Antibacterial Activity Of Nginang Herb Extract Mouthwash Formulation Against The *Streptococcus Mutans* Bacteria

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Abstract.

Nginang was chewing the raw green betel leaf combined with areca nut and gambier seeds, it was Indonesian people's habit in ancient times to maintain the dental health. Over time, the nginang mixture was developed into a mouthwash formula to make it easier and acceptable to use. Mouthwash is a liquid form preparation with a pleasant taste and smell, to keep clean the mouth. The phytochemical content in the nginang herbs was tannin which is a phenolic compound. The mechanism of action of phenolics was by inactivated the microbial cell adhesion and to avoid the attached to host cells by the cell surface. The purpose of this study was to determine the antibacterial activity by inhibitory zones diameter of the formula of nginang herb mouthwash against Streptococcus mutans bacteria. This type of research was descriptive and quantitative with an experimental research design. The experimental research by making mouthwash formulations of nginang herb extract (green betel leaf, areca seed, and gambier) in three formulas with a concentration ratio of green betel leaf: areca nut: gambir, that were Formula 1(3%:3% :3%), Formula 2 (3%:2%:1%), and Formula 3 (3%:1%:2%), the quality of the formulas was carried out by physical quality parameters (organoleptic, pH, clarity, and viscosity), and the antibacterial activity against Streptococcus mutans. The results of this study of the three formulas were had liquid form, clear to browny color, and slightly smell like mint and extract, the pH at 5.4, and had a viscosity value about 0.79. The results of antibacterial activity against Streptococcus mutans study was indicated that the formula which contain of green betel leaf, areca seed, and gambier had very strong category based of the inhibitory zone.

Keywords: *Green betel leaf, areca nut, gambier seed, nginang, mouthwash and Streptococcus mutans.*

I. INTRODUCTION

The World Health Organization data on 2020 was estimated, there were 2,3 billion people worldwide experience permanent dental caries as well as more than 530 million children experience caries in primary teeth. In Indonesia health problems teeth and mouth are still an interesting issue because of the high prevalence dental caries [1]. Dental caries is a disease of the tissue tooth hardness that can occur on one or more tooth surfaces and can spreads to the inside of the tooth from enamel to dentin to cementum [2]. Dental caries was caused by the interaction of various factors, including host factors (teeth and saliva), substrate (diet), microorganisms (bacteria), as well as time [3]. One of the triggers for dental caries is the bad hygiene of the mouth cavity, resulting the plaque buildup. Dental plaque was a thin, colorless film consisting of a collection of bacteria sticks tightly to the tooth surface that were not cleaned. Bacteria was playing an important role in plaque formation form extracellular polysaccharides, namely bacteria from the Streptococcus genuses [4]. The main bacteria species in dental plaque were plays an important role in the etiology of caries tooth was *Streptococcus mutanss*. This situation was due to ability of *Streptococcus mutanss* in producing acid rapidly or acidogenic [5]. One way to overcome the formation of dental plaque, namely by using mouthwash which contains antibacterial ingredients. The mouthwash was a solution formula, generally deep concentrated form that must be diluted before used for preventing or treatment throat infections [6]. Nginang was one of existing ancient culture, passed down from generation to generation until now betel or betel has become part of community life archipelago.

While the term beckons or betel itself is an activity chewing a mixture of betel leaves, areca nut, chalk and over time mix nginang then mix with gambier and tobacco [7]. Betel (*Piper betle* L.) is useful for dental health and Eliminates body odor and odor bad mouth, as medicine gargling, canker sores, nosebleeds, itching, scabs and treat vaginal discharge woman. Phenolic compounds contained in betel leaf essential oil had strong antimicrobial and antifungal and effectively inhibit growth several types of bacteria [8]. Areca nut (*Areca catechu* L.) contains alkaloid compounds, flavonoids, tannins, saponins, and polyphenols which is known to be efficacious as antibacterial [9]. That also commonly used with areca nut and betel leaf were had

high of calcium content, which able to prevent the demineralization teeth process and gave alkaline properties that important in maintaining Oral pH balance. The custom of betel is believed can prevent dental caries. This matter due to the influence the content of betel ingredients helps prevent dental caries [10]. Gambier (*Uncaria gambir* Roxb) were had many benefits for the physical health, but in general gambir in Indonesia was used for nginang. Another function of gambier is can be used as a dysentery, medicine mouthwash, canker sores, skin aches, burn wound medicine mixture, headaches, diarrhea, as well as a textile dye [11]

II. METHODS

This research was a quantitative research. The variables in this research were independent variable and the dependent variable. The independent variables in this research were variations of extract ratio the green betel leaf extract, areca nut extract, and gambier extract in each formula, there were respectively formula 1 (3:3:3%); formula 2 (3:2:1%); and formula 3 (3:1:2%) and formula 0 without exctracts for negative control.

