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Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some selected pathogenic bacterias

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ABSTRACT

Objective: To find the effectiveness of essential oil of lemongrass for the treatment of pathogenic organisms. **Methods:** Lemongrass oil was investigated for activity against *Staphylococcus aureus (S. aureus)* , *Bacillus cereus (B. cereus)* , *Bacillus subtilis (B. subtilis)* , *Escherichia coli (E. coli)*, *Klebsiella pneumoniae (K. pneumoniae)* and *Pseudomonas aeruginosa (P. aeruginosa)* , using Agar Diffusion Method and Broth Dilution Method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the Broth Dilution Method. The antibiotic susceptibility test against the test organisms was performed by Disc Diffusion Method. **Results:** Lemongrass was found effective against all the test organisms except *P. aeruginosa*. Gram positive organisms were found more sensitive to lemon grass oil as compared to gram negative organisms. The test organisms were found inhibited by Lemon grass oil at lower concentrations in Broth Dilution Method as compared to Agar Diffusion Method. **Conclusions:** The tested organisms, particularly gram-negative organisms had shown high resistance towards different antibiotics whereas they were found to be inhibited by lemongrass oil even at lower concentration. Thus lemongrass oil is effective against drug resistant organisms. It can be suggested that use of lemongrass oil would be helpful in the treatment of infections caused by multidrug resistant organisms.

1. Introduction

Cymbopogon citrates (DC.) stapf, commonly known as lemongrass and other *Cymbopogon* species is a tall, coarse grass with a strong lemontaste. lemongrass is a perennial herb widely cultivated in the tropics and sub-tropics, designates two different species, East Indian *Cymbopogn flexuosus* (DC.) stapf and West Indian, *Cymbopogon citratus* (DC.) stapf.

Cymbopogon citratus (DC) stapf. has been cultivated over many years for medicinal purposes in different countries through out the world. The use of lemongrass was found in folk remedy for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorders. Researchers have found that lemongrass holds antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties[1].

Further, many workers had reported about the antibacterial activity of lemongrass oil against a diverse range of organisms comprising gram positive and gram negative organism, yeast and fungi[2-9]. Onawunmi *et al*. had observed that gram positive organisms were more sensitive to the oil than gram negative organisms[10]. The lemongrass oil was found to be effective against *Acinetobacter baumanii (A. baumanii)*, *Aeromonas veronii (A. veronii)*, *Enterococcus faecalis (E. faecalis)*, *Escherichia coli (E. coli)*, *Klebsiella pneumonia (K. pneumonia)* , *Salmonella enterica (S. enterica)* serotype typhimurium, *Serratia marcesens (S. marcesens)*, *Proteus vulgaris (P. vulgaris)*, *Enterobacter aerogenes (E. aerogenes)* , *Corynebacterium equii (C. equii)* and *Staphylococcus aureus (S. aureus)* [3,4,6].

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.

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2. Materials and methods

2.1. Procurement of lemongrass oil

The essential oil of lemongrass (*Cymbopogon citratus*) was collected from Central Institute of Medicinal and Aromatic plants (CIMAP), Lucknow- India.

2.2. Test organisms/Bacterial organisms

The test organisms used in this study was obtained from the Culture Collections of the Department of Microbiology and Microbial Technology, College of Biotechnology & Allied Sciences. Allahabad Agricultural Institute-Deemed University. The organisms used in this study were: *S. aureus, B. cereus, B. subtilis, E. coli, K. pneumoniae*, and *P. aeruginosa.*

2.3 Propagation and maintenance of test organisms

The test organisms were streaked on the Nutrient Agar slants and were incubated overnight at (37 +1) ℃. The cultures were kept under refrigerated conditions and were subcultured after every fifteen (15) days.

2.4. Preparation of concentrations of lemongrass oil

The different concentrations (v/v) of lemongrass oil viz 5%, 10%, 15%, 20%, 25%, 30% were prepared aseptically in sterile tween-80.

2.5. Antibacterial activity

The testing of the bacterial cultures for the inhibitory effect of essential oil of lemon grass for different concentration (5 %, 10 %, 15%, 20%, 25% and 30 %) were performer by using agar well diffusion method as described by Southwell *et al* [11].

