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Antibiofilm activity and post antifungal effect of lemongrass oil on clinical *Candida dubliniensis* isolate

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Abstract

Candidal infections are often difficult to eradicate due to the resistance of biofilms to antifungal agents. This study aimed at determining the effects of lemongrass (*Cymbopogon citratus* DC) oil against *Candida dubliniensis* in both planktonic and biofilms form. The results from broth microdilution method revealed that the minimum inhibitory and minimum fungicidal concentration of lemongrass oil on *C. dubliniensis* were 0.43 and 0.86 mg/ml, respectively. Employing a formazan salt (XTT tetrazolium) reduction assay for biofilm study, the results showed that the average percentage (mean±SD) inhibition of lemongrass oil (0.43 mg/ml) on biofilm formation was 91.57±1.31%, while it exhibited more than 80% killing activity against *C. dubliniensis* in biofilm at concentrations of 1.7 mg/ml. In addition, a significant reduction ($P=0.03$) of candidal adhesion to acrylic occurred after a 15 min exposure to 1.7 mg/ml of lemongrass oil. Moreover, limited exposure of yeasts to lemongrass oil at subcidal concentration can suppress growth for more than 24 h. Altogether, the results obtained indicate that lemongrass oil possessed antifungal and antibiofilm activities and could modulate candidal colonization. Therefore, the efficacy of lemongrass oil merits further development of this agent for the therapy of oral candidiasis.

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1. Introduction

Biofilms of *Candida* species play a growing role in human medicine. Indeed, the majority of manifestations of candidiasis at both mucosal and systemic sites are associated in one way or another with the formation of biofilms on inert or biological surfaces (Ell, 1996; Cannon and Chaffin, 1999; Crump and Collignon, 2000). The prevalence of diseases caused by *Candida* spp. has increased dramatically, mainly due to an increase in the number of at-risk individuals, principally those with impaired immunity, such as transplant recipients, cancer patients receiving chemotherapy, and human immunodeficiency

virus-infected patients (Eggimann et al., 2003; Presterl et al., 2007; Ramirez-Amador et al., 2003; Singh et al., 2002). Oropharyngeal candidiasis (OPC) is the most common opportunistic infection in the immunocompromised. Although *Candida albicans* is a well-known etiological agent of OPC, *Candida dubliniensis* has emerged as another pathogen known for its azole resistance. *C. dubliniensis*, a new species of *Candida*, was recovered from oral cavities of HIV-infected patients, that is phenotypically and genotypically close to *C. albicans* (Sullivan et al., 1995). Therefore, it may be misidentified (Martinez et al., 2002). Evidence of the inducibility of a stable fluconazole resistance in vitro in *C. dubliniensis* strains may indicate that it is an emerging pathogen for immunocompromised patients receiving long-term fluconazole prophylaxis (Moran et al., 1998). In addition, *C. dubliniensis* can adhere to epithelial cells, which may indicate that this species is

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particularly adapted to colonize the oral cavity (Gilfillan et al., 1998).

Formation of biofilms by *Candida* spp. has been demonstrated on almost any medical device. The most commonly involved systemic devices include joint prostheses, cardiac valves, vascular and urinary catheters (Kojic and Darouiche, 2004; Ramage et al., 2006). In addition, there are many topical devices at risk, including contact lens and dentures. Different *Candida* spp. differ in their capacities to form biofilms (Hawser and Douglas, 1994; Kuhn et al., 2002). Formation of *Candida* biofilms has important clinical repercussions because of their increased resistance to antifungal therapy and the ability of cells within biofilms to withstand host immune defenses (Douglas, 2003; Ramage et al., 2006).

