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Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Areca Nut Ethanolic Extract Against *Streptococcus mutans* and *Escherichia coli* using Broth Dilution Method

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Abstract

Antimicrobial Resistance (AMR) is the global problems that continously develop. Two bacteria that are the most pathogenic are *Streptococcus mutans* (*S. mutans*) and *Escherichia coli* (*E. coli*). The AMR requires new strategies to address this health problem, including the development of new drugs from natural products. Through this study, we aimed to discovering the antibacterial activity of Areca Nuts against *S. mutans* and *E. coli* as potential new antibiotics from natural products. The study begin with extration process, then continued with characterization of extract, bacteria identification, and determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). This study obtained the yield percentage of extract was qualified the standard reference (28.95%). The characterization of extract showed the extract meet the qualification based on loss on drying, water content, ash content and total count plate parameters. The MIC values of Areca Nut ethanolic extract against *S. mutans* is 250 ppm and against *E. coli* is 62.5 ppm. The MBC values of Areca Nut ethanolic extract against *S. mutans* is 500 ppm and against *E. coli* is 62.5 ppm. The result show the ethanol extract of areca nut seeds has antibacterial activity with bactericidal properties, where the ethanol extract of areca nut seeds is more effective against *E. coli* bacteria than *S. mutans* bacteria.

Keywords: Areca Nut; *Escherichia coli*; Minimum Bactericidal Concentration; Minimum Inhibitory Concentration; *Streptococcus mutans*.

INTRODUCTION

When bacteria, viruses, fungi, and parasites stop responding to antimicrobial medications, it's known as antimicrobial resistance, or AMR. Drug resistance increases the risk of disease transmission, serious sickness, disability, and death by making antibiotics and other antimicrobial medications ineffective and making infections difficult or impossible to cure. AMR develops naturally as a result of genetic alterations in infections over time. Human activity, particularly the abuse and overuse of antimicrobials to treat, prevent, or control illnesses in humans, animals, and plants, accelerates its establishment and spread. The costs of AMR to national economies and health systems are substantial. For instance, it increases the need for more costly and intense care, reduces agricultural productivity, and impacts patient or caregiver productivity through extended hospital stays. The AMR is also causing an increase in the limited choice of antibiotics for treating infectious diseases. The quest for medicinal molecules to replace antibiotics that have developed resistance is still ongoing (World Health Organization, 2023).

The AMR requires new strategies to address this health problem, including the development of new drugs from natural products rich in secondary metabolites with medicinal properties. One potential medicinal plant is the areca nut (*Areca catechu* L.), which is commonly found in Papua. This plant has been extensively studied, and it has been reported that areca nuts are rich in secondary metabolites that can be used as medicines in the treatment of various diseases. Research conducted by (Shamim et al., 2023) analyzed the compound content of ethanol, methanol, and water extracts of areca nuts using HPLC. The results showed phenolic acid compounds, including quercetin, gallic acid, caffeic acid, syringic acid, cinnamic acid, sinapinic acid, vanillic acid, coumaric acid, and ferulic acid. A study led by (Tobi et al., 2022) discovered that areca nut ethanolic extract contains flavonoids, tannins, alkaloids, saponins, and steroids, which are a few groups of metabolite chemicals from plants that exhibit antibacterial activity. These organic antimicrobial substances may serve as a substitute therapy to lessen the prevalence of antibiotic or antimicrobial resistance (Bakri et al., 2025).

Two bacteria that are the most pathogenic are *Streptococcus mutans* (*S. mutans*) and *Escherichia coli* (*E. coli*). *S. mutans* is a Gram-positive, facultative anaerobic bacterium. *S. mutans* is part of the normal flora of the human oral cavity. This bacterium can thrive in acidic conditions or low pH. One health problem that *S. mutans* can cause is dental plaque or caries, as well as oral infections if overpopulated (Chismirina et al., 2021). Besides oral diseases, these oral microorganisms also cause cardiovascular diseases, strokes, premature birth, diabetes, and pneumonia, among others. (Fang et al., 2024). *Escherichia coli* is the microbiological organism that has been investigated the most globally. Its implications on human health are varied and include commensalism, gastrointestinal problems, and extraintestinal diseases. *E. coli* is a facultative anaerobic, Gram-negative bacterium. *E. coli* is a typical component of the intestinal flora. Because it is commonly discovered and can survive in living tissues, including soil, waste, and water, this bacterium is known as opportunistic. Numerous investigations have shown that specific *E. coli* strains can cause gastroenteritis or diarrhea epidemics, particularly in children. Urinary tract infections can also result from this bacterium's invasion of the urinary tract (Geurtsen et al., 2022).

