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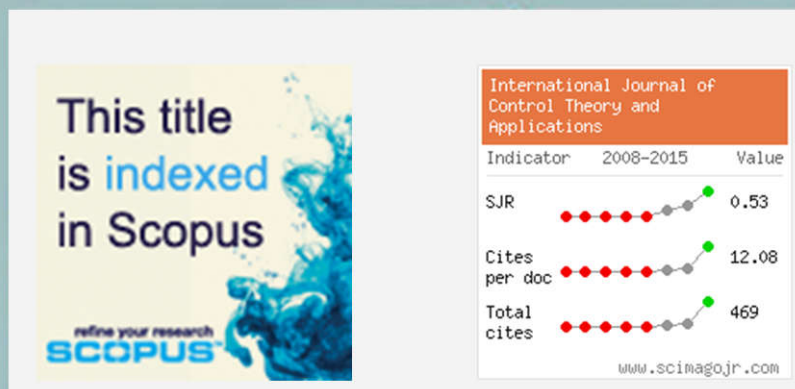
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In Vitro Study of Caries Process in Primary Teeth After Treated by Aloe Vera and Povidone-Iodine

Yetty Herdiyati* Eriska Riyanti* Eka Chemiawan* Diah Prastuti Warastri** Hening Tjaturina Pramesti***

Abstract : The primary teeth are reported to be relatively more susceptible to demineralization by acids than permanent teeth. *Streptococcus mutans* as acid producer bacteria are plausibly involved in demineralization of dental hard tissue. The high incidence of caries in Indonesian children has been an interesting topic of study. The objective of this study was to evaluate two kinds of antibacterial compound (natural and artificial) in inhibiting caries process in primary teeth model *in vitro*. Unfreshly extracted non-carious incisors of mandibular primary dentition were manipulated as tooth model and were divided into two groups, the control group and the group treated with povidone iodine or methanolic fraction of *Aloe vera* at MIC. Samples were then incubated and evaluated clinically after seven and 21 days and then observed using Scanning Electron Microscope (SEM) in order to measure carious lesion. Result showed significant differences in *S. mutans* ATCC 25175 growth between control group and the group given povidone iodine, while the comparison between control group and the group given *Aloe vera* extract showed no significant result in inhibiting *S. mutans* growth. Clinical and SEM analysis showed that povidone iodine was more effective than methanolic fraction of *Aloe vera* in inhibiting caries *in vitro*.

Keywords : Povidone iodine. Primary teeth. Caries. *Aloe vera*.

1. INTRODUCTION

Dental caries is a multifactorial disease. It is characterized by demineralization process in the dental hard tissues that are mainly triggered by microbial activity of dental plaque^{1,2}. Beside microbial activity, caries development process is also supported by host teeth structure (grooves, pits and fissures), time of exposure to caries, diet, salivary buffer capacity, salivary flow rate, carbohydrate intake frequency, the time of exposure to fluoride, and the use of dental sealants^{3,4}. Some other factors supporting the caries formation that are not directly related to oral health are education, lifestyle, dental health knowledge, and socioeconomic factor⁵⁻⁸.

The genus *Streptococcus* presented in the oral cavity includes *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus gordonii*, *Streptococcus sobrinus*, *Streptococcus salivarius*, and *Streptococcus mitis*. *Streptococcus mutans* are capable of synthesizing glucan from sucrose. Glucan is sticky, improves the efficiency of adhesion to the salivary pellicle and plaque bacteria, and ultimately increases the proportion of *Streptococcus mutans* in dental plaque. *Streptococcus mutans* is also an acid producer that makes the surrounding environment acidic thereby increases the risk of caries. Acid is able to break calcified dental tissues and in general

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begins the process of demineralization of enamel caries^{1,2}. The higher number of *Streptococcus mutans* in saliva was reported higher in the Decay Missing Fill Teeth (DMFT) score³.

A global survey found that dental caries is still a primary health problem worldwide, especially in developing countries⁹. Data obtained by Ministry of Health Republic of Indonesia in National Report of Basic Health Research 2013 showed that Indonesian children aged 12 year of age has DMFT index of 1.4. As many as 10,4% children one to four year of age, 28.9% children five to nine year of age, and 25.2% children 10- 14 year of age experienced oral problems¹⁰. So, the study focus on efforts to prevent or lowering caries prevalence are still needed.

Children with dental caries experience aches, pains, and tooth loss leading to difficulty of chewing and eating that influenced body weight and growth development, especially the growth and development of the jaw^{11,12}. Caries prevention can be done by using chemical compounds that contain antimicrobial agent. Some of them such as *Aloe vera* extract and povidone iodine have been reported to have the ability as an anti bacterial agent that change the composition of dental plaque flora, and reduce the incidence or severity of caries^{11,13,14}. According to above reasons explained, this study clinically and microscopically observed the caries process characteristics of primary teeth after conditioned in saliva with high sugar levels and cariogenic bacteria (*Streptococcus mutans*). Then, treated by methanolic fraction of *Aloe vera* and povidone Iodine as antibacterial agent. The objective was to evaluate two kinds of antibacterial compound (natural and artificial) in inhibiting caries process of primary teeth model *in vitro*.

