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ORIGINAL ARTICLE

Streptococcus Colonial Growth of Dental Plaque Inhibition Using Flavonoid Extract of Ants Nest (Myrmecodia pendans): An in Vitro Study

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Abstract

Objective: To know the activity of resistance of flavonoid content in ant nest plant in decreasing the number of colonies S. mutans oral cavity of children as a medic herbal material. **Material and Methods:** The subjects were plaque sample of 20 children aged 7-12 years. Research begins by making toothpaste from ant nest extract. Samples of children's dental plaque were inserted into BHIB media, after which incubated for 24 hours, 1/10 dilution with BHIB media three times, followed by TYC media planting and incubation of anaerob with temperature 37^{0}C for 48 hours. After that then count the number of colonies of S. mutans. **Results:** On ethyl acetate extract of ant nest incubated at room temperature with concentration 20%, 40%, 60%, 80%, 100% obtained a decrease from each treatment amount of Streptococcus mutans colony on TYC media with median value of each treatment was 89, 67, 64, 61, 59 and 51 for the ethyl acetate fraction, and 62, 61, 60, 59, 49 at the ethanol fraction. There was no significant difference between the six concentration groups ($p>0.05$). **Conclusion:** Flavonoids extract of ant nest plants have growth barrier on Streptococcus mutans bacteria, the greater the concentration given the greater the number of S. mutans colony.

Keywords: Plants, Medicinal; Flavonoids; Glucosyltransferases; Streptococcus mutans.

Introduction

Dental caries is a problem in oral health in Indonesia with a prevalence of 90.05%, the high prevalence is due to the lack of public awareness of the importance of oral health. Several factors as the cause of dental caries disease include host (tooth), environtment, substrate, microorganism, and time [1].

The first factor of host, concerning the habits of children where they are not diligent to brushing teeth after eating and leaving the rest of the food piled on the sidelines of the teeth causing the teeth easily demineralized thus causing caries. The second factor of environtment is the condition of pH of the oral cavity in children is low, demineralization process can occur because of low salivary pH / oral cavity (acid) [2]. The third factor of substrate is a carbohydrate and sweet food that is easily attached, attached to the tooth surface called debris. The fourth factor is microorganisms, that the formation of caries due to the dominance of a number of colonies of Streptococcus mutans and Lactobacillus which is able to form acids from carbohydrates and attached to the tooth surface due to the formation of extracellular polysaccharides [3].

One effort to inhibit the growth of Streptococcus mutans colony is to utilize one of the plants that are currently popular in the world of treatment namely ant nest plants in Latin called Myrmecodia pendans. Ant nest plant originating from Papua is one of the plants that have been utilized in the treatment of various diseases [4]. Based on some research results ant nest plants contain active compounds flavonoids, tannins, tocopherols and various types of minerals that are very useful. Flavonoids can play a direct role as antibiotics by interfering with the function of bacterial or viral microorganisms, but it also acts as an antioxidant that can form a cell defense mechanism against free radical damage [5].

Ant nest plants with flavonoid content can inhibit the growth of oral microorganisms and bacterial activity by the enzyme Glucosyltransferase (GTFs). Flavonoids act as potent inhibitors of GTF enzyme activity in solution, 5,7-trihydroxyflavone is the most effective inhibitor of the GTFs enzyme. Glucosyltransferase enzyme generated by Streptococcus mutans is known to play an important role as a virulence factor in the pathogenesis of dental caries [6,7]. In the GTF enzyme plays a role in the catalysis of the formation of α-linked glucan either soluble or insoluble from sucrose which further contribute significantly to the matrix composition of polysaccharide dental [8].

Flavonoids found in ant nest plants have activity against oral microorganisms in children. Flavonoids not only suppress the growth of plaque bacteria, but also have the potential to support the successful treatment of periodontal disease in children because it can increase the body's immune system thus it can accelerate the healing of damaged or wounded tissue, such as gum hemorrhage, postoperative wounds, or healing process after periodontal treatment [9].