The Nutrient agar media containing 0.5% tween-80 was melted and 20 mL of media was added to individual sterilized petriplates separately on a level plate form and allowed to solidify. 1 mL of active cell suspension of organisms was spread with the help of sterilized swabs on the agar surface uniformly. Three wells of 5 mm diameter each were made in agar patriplates of the solidified agar medium using sterilized hollow stainless steel gel cutter. The measure quantity of 25 μL of each concentration was pipetted out with a sterilized pipett and filed in the wells aseptically. In the control plate only Tween-80 was added into the well. The oil was allowed to defuse in the well for a period of one hour and plates were incubated at (37±1) ℃ for 24-48 hours. The zone of inhibition (mm) was measured with graduated scale after the period of incubation.

2.6. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The determination of MIC of the essential oil of lemongrass on the test bacterial strain was done using broth dilution method as explained by Hammer et al. with different concentrations of oil[9]. The cultures of the test strains were prepared by inoculating the test strain in sterilized test tube containing 5 mL nutrient broth. The tubes were incubated overnight at (37±1) ℃. The MIC was defined as the lowest concentration of the test compound to inhibit the growth of microorganisms and the MBC was defined as the lowest concentration of the test compound to kill the microorganisms. The test tubes containing 10 mL of sterilized tryptic soy broth (TSB) with 0.5% (v/v) tween-80 were inoculated with different concentration of lemon grass oil ranging from 0.5% - 0.015% (v/v). TSB with 0.5% (v/v) tween 80 without oil was used as positive growth control. An aliquot of bacterial suspension (25 μL) to each tube was added uniformly. The tubes were incubated at (37±1) ℃ for 24 hours then 48 hours. The tubes were observed for turbidity after the period of incubation. The lowest concentration at which no visible growth occurs in either culture tubes was taken as MIC. Then the tubes showing no increased in the turbidity at each time interval 24-48 hours were streaked on nutrient agar plates to check the bacterial growth. Each trial was repeated thrice.

2.7. Antibiotic susceptibility test

The antibiotic susceptibility test was performed by using the Bauer and Kirby Disc Diffusion Method[12] as per CLSI guidelines and the antibiotics (Hi Media) used in the present study were: azithromycin (15 mcg), ceftriazone (10 mcg), chloramphenicol (30 mcg), carbenecillin (100 mcg), gentamicin (10 mcg), kanamycin (30 mcg), tobramycin (10 mcg), nitrofurantoin (300 mcg), vancomycin (30 mcg), ciprofloxacin (30 mcg).

2.8. Statistical analysis

The data recorded during the course of investigation were statistically analysed by three way classifications and conclusion was drawn on the basis of analysis of variance technique. The calculated value of *F* was compared with the tabulated value at 5% and 1% level of significance for appropriate degrees of freedom.

3. Results

Lemongrass oil was found effective against all the test organisms except *P. aeruginosa*. Gram positive organisms (*S. aureus, B. cereus* and *B. subtilis*) were found more susceptible than gram negative organisms (*E. coli, K. pneumoniae, P. aeruginosa*). The antibacterial activity was found progressively increasing with the increase in concentration of oil. The maximum effect was found at 30% concentration and minimum effect was observed at 5% concentration of oil (Table 1).

In Broth Dilution Method the test organisms were found to be inhibited by lemongrass oil at very low concentration as compared to Agar Diffusion Method. Lemongrass oil was found to be effective against gram positive as compared to gram negative bacteria. *P. aeruginosa* was found to be highly resistant (even at neat). *S. aureus* and *B. cereus* was found to be more sensitive and got inhibited at 0.03% concentration (initial MIC) and at 0.06% concentration (final MIC). The final MIC and MBC was found to be the same. *B. subtilis* and *E. coli* was found to be inhibited at a concentration of 0.06% (MIC) with an MBC of 0.12% concentration. Compared to other test organisms K. *pneumoniae* showed a higher MIC (0.25%) and MBC (0.5%) (Table 2).

