International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 09, 2020
ISSN: 1475-7192

Extracts and Fractions of Green Tea (Camellia Sinensis L. Kuntze) as Antibiofilm on Staphylococcus Aureus ATCC 25923

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Abstract: *In this study, the potency of extract and fraction (water, n-hexane ethyl acetate) from green tea leaves to inhibit biofilm formation and to degrade of biofilm produced by S. aureus ATCC 25923 were evaluated. The total phenolic content of green tea leaves was determined using spectrophotometry UV visible on λ=646 nm. Green tea leaves were macerated with 96% ethanol then were fractionated with water, ethyl acetate and n-hexane solvents. DO value (optical density) optimization. inhibition and degradation of biofilms were carried out through the microtiter plate method (using 96 wells), followed by using a microplate reader on λ = 595 nm. Then, the total phenolic content of the extract and fraction by spectrophotometry were determined. The results showed that IC_{50} value from extracts, water fraction, ethyl acetate fraction and n-hexane to inhibit S. aureus ATCC 25923 biofilms were 2,32; 4,04; 2,55; and 2,30 mg/mL respectively. EC_{50} values of extract, water fraction, ethyl acetate fraction and n-hexane to degrade S. aureus ATCC 25923 biofilms were 3,33; 3,33; 4,14; and 3,28 mg/mL. Total phenolic contains from extracts, water, ethyl acetate, and n-hexane fractions were 8.97; 6.729; 7.26; and 7.82 %. These results indicate that green tea leave has the potential antibiofilm for S. aureus ATCC 25923.*

Keywords: *Antibiofilm, Green tea, Staphylococcus.aureus*

1. INTRODUCTION

Staphylococcus aureus is a major pathogenic bacterium for humans. In addition to infecting the respiratory tract, S. aureus bacteria can cause various types of infections, including infections of the skin, meningitis, infections of the urinary tract, and also endocarditis [1]. This infection caused by S. aureus is usually not easy to overcome because of frequent relapses. An important factor causing this is its ability to form biofilms. The ability of bacteria to produce biofilms is one of the virulence factors of S. aureus which will complicate treatment [2]. Biofilm production in S. aureus is facilitated by the presence of the ica gene, which is an operon gene consisting of ica A, B, C and D [3]. The nature of the structure and physiological attributes of the S. aureus biofilm that makes it difficult to overcome completely due to the attachment of strong bacterial cells to implanted medical devices, the production of endotoxins to counter the immune response of the host, as well as the existence of plasmid exchange in biofilms that carry genes resistant to antimicrobials. [4].

Biofilm is a product of the interaction results from quorum sensing (QS) of each microorganism. The process of biofilm formation begins when the microbes attach to a suitable surface, which then attaches and issues a QS signal. At the time of communication, bacteria will issue signals (autoinducers) for other bacteria [5].

Green tea leaves have been known by the people of Indonesia as a refreshing drink. Green tea leaves are also known to be good for health. The source of tea leaves is the same,

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namely a plant named C. sinensis L. Kuntze. This plant can produce green tea and black tea, the difference is where it grows and how it is processed [6].

Based on the discussion from the background, the formulation of the problem in this study is whether the extract, water fraction, ethyl acetate and n-hexane green tea (*C. sinensis* L. Kuntze) have the effect of inhibiting and destroying biofilms against *S. aureus* ATCC 25923, what is the concentration IC_{50} and EC_{50} from green tea extracts and fractions in inhibiting and destroying *S. aureus* ATCC 25923 biofilms, what are the total phenolic levels of extracts, water fractions, ethyl acetate, and *n*-hexane green tea by ultraviolet-visible spectrophotometry.

The purpose of this research is to find out: whether the extracts and fractions of water, ethyl acetate, and n-hexane green tea (*C. sinensis* L. Kuntze) have inhibitory and degradation effects of biofilms against *S. aureus* ATCC 25923 IC_{50} and EC_{50} concentrations of green tea extracts and fractions in the inhibition and degradation test of biofilms *S. aureus* ATCC 25923 based on concentrations of 2, 4, 6 and 8 mg / mL water fractions, ethyl acetate and *n*-hexane green tea (*C. sinensis* L. Kuntze) and finally the total phenolic content of extracts, water fractions, ethyl acetate, and n-hexane green tea (*C. sinensis* L. Kuntze) by visible ultraviolet spectrophotometry.

