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Original Article

The antifungal efficacy of essential oils in combination with chlorhexidine against *Candida* spp.

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Abstract

This study determined the chemical components of three essential oils (cinnamon oil, clove oil and lemongrass oil) by gas chromatography and mass spectroscopy. The *in vitro* antifungal activity of chlorhexidine (CHX) combined with essential oils was then assessed against planktonic *Candida albicans* ATCC10231, *Candida krusei* (STCK 1) and *Candida tropicalis* (STCT 1) and *C. albicans* biofilms using broth microdilution and chequerboard assays. The results demonstrated that CHX combined with either clove oil, cinnamon oil or lemongrass oil exhibited synergistic effect against planktonic *C. albicans* at FICI of 0.500, 0.375 and 0.312, respectively. Additive effects were recorded for combinations tested against *C. tropicalis* and *C. krusei*. Synergistic effects were observed for clove or cinnamon oil combined with CHX (FICI 0.500 and 0.375, respectively) against sessile *C. albicans* in biofilm**,** whereas the combinations of lemongrass oil and CHX showed only additive effect (FICI 1.062). In conclusion, the combination of CHX with either clove oil or cinnamon oil may prove useful as an alternative antifungal treatment for oral *Candida* spp.

Keywords: clove oil, cinnamon oil, lemongrass oil, oral *Candida* biofilm, chlorhexidine

1. Introduction

Oral candidiasis is an opportunistic infection in the oral cavity caused by overgrowth of *Candida* spp. The major causative organism of this disease is *Candida albicans* (*C. albicans*), but *Candida tropicalis* (*C. tropicalis*), *Candida glabrata* (*C. glabrata*), *Candida krusei* (*C. krusei*), and *Candida parapsilosis* (*C. parapsilosis*) are also found in oral candidiasis patients (Akpan & Morgan, 2002). Oral candidiasis can be hard to eliminate due to the ability of *Candida* spp. to form biofilms that increase resistance to antifungal agents and protect against the microorganism from host immune defences (Ramage, Walle, Wickes, & López–Ribot, 2001; Thein, Samaranayake, & Samaranayake, 2006). Biofilms are a community structure of microorganisms embedded in a matrix of extracellular polymeric substances (EPS) produced by the microorganism (Baillie & Douglas, 1998). This biofilm matrix consists of negatively charged exopolysaccharides, protein, nucleic acids and other components that tightly bind the microorganisms to the surface of biotic and abiotic materials (Baillie & Douglas, 2000; Douglas, 2003; Kumamoto, 2002; Seneviratne, Jin, & Samaranayake, 2008). Chlorhexidine (CHX) is a broad spectrum antimicrobial agent commonly used to inhibit fungi and biofilm formation in dental products. However, CHX has some side effects such as tooth staining, bitter taste and burning sensation (Filoche, Soma, & Sissons, 2005; Shim, Yim, Chung, & Hong, 2012). Therefore, there is a lot of interest in developing natural substances as alternative antimicrobial therapies and essential

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oils from various plants have been proven to have antifungal and antibacterial activity in the oral cavity (Chaieb *et al.*, 2007; Gupta, Kumari, Garg, Catanzaro, & Marotta, 2011; Prabuseenivasan, Jayakumar, & Ignacimuthu, 2006; Saeed & Tariq, 2008; Silva, Guterres, Weisheimer, & Schapoval, 2008; Taweechaisupapong, Aieamsaard, Chitropas, & Khunkitti, 2012a).

