

ANTIFUNGAL EFFECTIVENESS OF VIRGIN COCONUT OIL MOUSSE AGAINST *CANDIDA ALBICANS* BIOFILM IN CHILDREN WITH EARLY CHILDHOOD CARIES

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ABSTRACT

INTRODUCTION: Early childhood caries is defined as the presence of one or more decayed teeth, missing (due to caries) or filled teeth surface in a child 71 months of age or younger. The presence of *Candida albicans* will increase caries pathogenicity.

OBJECTIVES: The objective of this study was to analyze the antifungal effectiveness of virgin coconut oil (VCO) mousse against viability of *C. albicans* biofilm in children with early childhood caries.

MATERIAL AND METHODS: In this experimental laboratory study, *C. albicans* biofilm was exposed to three concentrations of VCO mousse, namely 0.8%, 8% and 80%. *C. albicans* biofilm viability was tested using crystal violet 0.5% and a colony forming unit assay. Statistical analysis was conducted using 1-way ANOVA.

RESULTS: VCO mousse 80% showed optical density and total viable colony count (CFU/ml) values significantly different compared to VCO mousse 0.8% and 8% ($p < 0.05$).

CONCLUSIONS: VCO mousse exhibited antifungal effect against viability of *C. albicans* biofilm in children with early childhood caries.

KEY WORDS: virgin coconut oil, *Candida albicans*, early childhood caries.

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INTRODUCTION

Dental caries is a complex disease that has multiple contributing factors [1]. One destructive form of tooth decay that commonly affects young children is early childhood caries (ECC) [2]. ECC is defined as the presence of one or more decayed teeth, missing (due to caries) or filled teeth surface in a child 71 months of age or younger [3, 4]. ECC is an infectious disease which depends on biofilm [5]. Biofilm will develop when bacteria

interact with dietary sugar and accumulate on the tooth surface forming microcolonies or densely packed cell clusters [6].

Candida is a normal commensal in the oral cavity of healthy individuals [2, 7]. In healthy individuals, the percentage of *Candida* colonization ranges from 20% to 40%. The most prevalent *Candida* in the oral cavity is *C. albicans*. The presence of *C. albicans* will increase caries pathogenicity [2]. De Carvalho and Molaic reported that children with ECC have a greater quanti-

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ty of *C. albicans* in dental plaque, infected dentin and saliva compared to caries-free children [7]. *C. albicans* could adhere and dissolve hydroxyapatite approximately 20-fold higher compared to *Streptococcus mutans* [3].

One of the natural coconut products without heating and chemical processing is virgin coconut oil (VCO) [8, 9]. The majority component of VCO is 93% saturated fats with 47-53% being medium chain fatty acids [10, 11]. Therefore, VCO exhibits antifungal, antibacterial and antiviral activity [10]. One form of ECC prevention is applying tooth mousse [12].

OBJECTIVES

The present study aimed to analyze the antifungal effectiveness of VCO mousse against *C. albicans* in ECC.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of the Faculty of Dentistry, Universitas Indonesia. This was an experimental laboratory study. The inclusion criteria were child age in the range 3-5 years, deciduous dentition, deft index score ≥ 5 and no systemic disease. The exclusion criteria were child above 5 years, caries free, medically compromised, received antibiotics treatment in the past 1 month, major and minor salivary gland disease and radiotherapy. Subjects were included in the study after informed consent was obtained. A plaque sample was swabbed from all tooth surfaces using a sterile toothpick and inserted into 1 ml Sabouraud dextrose broth (SDB) in an Eppendorf tube. The tube was kept in a 4°C cooler box. This study was conducted in the Oral Biology Laboratory of Universitas Indonesia. The antifungal effectiveness of VCO mousse extract against *C. albicans* was assessed using a crystal violet test and colony count (CFU/ml).

LABORATORY

The plaque sample was centrifuged then removed from supernatant and 100 μ l of saline was added to precipitate. After vortexing, a 10 μ l suspension was transferred into CHROMagar by the streak-plate method and incubated for 48 hours at 37°C. As a result, a *C. albicans* colony will exhibit a green color. A colony of *C. albicans* was extracted from the CHROMagar and placed into SDB. The broth was incubated for 24 hours at 37°C. After incubation, a DNA sample was identified using polymerase chain reaction (Dreamtaq Green PCR Master Mix) technique. This step was taken to ensure that the sample was *C. albicans*. After PCR, *C. albicans* was diluted in SDB medium and incubated for 24 hours. A laboratory strain of *C. albicans* (ATCC 10321) was also tested in this research.

