JOURNAL OF MEDICINAL FOOD
J Med Food 12 (1) 2009, 193–197

DOI: 10.1089/jmf.2008.0108

Short Communication

Effectiveness of *Cymbopogon citratus* L. Essential Oil to Inhibit the Growth of Some Filamentous Fungi and Yeasts

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ABSTRACT Lemon grass (*Cymbopogon citratus* L.) oil has been known as having therapeutic and antibacterial properties, and its antifungal activity is currently the subject of renewed interest. This study aimed to verify the effectivenesses of *C. citratus* essential oil to inhibit the growth/survival of some fungi (*Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium roquefortii*) and yeasts (*Candida albicans*, *Candida oleophila*, *Hansenula anomala*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Saccharomyces uvarum*, and *Metschnikowia fructicola*). *C. citratus* essential oil showed effectiveness in inhibiting the growth of all fungi by disc diffusion and broth dilution bioassay. Minimum inhibitory and minimum fungicidal concentrations between 0.062 and 20 μL/mL were determined. The Clinical and Laboratory Standards Institute agar-based method was also applied for *A. niger* and *C. albicans*. Data show the strong antifungal properties of lemon grass oil (*C. citratus*) *in vitro*.

KEY WORDS: ***• antifungal effect • essential oil • medicinal plant • lemon grass oil***

INTRODUCTION

FILAMENTOUS FUNGI AND YEASTS are widely distributed in nature and are able to spoil many foods such as cheese, beverages, juices, salads, sugar, vinegar, wines, and meat, causing changes in odor, color, taste, and texture.[1,2] *Candida*, *Saccharomyces*, and *Hansenula* are some important food-spoiling yeasts.[3] *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., and *Alternaria* spp. are particularly important wilt-producing fungi and attack important crops such as tomatoes, bananas, sweet potatoes, pears, and cereals can be responsible for considerable economic losses all over the world. In addition, some species of fungi may cause food and feed contamination, especially those fungi that are able to produce mycotoxins, which are known potent hepatocarcinogens in animals and humans.[4,5] Serious invasive fungal infections caused by yeasts, such as *Candida* spp. and molds, represent an increasing threat to human health. Incidence of infection by these microorganisms has risen dramatically during the last 20 years.[6,7]

Various antifungal additives may be used against harmful microorganisms in food products. However, there are increasing numbers of reports of people showing sensitivity to synthetic materials used as preservatives; also, food legislation has restricted the use of some synthetic antimicrobials based on a possible toxicity for consumers. Plants have emerged as effective compounds to provide microbiological safety of foods. Herbal products are reported to be safe, nonhazardous, relatively cheap, and ecofriendly.[8–10]

Lemon grass (*Cymbopogon citratus* L.) is an important aromatic grass. The crop is cultivated to obtain citral-rich essential oil used in perfumery, cosmetic, and pharmaceutical industries, and constituents are used extensively as a flavoring ingredient in a wide variety of foods, beverages, and confectionary products.[11,12] Recently, there has been considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or spoilage by fungi in foods.[13]

Although some researchers have found antimicrobial activity of these essential oils, there is a lack of information about their effect on the growth of food-spoiling fungi and yeasts.[14,15] The aim of this study was to investigate the antifungal activity (minimum inhibitory concentration [MIC] and minimum fungicidal concentration [MFC]) of lemon grass essential oil against various filamentous fungi (*Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium roquefortii*) and food-spoiling yeasts (*Candida albicans*, *Candida oleophila*, *Hansenula anomala*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Saccharomyces uvarum*, and *Metschnikowia fructicola*) at different concentrations *in vitro* with emphasis on its possible use as an alternative antifungal compound. Also, the an-

Manuscript received 24 April 2008. Revision accepted 27 May 2008.

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tifungal susceptibility test was used for *A. niger* and *C. albicans* with some chemical antifungal agents, and the effectiveness of *C. citratus* oil against these microorganisms was compared.

