Migration Letters

Volume: 21, No: S7 (2024), pp. 1833-1838

ISSN: 1741-8984 (Print) ISSN: 1741-8992 (Online)

www.migrationletters.com

Phytochemical Potentials And Antibacterial Activity Of Pineapple Peel Extract (Ananas Comosus L Merr) Against Streptococcus Mutans

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Abstract

The main cause of dental caries is Streptococcus mutans. The growth of S. mutans can be controlled by antibacterial agents. Antibacterial materials can be obtained from natural or synthetic materials. Pineapple skin contains bromelain enzymes and tannins which have antibacterial effects. This study aims to determine the phytochemical and antibacterial activity of pineapple peel extract against the growth of S. mutans. The phytochemical analysis indicated the presence of flavonoids and tannins in the crude extract. The results showed that the pineapple peel extract at concentrations of 25%, 50%, 75%, and 100% have a strong zone of inhibition against the growth of S. mutans, while povidone-iodine 1% has a weak zone of inhibition. Pineapple peel extract has antibacterial activity against S. mutans with an effective concentration of 25%. Further studies of isolation of antibacterial compounds from Ananas comosus L Merr against S. mutans should be conducted.

Keywords*: Antibacterial activity, pineapple peel, Streptoococcus mutans.*

Introduction

The most common oral disease with the highest prevalence rate compared to other oral diseases, which reached 90.5%, is dental caries[1]. Dental caries is a disease of hard tissues of the teeth in the oral cavity, due to the activity of acid-producing bacteria capable of fermenting carbohydrates consumed by humans. It involves several factors that interact with each other, which are, the interaction between teeth and saliva (host), form surface, general health, microorganisms, substrate, and time. A[1]lthough the cause is multifactorial, the main cause of dental caries is the Streptococcus mutans. S. mutans can grow well in an acidic environment as a result of carbohydrate fermentation. The acid produced by these bacteria can trigger tooth demineralization[2]. The growth of S. mutans can be controlled with antibacterial agents. Antibacterial as materials can be obtained from natural or synthetic materials[3]. The most used synthetic antibacterial is povidone-iodine. Povidone-iodine can kill various types of pathogenic bacteria. However, this material can cause allergies in certain individuals so antibacterial ingredients from natural ingredients are needed which prevent to cause allergic reactions in users. One of the natural ingredients that can be used is pineapple[4–6].

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Pineapple is a fruit that is much favored by Indonesian, both for direct consumption and in various processed forms such as juice, jam, syrup, and chips. Pineapple is easy to obtain and can be harvested all year round. Pineapple has the scientific name Ananas comosus (L) Merr. The Cayenne and Queen groups are types of pineapple that are widely grown in Indonesia. The waste parts of the pineapple include the skin which has an uneven texture, small thorns on the outer surface and is simply thrown away as waste. Pineapple peel contained vitamin C, carotenoids, fiber, anthocyanins, flavonoids, tannins, and bromelain enzymes[7–9]. There has been no utilization of pineapple peel even though it contained active substances such as bromelain enzymes, tannins, and flavonoids that function as antibacterial[10,11]. Bromelain is a proteolytic enzyme that can catalyze the hydrolysis of proteins. The function of bromelain is as a protein breaker by breaking peptide bonds and producing simpler proteins[12].

Pineapple peel is also known to have an inhibitory effect on the growth of salivary bacteria. These microorganisms are normal bacteria in the oral cavity but if there is a change in their environment, their population can increase and cause the caries process to take place more quickly. Pineapple is perishable and the shelf life is only about 7 days at a temperature of 21 ^{0}C, as a result when the harvest season comes, there is an oversupply. Previous research showed that pineapple peel extract was proven to be effective in inhibiting the growth of Staphylococcus aureus with Minimum Inhibitory Concentration (MIC) of 1.56%-0.78%[13]. From the description above, we are interested to study the antibacterial activity of pineapple peel extract (Ananas comosus (L) Merr) against S. mutans that causes dental caries. Pineapple peel Queen variant was collected from Rimbo Panjang, West Sumatera, Indonesia. Based on this study, we can use pineapple peel waste to reduce the incidence of dental caries. To the best of our knowledge, the study of pineapple peel extract (Queen variant) against S. mutans is reported for the first time.