Streptococcus mutans colony is a collection of gram-positive bacteria, non-motile (immobile), facultative anaerobic bacteria. It has the shape of a round or oval-shaped coccus, without spores, not encapsulated and arranged in rows. These bacteria grow optimally at ambient temperature 18^{0}C-

40^{0}C. Streptococcus mutans are usually found in the children's oral cavity, becoming the most invasive bacteria causing caries in tooth enamel. Streptococcus mutans are asidogenic, producing acidic acid, capable of living in acidic environments, and producing a sticky polysaccharide called dextran. The ability of this Streptococcus mutans to allow easy attachment with other bacteria and to mediate other bacteria attached to tooth enamel, and then produce acidic pruduk that can dissolve tooth enamel (demineralization) [10].

The purpose of this study is to know the activity of flavonoid content in ant plant herbs in reducing the number of colonies of Streptococcus mutans in the oral cavity of children as well as the development of new innovations in health care efforts of the oral cavity of children with the use of medicinal herbs in Indonesia.

Material and Methods

Study Design

This research was conducted in a pure laboratory experimental. The research location was at Phytochemical Laboratory of Faculty of Pharmacy of Hasanuddin University of Makassar.

Participants

Subjects were 20 children with 7-12 year age range in SDN Inpres Polejiwa Maros Regency, South Sulawesi Indonesia.

Ants Nest Extraction Stage

Fresh ant nest plant originated from Jayawijaya Papua as much as 900 grams, extracted using ethanol and then evaporated until produced concentrated ethanol extract. The ethanol concentrated extract was then dissolved in distilled water and subsequently partitioned in a separating funnel using n-hexane to obtain the n-hexane fractions and water (H_2O). The fraction of water obtained subsequently partitioned between water, ethyl acetate and ethanol yields ethyl acetate fraction and ethanol fraction. Dilution of ant nest extracts resulted in some concentration of ant plant extracts used for minimum inhibitory concentrations of ant nest plant extracts that could inhibit the growth of Streptococcus mutans bacteria. In this research it was made dilution as much as 6 concentrations they are: 10%, 20%; 40%; 60%; 80%; 100%.

Stage of making toothpaste: Weigh the Na laurylsulphate 2,5 g plus CMC Na 0,25 g aded with glycerin and CaCo3, then mix and stir until homogeneous. Weigh the propolis extract 1.25 g plus Na laurylsulphate 2,5g and CMC Na 0,25 g plus glycerin and CaCo3, then mix and stir until homogeny. Then calculated from the overall weight of gram (ad 25 gram x 2 = 50 g substracted by 48) obtained aquades required 2 cc. Add oleum mentha piperita 4 drops. Pour pasta paste into toothpaste packaging as needed

Data Collection

To minimize the error rate in the study, a meeting was conducted to equalize the perception among samples. Each child is given the same treatment namely the first 5 days of doing toothbrush without toothpaste, and the next 5 days to do toothbrush with ant flavonoid toothpaste. On the day leading to plaque taking, the sample is instructed not to eat anything or drink a sweet drink after brushing teeth at night. Stages and methods of plaque taking, the morning before the toothbrushing sample, plaque on the posterior tooth surface of the buccal section (first molar left and right) and anterior labial portion (first incisor right and left) and maxillary mandibe, were taken using excavator. With the aid of the excavator, the plaque was inserted into a test tube containing 5 ml sterile Brain Heart Infusion Broth (BHIB) solution, then incubated for 24 hours to dilute 1/10 diluted three times. Then from the dilution was taken as much as 5 ml with micropipette, planted on the media for Trypticase Yeast-Extract Cystine (TYC) and stored in anaerobic atmosphere at incubator with temperature 37^{0}C for 2 x 24 hours, after that we counted the number of colonies of S. mutans.

Data Analysis

All statistical analysis was undertaken using SPSS v. 18.0 (IBM Corp., Armonk, NY, USA) with significance value $p<0.05$.

