COMPARISON OF ANTIBIOFILM ACTIVITIES OF GREEN COFFEE BEANS (COFFEA CANEPHORA P.) AND ROASTED ROBUSTA COFFEE (COFFEA CANEPHORA L.) AGAINST STAPHYLOCOCCUS AUREUS ATCC 25923

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ABSTRACT

Biofilm is a collection of microbial cells that are irreversibly attached to a surface and encased in an EPS matrix (Extracellular Polymeric Substances). One of the infectious bacteria that produce biofilms is Staphylococcus aureus. Coffee contains compounds that are responsible for antibacterial activity, including alkaloids, flavonoids, phenolics, terpenoids/steroids and saponins among others. The purpose of this study was to determine the activity of the antibiofilm between Green Coffee (Coffea canephora P.) and Robusta Coffee (Coffea canephora L.) against Staphylococcus aureus bacteria. Extraction of green coffee beans and robusta coffee beans was carried out by maceration method, fractionation was carried out by liquid-liquid extraction method using water, ethyl acetate and n-hexane as solvents. Inhibitory activity and biofilm degradation were carried out using the crystal violet staining method which was read at a wavelength of 595 nm. Obtained inhibition and degradation were analyzed using the ANOVA statistical test. The method for testing anti-biofilm activity was determined by testing the inhibition of biofilm formation and biofilm degradation using extracts and fractions robusta green coffee bean (Coffea canephora P.) and roasted robusta coffee beans (Coffea canephora L.) at various concentrations of 2, 4, 8 and 16 mg/ml. Robusta roasted coffee bean extract has the greatest anti-biofilm inhibition effectiveness with IC50 biofilm inhibition of 2.13 ppm. The ethyl acetate fraction from roasted robusta coffee beans has the greatest effectiveness in destroying (degrading) anti-biofilms with the EC50 biofilm degradation of 1.93 ppm.

Keywords: antibiofilm; green coffee bean; robusta coffee; staphylococcus aureus ATCC 25923

INTRODUCTION

Increasing resistance to antimicrobials is a challenge because of the high mortality and morbidity rates in the treatment of infections. Biofilm formation one of the factor responsible for drug resistance in many pathogens especially Gram-positive bacteria. Bacterial biofilms can pose health risks in clinical settings, the food industry and drinking water systems (Mohammadi-Bazargani et al., 2017). The formation of biofilms and cell wrapping in a polymer-based matrix can reduce susceptibility to antimicrobials and immune defenses, making the problem of infection difficult to overcome. The spread of cells from the biofilm can spread to secondary sites that can exacerbate the infection (Lister & Horswill, 2014) One of the common bacteria that causes biofilm infection is Staphylococcus aureus (Patsilinakos et al., 2019) Staphylococcus aureus can cause various inflammatory diseases, and its biofilm formation is closely related to chronic infection and antibiotic resistance (Ahn et al., 2018). Staphylococcus aureus is able to attach and form biofilms, which can be largely regulated by the production of polysaccharide intercellular adhesin (PIA). PIA plays a key role in cell interactions or quorum sensing (QS) (Gowrishankar et al , 2016)

Drug development to see anti-biofilm activity is considered important as a strategy to avoid the emergence of drug resistance (Fair & Tor, 2014). Biofilm growth can be inhibited by using antimicrobials which have the ability to pass through the biofilm matrix (Roy et al., 2018). Coffee

has been used empirically as a treatment for diabetic ulcers by the community. In historical records, coffee is used as an ancient herb to treat wounds because coffee contains compounds that are responsible for antibacterial activity, including caffeine, trigonellin, glyoxal, methylglycosal, and chlorogenic acid. Robusta and Arabica coffees are the most widely used coffee species. The difference between the two is in the content of bioactive compounds which are responsible for the antibacterial activity. Previous studies have carried out antibacterial tests of the ethanol extract from robusta coffee beans (Coffea canephora L.) against Staphylococcus epidermidis bacteria and showed that the ethanol extract from robusta coffee beans has therapeutic potential for infection prevention. This was proven by being able to inhibit the growth of Staphylococcus epidermidis ATCC 12228 at concentrations of 50% and 100% with an average diameter of the inhibition zone of 6.8 mm to 9 mm. Green coffee extract (Coffea canephora P.) was chosen based on research conducted (Kiattisin et al., 2016) which proved that green coffee extract has higher levels of antioxidants compared to coffee that has gone through the roasting process (roasted). This study aims to determine the anti-biofilm activity between green coffee beans and robusta coffee against Staphylococcus aureus and to determine the most active extraction showing anti-biofilm activity against Staphylococcus aureus.

