Original Article

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Dentistry Section

Lemongrass-Incorporated Tissue Conditioner Against *Candida albicans* Culture

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ABSTRACT

Background: Tissue conditioner is applied popularly with dental prosthesis during wound healing process but it becomes a reservoir of oral microbiota, especially *Candida* species after long-term usage. Several antifungal drugs have been mixed with this material to control fungal level. In this study, lemongrass essential oil was added into COE-COMFORT tissue conditioner before being determined for anti-*Candida* efficacy.

Materials and Methods: Lemongrass (*Cymbopogon citratus*) essential oil was primarily determined for antifungal activity against *C. albicans* American type culture collection (ATCC) 10231 and MIC (minimum inhibitory concentration) value by agar disk diffusion and broth microdilution methods, respectively. COE-COMFORT tissue conditioner was prepared as recommended by the manufacturer after a fixed volume of the oil at its MIC or higher concentrations were mixed thoroughly in its liquid part. Antifungal efficacy of the tissue conditioner with/without herb was finally analyzed.

Results: Lemongrass essential oil displayed potent antifungal activity against *C. albicans* ATCC 10231and its MIC value was 0.06% (v/v). Dissimilarly, the tissue conditioner containing the oil at MIC level did not cease the growth of the tested fungus. Both reference and clinical isolates of *C. albicans* were completely inhibited after exposed to the tissue conditioner containing at least 0.25% (v/v) of the oil (approximately 4-time MIC). The tissue conditioner without herb or with nystatin was employed as negative or positive control, respectively.

Conclusion: COE-COMFORT tissue conditioner supplemented with lemongrass essential oil obviously demonstrated another desirable property as in vitro anti-*Candida* efficacy to minimize the risk of getting *Candida*l infection.

Keywords: Coe-comfort tissue conditioner, *Candida albicans*, Lemongrass essential oil, Minimum inhibitory concentration (MIC)

INTRODUCTION

Tissue conditioner is a soft lining material widely employed to distribute stress or force more evenly on any supporting tissues, especially the traumatic ones to decrease hard mastication. It thus has been used beneficially for many intraoral clinical purposes including tissue treatment, temporary obturator, baseplate stabilization, liners in surgical splint and functional impression material. After surrounded with multi-strain microbiota residing in oral environment for a while, it cannot neglect that the material has been contaminated and allowed these microorganisms to colonize, multiply and form their community. *Candida* species, especially *C. albicans*, has been reported as an opportunistic fungus frequently isolated from any appliances including denture, palatal obturator and so on inserted in oral cavity [1]. The fungus causing a wide range of diseases from mild mucocutaneous to mortal systemic ones among immunocompromised individuals including patients with advanced cancer; patients undergoing chemotherapy for malignancy, solid-organ transplantation, blood and marrow transplantation; immunosuppressive therapy; advanced age [2-6]. Morbidity and mortality of *Candida*l infection have increased continually during the past few decades. Therefore, several antifungal agents have been introduced and employed to control such opportunistic infection. Among herbs popularly used in the traditional medicines of eastern countries, lemongrass (*Cymbopogon citratus*) essential oil has been reported to express potent in vitro inhibitory effect against *C. albicans* and non-albicans *Candida* species [7]. An application of the essential oil extracted from this edible herb as an effective, safe and locally produced antifungal agent should be a choice of drugs to reduce the *Candida*l infection. Thus, this study aims to determine in vitro an inhibitory efficacy against the growth of *C. albicans* of the tissue conditioner that was primarily supplemented with lemongrass essential oil.

MATERIALS AND METHODS

Lemongrass (C. citratus) essential oil was purchased from Thai-Flavours and Fragrances Industry Co. Ltd., Bangkok, Thailand. Sabouraud dextrose and Mueller Hinton II agar were purchased from Becton, Dickenson and Company, NJ, USA. RPMI-1640 medium, 3-(N-morpholino) propanesulfonic acid/3-morpholinopropane-1-sulfonic acid (MOPS) and propylene glycol were purchased from Sigma-Aldrich Co. LLC. Nystatin oral suspension [100,000 International Unit (IU)/ml] (Tystatin), and COE-COMFORT™ tissue conditioner were purchased from T.O. Pharma Co., Thailand and GC America Inc., IL, USA, respectively. *C. albicans* ATCC 10231 and other ten clinical strains were kindly provided by Department of Oral Microbiology, Faculty of Dentistry, Mahidol University.

The tested lemongrass essential oil [10 µl/ml or 1% (v/v)] was primarily screened for its antifungal activities: inhibition zone and minimum inhibitory concentration (MIC) against *C. albicans* ATCC 10231 via agar disk diffusion and broth microdilution methods, respectively [8]. COE-COMFORT™ tissue conditioner was prepared according to the manufacturer's recommendation with modification by addition of lemongrass oil to yield final concentrations ranging from its MIC to higher values (two-fold increment) [9]. Briefly, the tested oil was serially diluted with propylene glycol to obtain 50% to 6.25% (v/v). In a separate cup, 0.5 ml of the undiluted and each concentration of lemongrass oil was mixed thoroughly with 1.5 ml COE-COMFORT liquid for 30 sec before slowly poured on the tissue

Final concentrations (µl/ml)	Appearance of yeast colony
0.313	+
0.625	+
1.25	+
2.5	-
5.0	-
10.0	-

[Table/Fig-1]: Inhibitory effect against *C. albicans* ATCC 10231 of the COE-COMFORT tissue conditioner supplemented with varied concentrations of lemongrass (C. citratus) essential oil
(-) : absence of yeast colony; (+) : presence of yeast colony

