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TOOTH PASTE FORMULATION FROM BETEL NUTS (*Areca catechu L.*) EKSTRACT AND ITS ANTI-BACTERIAL ACTIVITY AGAINST *Streptococcus mutans*

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Abstract

Dental caries is a demineralization of the tooth surface caused by the bacterium *Streptococcus mutans*. Betel nut is empirically believed to protect the teeth from dental caries. This study was done to determine the effect of the tragacanth and carbopol variations to the physical properties of the toothpaste and its antibacterial activity against *S. mutans*. Betel nuts was extracted with 70 % ethanol, the solvent was evaporated, and the extracts was made into five (I to V) formulas with carbopol and tragacanth variations in sequence of 0.5:0.5; 0.75:0.25; 0.25:0.75; 1:0; and 0:1 respectively. The physical properties of the toothpaste including homogeneity, pH, viscosity, foaming and extrudability, and antibacterial activity was analyzed. The results showed that formula III with variation of carbopol: tragacanth 0.25:0.75 not only had the best physical properties but also the most effective formula against *S. mutans*.

Keywords: Betel nuts, *Streptococcus mutans*, tragacanth, carbopol

A. Introduction

Dental caries, also known as tooth cavities and tooth decay is the condition that describes holes or other structural damage in the tooth. The oral bacterium, *Streptococcus mutans*, is the most responsible bacteria that cause the conversion of food carbohydrates to acids (Satcher 2000). According to Center for Disease Control and Prevention (CDC) 2014, children ages 6-19 years are suffered with dental caries about 15.6% (2007-2010) and adult ages 20-64 years with untreated dental caries are 23.7% (2005-2008). Proper brushing and using a tooth paste that can control the microbia in the mouth is one way to prevent dental carries (Ramos *et al*, 2010).

The areca nut is also popular for chewing throughout some Asian countries (George *et al*, 2006). The extracts from areca nuts have been proved to have antibacterial activity against mouth pathogenic bacteria (Cyriac *et al*, 2012; Chin *et al*, 2012) and antioxidant

(Wetwitayaklung *et al*, 2006; Amudhan *et al*, 2012). According to Knapsack database, this plant contains catechin, lauric acid, myristic acid, myristoleic acid, stearic acid, arecaidine, arecoline, guvacine, nicotine, procyanidin B3, procyanidin B7, procyanidin B1, arecatannin A1, arecatannin A2, arecatannin B1, procyanidin C4, and ethyl nicotinate, Ethyl N-methyl-1,2,5,6-tetrahydro-pyridine-3-carboxylate, Ethyl N-methylpiperidine-3-carboxylate, and Methyl nicotinate.

Anti-cariogenic toothpaste formulations should have characteristics such as cleansing action, feel and flavor, and desired in a toothpaste which has good consumer acceptability (Hefferren, 1967). Some binders which used to thicken toothpastes and prevent separation of the solid and liquid phases can affected the speed and volume of foam production, hence influenced the appearance of the toothpaste on the toothbrush, and the rinse ability of the product (Blanchford 2002). Carbopol and tragacanth variations were applied in this study to achieve the best toothpaste formula.

B. Methodology

1. Materials and equipments

The following chemicals were used: betel nuts seed, tragacanth, carbopol, glycerin, calcium carbonate, Sodium lauryl sulphate, sorbitol, nutrient agar media (NA), nutrient broth media (Mueller Hinton/MH) . Bacteria *Streptococcus mutans* ATCC 25175 were acquired from Microbiology laboratory of Dentistry Faculty of Universifat Gadjah Mada.

The SCAN500® Interscience was used to measure the diametre inhibition growth zone digitally.

2. Preparation of betel nut seed extracts

Unrippen betel nuts were collected from Sorong, West Papua, identified at the Laboratory of Pharmaceutical Biology laboratory, Pharmacy Department Universitas Islam Indonesia. The seeds were taken, dried, and grinded into powder. Seed powders were dissolved in ethanol for three days for three times, filtered, and evaporated to get crude ethanolic extracts. The extracts was the standardized according to General parameter for Plant Extract Standardization Guidelines (Depkes RI, 2000).

