Utilization of Bromelain Enzyme from Pineapple Peel Waste on Mouthwash Formula Against *Streptococcus mutans*

Utilization of Bromelain Enzyme from Pineapple Peel Waste on Mouthwash Formula Against *Streptococcus mutans*

H Rahmi[1,*], A Widayanti[1], and A Hanif[1]
[1]Department of Pharmacy and Science, Universitas Muhammadiyah Prof. DR. HAMKA, Delima II Street, Malaka Sari, Klender, Jakarta, Indonesia

*hanifah_rahmi@uhamka.ac.id

Abstract. The aim of this research was to apply bromelain enzyme from rough extract of *Ananas comosus* (*L.*) Merr. peel into the mouthwash preparation and investigate the enzyme activity to inhibit *Streptococcus mutans* growth.. The research method, bromelain was extracted by means of a blended pineapple skin, the extract was filtrated and centrifuged to obtain a supernatant. Enzyme activity was analyzed by spectrophotometer at 275 nm. Mouthwash formula which involved different concentration of bromelain (20%, 25%, 30%, 35%, 40%, 50%, and 60% (v/v)) was tested the antimicrobial activity by disc diffusion method. The results showed that the formula with 35% enzyme was an effective in restricting the growth of *Streptococcus mutans*, with a relative potential value of 100.40% compared to chlorhexidin *0.1% as* possitive control.

Keyword: bromelain enzyme, *Streptococcus mutans,*

1. Introduction

Dental caries is a disease in the hard tissues of teeth due to the activism of acid-producing bacteria capable of fermenting carbohydrates in the consumption by humans. One of the bacteria that is generally regarded as the main agent of dental caries is *Streptococcus mutans.* The dental plaque component of these oral microorganisms can become pathogenic if the population increases so that the caries process progresses more quickly [1]. *Streptococcus mutans* have an important role because of the ability to attach to the enamel through the saliva particle and as an acid-producing bacteria thereby creating an acidic environment that will be at risk of cavities [2].

Various methods to reduce the incidence of caries has been done, one of them by inhibiting the growth of caries-causing bacteria by utilizing a number of plants, especially pineapple. Pineapple includes fruit that has a very complex content, rich in minerals both macro and micro, organic substances, water and vitamins. The content of chlorine, iodine, phenol and bromelain enzymes on pineapple has an effect of suppressing bacterial growth [3]. Pineapple skin is an industrial processed product consisting of leftover flesh, skin, and outer shell. If pineapple skin is not used it can cause environmental pollution. Pineapple skin is a potential source for the utilization of bioactive compounds contained there in, especially bromelain enzyme [4]. Bromelain is one type of protease enzyme sulfhydryl capable of hydrolyzing peptide bonds on proteins or polypeptides into smaller molecules [5].

Bromelain is a common enzyme to evaluate the antibacterial activity of fruit or plant extracts. For example, Dutta and Bhattacharyya evaluated the antimicrobial activity of an aqueous extract of pineapple leaves which was active against strains of *E. coli*, *S. aureus* and *B. subtilis*. The aqueous extract inhibited 70 to 95% of microbial growth and its MIC varied from 1.65 to 4.95 µg/mL [6]. Beside that, in previous research, the result of Herliani study using the minimum inhibitory concentration test of the dilution method was found that the concentration of bromelain enzyme capable of inhibiting *Streptococcus mutans* was 30% (v / v) [7]. Considering that, this research was conducted pineapple peel extract to get bromelain enzyme that can be formulated on the mouthwash for against *Streptococcus mutans* . In this study used an alternative surfactant, which is tween 80 which can be used as a surfactant and cleaning agent. Tween 80 belongs to non-inonic surfactants that have low toxicity so widely used in the eating industry, cosmetics and oral drug formula [8].

2. Experimental Method

Pineapple peel was washed with aquades, cut into small pieces and weighed as much as 2000 grams. The pineapple peel was blended with PBS (phosphat buffer saline) cold pH 7 (1: 0,5) for 15 minutes. The pineapple peel extract was filtered and the filtrate was centrifuged for 20 min at a rate of 5,000 rpm at 4℃ to produce a supernatant [9].

Determination of protein content was determined by Warburg-Christian method with the standard curve of bovine serum albumin (BSA). The absorbance of the standard solution and the sample was measured using a UV spectrophotometer at λ 280 nm [10]. The bromelain activity test was performed by Horikoshi method at λ 275 nm against the sample along with the control with the standard of tyrosine standard.