2. MATERIALS AND METHODS

MATERIALS

Unfreshly extracted non-carious incisors of mandibular primary dentition without hypoplasia or calcified enamel, 10% Povidone-Iodine, pharmaceutical formulation (PI) dissolved in phosphate buffer saline pH 7 and methanolic fraction of *Aloe vera* as antimicrobial agent, artificial saliva (Oxoid), 2% sucrose, Mueller-Hinton Broth (MHB) (Oxoid).

3. BACTERIAL STRAINS AND CULTURE CONDITIONS

This study used *Streptococcus mutans* ATCC 25175. The bacteria were grown in MHB, and were incubated at 37°C for 24 hours, under anaerobic conditions enriched with 5% CO₂. The inoculum concentration used was equivalent to 0.5 McFarland solution (10⁷ CFU/mL).

4. METHODS

The protocol study was approved by the Health Research Ethics Committee, Faculty of Medicine University of Padjadjaran, Bandung-Indonesia.

Determination of Minimum Inhibitory Concentration (MIC) of Povidone-iodine solution and methanol extract of *Aloe vera* towards *Streptococcus mutans* ATCC 25175.

Preceding the caries model study, the MIC of PI solution towards *Streptococcus mutans* ATCC 25175 was conducted at the plate of 96 wells with slight modification^{15,16}. All wells were loaded by 0.1 mL MHB, then were divided into four categories *i.e* wells which were added by 0.01 mL inoculum and 0.1 mL PI at several different concentrations; added by 0.01 mL inoculum, and added by 0.1 mL PI, and last contained MHB only. After incubating at 37°C in anaerobic conditions for 24 hours, the optical density of solution in each well was measured using spectrophotometer methods ($\lambda = 600$). Because methanolic extract of *Aloe vera* was very viscous, the MIC of *Aloe vera* extract towards *S.mutans* was determined by inhibition zone method^{15,17}.

5. CARIES MODEL STUDY

Tooth samples were cut at its root until cervical region using diamond disc. The left soft tissues were then cleaned, polished by pumice, washed by 0,9% NaCl to dismiss pumice, keep in 0.9% NaCl and dried until used.

Dried crowns were polished using nail polish. About 3x3 mm of the labial areas were kept unpolished which was objected to caries process. After drying, crowns were put in wide-mouthed glass bottle which has been filled with artificial saliva, 2% sucrose, and 0.01 mL *S.mutans* inoculum¹⁸⁻²¹. Each bottle contained four teeth. The bottles containing teeth were divided into two groups. First group was control bottles containing medium, inoculum, and tooth only. Second group was treatment bottles which were added by 0.1 mL of PI at MIC. All bottles were then incubated for 48 hours at 37° C in anaerobic condition. Every 48 hours, the medium and inoculum were replaced to the new one to obtain cariogenic conditions.

The evaluation of caries occurrence was performed after seven and 21 days of incubation. In the seventh day, tooth samples from one bottle were washed with 0.9% NaCl, dried, and then examined clinically according to the criteria of the International Caries Detection and Assessment System (ICDAS)^{22,23}. After clinical examination, the teeth samples were prepared for Scanning Electron Microscope observation (JEOL JSM 636 @ 0 LA JAPAN). SEM examination conducted at the Laboratory of Polymer Physics Research Center Test-LIPI Bandung, Indonesia. Carious lesion was measured using SEM photograph. After 21 days of incubation, the measurement of caries was repeated.

6. RESULTS

Results of inhibition zone of methanolic extract of *A.vera* against *S.mutans* ATCC 25 175 showed that the ethanolic extract of *A.vera* at dilution of 50% was MIC. It is the lowest concentration of extract that can inhibit the growth of colonies of *S.mutans* ATCC 25175 on solid medium. Meanwhile according to optical density measurement, it was noted that the MIC of PI towards *S.mutans* ATCC 25175 was 0.313% or it was the lowest concentration of PI that inhibit cell growth of *S.mutans* ATCC 25 175 on liquid medium.

The characteristics of caries process in model study are presented in Table 1 and 2. There was a clinically significant difference between the control group on day 14 with the group treated with povidone iodine (p -value = 0.0001). Between the control group on day 21 and group treated with povidone iodine, significant difference was seen with p -value of 0.0028 (Table 1). In the SEM examination, the difference between the control group day 14 and the group of teeth treated with povidone iodine was not statistically significant (p -value = 0.0914). In the control group treated with povidone iodine, visible difference was highly statistically significant (p -value = $7,32 \times 10^{-6}$) (Table 2). The analysis indicated that there are significant differences between the clinical control group with the group given povidone iodine.

Aloe vera treatment did not show clinically significant difference between the control group on the 14th day and the group receiving treatment (p -value = 0.0628). In the control group on the 21st day, *Aloe vera* group also did not show significant difference (p -value = 0.3464). SEM examination was not visibly different statistically significant (p -value=0.5360) between the control group on day 14 and the group treated with *Aloe vera*. The same result was found in the control group on day 21, showing that *Aloe vera* group had no significant difference with the control group (p -value = 0.0962). These results indicate no clinically significant difference between the control group with the group given *Aloe vera*.

