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ORIGINAL ARTICLE

Antibiofilm Activity of Areca Nut (*Areca catechu* L.) Extract and Fractions against *Escherichia coli* ATCC 25922

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K E Y W O R D S

Areca catechu L.;
Biofilm;
Escherichia coli

A B S T R A C T

Escherichia coli is one of the pathogenic bacteria that is resistant to antibiotics. Antibiotic resistance does not only occur due to antibiotics failing, but changes in the bacteria themselves also play an important role. Biofilm formation is another effective way for bacteria to survive in the presence of antibiotics. One natural source that has the potential to address biofilm-related problems is Areca nut (*Areca catechu* L.). This study aimed to determine the anti-biofilm activity of areca nut seed by inhibiting and degrading biofilm parameters. The study procedures were extraction and fractionation, sample test and bacteria test preparation, bacteria test identification, optimization of bacteria test biofilm formation time, and inhibition-degradation biofilm growth test. The anti-biofilm activity test was conducted using the crystal violet staining method. Bacterium identification showed that the bacteria used were *E. coli*, a Gram-negative bacterium through the metallic sheen on the Endo agar media, no black sediment in SIM media, yellow colour in KIA media, and no black precipitation in LIA media. The optimal biofilm formation time of *E. coli* was 3 days at 595 nm wavelength. The greatest inhibition of biofilm activity was shown in the ethanol extract of Areca nut, with the IC_{50} value of 2.96 mg ml^{-1}. The greatest degradation of biofilm activity was shown in the water fraction of Areca Nut, with the IC50 value of 2.25 mg ml^{-1}. The extract and fraction of Areca nut have anti-biofilm activity that can be developed as an alternative source to prevent antibiotic resistance caused by biofilm bacteria.

Introduction

The long history of antibiotic use and antibiotic resistance cases began with the report of resistance cases in 1924. The first bacterium reported to be resistant was *Treponema pallidum*, which was resistant to the antibiotic arsphenamine (Li *et al*., 2023). Currently, the WHO antimicrobial resistance division has reported 15 families of bacteria that have become resistant to antibiotics. One of the bacteria that is included in the bacterial priority pathogens list is *Escherichia coli* (*E. coli*) (WHO, 2024; Ero-Omoighe *et al*., 2026). Based on its resistance level, *E. coli* is categorized as media (11-20%) and media-high (21-30%) level. Based on the number of incidents, reported cases of *E. coli* resistance categorized as media-high (5001-10,000 cases) and

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high (> 10,000 cases) per 1 million population (WHO, 2024).

Cases of antibiotic resistance do not only occur due to antibiotic failures, but changes in the bacteria themselves also play an important role. Biofilm formation is another effective way for bacteria to survive in the presence of antibiotics (Qian *et al*., 2022). Research of 60 bacterial isolates found a positive relationship between biofilm formation and antibiotic resistance (Donadu *et al*., 2021). *E. coli* biofilm, being the major causative agent for recurrent urinary tract infections, biofilm of *E. coli* is also responsible for indwelling medical device-related infectivity (Ballén *et al*., 2022).

Objectives

This study specifically aimed to measure the antibiofilm potential of areca nut extract and the fraction of one of the pathogenic bacteria that can form biofilms, *E. coli*. In this study, antibiofilm activity will be measured based on biofilm inhibition and degradation parameters.

Materials and Methods

Materials

The materials used in this study were fresh areca nut (*Areca catechu* L.), which were air-dried into simplicia. The materials used for extraction and fractionation were ethanol p.a 96% (Brataco®), ethyl acetate (Merck®), n-hexane (Merck®), and water. Other materials used were, crystal violet (Merck®), DMSO (Merck®), ciprofloxacin (KalbeMed), *E. coli* ATCC 25922 (bacteria obtained from the Microbiology Laboratory of the Faculty of Pharmacy, Sebelas Maret University, Surakarta) *Staphylococcus epidermidis* ATCC 12228, Nutrient Agar (NA) media (Merck®), Endo Agar media (Merck®), Citrate Agar media (Simmons), SIM (Motile Indole Sulphide) (HIMEDIA), KIA (Kligler Iron Agar) (HIMEDIA), and LIA (Lysine Iron Agar) (HIMEDIA).