2. METHODS

Tools and Materials

The tools used are autoclaves, incubators, freezers, flat-buttom polystyrene 96 well microtitterplate, *i Mark-Biorad* Microplate Reader, UV-Vis (shimadzu) spectrophotometer, rotary evaporator, Sterling-Bidwell device, moisture balance, UV 366. The sample material used in this study was green tea (*C. sinensis* L. Kuntze) which was fresh and dried and made powder. Tea leaves are taken from Kemuning, Karanganyar, Central Java, biofilms *S. aureus* ATCC 25923, VJA media (brand), PSA (brand), BHI (brand), 1% violet crystal (brand), gallic acid, Follin ciocaleteu (brand), slica gel GF254, SIM, KIA, LIA, Citrat, lugol iodine (brand), safranin (brand), Mc. Farland solution and DMSO 10%.

Making Green Tea Extractions and Fractions

Three hundred fifty-five grams of dried green tea samples were macerated with 95% ethanol as much as 3550 mL (1:10), then the filtrate obtained was then concentrated using an evaporator at a temperature below 45 ° C to obtain a thick extract [7]. Then liquid-liquid (ECC) was extracted with n-hexane (1: 1) so that two fractions were obtained, namely the water fraction and the n-hexane fraction. In the liquid-liquid (ECC) extracted water fraction with ethyl acetate (1: 1), shaken and allowed to separate so that the water fraction and ethyl acetate fraction are obtained. The three fractions namely water fraction, n-hexane fraction and ethyl acetate fraction were evaporated until the fraction was obtained and then tested the activity of antibiofilms against *S. aureus* ATCC 25923.

Total Phenolic Determination

Determination of Operating Time. 200 ppm gallic acid solution taken as much as 0.5 mL was added with a 1 ml Folin-Ciocalteu reagent of 2 ml then combined with 4 ml of sodium carbonate 1 M. Read the absorbance with a visible spectrophotometer at a wavelength of 650 nm for 15 minutes.

Determination of Maximum Wavelength. 200 ppm gallic acid solution taken as much as 0.5 mL was added with 1 ml of Folin-Ciocalteu reagent as much as 2 ml, then added with 4 ml of sodium carbonate 1 M. Leave it for operating time then read the absorbance at a wavelength of 400-800 nm.

Determination of the Raw Acid Gallic Curve. Gallic acid solution with a concentration of 80, 120, 140, 160, and 200 ppm taken as much as 0.5 mL added with 2% Folin-Ciocalteu reagent 2 ml, then added with 4 ml of sodium carbonate 1 M. Leave it for operating time then read the absorbance at maximum wavelength.

Total Phenolic Determination. Extracts, water fractions, ethyl acetate, and n-hexane 3 mg green tea leaves were dissolved with 96% ethanol 10 ml, then 0.5 ml each were taken with 2 ml of Folin-Ciocalteu 1% reagent, then added with 4 ml of 1 M sodium carbonate. Let stand for operating time then read the absorbance at maximum wavelength.

In Vitro Test of Antibiofilm Extract and Fraction of Green Tea Leaves (C. sinensis L. Kuntze)

Optimization of *S. aureus* ATCC 25923 Biofilm Formation Time. The test was carried out using a 96 wells microtitier flat-bottom polystyrene plate, by inserting 200 µL of bacterial suspension into each well then optimizing the incubation time. Biofilm optimization aims to obtain optimal incubation time in forming biofilms. The incubation time variations used are 1, 2, 3 days. After the incubation period, the microplate was washed using running water then added 200 µL of 1% violet crystal solution to each well and incubated at room temperature for 15 minutes then the microplate was rewashed using running water and added 96% ethanol solution of 200 µL added to each well and incubated at room temperature for 15 minutes. The optimization phase is the last reading was biofilm growth (OD_{595} absorption). The highest absorbance value is expressed as the optimal formation of *S. aureus* ATCC 25923 biofilm. Optimal incubation time is used for negative control in growth inhibition and destruction of *S. aureus* ATCC 25923 biofilms [8].

