Jurnal Penelitian Farmasi Indonesia
p-ISSN : 2302-187X | e-ISSN: 2656-3614
Homepage: https://ejournal.stifar-riau.ac.id/index.php/jpfi/
Vol. 14, No. 1, Hal. 10-15

RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY TEST OF METHANOL EXTRACT OF PULAI BASUNG STEM BARK (*Alstonia spatulata*) AGAINST *Escherichia coli* AND *Staphylococcus aureus*

Maslakha[1], Hilwan Y. Teruna[2*], Rudi Hendra[2]

[1]Postgraduate student, Department of Chemistry, Faculty of Mathematic and Natural Science, University of Riau, Pekanbaru

[2]Department of Chemistry, Faculty of Mathematic and Natural Science, University of Riau, Pekanbaru, 28293

*e-mail Correspondence: hyteruna@lecturer.unri.ac.id

Article History

Received:
2 Mei 2025

Accepted:
11 September 2025

Published:
31 Oktober 2025

ABSTRAK

Infeksi bakteri merupakan penyebab utama infeksi kronis yang terus mengancam kesehatan masyarakat. Penggunaan antibiotik yang tidak tepat merupakan salah satu penyebab utama terjadinya resistensi mikroorganisme patogen, sehingga pengobatan menjadi tidak efektif. Adanya peningkatakan prevalensi patogen yang resisten terhadap obat, maka diperlukan pengembangan obat yang manjur sebagai agen potensial antibakteri yang bersumber dari tumbuhan. Penelitian ini dilakukan untuk mengetahui aktivitas antibakteri fraksi aktif dari ekstrak metanol tumbuhan pulai basung (*Alstonia spatulata*) terhadap bakteri *Escherichia coli* dan *Staphylococcus aureus*. Kulit batang pulai basung diekstraksi dengan cara maserasi dengan pelarut metanol. Ektrak metanol diekstraksi dengan metode ekstraksi asam basa. Ekstrak metanol dilarutkan dengan NaOH hingga pH 9 dan ditambahkan HCl hingga pH 2. Fraksinasi dilakukan dengan metode cair-cair dengan pelarut *n*-butanol. Fraksi *n*-butanol pH 2 (sampel 2) dan pH 9 (sampel 1) diuji aktivitas antibakterinya dengan metode difusi cakram dengan konsentrasi 10, 100, 500 dan 1000 ppm. Hasil pengujian antibakteri menunjukkan bahwa hanya sampel 2 yang mempunyai aktivitas antibakteri terhadap bakteri *E. coli* dan *S. aureus*. Sampel 2 pada konsentrasi 500 dan 1000 ppm memiliki diameter zona hambat berturut-turut sebesar 10,50 mm dan 16,25 mm terhadap bakteri *E. coli*, dan *S. aureus* pada konsentrasi 100, 500 dan 1000 ppm berturut-turut 7,50 mm; 10 mm dan 15,50 mm.

Kata kunci: *Alstonia spatulata*, antibakteri, difusi cakram

ABSTRACT

Bacterial infections are the main cause of chronic infections that continue to threaten public health. Inappropriate use of antibiotics is one of the main causes of resistance among pathogenic microorganisms, resulting in ineffective treatment. With the increasing prevalence of drug-resistant pathogens, it is necessary to develop potent drugs as potential antibacterial agents from plant sources. The goal of this study was to find out how effective the active part of the methanol extract from pulai basung (*Alstonia spatulata*) is at fighting the bacteria *Escherichia coli* and *Staphylococcus aureus*. Pulai basung stem bark was extracted by maceration with methanol solvent. Methanol extract was extracted by the acid-base extraction method. Methanol extract was dissolved with NaOH to pH 9, and HCl was added to pH 2. Fractionation was carried out by the liquid-liquid partition method with *n*-butanol solvent. The *n*-butanol fractions at pH 2 (sample 2) and pH 9 (sample 1) were tested for antibacterial activity by the disc diffusion method with concentrations of 10, 100, 500, and 1000 ppm. The results of the antibacterial test showed that only the sample 1 indicated an antibacterial activity against *E. coli and S. aureus*. The sample 2 at concentrations of 500 and 1000 ppm demonstrated an inhibition zone diameter of 10.50 mm and 16.25 mm against *E. coli* and *S. aureus* at concentrations of 100, 500 and 1000 ppm; it showed diameters of 7.50 mm; 10.00 mm and 15.50 mm, respectively.

