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**THE CHANGE OF SALIVARY pH AFTER RINSING WITH FUJI APPLE
 (*Malus sylvestris*) EXTRACT 100% ON CHILDREN AGED 8-10 YEARS.**

(Review on students in SDN Pengambangan 3 Banjarmasin)

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ABSTRACT

Background: The age of 8-10 is the age group with highest number of dmft in children. The normal salivary pH can prevent the formation of caries. Fuji apple has contents that are antibacterial such as flavonoid, catechin and tannin that reduce the production of salivary acid by *Streptococcus mutans* bacteria. **Purpose:** To analyze the difference change of pH saliva before and after rinsing with Fuji apple (*Malus sylvestris*) extract 100% and Chlorhexidine 0,2% in oral cavity of children aged 8-10 years old. **Method:** This study used quasi experimental method with pre-post test control group design. The samples were determined by purposive random sampling on children aged 8-10 years old in SDN Pengambangan 3 Banjarmasin with the total of 63 people. Samples were divided to 3 treatment groups which are rinsing with Fuji apple (*Malus sylvestris*) extract 100%, Chlorhexidine 0,2% and aquades. **Result:** The result of One-way Anova test showed that there was difference on the three treatment groups ($p=0,0001$). The result of Posthoc Bonferroni test showed that there was significant difference between Fuji apple (*Malus sylvestris*) extract 100%, Chlorhexidine 0,2% ($p=0,016$). There was significant difference between Fuji apple (*Malus sylvestris*) extract 100% and Aquades ($p=0,0001$) and between Chlorhexidine 0,2% and aquades ($p=0,0001$). The increase of salivary pH was found in three groups treatment. **Conclusion:** There is significant difference between groups that rinse with Fuji apple (*Malus sylvestris*) extract and Chlorhexidine 0,2% in children aged 8-10 years old.

Keywords: Fuji apple extract 100%, Chlorhexidine 0,2%, salivary pH.

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INTRODUCTION

Caries is a disease on the hard tissue of a tooth (enamel, dentine and cementum). Caries is caused by four main factors: host, microorganism, substrate and time. One of the host factors that plays an important role in caries formation is salivary pH. Saliva acidity (pH) is one of the important factors that can affect the health of oral cavity. Under normal circumstances, salivary pH ranges from 6.8 to 7.2. According to Dwitha's study, a group of children with oral caries-free conditions (dmf-t value = 0) have higher salivary pH than oral cavities that have worse tooth decay. Maintaining salivary pH to remain at normal level is important because the acidic of oral cavity causes an increase in the number of cariogenic bacteria and an increase in resistance of the bacteria.^{1,2}

During caries formation there is an imbalance between the mineralization and demineralization process on the tooth surface. Demineralization of the tooth is caused by the acid conditions which is the result of substrate fermentation process by microorganisms. The main microorganism in the

formation of caries is *Streptococcus mutans*. *Streptococcus mutans* is one of the cariogenic bacteria that produce acid. The produced acid causes a decrease in the pH in the mouth to the level of 5.5 to 5, and increases the demineralization process to the formation of caries.^{3,4} According to Zhou's research, the pH level of the oral cavity has a correlation to tooth decay. DMF-T levels have shown to be higher in low pH conditions.⁵

Caries is often found in children, especially in children who are still in elementary school. Based on the research of Adhikari, the age of children who have the highest dmft (decayed, missing, filled teeth) is in children aged of 8-10 years. The high rate of tooth decay at the age of 8-10 years is caused by the lack of knowledge about oral health and also their diet is not good for oral health.⁶

Various ways can be done to prevent the formation of caries other than toothbrush, one of which is to use mouthwash. The mechanism of neutralization of pH by mouthwash is obtained from mechanical stimuli, chemical stimuli and antibacterial properties of the mouthwash.