METHOD

Tools and materials

The tools used in the research are Microplate flat-bottom 96 wells, autoclave, incubator, freezer, imark-Biorad Microplate Reader, rotary evaporator, moisture balance, waterbath, vacuum, toolvortex, micropipettes, analytical balance, evaporator cup, ose needle, bunsen, measuring cup (herma), erlenmeyer glass beaker (herma), test tube (iwaki). The plant materials used in the research are green coffee beans (Coffea canephora P.) and roasted Robusta coffee beans (Coffea canephora L.) dried. Bacteria Test used is Staphylococcus aureus ATCC 25923 bacteria taken from the Microbiology Laboratory of Setia Budi University. The material used in the extraction is 96% ethanol (Brataco). The solvent used for fractionation is ethyl acetate, nhexane, water, sterile aqua distillate. Other materials such as Media Nutrient Agar (NA), Brain Heart Infusion (BHI), crystal violet (1%), 25% ammonia, chloroform, Mayer's reagent, Dragondorf's reagent, distilled water, Mg powder, concentrated HCl, FeCl3 1%, H2SO4 concentrated, acetic acid anhydrous, lugol, safranin, solution Mc. Farland and 10% DMSO.

Extraction and fractionation

Extracts of green coffee beans and roasted robusta coffee beans are obtained by blending the green beans and roasted robusta coffee beans separately until they are small flakes and then ground to produce a finer texture. Furthermore, green seed powder and roasted robusta coffee were weighed and macerated for 5x24 hours with 2 liters of 96% ethanol solution while occasionally stirring. Then the maserate is filtered and evaporated withrotary evaporator at 50°C until a thick extract is formed. Furthermore, the thick extract was concentrated abovewaterbathuntil all the ethanol solvent evaporates to obtain a concentrated extract (Kiattisin et al., 2016). Fractionation using liquid-liquid extraction method of 96% ethanol extract of green coffee beans and roasted robusta coffee beans using several solvents, namely n-hexane, ethyl acetate, and water. 10 g ethanolic extract of two coffee bean that was taken then dissolved slightly in hot water, partitioned with 50 ml of water and 50 ml of n-hexane solvent into a separatory funnel and repeated 3 times. The n-hexane fraction is the filtrate located above and the water

fraction is the filtrate located below. The n-hexane fraction was separated from the water fraction and collected and concentrated using a rotary evaporator at a temperature of 50oC. The remaining water fraction from the n-hexane fraction was then fractionated again with 50 ml of ethyl acetate using a separatory funnel. This process was repeated 3 times. The ethyl acetate fraction is the filtrate which is located above and the water fraction is located below. The ethyl acetate fraction was separated from the water fraction and then concentrated using a rotary evaporator at a temperature of 500C. The remaining filtrate fractionated with ethyl acetate is the water fraction, which is then thickened with a water bath until thick.

Identification of chemical content of extracts and fractions

Identification of the chemical content of extracts and fractions of green coffee bean and roasted robusta bean was carried out using the methods in the book Phytochemical Methods: A Guide to Modern Methods of Analyzing Plants (Kiattisin et al., 2016).

Preparation of test sample and identification of bacteria

The experiment group were extracts, n-hexane fraction, ethyl acetate and water with various concentrations of 2, 4, 8 and 16 mg/ml. Preparation of the test solution begins with weighing each sample and then dissolving each in DMSO 10% to 10 ml and taking the volume required for the test. The negative control is Staphylococcus aureus ATCC 25923 bacteria which has been standardized with turbidity Mc. Farland 0.5. Meanwhile, the positive control in this study was Listerine Solution.