3. Minimum of Inhibitory Concentration (MIC) of betel nut extracts on S. *mutans*

The extracts was analyzed against *S. mutans* to determine MIC value. Extratc with concentration 195 µg/10 µl, 97.65 µg/10 µl, 48.8 µg/10 µl, and 24.41 µg/10 µl were used, using disk diffusion method. All the experiments were conducted in triplicate. For the agar diffusion test (double layer-well technique), MH culture medium was used for the *S. mutans*. The inoculum for bacterial strains were prepared in a saline suspension of the strains and adjusted to a density equivalent to the standard of the McFarland. In a laminar flow chamber, NA at 50 °C was poured into 25 x 150 mm sterile petri dishes. After solidification of the culture medium, 10^8 colony forming units (cfu) *per* milliliter of original inoculum, was poured onto the base layer. After solidification of microbia layer, the well (5 mm in diametre) were made, and filled with extracts. The plates were incubated at 37 ºC for 24 h. After incubation, the diameters of microorganism growth inhibition zones that formed around the disks were measured with digital SCAN 500® Interscience.

4. Toothpaste formulation

In this study, we developed areca nut extract into an anti-cariogenic toothpaste from Michael and Ash (1977) formula, using carbopol and tragacanth variations. Tragacanth, menthol, carbopol, and sorbitol were dilute in glycerin. Betel nut extracts was also diluted in glycerin and added with the first solution. $CaCO_3$ and Na lauryl sulphate were added into the mixture, mixed until homogene using a mixer until a semisolid toothpaste was formed. The composition of the formula was described as showed in table I.

Table 1. Formula of the tooth paste

Components	**Functions**	**F1**	**F2**	**F3**	**F4**	**F5**
Betel nut extracts (g)	Active substance	1.95	1.95	1.95	1.95	1.95
carbopol (g)	Binder	0.5	0.75	0.25	1	0
Tragacanth (g)	Binder	0.5	0.25	0.75	0	1
Glycerin (g)	Humectant	26	26	26	26	26
Na.lauryl sulphate (g)	Surfactant	0.5	0.5	0.5	0.5	0.5
$CaCo_3$ (g)	Abrasif	30	30	30	30	30
Sorbitol (g)	Sweetener	20	20	20	20	20
***Menthol* (g)**	Flavouring agent	0,4	0,4	0,4	0,4	0,4
***Aquadest* add (ml)**	solvent	100	100	100	100	100

All of the formula was the analyzed for its physical stability including organoleptic, homegeneity, spreadability, viscosity, pH, foaminess, and extrudability during 4 weeks of storage.

5. Antibacterial activity of the toothpaste against *S. mutans*

Media and *S. mutans* were prepared as described in the MIC determination of the areca nut extracts above. The best formula from the table 1 was test in triplicate, compare with herbal toothpaste from the market (X) that claimed to have anti cariogenic activity. Sample (100 mg) was applied in the well (10 mm in diametre) and plates were incubated at 37 ºC for 24 h. After incubation, the diameters of microorganism growth inhibition zones that formed around the disks were measured with digital SCAN 500® Interscience.

6. Statistical Analysis

Physical appearance and stability of the toothpaste were described descriptively and compare to reference and data on antimicrobial activity were evaluated by One Way Anova test (p = 0.05).

C. Results and Discussion

The areca nut extract yielded 26,83% of rendemen from its dried powder. The extracts was prune color (Colour Chart 701C1C) and water contain was 6,83 ± 0,34 %. Its Thin Layer Chromatograpy (TLC) profiling showed the flavonoid and alkaloid content (Figure 1).

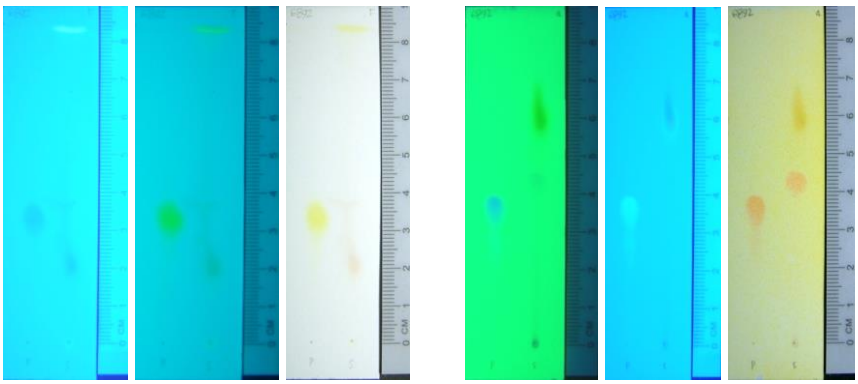

Figure 1. The TLC profiling of areca nut extract.