Based on the description above, the researchers were interested in conducting a study on the antibacterial activity of ethanol extract of areca nut seeds (*Areca catechu* L.) against *Streptococcus mutans* and *Escherichia coli*. The use of both Gram-positive and Gram-negative bacteria was intended to compare the antibacterial activity of the ethanol extract of areca nut seeds. It is known that these two groups of Gram-positive bacteria have different cell wall structures. This difference in cell wall structure can be a factor that can influence the activity of an antibacterial against both types of bacteria. This study used the broth dilution test. The principle of this method is to dilute the test material to obtain several concentrations, then add a bacterial suspension to each concentration in the medium. The broth dilution method was chosen because it has the advantage of creating a large surface area, resulting in greater contact between the test material and the bacteria.

MATERIALS AND METHODS

Research Site and Time

The research was conducted in the Pharmacy laboratory, Faculty of Mathematics and Natural Sciences, Cenderawasih University, from March 24 to September 26, 2025.

Materials and Equipment

The equipment used in this study were glass vessels, measuring cylinders, blenders, Whatman paper no. 1, rotary evaporators, stirring rods, glass containers, test tubes, water baths, test tubes, petri dishes, Erlenmeyer flasks, spirit lamps, round loops, incubators, micropipettes, and a Bio Safety Cabinet (BSC).

The materials used in this study were areca nut samples, 96% technical ethanol, distilled water, test bacteria *S. mutans* and *E. coli*, Brain Heart Infusion Broth (BHIBB), Nutrient Agar (NA), Blood Agar, physiological NaCl 0.9%, H2SO4 1%, BaCl2 1%, Ciprofloxacin 200 mg/100 ml, and DMSO.

Procedures

Sample Extraction

Areca Nuts were collected from the Arso region of Papua, fresh, as indicated by their deep green skin and unhardened flesh. The extract was first prepared by grinding the Areca Nut into a simple mixture, then pulverizing it using a blender. 500 grams of Areca Nut powder were taken and soaked in 96% ethanol (1:10) in a glass container. Maceration was carried out for 24 hours at room temperature, with occasional stirring for the first 6 hours. The macerate was filtered using Whatman No. 1 paper and then concentrated using a vacuum rotary evaporator at 55°C until a thick extract was obtained and the yield was calculated.

Extract Characterization

This procedure aimed to determine both specific and non-specific parameters, ensuring the quality, safety, stability, and consistency of the active compound content in the extract. The test consists of the Loss on Drying, Water content, ash content, and total plate count. The loss on drying test was conducted using the gravimetric method with the help of a moisture balance instrument. Determination of water content is carried out using an oven with repeated heating-cooling cycles until a constant weight is obtained. Determination of water content is done by heating the extract to a temperature of 600 °C in a furnace. In total plate count testing, bacterial colonies are counted after the extract is diluted in nutrient agar medium.

Bacteria Identification

Identification of *Streptococcus mutans* using blood agar. The bacteria are grown directly on blood agar using the streak method and incubated for 24 hours in an incubator at 37°C. A positive result for *Streptococcus mutans* is indicated by the formation of a transparent zone. For *Escherichia coli*, identification is performed by streaking on Endo agar. This is then incubated at 37°C for 24 hours. A positive result is indicated by the formation of a metallic sheen.

Sample and Bacteria Preparation

Pure cultures of *S. mutans* and *E. coli* were each taken using a sterile loop and inoculated by streaking in a zigzag pattern onto nutrient agar medium. They were then incubated at 37°C for 24 hours. The test solution

was prepared by weighing 20 mg of areca nut ethanol extract and dissolving it in 10 mL of 10% DMSO to obtain a stock solution of 2000 µg/mL. The test solution concentrations used were 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL Rejuvenated *S. mutans* and *E. coli* were suspended in 0.9% NaCl solution and equilibrated to a McFarland 0.5 solution. 0.5 mL of 200 mg/100 ml of ciprofloxacin and 0.5 mL of BHIB were added to a test tube and vortexed. Then, 0.1 mL of 106 CFU/ml of test bacteria was added. To 0.5 mL of BHIB in a test tube, 0.5 mL of 10% DMSO solution was added and vortexed. Afterward, 0.1 mL of the test bacterial solution (106 CFU/mL) was added.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Discovery

