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Short communication

Inhibitory effect of lemongrass oil and its major constituents on *Candida* biofilm and germ tube formation

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Abstract

Adhesion to a variety of host cells and the surface of biomaterials is a critical step in successful colonization and infection by *Candida* spp. Several essential oils are known to possess antifungal properties and are potentially used as antifungal agents. By studying the efficacy of essential oils against different pathogenic fungi in the genus *Candida*, we have evaluated the in vitro antifungal effects of eight essential oils used in aromatherapy, namely holy basil (*Ocimum sactum* L), lemongrass (*Cymbopogon citratus* DC), citronella grass (*Cymbopogon winterianus* Jowitt), kaffir lime (*Citrus hystrix* DC), sweet basil (*Ocimum basilicum* Linn), Plai (*Zingiber cassumunar* Roxb), Curcuma (*Curcuma longa* Linn), and ginger (*Zingiber officinale* Rose), against *Candida albicans* and *Candida krusei* in both planktonic and biofilm form. The results revealed that among the tested essential oils, lemongrass oil exhibited the most effective killing activity and possessed the strongest inhibitory effect on *Candida* biofilm formation. In addition, lemongrass oil and its major constituents can inhibit germ tube formation, which might affect adherence. The data in this study indicates that lemongrass oil possessed antibiofilm activity and could modulate candidal colonization. Therefore, it is a promising essential oil to combat candidal colonization and infection.

Keywords: Antifungal activity; Biofilm; Broth microdilution; *Candida*; Essential oils

1. Introduction

Candida spp. are the principle etiological agent of nosocomial fungal infections, with *Candida albicans* being the most common species. 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; Ramirez-Amador et al., 2003; Singh et al., 2002). 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 (Ramage et al., 2006). In addition, there are many topical devices at risk, including contact lenses and dentures. Different strains of *C. albicans* and different *Candida* spp. differ in their capacities to form biofilm (Kuhn et al., 2002). Formation of *Candida* biofilm has important clinical repercussions because of their increased resistance to antifungal therapy and the ability of cells within biofilm to withstand host immune defenses (Douglas, 2003; Ramage et al., 2006).

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Recently, interest in medicinal plants and their extracts as a new source of antimicrobial agents has grown dramatically. Essential oils have a wide application in folk medicine, food flavoring and preservation as well as in fragrance industries. In recent years, a large number of essential oils and their constituents have been investigated for their antimicrobial properties against some bacteria and fungi in more than 500 reports (Kalemba and Kunicka, 2003). However, little is known about the spectrum of action of essential oils against *Candida* biofilms. Recently, *Cymbopogon citratus* (lemongrass) oil was reported to exhibit effective killing activity and possessed a strong inhibitory effect on *Candida dubliniensis* biofilms (Taweechaisupapong et al., 2012). Beside *C. dubliniensis*, more than 17 different species of *Candida* are reported to cause invasive candidiasis in humans, with *C. albicans* and *Candida krusei* being the top five species (Lim et al., 2012). Therefore, in the present study we further investigated the antifungal and antibiofilm activities of the *C. citratus* oil against *C. albicans* and *C. krusei* and compared its activities with another seven essential oils used in aromatherapy, namely holy basil (*Ocimum sactum* L), citronella grass (*Cymbopogon winterianus* Jowitt), kaffir lime (*Citrus hystrix* DC), sweet basil (*Ocimum basilicum* Linn), Plai (*Zingiber cassumunar* Roxb), Curcuma (*Curcuma longa* Linn), and ginger (*Zingiber officinale* Rose).

2. Materials and methods

2.1. Essential oils

Eight essential oils were purchased from Thai China Flavours & Fragrances Industry Co. (Thailand). They were dissolved in 95% ethanol to an initial concentration of 900 μl/ml and further diluted with a solution containing 5% ethanol and 5% Tween 80 to a concentration of 64 μl/ml.