Crude enzyme of bromelain from pineapple peel was prepared with different concentration, there were 20% (F1), 25% (F2), 30% (F3), 35% (F4), 40% (F5), 50% (F6), and 60% (F7) (v/v). Mouthwash formula was contained tween 80, 15% sorbitol, 0.2% nipagin, 0.1% papermint oil, 0.1% menthol, aquades, and crude enzyme.

To investigate the antibacterial effectiveness, the mouthwash was tested using disc diffusion method. Positive control was used 0.1% (w/v) chlorhexidine and mouthwash formula without enzyme as negative control. Working stock preparation, *Streptococcus mutans* was taken from the stock of work to prevent the occurrence of contamination in primary stock. Bacteria derived from primary cultures taken as one ose. Thereafter, one ose of bacteria from the primary culture was scraped onto a TSA solid medium and incubated at 37 ° C. for 24 hours.

Bacteria from the working stock of one ose were transferred into 10 ml of sterile liquid TSB and incubated at 37℃ for 24 hours. Then take 50 µl from the culture is propagated into the petri dish, then added the medium for TSA at 45° C. After that the dish is shaken so the bacteria spread evenly. Furthermore, the bacteria culture is silenced at room temperature until solidified. After solidifying a 6 mm diameter disc paper with 0.02 ml absorption that has been previously immersed in samples F1, F2, F3, F4, F5, F6, F7, possitive control and negative control. Each experiment was done by repeating 3 times, then incubated at 37℃ for 24 hours. Response of antibacterial potency by measuring the diameter of the bacterial free zone around the clear disc-shaped paper and measured the area of its inhibition.

3. Results and Discussion

Results of determination of plant samples from LIPI Cibinong Biology Research Center, Bogor proved that the samples used in this study were true species *Ananas comosus* (L.) Merr., Family *Bromiliaceae.* Protein concentration of rough pineapple peel extract sample is about 43.71 mg/ml. The total bromelain activity value was 7.75 x 10^{-1} units and the specific activity was 1.77 x 10^{-2} units / mg.

Results of the antibacterial effectiveness test of mouth rinses on the growth of *Streptococcus mutans* cause dental caries resulted that all formulas have antibacterial effect on *Streptococcus mutans*. The results were seen after 24 sheets of disc paper (every 3 sheets) soaked into formulas 1, 2, 3, 4, 5, 6, 7 and negative controls (every 6 sheets) were immersed in positive controls. The disc paper is placed on TSA media that has been inoculated with a suspension of *Streptococcus mutans*. Observations were made after each medium was incubated at 37 °C for 24 hours to see whether or not the growth of *Streptococcus mutans* bacteria marked with a clear zone around the disc paper. The results are showed in **Figure 1**. The clear zone was formed in each formula, it interprets that bromelain from pineapple peel has the ability to forbid the growth of *Streptococcus mutans*.

The results obtained from **Figure 2** show that the formula 5 has the largest mean inhibitory zone diameter of 7.86 mm, formula 1 of 5.06 mm, formula 2 of 6.56 mm, formula 3 of 7.16 mm, formula 4 by 7.43 mm and as a positive control group has an average inhibition zone diameter of 7.40 mm while the negative control does not form the inhibit zone. The results of the analysis of one way ANOVA test showed a one way ANOVA test showed that the significance value (sig <0.00). This means there is a significant difference (sig <0.05) between formula 1, 2, 3, 4, 5, K+ and K-.

According to Davis & Stout, the classification of the bacterial growth resistance response seen on the inhibition zone diameter consists of 4 groups: weak response (≤5 mm diameter), medium (5-10 mm diameter), strong (10-20 mm), and very strong (diameter ≥20 mm) [11]. Based on the classification, it was found that all the formulas and positive controls had an antibacterial effect on the growth of *Streptococcus muta*ns in the medium category.

The results also show, the higher the concentration of the rough extract of the pineapple peel, the larger the inhibit zone that forms around the disc paper. This is in accordance with the statement Ulmursida A et al, that the higher the concentration of an antibacterial substance the antibacterial activity is stronger [12]. Increased concentrations of substances cause increased levels of active compounds that act as antibacterials, so the ability to kill a bacterium is also greater [13].