The MIC and MBC determination method used the broth dilution method. The extract was initially prepared at a concentration of 2000 µg/mL, which was then diluted to 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL. Several sterilized test tubes were prepared. Each tube was filled with 1 mL of BHIB medium. Then, 1 mL of the test solution was added to the first test tube and vortexed. 1 mL of the test solution was taken from the first tube and transferred to the second and final tubes. 1 mL of the solution from the last tube was removed and discarded, leaving each tube with 1 mL. 0.1 mL of the bacterial suspension was added to each tube and vortexed. All of the tubes were incubated in an incubator at 37°C for 18-24 hours. Turbidity was then observed and compared with the positive control (ciprofloxacin) and the negative control (media + bacterial suspension, without test solution). The lowest concentration that did not show turbidity was the MIC. The antibacterial activity of the most active Areca Nut sample was classified as strong if the MIC value was <100 µg/mL, moderate if 100 <MIC <625 µg/mL, and weak if the MIC value was >625 µg/mL (Tobi et al., 2024).

After obtaining the minimum inhibitory concentration, a further test was conducted to determine the MBC value. This test was performed on all tubes that did not show turbidity (clear) during the MIC determination by streaking on blood agar media, then incubating them for 18-24 hours at 37°C in an incubator. The lowest concentration that did not show bacterial colony growth on the media was determined as the MBC.

RESULTS AND DISCUSSION

Extraction Result

Extraction carried out using the maceration method yields an extract with the results shown in Table 1.

Table 1. Extraction Result.

Simplicia (g)	Solvent (ml)	Extract (g)	Yield (%)
500	5000	144,76	28,95

This value is almost comparable to (Andrifianie et al., 2025)study, which obtained a yield of 26.8%. The value obtained in this study also aligns with the Indonesian Herbal Pharmacopoeia, which requires a yield of concentrated areca nut extract of at least 16.50% (Kementerian Kesehatan Republik Indonesia, 2017). A yield approaching 30% indicates a relatively high potential for secondary metabolites, such as alkaloids, flavonoids, and tannins, thus suggesting the extract has the potential to provide significant biological activity (Buang et al., 2023). One parameter of extract quality is the yield. A higher yield indicates a higher extract quality.

Extract Characterization Result

Table 2. Extract Characterization Result.

Sample	Parameter	Method	Reference Value	Result	Unit
Areca nut extract	Loss on Drying	Thermogravimetric	-	12,63	%
	Water Content	Thermogravimetric	<30%	8,37	%
	Ash Content	Gravimetric	<5%	0,87	%
	Total Plate Count	Pour plate	$\leq 10^5$/g	<1 x 10 or 0	Colony/g

Determining drying loss in extracts is one of the requirements that must be met in the standardization of natural materials. Determining drying loss is a measurement of the remaining substance after drying at a temperature of 105°C for 30 minutes or until constant weight. The purpose of determining drying loss is to determine the maximum limit (range) of the amount of compounds lost during the drying process (Kementerian Kesehatan Republik Indonesia, 2020). Measurement of drying loss in this study used a Moisture balance tool. Based on the measurements, the ethanol extract of areca nut seeds had a drying loss of 12.63%.

Moisture content determination is carried out to determine the residual water after the thickening or drying process. This study used a thermogravimetric method for determining moisture content. According to (Kementerian Kesehatan Republik Indonesia, 2020), moisture content in extracts is divided into three types:

liquid extracts, which are still pourable, typically have a moisture content greater than 30%. Thick extracts have a moisture content between 5-30%, and dry extracts have a moisture content of less than 5%. The moisture content measurement results for the areca nut ethanol extract were 8.37%. This areca nut ethanol extract is a thick extract and falls within the range of 5-30% for thick extracts. The moisture content of this areca nut extract meets extract standards because it is below the specified value. A moisture content exceeding the limit or relatively high can encourage the growth of fungi and bacteria and can cause chemical changes in the substances contained, thus reducing the quality of the extract.

The next stage in extract characterization or standardization is determining the ash content. Ash is an inorganic substance left over from the combustion of organic materials. The purpose of determining the ash content is to obtain an overview of the mineral content of the extract from the beginning of the process until the extract is formed. The ash content determination process of the areca nut ethanol extract is carried out by heating until the organic compounds and their derivatives evaporate and are destroyed, leaving only the mineral and inorganic elements. The ash content of the areca nut ethanol extract obtained was 0.87%. This indicates that the ash content of the areca nut ethanol extract meets the specified standard of less than 5%.