Identification Staphylococcus aureus ATCC 25923 bacterial

Characterization Staphylococcus aureus ATCC 25923 bacteria was done by means of Gram staining. Gram staining was carried out with the aim of identifying bacterial isolates to be used in research. Bacterial characteristics were carried out by observing colonies by making preparations. Preparations can be seen under a microscope (Mulyadi, 2011). The result is Staphylococcus aureus ATCC 25923 bacteria Classified as Gram Positive bacteria with purple cell color.

Optimization of Biofilm Formation Time Staphylococcus aureus ATCC 25923

Optimization were carried out using microtitier plate flat-bottom polystyrene 96 wells by inserting 500 µl of bacterial suspension into each well. Biofilm optimization aims to obtain optimal incubation time in forming biofilms. Variation of incubation time were 1,2,3 days. After the incubation period microplate washed using running water then added 500 µl of 1% crystal violet solution to each well and incubated at room temperature for another 15 minutes microplate washed again using running water and added 96% ethanol solution as much as 500 µl put into each well and incubated at room temperature for 15 minutes. The last optimization step is to read the biofilm growth at 595 nm absorbance. Optimal incubation time is used for negative control in growth inhibition and biofilm destruction tests Staphylococcus aureus ATCC 25923 (Sandasi et al., 2010)

Biofilm Inhibitory Activity Test Staphylococcus aureus ATCC 25923

The objective of the biofilm growth inhibition test was to obtain the activity of green coffee beans and roasted robusta coffee beans in inhibiting biofilm growth Staphylococcus aureus. Biofilm

inhibition testing was carried out by adding green coffee bean extract and roasted robusta coffee meanwhile at the same time into each well 500 µl of BHI media was added, 500 µl of tested bacterial suspension and 500 µl of green coffee bean extract and roasted robusta coffee with various concentrations of 2, 4, 8, 16 mg/ml then incubated at the optimum time at 37OC next microplate washed with running water three times then added 500 µl of 1% crystal violet solution to each well incubated at room temperature for 15 minutes, the next step Microplate washed again using running water 3 times plus 96% ethanol solution as much as 500 µl was added to each well and incubated at room temperature for 15 minutes. Furthermore, biofilm growth readings were carried out at an absorbance of 595 nm using a tool iMark-Biorad Microplate Reader. The biofilm inhibition test was replicated 3 times (Sandasi et al., 2010). Positive control using Listerin. The IC50 value of Staphylococcus aureus was determined from the linear regression equation between sample concentration and the percentage of biofilm inhibition.

$$\%\ Biofilm\ inhibition = \frac{\text{OD Negative control} - \text{OD Positive control}}{\text{OD Negative control}}\ x\ 100\ \%$$

Information: OD (Optical Density)

Biofilm Destruction Activity TestStaphylococcus aureus ATCC 25923

The purpose of the biofilm destruction test was to obtain the activity of green coffee bean extract and roasted robusta coffee in destroying Staphylococcus aureus ATCC 25923 biofilm. This test was carried out in the same way as the biofilm growth inhibition test, except that green coffee bean extract and roasted robusta coffee were added to the biofilm that had formed. Biofilms form after each wells incubated for the optimal time with a temperature of 37OC with the amount of test bacterial suspension of 500 µl of BHI media after the formation of biofilms, the test bacterial suspension in microplate removed, then added 500 µl of green coffee bean extract and roasted robusta coffee with various concentrations of 2, 4, 8, 16 mg/ml then incubated at room temperature for the optimum time, then microplate washed with running water and added 96% ethanol solution as much as 500 µl was added to each well and incubated at room temperature for 15 minutes. Then read the biofilm growth at 595 nm absorbance using a tool iMark-Biorad Microplate Reader (Sandasi et al., 2010). Positive control using Listerin.

$$\%\ Biofilm\ destruction = \frac{\text{OD Negative control} - \text{OD sample control}}{\text{OD Negative control}}\ x\ 100\ \%$$

Information: OD (Optical Density)

The Staphylococcus aureus biofilm-destroying activity test was expressed by the parameter EC50 (Effective Concentration), namely the concentration of the test compound that destroyed the biofilm by 50%. The EC50 value was determined from the linear regression equation between sample concentration and the percentage of biofilm destruction.

