Antibacterial Effectiveness of Virgin Coconut Oil Mousse against *Streptococcus mutans* Biofilm in Early Childhood Caries

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Abstract

Early childhood caries (ECC) is one of the major oral health issues in children aged ≤71 months owing to multifactorial causes, including accumulation of *Streptococcus mutans* biofilm. This study aimed to analyze the effectiveness of virgin coconut oil (VCO) mousse at different concentrations against *S. mutans* biofilm in children with ECC.

Cell viability was analyzed to estimate biofilm growth on VCO at different concentrations (0.8%, 8%, and 80%) by crystal violet assay in 96-microwell plates and by measuring colony-forming units. *S. mutans* colonies were counted on brain heart infusion agar plates. Inter-group trials were analyzed by one-way analysis of variance (P<.05). The concentration of 80% VCO mousse showed significant results compared with that of VCO mousse at 0.8% and 8% concentrations, showing equal results obtained with the positive control which is casein phosphopeptide-amorphus calcium phosphate (CPP-ACP). VCO mousse (80%) is an effective antibacterial agent against *S. mutans* in children with ECC.

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Introduction

Early childhood caries (ECC) is one of the major oral health issues that commonly affects infants and pre-school children worldwide. Especially among children living in a socially disadvantaged environment, it has become a significant problem. ECC is defined as the presence of one or more decayed, missing, or filled tooth surfaces in any of the primary teeth in a child aged ≤71 months.[1] In children aged <3 years, presence of caries on the smooth surface of teeth is indicative of severe early childhood caries (**S-ECC**).[2] Caries remains one of the most challenging health problems for children and adults in Indonesia. According to a study in Jakarta, the prevalence of caries in children aged 3–5 years is 81.2%, with a national DMFT index of 4.6 in 2013.[3]

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Streptococcus mutans is a gram-positive facultative anaerobic bacteria that is associated with dental caries.[4] It plays a very important role in the etiology of caries, with its ability to attach to the enamel salivary pellicle and to other plaque bacteria. It is a strong acid producer that can create an acidic environment, increasing the risk of cavities. *S. mutans* possesses a higher ability than other gram-positive organisms to metabolize carbohydrates and synthesizes all necessary amino acids. Usually, the presence of *S. mutans* in the tooth cavities is followed by the appearance of caries after 6–24 months.[5,6] A recent study discovered that oral biofilm plays a significant role showing a higher level of tolerance against multi-drugs compared with its free-swimming planktonic counterpart. Previous studies have discovered that bacterial biofilms have 100 to 1000 times more tolerance to antibiotics compared to planktonic bacteria.[7]

Caries prevention and plaque control can be performed using mechanical, chemical, or a combination of both the methods. Toothpaste is used to mechanically clean the teeth surface of plaque and to eliminate bad breath. However, the use of toothpaste containing detergents can dry the oral cavity, which accelerates dental plaque growth, reduces saliva secretion and irritates the

oral mucose.[8] Recently, tooth mousse has gained popularity as a chemical agent that helps protect the teeth, with its basic ingredients being water. It is also sugar and fluoride free, thus making it safe for use by children aged <6 years.[9]

Presently, the use of traditional medicine derived from natural plants is in increasing demand that can be attributed to the human tendency of going back to the nature.[10] One of the natural plant derivatives that has beneficial effects on the human body is virgin coconut oil (**VCO**). Oil from coconut is saturated and has proven effect against bacterial, viral, and fungal infections. The saturated fats of VCO are dominated by lauric acid (44%–52%), followed by myristic acid (13%–19%) and palmitic acid (7.5%–10.5%).[11] Lauric acid can kill gram-positive or gram-negative bacteria, whose cell membranes contain fat, by destroying or inhibiting protein synthesis of the bacteria. Lauric acid has a nonpolar nature, enabling it to penetrate the bacterial cell membrane to cause damage to the phospholipid bilayers, resulting in cell lysis.[12] Lauric acid has been reported to be effective in inhibiting the growth of *Staphylococcus, Listeria monocytogenes*, and *Streptococcus.*[10,13]

The present study aimed to analyze the antibacterial effect of VCO mousse toward *S. mutans* in children with ECC.

Materials and methods

The present study was conducted at the pediatric dental clinic of RSKGMP Faculty of Dentistry in Universitas Indonesia from May to June 2018.

