Jurnal Farmasi Galenika (*Galenika Journal of Pharmacy*) (e-Journal) 2024; 10 (2): 234 – 243
ISSN: 2442-8744 (electronic); 2442-7284 (printed)
Journal Homepage: https://bestjournal.untad.ac.id/index.php/Galenika
DOI: 10.22487/j24428744.2024.v10.i2.16283

Antibacterial Activity Assay on *Escherichia Coli* of The Matoa Seeds Active Fraction

(*Uji Aktivitas Antibakteri terhadap Escherichia coli dari Fraksi Aktif Biji Matoa*)

Karnelasatri[1]*, Iren Anggreni Yacob[2], Pangeran Andareas[3], Feronia Reni Cyrena Santoso[4], Jessica Novia[5]

[1,2,5]DIII Farmasi, Fakultas Ilmu Kesehatan, Universitas Pelita Harapan
[3]DIV Teknik Laboratorium Medik, Fakultas Ilmu Kesehatan, Universitas Pelita Harapan
[4]SI Farmasi, Fakultas Ilmu Kesehatan, Universitas Pelita Harapan
**Email korespondensi: karnelasatri@uph.edu*

Article Info:
Received: 07 February 2024
in revised form: 16 May 2024
Accepted: 01 October 2024
Available Online: 01 October 2024

Keywords:
Escherichia coli
Antibacterial
Fraction
Pometia Pinnata J. R Forst and G. Forst..

Corresponding Author:
Saipul Maulana
Jurusan Farmasi
Fakultas MIPA
Universitas Tadulako
Palu
90245
Indonesia
email:
saifulmaulana011@gmail.com

ABSTRACT

Escherichia coli is one of the main bacteria that cause health problems. Antibiotic resistance in various medical therapies caused by bacteria has become a concern in the last few decades. Therefore, research on the antibacterial activity of the Matoa active fraction (*Pometia Pinnata* J.R Forst & G. Forst) against *Escherichia coli* is the aim of this research. Initially, this plant is an original plant from Papua that has been used traditionally by the community. The solvent used in the extraction was 70% ethanol, meanwhile the solvents used in the fractionation were n-hexane, ethyl acetate and water. Moreover, the positive control and negative control used were ciprofloxacin and dimethyl sulfoxide, respectively. Furthermore, the *Kirby Bauer* diffusion method was used in this study. Based on the results, the water fraction was determined as the active fraction. The antibacterial assay was continued on the water fraction with concentrations of 500 mg/mL, 400 mg/mL, 300 mg/mL, 200 mg/mL, and 100 mg/mL. The inhibition zones obtained from each of the above concentrations were 1,176 cm, 1,084 cm, 1,034 cm, 1,018 cm dan 0,889 cm, respectively. Based on the results of data analysis by *One Way ANOVA* and followed by the *post hoc Tukey HSD* test and a concentration of 500 mg/mL was concluded as the optimum concentration. Furthermore, the MIC study carried out by the liquid dilution method shows 50 mg/mL active fraction of Matoa seeds is the smallest concentration that can inhibit bacterial growth.

How to cite (APA 6th Style):
Karnelasatri, K., Yacob, I. A., Andareas, P., Santoso, F. R. C., Novia, J. (2024). Antibacterial Activity Assay on Escherichia Coli of The Matoa Seeds Active Fraction. *Jurnal Farmasi Galenika: Galenika Journal of Pharmacy (e-Journal), 10*(2), 234-243. doi:10.22487/j24428744.2024.v10.i2.16283

INTRODUCTION

In Indonesia, especially in eastern regions such as Papua, diseases caused by *Escherichia coli* are commonly found. *E. coli* is known as one of the coliform bacteria which is classified in the *Enterobacteriaceae* family. *Enterobacteriacea* is bacteria that can live and survive in the digestive tract or are known as enteric bacteria (Rahayu, Nurjanah and Komalasari, 2018). *E. coli* is the main etiology that causes diarrhea (Parashar et al., 2003). Currently, the most effective way to deal with bacterial infections is by antibiotic treatment. However, the rising cases of inappropriate uses of antibiotic have been concerning as of late, and this could lead to higher rate of antibiotic resistance. Moreover, some literature stated that one of the things that cause antibiotic resistance is its widespread and irrational use (Utami, 2011). Antibiotic resistance is a condition where the growth of bacteria is no longer inhibited when antibiotics are given systemically at the proper dose (Tripathi, 2003).