In vitro *S. aureus* ATCC 25923 biofilm inhibitory activity test. The biofilm inhibition test was carried out utilizing extracts, and each fraction namely water, ethyl acetate, n-hexane and media were added at the same time, 60 µL BHI media were put into

each well, 70 µL bacterial suspension test and 70 µL active extract and fraction 70 µL green tea leaves with various concentrations of 2, 4, 6 and 8 mg / mL, then incubated at an optimum time at 37 ° C then microplate was washed using flowing water three times, then added 200 µL of 1% violet crystal solution to each well and incubated at room temperature for 15 minutes, the next step Microplate was rewashed using flowing water three times plus 96% ethanol 96% solution was put into each well and incubated at room temperature for 15 minutes. The biofilm growth readings (AbsorbanODOD955) were read using the Mark-Biorad Microplate Reader tool (Inscription and Hahamani, 2010; Sandasi et al., 2010). Biofilm inhibition testing was replicated three times. The following formula can measure the percentage of inhibition of biofilm S. aureus ATCC 25923:

$$\frac{(DO\ control\ negative - DO\ control\ positive)}{DO\ control\ negative} \times 100$$

Note: DO (Optical Density)

Then the *S. aureus* ATCC 25923 biofilm inhibitory activity test was started with the IC_{50} (Inhibitory Concentration) parameter determined from the linear regression equation between the sample concentration and the percentage of biofilm inhibition.

In vitro *S. aureus* ATCC 25923 Biofilm Destruction Activity Test. This test was conducted as in the biofilm inhibition test, only extracts and fractions of green tea leaves were added to the formed biofilms. Biofilms were formed after each well were incubated for an optimum time at 37 ° C with 200 µL of BHI media test bacteria after the formation of biofilms, the suspension of test bacteria in the microplate was removed, then extracted and extracted green tea leaf fraction of 200 µL with concentration variations of 2, 4, 6 and 8 mg / mL were then incubated at room temperature for optimum time then microplate was washed using running water and added with 96% ethanol solution as much as 200 µL put into each well and incubated at room temperature for 15 minutes. The biofilm growth readings (Absorban OD_{595}) were read using the Mark-Biorad Microplate Reader tool (Inscription and Hahamani, 2010; Sandasi et al., 2010). Demolition Testing biofilm was replicated three times. The following formula can measure the percentage of destruction from S. aureus ATCC 25923 biofilms:

$$\frac{(DO\ control\ negative - DO\ control\ positive)}{DO\ control\ negative} \times 100$$

Then the test of the *S. aureus* ATCC 25923 biofilm activity expressed by the parameter EC_{50} (Effective Concentration) was determined from the linear regression equation between the concentration of the sample and the percentage of destruction of the biofilm.

3. RESULTS AND DISCUSSION

Based on the research obtained extracts and fractions of the table below.

Table 1. Yield results of green tea leaf extract

Sample weight	Extract Weight (g)	Yield extract (%)
355,0	44,93	12,65
345,0	40,65	11,78
362,0	42,45	11,72
Average percentage		12,05
SD		0,52

Table 2. Percentage of water fractions from green tea leaf extracts.

No	Maceration extract (g)	Water fraction (g)	Rendemen (%)
1	22,27	6,07	27,25
2	23,05	6,97	30,23
3	22,55	6,50	28,82
Average percentage			28,76
SD			1,49

Table 3. Percentage of ethyl acetate fraction from green tea leaf extracts

No	Maceration extract (g)	Ethyl acetate fraction (g)	Rendemen (%)
1	22,27	13,21	59,31
2	23,05	12,89	55,92
3	22,55	13,30	58,98
Average percentage			58,07
SD			1,86

Table 4. Percentage of n-hexane fraction from green tea leaf extracts

No	Maceration extract (g)	*n*-heksan fraction (g)	Rendemen (%)
1	22,27	2,81	12,61
2	23,05	2,99	12,97
3	22,55	2,76	12,23
Average percentage			12,60
SD			0,37

The results of the fractionation showed a difference in percent yield with the weight of the ethyl acetate fraction, which had the highest percentage of yield among the other fractions.

The difference in the results of the fractionation of green tea leaves is made possible by the difference in polarity of each class of chemical compounds and other factors that influence the percent yield of the fraction are the possibility that most of the compounds in green tea leaves are semi-polar. The yield obtained is less than 100%; this is likely due to extracts attached to the container and separating funnel.

