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## Research Article

### Antifungal activity of lemon grass oil against clinically *Aspergillus* spp. isolates

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#### Abstract

The aim of study was testing the biological activity of lemon grass essential oil against *Aspergillus* spp. Lemon grass oil was extracted by steam distillation of wilted leaves of lemon grass (*Cymbopogon citrates*). The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of this oil and citral against two clinical isolates of *Aspergillus* spp. were determined by agar dilution method. The mode of action of lemon grass oil and citral were proven to be fungicidal.

**Keywords:** Antifungal, lemon grass, *Aspergillus* spp.

## Introduction

Many of the plant species found that it can be used medically (Ali-Shtayeh and Abu,1999). Essential oils are aromatic substances which are obtained from various plant parts by steam distillation; many of which exhibit antibacterial (Ozcan *et al.*, 2006), antifungal (Chee and Lee, 2007) and antiviral (Khan *et al.*, 2005) activity. Lemon grass (*Cymbopogon citratus*) is widely used in traditional medicine in many countries around the world. Among its attributable properties are those related to antibacterial and antifungal activities (Bansod and Rai, 2008; Revathi *et al.*, 2012), as well as analgesic and anti-inflammatory properties (Negrelle and Gomes, 2007). A large amount of literature is also available on the medicinal properties of essential oil present in peppermint (Gulluce *et al.*, 2007; Rasooli, 2008). Aspergillosis results from the

inhalation of spores of *Aspergillus fumigatus*. Once in the lungs the spores of this fungus germinate to form a tangled mass of fungus fibers and blood clots. Fungus spreading increase gradually leading to the destruction of lung tissue, but they do not always spread to other parts of the body (Bansod and Rai, 2008). The majority of the clinically used antibiotics, used to cure this infection, suffer from various drawbacks relating to toxicity and drug drug interactions, lack of fungicidal efficacy, cost and finally the emergence of resistant strains caused by their frequent use. There is an urgent need towards the use of anti-fungal substances, especially with high efficiency and less toxic compared to currently used drugs (Rapp, 2004; Kauffman, 2006). The aim of this study was to assess the antifungal activity of Lemongrass oil against some *Aspergillus* spp. The

fungicidal and fungistatic effect of lemon grass oil and its known major components were determined.

## Materials and Methods

### Test organisms

*Aspergillus niger* and *A. flavus* were obtained from the Medical Laboratory Technology, College of Applied Medical Science, Hafr Al-Batin, Dammam University, Saudi Arabia and stock cultures were maintained on Czapek Dox agar slants, stored at 5°C.

### Chemicals

Citral (97%) and myrcene were purchased from Aldrich Chemicals. Lemon grass oil was prepared by steam distillation of leaves of lemon grass. Citral and myrcene were found to be the major components of the oil.

### Preparation of inoculums

*Aspergillus* spp. were grown on PDA at 30°C for 10 days. Suspensions of fungi were prepared in solution containing 0.1% Tween 80.

### Assessment of antifungal activity of lemon grass oil

Lemon grass oil was first assayed for its antifungal activity by the agar diffusion method. Seeded agar plates were prepared by pouring 20 mL of PDA into each sterile plate. After solidification of medium, each plate was overlaid with 5 mL of PDA which had been inoculated with 0.1 mL of inoculums. Lemon grass oil was applied onto filter paper discs (6 mm in diameter) with 20 µL/disc. These discs were then placed on the surface of seeded agar plates (1 disc/plate). All plates were incubated at room temperature for 10 days. The inhibition zone was determined by measuring the diameter of the clear zone around each disc.

### Assessment of minimum inhibitory concentration (MIC) and of minimum lethal concentration (MLC)

The MIC of lemon grass oil against test fungi was determined using agar dilution method. Stock

solution of lemon grass oil in various concentrations was prepared in 50% (v/v) dimethyl sulphoxide (DMSO). Test media was prepared by adding 1 mL of stock solution into 19 mL of PDA before boring. Inoculation was performed by applying 3 µL of the inoculums on each of sterile Millipore membrane (11 mm in diameter), which had been placed on the test media. The MIC was recorded as the lowest concentration which inhibited fungal growth. The MLC was determined by subsequently transferring the inoculated Millipore membrane showing no fungal growth into PD broth (PDB) tube. Tubes were incubated under the same conditions. The MIC was read as the lowest concentration that was lethal to the test fungi.

### Determination of the active component in lemon grass oil

To find out which of the major components was the active compound, citral and myrcene were comparatively screened for their antifungal activity using the agar diffusion method. The component possessing antifungal activity was subsequently studied for MIC by the method described above.

## Results and Discussion

In a preliminary test, the clear zone of 90 mm in diameter indicating a strong antifungal activity of lemon grass oil (Table, 1).

To determine if the mode of action of lemon grass oil as fungistatic or fungicidal, MIC and MLC were determined. The mean value of MICs and MLCs for *Aspergillus niger* and *Aspergillus flavus* tested are presented in Table 2. Comparison of MIC and MLC indicated that lemon grass oil was fungicidal, since MLCs were almost equal to MICs.

In this study, citral was found to be the major active component of lemon grass oil. The same conclusion was also reported previously in many experiments (Hemtanont *et al.*, 1991). Moreover, MIC and MLC of citral observed in this study revealed that citral functioned as a fungicidal agent. Kurita *et al.*, (1981) had proposed that citral was able to form a charge transfer complex with  $e^-$  donor of fungal cells resulting in fungal death.

**Table 1.** Antifungal activity of lemon grass oil and its components against *Aspergillus* spp.

Test fungi	Diameter of clear zone (mm)*		
	Lemon grass oil	Citral	Myrcene
<i>Aspergillus niger</i>	90.0	90.0	0.0
<i>Aspergillus flavus</i>	90.0	90.0	0.0

\*Mean of triplicate

**Table 2.** MICs and MLCs of lemon grass oil for *Aspergillus* spp.

Test fungi	Mean value	
	MIC ( $\mu\text{g/mL}$ )	MLC ( $\mu\text{g/mL}$ )
<i>Aspergillus niger</i>	64	64
<i>Aspergillus flavus</i>	128	128

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