Preparation

Before conducting the study, the research team obtained approval from the ethical commission. VCO mousse was processed by a chemist in Akademi Farmasi IKIFA while the research team prepared other materials needed for the research. Sample was obtained from subjects who fulfilled the inclusion criteria after checking the oral hygiene and the DMFT index. If the patient showed willingness to be a research subject, one of the parents was asked to provide signed and informed consent.

Plaque Sampling

Dental plaque was collected by rubbing across all upper and lower teeth using a sterile toothpick. Then, the toothpick was placed into 1.5-mL Eppendorf tube containing the Brain Heart Infusion Broth (BHIB). The tube was placed inside a cooler box with ice and then transported to the Oral Biology Laboratory to begin the process of bacterial incubation.

Bacterial Culturing

Eppendorf tubes containing the samples were vortexed, and 20-µL of each sample was spread across TYS20B agar plates, followed by incubation at 37°C for 24 h.

Identifying *S. mutans* using PCR Technique

After incubation for 24 h, the colonies on the agar plate were transferred into TYS20B broth, followed by DNA extraction by PCR. Identification of the bacteria was performed by PCR (Dreamtaq Green PCR Master Mix). After amplification, the PCR products were analyzed by electrophoresis on an agarose gel. The newly synthesized DNA fragments were visualized under ultraviolet light.

Biofilm Formation

BHIB containing samples were incubated at 37°C for 24 h. Two 96-microwell plates were prepared for the treatment groups: VCO mousse (8%, 0.8%, and 80%), mousse base without active ingredient, and positive and negative controls. The positive control is CPP-ACP and the negative control is the media. The unique combination between CPP and ACP creates a strong bond with biofilm on the surface of the tooth resulting in fulfillment of mineral balance and strengthen of the hard tissue. One-well plate was used for crystal violet assay and another for the colony-forming unit (CFU) assay. Next, 100 µL of the bacteria was inserted into each well (duplo) with prior dilution, followed by incubation into the well at 37°C for 24 h. After the incubation period, the well was rinsed twice with 100-µL of sterile PBS using multichannel pipettes. Next, 100 µL of each concentration and 50 µL of BHIB was added into each well, followed by incubation of the plate for 24 h. After incubation, the wells were rinsed once again with 100 µL of sterile PBS.

Viability Assay

Crystal Violet Assay

After incubation, the microwell content was removed by blotting with paper towels and washing twice thoroughly twice with 100-µL sterile PBS. Crystal violet (200 µL, 0.5%) was added to each well with a micropipette and then incubated at the room temperature for 15 min. Next, crystal violet was removed, and the wells were rinsed with 200 µL of sterile PBS. Ethanol (96%; 200 µL) was next added into each well. The viability of *S. mutans* biofilm (optical density; OD) was finally measured at 490 nm using a microplate reader.

CFU Assay

Biofilm was removed from the well plate by scrapping the well with a micropipette and transferred into 1.5-mL Eppendorf tube. In another Eppendorf tube containing 990 µL of sterile PBS, 10 µL of bacteria was added from the initial stock. The tube was homogenized, and the process was repeated once more for another dilution. From each dilution, 5 µL of the broth was spread onto BHI agar plate, and the plates were incubated in a 37°C incubator for 24 h. The colony formed on the plate was then counted to estimate the total viable colony counts from each plate (CFU/mL). The plate was put against a dark background for better viewing and the colony was counted manually by the researcher.

Statistical Analysis

Data analysis for the viability of *S. mutans* biofilm was performed using one-way analysis of variance (ANOVA) (P<.05). Statistical significance between the groups was analyzed using post-hoc LSD (P<.05).