Keywords: *Alstonia spatulata, antibacterial, disc diffusion*

INTRODUCTION

Infectious diseases are one of the main causes of world health problems that continue to grow from time to time, especially in tropical areas such as Indonesia. Dusty air, warm temperatures, and high humidity levels are optimal conditions for microbes to thrive (Katrin *et al.*, 2015; Rahman *et al*., 2023). Infectious diseases can be caused by bacteria. Some bacteria that often attack humans can cause various health problems, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Vibrio parahaemolyticus.* Bacteria can infect through various pathways, either through food, a poor environment, or even surrounding objects. The impact of these bacterial infections can include eye infections, acute diarrhea, meningitis, endocarditis, and even death (Desai *et al*., 2019; Esfahanian *et al.*, 2019; Ramamurthy and Nair, 2014) . In addition, inappropriate use of antibiotics is also one of the main causes of resistance among pathogenic microorganisms, resulting in ineffective treatment.

The World Health Organization reports that 1.27 million people died directly and 4.95 million people died indirectly worldwide in 2019 due to infectious diseases caused by microorganisms and antimicrobial resistance (Ranjbar and Alam, 2024). In 2019, the U.S. Department of Health and Human Services CDC reported that approximately 35,000 people die each year from bacterial and fungal infections accompanied by antimicrobial resistance (Flynn and Guarner, 2023). Antibiotic-resistant microorganisms contribute to increased mortality, as they are able to survive and recover through their ability to acquire and transmit resistance after exposure to antibiotic drugs (Marchese *et al.*, 2016). With the increasing prevalence of drug-resistant pathogens, it is necessary to develop potent drugs as potential antibacterial agents from plant sources.

The World Health Organisation (WHO) states that around 80% of the world's population is estimated to use traditional medicines derived from various plants because of the synergy of various secondary metabolite components contained in plants that are widely produced and needed by the body as a source of natural medicine (Altemimi *et al.*, 2017).

Many phytochemicals from plants have been shown to have antibacterial properties; the content of such phytochemicals can serve as a potential source for the development of new antibacterial agents. Combining traditional medicine with modern pharmacological research presents a significant opportunity to identify active compounds with novel therapeutic benefits. One such medicinal plant is *Alstonia spatula*.

A. spatulata, locally known as pulai basung, is widely distributed in Southeast Asia, including Indonesia, Malaysia and Thailand. This plant is traditionally used by the community to treat malaria, dysentery, fever, diarrhoea and diabetes (Dey, 2011; Pratap *et al.*, 2013). Although pharmacological research on *A. spatulata* remains limited, recent studies by Gumula *et al.*, (2024) have begun to explore the plant's biological potential. As a comparison within the *Alstonia* genus, *A. boonei* exhibits significant antibacterial effects: the ethanol root extract inhibits the growth of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*, with minimum inhibitory concentrations (MIC) as low as 0.39 mg/ml for *C. albicans* and 0.98 mg/mL for *S. aureus*. Ethanol root extracts showed inhibition zones of 5-10 mm against both Gram-positive and Gram-negative bacterial at a concentration of 10 mg/mL. In addition, pure compounds such as trans-fagaramide and lupeol were isolated from *A.boonei* leaves, demonstrating MICs of 125 µg/mL against *P. aeruginosa* and 250 µg/mL against *S. aureus, S. typhi* and *E. coli*. Therefore, with previous research on the *Alstonia* genus covering its excellent antibacterial activity and low MIC value, there is a strong scientific basis for further research on *A. spatulata*. Given its taxonomic similarity and traditional use within the same genus, *A. spatulata* has high potential as an antibacterial agent worthy of exploration.

Based on phytochemical analysis, plants of the genus *Alstonia* contain active compounds, such as steroids, flavonoids, terpenoids, saponins, and alkaloids (Zuraida and Sulistiyani, 2020). These compounds exhibited various activities, such as cytotoxic, anti-inflammatory, anti-microbial (antifungal and antibacterial), antidiabetic, and others (Zhao *et al.*, 2023).