Equipment used in this study were, beakers, measuring cups, rotary evaporators, glass stirrers, filter paper, glass containers, separating funnels, test tubes, Bunsen, 96-well polystyrene round-bottom microplates (Iwaki), 96-well polystyrene flat-bottom microplates (Iwaki), incubators (Memmert), micropipettes (Socorex), yellow tips, blue tips, round ose, ovens, Laminar air flow (LAF), UV lamps, and i-Mark- Biorad Microplate Reader.

Methods

Extractions and fractionation

Extraction was carried out using the maceration method using 96% ethanol solvent with a sample and solvent ratio of 1:10. The fractionation method is a liquid-liquid extraction of the ethanol extract of areca nut seeds using n-hexane, ethyl acetate, and water solvents. Ethanol extract of areca nut seeds, as much as 20 grams, was dissolved slightly with ethanol, then partitioned with 50 ml of water and 50 ml of n-hexane solvent into a separating funnel, which was repeated 3 times (Tobi *et al*., 2022).

Test Solution and bacteria suspension preparation

The test controls used were extracts and fractions. The concentrations made were 0.4 mg ml^{-1}, 0.6 mg ml^{-1}, 0.8 mg ml^{-1}, and 1 mg ml^{-1} using DMSO solvent.

The bacterial suspension was prepared by rejuvenating the pure culture of the test bacteria. After that, the preparation of a 0.5 McFarland standard solution was conducted. The preparation of bacterial suspension was done by taking ± 2 sterile loops of test bacteria *E. coli* from pure bacterial cultures, then planted in a tube containing 5 ml of Brain Heart Infusion (BHI) media, homogenized using vortex. After that, it is equalized with McFarland's standard solution. After that, 0.1 ml is taken and put into 100 ml of BHI media. The bacterial suspension that has been made has a dilution ratio of 1:1000.

Identification of test bacteria E. coli ATCC 25922

E. coli was identified by scratching or streaking on Endo agar media. Then, it was incubated at 37°C for 24 hours. Positive results if a metallic sheen was formed.

Biochemical identification of *E. coli* using the Citrate, SIM (Sulphide Indole Motile), KIA (Kligler's Iron Agar), and LIA (Lysine Iron Agar) tests. The test was done by inoculating the *E. coli* culture vertically with a puncture on the Citrate, SIM, KIA, and LIA media. Then, it was incubated at 37 °C for 48 hours. Testing on Citrate media: if there was a change in the media from green to blue, it indicated positive citrate. Testing on SIM Sulphide media was positive if a black precipitate formed, Indole was positive if a red ring formed after the addition of Kovac's reagent, and Motile was positive if there was turbidity, like a white membrane, on the inoculation puncture. The KIA test was positive if there was a change in the colour of the media from red to yellow in the puncture area. The LIA test was positive if no black precipitation formed.

Optimization of E. coli biofilm formation time

Optimization of *E. coli* biofilm formation time using procedures from research by (Tobi *et al*., 2022).

Anti-biofilm activity test

Inhibition and degradation of biofilm formation in this study were tested in vitro. The controls in this test were a positive control (bacteria + media + ciprofloxacin), a negative control (bacteria and media), and the test control (bacteria + media + extracts and fractions).