Results

Table 1 presents the distribution data of OD value and viable colony counts (CFU/mL). Based on the normality test (Shapiro–Wilk), the distribution data for both the assay were normal (P>.05). The *S. mutans* biofilm viability of each group was compared using one-way ANOVA. Figure 1 depicts comparison of the OD value between different groups using one-way ANOVA. The difference between the groups was statistically significant (P<.001). The mean OD for 80% VCO mousse was 0.115, whereas those for 0.8% and 8% VCO were 0.978, 0.127, and 0.051. Figure 1 also presents the comparison between the 2 groups using post-hoc Bonferroni test where (*) means P<.001 or statistically significant. Comparison between 80% VCO mousse and 0.8% and 8% VCO mousse was statistically significant (P=.001). However, difference between 80% VCO mousse and CPP-ACP was not significant (P=1.00). Similar outcomes were found between 80% VCO mousse and negative control (P=.917). Figure 2 presents the comparison of total viable colony counts (CFU/mL) between different groups using one-way ANOVA. The difference between the groups was significant (P<.001). Figure 2 also presents the comparison between 2 groups using post-hoc Bonferroni test where (*) means P<.001 or statistically significant. It was found that the mean colony counts for 80% VCO mousse was 167.25, whereas those for 0.8%, 8%, base, and negative control of VCO were 555.25, 533.75, 555.5, and 566, respectively. The difference among the groups was significant (P<.001). Significant difference was noted between 80% VCO mousse and 0.8% and 8% VCO mousse (P=.001). However, difference between 80% VCO mousse and CPP-ACP was not significant (P=1.00).

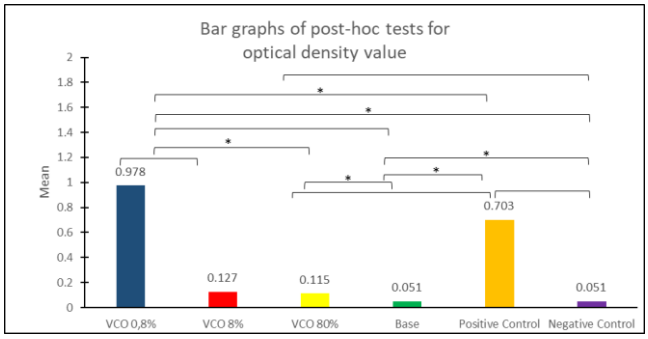

Figure 1. Bar graphs of post-hoc tests for optical density value between groups

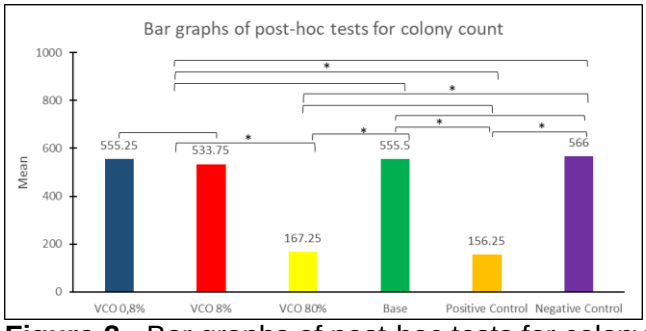

Figure 2. Bar graphs of post-hoc tests for colony count between groups

Treatment group	N	Shapiro–Wilk (P)	
		Optical density	CFU (CFU/mL)
VCO *mousse* 0.8%	4	.107	.740
VCO *mousse* 8			
VCO *mousse* 80%	4	.796	.139
Positive control (CPP-ACP)			
Negative control	4	.842	.107
Mousse base			
	4	.634	.207
	4	.071	.299
	4	.108	.910

Table 1. Shapiro–Wilk test for Optical Density and Colony Count (CFU/mL) of *Streptococcus mutans*

Discussion

Dental caries has a high incidence rate among other childhood chronic conditions. When compared with other common diseases, dental caries is 5 times as frequent as asthma and 7 times as common as hay fever.[14] Different names and terminologies have been used to refer to the presence of dental caries among very young children. However, the term ECC is becoming increasingly popular among dentists and dental researchers.[2] ECC has an extremely high prevalence among pre-school children, ranging from 17% to 94% in different countries. Overall, 90% of ECC cases are left untreated.[15] ECC is defined as the presence of one or more decayed (non-cavitated or cavitated lesions) teeth, missing teeth (due to caries), or filled tooth surfaces in any of the primary teeth in children aged ≤71 months. In children aged <3 years, any sign of smooth surface caries is indicative of S-ECC.[2]

S. mutans has been reported as the principal etiological agent of dental caries and a normal inhabitant of dental plaque.[16,17] Dental plaque is caused by a complex and dynamic process that involves the progressive destruction of teeth by bacteria. A recent study discovered that oral biofilm has a higher level of tolerance against multi-drugs and antibiotics than planktonic bacteria.[7]

