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In Vitro Analysis of Methanol Extract of Areca Nut (Areca Catechu) on the Inhibitory Potential of Streptococcus Mutans

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Areca catechu, betel nut palm methanol extract, Streptococcus mutans

ABSTRACT
Caries is an infectious disease caused by *Streptococcus mutans* that breaks down tooth tissue by forming a structure known as dental plaque. The aims are to determine the effects of betel nut palm (*Areca catechu*) methanol extract on the growth of *Streptococcus mutans* and to identify the most effective concentration of the extract in inhibiting the growth of *Streptococcus mutans*. The research used a randomized complete design with 7 treatments involved. The concentrations of betel nut palm methanol extract were 100%, 75%, 50%, 25%, and 5%; the positive control was amoxicillin, and the negative control was aqua. There were 5 replications for each treatment. This research used the disc diffusion method. The obtained data were analyzed statistically with one-way ANOVA followed by the Tukey test. Based on the ANOVA test, a significant difference was found ($p<0.05$) among the treatments of extract concentrations and controls. The analysis of the Tukey test shows that there are significant differences ($p<0.05$) among some treatments. Thus, the conclusion is that betel nut palm (*Areca catechu*) methanol extract has the ability to inhibit the growth of *Streptococcus mutans*.

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INTRODUCTION

Caries has become a problem in both developed and developing countries. Based on data from the World Health Organization (WHO), the average caries index is quite high. Similarly, in Indonesia, the incidence of caries is around 76.9%, so this can be considered a critical indicator of caries problems. Caries is one of the indicators of the lack of care for dental conditions in Indonesian society. Based on the 2004 Household Health Survey (*Survei Kesehatan Rumah Tangga*/SKRT), the prevalence of caries in Indonesia reached 90% and was higher than in other developing countries. According to the SKRT in 2011, public awareness in caring for teeth was still very minimal, at around 4-5%, while cases of tooth decay that had not been managed reached 82.5% (Arya & Usha, 2024). Caries disease is an infectious disease that attacks the hard tissues of the teeth, causing local damage to the teeth. Caries not only causes damage to tooth components but also to other components in the oral cavity and affects the organs of the body systemically (Al Akeel, Mateen, Janardhan, & Gupta, 2017). The presence of caries causes easy tooth loss and annoying malocclusion when chewing and can be one of the entry points for infection to enter the human body (Afni, Said, & Yuliet, 2015).

The causes of caries are multifactorial, including internal and external factors of the host and microorganisms (Bajalan, Rouzbahani, Pirbalouti, & Maggi, 2017). Internal host factors involved include the surface of the teeth, saliva, and pellicles (salivary protein), while diet or substrate and time are external factors of the host that play a role in the onset of caries (Santi, 2010). The most important causative microorganism is *Streptococcus mutans* because it has acidogenic properties and is resistant

to acids Czerkas, Olchowik-Grabarek, Łomanowska, Abdulladjanova, & Sękowski, 2024). The factors that affect the transmission of this bacterium are bacterial serotype, the number of *Streptococcus mutans* possessed by the transmitter, the number of bacteria that migrate with each contact and the frequency of contact, dietary factors, and host immunity factors (Binobead, Aziz, Ibrahim, & Aljowaie, 2024).

Areca nut (*Areca catechu*) is considered to have the ability to strengthen teeth by some people and has been used as a tooth cleaner traditionally. This has become a descending tradition for Southeast Asian nations, especially the Malays (Kashi, Ranjbar, Sabouri, Shabani, & Darbandi, 2025). According to Norhayati Mohd. Said in 1987, betel nut and areca nut are part of manners and mutual respect in Malay culture. In addition, areca nut is also used as a complement in traditional events such as engagements, weddings, and house-building ceremonies.

Areca nuts that have been extracted contain various substances that show antioxidant activity. One of the chemical compounds contained in areca nuts is flavonoids (Jain dkk., 2015). Flavonoids are natural compounds that are widespread in plants and can be found in almost all parts of plants. One of their functions is as an antibacterial agent. In addition, the alkaloid content in areca nuts also plays an important role in its function as an antibacterial agent. Flavonoids can function as antioxidants, anti-inflammatory agents, antimicrobials, and play a role in vascularization in the body (Hickl dkk., 2024).

