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Minimum inhibitory concentration and minimum bactericidal concentration of Areca nut ethanolic extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract

Background: Areca nut (*Areca catechu* L.) is a plant often found in Indonesia and it has become important part of the social, cultural and economic life of community in Papua and East Nusa Tenggara. Areca nuts have been widely studied and reported to contain various secondary metabolites with various pharmacological properties. This research aimed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of areca nut seed ethanol extract.
Method: The areca seeds were extracted using 96% ethanol by maceration method. Phytochemical screening in areca nut seeds extract using the reagent. MIC and MBC determination of areca nut seeds extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was employed by broth dilution.
Results: Results of secondary metabolite screening showed the presence of flavonoids, tannins, alkaloids, quinones, terpenoids and saponins. MIC value of areca nut seeds ethanol extract against *S. aureus* obtained was smaller than 62.5 µg/mL which was categorized as strong with MBC value was 250 µg/mL. MIC value against *P. aeruginosa* was 250 µg/mL which was categorized as moderate with MBC value was 1000 µg/mL.
Conclusion: MIC and MBC values obtained indicate that areca nut seeds ethanol extract has antibacterial activity against *S. aureus* and *P. aeruginosa.*

Keywords: *Areca catechu* L.; Broth dilution; Minimum Bactericidal Concentration; Minimum Inhibitory Concentration; *Pseudomonas aeruginosa*; *Staphylococcus aureus*

INTRODUCTION

Areca nut (*Areca catechu* L.) is a potential plant widely found in Papua. This plant has been widely studied and it is reported that Areca nut seeds are abundant with secondary metabolite content that can be used as medicine in the therapy of a disease. Main chemical content of areca nut are polyphenol (1), alkaloids (2), triterpenes, steroids, fatty acids, physcion, chrysophanol, and epoxyconiferyl alcohol (3). Type of alkaloid that most abundant in areca nut is Arecoline. The other type of alkaloid that found the most in areca nut are guvacine, arecaidine, and guvacoline. These four alkaloids not only constitute a significant portion of the alkaloids in areca nut but are also major active substances (4).

HPLC analysis in research by (5,6) has shown that the major components in the extract are catechins, quercetin, gallic acid, ellagic acid, epicatechin gallate, catechin, and epicatechin gallate ester of ellagic acid. Therefore, the phenolic compounds in the extract exhibit immunomodulatory activity against *Staphylococcus aureus* infection. The polyphenols in the ethanol extract, including catechins, epicatechin, and epicatechin gallate, possess infection-resistance activity against *Mycobacterium tuberculosis*, Gram-positive *S. aureus*, and Gram-negative *Escherichia coli* (7).

One of the most notorious and pervasive bacterial pathogens is Staphylococcus aureus, which causes an estimated hundreds of thousands to millions of more severe, invasive infections worldwide each year in addition to an incalculable number of simple skin infections It is a major cause of nosocomial bacteremia, surgical site, prosthetic joint, cardiovascular, and other respiratory tract infections, including

pneumonia. (8). In addition, *S. aureus* also the common cause of oral cavity infections, including canker sores (9).

P. aeruginosa is a rod-shaped Gram-negative bacterium of the class γ-proteobacteria and family Pseudomonadaceae. It is a facultative aerobe that prefers to use oxygen as the final electron acceptor during aerobic respiration. *P. aeruginosa* can also catabolize a wide-range of organic molecules for nutrients, making it one of the most biochemically versatile and ubiquitous bacterium found in many environments such as soil, water, vegetation, and even human skin and oral mucosa. The ability P. aeruginosa to thrive in diverse environments increases *P. aeruginosa* reservoirs and the possibility for exposure, leading to higher incidence of infections. In hospitals, *P. aeruginosa* has been isolated from respirators, physical therapy pools, sinks, and mops. Because of its metabolic versatility and an arsenal of virulence factors it possesses, *P. aeruginosa* is responsible for many serious and life-threatening acute and chronic infections, particularly in the setting of immunocompromised hosts with mortality rates reaching as high as 40%. *P. aeruginosa* is a killer of immunocompromised patients, a leading cause of bacteremia and sepsis in neutropenic cancer patients undergoing chemotherapy, the number one cause of hospital-acquired pneumonia and respiratory failure, and several other diseases (10).