Previous studies have demonstrated that combining essential oils with CHX reduced the amount of CHX required to inhibit biofilm cultures of *Streptococcus mutans*, *Lactobacillus plantarum* and *Staphylococcus epidermidis* by 4 – 16 fold (Filoche *et al*., 2005; Karpanen, Worthington, Hendry, Conway, & Lambert, 2008). Moreover, some combinations of CHX with phytocompounds have exhibited synergistic effects against *C. albicans* biofilm (Filoche *et al*., 2005; Khan & Ahmad, 2012). Thus, combining essential oils with CHX can lower the amount of CHX required to treat an infection and reduce side effects. Essential oils are a complex mixture of compounds and each essential oil is composed of different chemical components. Furthermore, the components of essential oils from the same species can vary due to changes in geographical sources and harvesting periods. Therefore, determining the major chemical components present in a tested essential oil is an important part of quality assurance.

The purpose of this study was to determine the chemical components and the antifungal activity of three essential oils: lemongrass (*Cymbopogon citratus* (DC) Stapf), clove (*Eugenia caryophyllata* L. Myrtaceae) and cinnamon (*Cinnamomum zeylanicum* L.). The anti-fungal activity of these essential oils was assessed alone and in combination with CHX against planktonic *Candida* spp. and sessile *C. albicans* biofilms.

2. Materials and Methods

2.1. Preparation of essential oil and chlorhexidine solutions

Essential oils (Thai China Flavours and Fragrances Industry Co.,Thailand), were dissolved in 95% ethanol (100 µl/ml) and then diluted in broth with a solubilizing solution containing 5% ethanol and 5% Tween 80® in distilled water to a concentration of 32 µl/ml. It was validated that this essential oil solubilizing solution had no effect on fungal growth in agreement with the study of Taweechaisupapong *et al*. (2012a). CHX (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water to a final concentration of 10 mg/ml and diluted to 1 mg/ml with broth before use in the experiments.

2.2. Chemical components of essential oils using gas chromatography-mass spectroscopy (GC/MS)

Chemical components of the three essential oils were analysed by gas chromatography (Model CN 10402086, Agilent, China) coupled with mass spectrometry (Model US 35120381, Agilent, USA). A DB-5ms capillary column (0.25 µm film thickness, 30 m × 0.25 mm) was used for separation. The essential oil (10 µl/ml) was diluted with dichloromethane. The injection temperature program was started at 70 ºC and held for 5 minutes, raised to 120 ºC (rate 3 ºC/min), then increased to 270 ºC at the rate of 5 ºC/min. Helium was the carrier gas (flow rate 1.0 ml/min). Injection volume was 1 µl in split mode (100:1). The scan rate and the scan range were 1388.2 amu/s and 35 – 550 amu, respectively. The components of essential oils were identified by comparison of mass spectra with MSD ChemStation software based on the Wiley 7nl. MS Search library and identify was confirmed with linear retention indices (LRI) related to C_{10}-C_{23} n-alkanes compared with authentic compounds (Taweechaisupapong *et al.*, 2012a).

2.3. Yeast strains

C. albicans ATCC10231, was obtained from the culture collection of the Faculty of Dentistry, Khon Kaen University. *C. krusei* (STCK 1) and *C. tropicalis* (STCT 1) were clinical isolates. All strains were reconstituted from lyophilized stock and maintained on sabourad dextrose agar (Becton, Dickinson and Company, Sparks, MD, USA). One colony of yeast was re-suspended in sabouraud dextrose broth (Becton, Dickinson and Company, Sparks, MD, USA) and incubated overnight at 37 ºC. The optical density of overnight cultures was adjusted to OD 0.1 at 600 nm (10^6 cfu/ml) with sabouraud dextrose broth (Taweechaisupapong *et al*., 2012a).

2.4. *In vitro* susceptibility test by broth microdilution assay

Essential oil and CHX solutions were serially two-fold diluted with sabouraud dextrose broth in microtiter plates. The final concentration in each well was in the range of 16 - 0.0078 µl/ml for essential oils and 500 – 0.005 µg/ml for CHX. A 50 µl inoculum of the *Candida* suspension (10^6 cfu/ml) was added and incubated at 37 °C for 24 hours. The lowest concentration of essential oil that inhibited the visible growth of *Candida* spp. was recorded as the minimum inhibitory concentration (MIC). Ten microliter aliquots of the wells without visual turbidity were inoculated onto sabouraud dextrose agar plates and incubated at 37 °C for 24 hours. The minimum fungicidal concentration (MFC) was defined as the lowest concentration of the tested agent that showed no growth after incubation (Taweechaisupapong *et al*., 2012a). Microorganism in broth without tested agents and sabouraud dextrose broth without microorganism were used as a positive control and a negative control, respectively. The experiments were performed in triplicate.