Inhibiting parameter

Liquid BHI media was added as much as 70 µL, bacterial suspension as much as 70 µL, and test solution as much as 70 µL in each well, except for the negative control well. The solutions were incubated for a predetermined time. After incubation, the contents of the microplate were removed and washed with running water. The test solutions were dried for 15 minutes by inverting the microplate at room temperature. A total of 200 µL of 1% crystal violet solution was added to each well and incubated for 15 minutes at room temperature. After incubation, the contents of the well were discarded, and the wells were washed again with clean water and allowed to dry at room temperature. After the microplate was dried, 200 µL of 96% ethanol was added to each well of the microplate and incubated for 15 minutes at room temperature. Then, it was measured using a microplate reader at optical density with a wavelength that was the result of optimization. Each of these tests was done 3 times. The percentage of biofilm inhibition can be calculated using the following formula:

$$\%\text{Inhibion} = \frac{\text{OD Negative Control} - \text{OD Test Control)}}{\text{OD Negative Control}} \times 100$$

*Note: OD (Optical Density)

After obtaining the average percentage of biofilm formation inhibition from each extract concentration and fractions, it is continued by determining the IC50 value using a linear regression line equation between the percentage of biofilm formation inhibition and the concentration of fractions. The regression was used to see the relationship between concentration and the percentage of inhibition of biofilm growth in inhibiting 50% of biofilm.

Degradation parameter

70 µL of media was put into each well, and then 70 µL of bacterial suspension in BHI was added, which was equivalent to $1.5x10^8$ CFU mL^{-1}. The microplate was then incubated at a temperature of ± 37°C for a predetermined time. After that, the contents of the well were removed, and the wells were washed with running water. Each well was filled with 70 µL of the sample. The microplate was re-incubated at a temperature of ± 37°C for 90 minutes. After incubation, the contents of the microplate were

removed and washed with running water, then dried for 15 minutes by inverting the microplate at room temperature.

A total of 200 µL of 1% crystal violet solution was added to each well and incubated for 15 minutes at room temperature. After incubation, the contents of the well were discarded, and the wells were washed again with clean water and allowed to dry at room temperature. After the microplate was dried, 200 µL of 96% ethanol was added to each well of the microplate and incubated for 15 minutes at room temperature. The microplate was then measured using a microplate reader at a predetermined wavelength.

The percentage of biofilm degradation can be calculated using the following formula:

$$\%\text{Degradation} = \frac{\text{OD Negative Control} - \text{OD Test Control)}}{\text{OD Negative Control}} \times 100$$

*Note: OD (Optical Density)

After obtaining the average percentage of biofilm degradation from each concentration of fractions, the IC50 value was determined using the linear regression line equation between the percentage of biofilm degradation and the concentration of fractions to see the relationship between concentration and the percentage of biofilm degradation in degrading 50% of the biofilm.

Data analysis

The results of the Optical Density OD value reading interpret the formation and degradation of biofilms. Data analysis using SPSS Statistics with a sig. p value <0.05. The data obtained, if normally distributed and homogeneous, then it were continued with the Two-Way ANOVA test to see the effects of comparison of several group test samples.

Results

Extraction is the process of separating dissolved components from insoluble components of a mixture with a suitable solvent. The results of making an ethanol extract of areca nut seeds can be seen in Table 1.

Table 1. Yield Percentation of Areca Nut Ethanol Extract

Simplicia (g)	Solvent (ml)	Extract	Yield (%)
500	5000	144.76	28.95

The extract yield value was expressed as a percentage (%). Based on the results of making areca nut seeds ethanol extract, it could be seen that the yield obtained was 28.95%. The comparison of the yield results of areca nut seed extract from the study conducted by (Poela & Hanafiah, 2014) was 63.879%. The study has a high yield value because the extraction method was carried out for 6 days, while in this study, the extraction process was only carried out for 24 hours.

The compounds contained in the extract will be separated according to their polarity (DiPiro *et al*., 2014). Fractionation in this study used three solvents that had different levels of polarity, namely polar, semi-polar, and nonpolar. The solvents used were water, ethyl acetate, and n-hexane. The results of fractionation can be seen in Table 2.

Table 2. Yield percentage of areca nut ethanol extract.