The use of pulai basung bark *(A. spatulata)* for testing antibacterial activity in this plant is described above. Therefore, this study was conducted to evaluate the antibacterial activity of methanol extract fractions of *A. spatulata* bark against *E. coli* and *S. aureus* bacteria. This study is important given the increasing resistance of bacteria to conventional antibiotics, therefore the development of new antibacterial agents. The results of this study are expected to contribute scientifically to development of new antibacterial drugs derived from plants and potentially serve as alternative antibacterial agents.

RESEARCH METHODS

This research is a type of experimental research to determine the antibacterial activity of the active fraction of the methanol extract from the stem bark of *A. spatulata* against *Escherichia coli* and *Staphylococcus aureus* bacteria. The research was conducted at the Chemistry Laboratory of Riau University.

Tools

The tools used in this research are laminar air flow (BBS-V800), autoclave (All America model 1925/KY-23D), analytical balance, rotary evaporator (Heidolph 2000), separatory funnel (Pyrex), incubator (Heraeus instrument), micropipettes and tip (Eppendrorf), measuring cup (Pyrex), erlenmenyer (Pyrex), beaker (pyrex), test tube (Pyrex), drop pipette (Pyrex), ose needle (Mico), petri dish (Pyrex), vial, and sterile cotton swab (Onemed).

Materials and Chemicals

The materials used in this study were pulai basung (*Alstonia spatulata*) bark samples, aluminium foil, *n*-butanol, methanol PA (*Merck*®), distilled water, sodium chloride (NaCl 0.9%), Nutrient Agar (NA) (*Merck*®) media, Mueller Hinton Broth (MHB) (*Merck*®), pure cultures of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538 bacteria, paper discs (Macherey-Nagel), positive

control (chloramphenicol 250 mg), negative control (distilled water) and tissue.

Procedure

Extraction of A. spatulata stem bark

The extraction process begins with the maceration method. The bark of the *A. spatulata* plant was cleaned, then dried without direct sunlight but with good air flow, after which it was mashed. The powder that has been mashed is macerated with methanol solvent for 3x24 hours until the macerate obtained is no longer coloured. The macerate was evaporated with a rotary evaporator at a temperature of ± 40ºC so that a thick brown-black methanol extract weighing 225 grams was obtained (Risal, 2023)

The viscous extract was extracted by a modified acid-base extraction method (Fadhli *et al.*, 2012). Some 20 grams of extract were dissolved in 5 N NaOH first, then the residue was filtered using filter paper to obtain a base layer with pH 9. The base layer was mixed with 5N HCl to lower the pH to 2, and then it was separated using n-butanol in a 2:1 ratio with water. The resulting n-butanol layer was evaporated using a rotary evaporator to obtain the n-butanol fraction pH 9 weighing 20 mg, while the n-butanol fraction pH 2 was 15 mg, so that fractions obtained were in a neutral state. The n-butanol fraction pH 9 was named sample 1 and pH 2 was named sample 2.

Sterilization and media preparation

The glassware used, such as Petri dishes, test tubes, and tweezers, was washed and dried, then wrapped using paper and aluminum foil and sterilized using an autoclave at 121ºC for 15 minutes. After sterilization, all tools were stored in laminar air.

Nutrient Agar (NA) and Mueller Hinton Broth (MHB) media were used in this study to facilitate bacterial growth. A total of 2.1 g MHB was suspended in 100 ml distilled water, and 5 g NA was suspended in 250 ml distilled water. Both media formulations were sterilized by autoclave.

Antibacterial activity test

Escherichia coli ATCC 25922 and *Staphylococcus aureus* ATCC 6538 bacteria were cultured on NA agar plates using the cross-scratch method. The rejuvenated bacterial cultures were incubated for 24 hours at 37ºC. Then a bacterial suspension was made by taking one ose of bacteria from the slanted agar and inserting it into 10 ml of MHB, incubating for 24 hours at 37ºC.

The antibacterial activity test was carried out using the disc diffusion method (Khotimah *et al.*, 2021). The first stage; the bacterial suspension that has been made is added with 0.9% NaCl until the turbidity level is the same as the McFarland standard. The concentration of each fraction was taken 2 mg and dissolved in 2 ml of methanol PA in different vials to make a concentration of 1000 ppm. Then diluted into 3 different concentrations, 500, 100 and 10 ppm, for positive control using *chloramphenicol* with concentrations of 1000 and 100 ppm and negative control using distilled water. Then pour sterile NA into a Petri dish as much as 10 ml, and let it stand until it hardens. Bacterial suspensions that are in accordance with the standard are spread on the surface of NA media using a sterile cotton swab until evenly distributed. Disc paper that has been dripped with 20 µl of test material was placed on the surface of the media and incubated for 24 hours at 37ºC. Observe the inhibition zone formed, which is marked by a clear zone, and measure using a transparent ruler with units of mm (Putri and Kurniatuhadi, 2023).

