Original Article

Fungicidal Effect of Lemongrass Essential Oil on *Candida albicans* Biofilm Pre-established on Maxillofacial Silicone Specimens

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ABSTRACT

Aims: This *in-vitro* study aimed to evaluate the efficacy of lemongrass (*Cymbopogon citratus*) essential oil in eradicating *Candida albicans* biofilm pre-established on the maxillofacial silicone specimens. **Materials and Methods:** Two maxillofacial silicones, namely, MDX4-4210 and Multisil Epithetik, were used for the fabrication of 6 mm diameter disks (n = 21 for each brand of silicone). A 48-h mature *C. albicans* ATCC 10231 biofilm was pre-established on sterile silicone specimen. These disks were then exposed to various concentrations of lemongrass essential oil ranging from 0.31% to 5% (v/v), 20% (v/v) nystatin, and RPMI-1640 medium for 18–20 h. After exposure, the remaining viable fungal biofilm was examined by the XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide]-reduction assay. All data were analyzed by using a regression coefficient and a *post hoc* Tukey HSD multiple comparisons test (α = 0.05). **Results:** Different brands of silicone used for fabrication did not significantly affect the formation of mature *C. albicans* biofilm (P =0.302). A 5% (v/v) lemongrass essential oil significantly eliminated fungal biofilm by approximately 95% (P =0.031). However, less than 50% of the fungal biofilm was eliminated by the tested oil at a concentration as low as 0.31% (v/v). Furthermore, the fungicidal efficacy against *C. albicans* biofilm of lemongrass essential oil at 2.5% (v/v) was as potent as that of 20% (v/v) nystatin suspension (P = 0.99). **Conclusion:** Lemongrass essential oil expressed fungicidal effect on *C. albicans* biofilm pre-established on the disks fabricated from different brands of silicone. Additionally, the fungicidal effectiveness of the oil against the mature fungal biofilm was dose-dependent.

Keywords: *Candida albicans biofilm, fungicidal effect, lemongrass essential oil, maxillofacial silicone*

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Introduction

Medical-grade silicone has been commonly used in some patients with complex facial disfigurement due to tumor surgical removal or partial-facial surgical reconstruction, especially in cases in which a more desirable complete autoplastic surgical repair by surgical reconstruction is inadvisable and/or contraindicated.[1] *Candida albicans* and *C. glabrata* are the species commonly isolated from medical indwelling devices and on used-silicone facial prostheses.[2-7] This dimorphic fungus not only colonizes on the silicone surfaces but also deeply penetrates inside this porous material through its filamentous form.[4,8] Expanding forces caused by the vegetative yeasts penetrating through the porosity resulted in softening of the edge, margin degradation, and permanent black discoloration

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of the silicones, and some needed to be replaced with a more comprehensive design.[2,3,9,10] The fungal infection may possibly cause a non-life-threatening mucocutaneous candidiasis or a life-threatening candidemia (*Candida* bloodstream infection) through the dissemination of single or clustered cells from the fungal biofilm in conjunction with its inherent drug-resistance capacity.[5,11] The *Candida* infection affects directly on people with immunocompromised conditions, including patients with cancer who have undergone a chemotherapy and/or radiotherapy.[5]

The immersion in disinfecting solution is a recommended procedure to clean the silicone prosthesis regularly to reduce the fungal biofilm as well as dispersal forms, to extend the life-span of the material, and to lower the risk of candidiasis, although undesirable effects reported.[12,13] Therefore, this study aimed to evaluate the capability of the lemongrass essential oil in eliminating *C. albicans* biofilm pre-established on silicone specimens and to compare with nystatin, an antifungal medication.

Materials and Methods

Silastic MDX4-4210 (Dow Corning Corporation Medical Products, USA) and Multisil Epithetik (Bredent GmbH, Senden, Germany) were two brands of room temperature vulcanizing (RTV) medical-grade maxillofacial silicones used in this study. Beyond silicone, the following materials were used in this study: separating medium F-901 (Factor II, Inc., USA), Sabouraud dextrose agar (SDA) (Difco, Becton Dickinson, Co.), yeast nitrogen base (YNB) medium (Difco, Becton Dickinson, Co.), XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] (Sigma, St Louis, MO, USA), menadione (Sigma), fetal bovine serum (FBS) (GE Healthcare Bio-Science), lemongrass essential oil (BOTANICESSENCE, Thailand), Tystatin oral suspension (T.O. Pharma Co., Thailand), and *C. albicans* ATCC 10231 (supplied by the Department of Oral Microbiology).