There are priority pathogens, known as the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and Enterobacter species) for which new antimicrobial development is urgently needed according to the World Health Organization (WHO) and the Center for Disease Control (CDC) (10). The development of new drugs from natural ingredients that contain many secondary metabolite compounds that have pharmacological properties may become a good choice to overcome the problem. One of these efficacious plants is areca nut. This study aimed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values as the antibacterial activity of areca nut seeds ethanol extract.

METHOD

The materials used for the extraction process and identification of secondary metabolites of the extract were 96% ethanol (Brataco®), distilled water, Dragendorf reagent, Liebermann Burchard reagent, HCL (Merck®), magnesium powder (Merck®), FeCl3 (Merck®), NaOH (Merck®), CHCl3 (Merck®), H2SO4 (Merck®) and K2CrO7 (Pudak®). The materials used for microbiological testing were *S. aureus* bacteria, *P. aeruginosa* bacteria, Brain Heart Infusion Broth (BHIB) (Merck®), Nutrient Agar (NA) (Merck®), Blood Agar (Oxoid®), physiological NaCl 0.9% (MJB Pharma®), H2SO4 (Merck®) 1%, BaCl2 (Merck®) 1%, Ciprofloxacin 200 mg/100 ml (KalbeMed®), DMSO 10% (Merck®) and distilled water.

The equipment used for the extraction process and identification of secondary metabolites of the extract were analytical scales, glass containers, measuring cups, blenders, Whatman paper no. 1, rotary evaporators, stirring rods, glass containers, test tubes and water bath. The equipment used for microbiological testing were test tubes, petri dishes, spirit lamps, needle holder inoculation loop (USBECK), 96-well polystyrene round-bottom microplates (Iwaki), 96-well polystyrene flat bottom microplates (Iwaki), incubators (Memmert), micropipettes (Socorex) and Laminar air Flow (LAF).

Areca Nut Seed Extraction

The obtained Areca nut seed sample was made into simplicia and powdered by blending to be extracted using the maceration method. A total of 500 grams of simplicial powder was soaked using 96% ethanol with a ratio of simplicia powder: 96% ethanol of 1:10. Maceration was carried out for 24 hours at room temperature with occasional stirring in the first 6 hours. Remaceration was carried out to obtain optimal extract. The macerate was filtered using Whatman paper no. 1 then concentrated with a vacuum rotary evaporator

at a temperature of 55°C until a thick extract was obtained and its yield was calculated (11). The extract was dried using a water bath and an ethanol-free test was carried out by reacting the extract with H_2SO_4 and $K_2Cr_2O_7$. The absence of a color change from orange to blue in the results of this reaction indicates that the extract does not contain ethanol.

Secondary Metabolite Identification

Identification of the content of ethanol extract of Areca nut seeds was carried out using a coloring method to confirm secondary metabolite compounds that act as antibacterials.

Bacterial Culture Rejuvenation

Pure cultures of test bacteria *S. aureus* and *P. aeruginosa*, each taken as much as one loop and inoculated by scratching in a zigzag scratch on nutrient agar media. Then incubated at 37°C for 24 hours (12).

Test Solution Preparation

20 mg of areca nut seeds ethanol extract was weighed and dissolved in 10 mL of 10% DMSO to obtain a stock solution of 2000 µg/mL. The concentrations of the test solutions made were 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL.

Bacteria Suspension Preparation

Rejuvenated cultures of *S. aureus* and *P. aeruginosa* were suspended in 0.9% NaCl solution and equilibrated to 0.5 Mc Farland.

Positive Control Solution Preparation

0.5 ml of Ciprofloxacin 200 mg/100 ml and 0.5 mL BHIB were put into a reaction tube and vortexed, then 0.1 ml of 106 CFU/ml test bacteria were added to the solution.

Negative Control Solution Preparation

0.5 mL of BHIB was added with 0.5 mL of 10% DMSO solution in a test tube and then vortexed. After that, 0.1 test bacteria solution of 106 CFU/mL was added.