Increasing awareness of hazards associated with the use of antibiotic and chemical agents has accelerated investigations into plants and their extracts as new sources of antimicrobial agents. Essential oils are odorous, volatile products of plant secondary metabolism, found on many leaves and stems. They have a wide application in folk medicine, food flavoring and preservation as well as in fragrance industries. The antimicrobial properties of essential oils have been known for many centuries. In recent years, more than 500 reports stated that essential oils and their constituents possessed antimicrobial properties against some bacteria and fungi (Kalemba and Kunicka, 2003). However, little is known about the spectrum of action of essential oils against *Candida* biofilm. Moreover, the post antifungal effect (PAFE) of lemongrass (*Cymbopogon citratus* DC) oil on *C. dubliniensis* has never been reported. The aim of current research was to investigate the antifungal activity of the lemongrass oil against both sessile and planktonic clinical *C. dubliniensis* isolate. The PAFE and the effect of lemongrass oil on the adhesion of *C. dubliniensis* to denture acrylic were also evaluated. With the results as reported in this paper, the lemongrass oil exhibited effective killing activity and possessed the strong inhibitory effect on *Candida* biofilm formation. A significant reduction of candidal adhesion to denture acrylic occurred after a 15 min exposure to lemongrass oil. In addition, limited exposure of *C. dubliniensis* to lemongrass oil at subcidal concentration can suppress growth for more than 24 h. Therefore, it is a promising essential oil to combat candidal colonization and infection.

2. Materials and methods

2.1. Essential oil

Lemongrass oil (Thai China Flavours & Fragrances Industry Co. Thailand) was dissolved in 95% ethanol to an initial concentration of 720 mg/ml and further diluted with the solution contained 5% ethanol and 5% Tween 80 to a concentration of 55 mg/ml before used.

2.2. Preparation of yeast suspension

C. dubliniensis kindly provided by Dr. P. Chongtrakool (Microbiology Unit, Ramathibodi Hospital, Faculty of Medi-

cine, Mahidol University, Bangkok, Thailand) was maintained on Sabouraud-dextrose agar (BBL Microbiology Systems, Cockeysville, MD) and grown in the yeast phase in Sabouraud-dextrose broth (Pronadisa, Hispanlab, S.A.) for 18 h.

Preparation of the yeast suspension was different in each assay according to the optimal concentration of the inoculum. For antifungal and antibiofilm assay, *C. dubliniensis* was adjusted to give an optical density (OD) at 600 nm=0.1 and plate count to determine the CFU/ml. The suspension of OD at 600 nm of 0.1 possessed 1×10^6 CFU/ml and was used as inoculum in antifungal and antibiofilm assay.

To determine the effect of the lemongrass oil and nystatin on candidal adhesion to denture acrylic, the yeast suspension of 1×10^8 cells/ml was used according to our previous study (Taweekaisupapong et al., 2006) which demonstrated that the optimal concentration of the inoculum for adhesion was 1×10^8 cells/ml. For this study, the organisms were washed three times in sterile phosphate buffered saline (PBS) by centrifugation at 3000 g for 10 min and finally suspended in PBS to 1×10^8 cells/ml determined by hemocytometer.

For post antifungal effect assay, a suspension of OD at 600 nm of 1.5 was used according to previous study (Ellepolo and Samaranayake, 1998).

2.3. Identification of lemongrass oils components

The chromatographic analyses were carried out with a Trace GC ultra gas chromatograph (Model K05200B20000070, Italy) coupled with a DSQ mass spectrometer (Model Trace DSQ-Mass spectrophotometry, USA). A Tr-5 capillary column (30 m \times 0.25 mm i.d.) coated with 0.25 μ m film 5%phenyl–95% dimethylpolysiloxane was used for separations. The GC was operated under temperature programmed condition started with 70 °C for 1 min, then programmed at 6 °C/min to 280 °C for 3 min. One μ l of 1% solution in hexane was manually injected in a purged split mode (1:100) using high-purity helium as a carrier gas at 1 ml/min flow rate. The scan range was 35–550 m/z and the scan rate was 1000 amu/s. The identification of the components was based on comparison of their mass spectra with those of a computer library (NIST MS Search library). Further confirmation was done by referring to retention index (RI) data generated from a series of alkanes (C₁₀–C₂₃).