The total plate count examination is the process of determining the number of bacteria in a sample (Yoelianto & Safari, 2025). The method used in this study is the pour plate method. The principle of the pour plate method is to grow bacteria evenly by mixing the diluted sample (extract) into a liquid agar medium. The process is carried out aseptically to avoid contaminants or unwanted organisms. After incubation, the culture is incubated at 37°C for 24 hours. After incubation, the growth of bacterial colonies in each plate containing 30-300 colonies is recorded. The total plate count was calculated in colonies/g of sample by multiplying the average number of colonies on the plate by the appropriate dilution factor (Kementerian Kesehatan Republik Indonesia, 2020). Based on observations, the media did not show any bacterial colony growth. This result could be due to the solvent used in the extraction process, ethanol, which can inhibit bacterial growth in the extract.

Bacteria Identification

The results of bacterial streaking on specific media were positive. It showed the growth of transparent white colonies. The color of the bacterial colonies on the blood agar media indicated that *S. mutans* ATCC 25175 was β-hemolytic (total hemolysis indicates a clear color on the blood agar medium around the colony), which was caused by the lysis of red blood cells due to the reduction of hemoglobin to methemoglobin. *S. mutans* produces hemolysin, which is an extracellular product that can lyse red blood cells. Based on the results, the media showed a metallic sheen. The basic fucshin substance in Endo Agar media was able to react with the *E. coli* ATCC 25922 isolate so that the fucshin substance was absorbed and caused the colonies to have a metallic sheen. The basic fuchsin and sodium sulfite substances in Endo Agar media were able to prevent the growth of Gram-positive bacteria so that only Gram-negative bacteria grew (Abu-Sini et al., 2023). The results of bacteria identification on specific media can be seen in Figure 1.

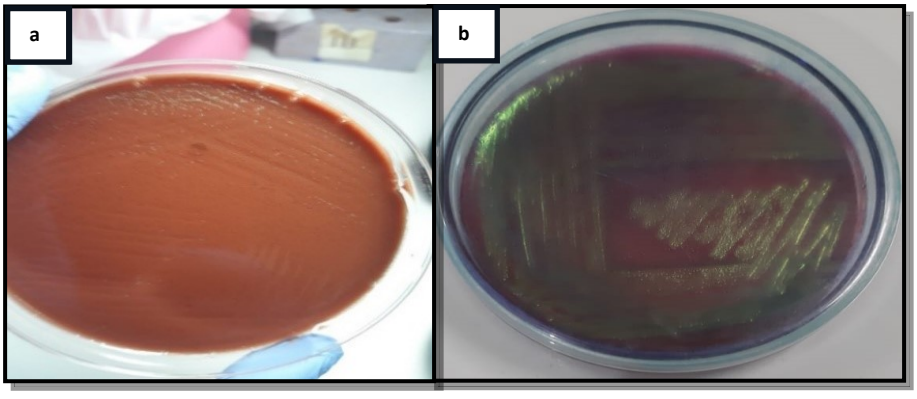

Figure 1. Bacteria Identification using Specific Media Result. *S. mutans* identification (a) *E. coli* identification (b).

MIC and MBC of Areca Nut Ethanolic Extract Result

The antibacterial properties of a material can be seen based on its ability to inhibit bacterial growth (bacteriostatic) or its ability to kill bacteria (bactericidal). Both cidal and statics are in vitro terms, which refer to the effect of antibiotic concentration affecting bacterial growth at a predefined threshold. Antibiotics targeting the organism's cell wall are mostly bactericidal, whereas those targeting protein synthesis are bacteriostatic. MIC (minimum inhibitory concentration) is the lowest antibiotic concentration that prevents visible growth at 24 hours. MBC (minimum bactericidal concentration) is the

minimal concentration of antibiotics that causes bacterial death. Breakpoints for antibiotics MIC's are set by the EUCAST and CLSI. A bactericidal antibiotic MBC is less than or equal to fourfold above the MIC. A bacteriostatic antibiotic MBC is eightfold above MIC (Neri et al., 2021). The MIC and MBC of Areca Nut ethanolic extract against *S. mutans* and *E. coli* can be seen ini Figure 2 and 3

