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THE COMPARISON OF PLAQUE INDEX BEFORE AND AFTER RINSING WITH FUJI APPLE (*Malus sylvestris*) EXTRACT0 100% AND CHLORHEXIDINE 0,2% IN CHILDREN AGED 8-10 YEARS

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ABSTRACT

Background:*The mixed dentition occurs in children aged 8-10 years, the change from deciduous teeth to permanent teeth. The children at this age is susceptible to oral disease because they favor to consume cariogenic foods that can cause the buildup of plaque on the teeth. Plaque is a gray-ish yellow substance that is firmly attached to the tooth surface. Plaque can be controlled with chemical treatment such as using a mouthwash. Chlorhexidine 0,2% is the most potential antimicrobial mouthwash in reducing plaque but chlorhexidine 0,2% has side effects to the oral cavity so herbal ingredients begin to be an alternative choice. Fuji applet (Malus sylvestris) contains flavonoids and tannins that are antibacterial to inhibit plaque growth.* ***Purpose:*** *To know the comparison of plaque index before and after rinsing with Fuji apple(Malus slyvestris) extract 100% solution and chlorhexidine 0,2% in children aged 8-10 years old.****Method:*** *This study used quasi experimental with pre-posttest with control group design The total sample was 62 people, consisted of 2 groups.* ***Results:*** *In the study, there was a greater decrease in plaque index score in rinsing group with Fuji apple(Malus slyvestri) extract 100% than with chlorhexidine 0.2%. The plaque index score decreased in the treatment group by 4.7% and in the control group only 3.1%.* ***Conclusions:*** *There are differences in plaque index scores before and after rinsing with Fuji apple(Malus sylvestris) extract 100% with chlorhexidine 0,2% in children aged 8-10 years old.*

Keywords*:Chlorhexidine 0.2%, Fuji apple (Malus sylvestris)*extract *100%, plaque index*

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INTRODUCTION

Plaque on the teeth is one of the causes of teeth problems that are common in children. Lack of knowledge in children to maintain oral hygiene can cause problems in the oral cavity, such as impaired speech function, mastication and confidence. The children are also susceptible to oral diseases because they favor to eat cariogenic snacks such as candy, ice cream and others.[1,2,3]

The age of 8-10 years is a mixed dentition phase, where the permanent anterior and molar teeth have erupted while the other teeth are still the deciduous teeth. Early prevention is needed to avoid problems during mixed dentition phase and new erupted teeth. One indicator for oral hygiene is the child's behavior from wrong maintenance, it can be seen from the presence of organic deposits, such as food debris, alba material, calculus and plaque buildup.[2,4]

Dental plaque is a gray-ish yellow substance composed of a collection of bacteria bound in the organic matrix and attached tightly on the tooth surface. The first stage of plaque formation is the formation of the pellicle on the transparent colored

tooth surface, then the color will turn yellowish because it is dominated by the proliferating coccus and bacillus bacteria, accompanied by the formation of inter bacterial matrix consisting of extracellular polysaccharide. Various types of other bacteria penetrate the plaque and the plaque matures with the change of environmental from aerobic to anaerobic conditions. Plaque can be controlled by mechanical action and chemical, such as brushing teeth and the use of mouthwashes.[1,5,6]

Chlorhexidine (CHX) is the most potential antimicrobial mouthwash in reducing gram-positive bacteria, one of them is *Streptococcus mutans*. The Sinaredi study states that the chlorhexidine with concentration of 0.2% is effective as anti-plaque. Chlorhexidine is not toxic, but it has disadvantages of brown staining on teeth, changes in temporal sensation of the oral cavity, difficult to clean the tongue surface, and erosion of the mucosa. The number of negative impacts from chemical mouthwash lead to the use of herbs by researchers.[7,8,9]

Herbal ingredients begin to be the society's choice because it is safe, cheap and effective. In some chemical content of mouthwash, there are antibacterial properties that can inhibit plaque formation. The content is also in herbal ingredients, one of them is Fuji apple (*Malus sylvestris*).[10,11]

The Fuji apple has the Latin name of *Malus sylvestris*, a fruit that is rich in polyphenols or phytomechanicals compound in it, such as flavonoids and tannins. Fuji apple (*Malus sylvestris*) contains 110 mg of flavonoids per 100 grams, which is the highest flavonoids compared to other apple varieties. A study by Hamrun et al. proved that Fuji apple (*Malus sylvestris*) extract 100% can reduce *Streptococcus mutans* bacteria, because of the work by flavonoids and tannins. Flavonoids and tannins have antibacterial properties that can kill the gram-positive bacteria such as *Streptococcus mutans* that play a role in plaque formation.[11,12]

The purpose of this study is to compare the plaque index before and after rinsing with Fuji apple (*Malus sylvestris*) extract 100% and chlorhexidine 0,2% in children aged 8-10 years. And hopefully the Fuji apple (*Malus sylvestris*) extract 100% can be used as an alternative herbal mouthwash with antibacterial properties especially for children as a prevention of oral cavity problems.