2.5. *In vitro* combination antimicrobial effect of three essential oils and CHX against planktonic cells by chequerboard method

This study was evaluated according to the chequerboard method of Jain *et al*. (2011). The essential oils were serially diluted two-fold along the columns of a microtiter plate (25 µl per well at final concentrations in a range of 2 - 0.0018 µl/ml). Serial two-fold dilutions of CHX in broth were prepared separately and added to the rows (25 µl per well, final concentration 31.25 – 0.039 µg/ml). A 50 µl inoculums of the yeast suspension (10^6 cfu/ml) was added and mixed with the combination agents before incubating at 37°C for 24 hours. The lowest concentration of the oil/CHX combination that inhibited the visible growth of *Candida* spp.

was recorded as the MIC. The microorganism in broth without tested agents was the positive control and sabouraud dextrose broth without microorganism was the negative control.

Interpretation of synergy or inhibition was categorized from the fractional inhibitory concentration index (FICI) which was derived from the summation of the FICs for each agent as follows:

FIC_{EO} = MIC of EO in combination / MIC of EO alone
FIC_{CHX} = MIC of CHX in combination / MIC of CHX alone
FICI = FIC_{EO}+ FIC_{CHX}

If the FICI was less than or equal to 0.5, the interaction was defined as synergistic; additive if the FICI was more than 0.5 but less than or equal to 4; and antagonistic was indicated when the FICI was more than 4 (Karpanen *et al*., 2008).

2.6. *In vitro* antifungal effects of essential oil combined with CHX on preformed *C. albicans* biofilm

The effect of essential oil combined with CHX on sessile *C. albicans* in biofilm was evaluated using the XTT – reduction assay (Taweechaisupapong *et al*., 2012a). A 100 µl suspension of *C. albicans* (10^6 cfu/ml) was added into a flat-bottomed microtiter plate and incubated at 37 °C for 48 hours. After biofilm formation, the medium was aspirated and non-adherent cells were removed by washing with sterile PBS. The biofilm was exposed to the combination agent (essential oil and CHX) accordingly; 100 µl of combination mixture was added to the biofilm in serially double-diluted concentrations at the final concentration of oil in the range of 4 – 0.0625 µl/ml and CHX in the range of 250 – 3.9062 µg/ml. The biofilm without tested samples served as positive control and the well without biofilm served as negative control. The plate was incubated at 37°C for one hour before the medium was aspirated and the biofilm was washed three times with PBS. In this study the exposure time was performed only one hour for simulation of the retained time of antiseptic in oral cavity. Cell viability was determined using the XTT-reduction assay. Briefly, 100 µl of 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2*H*-tetrazolium hydroxide or XTT solution (Sigma-Aldrich, St. Louis, MO, USA) was added to each well and incubated in the dark at 37°C for 2 hours. The colorimetric change was measured at 492 nm by Varioskan flash microplate reader (Thermo Scientific, Finland). Percentage of killing was calculated from the formula $[1 - OD_{sample}/OD_{control}]] \times 100$. The concentration of each component of the combination mixture that inhibited 50% of sessile *C. albicans* in the biofilm was recorded as the $SMIC_{50}$ (sessile minimum inhibitory concentration). The $SMIC_{50}$ was used to compare antifungal activity because the percent killing of some tested sample did not reach 90%. All experiments were performed in triplicate.