2.4. Determination of antifungal activities of the lemongrass oil and nystatin

Antifungal activities of the lemongrass oil and nystatin towards *C. dubliniensis* were determined by the broth dilution method (NCCLS, 2002). Fifty microliters of the lemongrass oil (55 mg/ml) and 50 μ l of nystatin (64 μ g/ml) were two-fold serially diluted with Sabouraud's dextrose broth in a microtiter plate. Fifty microliters of the *Candida* suspension was added and mixed with the lemongrass oil and nystatin. *C. dubliniensis* cultured in the broth without the tested agents served as a positive control and the mixture of broth and the tested agents without microorganism served as a negative control. The plates were incubated for 24 h, at 37 °C. Then *C. dubliniensis* growth

was examined and the lowest concentration of the tested agents which inhibited the visible growth of *C. dubliniensis* was recorded as the minimum growth inhibitory concentration (MIC).

Ten microliters of the mixture of the tested agents and *C. dubliniensis* which showed negative-visible growth after the first 24 h of incubation was dropped onto the surface of Sabouraud's dextrose agar. The lowest concentration of the tested agents giving negative growth of the *Candida* was recorded as the minimum fungicidal concentration (MFC). All experiments were repeated on three separate occasions, with triplicate determinations on each occasion.

2.5. Inhibitory effects of the lemongrass oil on *C. dubliniensis* biofilm formation

To determine the effects of the oil in inhibiting of biofilm formation, *C. dubliniensis* biofilm formation in wells of microtiter plates was performed as described previously (Taweekhaisupapong et al., 2010). One hundred microliters of the oil (55 mg/ml) was two-fold serially diluted with Sabouraud's dextrose broth in a microtiter plate. An equal volume of *C. dubliniensis* suspension was added and mixed with the oil. *C. dubliniensis* suspension in the broth without the oil served as a positive control and the broth without microorganism served as a negative control. The plates were incubated for 48 h at 37 °C. After biofilm formation, the medium was aspirated, and nonadherent cells were removed by thoroughly washing the biofilms three times in sterile PBS. The effect of the oil in inhibiting of biofilm formation was determined by using the 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium hydroxide (XTT)-reduction assay described below. The effect of nystatin on *Candida* biofilm formation was also determined by the same method as above. Percentage of inhibition of the tested agents was calculated using the formula $[1 - (\text{OD}_{492} \text{ sample} / \text{OD}_{492} \text{ control})] \times 100\%$. All experiments were repeated on three separate occasions, with triplicate determinations on each occasion.

2.6. Antifungal activities of the lemongrass oil against preformed *Candida* biofilm

Antifungal susceptibility testing of sessile cells was performed as described previously (Taweekhaisupapong et al., 2010). *C. dubliniensis* was grown in Sabouraud-dextrose broth for 18 h. Biofilms were formed on commercially available presterilized, polystyrene, flat-bottom 96-well microtiter plates (Corning Inc., Corning, N.Y.) by pipetting standardized cell suspensions ($100 \mu\text{l}$ of the $\sim 1 \times 10^6$ CFU/ml) into selected wells of the microtiter plate and incubating them for 48 h at 37 °C. The wells contained the broth without microorganism served as a negative control. After biofilm formation, the medium was aspirated, and nonadherent cells were removed by thoroughly washing the biofilms three times in sterile PBS. Residual PBS was removed by blotting with paper towels before the addition of the lemongrass oil. The oil was then added to the biofilms in serially double-diluted concentrations

(27.6 to 0.22 mg/ml) and incubated for a further 48 h at 37 °C. A series of the essential oil-free wells was also included to serve as positive control. The effect of the oil against preformed *Candida* biofilm was determined by using the XTT-reduction assay described below.

The effects of nystatin against preformed *Candida* biofilm were also determined by the same method as above. Percentage killing of each agent was calculated using the formula $[1 - (\text{OD}_{492} \text{ sample} / \text{OD}_{492} \text{ control})] \times 100\%$. All experiments were repeated on three separate occasions, with triplicate determinations on each occasion.

2.7. XTT-reduction assay

The XTT solution was prepared by dissolved 0.5 mg/ml of a water soluble tetrazolium salt, XTT (Sigma, St. Louis, USA) and 40 $\mu\text{g/ml}$ of coenzyme Q₀ (Sigma) in PBS and diluted 1:6 with PBS before used. A 100- μl aliquot of the XTT solution was added to each prewashed biofilm and to control wells (for the measurement of background XTT-reduction levels). The plates were incubated in the dark for 2 h at 37 °C. A colorimetric change in the XTT-reduction assay, a direct correlation of the metabolic activity of the biofilm, was measured in a microplate reader (Bio-tek instruments, Inc., Vermont, USA) at 492 nm.