Data analysis

The reading results are absorbance values that describe the quantity of biofilm formation and biofilm degradation. The research data obtained were in the form of quantitative data which were analyzed using IBM SPSS Statistics ver. software. 22.

RESULTS AND DISCUSSION

Results Green coffee bean extract and roasted robusta coffee

Dry powder obtained from green coffee bean simplicia and robusta coffee roasting from 198.20 grams. The crude extract obtained after evaporated from green coffee beans and roasted robusta coffee was 24.981 grams and 21.506 grams, respectively, with yields value were 12.60% and 10.85%. This value is in accordance with the determination of the yield of condensed extract of areca seeds in the Indonesian Herbal Pharmacopoeia, which is not less than 16.50%. Obtained yield value fulfilled the requirements of a good yield hence the percentage were > 10%. The percentage of extract yield is to determine the amount of simplicia needed to make a number of condensed extracts from simplicia. High yield values indicate the number of components of bioactive compounds that can be extracted from extracts.

Results of Identification of Chemical Compounds Extract of green coffee beans (Coffea canephora P.) and roasted Robusta coffee beans (Coffea canephora L.)

The results of identification of chemical compounds showed that the extract of the green coffee beans (Coffea canephora P.) and roasted Robusta coffee beans (Coffea canephora L.) contained alkaloids, flavonoids, fenolik, saponins and terpenoids. Results of identification of chemical may be presented in tables I.

Table 1.
Results of Identification of Chemical Compound

Identification	Green coffee bean	Ket.	Robusta coffee	Ket.
Alkaloid Extract add 5 ml of ammonia and chloroform Mayers reagent	Yellowish white precipitate	(+)	Brown precipitate	(-)
Alkaloid Extract add 5 ml of ammonia and chloroform Dragendroff's reagent	Brown precipitate	(+)	Brown precipitate	(+)
Flavonoid Mg powder	Greenish yellow	(+)	Red	(+)
Phenolic FeCl3 1%	Blue	(+)	Black	(+)
Steroid dan Terpenoid Extract add 3 ml chloroform, 2 ml H2SO4 concentrated and 2 ml of acetic anhydrous	Brownish red	(+) Terpenoid (-) Steroid	Brownish red	(+) Terpenoid (-) Steroid
Saponin Extract add 10 ml of hot water and then cool, after cold, shake vigorously for 10 seconds and (+) add HCl 2 N	Stable foam is formed	(+)	Stable foam is formed	(+)

The phytochemical screening in this study on extracts of green coffee beans and robusta coffee beans are in line with research conducted by (Mahajan & Kapoor, 2018) which explains that green coffee beans and robusta coffee beans have chemical compounds of alkaloids, flavonoids, phenolics, terpenoids and saponins. However, there is a slight difference in the results of previous research conducted by (Mahajan & Kapoor, 2018) which explains that in addition to alkaloid compounds, flavonoids, terpenoids, phenolics and saponins, green coffee beans and robusta coffee beans also contain steroid compounds. This difference may be due to differences in different growing places so that the levels of compound content found in green coffee beans and

robusta coffee beans will also be different.

Identification Staphylococcus aureus ATCC 25923 with gram stain

Gram staining of bacteria aims to determine whether the bacteria used are classified as gram positive or gram negative. Differences in the mechanism of gram staining on the structure and composition of the bacterial cell wall.

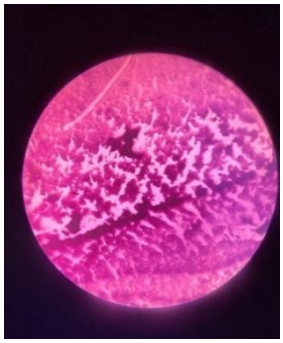

Figure 1. Coloring Staphylococcus aureus ATCC 25923

Gram stain results on Staphylococcus aureus ATCC 25923 bacteria in Figure 1 shows Gram positive because the cells appear purple, round and clustered. Administration of crystal violet, Lugol, and administration of ethanol in gram staining causes no lipids to be extracted thereby reducing the permeability of the gram-positive cell wall. The cell walls were dehydrated by alcohol treatment, causing the pores to shrink, the permeability of the cell walls and membranes decreased so that the safranin stain could not enter so that when observed using a microscope the cells would appear purple.