The results of the determination of total phenolic extracts, water fractions, ethyl acetate, and n-hexane green tea leaves were carried out employing 3 mg each diluted with 96% ethanol 10 ml then each 0.5 ml taken was added with a Folin-Ciocalteu reagent 1% as much as 2 ml, then added with 4 ml of sodium carbonate 1 M. Then read the absorbance at a wavelength of 646 nm and then calculated the total phenolic content from using the standard curve y = 0.0018x + 0.2076 with a correlation value of r of 0, 9637 and the total phenolic yield extracts, water fractions, ethyl acetate, and n-hexane can be seen in the image below.

Table 5. Total Phenolic Results of Green Tea Leaves.

Sampel	Absorbansi	Kadar % Fenolik Total
Ekstract	0,693	8,97
Water fraction	0,571	6,73
etil asetat fraction	0,600	7,26
n-heksan fraction	0,630	7,82

The diagram of the optimization time of *S. aureus* ATCC 25923 biofilm formation can be seen in Table 6.

Table 6. Results of optimization of *S. aureus ATCC 25923* biofilm formation.

NO	ABSORBANSI		
	Day-1	Day -2	Day -3
1	0,37	0,45	0,54
2	0,34	0,45	0,54
3	0,33	0,44	0,51
4	0,32	0,44	0,56
5	0,36	0,42	0,51
6	0,38	0,44	0,52
Average	0,35±0,02	0,44±0,01	0,53±0,02

The first-time biofilm formation began when bacteria were attached to surface conditions through organic molecules. The level of attachment of microbial cells is regulated by factors such as surface properties, surface layer conditions, characteristics and hydrodynamics of liquid media, various surface characteristics of microbial cells, gene regulation, and quorum sensing [9].

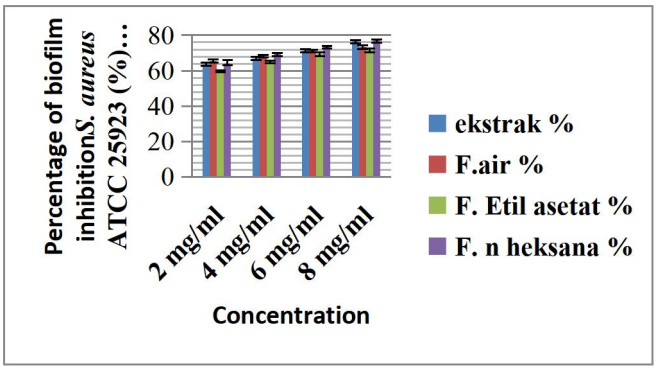

Figure 1. Results of% biofilm inhibition of *S. aureus* ATCC 25923

Then the IC_{50} values were determined from the extract, water fraction, ethyl acetate fraction, and *n*-hexane with linear regression. The price of IC_{50} is inversely proportional to the inhibitory activity of biofilms. The higher the IC_{50} value of biofilm inhibitory activity is smaller, meaning that the concentration needed to produce biofilm inhibitory activity is 50% greater.

Tabel 7. The result of IC_{50} biofilm *S. aureus* ATCC 25923.

Sample	Linear Regression	The result of IC_{50}biofilm *S. aureus* ATCC 25923. (mg/ml)
Extract	Y=0,0231X + 0,5905	2,32
Water fraction	Y=0,0122X + 0,6342	4,03
ethyl acetate fraction	Y=0,0194X + 0,5674	2,55
n-hexane fraction	Y=0,0125X + 0,6015	2,30

The results of % *S. aureus* ATCC 25923biofilm inhibition gave an IC_{50} value of 2,30 mg/ml from the *n*-hexane fraction. Statistical analysis of biofilm inhibition was started with normality test results showed OD data with biofilm inhibition *S. aureus* ATCC 25923 normal distribution. Then the homogeneity test was continued to show the biofilm inhibition OD data was homogeneous with a value ($P > 0.05$). The data was then tested by one-way ANOVA one-way test and found that there was a significant difference with a significance value of 0.00 ($p < 0.005$). Significance values between each treatment showed that there were significant differences in negative control OD with extract OD and fraction in inhibition of *S. aureus* ATCC 25923 biofilms. Destruction of biofilm activity of *S. aureus* ATCC 25923 results can be seen in the figure below.

