


# THE INHIBITORY POWER OF PINEAPPLE HUMP ETHANOL EXTRACT TOWARD THE GROWTH OF *STREPTOCOCCUS MUTANS*

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Info Article	Abstract
<p><b>Article History:</b></p> <p>Received: 3 Aug 2023 Revised: 23 Sep 2023 Accepted: 2 Oct 2023 Available Online: 19 Oct 2023</p> <hr/> <p><b>Keywords:</b> Inhibitory power, <i>Pineapple Hump</i> Ethanol extract, Inhibition zone diameter, <i>Streptococcus mutans</i></p> <hr/>  <p>This is an open access article under the <a href="https://creativecommons.org/licenses/by-sa/4.0/">CC-BY-SA</a> license. Copyright © 2023 by Author. Published by Politeknik Kesehatan Kemenkes Jakarta I</p>	<p>Background: This study aimed to investigate inhibitory powers of ethanol extract from pineapple hump with concentrations of 7,5%, 10%, 15%, 20%, 25%, and 30% toward the growth of <i>Streptococcus mutans</i> bacteria. Methods: Pineapple hump extract was made using maceration technique with ethanol 96% as a solvent. Agar well diffusion method was used in order to investigate the inhibitory ability of pineapple hump against <i>Streptococcus mutans</i>. Few (20-100 µl) volume of the pineapple hump extract solution from each of concentrations was placed into each of well on blood agar in petri dishes had contained <i>Streptococcus mutans</i> then incubated for 24 hours at 37°C temperature, lastly the measurement of inhibition power was measured with caliper. Results: The results showed that pineapple hump extract with concentrations of 7,5%, 10%, 15%, 20%, 25%, and 30% could inhibit the growth of <i>Streptococcus mutans</i> bacteria. Pineapple hump extracts with concentrations of 7,5% and 10% have moderate inhibition power (average diameter respectively are 7,45 mm and 8,58 mm), pineapple hump extracts with concentrations of 15%, 20%, 25%, and 30% have strong inhibition power (average diameter respectively are 10,16 mm, 11,33 mm, 13,33 mm, and 14,83 mm). Conclusions: The Conclusion is: ethanol extract of pineapple hump has inhibitory power toward the growth of <i>Streptococcus mutans</i> within range of moderate category and strong category</p>
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## Introduction

Dental caries is a demineralization process that can occur on any tooth surface that can penetrate into dentin & pulp hence it could develop into periapical infections. The processes are begun with dental plaque formation (Kidd, 2016). Dental caries is one of the most common oral health problem in the world and could affects all of ages (Apro et al, 2020). Riskesdas in 2018 shows that the amount of Indonesian citizens who have decay tooth and toothache is 45,3% (Litbang Kemkes, 2018).

*Streptococcus mutans* is a main bacteria that cause dental caries even though need sucrose to produce dental caries. *Streptococcus mutans* has a distinctive ability to generate much sticky extracellular polysaccharide that assist creating a strong bond of this bacteria on tooth enamel. Another characteristic of *Streptococcus mutans* are acidogenic and aciduric thus decrease plaque PH that initiate enamel demineralization (Samaranayake, 2012).

Since the dental caries incident has been high, thus it is necessary to conduct prevention right away and one of the ways is decrease the amount of *Streptococcus mutans* in oral cavity. Many researches have been conducted to decrease *Streptococcus mutans* in oral cavity utilizing herbal plants because these things have been utilized for many diseases since these plants have antimicrobe activities (Warganegara, 2016). Indonesia has been known has many plants and thousand amongs those could be utilized as herbal plants for traditional medicines (Yuslianti et al, 2022).

Pineapples is a fruit that mostly has been consumed by Indonesia 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 variety that has been cultivated in Indonesia (Minarni et al, 2022, Minarni and Hidayat AW, 2023).

The part of pineapple mostly utilized is fruitmeat, whereas other parts such as peels, leave, buds, and hump regarded as waste and have been disposed of, even though the others contain active components and one of it is bromelain enzyme. Bromelain is an enzyme generated from pineapple plant either from log, stick, leave, fruit, and peels in different amount. Bromelain is dominant in pineapple hump compared with other parts (Minarni, 2019) (Minarni et al, 2022)

According from previous researches conducted by other peers, bromelain could inhibit the growth of aerob bacteria and anaerob bacteria that generate acid. In West Sumatera, based on data in 2013 recorded pineapple production was 321 ton. Researcher also visited in buktinggi market and biaro, which pineapple is a cheap fruit, easy to find, and the taste is likeable by many people (Minarni et al, 2022) (Minarni, 2019).

