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Original Research Article

Effectiveness of Lime (*Citrus aurantifolia*) Extract Solution in Inhibiting Bacteria Streptococcus Mutans Case of Early Childhood Caries

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Background: **Early childhood caries is a dental health problem found in children. Mutans streptococci is a bacteria that causes dental caries. Lime fruit has essential oils which have an active antibacterial compound.** ***Aim:*** **This study aimed to see the effect of lime extract 40% for the growth of mutans streptococci in children with early childhood caries.** ***Materials and Methods:*** **This study used a cross-sectional study design and used experiments with pretest-posttest control group design. The study sample comprises 60 children who fit with the criteria. 30 subjects as a control group (rinsed with distilled water) and 30 subjects as a treatment group (rinsed with lime extract 40%). Each sample was given the same intervention, the first step was the collection of saliva before intervention (pretest), in the second step, subjects were given 10 ml of lime extract 40% to rinse their mouth for about 30 seconds. After that, saliva was collected twice in 15 minutes (post 1) and 30 minutes (post 2) after rinse. Furthermore, saliva was taken to the laboratory to count the number of colonies of mutans streptococci bacteria using the Colony Counter method and measured in CFU (Colony Forming Unit). Data processing and analysis was done using the SPSS 22.0 software for windows version.** ***Results:*** **The results of repeated ANOVA and paired t-test showed a significant reduction of the number of mutans streptococci colonies from before rinse to 30 minutes after rinse with lime extract 40%. The number of bacterial colonies on the pre was 72.23 CFU, on post 1 as 103.36 CFU, and the post 2 was 14.03 CFU. Statistical value showed p= 0.000, that means the reduction of mutans streptococci colonies was significant.** ***Conclusion:*** **Lime extract 40% is effective to reduce the growth of mutans streptococci colonies in the mouth.**

Keywords: Lime extract, Essential oils, Streptococcus mutans, Caries.

INTRODUCTION

Prevalence of caries in children is still high. Caries is influenced by several factors such as host, agents, environment, and time. Other factors such as environment and the fluoride level inside water and food like carbohydrate/ sugar, parent's culture and characteristics, and health service availability are also some other important factors for caries development. Research by Soeyoso et al, found that caries prevalence in one of the elementary schools in Indonesia, SD Negeri 161 Palembang is very high, which is 100% with mean DMFT 6.47.

ECC is growing fast and give bad impact to children's health. American Academy of Pediatric Dentistry (AAPD) defined ECC as one or more caries (without cavity or lesion), and the loss of teeth because of caries or deciduous tooth that has filling in children between 0-71 months. Early Childhood Caries is a dental health problem found in most children. Research by Febriana (2007) in five different areas at DKI Jakarta found that Early Childhood Caries prevalence in children below 3 years old is 52.7%

Prevalence of ECC is higher in developing countries. Research on ECC in several continents like Europe, Africa, Asia, and America showed that the highest prevalence is in Africa and South East Asia. In England and USA, the prevalence is around 6.8-12% and 11-53.1%. In the west, prevalence on 3 years old S-ECC is 19.9%, showed that this has strong connection with their socio-economic status.

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Streptococcus mutans is a microorganism which causes dental caries and contributes a lot in the beginning of the process. A lot of methods can be used to prevent caries, one of it is by inhibiting the growth of bacteria that cause caries, which is Streptococcus mutans

Streptococcus mutans produces carbohydrate polymers efficiently on plaque which makes it stick to tooth enamel and produces acid which causes demineralization of enamel where the plaque stuck on and make caries at the end.

There are several factors that contribute to the growth of bacteria. These factors are temperature, food supply, pH, ionic concentration and oxygen, especially obligate aerobic bacteria. Relationship between the amount of bacteria and growth time can be seen the growth curve. The growth curve is divided into four phases, which are; lag phase (initial phase), logarithm phase (quick growth phase), stationary phase (slowing down phase) and degrading phase (dying phase). Explanation of the phases is as follows:

Lag phase

Lag phase is a phase where the bacteria are just adapted to the new environment

Log phase

Log phase, also called the exponential phase. In this phase, there is increase of bacteria amount because it has already adapted well to its environment and its time to multiple has doubled (doubling time). Doubling time is the time needed by cells to multiply to two times their amount.