COE-COMFORT tissue conditioner			
C. albicans	C. citratus (2.5 µl/ml)	Positive control [Nystatin (5,000 IU/ml)]	Negative control (without supplement)
ATCC 10231	-	-	+
Clinical isolate #1	-	-	+
Clinical isolate #2	-	-	+
Clinical isolate #3	-	-	+
Clinical isolate #4	-	-	+
Clinical isolate #5	-	-	+
Clinical isolate #6	-	-	+
Clinical isolate #7	-	-	+
Clinical isolate #8	-	-	+
Clinical isolate #9	-	-	+
Clinical isolate #10	-	-	+

[Table/Fig-2]: Inhibitory effect against both reference and clinical strains of *C. albicans* of the COE-COMFORT tissue conditioner with C. citratus, nystatin or without supplement
(-): absence of yeast colony; (+): presence of yeast colony

conditioner powder primarily contained in each well of the 6-well plate. The mixture was stirred well to form slurry, spread evenly and allowed to set for 10 min. Then the tissue conditioner supplemented with lemongrass oil was covered with 4 ml of Sabouraud dextrose agar before 20 µl of fungal suspension (1-5x105 CFU/ml) either reference or clinical strain of *C. albicans* was spread on agar surface. The plate was incubated aerobically at 37°C for 48 h. The tissue conditioner without lemongrass oil or with nystatin suspension was designated as negative or positive control, respectively. Antifungal effect of the tested materials was determined from an absence of yeast colony. The experiment was performed for four different times; each time was done in triplicate.

RESULTS

Lemongrass essential oil illustrated vivid inhibition zone against *C. albicans* ATCC 10231 by agar disk diffusion method and its MIC value was 0.625 µl/ml [0.0625% (v/v)] by broth microdilution method. In contrast, neither reference nor clinical strain of *C. albicans* was inhibited by the tissue conditioner primarily supplemented with the tested oil at its MIC or without supplement (negative control). The growth of both strains of the tested microorganisms was completely inhibited after being exposed to the tissue conditioner supplemented with lemongrass oil at the concentration of 2.5 µl/ml [0.25% (v/v)] or at higher level. Similar result was also observed from the tissue conditioner supplemented with nystatin suspension (5,000 IU/ml) (positive control). All results are summarized in [Table/Fig-1,2].

DISCUSSION

Tissue conditioner, a type of soft relining material clinically used during wound healing period has been a predisposing area to the microbial accumulation due to its deterioration after longterm usage. *C. albicans* is the prominent species of *Candida* isolated from the surfaces of many materials placed in oral cavity and closely related to the inflammation of tissues known as candidiasis. Addition of either synthetic or herbal antifungal agent into the tissue conditioner was to overcome such disadvantage or to reduce the risk of getting this fungal infection. Medicinal herbs are of interest due to their effective antimicrobial activity, safe and affordability, especially for the developing countries [10-11].

Lemongrass (C. citratus), an edible herb composed in several eastern cuisine has been reported to display potent antibacterial and antifungal activities [12-14]. Here, the tested lemongrass essential oil as low as 0.06% (v/v) [MIC value] clearly inhibited the growth of floating *C. albicans*. This result agrees with the previous study by Taweechisupapong [14], which has demonstrated an impressive anti-*Candida* efficacy of this edible herb essential oil. Citral, the main component (approximately 70%) present in this herbal essential oil has been reported to express potent inhibitory effect against *Candida* suspension [15]. In contrast, the COE-COMFORT tissue conditioner being supplemented with the same concentration of the lemongrass essential oil as its MIC mentioned above was unable to cease the growth of both reference and clinical strains of the tested yeast. This discrepancy may be the consequence of some differences between these two experiments. In broth microdilution method (MIC determination), the contact between yeast and the oil was direct manner whereas the contact between the microorganism and the supplement in the tissue conditioner was indirect manner [16].

As a supplement in the tissue conditioner, lemongrass essential oil had to diffuse through the material and culture medium before contacted to the microorganism. The active component as citral of the supplement retaining in culture medium layer was probably not as high as that one in the tissue conditioner layer and its level was insufficient to eliminate the tested yeast. Diffusion rate of the supplement is mainly affected by several factors including its concentration, molecular size of active component and so on. Therefore, amount of the oil higher than its MIC value should be added into the material to reach sufficient level of active component to inhibit the growth of the tested fungus, as illustrated in this study. Here, the COE-COMFORT tissue conditioner supplemented with at least 0.25% (v/v) lemongrass essential oil (4-times of its MIC) vividly expressed potent antifungal efficacy against both reference and clinical isolates of *C. albicans*. This finding agrees with the previous study by Catalan [9]. In this study, volume of the oil added in the liquid part of tissue conditioner was fixed at 0.5 ml to affect the material's physical and chemical properties as less as possible [17]. The COE-COMFORT tissue conditioner itself without any supplement did not demonstrate anti-*Candida* effect so was used as negative control. It is quite different from the property in minimizing fungal growth of this soft relining material claimed by the manufacturer.

CONCLUSION

The COE-COMFORT tissue conditioner being supplemented with lemongrass essential oil has demonstrated an impressive in vitro anti-*Candida* efficacy similar to that of nystatin as supplement. Minimising or eliminating fungal growth is a necessary property of the tissue conditioner to overcome its disadvantage as a reservoir of microbial accumulation.

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