A research conducted by Novitasari et al in 2022 about effectivity of pineapple peels and hump with concentrations of 50%, 75%, and 100% in inhibit the growth of *Propionibacterium acnes* showed peels extract, fruit, and pineapple hump is able to inhibit the growth of *Propionibacterium acnes*. (Novitasari, 2022). The previous research that the researcher has conducted about an inhibitory test of etanol extract of pineapple hump against *Streptococcus mutans* has shown etanol extract of pineapple hump of concentration 100%, 75%, 50%, dan 25% be able to inhibit the growth of *Streptococcus mutans* with diameters of inhibitory zone respectively are 28,6 mm, 15,6 mm, 14,6 mm, and 13,6 mm (Minarni et al, 2022)

Based on the good result the researcher discovered before, the researchers aim to investigate again about the inhibitory power of etanol extract of pineapple hump against *Streptococcus mutans* with lower concentrations because in the previous research showed the inhibitory power of etanol extract of pineapple hump has strong category and very strong category against *Streptococcus mutans* growth.

## Method

This research was a laboratory experimental. Samples was used in this research was *Streptococcus mutans* bacteria. Sample in this research divided into 9 groups. The number of the samples was determined by Federer technique, from the calculation of formula of Federer, the total amount of the samples used were 3 at each of test groups.

The inhibitory power test of ethanol extract of pineapple hump was conducted in the Microbiology Laboratorium of Faculty of Medicine in Andalas University, and for the extract production was conducted in Pharmacy Laboratorium of Imam Bonjol Pharmacy Academy. Data collection time was from

june 2022 until november 2022. Data analyze used was One Way Anova

## 1. Materials and Equipments

Equipements utilized in this research:

Petri dish, neddle, vortex, ose, bunsen burner, test tube, rack, autoclave, inoculum tube, sterile cotton swab, micropipette, tip, inkcubator, hot plate, erlenmeyer, boor prop and vernier caliper.

Materials utilized in this research are pure isolation of *Streptococcus mutans* sample, pineapple hump, ethanol 96%, blood agar media, Nacl 0,9%, and Mc. Farland 0,5.

## 2. Prosedures

### a. The production of pineapple hump extract

2 kg Wet pineapple hump washed and cut into small pieces, then dried in oven for 4 hours. Pineapple hump dried made into powder, subsequently the extract was transferred to maseration vessel and extracted using 96% ethanol then soaked for 24 hours, after that the extraction was repeated 3 times, and then the extracted was collected and evaporated till was free from ethanol solvent using a rotary evaporator in 50°C temperature. The extract solutions concentration of 7,5%. 10%, 15%, 20%, 25%, and 30% was made using diluting with aquades.

### b. 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 and was heat using hot plate stier, after that it was sterilized using autoclave with 121°C temperature. for 15 minutes and 1 atm pressure and then it was poured into petri dish that had already had boor prop and it will be waited till solidified.

### c. Breeding of *Streptococcus mutans*

*Streptococcus mutans* colonies was taken with sterile oce needle, then transfered onto an agar media and incubated in an incubator at 37°C temperature for 24 hours.

### d. Inhibitory test of ethanol extract of pineapple hump using agar well diffusion method

1) The bacteria incubated before, taken its colonies from agar media using a sterile ose neddle and then placed into Nacl Broth media till the turbidity is same with Mc Farland standard. Mc Farland standar is equivalent with bacteria suspension with concentration of 1,5X 10<sup>8</sup>CFU/ ml. This turbidity is used as a suspension standard of bacteria colonies.

2) A cotton swab had been placed into bacteria suspension till wet, then cotton swab was pressed onto the inside part of a tube test and was spread over the entire of agar surface.

- 3) Several holes with a diameter of 6-8 mm was punched aseptically with a sterile corkborer or a tip on several areas on the blood agar, and few 20-100  $\mu$ l of the pineapple hump extract solution from each of concentrations was introduced into each of the well on the blood agar contain *Streptococcus mutans*
- 4) Incubate the petri dishes in incubator in 37°C temperature for 24 hours. Vertical and horizontal Inhibitory zones would be formed around the well was measured using venier caliper and the result of both of it was averaged

## Result

**Table 1.** The result of inhibitory zones of pineapple hump extract toward the growth of *S. mutans* colony on blood agar

Sample tests	The average of inhibitory zone diameter
Extract 7,5%	7,45mm
Extract 10 %	8,58 mm
Extract 15 %	10,16 mm
Extract 20 %	11,33 mm
Extract 25 %	13,33 mm
Extract 30 %	14,83 mm
Povidon iodine 1%	3,66 mm
Chlorhexidin 0,2%	11,25 mm
Aquades	0 mm

The data at table 1 shows each of 6 concentrations of Pineapple hump extract have inhibitory power toward the Growth of *S. mutans* and each of concentration has different average inhibitory diameter.