3. Results and Discussion

The major components of the tested oils were: eugenol (99.7 %) in clove oil, trans-cinnamaldehyde (68.1%) and eugenol (18.5%) in cinnamon bark oil and citrals (81.2%) in the form of neral (34.5%) and geranial (46.7%) in lemongrass oil (Table 1). These components and identification of chemical components proportions are similar to those previously reported for clove oil (eugenol 88.2%); cinnamon bark oil (trans-cinnamaldehyde 66.28 – 81.97%; and lemongrass oil (neral 28.0% and geranial 52.0%) (Li, Kong, & Wu, 2013; Velluti, Sanchis, Ramos, Egido, & Marín, 2003).

The antifungal activities (MIC and MFC) of the three essential oils and CHX when used alone against planktonic *Candida* are shown in Table 2. The results indicate that all three essential oils showed antifungal activity, with MIC/MFCs ranging from 0.125 – 1.0 µl/ml. Cinnamon oil exhibited the lowest MIC against all *Candida* spp. strains. For CHX, the MIC/MFCs ranged from 1.25 µg/ml for *C. tropicalis* to 3.91 µg/ml for *C. krusei* and 31.25 µg/ml for *C. albicans*. The MIC/MFCs for CHX in this study were higher for *C .albicans* ATCC10231 and the *C. krusei* clinical isolate and lower for the clinical isolate of *C. tropicalis* than those reported for CHX against different *Candida* strains in a previous study (Salim, Moore, Silikas, Satterthwaite, & Rautemaa, 2013).

These findings demonstrated that all three essential oils (clove oil, cinnamon oil and lemongrass oil) showed *in vitro* antifungal activity against *Candida* spp. The hydrophobic properties of essential oils and their components might be the important for antimicrobial activity enabling them to penetrate through the lipid bilayer of the cell membrane and disturb the membrane structure (Burt, 2004). The different types and proportions of the compounds present in essential oils could result in differences in antifungal activity depending on the cell wall composition of a fungal strain (Samaranayake & Samaranayake, 1994). The hydrophobic properties of essential oils can be expressed by logarithm of the octanol and water (Log P) which indicates the partitioning of compound into polar and non-polar phases. The higher the value of Log P, the better the compound partitions into the hydrophobic phase (de Bont, 1998). As shown in Table 1, the major components of lemongrass oil are citrals, clove oil is eugenol and cinnamon oil is eugenol and cinnamaldehyde. The partition coefficients of citral, eugenol and cinnamaldehyde are 2.76, 2.49 and 1.90, respectively (National Center for Biotechnology Information, 2016). Moreover, Minagi, Miyake, Fujioka, Tsuru, and Suginaka (1986) demonstrated that the cell-surface hydrophobicity of *C. tropicalis* was greater than *C. krusei* and *C. albicans*. It might be possible that lemongrass oil, which contains citrals as the major components, and has the highest partition coefficient, may penetrate through the cell surface of *C. tropicalis* more easily than *C. krusei* and *C. albicans*, respectively. As a result, lemongrass oil appeared to be more effective against *C. tropicalis* than against *C. krusei* and *C. albicans*, respectively. In fact, cinnamon oil was the most effective against *C. tropicalis*, higher than against *C. krusei* and *C. albicans*. Thus might be the synergistic effect of eugenol and cinnamaldehyde even though their log P was lower than that of citrals.

In addition, several studies have demonstrated that citrals may cause membrane interference by forming a charge transfer complex with fungal cell tryptophan and cross linking with an amino group on the cell wall and cytoplasm. Moreover, citrals also inhibit enzymes with thiol groups at the cytoplasmic membrane. Microscopic examination of *C. albicans* treated with lemongrass oil reveals morphological changes including cell shrinkage and cell surface alteration

Table 1. Chemical components of essential oils.