Initially, *Pometia pinnata* J.R Forst & G. Forst (matoa) is a typical plant originating from Papua. Matoa is utilized in various parts such as leaves, bark, fruit skin, fruit flesh, and seeds. Further, Matoa seeds contain flavonoids, saponins, and tannins which act as antibacterials. Moreover, research by Ngajow et al (2013) showed that Matoa stem bark contains tannins, flavonoids, terpenoids, and saponins that can inhibit the growth of *Staphylococcus aureus*. In addition, other research by Setyawan (2019) showed that the ethanol extract Matoa flesh contained saponins and was able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. Likewise, other research conducted by Theopanny (2019) showed that Matoa leaves contain alkaloids, flavonoids, tannins, glycosides, saponins, and steroids. Matoa leaves were extracted in various solutions such as n-hexane, ethyl acetate and 96% ethanol. 25 mg/mL both ethyl acetate and 96% ethanol extract inhibited both *Propionibacterium acnes* and *Escherichia coli*, nevertheless, n-hexane extract did not show antibacterial activities (G. Maria, 2019).

Based on the studies above, Matoa contains diverse compounds which are extracted in various solvent types of polarity. Owing to it, a fractioned compound based on their polarity is significant in studying their antibacterial activities. This study conducted n-hexane, ethyl acetate, and water as non-polar, semi-polar, and polar solvents, respectively. The most active fraction of Matoa seed was then tested by phytochemical screening, meanwhile, antibacterial assay was conducted by the Kirby Bauer method.

MATERIAL AND METHODS

Materials

The chemicals in this study were ethyl acetate (Smart-Lab), 70% ethanol, 96% ethanol (Smart-Lab), n-hexane (Smart-Lab), dimethyl sulfoxide (DMSO) (Merck), 1% iron (III) chloride solution (Merck), H_2SO_4 2M, chloroform (Merck), ammonia (Merck), 0,5M Mc Farland's solution (Oxoid), nutrient agar (Himedia), nutrient broth (Himedia), dragendorf reagent, sulfuric acid (Merck), magnesium powder

(Merck), methanol (Merck), NaCl 10% (Merck), aquadestillata, Mueller Hinton Agar (MHA) medium (Oxoid), crystal violet paint, iodine, safranin, hydrogen peroxide (H_2O_2) 10% (Merck), ciprofloxacin tablets, paper disc blanks (Oxoid).

Methods

Extraction And Fractionation of Matoa Seeds

The determination of the sample was conducted by the Research Center, BRIN. It dried in an oven at a temperature of 30-50ºC and macerated in 96% ethanol solvent with a ratio of 1:5. The mixture was then left to soak for 24 hours and occasionally stirred. Moreover, the precipitate separated and macerated one more time with equal solvent and method. Finally, the macerates obtained were then evaporated to obtain a thick extract (Erlyn, P. 2016).

Fractionation conducted by Liquid-Liquid Extraction method. First, the fractionation was carried out by dissolving the 96% ethanol extract in 200 mL of distilled water and then placed in a separating flask, then 200 mL of n-hexane solvent was added, shaken gently, and left until the n-hexane and aquadest fractions separated. The n-hexane fraction was set aside, then repeated several times until it became clear. Likewise, fractionation continued with ethyl acetate and water, which worked in a similar procedure. The liquid n-hexane fraction, liquid ethyl acetate fraction, and liquid water fraction respectively, were evaporated using a rotary evaporator and an oven to obtain a viscous fraction (Pakpahan & Sutriningsih, 2020).

