acetate, however, did not show any impressive antifungal activity against the fungi used (**Table 2**). Although we have evaluated the antifungal activity of lemongrass EOs against only two fungi, the results of the study are in full agreement with those reported previously revealing that citral is largely responsible for the antifungal property of lemongrass EOs (Asthana *et al.* 1992; Rice and Coats 1994; Rodov *et al.* 1995). The variation in the antifungal activity of the EOs of four lemongrass varieties is also most likely due the differences in the chemical composition of the EOs. Sakia *et al*. (2001) suggested that the differences in the EO composition are mainly responsible for variation in their antifungal activity of three elite *Cymbopogon* spp., *C. flexuosus*, *C. martinii* and *C. winterianus* EOs as well as the major components, citral, geraniol, citronellol and citronellal against four human pathogenic fungi. Previously, citral had been reported to possess antibacterial, antifungal, and insecticidal properties (Asthana *et al.* 1992; Rice and Coats 1994; Rodov *et al.* 1995). Citral strongly inhibits the growth of *Candida albicans* (Abe *et al.* 2003). The amount of citral present in the EO is correlated with the antimicrobial potential of the EO of *Cymbopogon* spp. (**Fig. 1A, 1B**). In addition to citral, different constituents present in the EOs of different *Cymbopogon* species, such as geraniol, geranyl acetate, α-bisabolol, and isointermedeol have been individually reported to have useful bioactivities, including anticancer activity (Kumar *et al.* 2008).

In conclusion, the antimicrobial potential of the EOs largely depends on their chemical composition and proportion of different chemical constituents present therein. Second, the antimicrobial potential of the EOs and constituents are also greatly influenced by morphological features like cell membrane structures of the microorganisms used in the study. Thus, bacteria with only an outer layer of peptidoglycan will be more susceptible than those surrounded by impermeable membrane to the EOs and constituents (Priya and Ganjewala 2008).

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