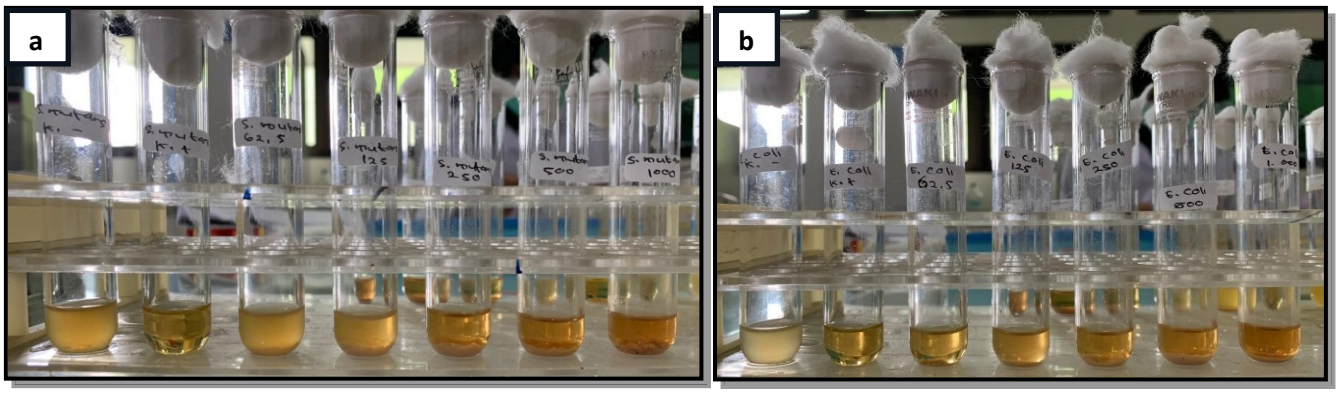

Figure 2. MIC Determination Result of Negative Control, Positive Control, Areca Nut Ethanolic Extract 62.5; 125; 250; 500; 1000 ppm (From Left to Right). MIC againts *S. mutans* (a) MIC against *E. coli* (b).

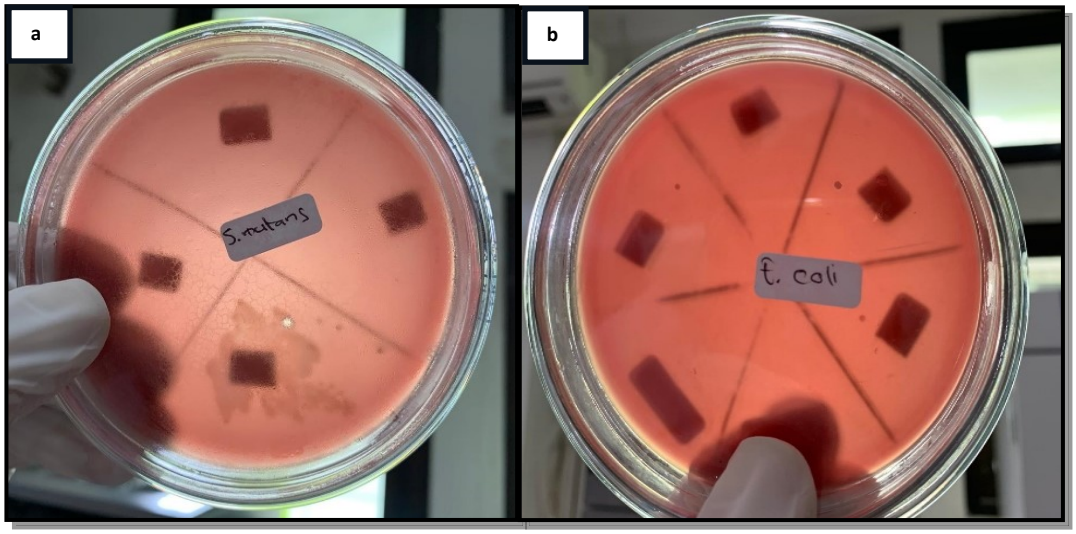

Figure 3. MBC Determination Result of Areca Nut Ethanolic Extract 1000 ppm; 500 ppm; 250 ppm; Positive Control (From Top-Clockwise) against *S. mutans* (a) and MBC of Areca Nut Ethanolic Extract 250 ppm; 500 ppm; 1000 ppm; Positive Control; 125 ppm; 62.5 ppm (From Top-Clockwise) against *E. coli* (b)

The MIC results for *S. mutans* bacteria showed turbidity in the negative control group, the areca nut extract group at concentrations of 62.5 ppm and 125 ppm. Meanwhile, in the positive control group, the areca nut extract groups at concentrations of 250 ppm, 500 ppm, and 1000 ppm showed clarity in the test solution. Based on these results, we can interpret the MIC value of the areca nut ethanol extract against *S. mutans* as 250 ppm.