Recently, natural plants have gained popularity in society for their medical purposes. These have important therapeutic properties that can be used in the treatment of emerging and re-emerging diseases. There has been increasingly justified assumption which claims traditional medicine is more effective and economic than modern medicines.[13] Therefore, studies on medicinal plants have been gaining much attention in recent years. Scientists have been consistently trying to find solutions to the problems of multiple resistances to the existing synthetic and conventional antimicrobial agents. Coconuts possess numerous nutritional benefits for the body, some of them being its antibacterial and anti-inflammatory properties. According to a few studies, VCO contains 3 medium-chain fatty acids, lauric, caprylic, and capric acids, all of which possess antibacterial and antifungal effects against lipid-coated bacteria and fungi. Lauric acid is found in high concentrations in breast milk to help protect the developing baby [13,18,19] This statement is consistent with the results stated in the present research. From the results presented in Figure 1 and 2, it can be noted that the viability of *S. mutans* biofilm decreases with higher doses of VCO mousse. The VCO mousse significantly reduced the number of colonies of *S. mutans* when treated with 80% VCO, with results comparable with that of the positive control (CPP-ACP), suggesting its function as an antibacterial substance.

Medium-chain fatty acids have a broad spectrum of microbicidal activity, although the mechanism by which the lipids kill the bacteria remain unknown. However, electron microscopic studies have indicated that they disrupt cell membranes.[20] The authors also demonstrated that a 15-min exposure to monolaurin-a monosaccharide present in coconut oil causes cell shrinkage and cell disintegration of gram-positive cocci.[18] Lauric acid could also inhibit protein synthesis of RNA at the transcription level, which resulted in the inhibition of the translation process. The inhibition of protein synthesis can result in the disruption of the bacterial cell growth metabolism, eventually leading to cell death.[10]

Lauric acid possesses antibacterial activity against a range of gram-positive bacteria but not against many gram-negative bacteria. Collectively, Ja-Hyung et al. suggested that lauric acid is effective as an antimicrobial medication. In addition, lauric acid has extremely low cytotoxicity against host cell. The authors also reported that lauric acid manifested significantly high antimicrobial activity by inhibiting microbial survival and biofilm growth against *S. mutans*.[21]

Padget et al. reported that the high level of lauric acid significantly lowers the film water permeability, which conforms to the assertion that higher the concentration, greater is the antimicrobial effect of an agent against an organism.[20] As observed in figure 2, higher the concentration of the VCO mousse, fewer is the colony count on the agar plate. The difference between VCO mousse at 0.8% and 8% concentrations was not significant. However, the outcomes with these concentrations and 80% concentration of VCO mousse were significantly different. The cell counts of *S. mutans* in the 80% concentration were significantly low compared with those in 0.8% and 8% concentrations. Suhartono et al. found that the minimum inhibitory concentration for VCO against *S. mutans* is 80%,[10] which agrees with our finding that the colony count and viability of the biofilm decreased significantly in 80% VCO concentration. In this study, the 80% VCO mousse concentration did not show any significant difference with the negative control in the crystal violet assay. The well plate showed cloudy appearance despite washing it twice. This may be due to the mousse content, which consisted of aquades, Na-CMC, guar gum, glycerol, propylene glycol, D-sorbitol, titanium dioxide, zinc oxide, Na-sakarin, xylitol, phosphoric acid, and natrium benzoat, leading to bias in the OD results. Similar results were also found in the CPP-ACP group comparison with the negative control. However, based on the viable colony counts, the difference in both 80% VCO mousse and CPP-ACP was significant compared with that of negative control. Therefore, VCO mousse was proven to possess antibacterial ability against *S. mutans* biofilms. Among the 3 concentrations tested in this research, VCO mousse at 80% concentration proved to be the most effective antibacterial agent against *S. mutans.*

Conclusions

VCO mousse demonstrated antibacterial effect against the viability of *S. mutans* biofilm in the plaque of children with ECC. Based on the OD value and CFU assay, VCO mousse at 80% concentration reduced the viability of *S. mutans* biofilm and it also exhibited no statistical difference as compared to the positive control (CPP-ACP), which is the gold standard in ECC prevention treatment.

Declaration of Interest

The authors declare no conflict of interest.

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