RESULTS AND DISCUSSIONS

Extraction of pulai basung stem bark

The results of the partition of methanol extract of pulai basung stem bark using *n*-butanol solvent obtained two *n*-butanol fractions. These two fractions obtained are already in a neutral state, and these two samples are named sample 1, weighing 20 mg and sample 2, as much as 15 mg.

Antibacterial activity test

The results of the antibacterial activity test found that the *n*-butanol fraction at sample 2 has antibacterial activity against *E. coli* and *S. aureus* bacteria characterised by the presence of a clear zone, which can be seen in **Figure 1**. The results of the inhibition zone against *E. coli* and *S. aureus* bacteria can be seen in **Table 1.**

The antibacterial test of the active fraction of the methanol extract of pulai basung bark against *Escherichia coli* and *Staphylococcus aureus* bacteria began with the preparation of the tools and materials used. The media and tools used are sterilized first in an autoclave so that when growing test bacteria, they are not contaminated by the growth of other microorganisms.

The production of the test bacterial suspension was carried out by adding 0.9% NaCl, and then the suspension was compared with the *McFarland* standard, which aims to adjust or equalise the turbidity of the suspension bacteria. Then the test bacteria were spread into the solid media using a sterile cotton swab evenly.

The method of testing antibacterial activity was carried out using the disc diffusion method with the working principle of the test material being saturated into paper discs. Each paper disc is dripped with

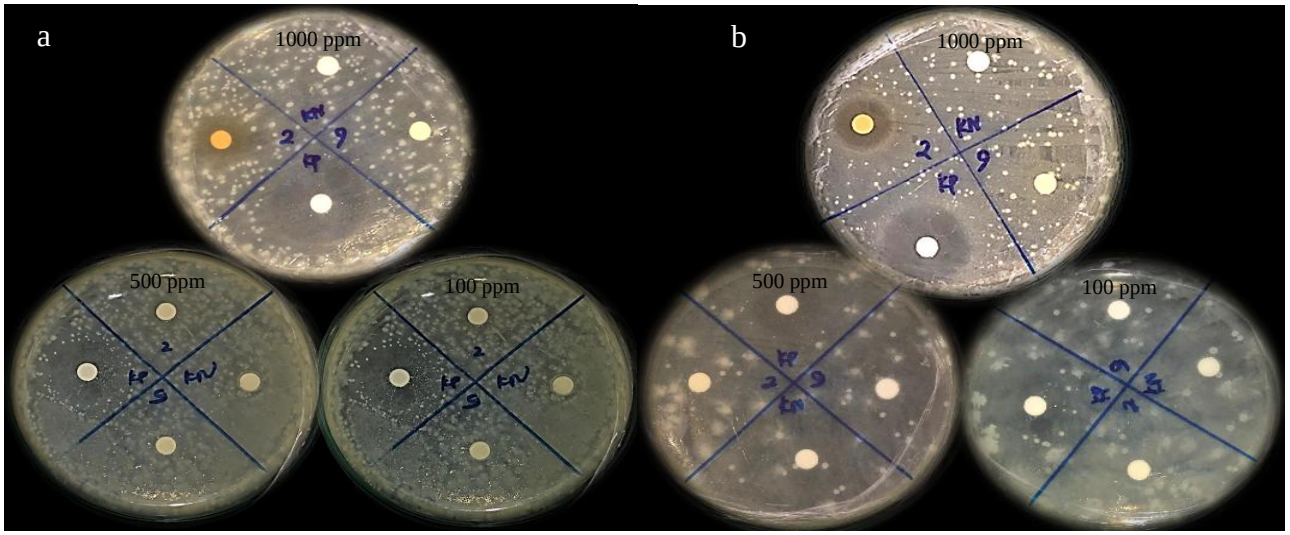

Figure 1. The inhibition zone of *n*-butanol fraction pH 9 (sample 1), pH 2 (sample 2), control positive (KP), and control negative (KN) at a concentration of 1000 ppm against (a). *E. coli*; (b). *S. aureus*