Material and Methods

Sample preparation

The first step of this research was the extraction of pineapple peel. Samples of wet pineapple skin as much as 3 kg were dried for 6 days obtaining a dry sample of 114.28 g. Then the dry samples were extracted by maceration. The maceration technique was carried out by stirring several times at room temperature 7 times for 21 days. The filtrate was concentrated to obtain crude extract (48.38 g). Crude extract was diluted to give several concentrations of extract (100%, 75%, 50%, and 25%).

Phytochemical screening

Qualitative phytochemical screening

Crude extracts of A. comosus L Merr was evaluated for their chemical constituents following the previous method[14]. This step aims to determine the presence of from flavonoids and tannins from the crude extract.

Quantitative phytochemical screening

Flavonoid total of the crude extract

The total flavonoid content of A. comosus L Merr was determined using quercetin as standard with varying concentrations of 20, 40, 60, 80, and 100 ppm. The absorbance of standard solutions was measured using UV-visible spectrophotometer at 725 nm. The total flavonoid content was analyzed statistically and presented as ppm.

Tannin total of the crude extract

The total tannin content of A. comosus L Merr was determined using Folin-Ciocalteu assay technique. Tannin calibration curve was made using varying concentrations of 20, 40, 60, 80, and 100 ppm. The absorbance of standard solutions was measured using UV-visible spectrophotometer at 725 nm. The total tannin content was analyzed statistically and presented as ppm.

Media preparation

The powder of media was put into an Erlenmeyer, dissolved with distilled water, and heated using a hot plate stirrer. Media were sterilized using an autoclave at a temperature of 121 ^{0}C for 15 minutes at a pressure of 1 atm. Media was poured into a petri dish with a drill prop and allowed to freeze at room temperature. Especially for blood agar media, 10% of sheep's blood is added and homogenized.

Bacterial suspension

The variation of the bacterial suspension was started by inserting NaCl into the inoculum tube and inserted the S. mutans colonies into the tube. After that, it was homogenized using a vortex and the bacterial suspension was equalized to a turbidity of 0.5 Mc-Farland.

Screening for antibacterial activity using well diffusion method

The antibacterial test[15,16] was carried out by dipping a sterile cotton swab in the bacterial suspension and then rubbing it on the surface of nutrient culture medium in a petri dish until smooth, allowing it to dry for 3-5 minutes. Drops of sample in wells that have been formed by a boor prop. A petri dish consists of 3 wells containing an extract (100%, 75%, 50%, and 25%), a positive control (povidone-iodine 1%) and a negative control (sterile distilled water), respectively. The samples were incubated at 37 ^{0}C for 24 hours. Each experiment was performed in triplicate.

Zone diameter measurement

The measurement of the zone of inhibition using the Kirby Bauer method was to measure the inhibition zone around the well vertically and horizontally using a caliper with units of millimeters (mm).

Minimum inhibitory concentration (MIC) of the crude extract

The crude extract was evaluated for the MIC following microplate broth dilution method[15]. Tested bacteria (approximately 10^6 CFU) were seeded into the wells for a night. The crude extract was tested at serial concentrations from 400 to 3.125 µg/mL and then incubated at 37°C for 24 h. MIC was determined as the least concentration of the extract inhibiting the growth of the tested bacteria.

Data analysis

The diameter of the inhibition zone was converted into area using the formula for the area of a circle, namely $L= \pi \times r^2$ and the total area and area of the well will be obtained. The area of inhibition was obtained by subtracting the total area with the well area. Then, these results were analyzed using one way analysis of variance (ANOVA) statistics at the 5% level for comparison of the results of the broad bacterial inhibitory test.

Results and Discussion

This study aimed to observe the activity of pineapple peel extract (A. comosus (L.) Merr) in inhibiting the growth of S. mutans with 1% povidone-iodine as positive control. The results of this

study showed that there was a significant difference between the antibacterial activity of pineapple peel extract with a concentration of 100% compared to a concentration of 50%, 25%, and 1% povidone-iodine and a 75% concentration of pineapple peel extract group compared to a concentration of 50%, 25%, and 1% povidone-iodine with p-value < 0.001 (Table 1). The concentration of 100% pineapple peel extract had the largest average inhibition zone of 18 mm, belong to the strong category. Then followed by pineapple peel extract with 75% concentration of 12.8 mm, 50% concentration of 11 mm, and 25% concentration of 6.2 mm. Povidone-iodine as a positive control had the smallest inhibition zone of 4 mm. Positive control (povidone-iodine 1%) was also able to inhibit the growth of S. mutans because iodine compounds have cytotoxic properties that can kill bacteria[13].