The VCO used in this research has been certified by the Indonesian government (Depkes P-IRT No. 207340101143). The VCO was processed to mousse by Akademi Farmasi IKIFA. Three VCO mousse concentrations were tested in this research, which were 0.8%, 8% and 80%.

A *C. albicans* biofilm was obtained by placing 100 μ l of diluted *Candida* (clinical and laboratory) into a 96-microwell plate. The microwell was incubated for 24 hours. After incubation, microwell content was removed by blotting with paper towels. Nonadherent cells were removed by thoroughly washing biofilm with 100 μ l sterile PBS and 50 μ l of SDB was added to the microwell. Another 100 μ l of VCO mousse (0.8%, 8%, 80%) was placed into the microwell (duplo). Positive and negative controls in this research respectively were CPP-ACP and SDB (duplo). Two 96-microwell plates were incubated for both crystal violet and colony forming unit assay.

CRYSTAL VIOLET TEST

After incubation, microwell content was removed by blotting with paper towels and thoroughly washed twice with 100 μ l of sterile PBS. Crystal violet 0.5% (200 μ l) was inserted into each well using a micropipette. After 15 minutes at room temperature, the microwell was washed in 100 μ l of PBS sterile. *C. albicans* biofilm viability (optical density) was measured in a microplate reader at 490 nm.

COLONY FORMING UNIT ASSAY

Following antifungal challenge and subsequent washing, sessile cells were removed from the plate by scraping with a sterile micropipette. The sessile cells were added to sterile PBS and vortexed for 30 s. Each sample was diluted in sterile PBS and dispensed onto Sabouraud Dextrose Agar plates. Plates were incubated for 24 h at 37°C. Colonies were counted the following day to estimate the total viable colony counts from each plate (CFU/ml).

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS 20.00 (SPSS, Chicago, IL, USA). The Shapiro-Wilk test was used to assess the normality of variables. After descriptive analysis, the comparisons between variables for optical density (OD) value and viable cell counts were analyzed using the 1-way ANOVA test. The statistical level of significance was set at 95% ($p < 0.05$).

RESULTS

Table 1 presents distribution data of optical density (OD) value and viable colony counts (CFU/ml). Based

on the Shapiro-Wilk test, distribution data for both values were normal ($p > 0.05$). Each group of *C. albicans* biofilm viability was compared against one another by the parametric test 1-way ANOVA.

Figure 1 shows a comparison of optical density values between different groups using a 1-way ANOVA test. The difference between the groups was statistically significant ($p < 0.001$). It was found that the mean optical density for VCO mousse 80% was 0.110, whereas values for 0.8%, 8% and base respectively were 0.247, 0.248, 0.255. The difference between the groups was significant ($p < 0.001$). On the other hand, the comparison between VCO mousse 80% and CPP-ACP was not significantly different (p value 1.000). A similar outcome was also found for the comparison between VCO mousse 80% and the negative control (p value 0.184).

Figure 2 presents a comparison of total viable colony counts (CFU/ml) between different groups using the 1-way ANOVA test. The difference between the groups was statistically significant ($p < 0.001$). It was found that the mean colony count for VCO mousse 80% was 20.75, whereas for 0.8%, 8%, base and negative control the counts were 525.75, 515.75, 561.25, 567 respectively. The difference between the groups was significant ($p < 0.001$). On the other hand, the comparison between VCO mousse 80% and CPP-ACP was not significantly different (p value 0.573).

DISCUSSION

Almost 36% of the world population has experienced dental caries and about 9% of the infant population is affected [13]. The most common caries found in young children is ECC, previously known as nursing caries or baby bottle caries [14]. Dental caries is associated with the ecological oral environment, infectious bacteria and dietary sugar [15]. The crucial carious process is local enamel demineralization resulting in degradation of hydroxyapatites. This process takes place within bacterial biofilm or dental plaque that covers the tooth surface. Caries lesions will develop when oral biofilms mature and remain on the tooth surface for a long period [13].

Several studies showed that *Candida* has a role in microbial attachment to the tooth surface. *In vitro* study showed that *Candida* enhanced *Streptococcus mutans* adherence to the oral biofilm. Therefore, one caries etiology is *C. albicans*. *C. albicans* participates in biofilm formation and has the ability to ferment glucose and maltose, producing acid and gas [3, 16]. De Carvalho *et al.* reported that in biofilm of ECC *C. albicans* is twice as prevalent as in caries-free children [7].