Ethical Aspects

Subject or parents of subjects were requested to fill out the informed consent first. The research was approved by Ethics Committee of Faculty of Medicine, Hasanuddin University.

Results

The results of calculating the amount of colonies of Streptococcus mutans of dental plaque gave significant results before and after the ant flavonoid toothpaste treatment. In the data of the research results, there is a difference in the number of colonies of Streptococcus mutans after treatment. In research data, after brushing without using toothpaste, and after brushing teeth with flavonoid ant toothpaste, can be seen in Tables 1 and 2.

Table 1. Calculation of ants nest extension resistance to *S. mutans* bacteria after 24 hour incubation.

Treatment	Concentration of ants nest extract on Barriers of *S. mutans*				
	20%	40%	60%	80%	100%
Without toothpaste	1 : 89	1 : 88	1 : 90	1 : 94	1 : 92
	2 : 89.5	2 : 92	2 : 89	2 : 91	2: 90
	3 : 89.5	3 : 89	3 : 90	3 : 90	3 : 92
Without toothpaste	1 : 89	1 : 87.5	1 : 88	1 : 94	1 : 93
	2 : 88	2 : 86	2 : 89	2 : 93	2 : 90
	3 : 89.5	2 : 89.5	3 : 90	3 : 92	3 : 90
Without toothpaste	1 : 90	1 : 87.5	1 : 92	1 : 95	1 : 92
	2 : 88.5	2 : 89	2 : 94	2 : 93	2 : 93
	3 : 92	3 : 89	3 : 92	3 : 92	3 : 91

The calculation of *S. mutans* colonies on TYS medium before treatment with toothpaste extract of an nest flavonoids with concentration of 20%, 40%, 60%, 80%, 100% replication for 5 times yielded almost equal growth with the number of *S. mutans* colonies betweeen 80 and 95 colonies.

Table 2 shows that the calcium colonies of S. mutans on TYS growth medium with concentrations of 20%, 40%, 60%, 80% and 100% replication 5 times, yielded each growth constraint. As for the negative control of the sample almost did not experience the process of resistance of S. mutans bacteria on the media. In the concentration group of 20% seen the smallest percentage of growth of S. mutans is on ethanol fraction treatment 60 and in high control group by 87. At concentration 40% seen the smallest percentage growth percentage of S. mutans is on ethanol fraction treatment 60 and largest in control group 87. At concentration 60%, the smallest percentage growth percentage of S. mutans is found in the ethanol 58.5 and the largest fraction of treatment in the control group 90. At 80% concentration it is seen that the smallest percentage of S. mutans growth is in the ethanol 58 and the largest in the control group 92. At 100% concentration seen the smallest percentage growth percentage of S. mutans is at treatment of fraction ethanol 47 and largest in control group 91.5.

Table 2. Calculation of ants nest extension resistance to *S. mutans* bacteria after 48 hour incubation.

Treatment	Concentration of ants nest extract on Barriers of *S. mutans*				
	20%	40%	60%	80%	100%
Without toothpaste (Control)	1 : 87	1 : 87	1 : 88	1 : 92	1 : 91.5
	2 : 86.5	2 : 86	2 : 89	2 : 91	2 : 90
	3 : 89.5	3 : 85.5	3 : 90	3 : 90	3 : 89.5
Ethyl Acetate	1 : 66	1 : 64.5	1 : 60	1 : 62.5	1 : 50
	2 : 67	2 : 63.5	2 : 61	2 : 63.5	2 : 51
	3 : 68	2 : 64	3 : 62	3 : 61.5	3 : 52
Ethanol	1 : 60	1 : 61	1 : 59	1 : 58	1 : 47
	2 : 63	2 : 62	2 : 59	2 : 59	2 : 51
	3 : 61	3 : 60	3 : 58.5	3 : 58.5	3 : 48