**Table 2.** One Way Anova Test

	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			

The data on table 2, from the one way anova test shown on table 2, P value is 0,000. This means there are significant differences between test groups

**Table 3.** The Phytochemicals Result of flavonoid and tannin contained in pineapple hump extract

No	Components examined	Analytic result	Method used
1	Flavonoid	63.0740 ppm (in 100 gr sample)	UV-Vis Spectrophotometer
2	Tannin	4.5454 ppm (in 100 gr sample)	UV-Vis Spectrophotometer

Result regarding it shown in table 3. Based on the result, pineapple hump has flavonoid and tannin component. Total content of flavonoid is 63.0740 ppm in 100gr pineapple hump and total content of tannin is 4.5454 ppm in 100 gr sample.

## Discussion

The results of average diameter of inhibitory zone of ethanol extract from pineapple hump toward the growth of *Streptococcus Mutans* are shown in table 1 The data at table 1 has shown each of 6 concentrations of pineapple hump extracts have inhibitory effect toward the growth of *Streptococcus mutans* and each of concentration has different average inhibitory diameter.

From the one way anova test shown above, P value is 0,000. This means there are significant differences between test groups. David and Stout have divided the inhibitory power into 4 categories which are: a) inhibitory diameters 20 mm or more means very strong, b) inhibitory diameters 10-20 mm means strong, c) inhibitory diameters 5-10mm means moderate, d) inhibitory diameters less than 5 mm means weak (Minarni et al, 2022).

According the research conducted, has shown pineapple hump extract with concentrations of 7,5%, and 10% have moderate inhibitory power, pineapple hump extract with concentrations of 15%, 20%, 25%, and 30% have strong inhibitory power in inhibit the growth of *Streptococcus mutans*. Povidon iodine has weak inhibitory, chlorhexidin 0,2% has strong inhibitory power and for aquades doesn't have an inhibitory zone

Based on the results of this research has shown as higher concentration of pineapple hump, hence as bigger the inhibitory zone formed. It is caused by some factors that could affect the inhibitory power and one of the factors is a concentration of antibacterial herbal plants, because as bigger the concentration, thus as bigger active components contained inside herbal plants, therefore inhibitory power will be formed and as faster antibacterial

components kill bacteria and prevent the growth of bacteria, thus the results of this research is not as bigger as the previous research that the researcher conducted (Pelczar et al, 2008), (Lasmayanti, 2007).

In this research, beside the research about the inhibitory power of ethanol extract of pineapple hump against *Streptococcus mutans* bacteria, researchers have conducted an investigation related with flavonoid and tannin phytochemicals contained in pineapple hump extract. Result regarding it shown in table 3. Based on the result, pineapple hump has flavonoid and tannin component. Total content of flavonoid is 63.0740 ppm in 100gr pineapple hump and total content of tannin is 4.5454 ppm in 100 gr sample.

Ethanol extract of pineapple hump could inhibit the growth of *Streptococcus mutans* because it has flavonoid, and tannin that act as antibacteria agents which is from the phytochemical test conducted, both of it have been found from pineapple hump, beside flavonoid and tannin, pineapple hump has bromelain enzyme, saponin and alkaloid that could act as an antibacterial agent too (Soulissa et al, 2021, and Minarni et al, 2022), but at the phytochemical test that the researcher and the author conducted before, we didn't examined bromelain enzyme, saponin and alkaloid test.

Mechanism of Bromelain enzyme acts as an antibacterial are digest surface proteins peptidoglikan, dan polysaccharide in bacteria cell wall, hydrolysis peptide bond in the bacterial cell wall, thus the cell wall is damaged, allowing the component inside cell to leak or otherwise to swell. Second, preventing bacterial adhesion to specific glycoprotein receptor therefore has potential to reduce plaque formation in the teeth Bromelain shows antimicrobial activity on gram positive bacteria and gram negative bacteria and bromelain enzyme also remains a promising antibacterial against oral pathogens. Moreover bromelain enzyme also has other functions in medical applications such as inhibiting platelet aggregation, improving cardiovascular conditions, boosting immune system, aiding in digestion, accelerating healing from injuries, antiinflammatory, and antitumor (Jancic, 2022 and liliyani et al, 2023).

