



Inhibitory Power of Mouthwash Containing Pineapple Cobs (*Ananas Comosus* (L.) Merr.) Ethanol Extract toward the Growth of *Streptococcus Mutans*

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ABSTRACT

Aim: This study aimed to investigate the inhibitory power of mouthwash containing pineapple cobs ethanol extract with concentrations of 7,5%, 10%, 15%, 20%, 25%, and 30% against *Streptococcus mutans*.

Materials and Methods: The agar well diffusion method was employed in order to investigate the inhibitory power of mouthwash containing pineapple cob extract against the growth of *Streptococcus mutans*. A Few (20-100 µl) volumes of the mouthwash containing pineapple cobs ethanol extract from each of the concentrations was placed into each of the wells on blood agar in petri dishes had contained *Streptococcus mutans* then incubated for 24 hours at 37°C temperature and after these the measurement of inhibition power was measured with a caliper.

Results and conclusions: The results showed that mouthwash containing pineapple cobs ethanol extract with concentrations of 7,5%, 10%, 15%, 20%, 25%, and 30% could inhibit the growth of *Streptococcus mutans*. Mouthwash containing Pineapple cobs extracts with concentrations of 7,5%, 10%, and 15% have moderate inhibition power, and mouthwash containing pineapple cobs extracts with concentrations of 15%, 20%, 25%, and 30% have strong inhibition power. The conclusion is mouthwash containing pineapple cobs ethanol extract has inhibitory power against *Streptococcus mutans*.

Keywords: Ethanol Extract, Inhibitory Power, Mouthwash, Pineapple Cobs, *Streptococcus Mutans*.

Introduction

Dental caries is a main dental health problem for children, adults, pregnant women, and elderly people in the world. The rate of dental caries incidence in developed countries is still high.¹ Riskesdas in 2018 shows that the number of Indonesian citizens who have decayed teeth and toothache is 45,3%.^{1,2}

Dental caries is a multifactorial chronic disease as a result of multifactor interactions between host, bacteria, food, and time that cause cavitation on the tooth. A group of main bacteria that initiates dental caries is *Streptococcus mutans*.¹ The habitat of *Streptococcus mutans* is in the oral cavity, especially on dental plaque, multi-species biofilm formed on teeth surfaces. Three characteristics of *Streptococcus mutans* are generating many sticky

extracellular polysaccharides that assist in creating a strong bond of this bacteria on tooth enamel and play an important role in dental plaque formation, also it is acidogenic and aciduric thus decreasing plaque PH that initiates enamel demineralization.^{3,4} the growth of *S. mutans* must be inhibited in order not to cause dental caries with utilize antibacterial agents. One of the ways to inhibit dental caries formation is to restrict dental plaque formation on the tooth surface or regularly cleansing (plaque control). Plaque control could be conducted mechanically or chemically, chemical way uses antibacterial agent.⁵

Chemical control is conducted by rinsing with an antiseptic solution and the antiseptic often used is chlorhexidine gluconate, however, there are adverse effects appeared from long-term consumption which are increased stain formation, dental calculus formation, alteration of taste sensation, and gingiva irritation. Recently, many types of mouthwash containing herbal plants investigated their antibacterial properties with minimal adverse effects.⁶ Indonesia has been known to have many plants and a thousand amongst them could be utilized as herbal plants for traditional medicines.⁷ Pineapple is a fruit that mostly has been consumed by Indonesian citizens, it could be consumed either directly or in many forms such as juice, jams, syrups, and crackers. Pineapple has a scientific name which is *Ananas comosus* (L) Merr. Cayenne and queen are the most varied that has been cultivated in Indonesia.⁸

The part of pineapple mostly utilized is fruit meat, whereas other parts such as peels, leaves, buds, and cobs are regarded as waste and have been disposed of, even though the others contain active components and one of them is bromelain enzyme.^{8,9}

Bromelain is an enzyme generated from the pineapple plant either from the log, stick, leaf, fruit, or peels in different amounts. Bromelain is dominant in pineapple cobs compared with other

parts. According to previous research conducted by other peers, bromelain could inhibit the growth of aerobic bacteria and anaerobic bacteria that generate acid. In West Sumatra, based on data in 2013 recorded pineapple production is 321 tons. The researcher also visited the Bukittinggi market and Biaro, where pineapple is a cheap fruit, easy to find, and the taste is likable by many people.^{8,9}