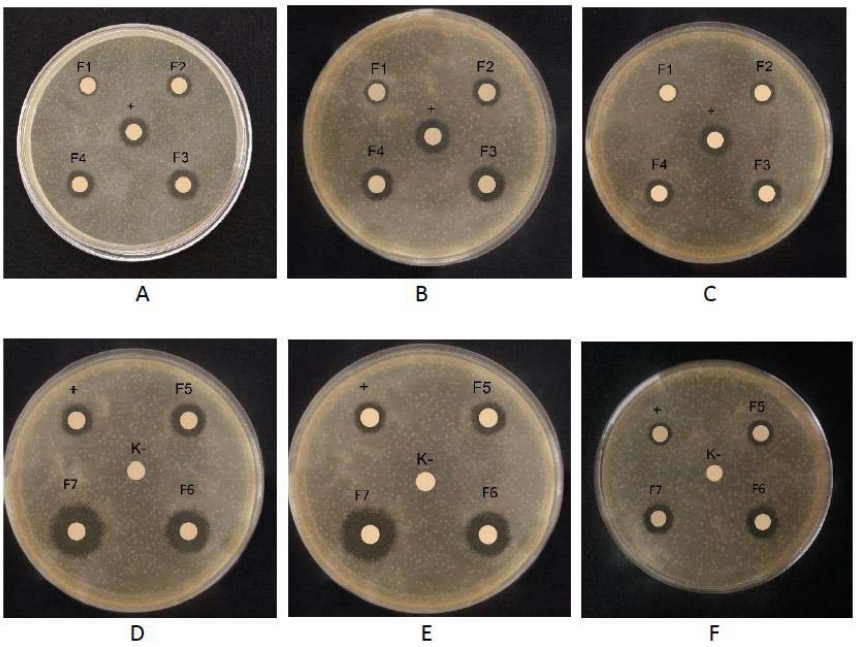

Figure 1. Inhibition zone of control and mouthwash formula. (K+ : possitive control, K- : negative control, F1 : formula 1 with 20% extract, F2 : formula 2 with 25% extract, F3 : formula 3 with 30% extract, F4 : formula 4 with 35% extract, F5 : formula 5 with 40% extract, F6 : formula 6 with 50% extract, and F7 : formula 7 with 60% extract).

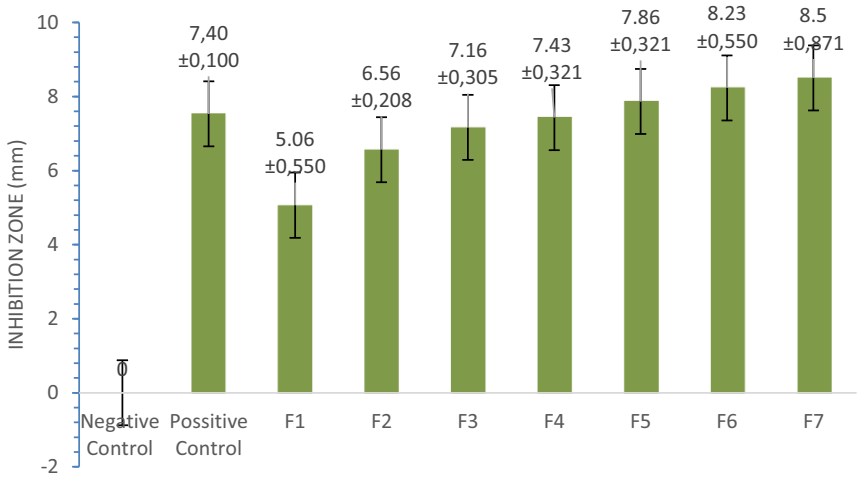

Figure 2. The diameter of inhibition zones of bromelain enzyme against *Streptococcus mutans*. F1 : formula 1 with 20% extract, F2 : formula 2 with 25% extract, F3 : formula 3 with 30% extract, F4 : formula 4 with 35% extract, F5 : formula 5 with 40% extract, F6 : formula 6 with 50% extract, and F7 : formula 7 with 60% extract).

The content in pineapple skin that becomes antibacterial substance is bromelain enzyme. The enzyme bromelain is a proteolytic enzyme that plays a role in protein breakdown [14]. The way bromelain enzyme works is to reduce the surface tension of bacteria by hydrolyzing salivary proteins and glycoproteins which are bacterial mediators to adhere to tooth surfaces [15]. In this research, it was found that formula 4 with pineapple crude extract concentration of 35% was an effective concentration in inhibiting the growth of *Streptococcus mutants* bacteria because it resulted in a better drag zone of positive control. Formula 4 had minimum inhibitory concentration of extract that was inhibit the growth of *Streptococcus mutans* after incubation [16]. A relative potential value of formula 4 compared to 0.1% (w/v) chlorhexidine was 100.40%.

