

Formulation of Ethanol Extract (*Myrmecodia pendans*) as an Antibacterial *Streptococcus mutans* in Chewable Lozenges for Children with Early Childhood Caries

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ABSTRACT

Background: Riskesdas showed an increase in the prevalence of dental caries in Indonesia, which was 43.4% in 2007 to 53.2% in 2013. Not only among adults affected by caries but also children. One of the caries that often occurs in children under five. Early Childhood Caries. To overcome this, a solution using herbal plants, one of which is the ant nest (*Myrmecodia pendans*) which can reduce the number of *Streptococcus mutans* bacteria as the main bacteria that causes caries. Chewing lozenges is one preparation that can be used as an alternative to antibacterial treatment in the mouth.

Objectives: aims to determine the effectiveness of ether extract from ant nests (*Myrmecodya pendans*) as an antibacterial *Streptococcus mutans* in the preparation of chewing lozenges in the case of early childhood caries.

Materials and Research Methods: This study used field and laboratory experiments with a pretest-posttest control group research design. The sample consisted of 30 children consisting of 3 groups, namely 10 children chewing xylitol market, 10 children chewing chewable moisturizing tablets 3.75%, and 7.5%. Data processing and analysis using SPSS version 22.0 for windows.

Results: The results of the paired t-test showed a significant reduction

in the number of *Streptococcus mutans* colonies before and after chewing the ant nest of chewable tablets, 3.75% and 7.5%. From the paired t test results obtained p-value of 0.004. This shows that the p-value obtained is smaller than 0.05 so it can be concluded that the treatment of chewable nest tablet concentration of 7.5% has a significant effect in reducing the number of bacteria in the mouth.

Conclusion: The extract of the ant nest lozenges saffron (*Myrmecodia pendans*) etiquette was 7.5% more effective than the chewed lozenges nest (*Myrmecodia pendans*) 3.75%, so in this study the nesting lozenges chewable tablets.

Keywords: Ant nest (*Myrmecodia pendans*), Chewable lozenges, Early Childhood Caries (ECC)

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INTRODUCTION

Dental and oral health have not received special attention among the Indonesian people, according to Riskesdas data¹, an increase in the prevalence of dental caries in Indonesia, namely patients with active dental caries increased by 9.8% from 43.4% in 2007 to 53.2% in 2013. Not only among adults affected by caries but also children and even toddlers can also be infected with caries, according to data from the PDGI (Indonesian Dental Association)² states that at least 89% of caries sufferers are children. Based on the results of the characteristics of the health survey, the prevalence of dental caries in children aged 3-5 years is 81.7%. The prevalence of dental caries according to their age group, age 3 years (60%), age 4 years (85%) and age 5 years (86.4%), thus the toddler age group is a vulnerable group of dental caries.

One of the caries that often occurs in children under five, namely Early Childhood Caries. Children with ECC have a history of consuming sugar in the form of fluid for a long time and often. Sugar as the cause of caries such as sucrose, glucose and fructose contained in fruit juices and some baby formula foods, this type of feeding during sleep will increase the risk of caries, because cleansing the oral cavity and reduced flow rate of saliva.

To overcome the high prevalence of caries among children, especially Early Childhood Caries, a solution is needed by the

use of herbs, fruits, etc., which can reduce the number of *Streptococcus mutans* bacteria one of the typical Indonesian plants which is an epiphytic plant that hangs or attaches to another larger plant, has a bulging trunk and inside it contains small spaces or cavities inhabited by ants. Ant nest plants are often found in Kalimantan, Sumatra, Papua New Guinea, Philippines, Cambodia, Malaysia, Cape York, Solomon Islands and Papua.

Ant nest plants (*Myrmecodia pendans*) contain chemical compounds from flavonoids, tannins, polyphenols, multiminerals and polysaccharides are known to be able to cure various diseases. Flavonoids act as antibiotics, antivirals for the HIV virus and herpes.⁵ Several studies have reported that ant nest extracts have anti-inflammatory, antidiarrheal, and can reduce uric acid levels⁶, anticancer⁷, antioxidants⁸, and it can inhibit bacterial growth.⁷ The effect is because ant nest plants have active substances terpenoids/steroids, phenolic⁹, flavonoids, tannins, and polyphenols.⁷ As an effort to supply natural antibacterial compounds which are efficacious, inexpensive and safe as well as increasing the usability of Indonesia's abundant natural resources,¹⁰ ant nest plants are used as an alternative ingredient for root canal irrigation solutions to reduce the number of bacteria in the root canals.^{11,12,13}

Chewable lozenges is one of the preparations that can be used as an alternative for local antibacterial treatment in the mouth because chewable lozenges can directly dissolve the active substances in the mouth.¹⁴ The shape and taste of chewable lozenges is expected to be preferred over other dosage forms such as tablets, syrups, or solutions because it is easier to use and more attractive. The active ingredient used in this study was the ant nest ethanol extract (*Myrmecodya pendans*).