MIC Value Determination

Determination of MIC and MBC using the broth dilution and agar dilution method. Several sterilized test tubes were prepared. 1 mL of BHI medium was filled to each tube. Then 1 mL of test solution was added to the first test tube and vortexed. 1 mL of test solution was taken from the first tube and transferred to the second tube to the last tube. 1 mL of solution was taken from the last tube and thrown away so that each tube only contained 1 mL solution. 0.1 mL of bacterial suspension was added to each tube and vortexed. All tubes were incubated at 37 °C for 18-24 hours. Then the turbidity was observed and compared with the positive control (ciprofloxacin) and negative control (media + bacterial suspension, without extract solution). The smallest concentration that did not show turbidity was determined as the MIC. The antibacterial activity of the most active sample of areca nut seeds is classified as strong if the MIC value is <100 µg/mL, moderate if 100 <MIC <625 µg/mL and weak if the MIC value is >625 µg/mL (13).

MBC Value Determination

Further tests were conducted to determine the MBC value. The solution used in the MBC test was solution in the tube that did not show turbidity in MIC determination. The solution was streaked on blood agar media, then incubated at 37 °C for 18-24 hours. The smallest concentration that did not show bacterial colony growth on the media was determined as the MBC.

RESULTS

The extraction result of 500 grams of areca nut powder produced a thick extract of 141.25 grams with a yield percentage of 28.25% (**Table 1**). The yield value of the areca nut extract obtained was in accordance with the Indonesian Herbal Pharmacopoeia, which was not less than 16.50%. The yield value describes the percentage of extract results so that the amount of simplicia needed to make a certain amount of thick areca nut extract can be known. The yield value also shows the amount of bioactive components contained in the extract (11). Ethanol-free test was carried out on the extract to prove that the extract did not contain ethanol solvent which is known to have antibacterial activity.

Table 1. Result of Areca Nut Extraction

Simplicia (gram)	Solvent (ml)	Exstract (gram)	Extract Yield (%)
500	1000	141,25	28,25

The results of identification of secondary metabolite content in areca nut seeds ethanol extract using the reagent method can be seen in **Table 2**. The reagent method is often used as an initial screening in the identification of secondary metabolite content in a plant. This method was used in this study to confirm the presence of several groups of secondary metabolite compounds in the ethanol extract of areca nut seeds that have the potential to be antibacterial.

Table 2. Result of Secondary Metabolite Identification

Bioactive Compound	Reagent	Result	Interpretation
Flavonoid	Mg + Concentrated HCl	Red	Positive
Alkaloid	HCl 2N + Dragendorf	Orange Precipitation	Positive
Tannin	Hot Water + FeCl 1%	Blackish Green	Positive
Quinone	Hot Water + NaOH 1N	Red	Positive
Terpenoid	CHCl3 + Lieberman Burchard	Red	Positve
Saponin	Hot Water + HCl 2N	stable foam in ± 5 minutes	Positive

The MIC and MBC tests aimed to see the antibacterial activity of areca nut ethanol extract against *S. aureus* and *P. aeruginosa*. This test uses the broth dilution and agar dilution method. The principle of this method is to make a series of dilutions of ethanol extract in BHIB media and add test bacteria then incubate for 18-24 hours at 37 °C. Concentrations of the test solutions made were 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL. All concentrations that showed clarity in broth media were recultured by streaking on blood agar media and then re-incubated to determine MBC. The smallest concentration that did not show bacterial colony growth on solid media was determined as MBC. The results of the MIC and MBC tests of Areca nut seeds ethanol extract against *S. aureus* and *P. aeruginosa* can be seen in **Figure 1, Figure 2, Figure 3, Figure 4** and **Table 3.**