Table 1. Antibacterial activity test results

Sample	Zone Inhibition (mm)							
	E. coli				***S. aureus***			
	10 ppm	100 ppm	500 ppm	1000 ppm	10 ppm	100 ppm	500 ppm	1000 ppm
Sampel 1	-	-	-	-	-	-	-	-
Sampel 2	-	-	10.50	16.25	-	7.50	10	15.50
Chloramphenicol		15.50		23.50		11.50		24
Aquadest			-				-	

sample 1 and sample 2 fractions (samples in a neutral state and no pH influence on the test), chloramphenicol and sterile distilled water; wait until it absorbs and dries. Then place each paper disc on the media with a predetermined distance; this approach aims not to affect the inhibition zone area formed and facilitate the measurement of the inhibition zone. Thereafter, incubate at 37ºC for 24 hours, and measure the clear zone formed.

Figure 1 illustrates that the presence or absence of clear zones on bacterial growth in solid media indicates antibacterial activity. The results showed that some active fractions of the stem bark of *Alstonia spatulata* have antibacterial activity. Such activity is indicated by the formation of an inhibition zone characterized by the presence of a large clear zone against *E. coli* and *S. aureus* bacteria.

The results of the inhibition zone on positive controls using chloramphenicol showed the presence of inhibition zones with a forceful category at a concentration of 1000 ppm and at a concentration of 100 ppm, a medium category with an average diameter of 23.75 mm and 13.50 mm, respectively. The use of positive control is to show that the test is accurate and produces changes characterized by the formation of an inhibition zone on the disc paper (Utami *et al.*, 2020). The negative control using distilled water did not form an inhibition zone on the disc paper. The use of distilled water in the negative control is because distilled water is a solvent that does not have antibacterial activity, so it has no effect on bacterial growth (Putri and Kurniatuhadi, 2023).

Based on the data in **Table 1**, the largest inhibition zone was produced by *n*-butanol fraction at sample 2 against *E. coli* bacteria at concentrations of 1000 ppm, 500 ppm, 16.25 mm, and 10.50 mm, respectively, and the inhibition zone against *S. aureus* bacteria at concentrations of 1000 ppm, 500 ppm, and 100 ppm, 15.50 mm, 10 mm, and 7.50 mm, respectively. In general, the inhibition zone formed shows that the average diameter of the inhibition zone increases as the concentration given increases. These results are in accordance with the reference which states that the higher the concentration of the fraction, the larger the inhibition zone formed against the test bacteria. This increase in antibacterial activity is closely related to the content of secondary metabolites present in the fraction. Secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids,

and phenolics are known to have the ability to disrupt the integrity of bacterial cell walls or membranes, inhibit protein synthesis, or interfere with the activity of essential bacterial enzymes (Cowan, 1999; Evans and Cowan, 2016; Cushnie and Lamb, 2005). The higher the concentration of the fraction the more metabolites it contains, and thus the more active compounds are available to inhibit or kill bacteria growth (Michael *et al*., 2005). According to the level of antibacterial strength, with an inhibition zone of 0-5 mm, it is categorized as having weak inhibition; 5-10 mm, as moderate; 10-20 mm, as strong; and more than 20, as forceful. Thus, the *n*-butanol fraction at sample 2 at a concentration of 1000 ppm has an inhibitory response with a strong category; at concentrations of 500 and 100 ppm, it is categorized as moderate, and at a concentration of 10 ppm, it is categorized as weak (Morales *et al.*, 2003; Sanam *et al*., 2022). These results suggest that the *n*-butanol fraction at sample 2 has the potential to be further investigated to ascertain the agent responsible for the antibacterial properties detected. It is imperative to purify and separate the active compounds present in this fraction.