Several studies explain the antimicrobial activity found in plant extracts containing flavonoids in several types of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Clostridium perfringens*. Udiana proved that areca nut extract is able to inhibit the growth of acid-forming bacteria in the oral cavity using the Agar Well Diffusion method. In addition, Sabir proved the activity of flavonoid compounds in inhibiting the growth of *Streptococcus mutans* bacteria. The concentration of 0.5% was the most effective concentration in inhibiting the growth of these bacteria, and the concentration of 0.1% was the lowest inhibitory concentration.

Based on preliminary studies, no separation of compounds in areca nuts was carried out, but the resulting extracts were tested thoroughly with varying concentrations of 100%, 75%, 50%, 25%, and 5%. These concentrations have different inhibitory effects on the growth of *Streptococcus mutans*, so this is relevant to previous studies. Thus, this study was conducted to determine the effect of areca nut methanol extract (*Areca catechu*) in inhibiting the growth of *Streptococcus mutans*, which is the main cause of dental caries.

The study provides practical benefits by offering a natural, alternative method for caries prevention and treatment, contributing to innovation in oral healthcare, providing insights into the dose-dependent antibacterial activity of areca nut, stimulating further research on traditional remedies, and serving as a reference for dental education and clinical applications.

METHOD

This study is a laboratory experimental research using a Completely Randomized Design (*Rancangan Acak Lengkap*/RAL) with 7 treatments, namely 5 concentrations of 100%, 75%, 50%, 25%, 5%, negative control in the form of *aqua destillata* and positive control in the form of amoxicillin antibiotic discs. This research was carried out from July 2010 to January 2011. The preparation of areca nut methanol extract (*Areca catechu*) was carried out at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences (*Matematika dan Ilmu Pengetahuan Alam*/MIPA), Syiah Kuala University. The cultivation of *Streptococcus mutans* bacteria that had been isolated was carried out at the Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University. Testing of the antibacterial activity of areca nut methanol extract against *Streptococcus mutans* bacteria was carried out at the Microbiology Laboratory, Faculty of Medicine, Syiah Kuala University.

Tools and Materials

The tools used in this study include a rotary vacuum evaporator, separatory funnel, Erlenmeyer flask, beaker, measuring cylinder, volumetric flask, petri dish, extraction flask, analytical balance, incubator, inoculating loop, test tube rack, test tube, clinical pipette, sterile cotton swab, sterilizer oven, and filter paper.

The materials used in this study are areca nut (*Areca catechu*) in the form of extracts, blank paper discs, antibiotic discs (amoxicillin), sterile *aqua destillata*, methanol, nutrient agar (Oxoid), Mueller Hinton Agar (MHA), and Trypticase Yeast Extract Sucrose Bacitracin Medium (TYS20B).

Research Work Procedure

Sterilization of equipment

The equipment to be used in the study was sterilized first to avoid microbial contamination. The tools were wrapped in paper so that there were no gaps that allowed air to enter. After the tools were packaged appropriately, they were then placed in the sterilizer oven until the temperature reached 150°C, then the tools were removed and ready for use.

Sterilization of materials

Some of the materials used in the study, such as Mueller Hinton Agar (MHA), Nutrient Agar (NA), and *aqua destillata*, were sterilized first by placing them in an autoclave until the temperature reached 121°C for ± 90 minutes. Once the desired temperature was reached, the materials were left for 15 minutes in the autoclave, then removed and ready for use.

Preparation of areca nut (Areca catechu) material

The areca nut (*Areca catechu*) was picked directly from the tree and collected in July 2010 from the Banda Aceh City Park, Aceh Province. After collection, the fresh fruit was sorted to select those in good and intact form. The areca nut used was mature areca nut whose outer skin was dark yellow, then the outer skin was peeled. After obtaining the seeds, the areca nuts were washed and dried at room temperature. After drying, the areca nuts were ground and sifted. The sifted areca nut powder was dried again to obtain areca nut powder and stored in a closed container.