Previous research that one of the researchers conducted about an inhibitory test of ethanol extract of pineapple cobs against *Streptococcus mutans* showed the ethanol extract of pineapple cobs of concentrations 100%, 75%, 50%, and 25% are able to inhibit the growth of *Streptococcus mutans* with diameters of inhibitory zone of them are 28,6 mm, 15,6 mm, 14,6 mm, and 13,6 mm, respectively. The next research that researchers conducted inhibitory power of ethanol extract from pineapple cobs toward the growth of *Streptococcus mutans* with smaller concentrations which is from the research, ethanol extract of pineapple cobs at concentrations of 30%, 25%, 20%, 15%, 10%, and 7,5% could inhibit the growth of *Streptococcus mutans*, diameters of inhibitory zone of them are 14,83 mm, 13,33 mm, 11,33 mm, 10,16 mm, 8,58 mm, and 7,45 mm., respectively. Based on the explanations above, the purpose of this research is to investigate the inhibitory power of mouthwash containing pineapple cobs ethanol extract toward the growth of *Streptococcus mutans*.

Methods and Materials

This research was a laboratory experiment. The sample used pineapple cobs obtained from a pineapple farm in Rimbopanjang, Riau province. In this research, there are 9 sample groups which are mouthwash containing pineapple cobs extract with concentrations of 7,5 %, 10%, 15%, 20%, 25%, and 30%, 1% povidon iodine as a control positive, 0,2% chlorhexidine as a control positive too, and equates as a control negative. The number of samples was determined by the Federer formula, from the calculation of the formula of Federer, the number of the samples used was 3 at each of the

sample groups which is there are repetitions at least 3 times for each concentration, thus a total number of samples is 27.

The inhibitory power test of ethanol extract of pineapple cobs was conducted in the Microbiology Laboratorium of the Faculty of Medicine at Andalas University, and the extract production was conducted in the pharmacy laboratorium of imam Bonjol pharmacy academy. The research data were analyzed with one-way anova.

1. Materials and Equipments Equipements utilized in this research which are: Petri dish, needle, vortex, ose, bunsen burner, test tube, rack, autoclave, inoculum tube, sterile cotton swab, micropipette, tip, incubator, hot plate, erlenmeyer, rotary vacuum evaporator boor prop and vernier caliper. Materials utilized in this research are pure isolation of Streptococcus mutans sample, pineapple cobs, blood agar media, filter paper, Nacl 0,9%, and Mc. Farland 0,5. 2. Procedures.

- i. The production of mouthwash containing pineapple cobs ethanol extract 2 kg wet

pineapple cobs washed and cut into small pieces, then dried in an oven for 4 hours. Pineapple cobs were dried and made into powder, subsequently, it was transferred to a maceration vessel to be extracted using 96% ethanol and soaked for 24 hours, the extraction process was repeated 3 times, and the extract was collected and evaporated until was free from ethanol solvent using a rotary evaporator in 500C temperature. Lastly, the extract was diluted with sterile aquades for obtaining extract with various concentrations of 7,5%. 10%, 15%, 20%, 25%, and 30%. Each of the concentrations was added up sorbitol, aquades and was mixed till homogeneous, subsequently added up peppermint to the solutions and mixed up until homogeneous to obtain mouthwash solutions containing pineapple cobs ethanol extract with different concentrations. The formulation of mouthwash could be looked at in table 1. Below.

Compositions	F1	F2	F3	F4	F5	F6
	7,5%	10%	15%	20%	25%	30%
Pineapple cobs ethanol extract	0,75 ml	1 ml	1,5 ml	2 ml	2,5 ml	3 ml
Corbitol	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml
Peppermint oil	0,1 ml	0,1 ml	0,1 ml	0,1 ml	0,1 ml	0,1 ml
Aquades	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml

Table 1: The Formulation of Mouthwash Containing Pineapple cobs Ethanol Extract.