RESEARCH METHOD

The type of research used is field and laboratory experiments. This study used a Pretest-Posttest with Control Group Design. The research was conducted in 3 places, namely Biopharmaca Laboratory, Faculty of Pharmacy, Hasanuddin University, Microbiology Laboratory, Faculty of Medicine, Hasanuddin University, Pharmaceutics Laboratory, Faculty of Mathematics and Natural Sciences, Makassar Islamic University, and Kindergarten of *Dharma Wanita* of Hasanuddin University. This study has obtained information on ethical qualifications number: 0144/PL.09/KEPK FKG-RSGM UNHAS/2019 and registration number UH 17120154 dated 16 May 2019. Inclusion criteria in this study are: Children aged at least 3 years and a maximum of 5 years, Children with caries at least 2 teeth. Willing to be the subject of research by filling out informed consent. Brush teeth twice a day. Do not have periodontal disease. Do not consume drugs. As for the exclusion criteria in the process of sampling the child suddenly refused to be the subject. not compliant with the treatment process.

Tools and Materials

Tools: Mirror, Sonde, Tweezers, Gargle, Nierbecken, Writing stationery, Headlamp, Informed consent sheet, Incubator, Vial bottle, Toothbrush, Autoclave, Petri dish, Spoit, Toothpaste tube, Tooth model, Small towel, Inoculum needle, Colony counter, Pipette, Laminary Air Flow (LAF), Oven, Test tube, Test tube rack, Petri dish, Chemical cup, Measuring cup, Ice pack, Sprayer.

Materials: Mask, Handschoen, Aquadest, Cotton, Alcohol, Chewable Lozenges ant nest (*Myrmecodya pendans*), Cutton

swab, Label, Spirtus, Medium NaCl 0.9%, TYSB20 selective Medium (*Tryptone Yeast Extract Cystein Sucrose Bacitracin 20*), Aluminum foil, *Streptococcus mutans* bacteria, DMSO, Weigh paper, 70% Ethanol, Menthol, Xyilitol sugar, Gum wax, Honey, Mint, Xyilitol gum.

Research Procedures

Ant nests are stored in plastic bags at room temperature (28 ± 2°C). Ant nests in a coarse blender then put into a jar and maceration for 72 hours using 70% Ethanol solvent. Then filtered with Buchner Funnel using filter paper. After that, it is put into a tube and evaporated at a temperature of less than 50°C to concentrate.

Making of Chewable Lozanges

After the ant nest (*Myrmecodya pendans*) is processed, mix it with as much as 0.5 grams as an anticariogenic material for chewable lozenges. As for the chewable lozenges formula, namely: ¹⁴ Bacterial sampling in children, Dilution of *Streptococcus mutans*, *Streptococcus mutans* identification test, calculation of the number of *Streptococcus mutans* bacteria colonies,

Research Data

The type of data used is primary data. Data is presented in tabular and descriptive form. Data management used SPSS 22.0 software application. Data were analyzed using paired t test variance to determine differences in the number of *Streptococcus mutans* bacterial colonies at each period in the group.

RESULTS

The ant nest ethanol extract (*Myrmecodia pendans*) was obtained by maceration (immersion) of 70% ethanol and evaporated at the Biopharmaca Laboratory of the Faculty of Pharmacy, Hasanuddin University. The extract results obtained in the form of concentrated brown extract 30 gr. After that, the research data were recorded and processed by using SPSS version 22 for windows. The results of the study are shown in the following table:

Table 1: Descriptive of Each Treatment of Children

Treatment	Average	N	Standard Deviation	P-Value
Pre <i>chewable lozenges</i> ant nest 3.75%	64.4000	10	58.83159	0,200
Post <i>chewable lozenges</i> ant nest 3.75%	59.9000	10	57.83012	
Pre <i>chewable lozenges</i> ant nest 7.5%	64.4000	10	58.83159	0,200
Post <i>chewable lozenges</i> ant nest 7.5%	43.8000	10	51.06597	

Pre Xylitol	22.9000	10	19.48475	0,183
Post Xylitol	50.7000	10	29.46203	

Based on the normality test results obtained from the three treatments given have normally distributed data. This can be seen from the significance value of the three treatments greater

than 0.05. To see the effect of the treatment given, testing was done using the paired t test as follows:

Table 2: Paired Test Concentration 3,75%

	Difference in Couple	T	P-value	
				Average
Pair 1 Pre <i>chewable lozenges ant nest 3,75%</i> - Post <i>chewable lozenges ant nest 3,75%</i>	4,5000	14,18332	1,003	0,342

Table 3: Paired Test Concentration 7,5%

Paired Samples Test					
		Difference in Couple		T	P-value
		Average	Standard Deviation		
Pair 1	Pre <i>chewable lozenges ant nest 7,5%</i> - Post <i>chewable lozenges ant nest 7,5%</i>	20,6	17,3025	3,765	0,004

