FORMULATION AND ANTIBACTERIAL ACTIVITY OF HERBAL TOOTHPASTE BASED ON ACEH TRADITIONAL MEDICINE

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ABSTRACT

This research is based on Acehnese traditional medicine containing betel leaf, areca nut, kencur rhizome, gambier sap, and clove buds. Traditionally, all parts of the plant are wrapped in betel leaf folds and chewed to maintain oral health. This study aimed to incorporate these ingredients into an herbal toothpaste and test its efficacy against bacteria that cause tooth decay, such as *S. mutans* and *E. faecalis*. Extracts from betel leaf, areca nut, aromatic ginger, gambier sap, and clove buds were used in this study. Based on variations in extract concentrations, three formulations were developed: F1, F2, and F3. In addition, F0 was the toothpaste base. The herbal toothpaste was light brown in color, homogeneous, semisolid, and had a characteristic betel leaf aroma, according to the evaluation results. The final product had a pH of 7.52–8.59, viscosity of 238.9–242.3 cP, and a foam height of 20 mm (F0), 11 mm (F1), 9 mm (F2), and 6 mm (F3). These values meet the toothpaste quality standard (SNI 112-3524-1995). While F1 showed moderate inhibition against *S. mutans* and *E. faecalis* in the antibacterial test, F2 and F3, showed high inhibition against *S. mutans* and moderate inhibition against *E. faecalis*. Based on these results, the optimal formula is F3 which has a larger inhibition diameter of 12.23 mm for *S. mutans* and 9.10 mm for *E. faecalis*. This herbal toothpaste can be developed to prevent oral candidiasis and gingivitis.

Keywords: herbal toothpaste, antibacterial activity, *Streptococcus mutans*, *Enterococcus faecalis*

INTRODUCTION

In Acehnese, the betel nut combination is known as *ranup*. The ingredients for this combination include coarsely powdered areca nut seeds (*Areca catechu* L.), dried gambier sap (*Uncaria gambir* (Hunt) Roxb), aromatic ginger (*Kaempferia galanga* L.) wrapped in betel leaves (*Piper betel* L.), and cloves (*Syzygium aromaticum* L.) connected. Chewing this concoction at various events is a practice that fosters intimacy and camaraderie among community organizations, as well as being beneficial to oral and dental health (Suryo, 2009).

Several studies have been conducted to demonstrate the efficacy of the components found in these traditional remedies against oral pathogen microbes that cause candidiasis, gingivitis, and dental caries. Crude ethanol extract from betel leaf has been shown to have antibacterial activity with inhibition zones of 22.30 ± 2.10 mm and 17.30 ± 0.67 mm for *C. albicans* DMST 8684 and *C. albicans* DMST 5815, respectively. Also, 7.80 ± 0.30 and 7.10 ± 0.00 mm for *S. gordonii* DMST 38731 and *S. mutans* DMST 18777, respectively (Phumat *et al*., 2017). In a concentration-dependent way, areca nut extracts reduced the growth of *S. mutans*, *S. salivarius*, *F. nucleatum*, *E. coli*, and *S. aureus* (Faden, 2012; Senthil *et al*., 2012). Gambier is a well-known herbal remedy for halitosis. Gambier extract and aromatic ginger oil can inhibit *C. albicans*. Using the agar disc diffusion method, the essential oil of *Kaempferia galanga* rhizome showed potent inhibition against *C. albicans* (31 mm) compared to the standard antifungal clotrimazole (25 mm) (Achmad *et al*., 2021; Kumar,

2020). Aqueous extract and ethanol extract of *Syzygium aromaticum* L. can inhibit *S. mutans* and *P. gingivalis* (Rosas-Piñón *et al*., 2012).

Researchers are interested in developing these herbal ingredients into a convenient preparation based on this information. One of the appropriate preparations is toothpaste, because this is more appropriate to clean deposits and polish the entire surface of the teeth, providing a comfortable and refreshing feeling in the oral cavity. The material used was obtained from the Aceh Besar district. The dried sap of gambier was obtained from the province of West Sumatra. All ingredients will be extracted and then designed into several toothpaste formulas with various concentrations. The agar diffusion well method was used to evaluate the toothpaste's efficacy.

