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Research Article

ANTIBACTERIAL EFFECT OF VIRGIN COCONUT OIL ON (*ACTINOMYCES SP.)* THAT CAUSES DENTAL BLACK STAIN IN CHILDREN

PRISCILLA LAVINE, EVA FAUZIAH*, MOCHAMAD FAHLEVI RIZAL, SARWORINI BAGIO BUDIARDJO

Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. Email: eva_dens@yahoo.com

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ABSTRACT

Objective: This study aimed to assess the effectiveness of virgin coconut oil (VCO) as a natural antibacterial agent to reduce the viability of the chromogenic bacteria *Actinomyces* sp., which causes dental black stains.

Methods: *Actinomyces* sp. was isolated from the saliva of a child diagnosed with black stain. Each streak of bacteria was cultured on a selective medium *Actinomyces* agar and confirmed visually and through a gram staining procedure. Each bacterial culture was exposed to VCO in concentrations of 12.5%, 25%, 50%, and 100%. Afterward, viability testing with a methyl-thiazolyl-tetrazolium assay was conducted, and the results were read using an enzyme-linked immunosorbent assay reader.

Results: The reduction of bacterial viability of *Actinomyces* sp. showed a significant difference between the negative control group and the groups treated with various concentrations of VCO 12.5%, 25%, 50%, and 100%.

Conclusion: The minimum concentration of VCO necessary to kill *Actinomyces* sp. is VCO 12.5%.

Keywords: *Actinomyces* sp., Dental black stain, Virgin coconut oil.

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INTRODUCTION

Dental black stain is a type of extrinsic discoloration related to clinical and esthetic problems that can occur at any age on primary and permanent teeth [1]. This type of pigmentation is a special form of dental plaque that contains insoluble iron salt and a high content of calcium and phosphate. The black material that is seen is a ferric salt (ferric sulfide) formed by the reaction between hydrogen sulfide that is produced by bacterial action and the iron present in saliva or gingival exudates [1,2]. Chromogenic bacteria such as *Actinomyces* sp. and *Prevotella melaninogenica*, which produce black color pigmentations, are suspected to be the cause of the staining. Of the bacteria that can be isolated from black stains, 90% are facultative aerobic and anaerobic Gram-positive rods, which are identified as *Actinomyces* sp. [3]. Black stain is defined as a thin, black deposit in a narrow line above the free gingiva. It can also present as distinct, dark dots that extend beyond the cervical third of the crown, sometimes affecting pits and fissures [4].

A previous study was conducted in 2012 on saliva samples from 15 children with black stain and saliva samples on 15 children without black stain in Jakarta. This study showed that the quantity of *Actinomyces* sp. in the saliva of children with black stain is higher than it is in children without black stain. However, it was also found that the quantity of *Actinomyces* sp. in the saliva of children did not differ significantly between those with black stain and those without black stain [5].

The cleaning procedure required for the treatment of black stain can be challenging to dentists, especially when the black stain is deposited on pit and fissure areas of the tooth. Even when removal through ultrasonic scaling and polishing with a rubber cup and fluoride pumice have been performed, black stain tends to recur, regardless of the patient's good oral hygiene [3]. Therefore, an antibacterial agent is required to inhibit the growth of the chromogenic bacteria that cause black stain.

Antibacterial mouthrinse has been considered an effective method in controlling dental plaque. These antibacterial agents may be derived from chemical compounds or natural ingredients. Evidence in the dental literature supports chlorhexidine as the gold standard of biofilm-preventing antiplaque and antigingivitis agents [6]. An alternative antibacterial agent can be obtained from coconuts, allowing for the use of natural ingredients to promote dental health. Virgin coconut oil (VCO) is taken from the flesh of fresh coconuts (*Cocos nucifera*) without the application of heat, a process which avoids any alteration of the oil so that the main components such as vitamins, antioxidants, and polyphenols remain in the VCO. Unlike any other cooking oil, VCO does not undergo chemical refining, bleaching, and deodorizing process. VCO appears as clear oil, which has a distinct coconut scent [7,8]. A number of fatty acids were detected within the oil, namely caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, and linoleic acid. However, lauric acid has highest percentage of about 47% in VCO [8]. Lauric acid, the main component of VCO, has antibacterial, antivirus, and antiprotozoal effects. Lauric acid can kill gram-positive bacteria by damaging the bacterial cell membrane, resulting in membrane lysis and inhibited bacterial growth [9,10].