Sampel	Extract (g)	Fraction (g)		
		Water	Ethyl acetate	N-hexane
Areca nut	20	11.13	4.33	1.02

The fractionation results showed that the water fraction obtained was 11.13 grams, the ethyl acetate fraction was 4.33 grams, and the n-hexane fraction was 1.02 grams. The water fraction results obtained were more than the ethyl acetate fraction and the n-hexane fraction because most of the compounds

contained in the areca nut seed extract were polar. The total of the three fractions was 16.48 g or 82.4%. If calculated, the weight loss was 3.52 g or 17.6%. The loss of weight indicated that the fractionation process was not running optimally. This was caused by the fractionation process being carried out only once, so that the absorption of compounds by the fractions was reduced.

Identification testing with a specific medium using Endo Agar media. Identification results can be seen in Fig. 1.

Fig. 1. Result of *E. coli* colony identification.

Based on the results, it could be seen that there was a metallic sheen on the media. The basic fuchsin and sodium sulphite substances in Endo Agar media can prevent the growth of Gram-positive bacteria, so that only Gram-negative bacteria grow (Abu-Sini *et al*., 2023).

Biochemical test for identification of *E. coli* using Citrate test media (Simmon's Citrate Agar), SIM (Sulphide Indole Motil), KIA (Klingler Iron Agar), and LIA (Lysine Iron Agar). Based on the results, the isolate used was positive for *E. coli* ATCC 25922, which was indicated by a change in the colour of the media from green to blue. The second biochemical test used SIM (Sulphide Indole Motility) media. Based on the test results on SIM media, it did not produce black sediment, which means that *E. coli* ATCC 25922 did not produce H2S. The next biochemical test used was KIA (Klingler Iron Agar) media. The results indicated conformity with the theory, which was marked by a change in the colour of the KIA media from red to yellow (Al-Khafaji *et al*., 2024).

The next biochemical test used was the LIA (Lysine Iron Agar) media. The results of the biochemical test of *E. coli* ATCC 25922 bacteria on the LIA media showed no black precipitation. The results of the *E. coli* biochemical test could be seen in Fig. 2.

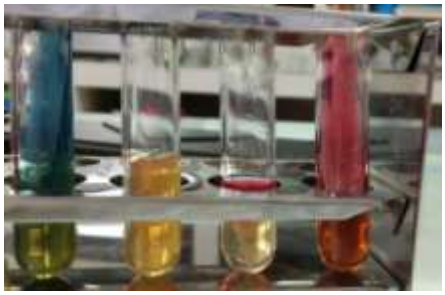

Fig. 2. Result of *E. coli* biochemistry identification.

Optimization of *E. coli* ATCC 25922 biofilm formation was carried out for 4 days using OD_{490}, OD_{595}, and OD_{655} wavelengths. The purpose of the optimization was to determine the optimal time for biofilm growth. The results of the optimization of *E. coli* ATCC 25922 biofilm formation could be seen in Table 3.

Table 3. Results of optimization of biofilm formation of *E. coli* ATCC 25922

Wavelength	Mean of DO ± SD			
	Day-1	Day-2	Day-3	Day-4
490 nm	0.048 ± 0.0017	- -	0.125 ± 0.0037	- -
595 nm	0.060 ± 0.0027	0.541 ± 0.1520	0.839 ± 0.0217	0.665 ± 0.0281
655 nm	0.053 ± 0.0038	- -	0.071 ± 0.0020	- -

Based on the optimization of the *E. coli* ATCC 25922 biofilm formation results in Table 3, it showed that the optimal time was the third day and the OD595 wavelength with an absorbance value of 0.839. The third day, with an absorbance value of 0.839, was considered the most optimal because on the fourth day, the absorbance value decreases. Furthermore, for testing the inhibition and degradation activity of *E. coli* ATCC 25922 biofilm, the optimal time and wavelength can be used. The media used was BHI media. This also showed that BHI media can be used for further testing the inhibition and degradation activity of *E. coli* ATCC 25922 biofilms. Brain Heart Infusion (BHI) media is a liquid medium containing carbohydrates and proteins, which are used as a fertilizer for bacterial growth. BHI media provides the nutrients needed by bacteria for biofilm formation. Optimization of biofilm formation of *E. coli* ATCC 25922 was incubated at the 37 ºC. *E. coli* is a Gram-negative bacterium that can grow at temperatures ranging from 10-40 ºC, with the most optimal temperature of 37ºC, so that the determination of treatment related to the incubation temperature of biofilm formation optimization was correct.