Mechanical stimulation occurs because the pressure from rinsing and chemical stimuli is obtained by the stimulation of taste, especially the taste of acid. Chemical and mechanical stimuli able to activate the salivary secretory cell receptors in the oral cavity and increase the salivary secretion. Increased in salivary secretion rises the content of bicarbonate in saliva which acts as a neutralizer of salivary pH.

The commonly used mouthwash in the community is Chlorhexidine gluconate 0.2%. Chlorhexidine gluconate has a wide spectrum antibacterial properties. In Mangundjaja research shows that rinsing using Chlorhexidine gluconate 0.2% can neutralize pH which initially has an acid pH induced by *Streptococci* bacteria. Based on a research by Ayub, Chlorhexidine as an effective mouthwash for salivary bufferisity capacity which effective in neutralizing the pH of the oral cavity. Lack of Chlorhexidine gluconate as a mouthwash is it causes burning sensation, changes in taste sensation, discoloration of teeth and tongue, erosion of the oral mucosa, and its uncomfortable taste.^{7,8}

Many people choose natural ingredients as their choice because they are effective, safe and inexpensive. At concentration of 100% Fuji apple extract (*Malus sylvestris*) is proven to reduce the bacteria *Streptococcus mutans*.⁹ Content of Fuji apple extract (*Malus sylvestris*) such as flavonoids, catechins and tannins have shown to have antibacterial properties. Antibacterial mechanisms of flavonoids, catechins and tannins are by destroying the cell wall and disrupting metabolism in bacteria. As the number of gram-positive bacteria decreases in an acidic oral cavity, there salivary pH neutralizes. Reduction of gram-positive bacteria and salivary neutralization can prevent the formation of plaque and caries on tooth surfaces.^{10,11,12}

MATERIAL AND METHOD

This research used quasi experimental with pre-posttest with control group design. The sample was taken by purposive random sampling technique. This research began by taking care of the research permit and ethical clearance issued by the Committee of Medical Research Ethics Faculty of Dentistry Lambung Mangkurat University No. 042 /KEPKG-FKGULM/EC/IX/2017. The population in this study were SDN 3 Banjarmasin students aged 8-10 years who met the inclusion and exclusion criteria such as children aged 8-10 years in good health or not taking any medicine. Children aged 8-10 years who have no gingivitis and stomatitis. Children aged 8-10 years who are not in orthodontic treatment which could affect the results of the study. The sample determined using Slovin formula and the total sample obtained was 63 people which then divided into 3 treatment groups which are rinsing with Fuji apple (*Malus sylvestris*) extract 100%, Chlorhexidine 0,2% and aquadest.

The process of making the Fuji apple (*Malus sylvestris*) extract 100% began with washing apples

then dried with room temperature. Apples were then cut into small pieces. Next, the pieces of Fuji apple were blended. The 600 gram sample weight was put into a sterile jar and added with 6 liters of 96% ethanol using a 1:10 ratio, then macerated for 72 hours (3 x 24 hours) in a cool and sheltered place from light with a stirring every 4 hours. After 72 hours, the maseration results were filtered using a funnel coated with filter paper. Then, the liquid was taken evaporated using a vacuum rotary evaporator at a temperature of 30 ° C until the ethanol evaporated. The obtained extract was thick and dark brown with a weight of 400 grams. The extract is then added with 400ml aquadest solution to obtain 100% extract solution.

Subjects who have agreed to informed consent were given a description of the research. For pretest, the samples' saliva was collected by passive drool method, which was by the samples were asked to stand upright and still on floor. The head bent slightly, leaning forward and the mouth remained open and let the saliva flew into the vial bottle for 5 minutes until saliva is obtained as much as 5ml. Saliva pH was then measured using a pH meter

After pretest data collected, the subjects were asked to rinse using all three mouth rinses as much as 15ml for each sample. Fuji apple (*Malus sylvestris*) extract 100% for group 1, Chlorhexidine Gluconate 0.2% in group 2 and aquades in group 3 for 30 seconds. After 10 minutes from rinsing, saliva from the samples was collected. Saliva was collected using the passive drool method (Posttest). Saliva pH was then recorded and tabulated and the data were analyzed.