2.2. Chemical analyses of the essential oils

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 × 0.25 mm i.d.) coated with 0.25 μm film 5%phenyl–95%dimethylpolysiloxane was used for separation. The GC injector was maintained at 250 °C, the GC transfer line at 250 °C, and the ion source at 220 °C. The column temperature started with 70 °C hold for 1 min, then increased the temperature at the rate of 6 °C/min to 280 °C hold for 3 min. Manual injection (1 μl) in a purged split mode (1:100) was conducted and high-purity helium was used as carrier gas at a 1 ml/min flow rate. The scan range was 35–550 m/z and the scan rate was 1000 amu/s. The volume injected was 1 μl of 1% solution (diluted in hexane). The identification of the components was based on a 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 (C_{10}–C_{23}) and comparison with literature data (Aiemsaard et al., 2010; Babushok and Zenkevich, 2009).

2.3. Microorganisms

C. albicans and *C. krusei* from clinical isolates were maintained on Sabouraud-dextrose agar (BBL Microbiology Systems, Cockeysville, MD) and grown in the yeast phase in Sabouraud-dextrose broth (Pronadisa, Hispanlab, South Africa) for 18 h. The organisms were adjusted with Sabouraud-dextrose broth to give a final absorbance at 600 nm = 0.1 (~1×10^6 CFU/ml).

2.4. Determination of antifungal activities of the essential oils and some major constituents

Antifungal activities of the eight essential oils towards *C. albicans* and *C. krusei* were determined by the broth dilution method (NCCLS, 2002). Briefly, 50 μl of the essential oils (64 μl/ml) was two-fold serially diluted with Sabouraud's dextrose broth in a microtiter plate. An equal volume of the *Candida* suspension was added and mixed with the oils. The plates were incubated for 24 h, at 37 °C. The *Candida* growth was examined by eyes and the lowest concentration of the oils which inhibited the visible growth of the *Candida* was recorded as the minimum growth inhibitory concentration (MIC). The positive growth of each microorganism cultured in the broth without oils served as a positive control and the negative growth found in the mixture of broth and oils without microorganism served as a negative control.

Aliquots of the mixture of oils and the *Candida* suspension which showed negative-visible growth after the first 24 h of incubation, were inoculated onto the surface of Sabouraud's dextrose agar. The lowest concentration of the oils giving negative growth of the *Candida* was recorded as the minimum fungicidal concentration (MFC). Antifungal activities of some major constituents of the most effective essential oil were also determined by the same method as above. All experiments were repeated on three separate occasions, with triplicate determinations on each occasion.

2.5. Inhibitory effects of the essential oils and selected major constituents on Candida biofilm formation

To determine the effects of the essential oils in inhibition of biofilm formation, the *Candida* biofilm formation in wells of microtiter plates was performed as described previously (Taweechaisupapong et al., 2010). Briefly, 100 μl of the essential oils (64 μl/ml) was two-fold serially diluted with Sabouraud's dextrose broth in a microtiter plate. An equal volume of the *Candida* suspension (~1×10^6 cells/ml) was added and mixed with the oils. The plates were incubated for 48 h at 37 °C. After biofilm formation, the medium was aspirated, and non-adherent cells were removed by thoroughly washing the biofilm three times in sterile phosphate buffered saline (PBS). A series of the essential oil-free wells and biofilm-free wells was also included to serve as positive and negative controls, respectively. The effect of each essential oil in inhibition of biofilm formation was determined by using the 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2*H*-tetrazolium hydroxide (XTT)-reduction assay described below.

The effects of some major constituents of the most effective essential oil in inhibition of biofilm formation were also determined by the same method as above. Percentage of inhibition of each agent was calculated using the formula [1 − (OD_{492} sample/OD_{492} control)] × 100%. All experiments were repeated on three separate occasions, with triplicate determinations on each occasion.

2.6. Antifungal activities of the essential oils and selected major constituents against preformed Candida biofilms

Antifungal susceptibility testing of sessile cells was performed as described previously (Taweechaisupapong et al., 2010). Briefly, *Candida* isolates were 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, NY) by pipetting standardized cell suspensions (100 μl of the ~1×10^6 cells/ml) into selected wells of the microtiter plate and incubating them for 48 h at 37 °C. After biofilm formation, the medium was aspirated, and non-adherent cells were removed by thoroughly washing the biofilm three times in sterile PBS. Residual PBS was removed by blotting with paper towels before the addition of the essential oils. The essential oils were then added to the biofilms in serially double-diluted concentrations (32 to 0.25 μl/ml) and incubated for a further 48 h at 37 °C. A series of wells free of the essential oils and biofilms, respectively, were also included to serve as positive and negative controls. The effect of each essential oil against preformed *Candida* biofilm was determined by using the XTT-reduction assay described below.