A was the flavonoid detection system and B was the alkaloid detection system. In the A we can see the left spot was rutin, and the right spot was area nut extract, under 1. UV 254 nm; 2. UV 366 nm; and 3. visibe light with $AlCl_3$ spray reagents with Silica Gel F_{254} as stationary phase and chloroform : methanol : formic acid (1:4:0,5) v/v as mobile phase
In B we can see the left spot was quinine and the right spot was areca nut extract, under 1. UV 254 nm; 2. UV 366 nm; and 3. visibe light with Dragendorff spray reagents with Silica Gel F_{254} as stationary phase and methanol : NH_4OH (100:1,5) v/v as mobile phase.

The extracts that contain flavonoid and alkaloid also active against *S. mutans*. The MIC value from the extract was 1,95% (Figure 2). This dose then was applied as active ingredient concentration for the toothpaste formulation.

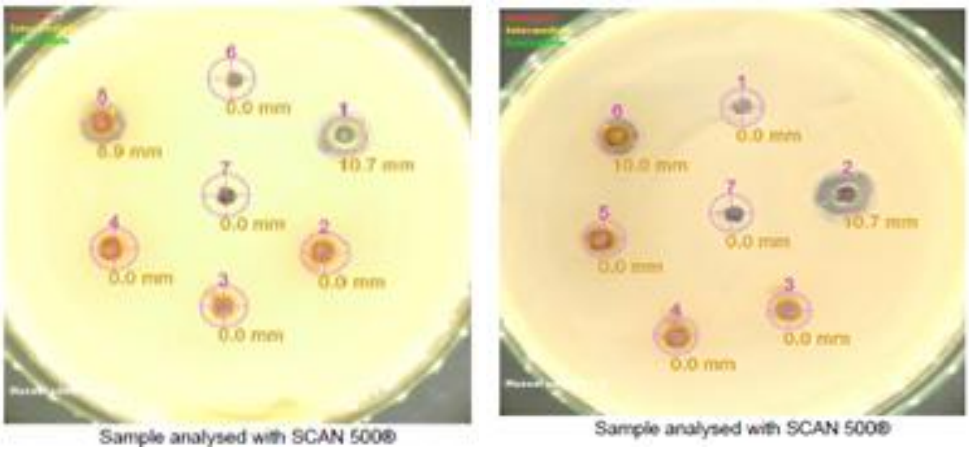

Figure 2. The two replicate results of the Minimum Inhibitory Concentration (MIC) of Areca nut extracts against *S. mutans.* The diameters of microorganism growth inhibition zones that formed around the disks were measured with digital SCAN 500® Interscience (diametre of the well was 5 mm). 1. DMSO 75%, 2. tymol 5 mg, 3. extract 24,41µg/10 µl, 4. extract 48,8µg/10 µl, 5. Extract 97,65µg/10 µl, 6. extract 195µg/10 µl, 7. DMSO 5%.

The physical characteristic of the toothpaste after 4 weeks storage was describe in table 2. From the table we can see that the best physical properties was formula 3 (F3) with carbopol: tragacanth 0.25:0.75. During storage, all formula tends to gain increment in pH but tend to decrease the foaminess, extrudability, and spreadability. According to the consumer satisfaction review (data not shown), formula 3 achieved the highest acceptance hence the formula 3 was chosen as the best formula. Formula 3 then compared with the herbal toothpaste available in the market (X) for its antibacterial activity against *S. mutans*.