Antibacterial Activity Assay

The extract was tested in various concentrations to inhibit *Escherichia coli* bacteria. Bacterial culture was grown in MHA (Mueller Hinton Agar) media and incubated in an incubator at 37°C for 1 day. Paper discs with a diameter of 0.55 cm were placed on other clear media and dripped with the extract and then transferred to a petri dish that contained the media and culture for each concentration. DMSO 10% and ciprofloxacin were used as a negative control and positive control, respectivelly. Petri dish incubated at 37°C for 1 day. The inhibition assay of the active fraction was carried out with four repetitions. The diameters of inhibition are measured with a ruler or caliper (Brooks et al., 2005). The results will be converted and grouped based on CLSI (Clinical and Laboratory Standards Institute®) compared to the positive control.

Minimum Inhibitory Concentration (MIC) Determination

The active fraction was prepared with diluted concentrations into 50, 25, 12.5, 6.25, and 3.13 mg/mL (Jahan et al 2011). Each test tube has filled with Nutrien Broth media and then inoculated with a mixture of 1 mL of both bacterial suspension and the active fraction. Then the media were incubated again for

up to 48 hours. Nutrient Broth media at the concentration that shows turbidity reduced indicated bacterial inhibition is designated as the final MIC. The results of the MIC determination obtained will be converted and grouped based on CLSI (Clinical and Laboratory Standards Institute®).

Statistical Data Analysis

The data taken is the calculations of the diameter inhibition zone from the active fractionation against *Escherichia coli* and it was processed using SPSS (Statistical Program for Social Science). Firstly, the normality and homogeneity tests were carried out. The data obtained is normally distributed and known as homogeneous, then continued to One way ANOVA data analysis to determine the ability of the Matoa seed fraction to work on the growth of *E. coli*. Contrariwise, the data obtained is not normally distributed, then continued with the Kruskal Wallis test.

RESULTS AND DISCUSSION

Results of Extraction and Fractionation of Matoa Seeds

The simplicia weight obtained was 2.7 kg from 2.91 kg of fresh matoa seeds. Respectively Table 1 and Table 2 below shows the extraction and fractionations results are shown.

Table 1 Extraction Results of Matoa Seeds in 96% ethanol

Ekstract	Weight (g)	Result (g)	% Yield	Characteristics
Matoa seeds	600	68.84	11.47%	Viscous extract, brown

Table 2 Fractionation Results of Matoa Seeds

Fraction	Weight (g)	Result (g)	% Yield	Characteristics
n-hexane	60 g	11.4 g	19%	Dry fraction, brown
Ethyl acetate	60 g	9.6 g	16%	Dry fraction, brown
Water	60 g	10.9 g	18.2%	Dry fraction, brown

Table 3 Phytochemical screening of the solvent fractions from Matoa seeds

Phytochemical screening	*n*-heksan	Ethyl acetate Fraction	Water Fraction
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Terpenoids	-	+	-
Steroids	+	-	+

Description: (+) = Positively identified, (-) = Unidentified

Table 4. The diameter of the inhibition zone of the solvent fractions

Fractions concentrations (mg/mL)	The inhibition zone (mm)		
	n-heksan	Ethyl acetate	Water
50	-	-	6.83
100	7.89	6.94	8.11

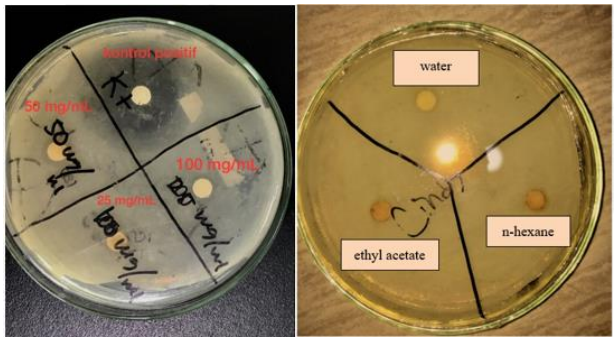

Figure 1. Comparison of antibacterial activity among the fractions (right); initial antibacterial activity assay of water fraction (left)