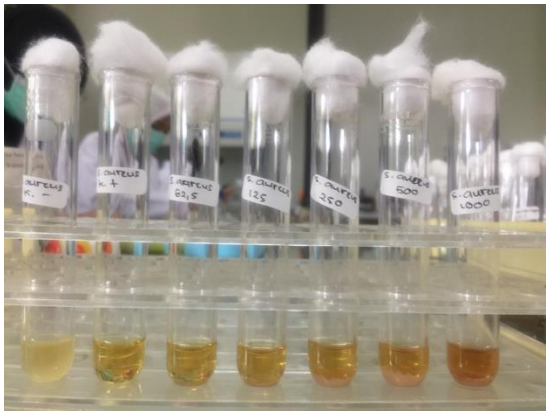

Figure 1. Result of MIC determination of areca nut seeds ethanol extract against *S. aureus* (left to right: negative control, positive control, extract of 62,5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL)

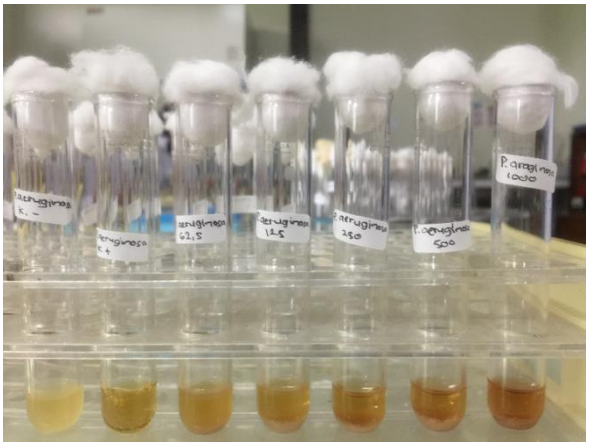

Figure 2. Result of MIC determination of areca nut seeds ethanol extract against P. aeruginosa (left to right: negative control, positive control, extract of 62,5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL).

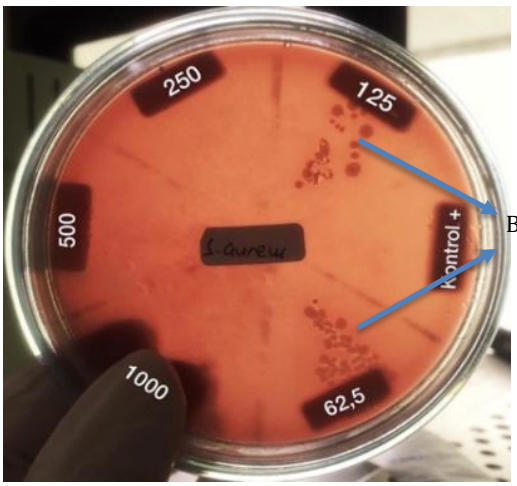

Figure 3. Result of MBC determination of areca nut seeds ethanol extract against *S. aureus* in concentration of 62,5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL and positive control

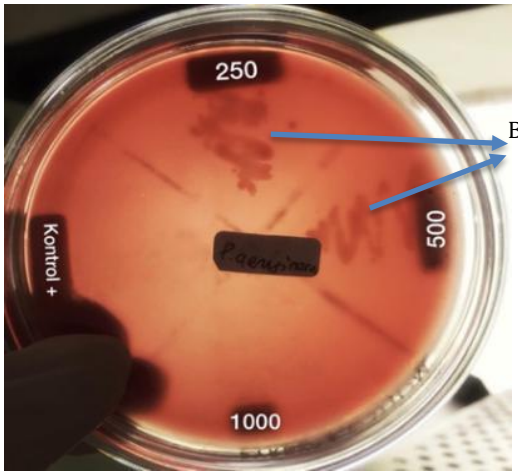

Figure 4. Result of MBC determination of areca nut seeds ethanol extract against *P. aeruginosa* in concentration of 62,5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL and positive control

The results of the MIC test of the extract against *S. aureus* (**Figure 1**), all tubes, namely 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL and positive control (ciprofloxacin), showed clarity in broth media. MIC cannot be determined in this test but it can be assumed that the MIC value was less than 62.5 µg/mL and categorized as having strong inhibition (MIC <100 µg/mL). All concentrations that showed clarity in the broth media were re-cultured on solid media to determine the MBC. The results of the MBC test showed the growth of bacterial colonies in the areas of 125 µg/mL and 62.5 µg/mL. The areas of 1000 µg/mL, 500 µg/mL and 250 µg/mL showed no bacterial colony growth so that the concentration of 250 µg/mL was determined as the MBC (**Figure 3**).