Preparation of silicone specimens

A rectangular plastic matrix (9 mm × 7 mm × 2.5 mm) was placed on a gypsum mold that was pre-coated with a separating medium. Two brands of RTV medical-grade maxillofacial silicones were individually prepared according to the manufacturer's recommendation and poured into the confined matrix before the surface was smoothed with a spatula to obtain an even thickness. Polymers were allowed to complete the vulcanization process at room temperature before silicone disks (6 mm in diameter) were obtained by using a puncher. Twenty-one specimens per brand of silicone (15 for lemongrass essential oil tested, 3 for nystatin-treated or positive control, and 3 for untreated or negative control) were washed under running water, dried, and cleaned with 70% ethanol before being sterilized with ethylene oxide gas.

C. albicans biofilm formation on silicone specimens

A fungal biofilm was pre-established on silicone specimen as described previously with some modifications.[14,15] A loop-full of *C. albicans* ATCC10231 freshly subcultured on SDA was inoculated in YNB supplemented with 50 mM glucose (YNB-50) and incubated at 37°C with agitation (75 rpm) for 24 h. Yeast cells were harvested by centrifugation (3000*g*, 5 min) and washed twice before being resuspended in YNB medium supplemented with 100 mM glucose (YNB-100) to a final cell density of 1×10^7 CFU/mL. The 6-mm diameter silicone disks were conditioned with 50% FBS and agitated at 75 rpm overnight before being used to form the fungal biofilm base. Silicone disks were washed with phosphate-buffered saline (PBS) before being transferred to a new six-well plate containing 6 mL of *C. albicans* inoculum previously prepared. The plates were incubated at 37°C with agitation for 90 min (adhesion phase). After adhesion phase, each specimen was picked up and washed gently with PBS to remove any non-adherent cells. These disks were then transferred to new six-well plates containing RPMI-1640 medium for the biofilm formation phase. The plates were incubated at 37°C with agitation for 48 h. Fresh RPMI-1640 medium was replenished at 24-h intervals. Silicone disks with fungal biofilm formation were washed with PBS and transferred individually to the 48-well plates ready to be treated with lemongrass essential oil.

Fungicidal effect of lemongrass essential oil against *C. albicans* biofilm

Five concentrations of lemongrass essential oil including 0.31%, 0.62%, 1.25%, 2.5%, and 5.0% (v/v) were prepared in RPMI-1640 medium. A 20% (v/v) nystatin in RPMI-1640 medium and RPMI-1640 medium were used as antifungal drug-treated (positive) and untreated (negative) controls, respectively. Silicone disks with 48-h mature *C. albicans* biofilm were individually submerged in various concentrations of lemongrass essential oil, 20% (v/v) nystatin solution, and RPMI-1640 medium for 18–20 h. The XTT-reduction assay was used to determine the viability of the fungal biofilm remaining on the silicone disks.[16] Each silicone specimen was washed gently in PBS to remove the remnant antifungal agents and transferred individually to a new 48-well plate that each well contained 1 mL

of working XTT solution (a mixture of 790 µL of PBS supplemented with 200 mM glucose, 200 µL of XTT in PBS, and 10 µL of 0.4 mM menadione in acetone). After being incubated at 37°C in the dark for 3 h, 200 µL of the metabolic reaction was individually transferred to a 96-well flat-bottom microplate, and then the optical density (OD) was measured at a 490-nm wavelength with a microplate reader (µ-Quant; MQX200 Bio-Tek). The percentage of *C. albicans* biofilm reduction was interpolated using the following equation[17]:

$$\%\ \text{biofilm reduction} = \left[1 - \left(\frac{\text{OD490 of the treated specimen}}{\text{OD490 of the untreated}}\right)\right] \times 100.$$

Statistical analysis

In this study, each test was performed in triplicate and repeated five times at different time points. SPSS Windows software (IBM SPSS 27 Inc., Chicago, IL, USA) was used to analyze all the data. A coefficient of regression was first used to analyze the fungicidal effects of lemongrass essential oil on *C. albicans* biofilm pre-established on silicone disks and followed by a *post hoc* Tukey HSD multiple comparisons test with the confidence level at $\alpha = 0.05$.