Results of Phytochemical Screening and Determination of Active Fractions

The fractionation results are shown in Table 3. Initially, the antibacterial activity assay shows that both the ethyl acetate and n-hexane fractions were less effective at inhibiting *Escherichia coli* growth. Contrariwise, the water fraction shows strong inhibition activity. The result is shown in Table 4 and Figure 1. The water fraction contains flavonoids, tannins, alkaloids, and steroids. Tannins and flavonoids are known to have antibacterial activity because of their polarity. Based on the research conducted by Sabir (2005) are knowing that the hydroxyl groups present in the flavonoid structures not only cause changes in both nutrient transport and organic components but also caused toxic effects on bacteria. Moreover, the study of tannin effect incorporated into hydrogel-based nanoparticles shows various mechanisms in inhibiting bacteria such as inhibition of cell wall synthesis, iron chelation, disrupting cell membranes, and inhibiting of the biosynthetic pathway of fatty acids (Farha et al, 2020). Hence, tannin obtained from the extract of *Musa balbisiana* showed positive results in inhibited *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acnes*.

Steroids are another classification of secondary metabolites. They also act as antibacterial by inhibiting bacterial growth, it interacts with the permeable phospholipid membrane of bacterial cells, which can cause the integrity of the cell membrane to decrease, resulting in cell lysis. Cantaloupe (*Cucumis melo* L. var. cantalupensis) contains steroids that have antibacterial properties against the growth of *Escherichia coli* bacteria (Anggraini et al, 2019). Alkaloids can act as antibacterial (Evan and Cowans, 2016), its damage the bacterial cell walls by impairing the peptidoglycan constituent components

therefore the formation of bacterial cell walls is inhibited which causes bacterial cell death (Sari et al, 2019).

Antibacterial Activity Assay of the Matoa Seeds Active Fraction

Based on the previous test, it was found that the active fraction inhibiting *Escherichia coli* was the water fraction. Hence, the water fraction became the fraction to be tested as an antibacterial. Table 5 and Figure 2 show the largest and smallest inhibition zone has been observed at 500 mg/mL, and 100 mg/mL, respectively. This result aligns with the literature which states the concentration is directly proportional to the inhibition produced, in other words, the inhibition obtained will be higher if the concentration used is high (Amrie et al., 2014).

The positive control used was the antibiotic Ciprofloxacin 5µg which produced an inhibition zone with an average of 39 mm. Based on CLSI (the Clinical and Laboratory Standards Institute), standards for the activity of Ciprofloxacin 5 µg against *Enterobacteraleas* bacteria grouped based on the inhibition zone divided into 3 categories, namely susceptible, intermediate, and resistant. Classification as susceptible if the diameter of the inhibition zone formed was ≥31 mm, intermediate if it was 21 to 20 mm in diameter, and resistant if it was ≤20 mm in diameter. Overall, the positive control is categorized as a susceptible category based on the diameter of the inhibition zone formed has an average of ≥31 mm. The results of the diameter of the inhibition zone in this test were adjusted to the CLSI standard for Ciprofloxacin 5 µg on *Enterobacterales* so that the overall concentration of the active fraction of Matoa seeds is equivalent to the resistant category.

Table 5. Inhibition Zone Diameter of the Matoa seeds Active Fraction against *Escherichia coli.*

Water fractions concentrations (mg/mL)	The inhibition zone (mm)	CLSI Ciprofloxacin 5 µg category (2020)	Greenwood category
100	8.89	*Resistant*	No drag
200	10.18	*Resistant*	Week
300	10.34	*Resistant*	Week
400	10.84	*Resistant*	Week
500	11.76	*Resistant*	Week
DMSO 10%	5.5	*Resistant*	No drag
Positive control Ciprofloxacin 5µg/µl	39.00	*Susceptible*	Strong