Essential oils	Part Botanical name	Chemical components	Retention time (min)	Area %	LRI
Clove oil	Bud	Eugenol	23.4	99.7	1351
	Eugenia caryophyllata	Caryophyllene oxide	32.0	0.3	1578
Cinnamon oil	Bark	Alpha-pinene	5.4	0.3	NI
	Cinnamomum zeylanicum	Camphene	5.9	0.4	NI
		Beta-pinene	6.8	0.6	NI
		O-Cymene	8.6	1.3	1024
		Limonene	8.8	1.4	1028
		L-Linalool	11.8	3.8	1000
		Trans-cinnamaldehyde	19.8	68.1	1273
		Eugenol	23.3	18.5	1349
		Caryophyllene	26.4	3.7	1416
		Alpha-humulene	27.8	0.4	1454
		O-methoxycinnamaldehyde	30.4	0.9	1528
		Caryophyllene oxide	32.0	0.6	1578
		6-methyl-5-heptan-2-one	7.0	1.3	NI
Lemongrass oil	Leaf	Beta-myrcene	7.2	6.1	NI
	Cymbopogon citratus	Limonene	8.8	1.0	1028
		Cis-ocimene	9.0	0.6	1034
		L-Linalool	11.8	1.0	1000
		Ethenyl-cyclohexane	14.6	0.9	1161
		Alpha-thujone	15.5	1.8	1180
		Beta-citral (Neral)	18.1	34.5	1238
		Geraniol	18.7	2.6	1250
		Alpha-citral (Geranial)	19.5	46.7	1268
		Geranyl acetate	24.7	2.0	1378
		Trans-caryophyllene	26.4	0.5	1416
		Selina-6-en-4-ol	33.1	0.9	1620

Percentage of three essential oils were calculated from results obtained on DB-5ms column (unidentified compounds are not shown); LRI = linear retention index; Mode of identification: a = mass spectra; b= LRI; c = comparing with authentic compounds. Eugenol (RT=23.4); trans-cinnamaldehde (RT=19.8); beta-citral (RT = 18.1); alpha-citral (RT = 19.5)
LRI: linear retention index generated from a series of n-alkanes ($C_{10} - C_{23}$).
NI: not identified.

Table 2. MICs and MFCs of essential oils and CHX against planktonic cells of *Candida* spp.

Strain	MIC/MFC			
	Clove oil	Cinnamon oil	Lemongrass oil	CHX
C. albicans	0.5/1.0	0.25/0.5	0.5/1.0	31.25/31.25
C. krusei	0.5/1.0	0.25/0.25	0.5/0.5	3.91/3.91
C. tropicalis	0.25/1.0	0.125/0.25	0.25/0.5	1.25/1.25

*Concentration of essential oils expressed in µl/ml
**Concentration of CHX expressed in µg/ml
The results were evaluated from three independent experiments

(Kurita, Miyaji, Kurane, & Takahara, 1981; Leite, Bezerra, Sousa, Guerra, & Lima, 2014; Lima et al., 2012; Tyagi & Malik, 2010).

The *in vitro* antifungal activity of cinnamon bark oil is likely to be due to the synergistic effect of cinnamaldehyde and eugenol. Trans- cinnamaldehyde and eugenol are believed to exhibit fungicidal activity through a mechanism that alters both the cell membrane and the cell interior. The carbonyl group of cinnamaldhyde binds with proteins in the cell membrane causing cell membrane damage (Burt, 2004; Taguchi, Hasumi, Abe, & Nishiyama, 2013). Eugenol targets ergosterol biosynthesis in the membrane causing the destruction of cell integrity (Burt, 2004; National Center for Biotechnology Information, 2016). These compounds then accumulate in the cell membrane and disorganize the cytoplasm, which disturbs the osmotic balance leading to ineffective protein and cellular function ultimately causing cell death (Khan, Ahmad, & Cameotra, 2013).