MATERIAL AND METHOD

This research began with the obtaining research permit and ethical clearance issued by Dentistry Faculty of Lambung Mangkurat University no. 041 / KEPKG-FKGULM / EC / IX / 2017. The research method used was quasi experimental with cross-sectional approach with pre-posttest control group design. This study used purposive random sampling consisted of 1 treatment group and 1 positive control group with total sample of 62 people. 31 students rinsed with 10 ml of Fuji apple (*Malus sylvestris*) extract 100% and 31 other students rinsed with 10 ml chlorhexidine 0.2%. The research was done in SD Negeri Pengambangan 3 Banjarmasin. The preparation of Fuji apple (*Malus sylvestris*) extract was done in Pharmaceutical Laboratory of Mathematics and Natural Sciences Faculty in Lambung Mangkurat University Banjarbaru.

The initial procedure of this research was determination of Research place in Banjarmasin, SD Negeri Pengambangan 3 Banjarmasin City was selected. Research permission was requested from the Education Office of Banjarmasin through the usage of research permit letter and given to the Principal of SD Negeri Pengambangan 3 Banjarmasin to arrange a research in the school. Pre-survey was done to collect initial data and gave explanation about the research procedure to the headmaster of SD Negeri Pengambangan 3 Banjarmasin. The next stage was to determine the division of the sample into 2 groups, the treatment group and the control group. The parents of the sample was given explanation and procedure and time of the research, then given informed consent as consent for their child as research subject.

This research started with the preparation of Fuji apple extract with approximately 5 kilograms of apples. First, the fruit selected according to inclusion criteria which were not rotten, ripe, not broken and odorless while for exclusion criteria were rotten fruit and changed in color. After the selection according to inclusion and exclusion criteria, Fuji apple was washed and wiped for drying and then cut into small pieces. After the apple was cut then the apple smoothened by blender. Next, the blended apple was inserted into a sterile jar and added with 1000 mL of 96% ethanol, then macerated for 72 hours (3 x 24 hours) in a cool and protected from light while being stirred every 4 hours. After 72 hours, the maceration results were filtered using a funnel lined with a filter cloth. Then the filtrate was evaporated using a vacuum rotary evaporator at a temperature of 30 °

C until the ethanol evaporated and only remained 600 ml of liquid Fuji apple extract. The extract was stored in a sterile jar and kept at room temperature protected from the light.

The research was done by gathering the parents and students aged 8-10 years to explain the research procedure and its benefit. Parents were asked to sign the informed consent sheets. The initial clinical examination was performed by calculating the plaque index of 8-10 year old students who had agreed to be the subject of this study. The accumulation of plaque formed on the tooth surface of all samples was examined using a disclosing solution. The disclosing solution was placed on a deppen glass and applied using a cotton bud when there is accumulation of plaque then tooth surface the colored.

Calculation of plaque index was done by using O'leary method. The calculation method was to determine the index of O'leary plaque, that is with the formula below and the resulting value is a number.

$$\textbf{IP O'Leary} = \frac{\text{Total of teeth surface with plaque}}{\text{Total of all teeth surface}} \times 100\%$$

Rinsing activity was performed after initial plaque index score data was collected. The rinsing activity in group 1 was carried out using 10 ml of Fuji apple extract which had been prepared by the researcher. Then the sample rinsed with Fuji apple extract solution for 30 seconds and kept it for awhile and instructed to not swallow he Fuji apple extract in order to avoid undesirable factors. In group 2, instructed as group 1 but using 10 ml of chlorhexidine 0.2%. Researchers and also parents accompanied the samples during rinsing so that the expected results will be maximal and the goal of rinsing with Fuji apple extract solution can decrease the plaque attained. Final examination was done after the students aged 8-10 years were instructed to rinse with Fuji apple extract solution. Clinical examination was done the same as the initial examination.

RESULT

The results showed that there was difference of plaque index on each student before and after rinsing with Fuji apple (*Malus sylvestris*) extract solution 100% and chlorhexidine 0,2%. The mean value of plaque index before and after rinsing on each sample can be seen in Table 1.

Table 1 Mean value of plaque index before and after rinsing with Fuji Apple (*Malus sylvestris*) extract 100% and Chlorhexidine 0,2% on children aged 8-10 years.

Group	**Mean ± SD (mm)**
Apple Extract Before	32,59 ± 0,39
Apple Extract After	28,89 ± 0,60
Chlorhexidine Before	33,72 ± 0,41
Chlorhexidine After	30,62 ± 0,36

The collected data was tested by *Kolmogorov-Smirnov* normality. Significant value from the normality test of the initial plaque index score group treated with Fuji apple (*Malus sylvestris*) extract 100% was p = 0,200 and initial plaque index score of control group with chlorhexidine 0,2% was p = 0,200. And p = 0,200 obtained for the final score of plaque index with Fuji apple (*Malus sylvestris*) extract 100% and p value = 0,200 for final score of plaque index control group with chlorhexidine 0,2%.