Results

Lemongrass essential oil demonstrated a fungicidal effect against *C. albicans* pre-established on both brands of medical silicone disks without a statistical difference between them (*P*-value = 0.436). Lemongrass essential oil at the concentration of 5% (v/v) eliminated the fungal biofilm by approximately 95%, but nearly 50% of the fungal biofilm was eliminated with the oil at the concentration of 0.31% (v/v). Moreover, the fungicidal effect of the oil at the concentration of 2.5% (v/v) was as potent as 20% (v/v) nystatin suspension. More than 80% of the *C. albicans* biofilm was reduced after it exposed to 2.5% (v/v) lemongrass essential oil or 20% (v/v) nystatin. Factors influencing the fungicidal effect of lemongrass essential oil against *C. albicans* biofilm formed on each brand of silicone disks were summarized in Tables 1 and 2.

Discussion

Available medical-grade silicones are mostly biocompatible but differ in their physical properties according to the multivariant contents of the polymer that have been formulated suitably for certain purposes such as the polymer's length, nano-fillers, and opacifier.[12,18] As reported previously, different brands of facial silicone had been differently susceptible to the colonization of *C. albicans*.[19] However, the fungal biofilm formation on these two brands (Silastic MDX4-4210 and Multisil Epithetik) silicone was not significantly different (*P*-value = 0.436). This implies that the surface of the specimens fabricated from these two brands is similar, or the compositions of these two silicone brands may not be different significantly, further evaluation of which is required. Silicone disks used in this study were fabricated on a gypsum mold pre-coated with separating medium to simulate the preparation of the material employed in maxillofacial clinics. It was different from previous investigations that mostly used a metal mold to fabricate the silicone specimens to obtain a smooth glossy surface, which does not imitate the intaglio surface of the material employed in the clinic.[12,13,20-22] In some clinical practice, to minimize the silicone surface roughness derived from laboratory processing, a silicone sealant (Multisil-sealant) has been

Table 1: Factors influencing on the fungicidal effect of lemongrass essential oil against *C. albicans* biofilm pre-established on two different brands of medical grade silicone disks analyzed by multiple linear regression analysis

Sources	Unstandardized coefficients		95% Confidence interval for *B*	*p*-value
	B	SE	(Lower, upper bounds)	
(Constant)	46.045	3.919	(38.311, 53.779)	0.001
Silicone brands	−1.622	2.080	(−5.727, 2.483)	0.436
Conc.	8.450	0.609	(7.248, 9.651)	0.001

B: regression coefficient; SE: standard error; Conc.: concentrations of solution

Table 2: Fungicidal effect of various concentrations of lemongrass essential oil against *C. albicans* biofilm pre-established on two different brands of medical-grade silicone disks (each brand of silicone and concentration of the oil: *n* = 15) expressed as mean percentage of fungal biofilm reduction with standard error (SE)

LG concentrations [% (v/v)]	Percentage of fungal biofilm reduction [mean (SE)]	
	Silastic MDX 4-4210	Multisil Epithetik
0.31	47.60 (3.275)^{a}	46.08 (3.275)^{a}
0.62	64.87 (3.275)^{b}	59.18 (3.275)^{b}
1.25	69.74 (3.275)^{b}	68.24 (3.275)^{b}
2.5	83.05 (3.275)^{c}	83.46 (3.275)^{c}
5.0	93.81 (3.275)^{d}	94.45 (3.275)^{d}
20% (v/v) nystatin	84.91 (3.275)^{c}	82.84 (3.275)^{c}

Different superscript letters indicated significant difference at each concentration; LG: lemongrass essential oil

applied to the silicone prosthesis surface in order to reduce microbial colonization, to prevent dirt adhesion, and to be cleaned easily. However, the efficacy of the sealant applied remains questionable as a study found that the use of sealant was not successful in preventing microbial colonization on specimens.[23]