The effects of some major constituents of the most effective essential oil against preformed *Candida* biofilm were also determined by the same method as above. Percentage killing of each agent was calculated using the formula [1 − (OD_{492} sample/OD_{492} control)] × 100%. All experiments were repeated on three separate occasions, with triplicate determinations on each occasion.

2.7. XTT-reduction assay

The XTT stock solution was prepared by dissolving 0.5 mg/ml of XTT (Sigma, St. Louis, USA) and 40 μg/ml of coenzyme Q_0 (Sigma) in PBS. To prepare working solution, the XTT stock solution was diluted in PBS at a ratio of 1:6 before use. A 100-μl aliquot of the XTT solution was added to each pre-washed biofilm and to control wells. The plates were incubated in the dark for 2 h at 37 °C. A colorimetric change in the XTT-reduction assay was measured in a microplate reader (BioTek instruments, Inc., Vermont, USA) at 492 nm.

2.8. Determination of effect of lemongrass oil and its major constituents on germ tube formation

The concentrations of lemongrass oil and its major constituents were selected based on the concentration of these agents that exhibited more than 80% inhibition on biofilm formation in the previous tests. The 100 μl aliquots of the yeast suspension (OD_{600} = 0.1) was added to 100 μl of each agent reaching a final concentration of 1, 0.5, 0.25 and 32 μl/ml for lemongrass oil, citral, geraniol, myrcene and geranyl acetate, respectively. The tubes containing yeast suspension in PBS served as the controls. Then the tubes were mixed gently and incubated at 37 °C for 15 min with gentle agitation. Following this limited exposure, all agents were removed by two cycles of dilution (with sterile PBS) and centrifuged for 10 min at 3000 × *g*. The supernatant was completely decanted and the pellets re-suspended in PBS to give a final absorbance at 600 nm = 0.1 for germ tube induction assay. The yeast suspension (50 μl) was added to 200 μl of human serum (obtained from the Blood Bank, Faculty of Medicine, Khon Kaen University) and incubated at 37 °C for 90 min. Then the cells were washed three times and resuspended in PBS. The tube was vortexed for 10 s and a drop of each cell suspension placed on a haemocytometer chamber for quantification of the germ tubes. Thereafter, 300 yeast cells in contiguous fields were counted and the percentage of germ tube forming cells was calculated as previously described (Taweechaisupapong et al., 2005). The counting criteria comprised: 1) yeast cells with a germ tube, without any constriction at the junction between the cell and the elongation; 2) clumped cells with germ tubes were excluded; and 3) pseudohyphae-forming yeast cells were excluded. The experiments were repeated on two separate occasions with quadruplicate determinations on each occasion. The effects of lemongrass oil and its major constituents on germ tube formation were analyzed using Student's *t*-test to evaluate the differences between the test and control groups. The level required for statistical significance was $P < 0.05$.

3. Results

According to the chemical components of the tested essential oils, they are characterized by a few major components at practically high concentrations (Table 1). Methyl eugenol, geranial, citronellal, limonene, methyl chavicol, α-phellandrene, tumerone and zingiberene were the highest percentage composition of *O. sanctum*, lemongrass, *C. winterianus*, *C. hystrix*, *O. basilicum*, *Z. cassumunar*, *C. longa* and *Z. officinale* oil, respectively.

Determination of the MIC and MFC of the eight essential oils by the broth microdilution method revealed that lemongrass oil exhibited the lowest MIC and MFC, followed by *C. winterianus* oil (Table 2). The MIC and MFC of lemongrass oil against both *Candida* isolates were 0.5 and 1 μl/ml (or 0.4 and 0.8 mg/ml), respectively. The essential oils from *Z. officinale* and *C. longa* were inactive against both isolates at the tested concentrations (0.06–32 μl/ml). Among the major constituents of lemongrass oil, geraniol exhibited the lowest MIC against *C. albicans*, while myrcene and geranyl acetate were inactive against both isolates at the tested concentrations (0.06–32 μl/ml).