Preparation of areca nut extract (Areca catechu)

The areca nuts that had been formed into powder were macerated using methanol solvent. The first extracts obtained were filtered and concentrated with a rotary vacuum evaporator. This method was repeated until the dissolved compounds were extracted, which was characterized by the clarity of the solution color. The residue obtained was macerated again using methanol solvent. The extracts obtained were filtered and concentrated with a rotary vacuum evaporator (appendix 2). Furthermore, pure areca nut methanol extract was divided into 5 concentrations, namely group 1 (P1) with a concentration of 100%, group 2 (P2) with a concentration of 75%, group 3 (P3) with a concentration of 50%, group 4 (P4) with a concentration of 25%, group 5 (P5) with a concentration of 5%, a positive control group (P6), and a negative control group (P7).

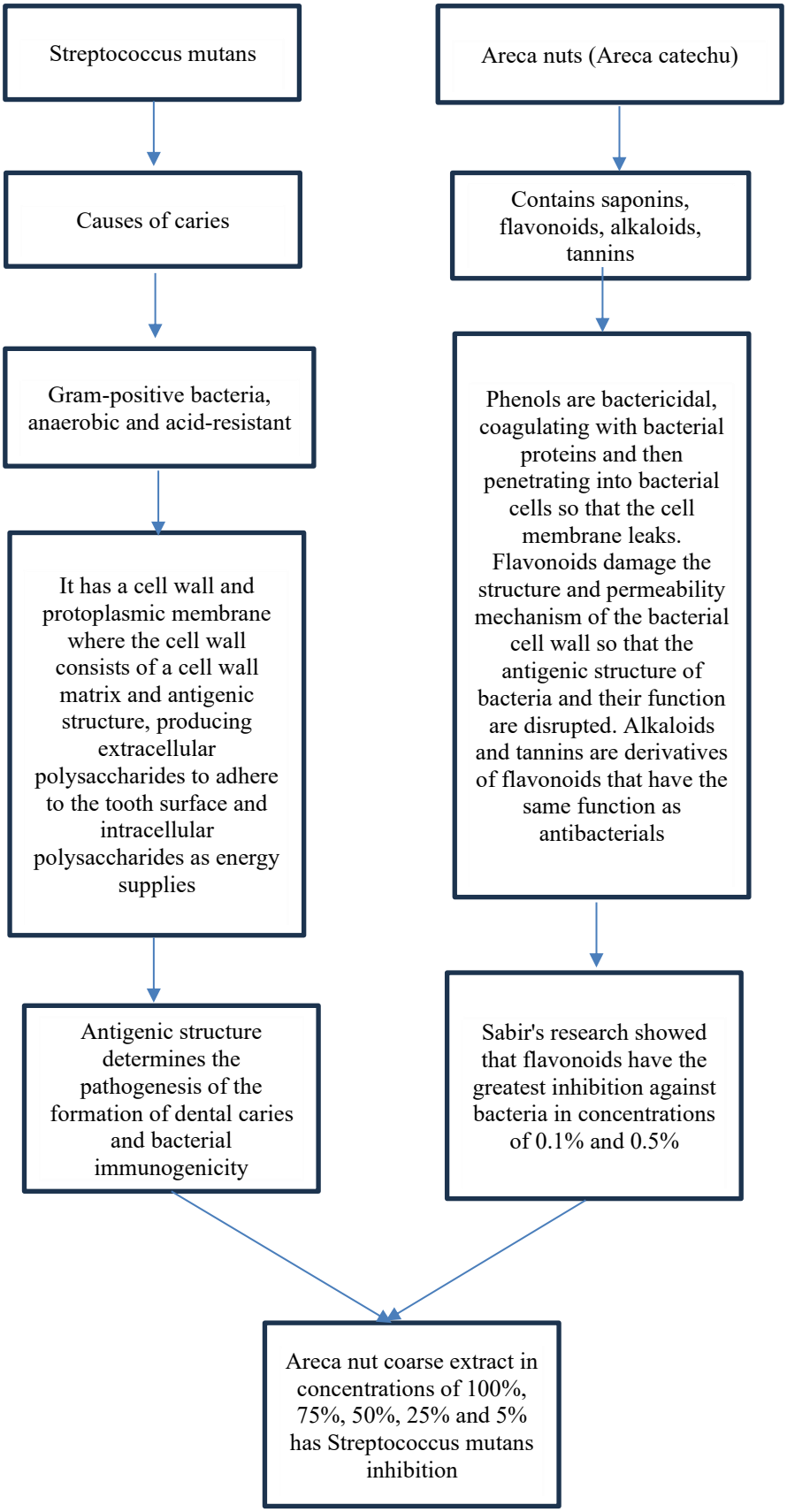

Figure 1. Concept framework

RESULT AND DISCUSSION

Phytochemical extraction and test results

The results of the extraction of 1000gr areca nuts with methanol solvent are evaporated using a vacuum rotary evaporator, then weighed. The result of pure extraction was 199.93 gr. These results were then used for antibacterial tests against Streptococcus mutans. After phytochemical tests were carried out, the results were obtained as seen in table 1 below

Table 1. Phytochemical test results from areca nut methanol extract

No.	Parameter	Hasil
1.	Alkaloid	+
2.	Flavonoid	+
3.	Tanin	+
4.	Saponin	+
5.	Steroid	-
6.	Terpenoid	-

Remarks: + give results, - do not give results

Based on the results of phytochemical tests from areca nut methanol extract, the results show that it contains chemical compounds of the alkaloids, flavonoids, tannins and saponins (appendix3). However, steroid and terpenoid compounds do not show positive results. Tannin and flavonoid compounds are a class of polyphenol compounds that are antibacterial. In addition to the chemical compounds above, according to Suprastiwi, areca nuts also contain phenolic acid which is also a class of polyphenol compounds that are antibacterial.

Test results of the effect of areca nut methanol extract on the growth of Streptococcus mutans