2.8. Effect of the lemongrass oil on candidal adhesion to denture acrylic

Heat-cured denture acrylic sheets were fabricated according to conventional prosthodontic technique. Subsequently, the acrylic sheet formed was cut into 5 × 5 mm square strips, the average thickness of the sheet being 2 mm. These strips were immersed for up to 4 weeks in water to leach the excess monomer then washed in running water for 3 h. Finally, they were sealed and sterilized in petri dishes before used.

For experiment examining the effect of the lemongrass oil and nystatin on candidal adhesion to denture acrylic, the acrylic strips were placed vertically in Eppendorf tubes and 0.4 ml of the yeast suspension (1×10^8 cells/ml) was added to each tube, mixed gently and incubated at 37 °C for 4 h with gentle agitation. After incubation, the acrylic strips were removed from the tubes and washed three times with 20 ml sterile normal saline solution (NSS) to remove non-adherent cells. Then the acrylic strips were placed in new Eppendorf tubes, each with 0.4 ml of the tested agents at concentration that exhibited more than 80% killing activity against *C. dubliniensis* in biofilm. The acrylic strips which were placed in tubes with 0.4 ml of sterile PBS served as a control. The tubes were incubated at 37 °C for 15, 30, 60, 120 min and 24 h. After incubation, the acrylic strips were removed from the tubes and washed with 20 ml sterile NSS. The effect of the tested agents on candidal adhesion was determined by using the XTT-reduction assay described above.

Table 1
Chemical compositions of the lemongrass oil.

Chemical composition	Retention time (min)	Area %	RI	Identification method
6-methyl-5-hepten-2-one	6.77	1.405	NI	MS
Beta-myrcene	6.94	9.470	NI	MS
Beta-ocimene	8.73	0.450	1034	RI, MS
Linalool	11.47	0.725	1000	RI, MS
Ethenyl-cyclohexanone	15.13	1.466	1180	RI, MS
Beta-citral (Neral)	17.79	32.518	1238	RI, MS
Geraniol	18.32	4.362	1250	RI, MS
Alpha-citral (Geranial)	19.16	43.377	1268	RI, MS
Geranyl acetate	24.31	2.154	1378	RI, MS

RI: retention index data generated from a series of n-alkanes (C₁₀–C₂₃).

MS: mass spectrum.

NI: not identified.

2.9. Post antifungal effect assay

The PAFE assay was performed as described previously (Ellepolu and Samaranyake, 1998) with minor modifications. A cell suspension was prepared in sterile PBS at 600 nm to an OD of 1.5. From this cell suspension, 0.5 ml was added to tubes containing 2 ml of RPMI broth (control) and 2 ml of RPMI/tested agents solution (test) in which the tested agents' concentrations varied from 1 to 2 times the MIC. The tubes were then incubated at 37 °C for a period of 1 h in a rotary incubator. Following this limited exposure, the tested agents were removed by two cycles of centrifugation for 10 min at 3000 g. Afterwards the supernatant was completely decanted and the pellets were resuspended in 2.5 ml of sterile PBS. Then aliquots of 100 µl from each cell suspension were added to a microtiter well containing 150 µl of RPMI broth. Then the microtiter plate was placed in a computerized spectrophotometric incubator (Varioskan Flash, Thermo Fisher Scientific, USA) and incubated at 37 °C for 24 h. Growth of yeast cells was automatically monitored by the computerized instrument in terms of the change in the turbidity (absorbance at 595 nm), at 30-min intervals, for a period of 24 h. The duration of PAFE was calculated by using the formula $PAFE = T - C$ (Craig and Gudmundsson, 1996; Lowdin et al., 1993; Scalarone et al., 1991) where T was the time required for the relative OD of the tested agents-exposed cell suspension to reach the 0.05 absorbance level after removal of the tested agents and C was the time required for the relative OD of the tested agents free control cell suspension to reach the same absorbance level. Thus T – C expressed the time in which the antifungal agent was capable of causing growth suppression of the organism following limited exposure to the tested agents (i.e. post antifungal effect).