Employing a formazan salt reduction assay for biofilm study, the results revealed that the inhibitory effect of the essential oils on biofilm formation and against preformed *Candida* biofilm appeared to be dose-related (Figs. 1 and 2). Among the eight essential oils, lemongrass oil showed the strongest inhibitory effect on biofilm formation of both *Candida* isolates. It exhibited

Table 1
Percentage composition of the eight essential oils.

Component	95% RI range	RI	Area %							
			1	2	3	4	5	6	7	8
Sabinene	957–979	–				15.21	6.7			
Myrcene	973–993	–		10.3		1.5				
α-Phellandrene	989–1013	1008				0.9		34.2	4.4	
α-Terpinene	1000–1025	1020				5.6		3.9		
β-Phellandrene	1005–1045	1027							0.8	
1-Methyl-3-(1-methylethyl)-benzene	1023 (Le Quéré and Latrasse, 1990)	1028						2.6		
Limonene	1009–1029	1033				24.8				
1,8-Cineole	1005–1039	1035					1.7		3.0	
α-Terpinolene	1075–1083	1062				6.3		7.7		
Linalool	1083–1089	1076				1.6				
Terpinolene	1064–1092	1092				5.15		1.5	6.3	
Isopulegol	1150 (Ho et al., 2008)	1151				2.5				
Camphor	1095–1142	1153					1.0			
Citronellal	1156 (Qiao et al., 2008)	1156			31.0					
Borneol	1134–1180	1173	0.9							
Terpinen-4-ol	1147–1178	1184				9.5		26.2		
Linalyl propionate	1184 (Meshkatalsadat et al., 2010)	1197				10.8				
Methyl chavicol	1203 (Sajjadi, 2006)	1206					78.2			
Citronellol	1203–1229	1230			12.2					
Neral (Qiao et al., 2008)	1244	1247		33.7						
Geraniol	1228–1258	1258		4.6	19.5					
Geranial	1232–1267	1277		40.6						
Carvacrol	1271–1301	1300	6.7							
Citronellyl acetate	1354 (Qiao et al., 2008)	1355			4.0					
Eugenol	1320–1381	1364	16.7		1.2					
Geranyl acetate	1344–1385	1386		2.2	4.3					
β-Elemene	1370–1404	1399			2.9		1.3			1.0
Methyl eugenol	1404 (Ho et al., 2008)	1410	37.7							
Trans-caryophyllene	1397–1449	1425	26.1				1.5		1.9	
Trans-α-bergamotene	1435 (Ho et al., 2008)	1435					3.0			0.5
β-Farnesene	1435–1488	1443								1.5
α-Humulene	1454 (Kiran Babu et al., 2007)	1454	1.5							
Germacrece D	1457–1493	1477	2.3				1.1			0.7
AR-curcumene	1481 (Ho et al., 2008)	1480								17.27
Zingiberene	1494 (Ho et al., 2008)	1485							7.0	30.4
α-Muurolene	1473–1506	1495			1.0					
β-Bisabolene	1506 (Ho et al., 2008)	1496							1.2	
γ-Elemene	1494 (Natta et al., 2008)	1498	0.9							
α-Amorphene	1482 (Djarri et al., 2008)	1505			0.7					
α-Farnesene	1479–1518	1510								14.6
β-Sesquiphellandrene	1523 (Ho et al., 2008)	1513						2.4	6.9	15.3
γ-Cadinene	1480–1526	1515			3.2	1.6				
Hedycaryol	1559 (Krauze-Baranowska et al., 2002)	1548			3.7					
Elemol	1550 (Ho et al., 2008)	1560								0.7
Cis-nerolidol	1563 (Ho et al., 2008)	1563								0.8
Epi-α-cadinol	1605–1643	1606			0.8					
(E and Z)-1-(3,4-Dimethoxyphenyl)butadiene (DMPBD)	1662 (Sabulal et al., 2007)	1639						9.7		
α-Cadinol	1618–1669	1652					1.2			
Dihydro-cis-α-copaene-8-ol	1608 (Ivanovic et al., 2010)	1667			1.4					
Tumerone	1643 (Natta et al., 2008)	1681							42.0	
α-Bisabolol	1690 (Sivasothy et al., 2011)	1686								0.6
Curlone (Natta et al., 2008)	1645 (Natta et al., 2008)	1713							11.6	
Cis-farnisol	1717 (Sivasothy et al., 2011)	1715								1.58
Others			8.1	8.6	14.1	14.54	4.3	11.8	14.9	15.05