These MIC results were then followed by the determination of the MBC value. According to Figure 3, the areas of the group that did not grow bacteria after 24 hours of incubation were the positive control group, the areca nut ethanol extract groups at concentrations of 500 ppm and 1000 ppm. The areca nut ethanol extract group at a concentration of 250 ppm was observed to have bacterial growth as seen from the spots formed on the blood agar medium. This can be interpreted as indicating that the MBC value of the areca nut ethanol extract was 500 ppm. The MIC and MBC values of areca nut ethanol extract against *S. mutans* can be used to determine whether the areca nut ethanol extract is bactericidal or static. The MBC value of areca nut ethanol extract against *S. mutans* is 2 times higher than its MIC value. Based on the theory previously stated, areca nut ethanol extract is bactericidal against *S. mutans*.

In the *E. coli* test, the turbidity observed in the negative control group, the positive control group, and all the areca nut ethanol extract groups indicated clarity in the test solution. The 62.5 ppm concentration of the areca

nut ethanol extract group can be interpreted as the MIC value of the areca nut ethanol extract.

The test solution that showed clarity was then streaked onto blood agar, and the results showed no visible staining on the blood agar in all test groups. This can be interpreted as the MBC value of the areca nut ethanol extract, which was 62.5 ppm. Based on the theory presented, the areca nut ethanol extract is bactericidal (MBC value equals MIC value) against *E. coli*.

As explained in the introduction, previous research on the secondary metabolite content of areca nut ethanol extract showed the presence of hydroxybenzoic acids (gallic, syringic, vanillic acid), hydroxycinnamic acids (caffeic, cinnamic, sinapinic, coumaric, ferulic acid), flavonoids, tannins, alkaloids, saponins, and steroids. These compounds work singly, synergistically, or complementarily in producing bactericidal properties in the ethanol extract of areca nut seeds against the tested bacteria. Several mechanisms of action of flavonoids as antibacterials are depicted in Figure 4 below.

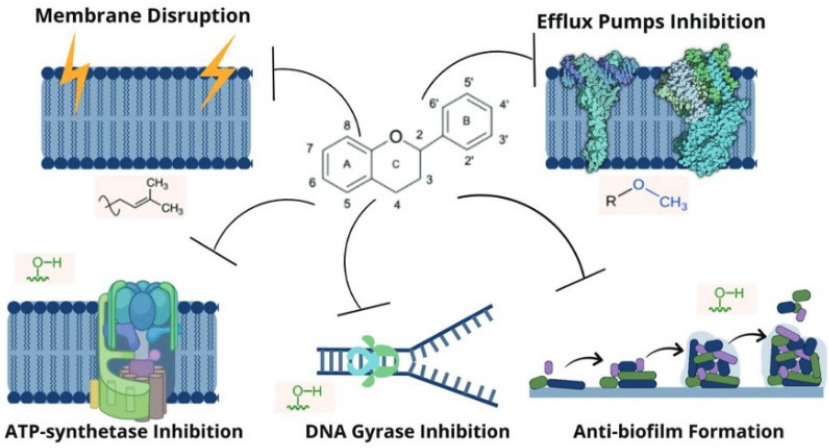

Figure 4. Mechanism of Action of Flavonoid as Antibacterial (Górniak et al., 2019; Rodríguez et al., 2023).

Bacterial membrane integrity is ensured by processes that make it crucial for bacterial life; any damage might result in metabolic dysfunctions and bacterial death. The idea that antibiotic activity and membrane interference cause bacterial membrane fluidity to decrease is supported by a number of studies. Unique structure of Gram-negative cell wall contributes to the Gram-negative cell wall's increased complexity and serves as a nearly impermeable barrier, limiting the entry of foreign molecules. In contrast, Gram-positive bacteria possess only peptidoglycans in their cell wall, making them more permeable and thus rendering them more sensitive to external influences. Consequently, Gram-positive bacteria are more susceptible, and flavonoids are found to have higher efficacy against Gram-positive bacteria than against Gram-negative (Yuan et al., 2021). This theory is contradictory with the research results obtained, where the MBC value of areca nut ethanol extract against Gram-negative bacteria *E. coli* is smaller than the MBC value against Gram-positive bacteria *S. mutans*. This is assumed to be caused by the compounds in areca nut ethanol extract being dominated by lipophilic compounds that can penetrate the cell walls of Gram-negative bacteria that are lipophilic more easily.

