

Formulation of Toothpaste Gel Containing Mixture of Aloe Vera (*Aloe barbadensis* mill.) and Red Betel (*Piper crocatum*) Extract in Prevention of Dental Caries

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Abstract :

Aim : Tried to give the scientific evidence how the antibacterial activity effect of aloe vera (*Aloe barbadensis* Mill.) and red betel (*Piper crocatum*) and mixed of those herbals in toothpaste gel product against to dental carries bacteria such as *Streptococcus mutans*.

Background : To better understanding and improve product trusting of the toothpaste gel with those herbals contents the scientific evidence must do. As literally found that aloe vera and red betel contents has many compound to maintain healthy dental.

Methods : Firstly a qualitative phytochemical screening among those extract herbals was done with some reagent. The colors from reaction proven that all information same as a previous publication. The aloe vera extract got from crumbling fine of flesh leaves, then squeezed on the filter to get some liquid, meanwhile, the red betel extract came from the maceration of simplisia leaves in ethanol 70% for 3 x 24 hours, then drying in a rotary evaporator at 40°–50°C. Further investigation to determine minimum growth inhibitory concentrations (MGIC) from each extracted and mixed with extract (aloe vera: red beetle = 1:1, 2:2.1:2, and 2:1) as an active ingredient in the toothpaste gel dosage form. The best MGIC from extract mixed will be blend with the chosen basis of toothpaste gel, then the value of MGIC was compared between gel without extract (basis only), gel with mixed extract (finished goods), and gel from the market. The effective time for antibacterial activity contact was tested with various time in 30, 60, 90, and 120 seconds.

Result and Discussion : The MGIC value for aloe vera extract in concentration 30% (1.8 cm), from red betel extract in concentration 9% (1.9 cm) and the best value MGIC came from extract mixture of red betel : aloe vera = 2 : 1 (17% : 30%) (1,95 cm). It means that mixture of those herbals effectively proven to reduce the growth of the bacteria. However, comparing of value MGIC between our product with the market product visually from their inhibitory diameter found that our product at 1.5 cm while market product at 1.9 cm. It was proven that growth inhibitory of our product almost like as market product. The antibacterial activity test was found that inhibitory activity of our product taken after 120 seconds (2 minutes) contacted.

Conclusion : Based on the evidence from the investigation, those herbals mixture effectively proved to against of bacteria growth and active around 2 minutes after application.

Keywords: Toothpaste gel, aloe vera, red betel, antibacterial activity

INTRODUCTION

Toothpaste gel with natural ingredients such as citrus fruit, aloe vera, and red betel has widespread in the market. But there is a little investigation to prove effectivity the dosage form. So, to better understanding and improve product trusting the scientific evidence must do. As literally found that aloe vera and red betel contents have many compounds to maintain healthy dental. In aloe vera found glycosid, alkaloid, tannin, calcium, magne-sium and a lot vitamin as B₁, B₂, and C (Claus, 1961). Meanwhile in red betel found flavonoid, tannin, alkaloid, a polyphenolic compound and some essential oil (Sudewo, 2005). In previous research, those ingredients have proven to against bacterial growth such as *Staphylococcus aureus* and other five strains *Streptococcus* mutant (Furna-wanthi, 2002; Fly, 1963; Sudewo, 2005).

To maintain healthy dental there have many dosage forms used to cleaned and teeth polished such as tooth powder, toothpaste and toothpaste gel (Carranza, 1996). Toothpaste type was widespread utilized cause has low density and small abrasive. The toothpaste gel with high humectant and smooth polishing was choosen more (Haywood, 1996). For pleasant application the toothpaste gel formulation then used in this investigation.

MATERIALS AND METHODS

Materials

Plant Materials

The leaves simplisia of Red Betel (*Piper crocatum* Ruiz&Pav.) and Aloe vera (*Aloe barbadensis* Mill.) comes from experiment garden *Manoko*, in sub district Lembang – West Bandung Regency, West Java, Indonesia which was determined in Taxonomy Labo-ratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia.

Bacterial Speciment

The bacterial used in this research was *Streptococcus mutans* comes from Faculty of Dentistry, Universitas Padjadjaran. The medium growth of bacteria was *Mueller-Hinton Broth* (Pronadisa), Fresh Blood (Faculty of Medicine) and *Blood Agar Base* (Pronadisa).

Extraction

The extract of aloe vera gained from blendering of flesh leaves until fines then pressed on the filter to get some liquid. Whereas, the red betel extract came from the maceration methods with soaked in ethanol 70% for 3 x 24 hours, then concentrated in a rotary evaporator at 40°–50°C until getting a thick extract with a constant weight. The

subsequent of extract result then standardized phytochemical screening and water content.

Antibacterial activity

Agar diffusion well-variant

Amount of 20 µl inoculum of bacterial was added in Blood Agar Plate (BAP) which made from 1000 µl fresh blood on a sterile Petri dish with 19 ml Blood Agar Base (BAB). After coagulation made four wells (50 µl) with perforator in each of Petri dish. Fill all wells with concentration 7.5, 15, 30 and 60% w/v of each extract which has dissolved in DMSO (Dimethyl Sulphoxide) before. As a positive control, BAP was used with the bacterial inoculum and for a negative control, used only BAP. All of Petri dish was incubated at 36°C ± 1°C for 18 – 24 hours. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in cm.