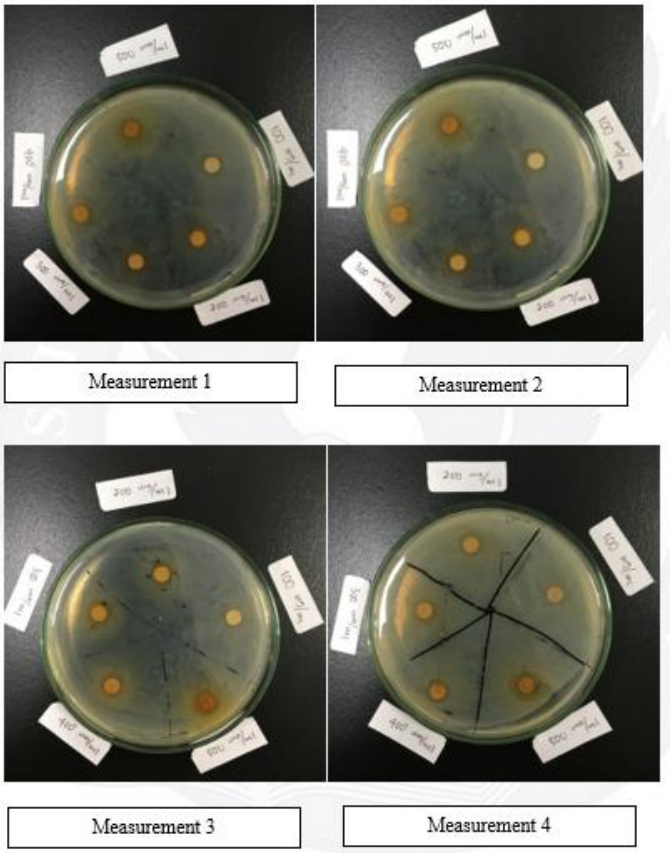

Figure 2. The inhibition zone diameter of the Matoa seeds active fraction against *Escherichia Coli* with four times measurement.

Minimum Inhibitory Concentration (MIC) by Liquid Dilution Method

The liquid dilution method was used to determine the minimum inhibitory level. The active fraction was prepared in five concentrations 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL. Further, the concentrations of 6.25 mg/mL, 12.5 mg/mL, and 25 mg/mL showed a very cloudy color aligned with negative controls. Conversely, the concentration of 50 mg/mL showed clearer color aligned to the positive controls indicating there is inhibition of bacterial growth. Hence, it can be concluded that the smallest concentration that can inhibit bacterial growth is a concentration of 50 mg/mL. The MIC result is shown in the Table 6 and Figure 3.

Based on the CLSI (the Clinical and Laboratory Standards Institute) standards, the activity of ciprofloxacin against Enterobacteraleas bacteria is grouped into 3 categories, namely susceptible, intermediate, and resistant rooted in the concentration used to determine Minimum Inhibitory Concentration (MIC). Classification as susceptible, intermediate, and resistant if MIC concentration achieved ≤0.06 µg/mL, 0.12 to 0.5µg/mL, and 1 µg/mL, respectively. Grouping of MIC was also carried out by (Sartorrato, 2004) who stated that strong, medium, and weak activity if MIC concentration achieved 0.05-0.50 mg/mL, 0.6-1.50 mg/mL, and 1.50 mg/mL, respectively.

Table 6. The Minimum Inhibitory Content of the Matoa Seeds Active Fraction gainst *Escherichia coli*

Concentrations (mg/mL)	Observation result	CLSI Ciprofloxacin (2020)	MIC Interpretation (Sartoratto, 2004)
6.25	cloudy	*Resistant*	week
12.5	cloudy	*Resistant*	week
25	cloudy	*Resistant*	week
50	cloudy but slightly clear	*Resistant*	week
100	cloudy but slightly clear	*Resistant*	week
negative control	cloudy	*Resistant*	-
positive control Ciprofloxacin 5µg/µl	transparent	*Susceptible*	strong

The normality test was carried out using the Shapiro-Wilk technique and the homogeneity test with the Barlet test technique. The result of subsequently mention is p> 0.05, hence it can be concluded that the data is normally distributed and homogeneous. The results of the analysis of the entire data using One Way ANOVA get a significance value of 0.001 (p≤0.05), hence, it can be concluded that there is a significant difference between the concentrations of the active fraction used in the inhibition zone against *Escherichia coli*. The data analysis then continued with the Tukey HSD Post Hoc and the concentration of 500 mg/mL was concluded as the optimum concentration.