- ii. The production of blood agar media At first, weigh powder of a blood agar media, then the powder was placed into Erlenmeyer and dissolved into sterile aquades. Secondly, it was heated using a hot plate stier, afterward, it was sterilized using an autoclave at 1210C temperature. for 15 minutes and 1 atm pressure. Thirdly, it was poured into petri dish that
- had had boor prop and it waited until solidified.
- iii. Breeding of Streptococcus mutans Streptococcus mutans colonies was taken with a sterile oce needle, then transferred onto an agar media, and incubated in an incubator at 370C temperature for 24 hours.

- iv. Inhibitory power test of mouthwash containing pineapple cobs ethanol extract employing agar well diffusion method:
 - a. The bacteria incubated before, taken colonies from agar media using a sterile ose needle, and then placed into Nacl Broth media till the turbidity is the same with McFarland standard. McFarland standar is equivalent to a bacteria suspension with a concentration of $1,5 \times 10^8$ CFU/ ml. This turbidity is used as a suspension standard for bacteria colonies.
 - b. A cotton swab was placed into bacteria suspension till wet, then the cotton swab was pressed into the inside part of a tube test and was spread over the entire agar surface.
 - c. Several holes with a diameter of 6-8 mm was punched aseptically with a sterile cork borer or a tip on several areas on the

blood agar, and a little of volume (20-100 μ l) of mouthwash solution containing the pineapple cobs ethanol extract from each of concentrations was introduced into each of the well on the blood agar contain *Streptococcus mutans*.

- d. Incubate the Petri dishes in an incubator at 37°C temperature for 24 hours. Vertical and horizontal Inhibitory zones would be formed around the well were measured using a venier caliper and the result of both of them was averaged.

Research Results and Discussions

From the one way anova test shown in table 2 below, P value is 0,000. This means mouthwash containing pineapple cobs ethanol extract with each of concentrations has inhibitory power toward the growth of *Streptococcus mutans* and there is a difference in inhibitory power.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	530,034	8	66,254	339,282	,000
Within Groups	3,515	18	,195		
Total	533,549	26			

Table 2: One Way Anova, *Significant Level Based On $P < 0,05$ *

The data in table 3 below has shown each of the 6 concentrations of mouthwash containing pineapple cobs extracts has an inhibitory effect toward the growth of *Streptococcus mutans* and each concentration has a different average inhibitory diameter.

David and Stout have divided the inhibitory power into 4 categories corresponding to diameters of the inhibitory zone which are: a) diameter > 20 mm means very strong, b) diameters 10-20 mm means strong, c) inhibitory diameters 5-10mm means moderate, d) inhibitory diameters < 5 mm means weak.¹⁰

Sample tests	The average of inhibitory zone diameter
Mouthwash 7,5%	7,45mm
Mouthwash 10 %	8,58 mm
Mouthwash 15 %	10,16 mm

Mouthwash 20 %	11,33 mm
Mouthwash 25 %	13,33 mm
Mouthwash 30 %	14,83 mm
Povidon iodine 1%	3,66 mm
Chlorhexidin 0,2%	11,25 mm
Aquades	0 mm

Table 3: The Result of Average Diameter of Inhibitory Zones on the Blood Agar Contained S. Mutans Colony After Had Been Incubated for 1X24 Hours.

According to the research conducted, has shown mouthwash containing pineapple cobs ethanol extract with concentrations of 7,5%, 10%, and 15% have a moderate inhibitory power, whereas mouthwash containing pineapple cobs ethanol extract with concentrations of 20%, 25%, and 30% have a strong inhibitory power in inhibit the growth of Streptococcus mutans.

1% Povidone-iodine has a weak inhibitory power in inhibiting the growth of Streptococcus mutans..0,2% Chlorhexidine has a strong inhibitory power and aquades doesn't have an inhibitory power in inhibiting the growth of Streptococcus mutans.