Studies that focus on black stain and its treatment are rarely found in the dental literature. The aim of this study was to assess the antibacterial effects of various concentrations of VCO on the viability of *Actinomyces* sp., which causes dental black stain in children.

METHODS

This study was an *in vitro* laboratory experiment that tested the viability of *Actinomyces* sp. after the administration of VCO in various concentrations. With approval from the ethical committee of the Faculty of Dentistry Universitas Indonesia, the subjects, children diagnosed with dental black stain, were identified. The inclusion criteria were as follows: children aged 4–11 years with a good oral condition, the presence of black stain on at least 10 teeth enamel surfaces, and

parental consent. The exclusion criteria included poor oral hygiene, high incidence of caries (deft >5), and subjects under medical care or taking medicine. A sampling of saliva was performed after parents signed the informed consent.

Saliva collection

Subject was aksed to sit in upright position to collect unstimulated saliva at the bottom of the mouth for 1 min. The collected saliva was then spit into a centrifuge tube.

***Actinomyces sp.* colonization and identification; viability test using an methyl-thiazolyl-tetrazolium (MTT) assay**

The experimental and laboratory study with sample measurement used the Federer formula to obtain 3 samples. Then, duplo was conducted so that each treatment provided 6 samples. The bacterial suspension was transferred to *Actinomyces* agar, which was used as a selective medium for *Actinomyces* sp. The agar plate was placed inside an anaerobic jar that contained 80% N_2, 10% H_2, and 10% CO_2 and was incubated for 48 h at 37°C. The identification of *Actinomyces* sp. culture was performed by visual inspection and a gram staining procedure. Following identification, 200 uL of bacteria was suspended on a 96-well plate and incubated in an anaerobic setting for 20 h at 37°C.

While the bacteria were in the incubator, a VCO testing solution was created in the following concentrations: 12.5%, 25%, 50%, and 100%. Dilution of the VCO was completed using a phosphate buffer solution. Then, the VCO solutions were homogenized to obtain a homogenous solution.

After 20 h, the 200 uL of *Actinomyces* sp. suspended on the 96-well plates were exposed to 100 uL of VCO in various concentrations. Bacteria exposed to the positive control (0.2% chlorhexidine gluconate) and the negative control (without testing material) were also prepared. Then, the bacteria were incubated for 60 min in an anaerobic setting at 37°C. Finally, an MTT solution was poured into each well of the tested material and the tested material solutions were incubated for 3 h. The results of the MTT testing were read using an enzyme-linked immunosorbent assay reader at wavelength of 490 nm.

Statistical analysis

One-way analysis of variance (ANOVA) tests were used to compare the bacterial viability of *Actinomyces* sp. (based on optical density [OD] measures) treated with various VCO concentrations of 12.5%, 25%, 50%, and 100%, a positive control (0.2% chlorhexidine gluconate), and a negative control (without testing material) with a significance value of $p<0.05$.

RESULTS

Data normality was tested using the Shapiro–Wilk test, and the data's score showed a normal distribution. The data for *Actinomyces* sp. after the administration of various concentrations of VCO were analyzed using one-way ANOVA to identify any discrepancies between each bacterial viability score.

Table 1 shows the differences in the *Actinomyces* sp. viability scores after the administration of the antibacterial agent in various concentrations. The ANOVA significance was calculated as 0.003 ($p<0.05$), and at least two groups had significantly different viability means. Since the results of the one-way ANOVA test revealed significant differences, *post hoc* testing was conducted to determine which intergroup had different means of viability scores.

Table 2 shows the results of the *post hoc* analysis of *Actinomyces* sp. Statistically significant differences in the *Actinomyces* sp. viability scores were found between the negative control group and the positive control (0.2% Chx) group, the negative control group and the group treated with VCO 100%, the negative control group and the group treated with VCO 50%, the negative control group and the group treated with VCO 25%, and the negative control group and the group treated with VCO 12.5% ($p<0.05$).