Minimum Growth Inhibitory Concentrations (MGIC)

The bacterial was suspended with *Mueller-Hinton Broth* (MHB) steril in reaction tube until the incubation (36°C ± 1°C for 24 – 48 hours) resulted in an inoculated suspense with McFarland 0.5 turbidity (10⁸ CFU/mL or 10⁵ CFU/well). After getting the standardized inoculum added 1 µl of the inoculum to each of MHB steril media on variation dilution concentration of extract. MGIC values were defined as the lowest concentration of each extract product, which completely inhibited microbial growth. The results were expressed in weight per volumes percentage. Beside each of extract product, a mixture of extract composition (aloe vera: red betel = 1:1, 2:2.1:2, and 2:1), formulation product and market product (as a comparator) were examined also.

Disinfection Contact time of Formulation Product

About 10 ml of the formulation product was mixed with 100 µl inoculum bacteria. After 30, 60, 90, and 120 seconds an ose of the mixture was inoculated on sterile Petri dish BAP. Incubate all Petri dish at 36°C ± 1°C for 18 – 24 hours. Observed the bacterial colony growth on Petri dish after incubation.

Toothpaste gel Formulation

After found the best composition of mixture which have to blocked bacterial growth. The formulation was arranged as like in table 2. The quality performance of the product has been compared with market product, especially for pH and viscosity perform as shown in table 6.

RESULT AND DISCUSSION

From 10 kg of aloe vera fresh flesh leaves found 6.5 litres of liquid extract. Meanwhile maceration of red betel obtained with a yield of 13.9% of a thick extract. According on phytochemical screening as shown in table 1. the differences of red betel extract and aloe vera extract were only at alkaloid content. The result of water content of Red Betel extract showed at 3.5%, the Aloe vera has a lot of water content and usually not to examined it.

Antibacterial Activity

Found that red betel extract has activity start from 15%, and aloe vera extract start from 30%, as shown in table 3. below. From this conc. the extracts dilution for MGIC values was determined.

Table 1: Phytochemical Screening

Compound Group	Red Betel	Aloe vera
Alkaloids	+	-
Flavonoids	+	+
Polyphenols	+	+
Tannin	+	+
Monoterpenoids and Sesquiterpenoids	+	+
Steroids and Triterpenoids	-	-
Quinones	+	+
Saponines	+	+

Note : (+) detected (-) undetected

Table 2: Formulation of Toothpaste Gel with Aloe vera and Red Betel Extract

Composition	Formula (%)	
	A ₀	A ₁
Sorbitol 70%	54.4	54.4
CaCO ₃	18	18
Glycerine	5	5
Sod. Lauryl Suphate	1.2	1.2
Sacharrine	0.35	0.35
Carboxy Methylcellulose	0.35	0.35
Mentholum	0.9	0.9
Aerosil	5.5	5.5
Red Betel Extract	-	17
Aloe vera Extract	-	30
Aquades hingga	100	100

Note : A₀ = Product without extract A₁ = Product with extract

Table 3: Antibacterial Activity Test

Red betel Conc. (% w/v)	Result	Aloe vera Conc. (% w/v)	Result
9.5	-	30.5	-
9	-	30	-
8.5	+	29.5	+
8	+	29	+
7.5	+	28.5	+

Table 4: MGIC against *Streptococcus mutans*

Extract Conc. (%w/v)	Zone Diameter (cm)	
	Aloe vera	Red Betel
60	2.5	2.6
30	1.8	2.2
15	-	1.9
7.5	-	-

Shown at table 4. as MGIC value of both extract that red betel extract has reduce bacterial growth from conc. 7.5 – 9% w/v and aloe vera from 28.5 – 30 %w/v. This result was draw the line for mix both extract to determine which has the best mixture composition. Then the best composition will used in toothpaste gel formulation. Found that the best composition came from extract mixture of red betel : aloe vera = 2 : 1 (17% : 30%). After formulation found performance quality (pH and viscosity) the product from formulation was almost like as market product. To find out when the antibacterial activity was begin in the formulation product, the contact time test was carried out. From experiment clarify that activity was start from 120 seconds after applicaton.

Table 5: MGIC against *Streptococcus mutans*

Mixture Composition (red betel: aloe vera) (% w/v)	Inhibitory zone diameter (cm)		Average (cm)
	Petri 1	Petri 2	
2 (17%) : 1 (30%)	1.92	1.98	1.95
2 (17%) : 2 (60%)	1.54	1.57	1.55
1 (8.5%) : 1 (30%)	0.91	0.83	0.87
1 (8.5%) : 2 (60%)	-	-	-

Table 6: Comparison of Various Products

Product	Physical Quality		Antimicrobial Activity (MGIC)				
			Inhibitory zone diameter (cm)	Contact time (seconds)			
	pH	Visc. (cP)			30	60	90
A ₀	6.91	190.30	-	+	+	+	+
A ₁	6.72	195.50	1.6	+	+	+	-
S	6.50	170.00	1.9				

Note : A = Formulation product S = Market product

CONCLUSION

Antimicrobial activity against *Strepto-coccus mutans* of red betel and aloe vera has carried out. The best result came from mixture of red betel : aloe vera = 2 : 1. The quality of our product has relatively as like as the market product. Shown from contact time test that our product was effective after 2 minutes application.

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