Stationary phase

Stationary phase is the phase where growth becomes null. In this phase, there is no additional in the amount of bacteria

Degrading phase

Degrading phase or dying phase. In this phase, most of bacteria stopped multiply and dying bacteria increases.

Citrus (*Citrus aurantifolia*) is one of the herbs that is commonly used, both in kitchen and for medication. For medication, citrus are used as appetizers, antipyretic, and antibacterial. Citrus has chemical composition that has many benefits, such as citric acid, amino acid (tryptophan and lysine), atsiri oil (limonene, lacetate lynalin, geranile acetate, fellandren, citral, chamfer lemon, cadinen, acetaldehyde, and aldehyde), vitamin A, B1 and vitamin C. Several studies also showed that citric acid extract also has very high antimicrobial activity.

Citric extracts also have antibacterial activity that has atsiri oil that has phenol that can inhibits staphylococcus aureus. Bactericidal of the phenol will denature protein and destroy cell cytoplasmic membrane. The instability of the cell wall and bacteria cytoplasmic membrane cause selective permeability, active transport function, and bacteria cell protein control are disturbed. Disturbance to cytoplasmic integrity will affect macromolecule and cell ion. Bacteria cell will loose its form and lysis occurs.

PURPOSE OF RESEARCH

This research is to see the effect of gargling with 40% citrus extract to Streptococcus mutans growth in child saliva that has Early Childhood Caries.

BENEFIT OF RESEARCH

1. Help writer to develop knowledge in Dentistry and gaining new information on the effect of citrus extract to Streptococcus mutans bacteria in children that has Early Childhood Caries.
2. Provide information for people for the effect of citrus extract to the growth of streptococcus mutans, which is one of the main cause of decayed teeth in children.
3. As a source of information that can be used to make another research in the effect of citrus (Citrus aurantifolia)

HYPOTHESIS

Citrus extracts 40% can reduce Streptococcus mutans bacteria colony in children' saliva that has Early Childhood Caries.

METHOD

The experiment use cross sectional method with pre and post test control group design. The solution is made using soxhletation process. Sampling methode is purposive in TK Nurul An-Nisa Antang and TK Kartini UNHAS student population. Sample consists of 60 children that match the criteria and divided into 2 groups, 30 control and 30 treatment. Sampling criteria are as follows:

1. Inclusion criteria

a. Sample has minimum 2 dental caries
b. ~~Age~~ Maximum age of 71 months or 6 years old
c. Sample is healthy, and not using antibiotic

2. Exclusion criteria

a. Sample has systemic disease
b. Sample not willing to participate in the research

Each group is given the same treatment, saliva taken before gargling, gargling solution is given (aquades for control group and citrus 40% of treatment group), saliva sample taken 15 and 30 minutes after. Saliva samples were then taken to the laboratory to count the colony. Counting method was by using colony counter with CFU (Colony Forming Unit). Result of research was processed using SPSS version 22 for Windows and analysis done with ANOVA repeated with paired t-test.

RESULTS

Table 1 showed mean Streptococcus mutans bacteria colony count based on age and sex on control group gargle with aquades. Result showed bacteria colony count decreased on post to compared to pre treatment in both characteristics. On age characteristic, highest colony count is for 6 years old, 101.25 CFU for pre, 85.50 CFU for post 1, and 91.00 CFU for post 2. For sex characteristic, highest colony count is for girls, 92.23 CFU on pre, 76.69 on post 1 and 84.53 CFU on post 2.

Table 2 showed mean Streptococcus mutans bacteria colony count based on age and sex on treatment group gargle with citrus extract 40%. Result showed bacteria colony count decreased on post compared to pre treatment in both characteristics.