Table2.Result of Normality test*Kolmogorov Smirnov*on treatment group plaque index before and afterrinsing with Fuji Apple (*Malus sylvestris*) extract 100% and Chlorhexidine 0,2% on children aged 8-10 years

Group	**Value**
Before Rinsing Extract	.200*
After Rinsing Extract	.200*
Before Rinsing Chlorhexidine	.200*
After Rinsing Chlorhexidine	.200*

* = There is significant difference (p>0,005)

From table 2, the results showed that this research data was normally distributed because p≥0,05. Data analysis continued with parametric test, paired T test. The parametric test requirement is that data must be normally distributed. In paired T tests, the

data variance does not need to be tested because the data groups are paired. Then paired T test was done with value of p = 0.000 (p <0,05), from paired T test result it can be concluded that there was significant difference between initial score of plaque index and after score of plaque index in treatment group with Fuji apple (*Malus sylvestris*) extract 100% and initial score of plaque index and final score of plaque index in control group using 0.2% chlorhexidine also showed significant difference.

DISCUSSION

The results showed that there were differences between plaque index score in rinsing group with Fuji apple (*Malus sylvestris*) extract 100% and control group of chlorhexidine 0.2%. This is due to the high concentration of fuji apple extract used, so the content of flavonoids, catechins and tannins in the extract is higher. This make the fuji apple extract very effective to inhibit the formation of dental plaque.

The result is in accordance with research by Hamrun (2013) which proved that Fuji apple (*Malus sylvestris*) extract can inhibit the growth of *Streptococcus mutans* bacteria at concentration of 100%.[11]

Flavonoid is one of the contents in fuji apple extract that has strong anti-microbial activity, which works by denaturing and coagulating the bacteria's protein cell and damage the cytoplasm membrane. The process of protein denaturation causes the coagulation of bacterial cell wall protein so that the bacteria will die.[11]

Flavonoid compound can lead to the leaking of important metabolites and activate the bacterial enzyme system because it works by destroying the cytoplasmic membrane. This situation can also cause the bacterial death by allowing the nucleotides and amino acids to leak out. Carlo et al. and Estrela et al in their study stated that hydroxyl groups in the flavonoid compound structure can cause changes in organic components and nutrient transport causing toxic effects on bacteria.[10,11,13]

Flavonoid is easier to penetrate the peptidoglycan layer because of its polarity. Polar properties are also owned by gram-positive bacteria. The cell walls of Gram positive bacteria are water-soluble polymers, such as polysaccharides (teric acid) that act as in and out transport for positive ion. The soluble property that shows that the cell wall of gram-positive is more polar. Hamrun et al stated that flavonoids are able to inhibit the bacterial cell wall synthesis and cause disruption in cell wall function. *Streptococcus mutans* bacterial cell wall eventually undergoes lysis process.[10,14]

Catechin is a toxic compound that function to inhibit the activity of glycotransferase enzymes which inhibit the process of plaque formation and bacterial attachment with the pellicle. Catechin is also one of the ingredients found in Fuji apple extract. The mechanism of catechins in inhibiting the growth of *Streptococcus mutans* is in two ways which are as bactericidal and inhibits the glycosylation process. Catechin works as bactericidal by denaturing proteins in bacterial cells resulting in damaging biological activity which cause the proteins unable to functions.[2,6]

Catechins in inhibiting the glycosylation process will work competitively with glucosyltransferase (GTFs) in reducing the basic material of the glycosylation process such as saccharides, so that the formation of extracellular polysaccharides in bacteria is inhibited. Catechin activity in reducing glucose is much greater than the activity of GTFs in using glucose which cause the growth of bacteria to be inhibited.[6,14]

Tannin is one of the content in fuji apple extract that can damage the bacterial cell membranes and inhibit plaque formation. According to Brannen and Davidson, the mechanism of inhibition in bacterial growth by tannins is by inactivation of essential enzymes and destruction or inactivation of functions, and reacting with membrane cells. The toxicity of tannins can damage the cell membranes and wrinkle the bacterial cell walls, resulting in the disruption of permeability on bacterial cells. Finally, bacteria cannot carry out their activities so that the growth is inhibited and even die.[10,15]

Tannin is a compound produced during the growth process. Masduki states that tannins also have the same effect as phenolic compounds, which targets the cell wall polypeptides that cause damage to the cell walls. Phenol compound is easily form

the protein complexes through hydrogen bonds. [13,16]

Hageman et al. states that if hydrogen bonding between tannins and proteins, especially at pH, isoelectric (4-5), the protein becomes deposited. This is called protein denaturation, the enzyme becomes inactive because of the denatured bacteria, so that the bacterial metabolism is disrupted resulting in cell damage.[11,17]

Chlorhexidine gluconate 0.2% as a positive control in this study is a biocide cationic agent that has broad spectrum activities and has the ability to work as anti-bacterial substance against gram-positive bacteria including *Streptococcus mutans*. Chlorhexidine will increase the permeability of bacterial cell walls, and form bonds with components on the tooth surface which cause bactericidal effects of microorganisms on tooth plaque. These drugs have many side effects that include the hypersensitivity reactions and discomfort. Based on the result of this research, it can be concluded that there was difference of plaque index score before and after rinsing with Fuji apple (*Malus sylvestris*) extract 100% and chlorhexidine 0,2% in children aged 8-10 year old.[8,18,19]

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