2.10. Statistical analysis

The effect of the tested agents on candidal adherence was analyzed using Kruskal Wallis with Mann–Whitney U test to evaluate the differences in adhesion between the test and control groups. Bonferroni method was used to adjust for multiple comparisons. P-values <0.05 were considered as statistically significant.

3. Results

The components of lemongrass oil were identified by GC–MS and the results were shown in Table 1. The major constituents found in lemongrass oil were α- and β-citral (75.89%), myrcene (9.47%) and geraniol (4.36%). The MIC of lemongrass oil and nystatin on *C. dubliniensis* by the broth microdilution method were 0.43 mg/ml and 2 µg/ml, respectively, while the MFC were 0.86 mg/ml and 4 µg/ml, respectively. Employing a formazan salt

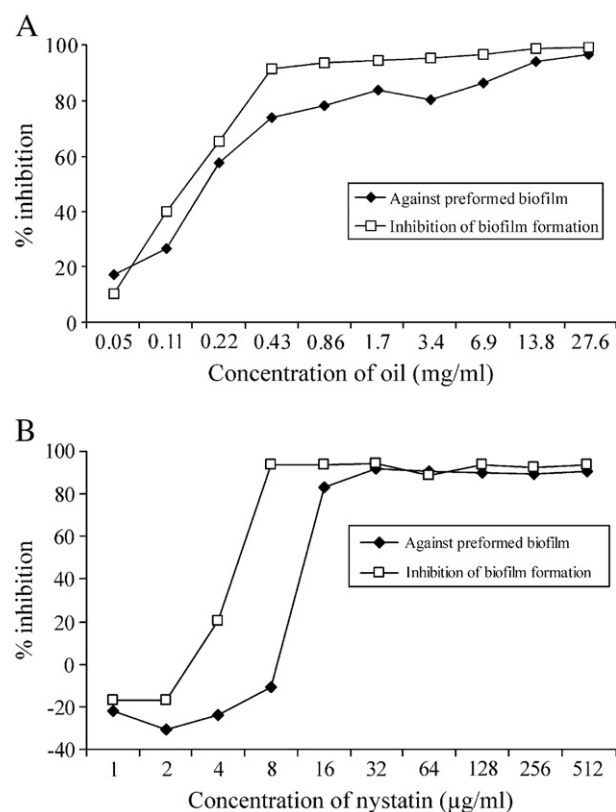


Fig. 1. Effects of different concentrations of lemongrass oil (A) and nystatin (B) on biofilm formation (□) and against preformed biofilm (◆) of *C. dubliniensis*. The presented percentage of inhibition was calculated from the formula $[1 - (OD_{492} \text{ sample} / OD_{492} \text{ control})] \times 100\%$. Results are from three experiments performed in triplicate (n=9).

reduction assay for biofilm study, the results revealed that the inhibitory effect of lemongrass oil and nystatin on biofilm appeared to be dose-related (Fig. 1). The lemongrass oil and nystatin at concentrations between 0.11–27.6 mg/ml and 4–512 μ g/ml exhibited 40–99% and 20–93% inhibition on biofilm formation, respectively, while the same concentrations of both agents showed less active against preformed biofilm of *C. dubliniensis*. The lemongrass oil and nystatin exhibited more than 80% inhibitory effect on biofilm formation at concentration of 0.43 mg/ml and 8 μ g/ml, respectively, while the MIC of lemongrass oil and nystatin against preformed *Candida* biofilm at 80% (SMIC₈₀) were 1.7 mg/ml and 16 μ g/ml, respectively. Therefore the SMIC₈₀ of both agents were selected to test their effects on the adhesion of *C. dubliniensis* to the acrylic strips at various time intervals. Compared with the control, a significant reduction of yeast adhesion to the acrylic strips was observed after exposure to lemongrass oil for 15 min ($P=0.03$), while a significant reduction of yeast adhesion to the acrylic strips was evident after exposure to nystatin for 30 min (Fig. 2).