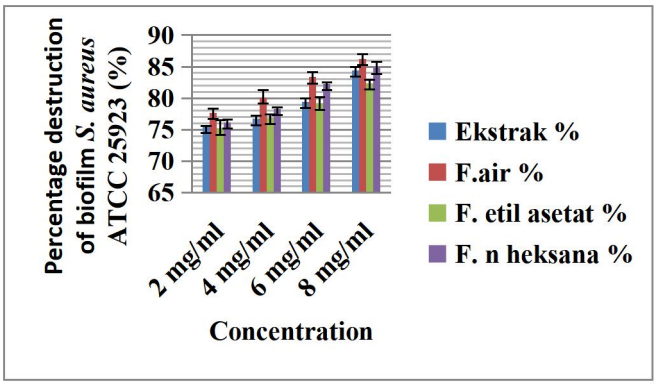

Figure 2. Results of % biofilm destruction *S. aureus* ATCC 25923

The EC_{50} value then determined the results of biofilm% destruction with linear regression, the linear regression table used to determine the EC_{50} value. The EC_{50} price is inversely proportional to the% of biofilm crushing. The higher the price of EC50 biofilm crushing activity is smaller, meaning that the concentration needed to produce biofilm crushing activity is 50%, the more significant the EC_{50} *S. aureus* ATCC 25923 results can be seen below:

Table 8. The result of EC_{50}biofilm *S. aureus* ATCC 25923

Sample	Linear Regression	The result of EC_{50}biofilm *S. aureus* ATCC 25923. (mg/ml)
Extract	Y=0,0148X + 0,7164	3,33
Water fraction	Y=0,0148X + 0,7464	3,33
etil asetat fraction	Y=0,0119X + 0,7247	4,14
n-hexane fraction	Y=0,015X + 0729	3,28

Table 8 shows that the ethyl acetate fraction has a smaller EC_{50} value than the water fraction, n-hexane fraction, and green tea leaf extract, meaning that the ethyl acetate fraction has higher anti-biofilm activity than the water fraction, n-hexane fraction, and leaf extract green tea with a concentration of 3,28 mg/ml The mechanism for destroying biofilms there are several ways including degradation of biofilm matrices, cell death and cell lysis. The content of green tea leaf extract compounds such as tannins, terpenoids, flavonoids, polyphenols which can be used to inhibit and destroy biofilms because it has a mechanism that can cause degradation of biofilm matrix, cell lysis, and cell death. Tannin compounds in green tea leaves have a cell death and cell lysis effect on biofilms. In addition, tannins also have a bacteriocidic impact, while flavonoid compounds have an inhibitory effect on adhesion molecules that are needed in the formation of biofilms [10].

4. CONCLUSION

First, extracts, water fractions, ethyl acetate, and n-hexane green tea (*C. sinensis* L. Kuntze) have inhibitory and degradation effects of biofilms against *S. aureus* ATCC 25923. Second, IC_{50} concentrations of extracts, water fractions, ethyl acetate fractions and n-hexane fraction of green tea leaves in inhibiting *S. aureus* ATCC 25923 biofilms was 2,32 mg / ml, 4,04 mg / ml, 2,55 mg / ml, and 2,30 mg / ml, EC_{50} concentration of extracts, water fraction, ethyl acetate fraction and The hexane n fraction of green tea leaves in destroying *S. aureus* ATCC 25923 biofilms was 3,33mg / ml, 3,33 mg / ml, 4,14 mg / ml, and 3,28 mg / ml. Third, the total phenolic content of the extract, water fraction, ethyl acetate, and n-hexane green tea were 8.97%, 6.729%, 7.26%, and 7.82%.

REFERENCES

[1] Sumpio, B. E., Cordova, A. C., Berke-Schlessel, D. W., Qin, F., & Chen, Q. H. (2006). Green Tea, the "Asian Paradox," and Cardiovascular Disease. *Journal of the American College of Surgeons*, *202*(5), 813–825. https://doi.org/10.1016/j.jamcollsurg.2006.01.018

[2] Kim, H.-S., Lee, S.-H., Byun, Y., & Park, H.-D. 2015. 6-Gingerol Jigisha, A., Nishant, R., Navin, K., & Pankaj, G. (2012). Green Tea: a Magical Herb With Miraculous Outcomes. *International Research Journal of Pharmacy*, *3*(5), 139–148.