Table 1. Standard Streptococcus mutans bacteria colony count Standard Deviation mean based on age and sex in control group (Aquades)

Aquades	Pre	Post 1	Post 2
	Mean ± SD	***Mean ± SD***	***Mean ± SD***
Age			
3	92,00 ± 0,00	69,00 ± 0,00	8,00 ± 0,00
4	66,99 ± 0,00	66,00 ± 0,00	78,00 ± 0,00
5	74,00 ± 30,41	72,83 ± 34,84	70,12 ± 34,44
6	101,25 ± 40,16	85,50 ± 34,25	91,00 ± 36,31
Total	77,96 ± 31,62	74,16 ± 33,26	73,63 ± 33,66
Sex			
Boy	66,29 ± 25,38	72,23 ± 36,38	65,29 ± 31,15
Girl	93,23 ± 33,35	76,69 ± 29,95	84,53 ± 34,90
Total	77,96 ± 31,62	74,16 ± 33,26	73,63 ± 33,66

Table 2. Streptococcus mutans bacteria colony count Standard Deviation mean based on age and sex in treatment group (citrus acid 40%)

Citrus Extract 40%	Pre	Post 1	Post 2
	Mean ± SD	***Mean ± SD***	***Mean ± SD***
Age			
3	106,00 ± 0,00	138,00 ± 0,00	29,00 ± 0,00
4	46,00 ± 1,73	84,66 ± 28,29	10,33 ± 3,21
5	75,57 ± 43,54	103,85 ± 39,25	14,28 ± 8,01
6	67,20 ± 33,11	105,60 ± 24,32	12,20 ± 6,97
Total	72,23 ± 39,77	103,36 ± 35,75	14,03 ± 7,84
Sex			
Boy	75,12 ± 39,52	109,62 ± 41,24	16,43 ± 8,72
Girl	68,92 ± 41,29	96,21 ± 28,02	11,28 ± 5,83
Total	72,23 ± 39,77	103,36 ± 35,75	14,03 ± 7,84

For age characteristic, highest colony count is for 3 years old, 106.00 CFU for pre, 138.00 CFU for post 1, and 29.00 CFU for post 2. For this characteristic there is an increase on bacteria colony count from pre to post 1 and decrease on post 2 for both sex. Highest colony counts found in boys, 75.12 CFU for pre, 109.62 for post 1 and 16.43 CFU for post 2. From these two characteristics, total mean Streptococcus mutans bacteria colony count after gargling with citrus extract 40% compared to pre treatment count is 72.23 CFU, 103.35 CFU for post 1 and 14.03 CFU for post 2. Table 3 showed Streptococcus mutans bacteria colony count mean of samples gargle with aquades and citrus extract 40%. The table showed decrease of colony count in every treatment time for control group.

Table 3. Difference of Streptococcus mutans bacteria colony count mean on every treatment between Aquades and Citrus Extract 40%

Solution	Pre	Post 1	Post 2	*P* value
	Mean ± SD	***Mean ± SD***	***Mean ± SD***	***Mean ± SD***
Aquades	77,96 ± 31,62	74,16 ± 33,26	73,63 ± 33,36	0,786*
Citrus Extract 40%	72,23 ± 39,77	103,36 ± 35,75	14,03 ± 7,84	0,000*

** Repeated-Measures Analysis of Variance (ANOVA) test: p < 0,05: significants.*

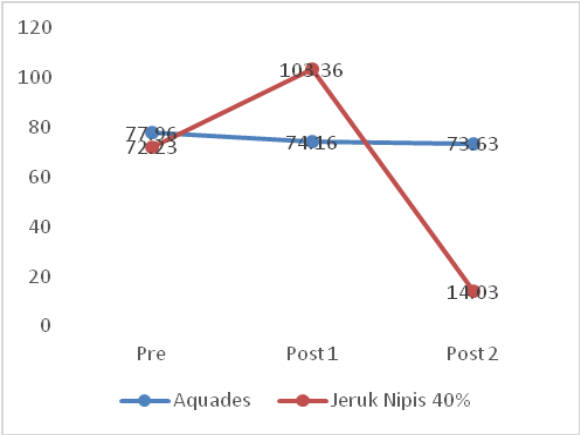

Figure 1. Paired t-test result for control and treatment groups

Table 4. Difference of Streptococcus mutans bacteria colony count mean on every treatment between Aquades and Citrus Extract 40% based on treatment time