RESEARCH METHODS

Equipment and Materials

Materials

The materials used in this study were betel leaves (*Piper betel* L.), areca nut seeds (*Areca catechu* L.), dried gambier sap (*Uncaria gambir* (Hunt) Roxb), rhizome of aromatic ginger (*Kaempferia galanga* L.), and clove buds (*Syzygium aromaticum* L.). These materials were obtained from local farmers' land in the provinces of Aceh and West Sumatra, Indonesia. The solvents used for extraction were ethanol (Merck) and n-hexane (Merck). The ingredients for the formulation of toothpaste are calcium carbonate (Merck), glycerine (Merck), menthol (Merck), sodium laureth sulfate/EMAL 270N (Kao–Japan), sodium carboxymethylcellulose (Aqualon™ and Blanos™), methylparaben (Sharon Laboratories), xylitol (NOW Foods), and Aquadest. The antibacterial effectiveness test used Mueller-Hinton Agar media (Oxoid), *Streptococcus mutans* ATCC 25175, and *Enterococcus faecalis* ATCC 29212.

Methods

Preparation of Extracts

The plant material was air-dried and processed to a fine powder at a temperature of 25°C. Ethanol was used as a solvent to extract materials. The n-hexane was used to extract *Kaempferia galanga*. The extracts were evaporated under reduced pressure in a vacuum rotary evaporator (Achmad *et al*., 2021; Mufidah, 2014; Musdja *et al*., 2020; Phumat *et al*., 2017; Rosas-Piñón *et al*., 2012).

Research Procedure

Formulation of Herbal Toothpaste

Herbal toothpaste was made using the trituration procedure. The formula was made based on research that has been done previously and modified. Carboxymethylcellulose (CMC) was sprinkled over hot water in a mortar and allowed to stand for 15 minutes to swell. Then it was crushed to form a homogeneous mass. The extracted materials were weighed and placed in the mortar in the appropriate amounts. Water was mixed with calcium carbonate ($CaCO_3$), sodium lauryl sulfate (SLS), glycerine, xylitol, and methylparaben. After a few drops of ethanol have been used to dissolve the menthol, the water was added. Then all of the ingredients were ground until a paste consistency was obtained (Gunaki *et al*., 2021). The formula for toothpaste is provided in Table I below.

Table I. Herbal Toothpaste Formula Based on an Aceh Traditional Medicine

Ingredients	Quantity (gram)			
	F0	F1	F2	F3
Betel extract	0	1	2	4
Areca nut extract	0	0.5	1	2
Kencur extract	0	0.3	0.6	1.2
Gambier extract	0	0.2	0.4	0.8

Clove bud extract	0	0.1	0.2	0.4
CMC	2	2	2	2
$CaCO_3$	50	50	50	50
SLS	1	1	1	1
Glycerine	30	30	30	30
Xylitol	1.3	1.3	1.3	1.3
Methyl paraben	0.3	0.3	0.3	0.3
Menthol	0.05	0.05	0.05	0.05
Aquadest	15.35	13.25	11.15	6.95
Total weight	**100**	**100**	**100**	**100**

Antibacterial Test Against *S. mutans* and *E. faecalis*

An antibacterial test of herbal toothpaste was carried out against two oral pathogen bacteria species: *S. mutans* ATCC 25175 and *E. faecalis* ATCC 29212. After 24 h of incubation, the bacterial suspension (inoculum) was diluted with a sterile physiological solution (turbidity = McFarland barium sulfate standard of 0.5). A sterile cotton swab was used to evenly disperse the bacterial inoculum on a sterile Mueller Hinton Agar (MHA) petri dish. Each of the four wells was filled with the toothpaste formulas F0, F1, F2, and F3. Chlorhexidine 0.2% was employed as a positive control. Under aerobic circumstances, the systems were incubated for 24 hours at 36°C. Confluent bacterial growth was detected after incubation. The bacterial growth inhibition was measured in millimeters (Saptawati *et al.*, 2019; Valgas *et al.*, 2007).

Data Analysis

The descriptive data from the observation findings of the preparatory evaluation were given. The one-way ANOVA (analysis of variance) approach was used to test the quantitative data of the diameter of the inhibitory zone created in the antibacterial effectiveness test.

RESULTS AND DISCUSSION

We employed five distinct extracts as active ingredients in herbal toothpaste based on an Aceh traditional remedy in this study. The extracts obtained were subjected to a phytochemical screening test (as shown in Table II). Phytochemical test results show the secondary metabolite content of the simplicia used.

Table II. The Results of Phytochemical Screening

Metabolite compound	Reagents	Betel extract	Areca nut extract	Kencur extract	Gambier extract	Clove bud extract
Alkaloids	Wagner	-	+	+	-	-
Steroids	Liebermann – Burchard	+	-	+	-	-
Terpenoids	Liebermann – Burchard	-	+	+	-	-
Saponin	Foam test	+	+	+	-	+
Flavonoid	HCl and Mg	+	+	+	+	+
Phenols	$FeCl_3$	-	+	+	+	+
Tannin	$FeCl_3$	-	+	+	+	+

+ : positive result; -: negative result

The findings of phytochemical screening of betel leaf ethanol extract revealed the presence of steroids, saponins, and no alkaloid chemicals when Wagner's reagent was used. These findings are consistent with earlier research (Patil *et al.*, 2015). The flavonoid molecules yielded different findings in the two studies, with Patil *et al.*, (2015) study

yielding negative results. In addition, in a study conducted by Patil *et al*., (2015), modest quantities of tannins were discovered. This disparity could be attributed to geographical origins and environmental conditions.