Clinically significant differences appear between the povidone iodine group at day 14 with the group treated with *Aloe vera* (p -value = 0.012). In the group treated with povidone iodine day 21 compared to group of *Aloe vera*, significant difference occurred (p -value = 0.0037). Significant difference was seen between the SEM examination of group treated with povidone iodine day 14 and the group treated with *Aloe vera* (p -value = 0.2652). On the day 21, comparison of povidone iodine group with group of *Aloe vera* showed a significant difference (p -value = 0.0144). The analysis showed clinically significant differences between groups treated with povidone iodine with the group given *Aloe vera*.

The difference in each treatment group was seen from the results of the Wilcoxon-Mann Whitney test in order to compare the value of significant level (p -value), the mean, and the median value of each treatment group. The analysis showed that there are high significant results among all treatment groups (p -value = $3,42 \times 10^{-5}$; $\alpha < 0.05$), as well as for SEM examination that showed a high significant results (p -value = 0.0007 ; $\alpha < 0.05$). Average rank showed that povidone iodine had better antimicrobial effects than *Aloe vera*.

7. DISCUSSION

The effectiveness of povidone iodine in inhibiting caries in this study was higher than the ethanol extract of *A. vera*. This can be explained because povidone-iodine as noted earlier is a material that has a broad spectrum of antibacterial properties, stable, has a range of activities suitable pH 3-6 with a pH where the bacteria *S. mutans* life. In the MIC test, it also appears that the povidone-iodine with 0.313% concentration has been able to inhibit the growth of *S. mutans* cells. The ethanolic extract of *A. vera* 50% was less effective in inhibiting dental caries in the model in this study, because the extract is a mixture of several compounds with only slight antibacterial compounds contained in the extract. SEM observation results also support that after administration of the *A. vera* extract in dental caries models, some samples were still on the ordinal scale 3 which means that enamel surface was eroded because of the opening of dentinal tubules.

Bactericidal effect of povidone-iodine contained in the existing concentration of free iodine. In pharmaceutical products containing iodine and iodide, the bactericidal effect remains dependent on the free iodine in the product. Active iodine reacts in electrophilic reactions with respiratory chain enzymes and amino acid bacterial cell membrane proteins found in the cell wall, thus damaging the structure of the respiratory chain and cell membranes. Povidone-iodine with a concentration of more than 0.5% are virucidal. In vitro studies showed that 0.25% Povidone-iodine solution can stop the activity of the HIV virus in a few seconds²⁴.

Aloe vera is a succulent plant (aqueous) of the family of Liliaceae. Trunk or midrib *A. vera* contains a lot of water for as much as 98-99% and 1-2% active ingredient. The active ingredients are owned by *A. vera*, including aloeferon, aloesin, aloin, alo-emodin, aloemannan, acemannan, aloeride, naftoquinones, methylchromones, flavonoids, saponins, sterols, amino acids and vitamins. Anthraquinone compound group serves as an anti-septic. However, the amount of active ingredient in each plant is different, depending on the type, species, and plant growth. In this study maybe methanolic fraction of *Aloe vera* used containing only a low concentration of anti bacterial components. It is concluded that povidone iodine is more effective than methanolic extract of *Aloe vera* in inhibiting caries in vitro.

ILLUSTRATION (TABLES)

Table 1. The result of clinical observation of caries process on tooth model.

<i>tooth sampel from bottle</i>	<i>control</i>	<i>50% Aloe vera methanolic fraction</i>	<i>0.313% povidone iodine</i>			
	C14	C21	A14	A21	P14	P21
A	2	2	2	2	0	0
B	2	2	1	2	0	1
C	2	2	1	1	1	1
D	2	2	1	2	1	1
E	1	1	1	2	0	1
F	1	2	2	2	1	1
G	2	2	2	2	1	1
H	2	3	1	2	1	2
I	2	3	1	2	1	2

Table 2. The result of SEM observation of caries process on tooth model.

<i>Tooth sampel from bottle</i>	<i>Control</i>		<i>50% Aloe vera methanolic fraction</i>		<i>0.313% povidone iodine</i>	
	C14	C21	A14	A21	P14	P21
A	2	3	1	3	2	2
B	1	3	2	3	1	1
C	3	3	3	2	1	1
D	1	3	2	3	2	2
E	1	3	1	1	1	1
F	2	2	2	2	2	2
G	2	2	1	2	1	1
H	2	3	2	3	1	1
I	3	3	1	1	1	1

Note: C14, Control bottle that was examined on the day of 14 after the beginning study

A14, The treatment bottle that was examined on the day of 14 after treatment of *Aloe vera* methanol extract

P14, The treatment bottle that was examined on the day of 14 after treatment of Povidone-iodine

Number 0, no clinical observation

Number 1, 2, 3 is a severe degree of caries according to ICDAS²².

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