RI, retention index data generated from a series of alkanes (C_{10}–C_{23}); 1, *Ocimum sactum* L; 2, *Cymbopogon citratus* DC; 3, *Cymbopogon winterianus* Jowitt; 4, *Citrus hystrix* DC; 5, *Ocimum basilicum* Linn; 6, *Zingiber cassumunar* Roxb; 7, *Curcuma longa* Linn; 8, *Zingiber officinale* Rose.

more than 90% inhibition on biofilm formation of *C. albicans* and *C. krusei* at concentrations of 1 and 0.5 μl/ml (or 0.8 and 0.4 mg/ml), respectively (Fig. 1A and C). In addition, lemongrass oil at concentrations less than the MIC values against both isolates (0.06–0.25 μl/ml) also possessed inhibitory effect on biofilm formation. The average percentage inhibition of lemongrass oil (0.06–0.25 μl/ml) on *C. albicans* and *C. krusei* biofilm formation ranged from 16 to 41% and 46–79%, respectively. For its major

Table 2
Minimum growth inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the eight essential oils and selected major constituents against *Candida albicans* and *Candida krusei* (n=9).

Tested agent	Common name	MIC (μl/ml)		MFC (μl/ml)	
		C. albicans	*C. krusei*	*C. albicans*	*C. krusei*
Ocimum sanctum Linn	Holy basil	4	2	32	16
Cymbopogon citratus DC	Lemongrass	0.5	0.5	1	1
Cymbopogon winterianus Jowitt	Citronella oil	1	1	2	2
Citrus hystrix DC	Kaffir lime	4	4	8	8
Ocimum basilicum Linn	Sweet basil	32	8	>32	32
Zingiber cassumunar Roxb	Plai	4	4	32	16
Curcuma longa Linn	Curcuma	>32	>32	>32	>32
Zingiber officinale Rose	Ginger	>32	>32	>32	>32
Citral		0.5	0.5	1	0.5
Geraniol		0.25	0.5	0.5	0.5
Myrcene		>32	>32	>32	>32
Geranyl acetate		>32	>32	>32	>32

constituents, citral and geraniol exhibited a strong inhibitory effect on biofilm formation of both isolates, while myrcene and geranyl acetate exhibited lower inhibitory activities (Fig. 1B and D). The effect on biofilm formation of myrcene (0.06–32 μl/ml) never reached 50% inhibition in both *Candida* isolates. In contrast, citral at a concentration of 0.25 μl/ml (0.2 mg/ml), which was less than the MIC values against *C. albicans* and *C. krusei*, showed 61% and 90% inhibition on biofilm formation, respectively.

Antibiofilm activities of the essential oils are shown in Fig. 2. Among the eight essential oils, lemongrass oil showed again the strongest antibiofilm activities against both isolates (Fig. 2A and C). It exhibited more than 80% killing activity against *C. albicans* and *C. krusei* in biofilms at concentrations of 2 and 4 μl/ml (or 1.7 and 3.4 mg/ml), respectively. However, these concentrations were higher than the planktonic MIC. Among its major constituents, citral and geraniol demonstrated higher killing activities than myrcene and geranyl acetate against both isolates (Fig. 2B and D).

The effects of lemongrass oil and its major constituents in inhibition of the germ tube formation in *C. albicans* are presented in Table 3 and Fig. 3. All tested agents were able to inhibit germ tube formation and reduced germ tube length. The suppression of germ tube formation was significant in comparison with that of the unexposed control ($P<0.01$).