Coconut products such as coconut oil have been used as diet and traditional medicine in Asian culture. One of the purest forms of coconut oil is VCO. Several reports suggested that VCO has antifungal, antibacterial,

TABLE 1. Shapiro-Wilk test for optical density and colony count (CFU/ml) value

Group	n	Shapiro-Wilk test (p)	
		Optical density	CFU/ml
VCO mousse 0.8%	4	0.900	0.613
VCO mousse 8%	4	0.517	0.970
VCO mousse 80%	4	0.955	0.976
Mousse base	4	0.492	0.798
Positive control (CPP-ACP)	4	0.180	0.213
Negative control	4	0.492	0.288

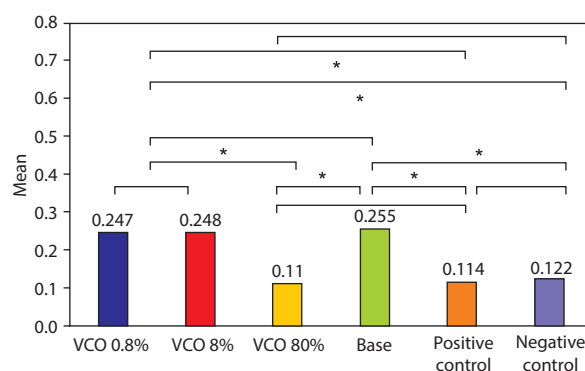


FIGURE 1. Bar graphs of post-hoc tests for optical density values between groups

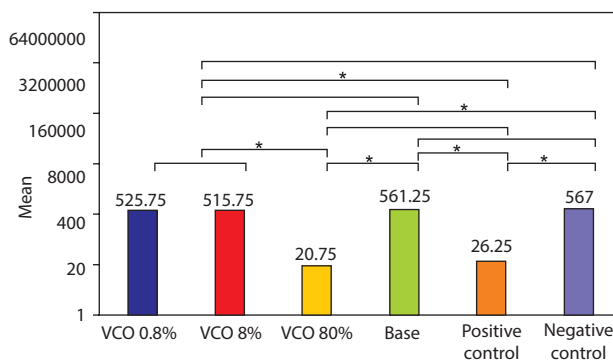


FIGURE 2. Bar graphs of post-hoc tests for colony count between groups

antiviral and antioxidant properties [17]. VCO has antimicrobial activity against *Candida albicans*, *Staphylococcus aureus*, *Streptococcus mutans*, *Actinomyces* spp., *Prevotella* spp. and *Cytomegalovirus* [8, 18, 19]. In this study, the optical density value of VCO mousse 80% showed no statistically significant difference compared to the negative control. The possible cause of this outcome was the cloudy ingredients of mousse such as Na-CMC, sodium saccharin, guar gum, titanium dioxide, glycerol, zinc oxide, propylene glycol, D-sorbitol, Aquades, sodium benzoate, phosphoric acid and xylitol. A similar result was also found in the CPP-ACP group

compared to the negative control. However, based on viable colony counts the difference of both VCO mousse 80% and CPP-ACP was statistically significant compared to the negative control. Therefore, VCO mousse was proven to possess antifungal ability against *C. albicans* biofilm. Among three concentrations of VCO mousse which were tested, VCO mousse 80% showed the most effective antifungal action. The Yusof *et al.* study showed that the minimum VCO extract concentration that exhibits *C. albicans* colony inhibition was 50%. The duration of extract exposure to *C. albicans* was 24 h [10].

VCO has many medical effects due to the high percentage of medium chain fatty acids (MCFA) [10, 17]. Several fatty acids have the highest antifungal activities, such as lauric acid, capric acid, monoglyceride monolaurin and monocaprin [8, 10, 17, 20]. The mechanism action of lauric acid against *Candida* is initiated by entering the lipid bilayer of fungal membranes and physically disturbing the fungal cell membrane, resulting in cell membrane disorganization due to increased fluidity of the membrane. This will lead to cell membrane destruction of *C. albicans* then intracellular components leakage, cytoplasmic disorder and protoplasmic lysis. As a result, *C. albicans* colony growth is inhibited [10, 17].

Other fatty acids that exhibit antifungal effect are capric acid, caprylic acid and myristic acid [8, 10]. Capric acid has been reported to be the fastest and most effective fatty acid eliminating *C. albicans*. This fatty acid inhibits *C. albicans* filamentation, and reduces biofilm formation and candidal adhesion, resulting in reducing candidal pathogenicity [17].

CONCLUSIONS

VCO mousse extract exhibited an antifungal effect against viability of *C. albicans* biofilm from clinical isolates of ECC dental plaque.

VCO mousse 80% showed no significant difference compared to tooth mousse (CPP-ACP), which was the gold standard in ECC prevention treatment.

Based on the optical density value and colony forming unit assay, VCO mousse with 80% concentration was proven to reduce *C. albicans* biofilm viability.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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