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THE EFFECT OF VCO TO THE GROWTH OF CANDIDA ALBICANS ON DENTURE BASIS OF ACRYLIC RESIN

Vivi Indah Fatmasari[1], Rebriarina Hapsari[2], Nadia Hardini[3], Endang Sri Lestari[2]
[1]Faculty of Medical Science, University of Diponegoro, Semarang, Indonesia
[2]Department of Microbiology, Faculty of Medical Science, University of Diponegoro
[3]Department of Dentistry, Faculty of Medical Science, University of Diponegoro

ABSTRACT

*Background***:** Acrylic resin was a material of denture base which frequently used, but it still had a weakness that it was porous, then, it was often the breeding ground of *Candida albicans.* Virgin coconut oil (VCO) contained fatty acid which was effective to hamper the growth of *C.albicans*. **Objective:** This research was to identify the effect of VCO on the growth of *C.albicans* on acrylic resin denture. **Methods:** This research was an experimental laboratory research which exerted post-test control group design. The total sample 24 which were divided into 3 groups of treatment and 1 group of control. The treatment covered acrylic plates soaking which have been contaminated with *C.albicans* suspension into VCO in concentration level 25%, 50%, 75%, and sterile aquades for 8 hours. The acrylic plate was vibrated to knock out *C.albicans* and was then continued by colony counting on the media of *Sabouraud Dextrose Agar* (SDA). The statistic test employed *Kruskal-Wallis* and continued to the Post-Hoc test which employed *Mann-Whitney*. **Findings:** The VCO in concentration level 25%, 50%, and 75% affected to inhibit the growth of *C.albicans*. In the *Kruskal-Wallis* test, it was found a significant difference between the number of *C.albicans* on the control group and the VCO in concentration level 25%, 50%, and 75% ($p<0,05$). The *Post-Hoc Mann-Whitney* test indicated no significant difference between VCO in concentration level 25% and 50%, 25% and 75%, also 50% and 75% ($p>0,05$). **Conclusion:** The VCO in concentration level 25%, 50%, and 75% affected was able to hamper the growth of *C.albicans*. Further, there was no significant difference in each VCO concentration.

Keywords: Acrylic Resin, Virgin Coconut Oil, *Candida albicans.*

INTRODUCTION

In this current era, people use denture to replace the missing teeth to substitute the chewing, swallowing, talking, aesthetic, function as well as to prevent anatomical and physiological damage of oral cavity. The removable denture is a denture that replaces one or more teeth or all missing teeth by the tooth proponent, mucosa, or combination from both, and once it can be removed by the patient. The removable denture is classified into two: partial removable denture (*GTSL/Gigi Tiruan Sebagian Lepasan*) and full denture *(GTL/Gigi Tiruan Lengkap*).

The type of removable denture is divided into three based on the material of denture base: the denture of a metal frame, denture of acrylic resin, and denture of thermoplastic (flexible denture). The denture base which is frequently used, acrylic resin. The advantages from the denture base of polymethyl methacrylate acrylic resin are similar color with the normal gingiva and light, therefore, it is comfortable to use. The material of acrylic resin however has a weakness, it is liquid absorbance and porous. Thus, the leftover easily attaches to the tooth and increase the bacteria colony and *C.albicans.*

C.albicans is a normal organism of fungi that colonizes on the intestine, oral cavity, respiratory tract, and vaginal tract. The excessive growth of *C.albicans* on particular conditions can affect opportunistic infection. Moreover, *C.albicans* can cause two kinds of main infection on humans: mild infection, for example oral or vagina candidiasis and systemic infection which can threaten human life.

The use of unclean denture can cause

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to the excessive growth of *C.albicans* which affect to the denture stomatitis. The denture stomatitis is defined as inflammation on oral cavity mucosa which contacts to the denture. The attempt to hamper the growth of *C.albicans* is needed, which covers to denture treatment and denture cleaning. The good denture cleaning contains content of bactericide and fungicide, biocompatible, and easy application.

The efficient technique of denture cleaning is through mechanical and chemical techniques. The cleaning with mechanical technique is done by *brushing* the denture, this step can relieve the leftover which attaches to the denture, but it still has a weakness, since this action cannot reach to undercut. While the denture cleaning with chemical technique is done through the soaking method in persulfate substance which benefits to the cleaning of the overall denture and minimal abrasion. However, the use of this substance can affect allergic reaction as itchiness, irritation, tissue damage, and gum tenderness.

Regarding this problem, it needs another alternative as an *attempt* to hamper the growth of *C.albicans* which is regarded to be more effective and affordable. Virgin coconut oil (VCO) is an alternative anti-bacterial or anti-fungus agent which can be applied to processed coconut products which contain saturated and unsaturated fatty acid content that is nutritious for the body. VCO is the potential to improve body immune system, reduce pain and exhaustion, solve skin problems, prevent osteoporosis, prevent heart disease, arteriosclerosis, reduce symptoms on diabetes disease, and prevent obesity. Moreover, VCO contains many active substances, as capric acid, caprylate, caproic, linoleic, myristate, palmitate, stearic, olerate, and lauric acid in the highest level up to 47%.