Table 1. Formulation of nginang mouthwash

No	Bahan	F1	F2	F3	F0	Function
1	Green betel leaf extract (%)	3	3	3	-	Active ingredients
2	Areca nut extract (%)	3	2	1	-	Active ingredients
3	Gambier extract (%)	3	1	2	-	Active ingredients
4	Glycerine (%)	2,5	2,5	2,5	2,5	Humectan
5	Tween 80 (%)	5	5	5	5	Surfactant
6	Saccharin (%)	0,2	0,2	0,2	0,2	Sweetener
7	Peppermint oil (drops)	2	2	2	2	Essence
8	Sodium Benzoate (%)	0,4	0,4	0,4	0,4	Preservatives
9	Aquadest (%)	*Ad* 100	*Ad* 100	*Ad* 100	*Ad* 100	Solvent

Notes:

F0 = Formula 0 (*mouthwash formula without extract*

F1 = Formula 1 (*mouthwash formula with green betel leaf , areca nut, and gambier extract 3:3:3)*

F2 = Formula 2 (*mouthwash formula with green betel leaf , areca nut, and gambier extract* 3:2:1)

F3 = Formula 3 (*mouthwash formula with green betel leaf , areca nut, and gambier extract* 3:1:2)

The dependent variable used in this research were physical quality parameters including organoleptic tests, pH tests, clarity tests, and viscosity tests and inhibition zone of antibacterial activity tests of the mouthwash formula.The materials in this research were betel leaf extract, areca nut extract, gambier extract, glycerine, tween 80, sodium saccharin, peppermint oil, sodium benzoate, distilled water, one of mouthwash brand product for positive control, BMH media (Blood Muller Hinton), 96% ethanol, handscoon, mask, and *streptococcus mutans* bacterial culture. The tools and instrument that used in this research were round osse, petri dishes, measuring cylinder, glass funnels, tubes, dropper pipette, micro pipette, disc paper, glass beaker, spiritus, caliper, autoclave, marker, filter paper, analytical balance, hotplate, rotary evaporator. The research was carried out by collecting and determinatings the green betel leaves (*Piper betle* L.), areca nut seeds (*Areca catechu* L.), and gambier (*Uncaria gambir* Roxb.) from Gede Hardjonagoro Market, Jebres District, Surakarta and determinates by biology laboratory of MIPA Sebelas Maret University. The collected materials then extracted using the maceration method using 96% ethanol solvent [12, 13, 14], after the extract was available then the nginang mouthwash formula was made with other additional ingredients [15]. The results analysis of this research was by descriptive analysis the results of the mouthwash formulation of nginang herb extract which had physical quality and analysis the quantitative inhibitory zone against *Streptococcus mutans* bacteria using the disk diffusion method [16].

III. RESULTS AND DISCUSSIONS

1. Extraction, formulation and physical quality of formula

Based on the research results, the obtained extracts were made from 300 grams of green betel leaf simplicia powder get 15.817 grams of green betle leaf extract, the yield of green betel leaf extract was

5.2723%, the 200 grams of areca nut simplicia powder was obtained 12.113 grams of extract with a yield of 6.0565%, and 200 grams of gambier simplicia powder produces 10.305 grams of crude extract with a yield as much as 5.1525%. Mouthwash preparations are made with green betel leaf extract, areca nut extract, and gambier extract. The stages carried out in the preparation of mouthwash formula was by mixing each extract with a additions ingredients in Table 1.

Fig 1. Formulation of nginang mouthwash

The quality test of mouthwash formula carried out by organoleptic, pH, clarity, and viscosity test. The organoleptic test of all formula had peppermint odors. All mouthwash formula was liquid, and had brown colors except the formula 0, because of doesn't contained any extracts were have been given the brown color. The pH test was carried out using a pH meter in triplo. The pH test results of formula 1 were found with an average in 5.57 ± 0.0152, formula 2 obtained an average of 5.51 ± 0.0264 and formula 3 obtained an average of 5.41 ± 0.03, meanwhile the negative control was obtained the average in 6.25 ± 0.0916. That were in accordance with the edition IV Indonesian Pharmacopeia, which the pH states of mouthwash in good ranges between 5-7. From this result the mouthwash has been acceptable on pH parameter. The formulation of mouthwash containing the betel leaf, areca nut, and Gambier extract, they had been given the acidic pH, this is influenced by the pH of the extract which can be showed in the results of formula 1, 2, and 3 were more acidic than the negative control which did not adding of any extracts [17].Clarity testing was carried out by visually using clean container and then observe it in good light illumination, using a white background, unobstructed to reflections by the eyes, the result must be completely free from small particles that be seen by the eyes [18]. The clarity results of control negative (formula 0), formulas 1, 2, and 3 were equally clear. There were no small particles that seen, it means the ingredients in the mouthwash formula was mixed and soluble with homogenously [18].Viscosity testing aims to see pourability of mouthwash formula. The closer of the mouthwash formula viscosity with water viscosity, that made the formula was easier to use, the viscosity of formula in 0,6440 -0,7862 cp, meanwhile the pure water viscosity was 0.89 cp [4].

