



A Study on Antimicrobial Activities of Essential Oils of Different Cultivars of Lemongrass (*Cymbopogon flexuosus*)

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ABSTRACT

Background: *Cymbopogon flexuosus* popularly known as lemongrass provides a lemon scented essential oil which is widely used in flavour and fragrance, perfumery, food and pharmaceuticals. The aim of the present study was to assess antimicrobial activities of essential oils of three lemongrass cultivars viz., Pragati, Praman and Suvarna.

Methods: Essential oils were isolated from one month old plants by hydro-distillation in mini Clevenger apparatus for 2 h. Antimicrobial activities were determined by agar well diffusion method

Results: Lemongrass oils exhibited strong antimicrobial activity against all the microbes except E coli. Mean inhibition zone diameter (mm) against bacteria was ranged 27-38 mm. B. Subtilis was the most sensitive bacterium to all essential oils. Essential oils also showed strong antifungal effects against both A. niger and C. albicans with mean inhibition zone diameter (mm) values 20-26 and 27-29 mm, respectively. Statistical analyses revealed that antimicrobial activity shown by essential oils were significant ($p < 0.05$) against all microorganisms except B. subtilis and C. albicans ($p \gg 0.05$).

Conclusion: The study revealed strong antimicrobial potential of the essential oil against pathogenic microbial strains which may be of high clinical importance in future.

Introduction

Medicinal and aromatic plants are important pillars of healthcare and traditional medicinal systems of the world.¹ A number of aromatics plants provide essential oils of unique aroma which is mainly used in flavours and fragrances, perfumery, cosmetics and pharmaceuticals.² Essential oils possess many useful bioactivities particularly the antimicrobial activities which have formed the basis for the development of new alternative remedies/therapeutics for the control and inhibition of human and food-borne pathogens and food spoilage microorganism's growth.³⁻⁹ Applications of essential oils are often considered quite safe and effective with no side effects. With increasing number of bacterial strains resistant to various antibiotics, many attempts to use antimicrobial potential of plants have been done. Antimicrobial compounds from plants with different mechanisms of action against resistant microbial strains are of clinical importance.¹⁰

Cymbopogon flexuosus popularly known as lemongrass is indigenous to India. It is generally used as an important ingredient in Ayurveda an oldest system of medicine practiced in India for

centuries to treat diseases and ailments.¹¹ It has been used to cure various ailments like cough, cold, spitting of blood, rheumatism, lumbago, digestive problems, bladder problems, leprosy, and as mouth wash for the toothache and swollen gums. Lemongrass provides an essential oil known as lemongrass oil which is characterized by the presence of monoterpenes such as, citral (a racemic mixture of geranial and neral), geraniol, citronellol, citronellal, linalool, elemol, 1,8-cineole, limonene, geraniol, methyl heptenone, geranyl acetate and geranyl formate.¹²⁻¹⁴ Citral is identified as the major constituent that imparts lemon like aroma to lemongrass oil. Lemongrass oil and citral are widely used in flavors and fragrance, cosmetics, food and pharmaceuticals. Citral is also used in soaps, detergents, and for the synthesis of vitamin and ionones.¹²⁻¹⁶ Lemongrass oil and citral possessed a number of bioactivities such as antibacterial, antifungal, anti-inflammatory, anticancer and allelopathic activities.^{13,15-16} Recently, we reported antibacterial properties of lemongrass essential oils separately and in combination with honey and of silver nanoparticles synthesized from lemongrass leaf extract against drug resistant pathogenic

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bacteria.¹⁷⁻¹⁸ According to previous studies a number of pathogenic bacteria have been continuously evolving resistance mechanism against currently available antibiotics and posing serious threats to human health. This trend has necessitated the discovery of new antimicrobial compounds against drug resistant bacterial and fungal pathogens. Lemongrass oils owing to their tremendous bioactive potential could be a good source of new antimicrobial agents of clinical importance and may useful in treatment of various forms of infections caused by pathogenic microorganisms. The aim of the present study was to determine antimicrobial properties of essential oils of three lemongrass cultivars namely Pragati, Praman and Suvarna against bacterial and fungal pathogens associated with human diseases.