Table 4: Paired Test Xylitol

	Difference in Couple	T	P-value	
				Average
Pair 1 Pre Xylitol Post Xylitol	27,80000	10,993	7,997	0,000

To see the most significant treatment group in reducing the number of bacteria, a comparison was performed before and

after using the one-way ANOVA test, with the average difference data as follows:

Table 5: ANOVA Control Group Test and Treatment

Treatment	Average Difference	F	Sig.
Pre <i>chewable lozenges ant nest 3,75%</i> - Post <i>chewable lozenges ant nest 75%</i>	4,5000	13,881	0,000
Pre <i>chewable lozenges ant nest 7,5%</i> - Post <i>chewable lozenges ant nest 7,5%</i>	20,600		
Pre Xylitol - Post Xylitol	-27,800		

Table 6: Difference the Mean of Treatment Group

		Mean Difference (I-J)	Std. Error	Sig.
Xylitol	Chewable lozenges ant nest,75%	32,30 [*]	6,4363	0,000
	Chewable lozenges ant nest 7,5%	7,20	6,4363	0,273

DISCUSSION

The natural plant products such as Neem, babool and miswak have been proven to be the most effective biologically active compounds that are used in traditional practices in order to maintain oral hygiene. Concerning the diseases caused by the micro organisms the increasing resistance in many pathogens to commonly used antibiotics has led to the development of anti- microbial compounds derived from herbs. The use of the herbal plants as a traditional medicine to maintain oral health is common in developed countries.¹⁵

Dental caries or cavities are often associated with the role of *Streptococcus mutans*. The process of caries infection begins with the attachment of *Streptococcus mutans* to the tooth surface. This is because *Streptococcus mutans* has the enzyme glucosyltransferase which can break down sucrose into glucans in large quantities. According to Cheung (2015), predominantly, *Streptococcus mutans* forms dextran chains that are insoluble in water and have a sticky power to colonize the tooth surface. Furthermore, these bacteria form organic acids from sucrose. Sucrose metabolism by *Streptococcus mutans* produces lactic acid which is an acid that can cause decalcification of teeth.¹⁶

According to Umashankar (2016), chewable lozenges is one of the preparations that can be used as an alternative for local antibacterial treatment in the mouth because chewable lozenges can directly dissolve the active substances in the mouth. The shape and taste of chewable lozenges is expected to be preferable to other dosage forms such as tablets, syrups or solutions because it is easier to use and more attractive.¹⁷

Chewable lozenges are chewable tablets that have a basic composition, namely glycerin, gelatin, and water. Chewable lozenges are easily consumed by all ages, including children and parents. In addition, chewable lozenges with unique preparations can extend the half-life of the drug in the oral cavity, so as to get the maximum effect of the drug. This preparation is easy to make, so that the bioavailability of chewable lozenges can be increased.¹⁴

The main purpose of chewable lozenges is to provide a convenient unit dose of the drug to make it easier for patients such as children or elderly people who have difficulty swallowing whole tablets. Chewable lozenges have several specific advantages, which are good taste, no need to drink

water to swallow chewable tablets, can substitute drugs in liquid preparations (syrups), and drug absorption faster, so that the drug can be distributed locally and systemically well.¹⁴

Myrmecodia pendans species used in this study contains essential nutrients, namely flavonoid and tannin. Flavonoid may role directly as antibiotic by disturbing the microorganism or viral function, and as antioxidant against free radical. Such phenolic compound is a potent antimicrobial agent, that could be extracted by several methods. Heat reflux is a common method for the extraction of bioactive compounds from natural products. This extraction method is chosen for the first preliminary study because of its simplicity and manageability.¹⁸

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.¹⁹

According to Achmad (2014), 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

According to the research from Gartika (2018), natural products are used in caries prevention research. The effect of natural substances are on bacterial growth inhibition through cell wall biosynthesis and/or cell membrane permeability, inhibition of protein synthesis or nucleic acid metabolism and inhibition of enzyme activity such as glucosyltransferase or transcription level of genes.^{20,21,22}

CONCLUSION

Based on research that has been done, it can be concluded as follows:

1. Chewable lozenges ethanol extract of ant nests (*Myrmecodia pendans*) 3.75% and 7.5% have an influence on *Streptococcus mutans*, which is an antibacterial that can reduce the number of *Streptococcus mutans* bacteria colonies
2. Chewable lozenges ethanol extract of ant nests (*Myrmecodia pendans*) 7.5% is more effective than Chewable lozenges ant nests (*Myrmecodia pendans*) 3.5%, thus in this study the chewable lozenges ant nests (*Myrmecodia pendans*) has a 7.5% more significant effect as an antibacterial in reducing the number of *Streptococcus mutans*.

CONFLICT OF INTEREST

There is no conflict of interest in this study

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