The results of the MIC test of the extract against *P. aeruginosa* (**Figure 2**), tubes of 1000 µg/mL, 500 µg/mL, 250 µg/mL and positive control (ciprofloxacin) showed clarity in broth media. The smallest concentration that showed clarity in broth media was 250 µg/mL and was determined as the MIC. The antibacterial activity of areca nut seeds ethanol extract was categorized as moderate with a value of 100 <MIC < 62.5 µg/mL. All concentrations that showed clarity in the broth media were re-cultured on solid media to determine the MBC. The results of the MBC test showed the growth of bacterial colonies in the areas of 250 µg/mL and 500 µg/mL. The area of 1000 µg/mL was the only concentration that showed no bacterial colony growth and was determined as the MBC (**Figure 4**).

The clear BHIB media in the test tube is caused by the presence of antibacterial substances that inhibit bacterial growth, while the cloudy BHIB media in the test tube is caused by the absence of antibacterial substances that cause bacteria to remain alive and distributed in the broth media. The results of the MIC and MBC tests of the ethanol extract of Areca nut seeds against *S. aureus* and *P. aeruginosa* are presented as a whole in **Table 3**.

DISCUSSION

The results of the MIC and MBC tests in this study showed that the areca nut seeds ethanol extract had greater antibacterial activity against *S. aureus* (Gram-positive) compared to *P. aeruginosa* (Gram-negative). This may be due to differences in Gram-positive and Gram-negative bacteria cell wall structure. According to (14), differences in bacterial cell wall structure determine the binding, penetration and activity of antibacterial compounds.

The cell wall of negative-Gram bacteria is more complex than positive-Gram. Negative-Gram cell walls are more complex, containing two distinct lipid membranes, the cytoplasmic and outer membranes, with a thin layer of peptidoglycans in between. The outer membrane works as an additional compound-selective barrier. It is highly permeable and contains lipopolysaccharides (LPS), the main lipid component, and a periplasmic space, where enzymes capable of degrading molecules introduced from the extracellular medium are present (15).

Various secondary metabolites found in the ethanol extract of areca nut seeds have antibacterial activity. The ethanol extract of areca nut seeds contains several bioactive compounds such as flavonoids, tannins, alkaloids, quinones, terpenoids and saponins as carried out in the previous phytochemical screening. These compounds can acts synergistically as antibacterials.

Luteolin and quercetin are examples of flavonoid compounds found in areca nuts. (16) reported that Luteolin inhibits the activity of DNA topoisomerase I and II, resulting in some reduction in nucleic acid and protein synthesis. (17) also reported that the bioactivity of quercetin is due to disruption of cell membranes and the formation of complexes with soluble proteins and extracellular proteins, resulting in protein inactivation.

Procyanidin and catechin are examples of tannin compounds present in betel nut. (18) reported that procyanidin significantly changed the morphology of bacteria. The cell wall and cell membrane were destroyed. The content of extracellular alkaline phosphatase, the conductivity of bacterial fluid, and the activities of Na^+/K^+-ATPase and Ca^{2+}-ATPase in the membrane system all increased. The activities of succinate dehydrogenase, malate dehydrogenase and adenosine triphosphatase (ATPase) all decreased in the energy metabolism system resulting in slowing metabolism and inhibition of bacterial growth. Changes in protein content and composition in bacteria indicate that the protein expression system will be affected. Procyanidin was also found to bind to DNA grooves to form a complex that resulted in the termination of bacterial growth. (19) also reported the bactericidal activity of catechin in the form of inhibition of bacterial toxin, inhibition of extracellular matrix, inhibition of plasmid transfer, inhibition of cell walls synthesis, cell membrane disruption, inhibition of membrane protein, inhibit several vital processes with involving enzyme such as DNA synthesis, fatty acid synthesis, and metabolism, DNA damage, iron chelation, modulation of gene expression.