Material and Methods

Plant Material

Lemongrass (*Cymbopogon flexuosus* Steud.) Wats plants were raised at Organic Farm House of Amity University Uttar Pradesh, Noida (U.P.) following standard agronomic practices. It took almost a year to acclimatize and grow well. At the time of study plants were harvested from 10 cm above the ground level and tagged. Plants were allowed to grow for one month and then were harvested for isolation of essential oils.

Isolation of essential oil

One month old lemongrass plants were harvested; leaves were separated, washed thoroughly and cut into small pieces. Leaves (100 g) were subjected to steam-distillation in mini Clevenger apparatus for 2 h.¹⁹ The volume of the essential oil accumulated in the burette of Clevenger apparatus was measured and collected in small vials. Anhydrous sodium sulphate was added to vials in order to remove last traces of moistures in essential oils. Essential oils were stored at 4°C in refrigerator till further use.

Culture of microorganism

Four bacterial pathogens including *Escherichia coli* (MTCC901), *Pseudomonas aeruginosa*, *Staphylococcus aureus* (MTCC96) and *Bacillus subtilis* (1429) and two fungal pathogens *Aspergillus niger* (MTCC2723) and *Candida albicans* were obtained from Microbiology Laboratory, Amity Institute of Biotechnology, Noida, Uttar Pradesh. The bacterial and fungal cultures were inoculated in Muller Hinton Agar (MHA) and Potato Dextrose Agar (PDA), respectively. The pure colonies were sub-cultured and preserved in agar slants at 4° C and 50 % glycerol stock at -20°C.

Antimicrobial assay

Antimicrobial activities of essential oils obtained from three cultivars of lemongrass namely, Suvarna, Praman and Pragati were determined by agar well diffusion method and compared.²⁰ The culture plates were prepared by first sterilizing the MHA and PDA in an autoclave at 121°C at 15 lb for 15 minutes and then by pouring 20 ml of media into each sterilized Petri dish used. One milliliter (1 ml) inoculum suspension was spread uniformly over the agar medium using sterile glass rod. Wells were made by using sterile cork borer (6 mm) in each plate. Essential oil samples (10 µl) were inserted aseptically with the help of micropipette into the well. The bacterial media plates were incubated at 37°C for 24 h and fungal plates for 48 h at 30°C. After incubation, microbial growth and the zone of inhibition (mm) were measured and compared to the controls. Antimicrobial assays were performed in triplicate.

Statistical analysis

The mean and standard deviation of mean of inhibition zone (MIZ) diameter (mm) were calculated. Statistical significance was measured by using one-way ANOVA followed by Tukey's post-hoc test. Analysis of data was done by IBM SPSS (statistical package for social science) version 19.0.

Results and Discussion

Antimicrobial activity profiles of essential oils of three cultivars of lemongrass namely Pragati, Praman and Suvarna are presented in Table 1. Antimicrobial activities were determined by agar well diffusion method and expressed in terms of MIZ diameter (mm). All the three essential oils showed good antimicrobial activities against bacteria and fungi used. Overall average values of MIZ against bacteria was ranged 27-38 mm and fungi 23-30 mm, respectively. All of essential oils were highly effective against *B. subtilis* and less effective against *E. coli*. However, Suvarna essential oil showed strong antibacterial activity against *B. subtilis* followed by essential oils of Praman and Pragati (Table 1). The essential oil of suvarna also exhibited highest bactericidal effect against *S. aureus* and *P. aeruginosa*. Essential oils of Praman and Pragati displayed almost similar bactericidal effects as that of suvarna against same set of bacteria but with little less values of MIZ 28-30 mm. The Essential oils of lemongrass cultivars tasted here also showed strong antifungal effects against *C. albicans* with values of zone of inhibition 29-30 mm. However, Suvarna essential oil showed strong antifungal activity against *A. niger* causing a zone of inhibition 28 mm while Praman and Pragati essential oils were comparatively lesser effective with values of the zone of inhibition 26 and 23 mm, respectively

Table 1. Antimicrobial activities of three different types of essential oils of lemongrass obtained from cultivars Pragati, Praman and Suvarna.