The content of the monolaurin compound within the lauric acid can ruin lipid components of the cell membrane because the effect of anti-microbial in this compound is great to kill bacteria or fungus. The content of medium-chain fatty acid in virgin coconut oil is effective to kill *C.albicans* fungi as the alternative to handle the fungal infection which is caused by resistant candida species. Besides, the unsaturated fatty acid is also influential to the *C.albicans* biofilm, since the increase of oxidative stress because of this unsaturated fatty acid is bound on the lipid component. Base on those explanations, the researchers are interested to identify the effect of VCO on the growth of *C.albicans* on the denture basis of acrylic resin.

MATERIALS AND METHODS

This research was a true experimental laboratory research which exerted post0test control group design. The sample of this research was 24 which were divided into 3 treatment groups and 1 control group. The treatment covered the acrylic plate soaking which has been contaminated with *C.albicans* in VCO with concentration level 25%, 50%, 75%, and sterile *aquades* for 8 hours. The breeding of *C.albicans* was in approximately 1 *ose* which inserted into 100 ml of *Sabouraud Dextrose Broth* (SDB) media, and then the acrylic plate which has been sterilized would be inserted in C.albicans suspension and incubated on shaker rotary for 24 hours in temperature 37 oC.

18 *samples* were divided into 3 groups with a concentration level of 25%, 50%, and 75%. They were treated by soaking for 8 hours in VCO, while 6 samples were soaked in *aquades* as control solvent. Each sample that has been soaked would be rinsed in sterile *aquades*, and then put into a test tube containing 10 ml sterile *aquades* to have defoliation of *C.albicans* by exerting vortex mixer for 1 minute and series of multilevel dilution in 10-1,10-2, 10-3. The result from each dilution was put into the Petri cup in about 1 ml and poured into SDA media. After the media has been condensed, the SDA media would be incubated in the incubator

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for 1x24 hours in temperature 37oC, next, it was continued to the calculation of *C.albicans* colony amount in CFU unit/ml by employing this following formula:

$$\text{Figure of Fungus} = \text{Total of Fungus Colony} \times \frac{1}{\text{Dilution Factor}} \Big/ \text{Volume}$$

This *research* exerted the normality test of *Saphiro Wilk*, because the total sample < 50 subjects. *The* data which was acquired have abnormal distribution, therefore, it needed to the non-parametric test of *Kruskal-Wallis* which aimed to analyze the difference between groups, and then continued to Post-Hoc test of Mann Whitney.

FINDINGS

The result of statistic data processing showed the average value standard deviation, median, maximum and minimum value, and value of normality test could be seen on table 1 below.

Table 1. Average Value, Standard Deviation, Median, Minimum, Maximum, and Normality Test on Various Groups

Group	Mean □ SD	Median (min-max)	*Shapiro-Wilk*
Sterile Aquades	9366,67± 6160,73	5850 (5000-19000)	0,020
VCO 25%	33,33 ± 51,64	0 (0 – 100)	0,001
VCO 50%	33,33 ± 81,65	0 (0 – 200)	0,000
VCO 75%	16,67 ± 40,82	0 (0 – 100)	0,000

* Shapiro-Wilk Test (it was significant if p>0,05)

The result of the normality test indicated that the data were abnormally distributed. The data was then tested in the non-parametric test of *Kruskal-Wallis* to identify the difference of VCO groups in several levels of concentration to the growth of *C.albicans* on denture basis of acrylic resin (Minor Hypothesis)

Table 2. Non-parametric Test of Kruskal-Wallis

Group	Median (min-max)	*p*
Sterile Aquades	5850 (5000 – 19000)	
VCO 25%	0 (0 – 100)	
VCO 50%	0 (0 – 200)	0,001*
VCO 75%	0 (0 – 100)	

* Kruskal-Wallis Test (it was significant if p<0,05)

The analysis of the *Kruskal-Wallis* test was aimed to identify a significant difference between VCO groups in several levels of concentration and the control group to the growth of *C.albicans* on the denture basis of acrylic resin with *p*-value = 0,001 (p<0,05). Further, the Kruskal-Wallis test was aimed to indicate the difference at least on two groups, next, it was continued to be tested in the *Post- Hoc* test by exerting the *Mann-Whitney* method which aimed to find out the difference among treatments.

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Table 3. *Post-Hoc* Test by Exerting *Mann-Whitney*

Group	*P-Value*	
	VCO 50%	VCO 25%
VCO 75%	0,902	0,523
VCO 50%	-	0,673
VCO 25%	-	-

* *Post-Hoc* of *Mann-Whitney* (it was significant of $p<0,05$)

Based on the *Mann-Whitney* test, it referred to the result of VCO in *concentration* level 25% up to 75% could hamper the growth of *C.albicans* on acrylic plate. This result was indicated from table 7 that there was not found any significant difference between VCO in concentration level 25% until 75%.

DISCUSSION

The use of unclean denture became a predisposition factor in the excessive growth of *C.albicans*. The use of denture which was not routinely cleaned every night or left overnight in the oral cavity could cause plaque accumulation which was the ground of microorganism breeding. This condition would increase the prevalence of *C.albicans* in the oral cavity.