[3] Sinija, V. R., & Mishra, H. N. (2008). Green tea: Health benefits. *Journal of Nutritional and Environmental Medicine*, *17*(4), 232–242. https://doi.org/10.1080/13590840802518785

[4] Scholl, C., Lepper, A., Lehr, T., Hanke, N., Schneider, K. L., Brockmöller, J., … Stingl, J. C. (2018). Population nutrikinetics of green tea extract. *PLoS ONE*, *13*(2), 1–22. https://doi.org/10.1371/journal.pone.0193074

[5] Kokubo, Y., Iso, H., Saito, I., Yamagishi, K., Yatsuya, H., Ishihara, J., … Tsugane, S. (2013). The impact of green tea and coffee consumption on the reduced risk of stroke incidence in japanese population: The Japan public health center-based study cohort. *Stroke*, *44*(5), 1369–1374. https://doi.org/10.1161/STROKEAHA.111.677500

[6] Kaur, G., & Kaur, P. (2019). *Green tea (Camellia sinensis): It's promising health benefits for the welfare of humans*. 299–302.

[7] Fritz, H., Seely, D., Kennedy, D. A., Fernandes, R., Cooley, K., & Fergusson, D. (2013). Green tea and lung cancer: A systematic review. *Integrative Cancer Therapies*, *12*(1), 7–24. https://doi.org/10.1177/1534735412442378

[8] Petta, S., & Marchesini, G. (2017). Coffee and tea breaks for liver health. *Journal of Hepatology*, *67*(2), 221–223. https://doi.org/10.1016/j.jhep.2017.04.014

[9] Jahani, S., Bazi, S., Shahi, Z., Sheykhzade Asadi, M., Mashhady, M. A., & Sohil Baigi,

G. (2016). Effects of Methanol Extract of Green Tea on Biofilm Formation of Staphylococcus aureus. *International Journal of Infection*, *4*(3), 3–8. https://doi.org/10.5812/iji.42394

[10] Causes, A. (2015). *Green Tea Consumption and Mortality Due to Cardiovascular Disease, 296*(10), 1255–1265.

[11] Vishnoi, H., Bodla, R., Kant, R., & Bodla, R. B. (2018). Green Tea (Camellia Sinensis) and Its Antioxidant Property: a Review. *Article in International Journal of Pharmaceutical Sciences and Research*, *9*(5), 1723. https://doi.org/10.13040/IJPSR.0975-8232.9(5).1723-36

[12] Lee, K. W., Lee, H. J., & Lee, C. Y. (2002). Antioxidant Activity of Black Tea vs. Green Tea. *The Journal of Nutrition*, *132*(4), 785–785. https://doi.org/10.1093/jn/132.4.785

[13] Nabi, B. N., Sedighinejad, A., Haghighi, M., Farzi, F., Rimaz, S., Atrkarroushan, Z., & Biazar, G. (2018). The anti-obesity effects of green tea: A controlled, randomized, clinical trial. *Iranian Red Crescent Medical Journal*, *20*(1), 1–7. https://doi.org/10.5812/ircmj.55950

[14] Young, J. F., Dragsted, L. O., Haraldsdóttir, J., Daneshvar, B., Kall, M. A., Loft, S., … Sandström, B. (2002). Green tea extract only affects markers of oxidative status postprandially: lasting antioxidant effect of flavonoid-free diet. *British Journal of Nutrition*, *87*(4), 343–355. https://doi.org/10.1079/bjn2002523

[15] Oliveira, R. M. M. De. (2012). Quantification of catechins and caffeine from green tea (Camellia sinensis) infusions, extract, and ready-to-drink beverages. *Food Science and Technology*, *32*(1), 163–166. https://doi.org/10.1590/s0101-20612012005000009

[16] Antibiofilm, A., Air, E., Pepaya, D., & Bakteri, L. (2017). *The In Vitro Antibiofilm Activity of Water Leaf Extract of Papaya (Carica papaya L.) against Pseudomonas aeruginosa*. *2*(3), 150–163. https://doi.org/10.29244/150-163