The inhibition test of *E. coli* ATCC 25922 bacterial biofilm growth was carried out based on the results of the optimization of the optimum time and wavelength for the growth of *E. coli* ATCC 25922 biofilms. The optimum incubation time was obtained for 3 days, and the wavelength of OD_{595}. The percentage results of the *E. coli* ATCC 25922 bacterial biofilm growth inhibition test could be seen in Fig. 3

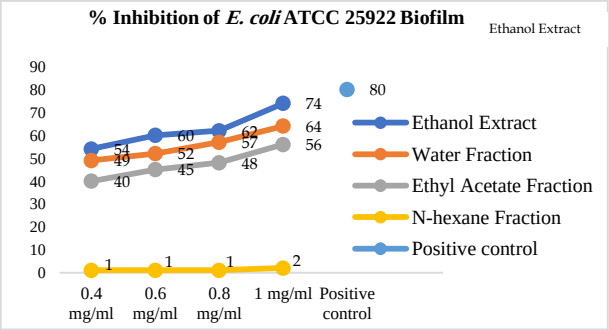

Fig. 3. Results of the biofilm inhibition activity of areca nut seed extracts and fractions against *Escherichia coli* ATCC 25922

The results of the IC_{50} value calculation for the inhibition of the growth of *E. coli* ATCC 25922 bacterial biofilm could be seen in Table 4.

Table 4. IC_{50} value of *E. coli* ATCC 25922 biofilm inhibition.

Sample	Equation Linear Regression	IC_{50} (mg ml^{-1})
Ethanol extract	50 = 3.1x+40.8	2.96^{bcd}
Water fraction	50 = 2.5x+38	4.8^{acd}
Ethyl acetate fraction	50 = 2.55x+29.4	8.07^{abd}
N-Hexane fraction	50 = 0.15x+0.3	331.33^{abc}

a = Significantly different from the ethanol extract group <0,05

b = Significantly different from the water fraction group <0,05

c = Significantly different from the ethyl acetate fraction group < 0,05

d = significantly different from the n-hexane fraction group < 0,05

The degradation test of *E. coli* ATCC 25922 bacterial biofilm growth was carried out based on the optimization result of time and wavelength for the growth of *E. coli* ATCC 25922 biofilms. The optimum incubation time was obtained for 3 days, and the wavelength of OD_{595}. The percentage results of the *E. coli* ATCC 25922 bacterial biofilm growth degradation test can be seen in Fig. 4.

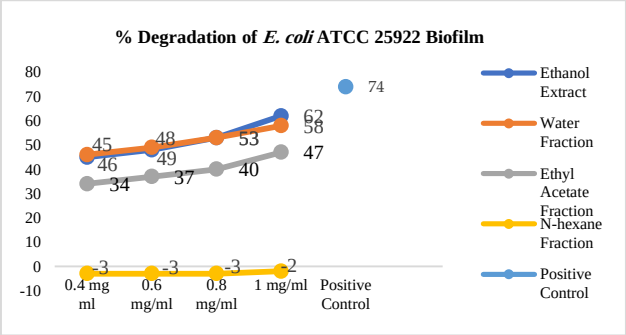

Fig. 4. Results of the biofilm degradation activity of areca nut seed extracts and fractions against *Escherichia coli* ATCC 25922

The results of the IC_{50} value calculation for the degradation of the growth of *E. coli* ATCC 25922 bacterial biofilm can be seen in Table 5.