All the test organisms showed difference in their sensitivity against different antibiotics (Table 3). Gram-positive organisms were found to be more susceptible as compared to gram-negative organisms. Among gram-positive organisms *S. aureus* was found sensitive to all the antibiotics except nitrofurantoin. *B. cereus* was also found sensitive to almost all antibiotics tested except kanamycin and tobramycin. *B. subtilis* was found to be more resistant to antibiotics as compared to *S. aureus* and *B. cereus*. Azithromycin, ceftriazone, chloramphenicol, carbenicillin were found ineffective against *B. subtilis*. Gram negative organisms like *K. peneumoniae* and *P. aeruginosa* showed maximum resistance to antibiotics. *E. coli* was found resistant to ceftriazone, chloramphenicol, carbenicillin and tobramycin.

Table 1
Antibacterial activity of lemon gross oil against various selected pathogenic bacteria.

Organisms	Zone of inhibition (mm)* Lemongrass oil					
	5%	10%	15 %	20%	25%	30%
Staphylococcus aureus	14.33	19.33	22.33	24.66	27.33	29.66
Bacillus cereus	12.66	15.66	18.66	21.00	24.00	28.00
Bacillus subtilis	8.33	10.33	12.66	16.00	19.66	24.66
Escherichia coli	8.33	11.33	14.00	16.33	19.33	22.33
Klebsiella pneumoniae	7.66	9.33	11.33	12.66	14.66	17.00
Pseudomonas aeruginosa	0.00	0.00	0.00	0.00	0.00	0.00

S.E. due to treatment = 0.72 C.D. due to treatment = 1.42 ; S.E. due to organisms=0.72,C.D. due Organisms=1.42; * Well size 5 mm included

Table 2
MIC (Initial, final) and MBC of the test organisms against lemongrass oil.

Test organisms	Initial MIC (%)	Final MIC (%)	MBC (%)
Staphylococcus aureus	0.03	0.06	0.06
Bacillus cereus	0.03	0.06	0.06
Bacillus subtilis	0.06	0.06	0.12
Escherichia coli	0.06	0.12	0.12
Klebsiella pneumoniae	0.25	0.50	0.50
Pseudomonas aeruginosa	ND	ND	ND

Table 3
Antibiotic sensitivity pattern of test organisms.

Antibiotics	Organisms					
	S. aureus	*B. cereus*	*B. subtilis*	*E. coli*	*K. pneumonia*	*P. aeruginosa*
Azithromycin	++	++	–	++	–	–
Ceftriazone	++	++	–	–	–	+
Chloramphenicol	++	++	–	–	–	–
Carbenicillin	++	++	–	–	–	++
Gentamycin	++	++	++	++	–	–
Kanamycin	++	–	++	+	–	–
Tobramycin	++	–	++	–	–	–
Nitrofurantoin	–	++	++	+	+	–
Vancomycin	++	++	++	++	–	–
Ciprofloxacin	++	++	++	++	–	++

Sensitive= ++, Intermediate= +, Resistant=–.

4. Discussion

From the present study it is clear that lemongrass oil possess a promising antibacterial activity against the test organisms. The results obtained from the Agar diffusion assay and broth dilution method support the general indication that gram positive organisms are more sensitive to the oil than gram negative bacteria. Similar observations were made by Onawunmi and Ongulana[7] and Cimanga *et al*[4]. *P. aeruginosa* were found resistant at all the concentration of lemongrass oil including neat. Similar results were reported by Pereira *et al*, Marta War *et al*., Torris *et al*, Alam *et al*, and Onawunmi *et al*, [6,13-15,10].

The test organisms were found inhibited by lemongrass oil at very low concentration in broth dilution method as compared to agar diffusion method, this is in accordance with the results of Tortorano *et al*,[16].The results obtained by each of these methods differ due to many factors between assays[17,18]. These include differences in microbial growth, exposure of microorganisms to the oil, the solubility of oil or oil components and the use and quality of an emulsifier etc.

The comparative effects of lemongrass oil and the standard antibiotic discs on the various test organisms are demonstrable indications of the oil as an antibacterial agent. Onawunmi and ongulana had also reported the similar antibiotic susceptibility pattern and had suggested that the test organisms particularly gram negative were found to be more susceptible to lemongrass than standard antibiotics[7]. Thus, we conclude that in present era of emerging multidrug resistance among gram positive and gram negative organisms lemongrass oil will be helpful in treating such infections.

Conflict of interest statement

We declare that we have no conflict of interest.

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