MATERIALS AND METHODS

Essential oils

Essential oil of lemon grass (*C. citratus* L.) was kindly supplied by Dr. Valerie Edwards-Jones, Research Development and Innovations Unit of Manchester Metropolitan University, Manchester, UK. Its quality parameters (appearance, color, purity, odor, density at −20°C, and refraction index at −20°C) were described in an accompanying technical report. The essential oil was assayed at concentrations of 0.062, 0.31, 1.25, 2.5, 5, 10, and 20 μL/mL (μL of essential oil/mL of methanol), with the solutions being prepared according to the methods of Rasooli and Abyaneh[16] and Sökmen *et al.*[17]

Organisms

A. niger, *P. roquefortii*, *A. alternata*, *F. oxysporum*, *S. cerevisiae*, *S. pombe*, *S. uvarum*, *H. anomala*, *M. fructicola*, and *C. oleophila* strains were isolated from foods in the Food Engineering and Plant Protection Departments, Uludag University, Bursa, Turkey, and identified using standard fungi determination procedures by the methods of Pitt,[18] Samson *et al*.,[19] and Korukluoglu *et al*.[20] *C. albicans* ATCC10231, a medically important yeast, was obtained from the Medicine Faculty of Uludag University. Stock cultures were maintained on malt extract agar (MA) (Merck, Darmstadt, Germany) slants at 4°C. All fungal cultures were maintained on MA slants. Ten milliliters of 1% (vol/vol) Tween 20 was added for conidia collection. Conidia were harvested by centrifugation at 1,000 *g* for 10 minutes and washed with sterile distilled water. This step was repeated three times. One milliliter of these conidia suspensions was added into 50 mL of sterile malt extract broth (MB) (Merck) and incubated for 24 hours at 30°C according to the procedure of Yin and Tsao.[21] MB suspension concentrations of filamentous fungi contained approximately 10^4 conidia/mL. Yeasts were grown in 50 mL of sterile MB for 18 hours on a shaker incubator (model AGCH-4103, Infors, Böttmingen, Switzerland) at 50 rpm at 30°C, producing yeast suspensions of approximately 10^6 colony-forming units/mL. All filamentous fungi and yeasts in MB broth were enumerated on MA at 22–25°C for 4–5 days, by using serial dilution methods.[22]

Antimicrobial assays

Disc diffusion method. The disc diffusion procedure was used to determine the antifungal activity of essential oils.[23,24] For this, 0.2 mL of the yeast or fungi suspension was uniformly spread on sterile MEA Petri dishes. After inoculum absorption by agar, sterile filter discs (catalog number 2668, 6 mm in diameter; Schleicher & Schüll, Dassel, Germany) were placed on the culture medium and impregnated with 50 μL each of various dilutions (0.62–20 μL/mL) of the essential oil. After 30 minutes, the plates were inverted and incubated at 30°C for 72 hours for fungi and 48 hours for yeasts. At the end of the incubation period, the inhibition halo diameters were measured using calipers and expressed in millimeters. When the inhibition halo observed was equal or higher than 10 mm, it was considered as positive antifungal activity.[25] Controls included in the assay were essential oil replaced by sterile water. All experiments were performed in triplicate.

Clinical and Laboratory Standards Institute (CLSI) method (agar-based testing). Antifungal susceptibility was tested by the CLSI method for the medically important microorganisms *A. niger* and *C. albicans* by the disc diffusion method.[26] For analysis, 0.2 mL of the yeast or fungi suspension was spread on Mueller-Hinton (MH) agar (Oxoid, Basingstoke, UK) supplemented with 2% glucose, and commercial disks (Bristol-Myers Squibb [New York, NY] or Pfizer Pharmaceutical [New York]) of voriconazole (1 μg), caspofungin (5 μg), amphotericin B (5 μg), and fluconazole (1 μg) were placed on MH agar. Petri dishes were incubated at 35°C for 24 hours for *A. niger* and for 48 hours for *C. albicans*. Pure methanol (5 μL) was impregnated on sterile discs for the control. The average diameter of inhibition zones was determined.

Table 1. Diameter of Inhibition Zones of *C. citratus* Essential Oil Against Selected Filamentous Fungi and Yeasts Tested Using the Disc Diffusion Assay