Table 1 indicated the pineapple peel extract with various concentrations of 25%, 50%, 75%, and 100% had strong antibacterial activity. The data also showed the higher the concentration, the larger the inhibition zone formed. Furthermore, crude extract of pineapple peel was also determined for the MIC value. Interestingly, had MIC value of crude extract was 25 µg/ml. It was only 4 times higher than positive control.
This result is supported by Suryelita et al. (2021) that the effectiveness of an antibacterial substance is influenced by the concentration of the substance. Increasing the number of bioactive metabolites will have a positive impact on the potential as antibacterial, so the ability to kill bacteria is also greater[15]. Some factors of antibacterial activity of pineapple peel are bromelain enzyme, flavonoids, and tannins.

Bromelain enzyme is found in stems, skins, fruit leaves, and stems in varying amounts. The amount of bromelain enzyme in pineapple skin is 32.2 U/mg. Bromelain is a hydrolase enzyme that can hydrolyze proteins thousands of times their weight. This enzyme has been shown to inhibit the growth of aerobic and anaerobic bacteria in vitro. Bromelain enzyme works by lowering the surface tension of bacteria through hydrolysis of salivary proteins and glycoproteins. This enzyme also inhibits bacterial growth by breaking protein bonds in bacteria.

Furthermore, pineapple peel (A. comosus (L.) Merr) is rich of flavonoids. The amount of flavonoid in pineapple skin is 106.7654 ppm. Flavonoids belong in the class of phenolic compounds which act as disinfectants and are very effective at inhibiting the growth of bacteria. Flavonoids of the flavanone type are the effect of the inhibiting growth of S. mutans that occur due to the reaction of a chemical compound. The mechanism of action of flavonoids contained in pineapple peel extract as antibacterial can be divided into three, which are inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism[17].

The main factor for antibacterial mechanism of flavonoids in pineapple peel (A. comosus (L.) Merr) to inhibit nucleic acid synthesis is ring A and B. Both rings cause damage to play an important role in the process of intercalation or hydrogen bonding by accumulating nucleic acid bases which will inhibit the formation of nucleic acids, DNA, and RNA. This is the permeability of the bacterial cell wall and lysosomes[17].

In addition, the mechanism action of antibacterial flavonoids in pineapple peel (A. comosus (L.) Merr) inhibits cell membrane function is to form complex compounds with extracellular proteins. It will damage cell membranes followed by the release of intracellular compounds. Another study has shown that the mechanism of flavonoids inhibits cell membranes by disrupting cell membrane permeability and inhibiting binding enzymes such as ATPase and phospholipase. Finally, the antibacterial mechanism of flavonoids inhibits energy metabolism by inhibiting cytochrome C

reductase. It will make the metabolic processes and biosynthesis of macromolecules inhibited. Energy is needed by bacteria for the biosynthesis of macromolecules[18].

Tannins have also an antibacterial activity. The total tannin in the crude extract was 8.23 ppm. The mechanism action of tannins as an antibacterial is to inhibit enzymes reverse transcriptase and DNA topoisomerase so that bacterial cells cannot be formed. Tannins have antibacterial activity related to their ability to inactivate microbial cell adhesion, inactivate enzymes, and interfere with protein transport in the inner layer of cells. Tannins damage the polypeptide components of the cell wall so that the formation of the cell wall becomes less than perfect. This causes the bacterial cell to lyse due to osmotic and physical pressure so that the bacterial cell will inactive[19, 20]

Conclusions

Based on the results of the study, it can be concluded that pineapple peel extract (A. comosus (L.) Merr) had antibacterial activity. The extract inhibits the growth of S. mutans in each treatment group with a significance value of $p=0.001$ ($p<0.05$).

Acknowledgements
This research was funded by Padang Health Polytechnic, Ministry of Health Padang in 2021.

Conflict of Interests
The authors declare that no conflict of interest is associated with this work.

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Table 1. Inhibition zone of pineapple peel extract against S. mutans

Concentration	P1	P2	P3	Average (mm)
25%	6.5	6	6	6.2
50%	11	11	11	11
75%	13.5	13	12	12.8
100%	18	18	18	18
Positive Control	4	4	4	4
Negative Control	0	0	0	0