The in vitro PAFE of the lemongrass oil and nystatin on *C. dubliniensis* after exposure for 1 h at 1, 1.5 and 2 times MIC in RPMI broth is shown in Fig. 3 and Table 2. There was continuous growth suppression following removal of lemongrass oil, which failed to show a growth rate comparable to that of the unexposed control during a 24 h period. In contrast, the PAFE of nystatin which ranged from 1 to 7.5 h was shorter compared to lemongrass oil.

4. Discussion

In this study, the conventional 96-well microtiter plates coupled to a colorimetric method were used to assess the effects of lemongrass oil against biofilm cells. Measurements of cellular mitochondrial dehydrogenase activity using tetrazolium salts have been employed with *Candida* cells for studies of biofilm formation on catheter materials and plastic in several studies (Hawser, 1996; Hawser and Douglas, 1994). This method is rapid, inexpensive, easy to use, accurate, and reproducible methodology for antifungal susceptibility testing of *Candida* biofilms (Ramage et al., 2001).

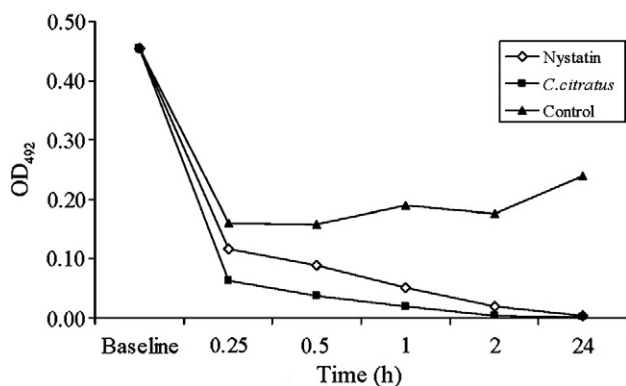


Fig. 2. Adhesion of *C. dubliniensis* to denture acrylic following differential periods of exposure to lemongrass oil and nystatin. Results are from three experiments performed in triplicate ($n=9$).

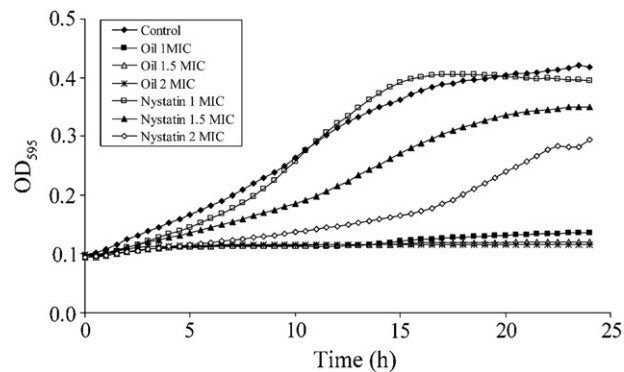


Fig. 3. Growth curves of *C. dubliniensis* after limited exposure to lemongrass oil and nystatin for 1 h. Results are from three experiments performed in triplicate ($n=9$).

The present study demonstrated that lemongrass oil exhibited the strong inhibitory effects against *C. dubliniensis* in both planktonic and biofilms form. However, preformed *Candida* biofilms were found to be more resistant to lemongrass oil than their planktonic cells. The observation in this study is consistent with previous reports that biofilm-associated *Candida* cells are resistant to antifungal agents relative to their planktonic counterparts (Bachmann et al., 2002; Chandra et al., 2001; Shuford et al., 2007). Although adherent populations were not completely eradicated by treatment with lemongrass oil, a >80% reduction in the metabolic activity of adherent cells was detected at concentrations of 1.7 mg/ml. Moreover, it is interesting to observe that lemongrass oil at the MIC values (0.43 mg/ml) can inhibit biofilm formation of *C. dubliniensis*, while the concentration of nystatin that can inhibit biofilm formation was 4×MIC (8 μ g/ml). These results indicated that exposure of *Candida* cells to subcidal concentration of lemongrass oil can reduce the adherence ability of the cells compared with the unexposed controls, whereas the subcidal concentration of nystatin did not exhibit inhibitory effect on biofilm formation. Since adherence represents a major step in biofilm formation, therefore, lemongrass oil might be used to prevent *Candida* biofilm-associated infection.