Concentration (μL/mL)	*Diameter of inhibition zone (mm)*[a]										
	Aa	*An*	*Fo*	*Pr*	*Ha*	*Co*	*Ha*	*Mf*	*Sc*	*Su*	*Scp*
20	>	>	>	>	56.3 ± 2.6	>	>	73.3 ± 1.3	21.8 ± 1.0	>	>
10	>	>	>	>	41.5 ± 1.0	>	>	61.3 ± 1.3	16.3 ± 0.5	>	>
5	>	>	>	>	34.0 ± 1.4	>	>	36.0 ± 1.4	15.0 ± 0.0	>	>
2.5	>	60.8 ± 1.0	55.3 ± 1.3	>	22.0 ± 2.2	16.8 ± 1.0	52.0 ± 1.4	26.8 ± 1.0	12.0 ± 0.8	26.5 ± 2.4	51.0 ± 0.8
1.25	>	46.3 ± 1.5	36.0 ± 1.2	>	17.0 ± 1.6	12.5 ± 0.6	35.5 ± 1.0	15 ± 1.6	0	0	25.0 ± 1.6
0.31	>	34.8 ± 2.1	21.5 ± 13	25.0 ± 0.8	14.5 ± 1.3	0	11.8 ± 1.0	14.3 ± 1.0	0	0	19.8 ± 2.1
0.062	42.5 ± 2.1	27.5 ± 2.1	18.8 ± 1.0	0	12.3 ± 1.3	0	0	0	0	0	0

Data are mean ± standard deviation values. Aa, *A. alternata*; An, *A. niger*; Fo, *F. oxysporum*; Pr, *P. roquefortii*; Ca, *C. albicans*; Co, *C. oleophila*; Ha, *H. anomala*; Mf, *M. fructicola*; Sc, *S. cerevisiae*; Su, *S. uvarum*; Scp, *S. pombe*.

[a]0 indicates not detectable; > indicates values equal to or greater than the diameter of Petri dishes.

Table 2. MIC and MFC of *C. citratus* Essential Oil Against Selected Filamentous Fungi and Yeasts

	Aa	*An*	*Fo*	*Pr*	*Ca*	*Co*	*Ha*	*Mf*	*Sc*	*Su*	*Scp*
MIC/MFC (μL/mL)	0.062/0.062	0.062/0.062	0.062/0.31	0.31/1.25	0.31/0.31	0.13/0.13	1.25/1.25	1.25/2.5	5/10	2.5/2.5	0.31/1.25

Aa, *A. alternata*; An, *A. niger*; Fo, *F. oxysporum*; Pr, *P. roquefortii*; Ca, *C. albicans*; Co, *C. oleophila*; Ha, *H. anomala*; Mf, *M. fructicola*; Sc, *S. cerevisiae*; Su, *S. uvarum*; Scp, *S. pombe*.

Broth dilution method (determination of MIC and MFC). In addition to the solid medium diffusion procedure, the broth dilution bioassay was used to study the antifungal activities of essential oils. The MIC and MFC were assessed according to the methods of Rasooli and Abyaneh[16] and Pyun and Shin.[23] MFC was determined by the broth dilution method as follows: 50 μL from each of various dilutions of the oils was added to 5 mL of MB tubes containing 10^4 colony-forming units/mL for fungi and 10^6 colony-forming units/mL for yeasts. The tubes were then incubated in a shaker incubator at 30°C for 72 hours for fungi and 48 hours for yeasts. The lowest concentration of essential oil that prevented visible growth in the tube was considered the MIC. The MFC was determined by culturing 0.2 mL from all tubes that lacked visible turbidity in the MIC assay on MA plates at 30°C for 72 hours. The MFC was defined as the lowest concentration at which the growth of fungal colony was completely inhibited on agar plates.

RESULTS AND DISCUSSION

The current necessity of discovering new antifungal compounds in all fields of fungal control has stimulated research regarding antifungal properties of plant compounds.[1,8] In this research the essential oil of lemon grass (*C. citratus* L.) was examined as an antifungal agent *in vitro*. Table 1 gives the mean ± standard deviation of inhibition zones determined by the disc diffusion method. The results showed that the lemon grass essential oil had an inhibitory effect on all fungi and yeasts assayed as shown by large growth inhibition halos. Most strains assayed showed an MIC of 0.062–0.31 μL/mL. The highest inhibitory activity was against *A. alternata*, *A. niger*, and *F. oxysporum*, which showed the lowest MIC (0.062 μL/mL) and the largest growth inhibition halos. In contrast, *S. cerevisiae*, *S. uvarum*, *M. fructicola*, and *H. anomala* were the least sensitive yeasts with MICs of 5, 2.5, 1.25, and 1.25 μL/mL, respectively, and *S. cerevisiae* showed the smallest growth inhibition halo diameter when compared to all other strains. This high antimicrobial activity of lemon grass essential oil supports the results found by other researchers.[4,8,9]

Table 2 gives MIC and MFC values of essential oil against the microorganisms tested by the broth dilution method. The MFC values found for the broth dilution assay were always higher than the MIC values found in the disc diffusion assay.