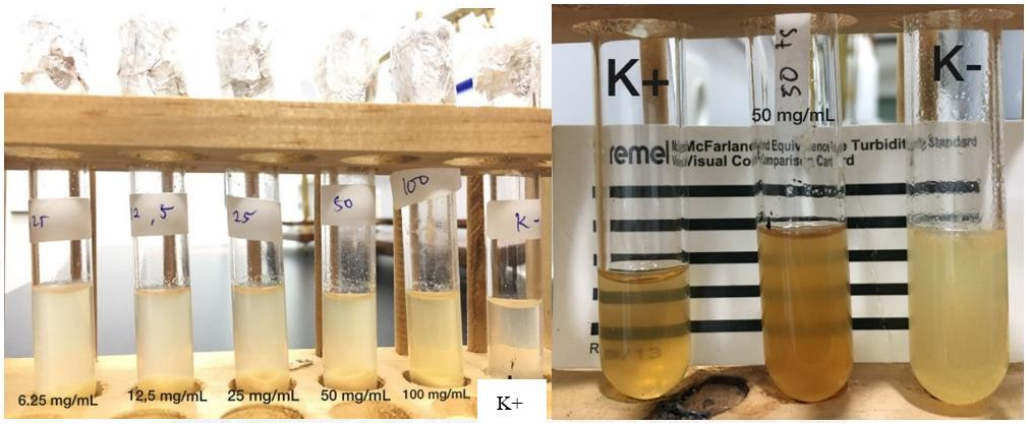

Figure 3. MIC of the Matoa active fraction

CONCLUSION

Overall, an 11,47 % yield of 96% ethanol extract was obtained and fractioned in three different solvents which were n-hexane, ethyl acetate, and water. All fractions contain metabolite secondary, such as terpenoids, steroids, alkaloids, tannins, saponins, and flavonoids. Further, the water fraction was then observed as an active fraction, and the antibacterial activity of this fraction was determined. The result

of inhibition zones in concentrations of 500 mg/mL, 400 mg/mL, 300 mg/mL, 200 mg/mL, and 100 mg/mL were 1,176 cm, 1,084 cm, 1,034 cm, 1,018 cm dan 0,889 cm, respectively. Moreover, the Minimum Inhibitory Concentration (MIC) was obtained at 50 mg/mL.

ACKNOWLEDGEMENT

Acknowledgments are given to LPPM Pelita Harapan University due to fund this research in No. P-48-FIKes/XII/2021 and the Faculty of Health Sciences, Universitas Pelita Harapan to support this research in various aspects, especially facilities and places.

CONFLICT OF INTEREST

The manuscript has been seen by all co-authors, and they agree with the contents. Moreover, there is no financial interest conflict. We certify that the submission is original work.

REFERENCES

Anggraini, W., Nisa, S. C., Ramadhani Da, R., & Ma'arif ZA, B. (2019). Aktivitas Antibakteri Ekstrak Etanol 96 % Buah Blewah (Cucumis melo L. var. cantalupensis) Terhadap Pertumbuhan Bakteri Escherichia coli. *Pharmaceutical Journal of Indonesia*, *5*(1), 61–66.

Amrie, A., Ivan, S., Anam., & Ramadhanil. (2014). Uji Efektivitas Ekstrak Daun dan Akar Harrisonia perforata Merr. Terhadap pertumbuhan Bakteri Vibro chlorae. *Journal of Science and Technology*. Vol 3 (3).

Brooks G. F, Janet S. B, Stephen A.M, Jawetz, Melnick, & Adelbergs. (2005). *Mikrobiologi Kedokteran Buku 1* (E. Mudihardi, Kuntaman, Wasito E.B, Mertaniasih N. M, Harsono S, & Alimsardjono L, Eds.). Jakarta: Salemba Medika.