RESULT

The result of differences in salivary pH change before and after rinsing with Fuji apple extract (*Malus sylvestris*) 100% and Chlorhexidine 0.2% in children aged 8-10 years can be seen in Table 1

Table 1. Mean valuedan standard deviation of saliva pH before and after rinsing with Chlorhexidine 0,2%, Fuji apple (*Malus sylvestris*) extract 100% and aquadeson children aged 8-10 years old.

| Treatment Group | Mean SDBefore | ±Mean ± SDAfter |
|--------------------|---------------|-----------------|
| Chlorhexidine 0,2% | 5,433 ± .323 | 6,157 ± .289 |
| Apple Extract | 5,377 ± .368 | 6,219 ± .358 |
| Aquades | 5,395 ± .482 | 5,738 ± .533 |

Based on the data in Table 1. shows that the group rinsing with Chlorhexidine 0.2% mouthwash, as the positive control group, has an average salivary pH before rinsing of 5.43 and after rinsing is 6.16. In the rinsing group with Fuji apple- (*Malus*

sylvestris) extract 100% had an average salivary pH before rinsing of 5.38 and after rinsing was 6.22. In the negative control group, the rinsing group using aquades had an average salivary pH before rinsing with aquades of 5.39 and after rinsing was 5.74. From the data, it is known that there is an increase in average salivary pH in each treatment group.

Salivary pH data of all treatment groups were analyzed by statistical tests using SPSS 24.0. Based on the test result, the data was normally distributed ($p = 0,073$) and homogenous ($p = 0,076$), so the data analysis continued by *One Way Anova* statistic test with 95% Confidence level. The result from *One Way Anovatest* was $p = 0.0001$ ($p < 0, 05$) which showed that there were differences between each treatment groups.

Based on *Bonferroni Posthoc* test result, it can be understood that there were significant differences between the three treatment groups. The result of *Bonferroni Post Hoc* test can be seen in table 2

Table 2. Result of *Bonferroni Post Hoc* test.

| Group | Chlorhexidine | Extract | Aquades |
|---------------|---------------|---------|---------|
| Chlorhexidine | --- | 0.016* | 0.00* |
| Extract | 0.016* | --- | 0.00* |
| Aquades | 0.00* | 0.00* | --- |

*Note: *= There is a significant difference ($p < 0,05$)

There were significant differences between Fuji apple (*Malus sylvestris*) extract 100% and Chlorhexidine group 0,2% ($p = 0,016$). There were significant differences between Fuji apple (*Malus sylvestris*) extract 100% group and aquades group ($p = 0.0001$) and among Chlorhexidine group 0.2% and aquades ($p = 0.0001$).

DISCUSSION

From this research, it can be concluded that rinsing with Fuji apple (*Malus sylvestris*) extract 100% is effective in increasing salivary pH of oral cavity in children aged 8-10 years. The mechanism of antibacterial action of the Chlorhexidine 0.2% mouthwash is by disrupting the action of dehydrogenase enzyme and adenosine triphosphates located on the surface of bacterial cell wall, thus destroying the metabolism of proteins in bacteria leading to the death of bacteria. In Fuji apple (*Malus sylvestris*) extract 100% antimicrobial properties obtained from the contents of Fuji apple which are flavonoids, catechins and tannins. According to a research by Hamrun (2013), Fuji apple (*Malus sylvestris*) extract 100% is effective against *Streptococcus Mutans* bacteria.