Based on MIC results, a classification for the antimicrobial activity of plant products was proposed as follows: strong inhibitors, MIC up to 0.5 μL/mL; moderate inhibitors, MIC between 0.6 and 1.5 μL/mL; and weak inhibitors, MIC above 1.6 μL/mL.[27] Thus, based on the MIC results found in these assays the lemon grass essential oil has potential as an antifungal compound because most fungal strains were inhibited with values higher than the 0.5 μL/mL MFC. Determination of the inhibitory effect of essential oil against microorganisms by the broth dilution assay is an effective method as the essential oil can contact directly the microorganism.

Lemon grass oil is rich with citral and/or citronellal aldehydes, which are known for their antibacterial and antifungal activity,[28,29] and its fungitoxic properties were reported to have high thermostability and were unaltered after 7 months.[4] Lemon grass oil was found to contain citral (43.8%), *z*-citral (18.93%), geranyl acetate (5.27%), and *trans*-geraniol (3.66%) as major constituents.[12] Aggarwal *et al*.[30] determined that (*R*)-(+)-limonene, an isomer of limonene, was highly effective in inhibiting growth of a variety of microorganisms that cause crop damage or food spoilage, including *A. niger*. Hammer *et al*.[31] previously found antifungal activity for thyme, clove, and lemon grass

Table 3. Diameter of Inhibition Zones of Voriconazole, Caspofungin, Amphotericin B, and *C. citratus* for *A. niger* and *C. albicans*

					C. citratus *oil/methanol*		
Organism	*Voriconazole (1 μg)*	*Fluconazole (1 μg)*	*Caspofungin (5 μg)*	*Amphotericin B (5 μg)*	*1 μL/mL*	*5 μL/mL*	*Methanol (control)*
A. niger	23 ± 1.2	20 ± 1.0	26 ± 0.8	30 ± 1.3	30 ± 1.3	42 ± 1.3	0
C. albicans	12 ± 1.4	30 ± 1.2	28 ± 1.3	32 ± 1.0	15 ± 2.1	32 ± 1.0	0

[a]0 indicates not detectable.

oils against some bacteria and *C. albicans.* Pandey *et al.*[12] reported that lemon grass oil and citral have fungicidal activity against *C. albicans*, *Fusarium solani*, and *Clostridium* spp. Also, they reported a very high fungitoxic effect of lemon grass oil against *Trichophyton mentagrophytes* and *F. oxysporum* as compared to ketoconazole nitrate. Amphotericin B, voriconazole, and caspofungin are approved for the treatment of aspergillosis, and fluconazole is used particularly against *C. albicans.* However, primary and secondary resistance among the strains causing human mycosis has been reported in some studies.[32,33] The results in Table 3 show that 1 μL/mL and 5 μL/mL concentrations of *C. citratus* oil and methanol showed a higher inhibitory effect against *A. niger* and *C. albicans* than these chemical antifungals tested.

Lemon grass oil is nonphytotoxic in nature; it did not exhibit any adverse effects on germination and seedling growth of wheat and rice. Application of chemical fungicides increases the risk of high-level toxic residues in the products. Also, they may produce side effects, environmental contamination, and the development of multiresistant fungal strains.[6]

The mechanism of antimicrobial activity of essential oils is mainly related to the presence of phenolic compounds, which are capable of dissolving in the microbial membrane and penetrating inside the cell. Thus cytoplasmic membrane disturbance and cytoplasm content coagulation are mechanisms for the antimicrobial properties of essential oil.[34]

The difference in the results when testing essential oils for antimicrobial activity could be related mainly to the assay technique, the growth medium, the microorganism being tested, and the composition of the essential oil, which depends on the plant species, the chemotypes, and the climatic conditions.[16,35,36]

The importance of the results of the present study is that commonly pathogenic and some important spoilage fungi can be controlled with *C. citratus* essential oil because this oil successfully inhibited the growth of these organisms. Thus lemon grass essential oil can be applied practically as an antifungal agent for foods and pharmaceutical products.[28]

ACKNOWLEDGMENTS

The authors thank to Dr. Valerie Edwards-Jones, Research Development and Innovations Unit of Manchester Metropolitan University, Manchester, UK, for supplying essential oil of lemon grass (*C. citratus* L.).

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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