Table 5. IC_{50} Value of *E. coli* ATCC 25922 Biofilm Degradation

Sample	Equation linear regretion	IC_{50} (mg ml^{-1})
Ethanol extract	50 = 2.8x+32.4	6.28^{cd}
Water fraction	50 = 2x+37.5	6.25^{cd}
Ethyl acetate fraction	50 = 2.1x+24.8	12^{abd}
N-Hexane fraction	50 = 0.15x+3.8	308^{abc}

a = Significantly different from the ethanol extract group <0,05

b = Significantly different from the water fraction group <0,05

c = Significantly different from the ethyl acetate fraction group < 0,05

d = significantly different from the n-hexane fraction group < 0,05

Discussion

One of the parameters of extract quality is the extract yield. The higher the yield value produced, the greater the value of the extract produced. The yield value produced can be influenced by several factors. One of the factors that affects the yield results is the contact time of the solvent with the powdered simplicial. Fractionation is a technique for separating and grouping the chemical compounds of an extract based

on their polarity or solubility using two or more immiscible solvents. The purpose of fractionation is to separate compounds based on the polarity of the solvent.

Bacteria identification is useful for making sure that the bacteria used are the right bacteria so that there are no errors or bias in the research results. On the colony test, the basic fuchsin substance in Endo Agar media is able to react with the *E. coli* ATCC 25922 isolate so that the fuchsin substance is absorbed and causes the colonies to have a metallic sheen. On the biochemical test, bacteria testing using the Simmon's Citrate Agar aims to determine the ability of bacteria to use citrate as the sole source of carbon. Simmon's Citrate Agar media contains Brom thymol blue as a colour indicator, Na citrate, and NH_4^+. The occurrence of a colour change from green to blue means that the *E. coli* ATCC 25922 isolate uses citrate as the sole source of carbon. The use of citrate as a carbon source by bacteria will cause the media to change to alkaline and change colour from green to blue (Abu-Sini *et al*., 2023). Indole was declared positive because a red ring was formed after the addition of Kovac's reagent. This is because *E. coli* is able to use the tryptophanase enzyme to form indole. The reagent will react with indole and produce a compound that is insoluble in water and is red on the surface of the media. Motility is characterized by turbidity at the puncture site due to bacterial growth; This indicates that the spread of the puncture site caused by bacterial movement. Testing on the KIA media aims to determine the occurrence of carbohydrate fermentation. The test results showed a change in the colour of the KIA media from red to yellow at the puncture site. This is because *E. coli* ATCC 25922 is able to ferment glucose and lactose. Testing with the LIA media aims to determine the deamination of lysine and the formation of sulphide. *E. coli* ATCC 25922 on the LIA (Lysine Iron Agar) media is known to be unable to produce hydrogen sulphide (H2S) from sodium thiosulfate (Al-Khafaji *et al*., 2024).

Biofilm is a collection of bacterial cells that are irreversibly attached to a surface and wrapped in an EPS (Extracellular Polymeric Substance) matrix. Biofilm has a microscopic structure so that it can only be seen with a special microscope. If the growth of this biofilm is fertile and fast, then the biofilm can be seen with the eye without any aids. Examples of visible biofilms are dental plaque and slimy layers on the surface of wounds. Some criteria for infections associated with biofilms are the adherence of a bacterium and increased resistance to antibiotics compared to bacterial infections in planktonic form (Flemming *et al*., 2023). *E. coli* bacteria can form many biofilms even in extreme conditions. The extreme conditions in question include reduced nutritional conditions, conditions outside the optimum pH, and adverse temperature (Wang *et al*., 2023). Biofilms formed from *E. coli* contain EPS, which functions to attach themselves and protect the bacterial cells. The EPS formed is more abundant and contains glucuronic acid and mannose (Das, 2022). The increase in the number of biofilm cells depends on the availability of media nutrients. Reduced nutrition can cause *E. coli* to adapt by rounding itself and shrinking its volume (dwarfing). When amino acids are synthesized, *E. coli* will use them for protein synthesis, producing purine and pyrimidine bases. Both bases can then be utilized by *E. coli* to form DNA and RNA. RNA helps ribosomes in the protein synthesis process, while DNA has two types, namely chromosomal DNA and extrachromosomal DNA (plasmids), which are useful when duplicated in the conjugation process. Plasmids do not regulate growth and metabolism but function as protection against antibiotics (antibiotic resistance), as well as the production of enzymes and toxins (Darby *et al*., 2023). In *E. coli*, there is a wcaF gene that regulates the release of acetyltransferase during the synthesis of cholanic acid, which will mature the biofilm (Mirani *et al*., 2021). *E. coli* is commonly found in the human large intestine as normal flora. *E. coli* is a bacterium that causes primary infections in the intestines and