Optimization of Biofilm Formation Time

Optimization of the time for Staphylococcus aureus biofilm formation was carried out for 72 hours or 3 days using the Elisa Reader with a wavelength of 595 nm.Based on the results in the table II, it can be seen that Staphylococcus aureus can form good and optimal biofilms at an incubation time of 3 days or 72 hours. Observed the growth curve of Staphylococcus aureus which consisted of several growth phases, namely the lag phase, the log phase, the stationary phase and the death phase. The stationary phase is reached when growth reaches 1220 hours, and the death phase is reached after 20 hours.

Table 2.
Optimization Results of S. aureus Biofilm Formation

ABSORBANCE		
Day 1	Day 2	Day 3
0,27	0,35	0,46
0,29	0,35	0,44
0,24	0,38	0,47
0,30	0,4	0,45
0,28	0,37	0,48
0,27±0,02	0,37±0,02	0,46±0,01

The results of optimizing the time for the formation of Staphylococcus aureus biofilm bacteria had an average absorbance value at 24 hours incubation, namely 0.27; at 48 hours of incubation is 0.37

and at 72 hours of incubation is 0.46. The results of optimizing the time of biofilm formation were used as a reference for the incubation period in the test for the inhibition and degradation of biofilms by Staphylococcus aureus bacteria. The maximum wavelength used in this study is 595 nm, referring to the journal Tobi et al., 2022 which optimizes the wavelength using three variations, namely 490 nm, 595 nm and 655 nm and the most optimal wavelength for reading biofilms is 595 nm. which is indicated by the largest absorbance or OD value on each reading. Based on this research, this study uses a wavelength of 595 nm in reading the biofilm of test bacteria on a microplate reader. The most optimal growth of Staphylococcus aureus biofilm bacteria was on day 3 with an average absorbance or OD value of 0.540. This time is the optimal time for biofilm growth which is then used as the incubation period in testing the activity of inhibiting biofilm formation and biofilm degradation in each of these bacteria.

Biofilm formation begins when bacteria adhere to surface conditions via organic molecules. The attachment level of microbial cells is regulated by factors such as surface properties, surface layer conditions, characteristics and hydrodynamics of the liquid media, various characteristics of the microbial cell surface, gene regulation and quorum sensing (Mahami & Adu-Gyamfi, 2011)Biofilm formation can occur on various types of surfaces and various environmental conditions where bacteria are present. Bacteria, organic and inorganic molecules that are on the surface then form a film state. These organic and inorganic substrates together with microorganisms move to the surface by diffusion or following the liquid flow. Nutrient transfer is higher in biofilms than in the liquid phase (Todd, 2014). The condition of the film is important in the bonding process. The organic polymer of the media whose surface is submerged affects the extent and strength of microbial attachment; films form within minutes of exposure, and continue to expand over several hours. Characteristics of the liquid medium, such as pH, nutrient level, ionic strength, and temperature, may also play a role in the degree of microbial attachment to surfaces.

Biofilm Formation Inhibition Activity

The test for the inhibition of biofilm formation was aimed to obtaining the activity of the two simplicias in inhibiting the formation of Staphylococcus aureus biofilms. Biofilm formation in this study was measured quantitatively using the crystal violet assay method. The data shown in the table III is data resulting from the calculation of the percentage of biofilm inhibition based on the results of absorbance or optical density (OD). The OD value obtained describes the thickness of the biofilm formed, the higher the OD value indicates that the thicker the biofilm is formed. The average percentage inhibition of biofilm formation as shown in the table increases with the increase in the dose of antibiofilm used, this is different from the absorbance/OD value where the increasing dose of antibiofilm decreases the absorbance value.