[17] Arjuna, A., Pratama, W. S., Sartini, S., & Mufidah, M. (2018). Uji Pendahuluan Anti-biofilm Esktrak Teh Hijau dan Teh Hitam Pada Streptococcus mutans melalui Metode Microtiter Plate. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy)*, *4*(1), 44. https://doi.org/10.22487/j24428744.2018.v4.i1.9965

[18] Ulyah, H., Ulfa, E. U., & Puspitasari, E. (2015). Uji Aktivitas Antibakteri dan Antibiofilm Minyak Atsiri Rimpang Bengle (Zingiber purpureum Roscoe) terhadap Bakteri Staphylococcus epidermidis. *E-Jurnal Pustaka Kesehatan*, *3*(2), 267–271.

[19] Piegerová, A., Koščová, J., Schusterová, P., Nemcová, R., & Kryvtsova, M. (2019). In Vitro Inhibition of Biofilm Formation by Staphylococcus Aureus Under the Action of Selected Plant Extracts. *Folia Veterinaria*, *63*(1), 48–53. https://doi.org/10.2478/fv-2019-0007

[20] Hamzah, H., Hertiani, T., Utami Tunjung Pratiwi, S., & Nuryastuti, T. (2019). The Inhibition Activity of Tannin on the Formation of Mono-Species and Polymicrobial

Biofilm Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. *Majalah Obat Tradisional*, *24*(2), 110. https://doi.org/10.22146/mot.44532

[21] Brambilla, L. Z. S., Endo, E. H., Cortez, D. A. G., & Filho, B. P. D. (2017). Anti-biofilm activity against staphylococcus aureus mrsa and mssa of neolignans and extract of piper regnellii. *Brazilian Journal of Pharmacognosy*, *27*(1), 112–117. https://doi.org/10.1016/j.bjp.2016.08.008

[22] Kerekes, E. B., Vidács, A., Takó, M., Petkovits, T., Vágvölgyi, C., Horváth, G., … Krisch, J. (2019). Anti-biofilm effect of selected essential oils and main components on mono- and polymicrobic bacterial cultures. *Microorganisms*, *7*(9). https://doi.org/10.3390/microorganisms7090345

[23] Homenta, H., (2016). Infeksi Biofilm Bakterial. *Jurnal E-Biomedik*, *4*(1), 1–11. https://doi.org/10.35790/ebm.4.1.2016.11736

[24] Moormeier, D. E., & Bayles, K. W. (2017). Staphylococcus aureus biofilm: a complex developmental organism. *Molecular Microbiology*, *104*(3), 365–376. https://doi.org/10.1111/mmi.13634

[25] Dewi, M., Helmi, T. Z., & Gani, B. A. (2019). 17. Oxytetracycline Activities to Staphylococcus aureus Biofilm Inhibition of Aceh Cattle Preputium Isolate. *Jurnal Medika Veterinaria*, *13*(1), 125–131. https://doi.org/10.21157/j.med.vet..v13i1.8884

[26] Purbowati, R. (2017). KEMAMPUAN PEMBENTUKAN SLIME PADA Staphylococcus epidermidis, Staphylococcus aureus, MRSA DAN Escherichia coli. *Florea : Jurnal Biologi Dan Pembelajarannya*, *4*(2), 1. https://doi.org/10.25273/florea.v4i2.1647

[27] Chen, Y., Liu, T., Wang, K., Hou, C., Cai, S., Huang, Y., … Chen, Y. (2016). Baicalein inhibits Staphylococcus aureus biofilm formation and the quorum sensing system in vitro. *PLoS ONE*, *11*(4), 1–18. https://doi.org/10.1371/journal.pone.0153468

[28] Avila-Novoa, M.-G., González-Gómez, J.-P., Iñíguez-Moreno, M., Gutiérrez-Lomelí, M., Solís-Velázquez, O.-A., & Guerrero-Medina, P.-J. (2018). Biofilm Formation by Staphylococcus aureus Isolated from Food Contact Surfaces in the Dairy Industry of Jalisco, Mexico. *Journal of Food Quality*, *2018*. Retrieved from http://dx.doi.org/10.1155/2018/1746139%0Ahttps://ezproxy.lib.uconn.edu/login?url=https://search.ebscohost.com/login.aspx?direct=true&db=agr&AN=IND606243538&site=ehost-live