Alkaloids' strong ability to obstruct the bacterial cells' production of proteins and nucleic acids is a crucial part of their antibacterial action. Alkaloids' ability to alter the permeability of bacterial cell membranes is another significant antibacterial effect. They can penetrate bacterial cell membranes, get to their intended sites, and exert antibacterial effects due to their high lipophilicity (Thawabteh et al., 2024). The study of structure-activity relationships can logically explain the antibacterial properties of a sample based on the tannin structure contained in the sample. Tannins have many phenolic hydroxyls that act as ring activators and electron donors. Electron-donating substituents can enhance antibacterial and other biological activities. The presence of OH will affect the hydroxylation process in converting lipophilic fat-soluble compounds into water-soluble or hydrophilic compounds so that the hydroxyl groups will interfere with the partition of the compound into biological membranes (Kurniawan & Zahra, 2021).

In addition to the cell wall as a target of action, ethanol extract of areca nut seeds has also been shown to work on biofilms formed by *E. coli* bacteria through inhibition and destruction mechanisms (Tethool et al., 2025)

Based on the research results obtained which are explained with existing theories, it can be concluded that the ethanol extract of areca nut seeds has antibacterial activity, where the ethanol extract of areca nut seeds is more effective against *E. coli* bacteria than *S. mutans* bacteria. The difference in MIC and MBC results is inseparable from the structure and properties of the cell walls and cell membranes of each bacterium (Maher &

Hassan, 2023). The linearity of the hydrophilic and lipophilic properties of the compounds in the ethanol extract of areca nut seeds against the bacterial permeable membrane is a factor that can affect the MIC and MBC values. However, further, more comprehensive studies are recommended from this research to examine the complexity of this barrier as a variable that influences the difference in MIC and MBC results in Gram-positive and Gram-negative bacteria.

CONCLUSIONS

The study of discovering MIC and MBC values of Areca Nut ethanolic extract against *S. mutans* and *E. coli* bacteria have been accomplished. The MIC values of Areca Nut ethanolic extract against *S. mutans* is 250 ppm and against *E. coli* is 62.5 ppm. The MBC values of Areca Nut ethanolic extract against *S. mutans* is 500 ppm and against *E. coli* is 62.5 ppm.

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Competing Interests: The authors declare that there are no competing interests.

REFERENCES

Abu-Sini, M. K., Maharmah, R. A., Abulebdah, D. H., & Al-Sabi, M. N. S. (2023). Isolation and identification of coliform bacteria and multidrug-resistant Escherichia coli from water intended for drug compounding in community pharmacies in Jordan. *Healthcare*, *11*(3), 299.

Andrifianie, F., Daffa, M. N., Anggraeni, R. E., Wardhani, O. R., Graharti, R., Ali, N. F. M., & Adjeng, A. N. T. (2025). Perbandingan Ekstrak Biji Pinang Terdelipidasi dan Non-Terdelipidasi: Karakterisasi, Fitokimia, dan Aktivitas Antibakteri terhadap Cutibacterium acnes. *Jurnal Mandala Pharmacon Indonesia*, *11*(2), 474–482.

Bakri, N. F., Pratiwi, M. E., Manemi, P. W., Gunawan, E., & Mende, J. (2025). Antibacterial Activity Test of Extract and Fraction of Jabon Stem Bark (Anthocephalus cadamba (Roxb) Miq.) Againts Staphylococcus aureus dan Escherichia coli. *Jurnal Mandala Pharmacon Indonesia*, *11*(1), 77–84.

Buang, A., Adriana, A. N. I., & Rejeki, S. (2023). Formulasi Tablet Ekstrak Etanol Biji Buah Pinang (Areca catechu L.) dengan Variasi Konsentrasi Gelatin Sebagai Bahan Pengikat. *Jurnal Mandala Pharmacon Indonesia*, *9*(1), 100–110.