Pineapple hump also contains flavonoid. Mechanism of flavonoid inhibits bacteria are inhibit nucleat acid synthesis, penetrate into peptidoglycan layer, inhibit cell membrane function, and inhibit energy metabolism (Umarudin, 2018 and Soulissa et al, 2021). Mechanism of inhibitory of nucleat acid synthesis is the B ring of the flavonoids may play a role in intercalation at nucleic acid bases thus inhibit action on DNA and RNA synthesis (Cushnie et al, 2005).

Mechanisms of inhibitory of cell membrane function are reduce membrane fluidity of bacterial

cell, increase permeability of the inner of bacteriaal membrane. Mechanisms of inhibitory of energy methabolism is inhibit NADH-cytochrome c reductase, a dissipation of the membrane potential, the electrochemical gradient of proton across the membrane is essential for to maintain capacity for ATP synthesis and membrane transport (Cushnie et al, 2005).

Tannin also has antibacterial activities which is mechanisms of tannin act as an antibacterial are inhibit reverse transcriptase enzyme and DNA topoisomerase, thus bacteria cell could not be formed, second mechanisms are imactivate bacteria enzyme, disrupt protein transport in cell layers, third mechanism is destroy peptide components of cell wall thus formations of cell wall get inhibited and this thing results in bacteria cell lysis Minarni et al, 2022).

Mechanism of saponin in pineapple hump acts as an antibacteria are damage the cell membrane and reduce membrane permeability, causing disruption of nutrient absorbsion into the bacteria cells and resulting in cell death. Mechanism of alkaloid acts an antibacterial are disrupt the formation peptioidoglycan, causing prevent complete formation of bacteria cell wall layer resulting in cell death (Soulissa et al, 2021).

The results of this study are supported by Soulissa et al who used agar well diffusion method to test the inhibitory power of pineapple hump toward the growth of *Porphyromonas gingivalis* and the results is the pineapple hump shows an antibacterial activity against *Porphyromonas gingivalis* bacteria starting at concentration 50% with the average inhibiton zone diameter are 9,85 mm (Soulissa et al, 2021). Other study by Alhaq et el in 2022 who used agar well diffusion method to test the inhibitory power of pineapple hump and pineapple peel toward the growth of *Enterococcus faecalis* and obtained the results which are pineapple hump water extract with maseration extraction is more effective against gram positive bacteria and has inhibitory zone against *Enterococcus faecalis* with 17,67 mm diameter

Other study by Marlina et al who used swab method to test the ability pineapple hump as disinfectan on milk can and use agar well diffusion method to test the inhibitory power of pineapple hump toward the growth of bacteria on milk can and the result are pineapple hump extract can inhibit the growth of bacteria on milk can, producing inhibitory zone with 7,25 mm and can be able to reduce bacteria on milk can (Marlina et al, 2018). Other study by Ujiani who conducted effectiveness of pineapple extract (*Ananas comosus*. L.) on *Streptococcus pneumococcus* growth used diffusion and dilution method and the results are pineapple hump extract was effective in inhibiting and killing *Streptococcus pneumococcus* at a concentration of 8% (Ujiani, 2021).

Another study by Liliyani et al who conducted antibacterial effect of bromelain enzyme from pineapple hump on *Enterococcus faecalis* and used diffusion and dilution test and the results are bromelain enzyme from pineapple hump extract showed effective inhibitory and bactericidal activity against *Enterococcus faecalis* and minimum concentration that could inhibit *Enterococcus faecalis* are 12,5% (Liliyani et al, 2023).

## Conclusions and Suggestions

There are several conclusions according to the research that the authors have conducted are firstly, ethanol extract of pineapple hump with lower concentrations compared with the previous research of the authors which are concentrations of 7,5%, 10%, 15%, 20%, 25%, and 30% have inhibitory power toward the growth of *Streptococcus mutans*. Secondly, Ethanol extract of pineapple hump with concentration of 7,5% and 10% have moderate inhibitory power toward the growth of *Streptococcus mutans*.

Thirdly, Ethanol extract of pineapple hump with concentrations of 15%, 20%, 25%, and 30% have strong inhibitory power toward the growth of *Streptococcus mutans*. The recommendation refer to the authors of this article and other researchers for testing the potential of the ethanol extract of pineapple cobs become mouthwash that could be as one of the ways for preventing dental caries.

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