Antifungal activities of lemongrass oil found in this study are consistent with several reports of lemongrass oil against various fungi (Abe et al., 2003; Cassella and Cassella, 2002; Hammer et al., 1999; Helal et al., 2006; Paranagama et al., 2003; Wannissorn et al., 1996). These activities can be attributed to the presence of various constituents such as citral, limonene, citronellal, β -myrcene, linalool and geraniol (Raubert Cda et al.,

Table 2
Postantifungal effect of lemongrass oil and nystatin on *C. dubliniensis*.

Concentration of tested agent	Postantifungal effect (h)	
	Lemongrass oil	Nystatin
1 MIC	>24	1
1.5 MIC	>24	2
2 MIC	>24	7.5

MIC: minimum growth inhibitory concentration.

2005; Schaneberg and Khan, 2002; Tognolini et al., 2006). It was observed that citral demonstrated inhibitory effects on both mycelial and yeast-form growth of *C. albicans* (Abe et al., 2003). Geraniol was found to inhibit growth of *C. albicans* and possessed antibiofilm activity (Bard et al., 1988; Dalleau et al., 2008). Moreover, several studies have demonstrated that terpenes (i.e. citral, geraniol, linalool, menthol, and thymol) which are the major components of essential oils, alter cell permeability by penetrating between the fatty acyl chains making up the membrane lipid bilayers, disrupting lipid packing and changing membrane fluidity (Bard et al., 1988; Braga and Dal Sasso, 2005). Braga and Dal Sasso (2005) suggested that these phenomena led to major surface alterations and morphological modifications, also reducing the adherence capacity of *C. albicans*. Since *C. dubliniensis* is phenotypically and genotypically close to *C. albicans* (Sullivan et al., 1995), the inhibitory effects on biofilm formation after exposure to lemongrass oil found in this study could be due to those effects of terpenes in the oil.

For PAFE assay in this study, a two washing step was used to reduce antimicrobial concentration of the tested agents because it has been found by previous investigators that removal of 90% of the supernatant with two washings reduces antimicrobial concentration 100-fold, while complete decanting of the supernatant with two washings (as carried out in the current study) reduces the concentration 10,000-fold (Craig and Gudmundsson, 1996). Hence this method virtually eliminates any 'carry-over effect' of the tested agents. Previous workers have evaluated the PAFE of antifungal agents either by using viable counts (Turnidge et al., 1994), or turbidometric measurement of fungal growth (Scalarone et al., 1991). In this study, a turbidometric measurement was used for this purpose albeit the evaluation was greatly facilitated due to an automated technique. The Varioskan Flash machine (Varioskan Flash, Thermo Fisher Scientific, USA) enabled easy, automatic and reliable quantification of yeast growth in terms of increased turbidity as reported for bacteria and fungi in previous studies (Lowdin et al., 1993; Scalarone et al., 1991). The sensitivity of the machine was good as replicate experiments yielded only minimal growth differentials in the tested organisms, and did not yield high standard errors of means (s.e.m.) usually witnessed in conventional techniques relying on CFU determinations (MacKenzie and Gould, 1993).

In the mouth the diluent effect of saliva and the cleansing action of the oral musculature often tend to reduce the availability of the drug below that of the effective therapeutic concentration. Thus the organisms undergo only a limited exposure to the antifungal agents during treatment and the concentration of the drug tends to vary in different niches of the oral cavity. The current observations suggest that limited exposure to lemongrass oil elicits a significantly high PAFE in *C. dubliniensis* even at the MIC value. Therefore, the efficacy of lemongrass oil merits further development of this agent for the therapy of oral candidiasis.

In conclusion, the results in this study demonstrated potent *in vitro* activity in inhibiting biofilm formation and against preformed biofilms of *C. dubliniensis* by lemongrass oil. These effects could modulate candidal colonization thereby

suppressing the invasive potential of the pathogen. Therefore, the efficacy of lemongrass oil merits further investigation of this agent for the therapy of *Candida* biofilm-associated infection.

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