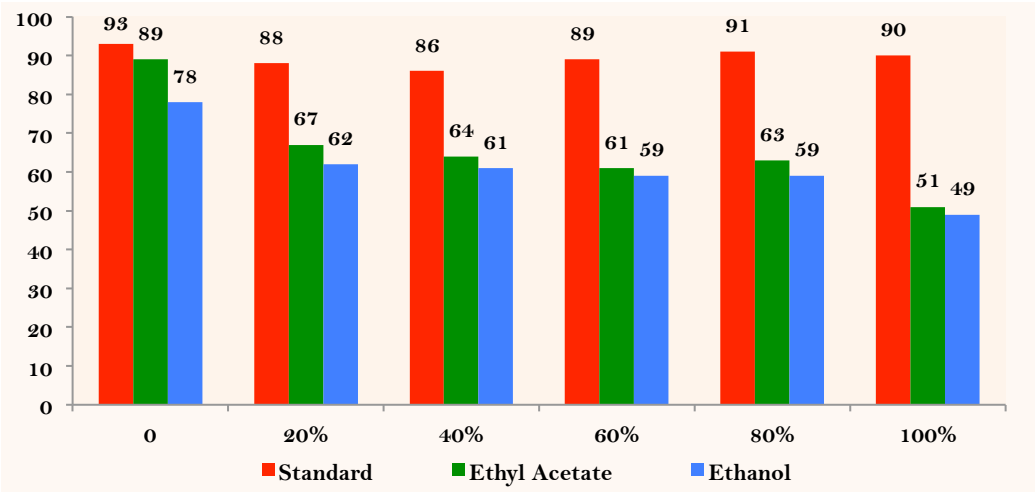

Figure 1. Colony of S. mutans calculation based on treatment of ethyl acetate extract concentration and ethanol plant ant nest.

Based on the Figure 1 it can be seen that Ant nest extract affects the growth and able to inhibit the growth of bacteria Streptococcus mutans, which can be seen at each concentration with

repetition of 5 times repetition. It was found that the extract of ethyl acetate of ant nest that incubated at room temperature with concentration of 20%, 40%, 60%, 80%, 100% showed the smallest percentage of growth of S. mutans ie the amount of 49 colonies at concentration 100% and the largest percentage growth of S. mutans is in the control group of 89 colonies at control concentration. While ethanol extract of ant nests at concentration 20%, 40%, 60%, 80%, 100% seen the smallest percentage of growth of S. mutans are 49 colonies at 100% concentration and the biggest growth percentage amounting to 78 colonies of S. mutans.

There was a significant difference in each group of ant plant nest extract concentration in inhibiting the growth of S. mutans bacteria (p<0.001) (Table 3).

Table 3. Test results of each concentration group.

Concentration	Group		
	Control Mean ± SD	Ethyl Acetate Mean ± SD	Ethanol Mean ± SD
20%	88.00 ± 0.96	67.00 ± 0.58	90.00 ± 1.07
40%	86.00 ± 1.38	64.00 ± 0.30	61.00 ± 1.25
60%	89.00 ± 0.60	61.00 ± 0.60	59.00 ± 0.51
80%	91.00 ± 0.43	63.00 ± 0.40	59.00 ± 0.75
100%	90.00 ± 0.43	51.00 ± 0.43	49.00 ± 0.43
p-value	0.000*	0.000*	0.000*

One Way ANOVA; *Statistically Significant (p<0.001).

Table 4 shows the comparison of two concentration groups indicating a significant difference between the two concentration groups. While in the concentration group of 20% with 40% (p = 0.319), the concentration of 40% with 60% (p = 0.218), and the concentration of 20% with 60% (p = 0.155), so there is no significant difference between the two concentration groups.

Table 4. The result of Post Hoc statistical test based on the relationship of five treatment groups of concentration.

%	Concentration				
	20%	40%	60%	80%	100%
20%		0.319	0.155	0.000*	0.000*
40%			0.218	0.000*	0.000*
60%				0.000*	0.000*
80%					0.000*
100%					0.000*

*Statistically Significant (p<0.001).