Various concentrations of areca nut methanol extract have the diameter of the growth inhibition zone of streptococcus mutans bacteria. This shows that the activity test of betel nut methanol extract that has been carried out has been proven to be able to inhibit the growth of bacteria (Raji, Samrot, Keerthana, & Karishma, 2019). This theory is in accordance with Udiana that areca nut methanol extract can be used as an antibacterial. The barrier zone formed is a clear area that does not show the growth of bacteria around the disc (Pallavi, Sahoo, Sen, & Raut, 2024). The clear area showed the antimicrobial power of the methanol extract of the areca nut methanol tested. The existence of an inhibition zone formed in the media is caused by the active substances of areca nut methanol extract which have activities that inhibit the growth of bacteria (bacterostatic) or kill bacteria (bactericidal). The results of this study show that areca nut has activities to inhibit the growth of bacteria due to the presence of clear zones around the disc that are not overgrown with bacteria. It is suspected that the active compounds contained in areca nut such as flavonoids, alkaloids, tannins and saponins have been proven to inhibit the growth of bacteria (Liu, Zhang, Li, Wang, Liu, & Song, 2023).

Flavonoids have antioxidant, antibacterial, anti-inflammatory, antitumorigenic and anticancer effects. Flavonoids can damage the structure and permeability mechanism of bacterial cell walls. Masduki said that tannins have antibacterial properties by precipitating proteins (Tzimas, Antoniadou, Varzakas, & Voidarou, 2024). Robinson said that the antibacterial effects of tannins can interfere with the formation of cell membranes, the inactivation of enzymes and the destruction or inactivation of the function of genetic material, especially active against growing cells. Its work inhibits DNA polymerase from microorganisms by suppressing the formation of chains in DNA synthesis, so that DNA synthesis

will stop (Samson dkk., 2024; Shie, 2014; Tong dkk., 2024; Vukić dkk., 2022). Phenol, which is a flavonoid derivative, has a function as an antiseptic and can kill germs bactericidal. In this case, it is suspected that flavonoids have a role in inhibiting the growth of Streptococcus mutans but the mechanism of action of several active compounds such as alkaloids, flavonoids, saponins and tannins is not yet known if they are combined at the same time, so further research is needed (Singh, Purohit, Tandon, Singh, & Saha, 2017).