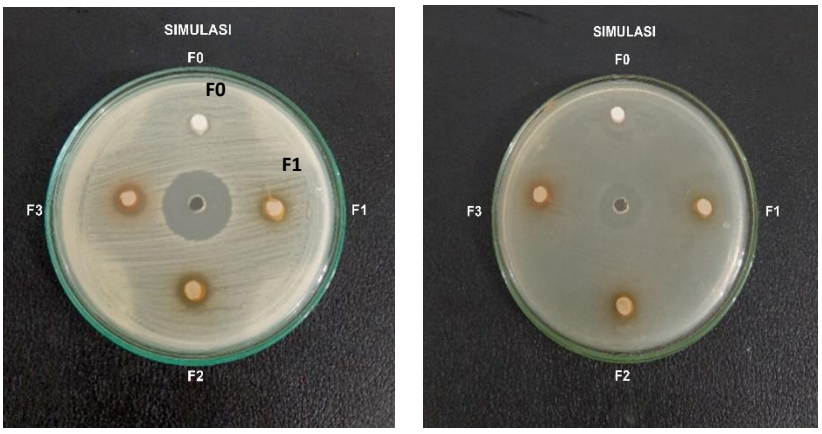
Secondary metabolites in the form of alkaloids, terpenes, and flavonoids were discovered in Areca nut extract. However, no steroid compounds were discovered. These findings are consistent with earlier research (Djohari *et al*., 2019). Other chemicals, such as saponins, phenols, and tannins, were discovered in the arecanut extract assay.

Terpenoids, flavonoids, and phenol are the primary components of kencur extract. While the ethanol extract of gambier contains flavonoids, phenols, and tannins. Secondary metabolites such as saponins, flavonoids, phenols, and tannins have been found in clove bud extract (Fatimatuzzahroh *et al*., 2015; Kumar, 2020; Viena & Nizar, 2018) All supported by the phytochemical analysis of the extracts used in this investigation.

A **B** **C** **D**

Figure 1. Herbal toothpaste of F0 (A), F1 (B), F2 (C), and F3 (D)

The results of organoleptic observations showed that the herbal toothpaste preparations (F1, F2, and F3) were semisolid, reddish-brown in color, and had a distinctive aroma of betel leaf, as shown in Figure 1. The color of the toothpaste probably comes from betel nut extract. In this study, young areca nut seeds were used, and it is known that young areca nuts contain high levels of tannins, which are around 30–47%. These tannins are natural dyes that give pink to reddish-brown colors (Isnaini *et al*., 2020).



(a) **(b)**

Figure 2. Inhibition zone of traditional Aceh herbal toothpaste against *S. mutans* (a) and *E. faecalis* (b).

Streptococcus mutans and *Enterococcus faecalis* were used to investigate the efficacy of herbal toothpaste against harmful bacteria in the mouth. Both of these bacteria are members of the gram-positive bacteria family. Oral candidiasis, gingivitis, and dental caries are all caused by these microbes (Williams & Lewis, 2011). *S. mutans* has the ability to swiftly generate acid. The high composition of *S. mutans* and other acidogenic bacteria and acid-tolerant bacterial species in the biofilm flora can trigger ecological alterations in the

biofilm flora. The aggressiveness of the *S. mutans* biofilm in causing dental caries will be affected (Fatmawati, 2011). Meanwhile, *E. faecalis* is routinely detected in root canals and survives therapy. This bacterium causes root canal infection and periapical disease in teeth (Nurdin & Satari, 2013). Table III shows the results of the efficacy test (5 replicates). We used chlorhexidine as a positive control. Chlorhexidine is an antiseptic for treating gingivitis (gingivitis), cleaning the skin in the wound area, the area to be given an injection, or the body surface area to be operated on, and disinfecting hands before surgery. Chlorhexidine works by killing bacteria.