Discussion

The aim of this research was to know the effectiveness of toothpaste containing ant flavonoid extract. Effectivity of ant flavonoid toothpaste extract is seen based on the high number of decreasing number of S. mutans colonies on TYC media containing dental plaque sample.

In this study, it shows that the use of two flavonoid fractions of ant plant extract, ethanol fraction and ethyl acetate ant nest extract (Myrmecodia pendans), has the ability to decrease the amount of Streptococcus mutans in vitro colony. In fractionation of ant plant extract, contains active

compound that is effective flavonoid inhibit growth of Streptococcus mutans colony by reacting to bacterial protein cell, thus procures denaturation of cell protein membrane. The content of compounds contained in flavonoids produced from tuber of ant nests is likely to cause osmotic increase of bacterial cells that can trigger damage to the cell wall and causes bacterial cell lysis and die, this is causing a decrease in the number of strains of Streptococcus mutans. From the results of this study, the two ant flavonoid extracts showed that ethanol fraction of ant nest plants are more likely to decrease the amount of Streptococcus mutans colony than ethyl acetate fraction at the same concentration, starting at concentrations of 10%, 20%, 40%, 60%, 80 %, up to 100%. This is probably by virtue of the ethanol fraction properties compared to ethyl acetate fractions that may have a specific content that can reduce the number of bacterial colonies more.

This study shows that the growth barrier of S. mutans formed continues to increase along with the increasing amount of concentration. The biggest growth constraint is found at concentration 100%, while the lowest growth constraint is at concentration 20%. The higher the concentration of flavonoid fraction of ant nest extract the higher the active substance content in it that the antibacterial activity will be greater and also the lower the concentration of ant nest extract, the less active substance content in it that the antibacterial activity will decrease. The results obtained showed significant differences in growth inhibition in each concentration.

The flavonoid component has the capability of inhibiting activity of Glucosyltransferase enzyme produced from Streptococcus mutans bacteria. The most active inhibitory power of Glucosyltransferase activity is flavanols and flavones, which are active components in flavonoids. Flavonoids inhibit the growth of Streptococcus mutans by reacting with protein Streptococcus mutans cells resulting in protein denaturation. The presence of protein coagulation in the cell wall of Streptococcus mutans results in malfunctioning of bacterial cell membranes and an increase in osmotic pressure within the cell. Thus, there was damage to the cell wall and causes bacterial Streptococcus mutans lysis cells and die [11-13].

Streptococcus mutans colonies have Glucosyltransferase enzymes that can form extra cell polysaccharides and attach together with dextrant, which helps the bacterial attachment to the tooth surface and trigger plaque formation [14]. The formation of this plaque can then be prevented by giving medicinal herbal ingredients such as ant nest plant extract (Myrmecodia pendans). Ant nest plant extract has a mechanism of glucosyltransferase enzyme inhibition, consequently bacterial proteins becomes inactive and lose its function in further process of demineralization [15]. The content of flavonoids in ant nest plant extracts can dissolve lipids in bacterial cell membranes until there is a decrease in lipid stress. As a result the permeability of bacterial membrane cells decreases its activity, consequently causing abnormal bacterial cell capability and causing bacteria lysis and obstructed, thus in the end there is a decreased activity of bacteria work [16-18].

Flavonoids are known to act as natural antibiotics. Flavonoids are potential inhibitors of enzyme activity of glycosyltransferases (GTFs). Flavonoids from some previous studies effectively inhibit the growth of Streptococcus mutans colonies by reacting with Streptococcus mutans protein

cells resulting in increased osmotic in cells. The presence of protein coagulation in Streptococcus mutans cell wall leads to decreased cell membrane function and cell membrane protein denaturation. Thus, cell damage occurs and leads to bacterial lysis resulting in the number of strains of Streptococcus mutans decreased [19].

Conclusion

Flavonoid extract of ant nest plants has growth barrier on Streptococcus mutans bacteria, the greater the concentration given the greater the amount of decrease of S. mutans colony.

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Conflict of Interest: The authors declare no conflicts of interest.

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