4. Conclusions

Rough extract of pineapple peel can be formulated into mouthwash. Protein concentration of pineapple peel extract is 43.71 mg/ml. The total bromelain activity value was 7.75 x 10^{-1} units and the specific activity was 1.77 x 10^{-2} units/mg. The formula that can be used as a mouthwash is a formula 4 with a rough concentration of pineapple peel extract of 35% (v/v), with a relative potential value of 100.40%.

References

[1] Daboor, S. M., Masood, F. S. S., Azab M.S., & Nori E.E. 2015. A review on *Streptococcus mutans* with its diseases dental caries, dental plaque and endocarditis *Indian J Microbiol Res* **2** 2 76-82.

[2] Forssten, S.D., Marika, B. & Arthur, C.O. 2010. *Streptococcus mutans*, *Caries and Simulation Models. Journal Nutrients* **2** 290-298.

[3] Kasa, T., Fistum, G., Yohanis. 2017. Chemical Composition and Nutritional Effect of Pineapple, Mango, Banana, Avocado and Orange: A Review Article *Chemical and Process Engineering Research* **54** 1-6.

[4] Ketnawa, S., Theppakorn, T., Chaiwut, P., & Rawdkuen, S. 2009. Partitioning of Bromealin from Peneapple Peel (Nang_Lae cultv.) by *Aquedous Two Phase System. Journal Ag-Ind* **2** 4 457- 468.

[5] Urumugam, A. & Ponnusami, V. 2013. Pineapple fruit bromelain recovery using recyclable functionalized ordered mesoporous silica synthesized from sugarcane leaf ash *Brazilian Journal of Chemical Engineering* **30** 3 477-486.

[6] Dutta, S. & Bhattacharyya, D., 2013. Enzymatic, antimicrobial and toxicity studies of the aqueous extract of Ananas comosus (pineapple) crown leaf *J. Ethnopharmacol* **150** 451–457.

[7] Herliani, H. 2015. Efektifitas Enzim Bromelin dari Limbah Kulit Nanas (*Ananas comosus*) dan Enzim Papain dari Getah Pepaya (*Carica papaya*) Sebagai Antiplak Dalam Obat Kumur. *Laporan Akhir Program Kreativitas Mahasiswa*. Universitas Muhammadiyah Prof. DR. Hamka. Jakarta.

[8] Rowe, C.R, Sheskey P.J., Cook, G.W., & Fenton, E.M. 2012. Handbook of Pharmacetical Excipients Seventh Edition. *Pharmacetical Press*. London.

[9] Manzoor, Z., Nawaz, A., Mukhtar H., & Haq I. 2016. Bromelain: Methods of extraction, purification and therapeutic applications *Braz. Arch. Biol. Technol* **59** 1-16.

[10] Bonjoch, N.P. & Tamayo, P.R. 2001. Protein Content Quantification by Bradford Method on *Handbook of Plant Ecophysiology Techniques*. **19** 283-295.

[11] Davis, W.W. & Stout, T.R. 1971. Disc plate method of microbiological antibiotic assay *Applied Microbiology* **22** 4 659-665.

[12] Ulmursida, A., Ambariyanto, A., Trianto, A. 2017. Antibacterial activity of mangrove *Avicennia marina* leaves extract against *Virgibacillus marismortui* and *Micrococcus luteus* bacteria. *AACL Bioflux* **10** 2 372-380.

[13] Roslizawaty, Ramadani, N.Y., Fakhrurrazi, & Herrialfian. 2013. Aktivitas Antibakterial Ekstrak Etanol dan Rebusan Sarang Semut (*Myrmecodia* sp.) terhadap Bakteri *Escherichia coli. Jurnal Medika Veterinaria* **7** 2 91-94.

[14] Arshad, M.S., Kwon, J.H., Imran, M., Sohaib, M., Aslam, A., Nawaz, I., Amjad, Z., Khan, U., & Javed M. 2016. Plant and bacterial proteases: A key towards improving meat tenderization, a mini review. *Cogent Food & Agriculture* **2** 1-10

[15] Ledder, R.G., Madhwani, T., Sreenivasan, P.K., Vizio, W.D., McBain, A.J. 2009. An in vitro evaluation of hydrolytic enzymes as dental plaque control agents. *Journal of Medical Microbiology* **58** 482-491.

[16] Andrews, J.M. 2002. Determination of minimum inhibitory concentrations. J Antimicrob Chemother **49** 6 1049