2. Antibacterial Activity Test Against Streptococcus mutans bacteria

Nginang mouthwash antibacterial activity test was carried out on the growth of *Streptococcus mutans* using the disc diffusion method. This research uses the disc diffusion method because it was had advantage of being able to provide high accuracy and easier to measure the area of the inhibitory area formed due to the penetration effect of active compounds not only on the top surface of the agar media but also down to the bottom (Hidayah et al., 2020). Antibacterial activity testing was carried out aseptically in Laminar Air Flow (LAF). The LAF has a blower which functions to blow air out so that microorganisms cannot enter the LAF. LAF is sprayed first with 70% alcohol disinfectant before use to increase workplace sterilization [19]. Before carrying out the antibacterial test, all tools to be used are sterilized first by placing them in an autoclave. The sterilization process was carried out using an autoclave at a temperature of 121 ºC for 15 minutes. The purpose of sterilization was to kill microorganisms on the tools that used, so they did not interfere on the test of this research [20].Bacterial cultures were obtained from the Microbiology Laboratory, Faculty of Medicine, Sebelas Maret University (UNS). The species of bacterial culture were rejuvenated was identified using gram staining, to see the morphology, shape and colony of bacteria using a microscope. The results obtained by the bacterial culture on the Mueller Hinton Blood media was the *Streptococcus mutans* species, characterized by purple-colored bacteria with cocci shape.In this study, one of the branded

commercial products was used as a positive control. The resulting inhibition zone was compared with the inhibition zone from formula 0 as a negative control, formulas 1, 2, and 3. The Antibacterial activity test result was showed in table 2:

Table 2. Antibacterial activity test result

Formula	Average ± SD	Category [21]	Antibacterial activity
Formula 0 (Control negative)	0,63±1,0969	> 20 mm: Very strong	Weak
Formula 1	21,06±0,7977	10-20 mm: Strong	Very strong
Formula 2	19,75±0,7784	< 5 mm: Weak	Strong
Formula 3	20,06±0,174		Very strong
Control positive	16,32±2,0567		Strong

Antibacterial test results on mouthwash formulas prove that all formulas containing extracts show strong to very strong inhibition zones, while formulas without extracts produce the lowest inhibition zones compared to other formulas.The extract content of green betel leaf, gambier and areca seeds has been proven to provide activity in inhibiting the growth of Streptococcus mutanss. This has also been proven by [22]., that the ethanol extract of green betel leaf has antibacterial activity against several gram-positive bacteria. The difference in concentration of gambier extract and areca nut extract affects the resulting inhibition zone. The greater the concentration of gambier extract, the greater the inhibition zone produced. In previous research proved that gambier contains Gambirine, Isogambirine, Gambirtannine, and and Roxburghine [23].Tannin compounds can damage bacterial cell membranes due to the toxicity of tannins and the formation of metal ion complex bonds from tannins [24]. The results of the antibacterial test showed that the higher the test, the larger the diameter of the inhibition zone produced.

According to category of inhibitory zones, antibacterial strength can be grouped as follows: inhibition zone 20 mm or more: very strong, inhibition zone 10-20 mm: strong, inhibition zone 5-10 mm: medium, inhibition zone 5 mm or lacking: weak. Based on these criteria, the antibacterial power of the nginang mouthwash formula against Streptococcus mutanss' bacteria in formula 1 and formula 3 was classified as very strong activity. Meanwhile, formula 2 was classified as strong. The positive control antibacterial inhibition zone had smaller inhibitory zone than the nginang mouthwash formulas 1, 2 and 3.The positive control as comparison that used was a one of mouthwash brand product with 1% povidone iodine content. The iodine content is able to quickly come into direct contact with the surface of bacterial cells which results in the loss of cytoplasmic material and deactivation of enzymes resulting in damage to the structure and function of bacterial cells. Povidone iodine 1% which only contains povidone and iodine to inhibit the growth of Streptococcus mutans [25].

IV. CONCLUSION

Based on the results, the conclusions of this research were physical quality of the nginang herb extract mouthwash preparations in formula 1, formula 2, and formula 3 had liquid form, brown color, pepermint and herbs extract odor. The pH results on all nginang mouthwash preparations in accordance with 4th edition of Indonesian Pharmacopoeia, that were between 5-7. The clarity result of all nginang mouthwash preparations have been proven to be clear because not a single particle was existing. The viscosity results on all mouthwash preparations was qualified, the viscosity is 0.89 cP. The antibacterial activity of the nginang herb extract mouthwash formula is capable inhibits the growth of *Streptococcus mutans* bacteria. formula 1 and 3 have very strong category of antibacterial activity, that were 20 mm or more. Meanwhile on formula 2 have strong category of antibacterial activity.

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