4. Discussion

In this study, the GC–MS was used just to assess the composition (as main constituents) of the tested oils as semi-quantitative results. Our data demonstrated that among the tested essential oils, lemongrass oil exhibited the strongest inhibitory effects against *C. albicans* and *C. krusei* in both planktonic and biofilm form. Although lemongrass oil possessed the same MIC and MFC on planktonic cells of *C. albicans* and *C. krusei* (Table 2), the inhibitory effect of lemongrass oil on biofilm formation of *C. krusei* was stronger than that of *C. albicans*. However, preformed *C. krusei* biofilms were found to be more

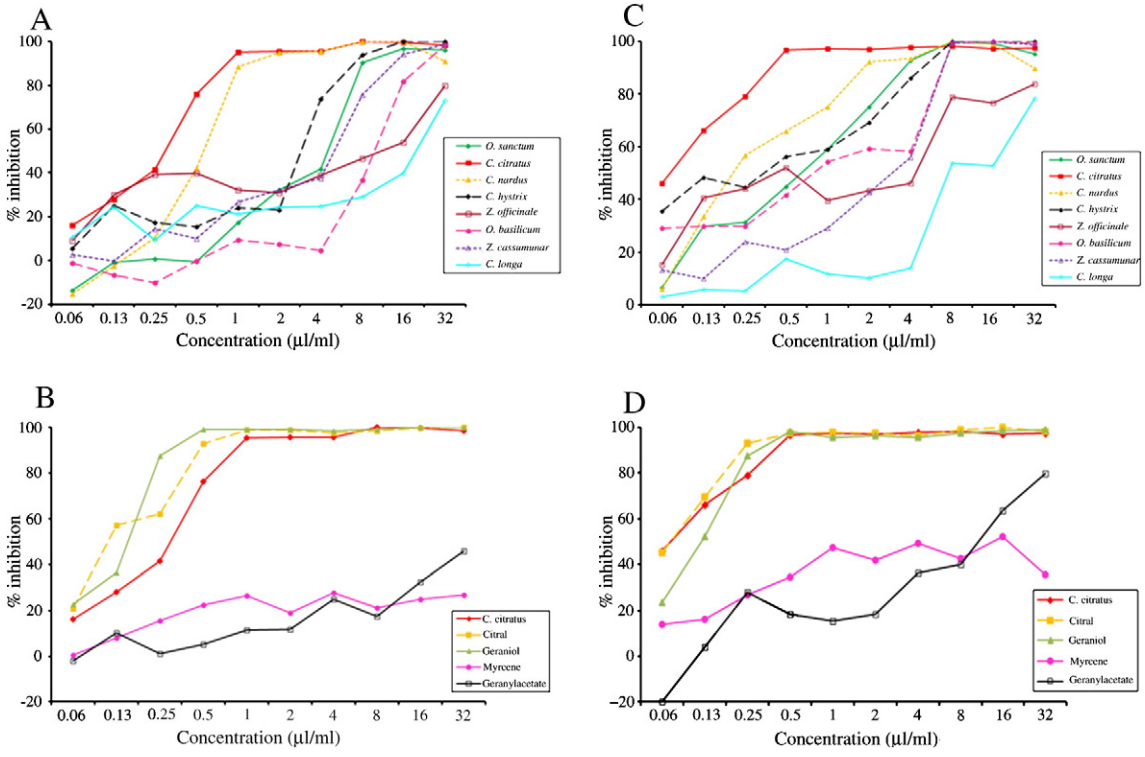

Fig. 1. Activities of the eight essential oils and selected major constituents at different concentrations in inhibition of *Candida albicans* (A–B) and *Candida krusei* (C–D) biofilm formation. Percentage of inhibition was calculated using the formula $[1-(OD_{492}\ \text{sample}/OD_{492}\ \text{control})]\times 100\%$. Results are from three experiments performed in triplicate.