Chismirina, S., Sungkar, S., Andayani, R., & Rezeki, S. (2021). Existence of Streptococcus Mutans and Streptococcus Sobrinus in Oral Cavity as Main Cariogenc Bacteria of Dental Caries. *1st Aceh International Dental Meeting (AIDEM 2019), Oral Health International Conference On Art, Nature And Material Science Development 2019*, 90–92.

Fang, Y., Chen, X., Chu, C. H., Yu, O. Y., He, J., & Li, M. (2024). Roles of Streptococcus mutans in human health: beyond dental caries. *Frontiers in Microbiology*, *15*, 1503657.

Geurtsen, J., de Been, M., Weerdenburg, E., Zomer, A., McNally, A., & Poolman, J. (2022). Genomics and pathotypes of the many faces of Escherichia coli. *FEMS Microbiology Reviews*, *46*(6), fuac031.

Górniak, I., Bartoszewski, R., & Króliczewski, J. (2019). Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews*, *18*(1), 241–272.

Kementerian Kesehatan Republik Indonesia. (2017). *Farmakope Herbal Indonesia Edisi II* (II, Vol. 1). Kementerian Kesehatan Republik Indonesia.

Kementerian Kesehatan Republik Indonesia. (2020). *Farmakope Indonesia Edisi VI*. Kementerian Kesehatan Republik Indonesia. Direktorat Jenderal Kefarmasian dan Alat Kesehatan.

Kurniawan, I., & Zahra, H. (2021). gallotannins; biosynthesis, structure activity relationship, anti-inflammatory and antibacterial activity. *Current Biochemistry*, *8*(1), 1–16.

Maher, C., & Hassan, K. A. (2023). The Gram-negative permeability barrier: tipping the balance of the in and the out. *MBio*, *14*(6), e01205-23.

Neri, V., Huang, L., & Li, J. (2021). *Infections and sepsis development*. BoD–Books on Demand.

Rodríguez, B., Pacheco, L., Bernal, I., & Piña, M. (2023). Mechanisms of action of flavonoids: antioxidant, antibacterial and antifungal properties. *Ciencia, Ambiente y Clima*, *6*(2), 33–66.

Shamim, A., Ali, A., Iqbal, Z., Mirza, M. A., Aqil, M., Kawish, S. M., Siddiqui, A., Kumar, V., Naseef, P. P., & Alshadidi, A. A. F. (2023). Natural medicine a promising candidate in combating microbial biofilm. *Antibiotics*, *12*(2), 299.

Tethool, A. M., Rahmawati, I., Saptarini, O., Pratiwi, M. E., & Tobi, C. H. B. (2025). Antibiofilm Activity of Areca Nut (Areca catechu L.) Extract and Fractions against Escherichia coli ATCC 25922. *Journal of Nuts*, *16*(4), 331–340.

Thawabteh, A. M., Ghanem, A. W., AbuMadi, S., Thaher, D., Jaghama, W., Karaman, R., Scrano, L., & Bufo, S. A. (2024). Antibacterial activity and antifungal activity of monomeric alkaloids. *Toxins*, *16*(11), 489.

Tobi, C. H. B., Bakri, N. F., & Pratiwi, M. E. (2024). Minimum inhibitory concentration and minimum bactericidal concentration of Areca nut ethanolic extract against Staphylococcus aureus and Pseudomonas aeruginosa. *Riset Informasi Kesehatan*, *13*(2), 170–177.

Tobi, C. H. B., Saptarini, O., & Rahmawati, I. (2022). Aktivitas antibiofilm ekstrak dan fraksi-fraksi biji pinang (Areca catechu L.) terhadap Staphylococcus aureus ATCC 25923. *J Pharm Sci*, *1*, 57.

World Health Organization. (2023, November 21). *Antimicrobial resistance*. Https://Www.Who.Int/News-Room/Fact-Sheets/Detail/Antimicrobial-Resistance.

Yoelianto, Y. A., & Safari, W. F. (2025). Nilai Total Plate Count (TPC) pada Jajanan Pasar di Pasar Pondok Gede Kota Bekasi. *Jurnal Analis Kesehatan Kendari*, *7*(2), 51–56.

Yuan, G., Guan, Y., Yi, H., Lai, S., Sun, Y., & Cao, S. (2021). Antibacterial activity and mechanism of plant flavonoids to gram-positive bacteria predicted from their lipophilicities. *Scientific Reports*, *11*(1), 10471.