	10	15	20	25	30	PV	CHX	Aquades
M7,5	.023	.000	.000	.000	.000	.000	.000	.000
M10		.000	.000	.000	.000	.000	.000	.000
M15			.015	.000	.000	.000	.000	.000
M20				.005	.000	.000	.059	.000
M25					.001	.000	.246	.000
M30						.000	.000	.000
PV							.000	.000
CHX								.000

Table 4: The Result of Post HOC LSD Test

Based on the Post Hoc LSD test shown in table 4, there is a significant difference in inhibitory power between each concentration from mouthwash solution with other Concentrations except at 20% concentration with 25% concentration because $P=0,05$. This research also showed the higher concentration of mouthwash containing pineapple cobs ethanol extract, hence the bigger the inhibitory zone formed. It is caused by some factors that could affect the inhibitory power and one of the factors is the concentration of antibacterial herbal plants, because the bigger the concentration, thus the bigger the active components contained inside herbal plants,

therefore inhibitory power will be formed and the faster antibacterial components kill bacteria and prevent the growth of bacteria. 11,12 From the result of postdoc LED also showed there is a significant difference in inhibitory zone diameter between each of the concentrations from mouthwash containing pineapple cobs ethanol extract with 1% povidone-iodine which is the inhibitory power of mouthwash containing pineapple cobs ethanol extract is better than 1% povidone-iodine and the inhibitory power of 1% povidone-iodine is weak. These things also have been shown in the previous research conducted before.⁸

There is a significant difference in inhibitory zone diameter between each of the concentrations from mouthwash containing pineapple cobs ethanol extract with 0,2% chlorhexidine, except between 25% concentration of mouthwash with 0,2% chlorhexidine, this means the inhibitory power of both of it is almost equivalent and belonging in the strong category. This thing has been aligned with the previous research conducted.⁸ There is a significant difference in inhibitory zone diameter between a mouthwash containing pineapple cobs ethanol extract with aquades which is aquades don't have an inhibitory power toward the growth of *Streptococcus mutans*. This thing has shown that aquades as a solvent of mouthwash containing pineapple cobs ethanol extract doesn't affect the result of the inhibitory power test. This thing is aligned with the previous research conducted and research from nugraha that stated sterile aquades don't affect the result of the antibacterial test and the growth of *Streptococcus mutans*.^{8,13}

Mouthwash containing pineapple cobs ethanol extract could inhibit the growth of *Streptococcus mutans* because it has bromelain enzyme, flavonoid, and tannin that act as antibacterial agents.⁸ Mechanism of Bromelain enzyme in inhibit *Streptococcus mutans* are digesting surface proteins in the bacteria cell wall, hydrolysis peptide bond in the bacterial cell wall, thus the cell wall is damaged, allowing the component inside a cell to leak or otherwise to swell. Second, preventing bacterial adhesion to specific glycoprotein receptors, and then bromelain shows antimicrobial activity on gram-positive bacteria and gram-negative bacteria.¹⁴

Pineapple cobs also contain flavonoids. The mechanism of flavonoid inhibition bacteria is to inhibit nuclear acid synthesis, inhibit cell membrane function, and inhibit energy metabolism.¹⁵ The mechanism of inhibitory nuclear acid synthesis is the B ring of the flavonoids may play a role in intercalation at nucleic acid bases thus inhibiting action on DNA and RNA synthesis. Mechanisms of inhibitory cell

membrane function are reduced membrane fluidity of bacterial cells, and increased permeability of the inner bacterial membrane. Mechanisms of inhibitory energy metabolism are inhibiting NADH-cytochrome c reductase, dissipation of the membrane potential, and the electrochemical gradient of a proton across the membrane is essential for maintaining the capacity for ATP synthesis and membrane transport.¹⁶

Tannin also has antibacterial activities which mechanisms of tannin act as an antibacterial inhibiting transcriptase reverse enzyme and DNA topoisomerase, thus bacteria cells could not be formed.¹⁷ Second mechanisms are inactivating microbial adhesion, bacteria enzymes, cell envelope transport protein, etc.¹⁸ and also target peptide components of the cell wall thus will destroy the cell wall.¹⁹

Conclusion

1. Mouthwash containing pineapple cobs ethanol extract with concentrations of 7,5%, 10%, 15%, 20%, 25%, and 30% have inhibitory power toward the growth of *Streptococcus mutans*.
2. Mouthwash containing pineapple cobs ethanol extract with a concentration of 7,5%, 10%, and 15% have moderate inhibitory power toward the growth of *Streptococcus mutans*.
3. Mouthwash containing pineapple cobs ethanol extract with concentrations of 20%, 25%, and 30% have strong inhibitory power toward the growth of *Streptococcus mutans*.

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