The synergistic interaction of cinnamaldehyde and eugenol against *E. coli* is reportedly due to their interaction with proteins and enzymes (Pei, Zhou, Ji, & Xu, 2009). Thus, the antimicrobial activity of different essential oils may be partly due to the different ability of the chemical components of the oils to partition into the lipid bilayer of the cell membrane and act on microbial target sites.

Table 3 shows that the combination of essential oils and CHX reduced the MICs of both the essential oil and CHX against all tested *Candida* spp. Synergistic effects (FICI ≤ 0.5) were found for all combinations of essential oil with CHX against *C. albicans* as well as the combination of lemongrass oil with CHX against *C. tropicalis*. Combinations of all essential oils and CHX only showed the additive effect against *C. krusei*.

The synergistic activity of essential oil in combination with CHX might be due to their common target site, the cell membrane (Filoche *et al*., 2005). CHX is believed to act by binding to proteins in the cell wall leading to a loss of cell integrity, the leakage of cell constituents and cell precipitation (Fathilah, Himratul-Aznita, Fatheen, & Suriani, 2012; Filoche *et al*., 2005; Machado *et al*., 2010). Thus, the hydrophobic properties of essential oils might enable them to penetrate the lipid bilayer of the cell membrane and alter the membrane structure, which may enhance cell permeability to CHX (Burt, 2004). Conversely, previous reports have noted that the synergistic effect of lemongrass oil in combination with CHX against *C. tropicalis* might be due to the different target sites of each agent, and the antifungal activity may depend on the ratio between lemongrass oil and CHX (Ellepola & Samaranayake, 2001; Lima *et al*., 2012). Furthermore, lemongrass oil consists of constituents other than citral, such as beta-myrcene, geranyl acetate and geraniol (National Center for Biotechnology Information, 2016) that could also enhance the penetration of lemongrass oil into the cell membrane of *C. tropicalis* (Taweechaisupapong, Ngaonee, Patsuk, Pitiphat, & Khunkitti, 2012b).

The antifungal effects of essential oils and CHX alone and in combination against sessile *C. albicans* in biofilm are expressed as the sessile minimum inhibitory concentration at 50 percent ($SMIC_{50}$) in Table 4. The $SMIC_{50}$ of CHX alone was 250 µg/ml, and the cinnamon oil, lemongrass oil and clove oil $SMIC_{50}$ were 2.0 µl/ml.

Synergistic effects against sessile *C. albicans* in biofilm were found for clove and cinnamon oil combined with CHX (FICI 0.500 and 0.375, respectively). These combinations reduced the $SMIC_{50}$ of clove oil 4-fold, cinnamon oil 8-fold and CHX 4-fold. An additive effect (FICI 1.062) was found in the combination of lemongrass oil and CHX. This combination did not reduce the $SMIC_{50}$ of lemongrass oil, but did reduce the $SMIC_{50}$ for CHX 16-fold. In the current study, cinnamon oil combined with CHX was the most effective against *C. albicans* biofilm (62.5 µg/ml CHX combined with 0.25 µl/ml cinnamon oil, which is equivalent to 263.5 µg/ml). The $SMIC_{50}$ of cinnamon oil alone was 2 µl/ml (equivalent to 2108 µg/ml), which is much higher than previously reported. Raut, Shinde, Chauhan, and Karuppayil (2014) found that the $SMIC_{50}$ of cinnamaldehyde and eugenol against *C. albicans* biofilm were 500 µg/ml and 1000 µg/ml, respectively. Moreover, He, Du, Fan, and Bian (2007) found that the $SMIC_{50}$ of eugenol was 500 µg/ml against *C. albicans* biofilm. This suggested that the combination of CHX and cinnamon oil was more effective against *C. albicans* biofilm than cinnamon oil alone.

Table 3. MICs and FICI of essential oils in combinations with CHX against planktonic cells of *Candida* spp.