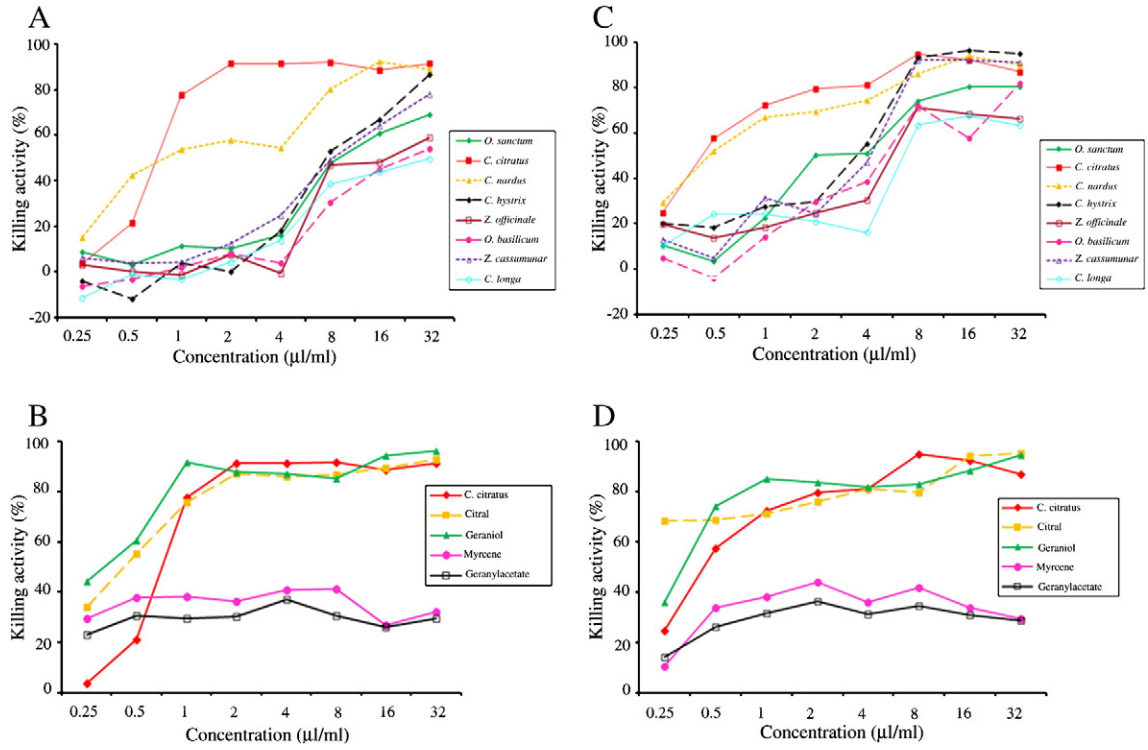

Fig. 2. Activities of the eight essential oils and selected major constituents at different concentrations against preformed biofilms of *Candida albicans* (A–B) and *Candida krusei* (C–D). Percentage killing was calculated using the formula $[1-(OD_{492}$ sample$/OD_{492}$ control$)]\times 100\%$. Results are from three experiments performed in triplicate.

resistant to lemongrass oil than those of *C. albicans* and *C. dubliniensis* from our previous study (Taweechaisupapong et al., 2012). These results indicated that antibiofilm activities of lemongrass oil depended on its concentration and the fungal species.

Similar to our previous observations toward *C. dubliniensis* (Taweechaisupapong et al., 2012), preformed biofilms of *C. albicans* and *C. krusei* 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; Shuford et al., 2007). Although adherent populations were not completely eradicated by treatment with lemongrass oil, a >90% reduction in the metabolic activity of adherent cells was detected at concentrations of 2 and 8 µl/ml against *C. albicans* and *C. krusei*, respectively. Moreover, it is interesting to observe that lemongrass oil and its major constituents at concentrations less than the MIC values can inhibit biofilm formation of both isolates. These results indicated that exposure of *Candida* cells to sub-inhibitory concentration of these agents can reduce the adherence ability of the cells compared with the unexposed controls. Since adherence represents a major step in biofilm formation, therefore, these agents might be used to prevent *Candida* biofilm-associated infection.

Antifungal activities of lemongrass oil against various fungi have been reported (Abe et al., 2003; Cassella and Cassella, 2002; Helal et al., 2006; Paranagama et al., 2003). These activities can be attributed to the presence of various constituents such as citral, limonene, citronellal, β-myrcene, linalool and geraniol (Rauber Cda et al., 2005; Schaneberg and Khan, 2002). 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). Our results demonstrated the strong antifungal activity of lemongrass oil, citral and geraniol, in agreement with observations made by other authors (Abe et al., 2003; Bard et al., 1988; Dalleau et al., 2008; Silva Cde et al., 2008). However, inhibitory effects on biofilm formation, antibiofilm activity and the effects on germ tube formation of myrcene and geranyl acetate have never been reported.