Microorganisms	Essential oil Zone of inhibition (mm)			F-factor	p- value*
	Pragati	Praman	Suvarna		
Bacteria					
<i>Escherichia coli</i>	26.9 (23.6)**	26.5 (23.0)	29.0 (26.0)	8.511	0.003
<i>Pseudomonas aeruginosa</i>	29.0 (21.5)	29.5 (25.0)	32.0 (27.0)	7.278	0.005
<i>Staphylococcus aureus</i>	28.0 (20.9)	30.0 (26.0)	32.0 (27.5)	18.733	0.000
<i>Bacillus subtilis</i>	34.0 (30.9)	36.0 (30.0)	38.0 (32.0)	5.043	0.018
Fungi					
<i>Aspergillus niger</i>	22.5 (19.5)	26.0 (20.0)	28.0 (24.0)	17.65	0.000
<i>Candida albicans</i>	29.1 (25.1)	30.0 (26.0)	29.0 (24.0)	1.965	0.169

* The mean difference is significant at the level of 0.05.

** Minimum measured values of the zone of inhibition is shown in the parenthesis.

Table 2. Turkey's post hoc analyses of antimicrobial activities of three different types of essential oils of lemongrass obtained from cultivars Pragati, Praman and Suvarna.

Microorganisms	Essential oil Mean of the zone of inhibition (mm)			Standard deviation	Turkey's post hoc
	Pragati (1)	Praman (2)	Suvarna (3)		
Bacteria					
<i>Escherichia coli</i>	26.5	24.8	27.4	1.32	3>1>2
<i>Pseudomonas aeruginosa</i>	21.5	27.5	29.8	4.28	3>2>1
<i>Staphylococcus aureus</i>	22.0	28.3	29.8	4.13	3>2>1
<i>Bacillus subtilis</i>	32.0	33.1	35.2	1.63	3>2>1
Fungi					
<i>Aspergillus niger</i>	19.5	23.1	26.1	3.29	3>2>1
<i>Candida albicans</i>	29.1	28.3	26.8	1.17	1>2>3

*Sample size N=7.00

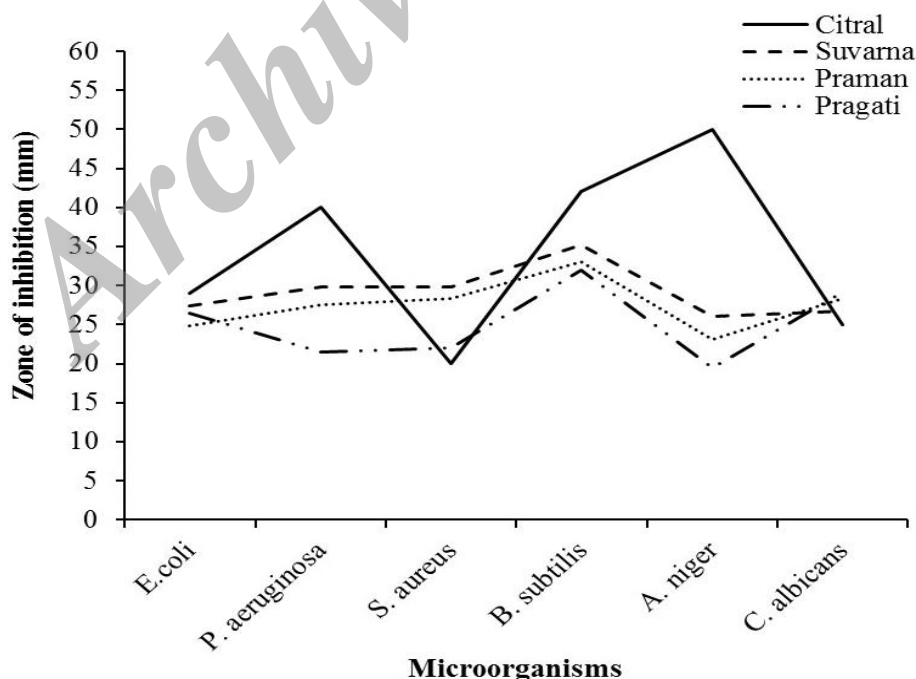
**Figure 1.** Antimicrobial activity profiles of the lemongrass oils isolated from cultivars Pragati, Praman and Suvarna and major constituent citral.

Table 3. Essential oil compositions of one month old lemongrass cultivars.