Flavonoids, catechins and tannins are found in many plants that have also been used by researchers as an extract for the creation of alternative ingredients in dentistry such as for mouthwash solution. Flavonoids that are phenol

compounds have antibacterial properties. Flavonoids have a ring B that can form hydrogen bonds and provide a hydrophobic effect on bacterial membrane cells that interfere with the work of bacterial cells resulting in the death of bacterial cells. The ability of hydrogen bonding in flavonoids binds nucleic acids to bacteria. Nucleic acid bonds inhibit mRNA synthesis in the process of transcription and inhibiting DNA replication in cell division processes. The hydrophobic effect affects the permeability of the cell membrane resulting in leakage resulting in the release of essential cell components which lead to the death of bacteria.¹⁰

Catechins have antibacterial properties by disrupting the glycosyltransferase enzymes in bacteria. Glycosyltransferase enzyme is an enzyme that plays a role in the processing of sucrose in the mouth into extracellular polysaccharide (PSE) which is a medium for forming a particle on the tooth surface. Surface attachment by glucosyltransferase enzyme causes the synthesis of glucose complexes from sucrose, where bacterial accumulation occurs on the tooth surface and created a biofilm. Furthermore, the production of acids by bacteria causes a low pH value, so that damage to glycosyltransferase enzyme can prevent the decrease of pH.¹⁰

Tannin has antibacterial action by inhibiting reverse transcriptase enzymes and cell DNA so that new cell divisions are disrupted. A decrease in the number of gram-positive bacteria can affect the pH of the oral cavity. This results in inhibition of acid production in the oral cavity which produced by gram-positive bacteria. This is in accordance with a research by BayyinBunayya, et al who research the effect of sweet starfruit juice on changes in plaque pH and saliva pH, the catechin and flavonoid content found in star fruit proved helpful with increasing salivary pH.¹³ The results of this study were also consistent with study by Manta Rosma. The green tea that also contains catechins and tannins has been shown to increase salivary pH to close to the normal pH level in oral cavity.¹⁴

The acidic flavor of Fuji apples is an effective stimulant to stimulate salivary flow, which also continues to increase salivary pH, because the increase in salivary flow rate is directly in line to the increase in salivary pH. This theory is supported by a research from Indriana which proves an increase in citrus acid impact on increased salivary secretion. The acidic flavor of the Fuji apple activates the central nervous system and the brain stimulated to release noradrenaline that binds with receptors on salivary glandular cells which resulting in increasing of salivary secretion in the oral cavity.¹⁵

The three treatment groups showed significant differences in pH change. In the rinsing group using aquades showed lowest difference in pH increase among the three treatment groups. The aquades group is a negative control group that has no effect on microbes. In a research by Juventus et

al shows that aquades do not form inhibit zones against *Streptococcus mutant* bacteria.¹⁶ Aquades has no antimicrobial ability such as Chlorhexidine 0.2% and Fuji apple (*Malus sylvestris*) extract 100% can be seen from the lowest increase in salivary pH than the increase in salivary pH from Chlorhexidine 0.2% mouthwash and Fuji apple (*Malus sylvestris*) extract 100%.

Based on the research data, the samples in the three treatment groups had salivary pH values below the normal saliva values of 5.43, 5.38 and 5.39. The average of 3 treatment group salivary pH value before rinsing is at a critical pH of <5.5. In critical pH conditions, the demineralization process in the oral cavity occurs more rapidly resulting in easier formation of caries. In the use of Fuji apple (*Malus sylvestris*) extract 100% as a mouthwash obtained an increased in salivary pH. Increased salivary pH occurs due to antibacterial properties in the content of Fuji apple such as flavonoids, tannins and catechins. A decrease in the number of gram-positive bacteria inhibits the formation of an acidic condition so that the oral cavity remains at normal pH conditions. The process of salivary pH increase also occurs due to an increase in salivary flow caused by mechanical and chemical stimulation during rinse. The increasing salivary pH to a normal pH is important in order to prevent an increase in the number of cariogenic bacteria and increased resistance of these bacteria. The effectivity of Fujiapple (*Malus sylvestris*) extract 100% in increasing saliva pH makes Fuji apple (*Malus sylvestris*) extract 100% can be used as an alternative ingredient for mouthwash that can be used as a preventive action of early caries in children. Based on the results of this study, it can be concluded that there is a significant difference between rinsing group using Fuji apple (*Malus sylvestris*) extract 100% and Chlorhexidine 0.2% in the oral cavity of children aged 8-10 years old

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