Candida strain	Combinations	MIC in combination Oil*/CHX**	FIC	FICI	Outcome
C. albicans	Clove oil/CHX	0.125/7.8125	0.25/0.25	0.500	Synergistic
	Cinnamon oil/CHX	0.0625/3.9063	0.25/0.125	0.375	Synergistic
	Lemongrass oil/CHX	0.0625/7.8125	0.0625/0.25	0.312	Synergistic
C. krusei	Clove oil/CHX	0.25/0.9766	0.50/0.25	0.750	Additive
	Cinnamon oil/CHX	0.125/0.1221	0.50/0.031	0.531	Additive
	Lemongrass oil/CHX	0.25/0.4883	0.50/0.125	0.625	Additive
C. tropicalis	Clove oil/CHX	0.25/0.312	1.00/0.25	1.250	Additive
	Cinnamon oil/CHX	0.062/0.312	0.50/0.25	0.750	Additive
	Lemongrass oil/CHX	0.062/0.312	0.25/0.25	0.500	Synergistic

*Concentration of essential oils expressed in µl/ml
**Concentration of CHX expressed in µg/ml
The results were evaluated from three independent experiments.

Table 4. $SMIC_{50}$ of CHX, essential oils and combinations against sessile *C. albicans* sessile in biofilm

Sample	$SMIC_{50}$	FIC	FICI	Outcome
CHX (µg/ml)	250			-
Clove oil (µl/ml)	2.0			-
Cinnamon oil (µl/ml)	2.0			-
Lemongrass oil (µl/ml)	2.0			-
Clove/CHX	0.50/62.5	0.25/0.25	0.500	Synergistic
Cinnamon/CHX	0.25/62.5	0.125/0.25	0.375	Synergistic
Lemongrass oil/CHX	2.0/15.62	1.0/0.062	1.062	Additive

*Concentration of essential oils expressed in µl/ml
**Concentration of CHX expressed in µg/ml
The results were evaluated from three independent experiments.

The $SMIC_{90}$ of clove oil or cinnamon oil against *C. alibicans* biofilms were 10 – 20 times the MIC in the planktonic cells. This might be due to the resistance of the biofilm to drug penetration. CHX is a highly cationic chlorophenyl bisbiguanide compound that may bind to the negatively charged extracellular matrix (ECM), thereby retarding penetration and reducing access of CHX to the sessile *Candida* sessile cells. The synergistic effect found in combinations of CHX with either cinnamon oil or clove oil against sessile *C. albicans* suggested that these combinations may change the chemical micro environment of the ECM, causing the cells in the biofilm to be exposed to higher concentrations of the components with antifungal activity (Al-Fattani & Douglas, 2004).

The increased drug resistance and decreased susceptibility to host immune mechanisms that is characteristic of biofilms is due to exopolysaccharides, protein and phosphorus in the ECM (Douglas, 2003; Kaomongkolgit & Jamdee, 2016) The composition of the extracellular polymeric substances surrounding *C. albicans* is significantly different between planktonic and sessile cultures with an increased hydrophobic population of dispersal cells in the biofilm (Bujdáková, Didiášová, Drahovská, & Černáková, 2013; Filoche *et al*., 2005). The synergistic effect of either clove oil or cinnamon oil in combination with CHX against sessile *C. albicans* in biofilm may be due to the penetration of the hydrophobic essential oil components through the charged extracellular matrix of the biofilm. If the cytoplasmic membrane of the sessile *Candida* cells was disturbed, this might increase uptake of CHX (and also the essential oil components) into the target sites at cell membrane causing cell membrane damage, loss of structural organization and integrity and coagulation of cytoplasmic constituents (Burt, 2004; Khan & Ahmad, 2012).

4. Conclusions

This study demonstrated that the combination of CHX with either clove oil or cinnamon oil had a synergistic effect against *C. albicans* in both planktonic and biofilm forms and additive effect against planktonic form of *C. tropicalis* and *C. krusei*. Therefore, the combination of CHX with either clove oil or cinnamon oil may prove useful as an alternative antifungal treatment for oral *Candida* spp.

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