Table 3.
Percentage Effectiveness of Robusta Roasted Coffee and Green Coffe Bean Inhibition of Biofilm S. aureus

Concentration (mg/ml)	Robusta Roasted Coffee				Green Coffee Bean			
	Ekstract (%)	n-heksan fraction (%)	ethyl acetat fraction (%)	Water fraction (%)	Ekstract (%)	n-heksan fraction (%)	ethyl acetat fraction (%)	Water fraction (%)
2 mg/ml	5.00	31.66	30.83	31.87	29.58	34.99	32.08	34.58
4 mg/ml	11.25	37.70	38.95	38.53	32.50	39.79	38.95	37.70
8 mg/ml	21.25	45.41	44.58	44.37	35.00	48.12	46.24	47.08
16 mg/ml	28.33	58.54	59.58	59.99	35.83	58.74	59.58	56.24

This study used variations of four concentrations, namely 2, 4, 8, and 16 mg/ml, where the concentration Robusta Roasted Coffee dan green coffee bean the highest in inhibiting biofilm formation was 16 mg/ml. The results of calculating the inhibition percentage of biofilm formation showed that increasing the concentration of the extract and fractions increased the inhibition of biofilm formation. In the biofilm inhibition test, all concentrations of green coffee bean extract and roasted robusta coffee had lower and different OD values compared to the negative control. This illustrates that all concentrations of green coffee beans and roasted robusta coffee as well as positive controls are able to inhibit biofilms. The process of forming biofilms on solid surfaces occurs in two stages. The first stage is cell growth and the formation of Extracellular Polymeric Substances (EPS), so that biofilm cells accumulate. The second stage is that release or reattachment can occur. Prevention of biofilm growth can be done by inhibiting or even killing the cells so they don't increase, and preventing the formation of EPS. each other (M. Kim, 2004)

Table 4.
IC50 value of biofilm inhibition

Sample	IC50 inhibition of biofilm formation (mg/ml)	
	Robusta Roasted Coffee	Green Coffee Bean
extract	2.13	4.14
n-hexane fraction	2.67	2.97
Ethyl acetat fraction	2.56	2.62
Water fraction	2.57	3.18

The results of the data on the percentage inhibition of biofilm formation obtained were then continued to calculate the IC50 value using the linear line equation between the percent inhibition of biofilm formation and the concentration of the fraction. IC50 Roasted coffee extract showed biofilm inhibition results S.aureus ATCC 25923 2,13 mg/ml. IC50 is the concentration at which the ethyl acetate fraction can inhibit biofilm formation by 50%. Use of IC50 It is generally used as a measure of inhibition of biofilm formation. The lower the IC50, the more effective the sample is in inhibiting biofilm formation. Test results One-Way ANOVA showed a significant difference with a significance value of 0.000 ($p<0.05$). The significance value between each treatment indicated a significant difference in terms of biofilm inhibitionS aureus ATCC 25923. Based on these data, the most active and greatest robusta roasted coffee extract has the potential to inhibit biofilms.

Robusta roasted coffee contains several chemical compounds, including tannins and flavonoids which have the potential to inhibit the intercellular adhesion genes icaA and icaD which are factors

for biofilm formation (Lee et al., 2013). This inhibition of ica gene expression causes tannins and flavonoids to also inhibit bacterial cell adhesion, both bacterial attachment to the surface of the substrate and attachment between bacteria, where adhesion is the main factor in biofilm formation. The biofilm inhibition ability of a compound is related to the penetration ability of the compound into the formed biofilm, which is capable of penetrating the Extracellular Polymeric Substance (EPS) layer or the mucus layer that covers the bacteria.

Biofilm Degradation Activity

Based on the results of the biofilm destruction test, all concentrations of green coffee extract and roasted robusta coffee had lower OD values and were significantly different from the negative control. This means that the concentrations of green coffee extract and roasted robusta coffee as well as the positive control were able to destroy the biofilm that had formed. The highest biofilm destruction activity of green coffee bean extract and roasted robusta coffee was seen at a concentration of 16 mg/ml, namely 27.11% and 19.55%. The results of biofilm destruction activity can be seen in table V.