[29] Savage, V. J., Chopra, I., & O'Neill, A. J. (2013). Population Diversification in Staphylococcus aureus Biofilms May Promote Dissemination and Persistence. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0062513

[30] Yarwood, J. M., Bartels, D. J., Volper, E. M., & Greenberg, E. P. (2004). Quorum Sensing in Staphylococcus aureus Biofilms. *Journal of Bacteriology*, *186*(6), 1838–1850. https://doi.org/10.1128/JB.186.6.1838-1850.2004

[31] Nuryastuti, T., Praseno, & Mustafa, M. (n.d.). Biofilm formation properties of Methicillin resistant Staphylococcus aureus (MRSA) strains isolated in Microbiology Laboratory, Faculty of Medicine , UGM , Yogyakar ta. *Department of Microbiology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta*, 1–11.

[32] Wang, Y., Tan, X., Xi, C., & Phillips, K. S. (2018). Removal of Staphylococcus aureus from skin using a combination antibiofilm approach. *Npj Biofilms and Microbiomes*, *4*(1). https://doi.org/10.1038/s41522-018-0060-7

[33] Asli, A., Brouillette, E., Ster, C., Ghinet, M. G., Brzezinski, R., Lacasse, P., … Malouin, F. (2017). Antibiofilm and antibacterial effects of specific chitosan molecules on Staphylococcus aureus isolates associated with bovine mastitis. *PLoS ONE*, *12*(5), 1–23. https://doi.org/10.1371/journal.pone.0176988

[34] Kong, C., Chee, C. F., Richter, K., Thomas, N., Abd Rahman, N., & Nathan, S. (2018). Suppression of Staphylococcus aureus biofilm formation and virulence by a benzimidazole derivative, UM-C162. *Scientific Reports*, *8*(1), 2758. https://doi.org/10.1038/s41598-018-21141-2

[35] únior, S. D. da C., Santos, J. V. de O., Campos, L. A. de A., Pereira, M. A., Magalhães, N. S. S., & Cavalcanti, I. M. F. (2018). Antibacterial and antibiofilm activities of quercetin against clinical isolates of Staphyloccocus aureus and Staphylococcus saprophyticus with resistance profile. *International Journal of Environment, Agriculture and Biotechnology*, *3*(5), 1948–1958. https://doi.org/10.22161/ijeab/3.5.50

[36] Song, Y. J., Yu, H. H., Kim, Y. J., Lee, N. K., & Paik, H. D. (2019). Anti-Biofilm Activity of Grapefruit Seed Extract against Staphylococcus aureus and Escherichia coli. *Journal of Microbiology and Biotechnology*, *29*(8), 1177–1183. https://doi.org/10.1041/jmb.1905.05022

[37] Nair, S., Desai, S., Poonacha, N., Vipra, A., & Sharma, U. (2016). Antibiofilm activity and synergistic inhibition of Staphylococcus aureus biofilms by bactericidal protein P128 in combination with antibiotics. *Antimicrobial Agents and Chemotherapy*, *60*(12), 7280–7289. https://doi.org/10.1128/AAC.01118-16

[38] Archer, N. K., Mazaitis, M. J., William Costerton, J., Leid, J. G., Powers, M. E., & Shirtliff, M. E. (2011). Staphylococcus aureus biofilms: Properties, regulation and roles in human disease. *Virulence*, *2*(5), 445–459. https://doi.org/10.4161/viru.2.5.17724

[39] Wu, S., Huang, F., Zhang, H., & Lei, L. (2019). Staphylococcus aureus biofilm organization modulated by YycFG two-component regulatory pathway. *Journal of Orthopaedic Surgery and Research*, *14*(1), 1–8. https://doi.org/10.1186/s13018-018-1055-z

[40] Mirzaee, M., Peerayeh, S. N., & Ghasemian, A.-M. (2014). Detection of icaABCD Genes and Biofilm Formation in Clinical Isolates ofOriginal Article. *Iranian Journal of Pathology*, *9*(4), 257–262. https://doi.org/10.1310/hpj4902-170