	Interval Waktu	***Mean ± SD***	***Mean Difference***	Nilai *p*
1	CFU Pre CFU Post 1	72,23 ± 39,77 103,36 ± 35,75	31,13	0,000*
2	CFU Pre CFU Post 2	72,23 ± 39,77 14,03 ± 7,84	58,2	0,000*
3	CFU Post 1 CFU Post 2	103,36 ± 35,75 14,03 ± 7,84	89,33	0,000*

**Paired sample t-test: p < 0,05; significants*

The count value is 77.96 CFU on pre, 74.16 CFU on post 1, and 73.63 CFU on post 2. Based on ANOVA repeated test, p value is 0.786 (p<0.05; significant). Aquades do not have a significant affect on the growth of Streptococcus mutans bacteria colony count

On treatment group gargle with citrus extract 40% showed an increase on bacteria colony count from pre to post 1, then decreased on post 2. Colony count is 72.23 CFU for pre, 103.356 CFU for post 1 and 14.03 CFU for pot 2. Based on ANOVA repeated test, p value is 0.786 (p<0.05; significant). Citrus extract 40% has significant effect to decrease Streptococcus mutans bacteria colony count. Because ANOVA test showed significance, paired t-test made and the result is showed on Figure 1.

Table 4 showed a difference of Streptococcus mutans bacteria colony count mean on every treatment between Aquades and Citrus Extract 40% based on treatment time using paired sample t-test. First result comparing pre to post 1 bacteria colony count, showed growth increased. Second result comparing pre to post 2 bacteria colony count, showed bacteria growth decreased. Third result comparing post 1 to post 2 bacteria colony count, showed growth decreased.

DISCUSSION

Data shows difference of Streptococcus mutans colony count before and after intervention (pre-post 1-post 2). Table 3 showed that gargle with aquades will cause decrease, but not significant, while gargle with citrus extract 40% can decrease colony count significantly on 30 minutes after gargle (post 2), but the count has an increase on the first 15 minutes. Figure 1 showed the colony count of the bacteria which match bacteria growth phase.

Pre-result showed lag phase (bacteria adapt to the environment), post 1 showed logarithm phase (bacteria adapt to the environment and growth doubled) and post 2 on dying

phase. The result means that gargle with citrus extract 40% can accelerate decrease or dying of streptococcus mutans bacteria.

Decrease of colony count after gargle with citrus extract 40% might be caused by its pH. In this experiment, pH was measured using pH-indicator strips non-bleeding and the pH of the extract is 3 so its acidic. Manta Rosma and Netty Jojor in their journal stated that the lower pH saliva the more acidic it will be and Streptococcus mutans is actually growing better in an acidic environment.

Citric acid has other active ingredients such as atsiri oil, phenol that has bactericidal properties, which can inhibit bacterial growth. Acidity in citrus acid coming from organic acid, content citric acid. In this research, acidity accelerates bacterial growth from pre to post 1, and active ingredients that has bactericidal properties working in minutes 15 to 30 (post 1 to post 2) after gargle which leads to significant decrease of bacteria count. The decrease in control group is not significant may be because of the environment that kept neutral or base.

CONCLUSION

Based on the result of the research, gargle with citrus extract 40% gives significant effect of the decrease of growth of Streptococcus mutans in saliva of children who has early childhood caries. Some things to put attention are as follows:

1. Citrus extract 40% has significant effect to decrease of bacteria 30 minutes after gargle.
2. Citrus extract 40% is more effective to inhibit bacteria growth compared to aquades

SUGGESTION

From research some suggestions are as follows:

1. Further analysis is needed to see the effect of citrus extract 40% to Streptococcus mutans in every phase of bacteria growth
2. Further research with bigger sample to get a more accurate result
3. Consultation from pharmacist is needed to improve flavor of the extract without decrease concentration of the active ingredients.

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