other tissues outside the intestines, such as diarrheic in children and urinary tract infections. Biofilm control can be done in several ways, such as chemical, physical, and biological control. It can also be done by utilizing natural materials using chemical compounds from plants (Bonten *et al*., 2021).

The ability of areca nut seeds to inhibit and degrade *E. coli* ATCC 25922 biofilms is thought to be due to the presence of tannins and flavonoids. Research by (Neumann *et al*., 2022) stated that phytochemical compounds in plant extracts, in general, that act as antibiofilm are flavonoids and tannins. Flavonoids and tannins have the potential to reduce bacterial adhesion and can inhibit bacterial protein synthesis. According to (Xiao-Quan *et al*., 2025), flavonoids can suppress biofilm formation by inhibiting quorum sensing, and flavonoids can also inhibit the expression of the IsrACDBF, csgA, and csgB genes, which are regulators of fimbria formation in *E. coli* biofilms, thereby disrupting metabolism in the extracellular matrix and inhibiting nutrient transport.

Research by (Chevallier *et al*., 2023) significantly shows that tannin compounds can reduce biofilm metabolic activity. Tannins have the property of forming complexes with metal ions that can cause tannin compounds to be toxic to microbial membranes. In addition to flavonoids and tannins, alkaloids are also thought to play an important role in inhibiting and degrading biofilms. According to (Liu *et al*., 2023), Alkaloids can disrupt protein components in the extracellular matrix, where alkaloids contain nitrogen base groups that can react with amino acid compounds, resulting in changes in the structure and composition of amino acids that can cause changes in genetic balance, causing damage to the extracellular matrix of the biofilm. SIM media is a semi-solid medium.

Conclusions

Areca nut extract and fraction have anti-biofilm activity against bacteria that can form biofilms, namely *E. coli*. The anti-biofilm activity is shown from the parameters of inhibition and degradation of biofilm growth. The greatest anti-biofilm activity is shown by the ethanol extract and water fraction group.

Conflict of interest

The authors declare that there is no conflict of interest in this research

References

Abu-Sini MK, Maharmah RA, Abulebdah DH., Al-Sabi MNS (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.

Al-Khafaji NSK, Almjalawi BSA, Ewadh RMJ, Al-Dahmoshi, H. O. M., Abed, S. Y., Nasrolahi, A., Nwobodo, D. C., Kanaan, M. H. G., Abdullah, S. S., & Saki, M. (2024). Prevalence of plasmid-mediated quinolone resistance genes and biofilm formation in different species of quinolone-resistant clinical Shigella isolates: a cross-sectional study. European Journal of Medical Research. 29(1), 419.

Ballén V, Cepas V, Ratia C, Gabasa Y, Soto SM (2022) Clinical Escherichia coli: from biofilm formation to new antibiofilm strategies. Microorganisms. 10(6), 1103.

Bonten M, Johnson JR., van den Biggelaar AHJ, Georgalis L, Geurtsen J, de Palacios PI, Gravenstein S, Verstraeten T, Hermans P, Poolman JT (2021) Epidemiology of Escherichia coli bacteremia: a systematic literature review. Clinical Infectious Diseases. 72(7), 1211–1219.

Chevallier P, Wiggers HJ, Copes F, Zorzi Bueno C, Mantovani D (2023) Prolonged antibacterial activity in tannic acid–iron complexed chitosan films for medical device applications. Nanomaterials. 13(3), 484.

Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, Webber MA, Blair JMA (2023) Molecular mechanisms of antibiotic resistance revisited. Nature Reviews Microbiology. 21(5), 280–295.

Das S (2022) Genetic regulation, biosynthesis and applications of extracellular polysaccharides of the biofilm matrix of bacteria. Carbohydrate Polymers. 291, 119536.

DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM (2014) Pharmacotherapy: a pathophysiologic approach.

Donadu MG, Mazzarello V, Cappuccinelli P, Zanetti S, Madléna M, Nagy ÁL, Stájer A, Burián K, Gajdács M (2021) Relationship between the biofilm-forming capacity and antimicrobial resistance in clinical acinetobacter baumannii isolates: results from a laboratory-based in vitro study. Microorganisms. 9(11), 2384.

Ero-Omoighe DE, Ngwoke UN, Igiebor FA, Edegbai BO, Ikhajiagbe B (2026) Anion-mediated modifications in rhizospheric bacterial composition and morphological plasticity of *Celosia argentea* L. in sodic-saline soil conditions. International Journal of Horticultural Science and Technology. 13(3), 445-466.

Flemming HC, van Hullebusch ED, Neu TR, Nielsen PH, Seviour T, Stoodley P, Wingender J, Wuertz S (2023) The biofilm matrix: multitasking in a shared space. Nature Reviews Microbiology. 21(2), 70–86.

Li T, Wang Z, Guo J, de la Fuente-Nunez C, Wang J, Han B, Tao H, Liu J, Wang X (2023) Bacterial resistance to antibacterial agents: Mechanisms, control strategies, and implications for global health. Science of the Total Environment. 860, 160461.

Liu S., Zhang T, Li Z, Wang Y, Liu L, Song Z (2023) Antibacterial mechanism of areca nut essential oils against Streptococcus mutans by targeting the biofilm and the cell membrane. Frontiers in Cellular and Infection Microbiology. 13, 1140689.

Mirani ZA, Urooj S, Ullah A, Khan MN, Rauf N, Mehmood A, Fenghuan W, Shaikh IA, Khan AB(2021) Phenotypic heterogeneity in biofilm consortia of *E. coli.* Microbiology. 90, 237–246.

Neumann N, Honke M, Povydysh M, Guenther S, Schulze C (2022) Evaluating tannins and flavonoids from traditionally used medicinal plants with biofilm inhibitory effects against MRGN *E. coli*. Molecules. 27(7), 2284.

Poela S, Hanafiah A (2014) Uji Aktivitas Anti Radang Ekstrak Etanol Biji Pinang (*Areca catechu* L.) Pada Tikus Putih Jantan. Indonesian Journal of Pharmaceutical Science and Technology. 3(1). 10.58327/jstfi.v3i1.32

Qian W, Li X, Yang M, Liu C, Kong Y, Li Y, Wang T, Zhang Q (2022) Relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance in *Escherichia coli* isolates from Ningbo, China. Infection and Drug Resistance. 2865–2878.

Tobi CHB, Saptarini O, Rahmawati I (2022) Aktivitas antibiofilm ekstrak dan fraksi-fraksi biji pinang (*Areca catechu* L.) terhadap Staphylococcus aureus ATCC 25923. Journal of Pharmaceutical Sciences. 1, 57.

Wang S, Zhao Y, Breslawec AP, Liang T, Deng Z, Kuperman LL, Yu Q (2023) Strategy to combat biofilms: a focus on biofilm dispersal enzymes. Npj Biofilms and Microbiomes. 9(1), 63.

WHO (2024) Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. World Health Organization: Geneva, Switzerland, 24.

Xiao-Quan Y, Jian-Ting H, Han-Zhong F, Jun H, Zhi-Ping W, He YX (2025) The flavonoid-sensing regulator AefR is involved in modulating quorum sensing through repressing the MexEF-OprN efflux pump in *Pseudomonas fluorescens*. MSystems. e00915-24.