Arecoline, arecaidine, guavacoline, guavasine, arecolidine are included in the type of pyridine alkaloids. (20) reported that the first stage of bacterial destruction by pyridine is the formation of holes caused by the alkyl chain followed by penetration of the entire molecule into the bacterial cell. Accumulation of pyridine compounds and transfer of oxidative chlorine (or other halide anions) to biological receptors that enhance the antibacterial effect. The hydrophobic region of pyridine begins to penetrate the hydrophobic bilayer of the membrane to cause membrane leakage resulting in the release of K^+ components from the cytoplasm, and ultimately cell death.

Quinone compounds will undergo a process of change, namely electron reduction, which changes quinone into half hydroquinone (semiquinone) and produces free radicals such as reactive oxygen species (ROS) and hydroxyl groups. These free radical products will later interfere with the development of bacterial cells through the genetic pathway and the resulting proteins. Hydroxyl groups can covalently bond with DNA to form DNA adducts or damage DNA, causing inhibition of the mitotic process of microbial cells, and the ROS produced, especially hydroxyl radicals, will bind irreversibly to lipids and proteins in microbial cells. This reaction can interfere with surface-exposed adhesins, cell wall polypeptides and membrane-bound enzymes in the bacterial cell membrane (21).

The mechanism of action can be attributed to the properties of cell-terpenoid adhesion and the calcium stress generated along the membrane (22). Terpenoids react

with porins (transmembrane proteins) on the outside of the bacterial cell wall, forming strong polymer bonds that result in protein damage (23). The mechanism of saponin as an antibacterial is that it can cause leakage of proteins and enzymes from within the cell. Saponin can be antibacterial because its surface-active substance is similar to a detergent, as a result saponin will reduce the surface tension of the bacterial cell wall and damage membrane permeability. Damage to this cell membrane greatly disrupts the survival of bacteria. Saponin diffuses through the outer membrane and vulnerable cell wall then binds to the cytoplasmic membrane, disrupting and reducing the stability of the cell membrane. This causes the cytoplasm to leak out of the cell resulting in cell death. Saponin is an antimicrobial compound that disrupts the cytoplasmic membrane and have bactericidal properties (24).

CONCLUSIONS

Determination of MIC and MBC values of ethanol extract of Areca nut seeds against *Staphylococcus aureus* and *Pseudomonas aeruginosa* has been done. The MIC and MBC values obtained indicate that ethanol extract of Areca nut seeds has antibacterial activity against both types of pathogenic bacteria from Gram-positive and Gram-negative. Further research suggested is isolation of chemical compounds in areca nut seeds and antibacterial activity test of the isolate against both types of Gram bacteria to test the reliability of single isolate compounds.

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REFERENCES

1. Celimuge XU. Research progress in chemical composition, pharmacology and toxicity of Areca Semen and prediction and analysis of its quality markers. NATURAL PRODUCT RESEARCH AND DEVELOPMENT. 2023;35(8):1431.
2. Cao M, Yuan H, Daniyal M, Yu H, Xie Q, Liu Y, et al. Two new alkaloids isolated from traditional Chinese medicine Binglang the fruit of Areca catechu. Fitoterapia. 2019;138:104276.
3. Liu PF, Chang YF. The Controversial Roles of Areca Nut: Medicine or Toxin? Int J Mol Sci. 2023;24(10):8996.
4. Oliveira NG, Ramos DL, Dinis-Oliveira RJ. Genetic toxicology and toxicokinetics of arecoline and related areca nut compounds: an updated review. Arch Toxicol. 2021;95(2):375–93.
5. Sari LM, Hakim RF, Mubarak Z, Andriyanto A. Analysis of phenolic compounds and immunomodulatory activity of areca nut extract from Aceh, Indonesia, against Staphylococcus aureus infection in Sprague-Dawley rats. Vet World. 2020;13(1):134.
6. Xing JH, Guan D, Zhu XY. Determination of polyphenol in areca extract. Chem Res Toxicol. 2019;30(1):38–41.
7. Raju A, De Subrata S, Ray MK, Degani MS. Antituberculosis activity of polyphenols of Areca catechu. The International Journal of Mycobacteriology. 2021;10(1):13–8.
8. Cheung GYC, Bae JS, Otto M. Pathogenicity and virulence of Staphylococcus aureus. Virulence. 2021;12(1):547–69.
9. Pratiwi ME, Tobi CHB. Formulasi dan Evaluasi Edible Film dari Ekstrak Terpurifikasi Daun Beluntas (Pluchea indica L.) Sebagai Anti-Sariawan. MPI (Media Pharmaceutica Indonesiana). 2023;5(2):178–87.
10. Wood SJ, Kuzel TM, Shafikhani SH. Pseudomonas aeruginosa: infections, animal modeling, and therapeutics. Cells. 2023;12(1):199.
11. Tobi CHB, Saptarini O, Rahmawati I. Aktivitas antibiofilm ekstrak dan fraksi-fraksi biji pinang (Areca catechu L.)