Table III. Results of Antibacterial Activity Testing

Test strains	Zone of inhibition (mm)				
	F0	**F1**	**F2**	**F3**	**PC**
S. mutans ATCC 25175	6.10±0.05	8.62±0.25	10.85±0.29	12.23±0.57	23.04±0.74
E. faecalis ATCC 29212	6.03±0.02	7.19±0.33	8.18±0.25	9.10±0.46	15.33±0.10

PC: positive control (0.2% chlorhexidine)

A one-way ANOVA analysis was carried out on the results of the effectiveness test of herbal toothpaste preparations. Based on the test of homogeneity of variances conducted on *S. mutans*, the test results showed that the variants of the three groups (F1, F2, and F3) were the same (P-value = 0.779). The ANOVA test to see the difference in the inhibitory power of the three concentration groups obtained a P value (P-value) of 0.000 with a significant level of 0.05. There was a significant difference in the average inhibition formed based on each of these concentrations. The post-hoc test showed that the three concentration groups (F1, F2, and F3) showed a difference in average inhibition ($P < 0.05$). Hence, it was concluded that the concentration had an effect on the inhibitory power.

Analysis of *E. faecalis* based on the test of homogeneity of variances showed that the variants of the three concentration groups (F1, F2, and F3) were the same (P-value = 0.136). The ANOVA test obtained a P-value of 0.000. Thus, at the level of significance of 0.05, there is a significant difference in the average inhibition formed based on each of these concentrations. The three concentration groups, namely F1, F2, and F3, showed differences in the average inhibition power. Based on the ANOVA test, there was a significant difference ($p < 0.05$) between the concentration and the inhibitory power formed, so it could be concluded that the concentration effected the inhibitory power. Based on the univariate analysis of variance, it shows a sig value of 0.102, so it can be concluded that the variance between groups is not significantly different. The results of the Tukey test showed a significant difference ($p < 0.05$) in the antibacterial activity of *S. mutans* and *E. faecalis* at each concentration.

F1 has moderate inhibition against *S. mutans* and *E. faecalis*, as shown in the Figure 2 and Table III. The inhibitory strength of the F2 preparation against *S. mutans* was in the strong category, while the inhibitory power against *E. faecalis* was moderate. Meanwhile, F3 has a considerable inhibitory capacity against *S. mutans*, but only a modest inhibitory power against *E. faecalis*. The content of secondary metabolites has the power to impede the growth of these bacteria. Flavonoids found in the extract of betel leaf, areca nut, kencur, gambier, and clove bud can suppress the growth of these bacteria. It also derives from the activity of saponin chemicals found in betel and kencur extracts, as well as eugenol compounds, which are the major components of these plants (Djohari *et al*., 2019; Fatimatuzzahroh *et al*., 2015; Kumar, 2020; Patil *et al*., 2015; Viena & Nizar, 2018). Gram-positive bacteria do not have a cell wall with an outside membrane. As a result, the

antibacterial mechanism of action involves destroying the bacterial cell wall and cytoplasmic membrane, resulting in cytoplasm leakage and coagulation (Rosas-Piñón *et al*., 2012).

The antibacterial activity of betel nut extract is related to numerous biologically active compounds such as eugenol, allyl pyrocatechol, chavibetol, chavibetol acetate, caryophyllene, and hydroxychavicol. The killing kinetic pattern is considered to be dose- and time-dependent (Bhalerao *et al*., 2013; Dwivedi & Tripathi, 2014; Phumat *et al*., 2017). The key antibacterial principles against a primary cariogenic bacterium, *S. mutans*, and the predominant inhibitory action against *S. mutans* glucosyltransferase were shown to be arecanut fatty acids (myristic and oleic acids) and procyanidins from betel nuts, respectively (Senthil *et al*., 2012). Also in vitro, ethyl p-methoxycinnamate, derived from the essential oil of *Kaempferia galanga* rhizome, demonstrated significant antibacterial and antifungal activity (Kumar, 2020). The presence of significant amounts of eugenol in the clove bud accounts for the strong antibacterial activity (Hemalatha *et al*., 2016). Catechins, which are polyphenolic chemicals found in gambier extract, have been shown to exhibit biological action as antimicrobials and antioxidants. Polyphenols' propensity to bind to other organic molecules, particularly proteins, through a denaturation process disrupts protein function, destroys cell walls, and deactivates enzymes, making them antibacterial (Achmad *et al*., 2021).

CONCLUSION

Herbal toothpaste containing extracts from traditional Acehnese herbal ingredients consisting of betel leaf, young areca nut, kencur rhizome, gambir sap, and clove bud has been shown to inhibit the growth of pathogenic bacteria in the mouth, like *S. mutans* and *E. faecalis*. The diameter of the inhibition that results is the dosage of Chlorhexidine (0,2%). This herbal toothpaste can be developed to prevent oral candidiasis, gingivitis, and dental cavities.

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