Without proper disinfection, silicone prosthesis may become a reservoir or source of microbial accumulation including *Candida* species that probably causes health problem. Therefore, a simple and effective procedure to keep the silicone prostheses hygienically clean and free from debris and microbes is recommended to decrease the risk of infectious disease as well as to prolong the life-span of the prosthesis.[24] The mechanical cleaning procedure, especially brushing, is not recommended as some undesirable effects have been observed.[25] A routine mechanical washing may dissolve and dislodge color or pigment from the superficial layers of silicone prosthesis as well as abrade and roughen the material surfaces, which make the material more susceptible to microbial colonization.[21,26] An alternative option proposed for cleaning the silicone prosthesis is to immerse the material in chemical disinfectants such as oxidizing agents (sodium hypochlorite, peroxide) and non-oxidizing agents (alcohol, chlorhexidine). The chlorhexidine gluconate solution was a potent disinfectant for silicone prostheses in the previous studies.[20,23] However, undesirable effects including silicone degradation and discoloration have been reported when oxidizing agents or chlorhexidine solution was used to disinfect the prostheses.[12,13] Hence, new and naturally derived disinfectants are of interest.[13,14,17,22]

Lemongrass is a popular culinary herb, which also has several medicinal properties.[27] This *in-vitro* study demonstrated that lemongrass essential oil has a potent fungicidal effect on *C. albicans* biofilm established on silicone disks. More than 90% of the fungal biofilm pre-formed on silicone disks was eliminated or reduced after being exposed to the lemongrass essential oil at the concentrations of 2.5% and 5% (v/v). These concentrations of the oil used to eliminate the fungal biofilm significantly were much higher than the minimum inhibitory concentration (MIC) value [ranging from 0.03% to 0.06% (v/v)] against the planktonic fungus.[28,29] The results also supported that the biofilm form was more tolerant to the disinfectant than its planktonic counterpart. Furthermore, the fungicidal effect of lemongrass essential oil [5.0% (v/v)] against fungal biofilm was significantly higher than 20% (v/v) [20,000 IU] nystatin suspension (positive control). Nystatin suspension is an antifungal in the polyene family commonly used to treat oral candidiasis because the drug adversely affects fungal cells by causing permeability loss of cell membrane. The fungicidal efficacy of lemongrass essential oil against fungal biofilm formed on silicone specimens only depended on the concentration of the oil used (P-value = 0.001). In other words, the killing or eliminating effectiveness of lemongrass essential oil against fungal biofilm was dose-dependent. In contrast, brands of medical-grade silicone used for fabrication had no influence on the fungicidal effect of the oil and the formation of fungal biofilm on the specimens. Consequently, lemongrass essential oil may be proposed as a potent disinfectant used to clean *Candida* biofilm formed on silicone prosthesis.

As with other *in-vitro* study design, *C. albicans* biofilm formed on silicone specimens in this study was established from a single species of yeast. However, the fungal biofilm naturally formed on silicone prosthesis's surfaces or human skin found in clinic is a multispecies community and frequently found to be a coexistence of *Candida* species and bacteria including *Staphylococcus aureus* and *S. epidermidis*. The complexity of the architectural structure of microbial biofilms, including synergistic and antagonistic interactions, metabolic cooperation, and quorum sensing, causes the microbial community more resistant to drugs and antimicrobial agents.[30] Therefore, the fungicidal effect of lemongrass essential oil against the *in vivo* fungal biofilm with multispecies will be evaluated inevitably. Future studies are also necessary to investigate the effect of the lemongrass essential oil on physical properties including color, hardness, and surface roughness of the silicone before the oil can be introduced as an effective disinfectant for cleaning the silicone prosthesis.

Conclusion

Lemongrass essential oil eliminated the *C. albicans* biofilm pre-established on medical-grade silicones substantially. Additionally, the fungicidal effectiveness of the lemongrass essential oil against the fungal biofilm formed on silicone specimens was dose-dependent.

Future scope/clinical significance

In conjunction with cytotoxicity testing and clinical study, lemongrass essential oil, the natural-derived product, can be used safely and effectively as alternative disinfectant to eliminate *Candida* biofilm frequently formed on prosthesis including maxillofacial prosthesis. Prosthesis free from fungal biofilm will keep the patients far from candidiasis and extend the material life-span.

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Conflicts of interest

No conflict of interest to be declared.

Authors contributions

Shamsiahwati Mat-Rani: Investigation, data analysis, writing original draft; Natdhanai Chotprasert: Data analysis, statistical analysis; Natchalee: Data analysis, statistical analysis; Suwan Choonharuangdej: Conceptualization, experimental design, data analysis, writing-original draft, writing-review and editing, validation.

Ethical policy and institutional review board statement

Not applicable.

Patient declaration of consent

Not applicable.

Data availability statement

Not applicable.

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