Table 5.
Percentage of Green Coffee Destruction to S. aureus Biofilm

Concentration (mg/ml)	Robusta Roasted Coffee				Green Coffee Bean			
	Ekstract (%)	n-heksan fraction (%)	ethyl acetat fraction (%)	Water fraction (%)	Ekstract (%)	n-heksan fraction (%)	ethyl acetat fraction (%)	Water fraction (%)
2 mg/ml	13.33	32.50	32.01	31.45	16.44	29.99	29.99	27.91
4 mg/ml	15.55	37.91	38.54	39.58	19.11	38.33	38.33	32.70
8 mg/ml	16.89	44.58	45.83	45.41	24.00	43.75	43.75	46.45
16 mg/ml	19.55	59.99	60.41	60.62	27.11	62.29	62.29	63.12

Yield % biofilm destruction was then determined by the EC50 from green coffee bean extract and roasted robusta coffee with linear regression, the linear regression table was used to determine the EC50. EC50 results green coffee bean extract and roasted robusta coffee can be seen in table VI

Table 6.
EC50 value of biofilm destruction

Sample	EC50 destruction of biofilm formation (mg/ml)	
	Robusta Roasted Coffee	Green Coffee Bean
extract	19.32	10.18
n-hexane fraction	2.58	10.43
Ethyl acetat fraction	2.53	1.95
Water fraction	2.52	2.34

Based on the results of biofilm degradation tests using green coffee bean extract fractions, it appears that all fractions show effectiveness in destroying or degrading biofilms inStaphylococcus aureus ATTC 25923. The ethyl acetate fraction showed higher effectiveness than the extract, water fraction and n-Hexane fraction. While the results of biofilm degradation tests using roasted robusta coffee bean extract fractions showed that all fractions showed effectiveness in destroying or degrading biofilms inStaphylococcus aureus ATTC 25923. The water fraction showed higher effectiveness than the extract, ethyl acetate fraction and n-Hexane

fraction. The higher the EC value50, the lower the biofilm destroying activity, which means the higher the concentration required to achieve 50% biofilm destruction (jagani, 2012). Several mechanisms in destroying biofilms include biofilm matrix degradation, cell death and cell leakage. The content of chemical compounds in the coffee bean are saponins, tannins, alkaloids, terpenoids and flavonoids.

These compounds inhibit and destroy biofilms because they have mechanisms that can cause biofilm matrix degradation, cell death and cell leakage. The ability of biofilm degradation of a compound is related to the ability of the compound to penetrate into the biofilm that is formed, namely being able to penetrate the Extracellular Polymeric Substance (EPS) layer or the mucus layer that covers bacteria. In addition, the ability of these compounds to degrade biofilms is to remove EPS from existing biofilms (Cosmo Andrade et al., 2019). The mechanism of saponins in destroying biofilms is by influencing the extracellular polymer matrix present in the bacterial biofilm matrix so that polymer substances are reduced and changing the integrity of the bacterial cell membrane which causes instability in the bacterial cell wall. Terpenoid compounds in degrading biofilms can reduce existing biofilms and kill bacteria in biofilms (Cosmo Andrade et al., 2019). Tannin compounds have the effect of cell death and cell leakage in biofilms, besides that tannins also have a bactericidal effect. Flavonoid compounds have the effect of inhibiting adhesin molecules which are needed in the formation of biofilms (Karatan & Watnick, 2009). Tannins and flavonoids work by binding to a bacterial adhesin protein which is used as a bacterial surface receptor, resulting in a decrease in bacterial adhesion and inhibition of protein synthesis for cell wall formation (Agnol et al., 2003).

CONCLUSION

Green Coffee extract, water fraction, ethyl acetate and n-hexane (Coffea canephora P.) and Robusta Coffee (Coffea canephora L.) can inhibit the formation and degradation of Staphylococcus aureus bacterial biofilms. Robusta roasted coffee bean extract has the greatest anti-biofilm inhibition effectiveness with IC50 biofilm inhibition of 2.13 ppm. The ethyl acetate fraction from roasted robusta coffee beans has the greatest effectiveness in destroying (degrading) anti-biofilms with the EC 50 biofilm degradation of 1.93 ppm.

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