The present investigation showed that *C. albicans* cells treated with lemongrass oil and its major constituents had a reduced ability for germ tube formation. The inhibition of germ tube formation by lemongrass oil and its major constituents is important, since it is well known that germ tube and mycelial forms of *C. albicans* adhere more efficiently to host cells than do yeast form cells and they are a pathogenic factor of the yeast (Kimura and Pearsall, 1980; Pendrak and Klotz, 1995). The observed suppression of germ tube formation elicited by lemongrass oil and its major constituents may be related to the effect of these agents on the *Candida* cell wall. Others 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

Table 3
Effect of *Cymbopogon citratus* oil and its major constituents on germ tube formation of *Candida albicans* after incubation with the tested agents for 15 min.

Tested agent	Concentration of tested agents (μl/ml)	Number of germ tubes formed/300 yeasts (mean±SD) (n=8)	% inhibition
C. citratus oil	1	40.3±6.9 *	84.35
Citral	0.5	54.5±16.8 *	78.81
Geraniol	0.25	86.3±33.6 *	66.45
Myrcene	32	130.6±91.0 *	49.22
Geranyl acetate	32	1.3±1.2 *	99.51
Control (PBS)	–	257.3±21.5	–

* $P<0.01$ compared with control.

(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*. Therefore, the inhibitory effects of lemongrass oil on biofilm formation found in this study could be due to the activities of those constituents.

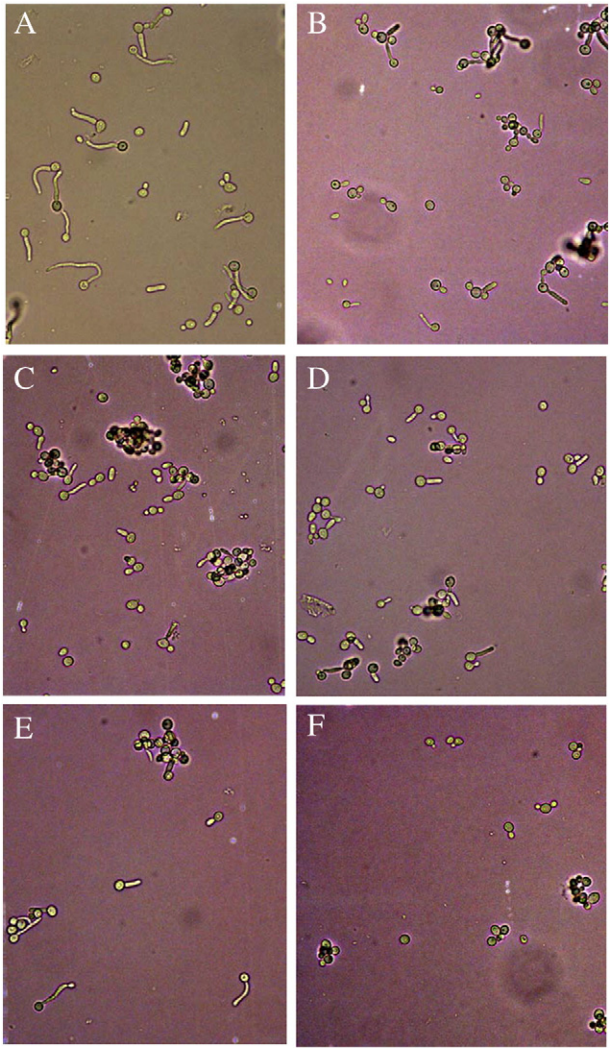

Fig. 3. Illustration of (A) control; (B) lemongrass oil; (C) citral; (D) geraniol; (E) myrcene and (F) geranyl acetate on germ tube formation of *Candida albicans* after incubation with the tested agents for 15 min (400× magnification).

In conclusion, our results demonstrated that lemongrass oil possessed potent in vitro activity in inhibiting biofilm formation and against preformed biofilms of both *C. albicans* and *C. krusei*. These effects could modulate candidal colonization, thereby suppressing the invasive potential of the pathogen. Therefore, the efficacy of lemongrass oil merits further investigation for the therapy of *Candida* biofilm-associated infection.

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