Tabel 2. Physical properties of the toothpaste formula with carbopol: tragacanth variations after 4 weeks of storage

Formula		**F1**	**F2**	**F3**	**F4**	**F5**
	a	4,0	2,7	2,53	2,5	2,53
Spreadability (cm)	b	3,56	2,0	2,4	1,86	1,8
	a	2,9	2,6	3,2	3	2,85
Extrudability	b	3,1	2,45	3,1	2,8	3
	a	2,55	2,43	2,72	2,82	2,98
Foaminess (s)	b	2,31	2,64	2,55	2,75	2,56
	a	7	7	7	7	8
pH	b	8	8	8	8	8
	a	424	367.3	411.6	371	512
Viscosity (dPas)	b	423,3	350	419,5	333,3	540

F1, F2,F3,F4,and F5 were formulas with carbopol and tragacanth variations in sequence of 0.5:0.5; 0.75:0.25 ; 0.25:0.75 ; 1:0 ; and 0:1 respectively (a: first week of storage, b: 4 weeks of storage).

Figure 3 showed the result of antibacterial activity between formula 3 and toothpaste X against S. *mutans*. Formula 3 gives inhibitory growth zone 20 ± 0,4 mm and toothpaste X has inhibitory growth zone 20 ± 0,4 mm. According to One Way Anova statistical analysis, the activity between those toothpaste was significantly different, and formula 3 has better activity against S. *mutans*.

This result give indications that the formula using carbopol and tragacant combination with active ingredient of betel nut extract is really promising product. Many research need to be done to esnsure the long term stability and to improve the appearance of the toothpaste from betel nut extract.

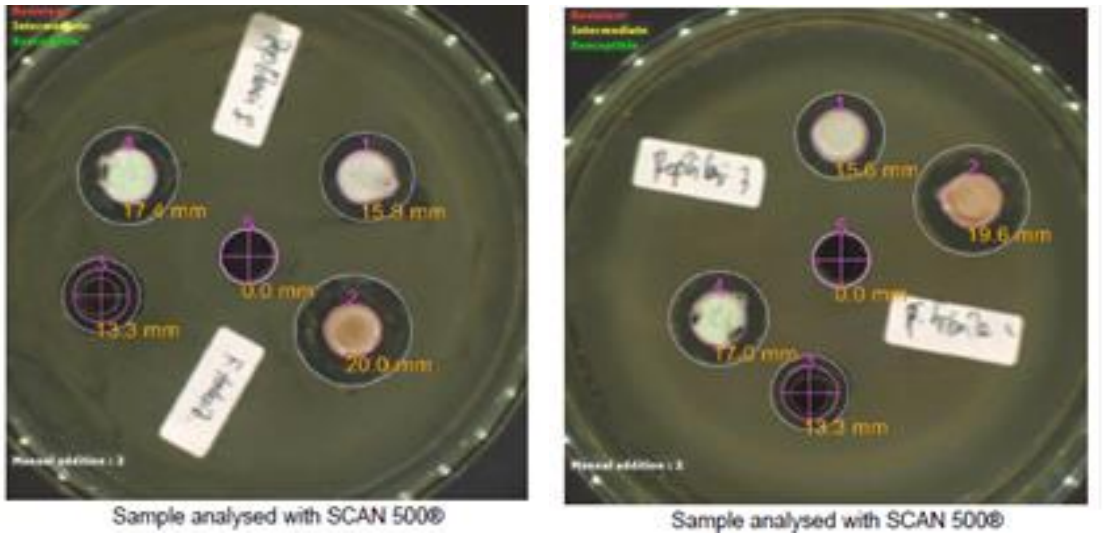

Figure 3. The two replicate results of the antibacterial activity of formula 3 and toothpaste X against *S. mutans.* The diameters of microorganism growth inhibition zones that formed around the disks were measured with digital SCAN 500® Interscience (diametre of the well was 10 mm). 1. Formula without extract, 2. Formula 3, 3. Areca nut extract, 4. Toothpaste X, 5. DMSO 5%.

D. Conclusion

From the study we can make conclusion that the aethanolic extract of Areca *catechu* seed can be made into anti-cariogenic toothpaste with carbopol :tragacant (0.25:0.75). The activity of areca nut toothpaste against *S. mutans* was better compare to branded herbal toothpaste that available in the market. Some development need to be done to improve the quality of the areca nut toothpaste formulation.

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