Generally, the denture could be cleaned in several ways, in mechanical and chemical techniques. The denture cleaning in mechanical technique was done by brushing the false denture, this treatment was able to relieve the leftovers which attached on the denture, however, this technique has a weakness, since this technique could not reach to undercut. Next, the denture cleaning in the chemical technique was done by soaking the false denture within a disinfectant solution. This way was the most effective protection towards the infection from the growth of *C.albicans* on denture. The disinfectant was an attempt which could be used to reduce the pathogen amount on denture.

Based on this research conducted from the immersion of acrylic plates in VCO with various concentrations and immersion of acrylic plates in *aquades* showed that VCO concentrations of 25%, 50%, and 75% could affect the decrease of *C.albicans* colony amount on the denture basis of acrylic resin if it was compared to the soaking process in sterile *aquades.* In short, this research finding referred that VCO resulted in an anti-bacterial effect to *C.albicans.*

This research finding was in line with the previous research done by Arina Novilla, et al (2017) under the title "The Composition of Fatty Acid of VCO which Was Potential as Anti-Candidiasis ". She has demonstrated that the gas chromatographic result of mass spectrometry, fatty acid content in VCO as caproic acid (0,187%), cyclopropane pentanoate acid (0,54%), octanoate acid (1,12%), stearate acid (5,68%), oleic acid (14,09%), palmitic acid (17,16%), myristic acid (28,55%), and lauric acid (32,73%). VCO was able to obstruct the growth of *C.albicans*, since the viscosity of oil contained the main component, as lauric acid 32,73% and myristic acid 28,55% which were able to hamper bacteria or fungus adhesion and plaque congregation.

The main target of fatty acid was the cell membrane which attempted to hamper the growth of fungus. The content of the monolaurin compound in the lauric acid was able to ruin the lipid component of the cell membrane, because of the antimicrobial effect on this compound was great to kill either bacteria or fungus. Furthermore, the caproic acid was able to hamper the hyphae and biofilm formation which functioned to ruin biofilm to obstruct the growth of *C.albicans*. The other component within VCO was unsaturated fatty acid as

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cyclopropane pentanoate acid, oleic acid, and octadecanoic acid which have antimicrobial effectiveness, because the unsaturated fatty acid contained C=C bond content which could get into the cell membrane. This result indicated that saturated and unsaturated fatty acid content in VCO was proven to be able to hamper the growth of *C.albicans.*

The previous research done Burhanuddin, et al (2017) on the inhibitory power of VCO to the growth of *C.albicans* fungi isolated from vaginal swab. The VCO power to inhibit the growth of *C.albicans* was proven on concentration level 90% which indicated to the highest inhibitory power and was shown on the minimum zone score 24,0 mm, while on the concentration level 75% (20 mm), 50% (9,7 mm), 25% (1,9 mm), and negative control (0,0 mm). This result was in line with the recent research that the higher concentration of VCO would result in the greater inhibitory power. This research supported the recent research finding that VCO was effective in the growth of *C.albicans* on the denture basis of acrylic resin and potential as an alternative of denture cleaning.

Based on the advanced test of *Post-Hoc LSD*, it showed that each concentration of VCO (25%, 50%, and 75%) did not result in a significant difference, where *p*-value > 0,05. On the calculation of the fungus barrier, the VCO in concentration level 25% and 50% were found a decline of fungus colony amount in approximately 99,64%, while on VCO in concentration level 75%, the decline of fungus colony in approximately 99,82%. Regarding this research finding, it was referred that VCO resulted in inhibitory power to *C.albicans.*

The research restriction was that the sample in this research was taken from the acrylic plate in square shape and small size, then, the attachment of fungus which found was lesser, which this research did not exert the denture from the patient. This condition was because of the difficulties in sample searching. Moreover, the use of denture at night, poor oral hygiene, and saliva with low PH showed significant relation to the growth of *C.albicans* or bacteria. It was required to the advanced research which exerted the denture of patients in poor oral hygiene to be able to result in more various microorganisms.

The variety of fatty acid and lauric acid content in VCO was affected by the ripeness level of coconut, the height of the growing ground, and the VCO extraction method. On the research done by *Damin*, et al., they have said that the higher growing ground would result in the higher fatty acid content, but the clarity level of VCO would be lower. Next, the research was done by *Prapun*, et al., they have asserted that the result of oil yield and highest fatty acid content were found more in the extraction process in the help of protease rather than in fermentation technique and heat extraction. The ripeness of coconut in the middle time range would contain higher coconut amount than in earlier time and late time. Further research was required to find out the standardization of VCO composition as a disinfectant.

Based on the research findings about the effect of VCO to the growth of *C.albicans* on the denture basis of acrylic resin, the researchers summed up these following points VCO could affect the growth of *C.albicans* on the denture basis of acrylic resin in all levels of concentration (25%, 50%, and 75%) and this research did not find any significant differences in VCO which was able to inhibit or hamper the growth of *C.albicans* in concentration level 25%, 50%, and 75%.

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