terhadap Staphylococcus aureus ATCC 25923. J Pharm Sci. 2022;1:57.
12. Tobi CHB, Pratiwi ME. Identifikasi Senyawa Flavonoid dan Uji Aktivitas Antibakteri Ekstrak Terpurifikasi Daun Beluntas (Pluchea Indica L.) terhadap Staphylococcus aureus: Qualitative-Quantitative Test of Flavonoid Compound and Antibacterial Activity of Beluntas Leaves Purified Extract against Staphylococcus aureus. Jurnal Sains dan Kesehatan. 2023;5(5):766–76.
13. Dewi FI, Wahyunitisari MR. Journal of Vocational Health Studies www.e-journal.unair.ac.id/index.php/JVHS Journal of Vocational Health Studies. 2018;01:2580–717.
14. Fisher JF, Mobashery S. Constructing and deconstructing the bacterial cell wall. Protein science. 2020;29(3):629–46.
15. Tavares TD, Antunes JC, Padrão J, Ribeiro AI, Zille A, Amorim MTP, et al. Activity of specialized biomolecules against gram-positive and gram-negative bacteria. Antibiotics. 2020;9(6):314.
16. Qian W, Liu M, Fu Y, Zhang J, Liu W, Li J, et al. Antimicrobial mechanism of luteolin against Staphylococcus aureus and Listeria monocytogenes and its antibiofilm properties. Microb Pathog. 2020;142:104056.
17. Nguyen TLA, Bhattacharya D. Antimicrobial activity of quercetin: an approach to its mechanistic principle. Molecules. 2022;27(8):2494.
18. Mattos MMG, Filho SA, Martins GR, Venturi LS, Canetti VB, Ferreira FA, et al. Antimicrobial and antibiofilm properties of procyanidins: potential for clinical and biotechnological applications. Crit Rev Microbiol. 2024;1–24.
19. Renzetti A, Betts JW, Fukumoto K, Rutherford RN. Antibacterial green tea catechins from a molecular perspective: Mechanisms of action and structure–activity relationships. Food Funct. 2020;11(11):9370–96.
20. De S, SK AK, Shah SK, Kazi S, Sarkar N, Banerjee S, et al. Pyridine: the scaffolds with significant clinical diversity. RSC Adv. 2022;12(24):15385–406.
21. Sahoo PMS, Behera S, Behura R, Acharya A, Biswal D, Suna SK, et al. A brief review: antibacterial activity of quinone derivatives. Biointerface Res Appl Chem. 2022;12:3247–58.
22. Lahiri D, Dash S, Dutta R, Nag M. Elucidating the effect of anti-biofilm activity of bioactive compounds extracted from plants. J Biosci. 2019;44(2):52.
23. Guimarães AC, Meireles LM, Lemos MF, Guimarães MCC, Endringer DC, Fronza M, et al. Antibacterial activity of terpenes and terpenoids present in essential oils. Molecules. 2019;24(13):2471.
24. Dong S, Yang X, Zhao L, Zhang F, Hou Z, Xue P. Antibacterial activity and mechanism of action saponins from Chenopodium quinoa Willd. husks against foodborne pathogenic bacteria. Ind Crops Prod. 2020;149:112350.