DISCUSSION

This study was conducted to identify the effect of VCO as an alternative antibacterial agent on the viability of *Actinomyces* sp., which causes dental black stain in children. In this *in vitro* laboratory experiment, VCO that was 61.07% lauric acid [11] was used as antibacterial agent in the following concentrations: 12.5%, 25%, 50%, and 100%. Lauric acid is a powerful Gram-positive bacteria destroyer, whose non-polar properties enable it to penetrate the cell membrane, destroying the phospholipid bilayer and resulting in membrane lysis [12].

A cytotoxicity assay can be used to assess the antibacterial effects of certain substances. The cytotoxicity testing used in this study was an MTT assay, which is a standard colorimetric laboratory test that measures viable cells and is stated in OD [13].

In this study, exposure to VCO was completed during the 20-h biofilm formation phase (active accumulative phase), a process which was performed in previous studies in which active growth occurred, enabling antibacterial agents to kill bacteria. In the 4-h biofilm formation phase (adhesive phase), exposure to antibacterial agents did not reveal bacterial growth, and bacterial viability was not detected. In the 24-h biofilm formation phase (maturation phase), bacterial growth slows, increasing the formation of extracellular polysaccharides and the bacteria's resistance to antibacterial agents [14].

The statistical analysis (Table 1) shows that each increase in VCO concentration causes a decline in the bacterial viability value compared to the negative control where there is no testing material. Compared to the positive control, which was a commercially available 0.2% chlorhexidine gluconate mouthrinse, various concentrations of VCO showed the same effect as the gold standard antibacterial agent.

The statistical analysis (Table 2) shows a significant difference in the bacterial viability of *Actinomyces* sp. between the negative control group and the groups treated with VCO 12.5%, 25%, 50%, and 100%. Therefore, in this study, the minimum concentration of VCO necessary to kill *Actinomyces* sp. was VCO 12.5%.

CONCLUSION

Based on our findings, it is concluded that each progressive augmentation of VCO concentrations 12.5%, 25%, 50%, and 100% cause reduction in the bacterial viability of *Actinomyces* sp. The reduction of bacterial viability of *Actinomyces* sp. is significantly different between a negative control group and groups treated with VCO. The minimum concentration of VCO necessary to kill *Actinomyces* sp. is VCO 12.5%.

This study is preliminary and involves direct contact between the antibacterial solutions and *Actinomyces* sp., so it does not accurately describe the situation in an actual oral cavity. Therefore, further *in vivo* study is needed to assess the antibacterial effect of VCO on dental black stain in conditions that resemble the actual oral environment.

Table 1: Differences in *Actinomyces* sp. viability scores after administration of VCO in various concentrations

Treatment group	n	Bacterial viability (OD) means±SD	p value
Negative control	3	0.221±0.003	0.003*
Positive control (0.2% Chx)	3	0.193±0.008	
VCO 100%	3	0.195±0.006	
VCO 50%	3	0.195±0.014	
VCO 25%	3	0.190±0.004	
VCO 12.5%	3	0.193±0.003	

One way ANOVA test; *significant score based on *P*<0.05, OD: Optical density, SD: Standard deviation, VCO: Virgin coconut oil

Table 2: *Post hoc* analysis of differences in *Actinomyces* sp. viability scores in treatment intergroups

Treatment group	Difference in viability means	p-value
Negative control versus positive control (0.2% Chx)	0.271	0.011
Negative control versus VCO 100%	0.253	0.019
Negative control versus VCO 50%	0.255	0.018
Negative control versus VCO 25%	0.305	0.004
Negative control versus VCO 12.5%	0.278	0.009
Positive control (0.2% Chx) versus VCO 12.5%	0.001	1.000
VCO 100% versus 12.5%	0.002	1.000
VCO 50% versus 12.5%	0.002	1.000
VCO 25% versus 12.5%	0.002	1.000

Post hoc Bonferroni test; significant score based on $P<0.05$, VCO: Virgin coconut oil

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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