CLSI (Clinical and Laboratory Standars Institute). (2020). *Performance Standars for Antimicrobial Susceptiblity Testing 30 th Ed.* Diakses pada 7 Maret 2022.

Erlyn, P. (2016). Efektivitas Antibakteri Fraksi Aktif Serai (Cymbopogon citratus) terhadap Bakteri Streptococcus mutans. *Syifa Medika Jurnal Kedokteran dan Kesehatan*. 6 (2). 111-125.

Evans, S. M., & Cowan, M. M. (2016). Plant products as antimicrobial agents. *Cosmetic and Drug Microbiology*, *12*(4), 205–231.

Farha, A. K., Yang, Q. Q., Kim, G., Li, H. Bin, Zhu, F., Liu, H. Y., Gan, R. Y., & Corke, H. (2020). Tannins as an alternative to antibiotics. *Food Bioscience*, *38*(September), 100751.

G. Maria Theopanny D. (2019). *Uji Aktivitas Antibakteri Ekstrak N-Heksana, Etil Asetat, Serta Etanol 96% Dari Daun Matoa (Pometia Pinnata J.R & G.Fors) Terhadap Propionibacteri Acnes Dan Escherichia Coli* (Unpublished undergraduate thesis), Unversitas Sumatera Utara, Indonesia.

Jahan, F., Lawrence, R., Kumar, V., & Junaid, M. (2011). Evaluation of antimicrobial activity of plant extracts on antibioticsusceptible. *Journal of Chemical and Pharmaceutical Research*, 777-789.

Ngajow, M., Abidjulu, J., & Kamu, V. S. (2013). Pengaruh Antibakteri Ekstrak Kulit Batang Matoa (*Pometia pinnata*) terhadap Bakteri *Staphylococcus aureus* secara In vitro. *Jurnal Mipa Unsrat Online*. 2(2). 128-132.

Pakpahan, D.T & Sutriningsih. (2020). Uji Aktivitas Antibakteri Fraksi n-heksana, Etil asetat, dan Butanol Daun Petai Cina (*Leucaena leucocephala* (*Lam*.) *de Wit*) terhadap Bakteri *Propionibacterium acnes* dan *Staphylococcus epidermidis* Secara In Vitro. *Indonesia Natural Research Pharmaceutical Journal*. 5(2). 12-19.

Parashar, U. D, Hummelman, E. G, Bresee, J.S, Miller, M. A, & Glass, R. I. (2003). Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Disc*. 9(5). 565–572.

Rahayu, W. P., Nurjanah, S., & Komalasari, E. (2020). *Escherichia Coli: Patogenitas, Analiss dan Kajian Risiko*. Bogor: IPB Press.

Sari, R. C., Wijayanti, I., & Agustini, T. W. (2019). The Effectiveness of Melanin from Squid Ink (Loligo sp.) as Antibacterial Agent Against Escherichia coli and Listeria monocytogenes. *IOP Conference Series: Earth and Environmental Science*, *246*(1).

Sabir, A. (2005). Aktivitas Antibakteri Flavonoid Propolis Trigono sp terhadap bakteri *Streptococcus mutans* (in vitro). *Majalah Kedokteran Gigi*. 38(3). 135.

Setiawan, M., Retroningrum,M.,Yahya F. (2020). Aktivitas Antibakteri Ekstrak Biji Jeruk Siam (*Citrus reticulata*) pada Bakteri *Escherichia coli. Jurnal Bioteknologi & Biosains Indonesia*. 2(7). 289-295.

Tripathi, K. D. (2003). Antimicrobial Drugs: General Consideration. Essentials of Medical Pharmacology. Fifth Edition. *Jaypee Brothers Medical Publishers*.

Utami, E. R. (2011). Antibiotika, Resistensi, dan Rasionalitas Terapi. In *El-Hayah Jurnal Biologi*. 1(4). 124-138.