<i>C. flexuosus</i> cultivars	Relative % in the oil				
	Geraniol	Geranyl acetate	Geranial (citral a)	Neral (citral b)	Others
Pragati	2.8	1.1	53.0	28.8	14.3
Praman	2.0	1.0	53.5	29.8	13.7
Suvarna	2.2	0.6	53.9	30.3	13.0

Overall, the results revealed that the essential oil from suvarna had powerful antimicrobial effects against microorganisms used. Statistical analysis of the data of antimicrobial activities revealed that all three different essential oils displayed significant antimicrobial activity ($p < 0.05$) against all microorganisms except *B. subtilis* and *C. albicans* ($p \gg 0.05$). On the statistical basis *S. aureus* and *A. niger* were found highly susceptible to essential oils tested (p value- 0.000). Turkey's post-hoc test (Table 2) suggested that the essential oil from Suvarna possessed strongest antimicrobial activities compared to the others cultivars against microorganisms used except *C. albicans* while the essential oil from cultivar Pragati was the most active against *C. albicans* (Table 2). Antimicrobial activity profiles of citral are presented in Figure 1. Citral showed strong fungicidal activity against *A. niger* with zone of inhibition measured 50 mm while less effective against *C. albicans* with value of the zone of inhibition 25 mm. It also strongly inhibit the growth of *P. aeruginosa* (40 mm) and *B. subtilis* (42 mm), moderate against *E. coli* (29 mm) and least effective against *S. aureus* (20 mm). Essential oils are defined as the complex mixture of cyclic and acyclic monoterpenes which possessed many useful properties like antibacterial, antifungal, antioxidant and anti-carcinogenic.²¹ Bioactivities of essentials have been implicated in controlling pathogens and/or toxin producing microorganisms.²²⁻³⁴ Previously we have also reported antimicrobial activities of lemongrass essential oil and its major constituents, citral, geraniol and geranyl acetate.²⁰ Antimicrobial activities of the three lemongrass oils reported here was highly expected and in accordance to several previously published reports. Our recent report has documented antibacterial potential of lemongrass essential combined with honey against the multi-drug resistant bacteria.¹⁷ Silver nanoparticles synthesized using lemongrass leaf extract also have potential antibacterial activities.¹⁸ The antimicrobial potential of lemongrass oil are often attributed to the percentage of citral (3,7-dimethyl-2,6-octadienal).¹³⁻¹⁶ However, other constituents may also be important in determining bioactivity of lemongrass oils. The GC-MS analyses of essential oils of three lemongrass cultivars studied have identified citral as the major oil constituents which accounted for 80-85% of the total monoterpenes (Table 3). It is reported that the level

of citral varied markedly in the essential oil isolated from different lemongrass species and cultivars.¹² The differences in the antimicrobial potential of essential oils are often correlated with their chemical compositions.^{35,36} Therefore the differences in the antimicrobial potential of the three lemongrass cultivars tasted may be attributed to the amount of citral in essential oils. High volatility and lipophilicity of lemongrass oils help them to easily attach and penetrate in to cell membrane of the pathogen and cause cell lysis.³⁷ Cristiane et al. have reported that lemongrass oil displayed higher anticandidal activity against strains isolated from superficial mycoses as compared to citral.³⁸ Water soluble essential oil of lemongrass recovered by redistilling the hydrosol showed significant antifungal activity against *A. Niger*.³⁰ The water soluble essential contain high percentage of citral. In the present study lemongrass oils were found more effective against *C. Albicans* than *A. Niger* similar to that reported by Adinarayana et al.³⁰ In accordance to the previous reports our study has correlated antimicrobial potential of essential oils with their chemical composition and particularly with dominating constituent/s. The efficacy of the essential oil is also determined by the morphology and membrane structure of the microorganisms used. The poor activity of lemongrass oils against Gram negative *E. coli* is because of the presence of an outer membrane which possesses hydrophilic polysaccharides chains that serve as a barrier to hydrophilic essential oils.³⁵ However, at present mechanism of antimicrobial action of lemongrass oil is not fully known and need to be worked out.

Conclusion

Lemongrass oils have demonstrated significant antibacterial and antifungal activities against pathogenic bacteria and fungi associated with various forms of infection in human. Also, the study concluded that efficacy of essential oils is correlated with their chemical compositions, mainly the most abundant constituent, citral. Morphological features and membrane structure of the microorganisms also play important roles in determination of resistance or susceptibility of the microorganisms against lemongrass oils. However, this point has not been addressed in the present study. Nevertheless, the study highlighted prospects of lemongrass essential oils in the treatment of bacterial and fungal diseases.

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Conflict of interests

The authors claim that there is no conflict of interest.

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