Results of the concentration test of areca nut methanol extract on the area of the growth inhibition zone of Streptococcus mutans

Based on the results of the research that has been carried out, it can be seen that the widest diameter of the inhibition zone of amethanol extract is at 100% in inhibiting the growth of Streptococcus mutans bacteria and the smallest is at a concentration of 5%, which can be seen in table 4.2 and table 4.1. The results of the observations showed a linear decrease from 100% concentration to 5% concentration

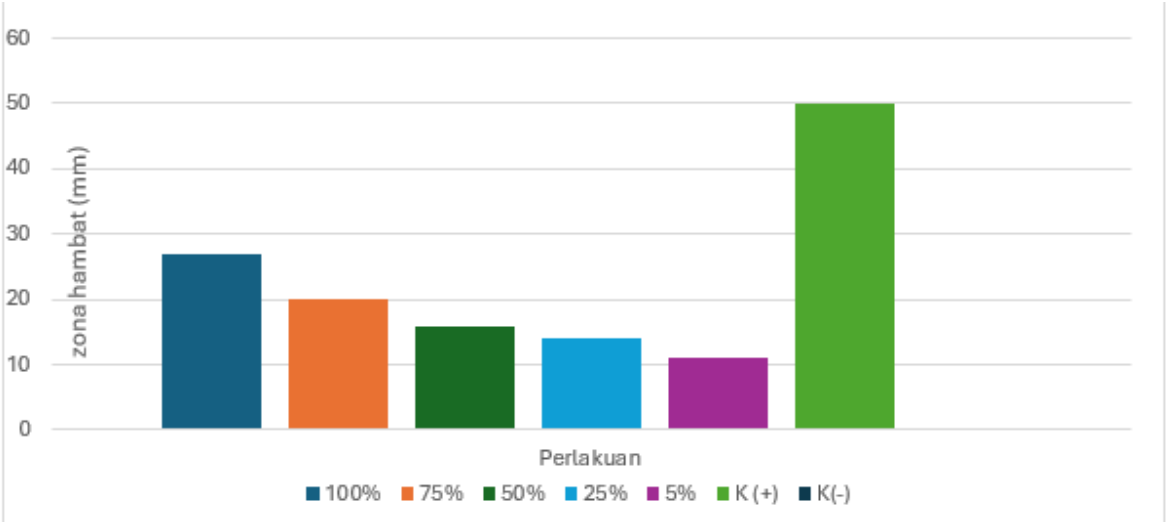

Figure 2. Graph of observation results of treatment of concentration of amethanol extract of areca nut (Areca catehu) and control of the growth of Streptococcus mutans

Sabir stated that the ability of an antibacterial ingredient in inhibiting the growth of a microorganism depends on the concentration of antibacterial ingredients. Then, the smaller the concentration, the less active substances are contained in it, so the lower the ability to inhibit bacterial growth and vice versa. However, the results of this study are not in accordance with this statement, namely the greater the concentration of areca nut methanol extract tested against bacteria, it does not always show a larger inhibition zone and the smaller the concentration does not necessarily indicate a smaller inhibition zone. This can be seen in figure 4.2 where the concentration of 50% is the same as the concentration of 25% and the concentration of 25% is the same as the concentration of 5%.

CONCLUSION

The results of this study indicate that methanol extract of areca nut fruit (*Areca catechu*) is effective in inhibiting the growth of *Streptococcus mutans*, with the largest inhibition observed at 100% concentration and the smallest at 5% concentration. Phytochemical analysis of the extract revealed the presence of active compounds, including flavonoids, alkaloids, tannins, and saponins, which are likely

responsible for the antibacterial activity. Based on these findings, it is suggested that areca nut methanol extract could be further developed as a natural alternative for the prevention and treatment of dental caries. Future studies are recommended to explore its efficacy *in vivo*, evaluate optimal dosage and safety profiles, and investigate potential formulations for practical oral healthcare applications, such as toothpaste, mouthwash, or chewing gum.

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