CONCLUSIONS

Based on the results of the research conducted, it can be concluded that the extract from the stem bark of *A. spatulata* has antibacterial activity against *E. coli* and *S. aureus* bacteria. The most active fraction from the test results is the *n*-butanol fraction at sample 2, which is characterised by the presence of inhibition zones formed in the antibacterial activity test against the bacteria *E. coli* and *S. aureus*. *n*-butanol fraction sample 2 with a concentration of 100 ppm is the lowest concentration that can inhibit the growth of *S. aureus* bacteria and a concentration of 500 ppm against *E. coli* bacteria. In order to gain a comprehensive and effective understanding by utilizing the antibacterial properties of *A. spatulata*, it is imperative to purify and separate the active constituents present in the fractions. To confirm the exact agents responsible for the detected antibacterial properties, it is imperative to identify and isolate these active compounds. Using the bioactive components of *Alstonia spatulata*, this procedure will not only elucidate the underlying mechanism of action but also facilitate the development of targeted antibacterial therapies.

ACKNOWLEDGMENTS

The authors would like to express their sincere appreciation to the Research and Community Development Centre of Riau University and the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for their indispensable assistance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in writing this article.

REFERENCES

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G. and Lightfoot, D.A. 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, **6(4)**.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, **12(4)**: 564-582.

Cushnie, T.P.T. and Lamb, A.J. 2005. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, **26(5)**: 343-356.

Desai, S., Sanghrajka, K. and Gajjar, D. 2019. High adhesion and increased cell death contribute to strong biofilm formation in *Klebsiella pneumoniae*. *Pathogens*, **8(4)**: 277.

Dey, A. 2011. *Alstonia scholaris* R.Br. (Apocynaceae): Phytochemistry and pharmacology: A concise review. *Journal of Applied Pharmaceutical Science*, **1(6)**: 51–57.

Esfahanian, E., Adhikari, U., Dolan, K. and Mitchell, J. 2019. Construction of a new dose–response model for *Staphylococcus aureus* considering growth and decay kinetics on skin. *Pathogens*, **8(4)**: 253.

Evans, S.M. and Cowan, M.M. 2016. Plant products as antimicrobial agents, in *Cosmetic and Drug Microbiology*.

Fadhli, H., Teruna, H.Y. and Jose, C., 2012. Uji toksisitas ekstrak kulit batang pulai basung (*Alstonia spatulata* BL) dengan metode brine shrimp lethality test. *J. Ind. Che. Acta*., **3**: 1.

Flynn, C.E. and Guarner, J. 2023. Emerging antimicrobial resistance. *Modern Pathology*, **36(9)**: 100249.

Gumula, I., Kyarimpa, C., Nanyonga, S.K., Kwesiga, G., Busulwa, G., Opio, B., Heydenreich, M. and Omara, T. 2024. Antibacterial properties of phytochemicals isolated from leaves of alstonia boonei and aerial parts of *Ipomoea cairica*. Natural Product Communications, **19(9)**.

Katrin, D., Idiawati, N. and Sitorus, B. 2015. Uji Aktivitas Antibakteri dari Ekstrak Daun Malek (*Litsea graciae* Vidal) Terhadap Bakteri *Stapylococcus aureus* dan *Escherichia coli*. *Jurnal Kimia Khatulistiwa*, **4(1)**: 7–12.

Khotimah, T.K., Krisridwany, A., Orbayinah, S. and Harimurti, S., 2021. Antibacterial Activity of Fractions from Extract Ethanolic of *Hylocereus Polyrhizus* Peel Against *E. coli* and *S. aureus*. *Journal of Fundamental and Applied Pharmaceutical Science*, **1(2)**: 65-71.

Marchese, A., Barbieri, R., Sanches-Silva, A., Daglia, M., Nabavi, S.F., Jafari, N.J., Izadi, M., Ajami, M. and Nabavi, S.M. 2016. Antifungal and antibacterial activities of allicin: A review. *Trends in Food Science & Technology*, **52**: 49-56.

Morales, G., Sierra, P., Mancilla, A., Paredes, A., Loyola, L.A., Gallardo, O. and Borquez, J., 2003. Secondary metabolites from four medicinal plants from northern Chile: antimicrobial activity and biotoxicity against *Artemia salina*. *Journal of the Chilean Chemical Society*, **48(2)**: 13-18.

Pratap, B., Chakraborthy, G.S. and Mogha, N. 2013. Complete aspects of *Alstonia scholaris*. *International Journal of PharmTech Research*, 5(1): 17–26.

Putri, M., W, E.R.P. and Kurniatuhadi, R. 2023. Potensi Ekstrak Metanol Akar dan Batang Kratom (*Mitragyna speciosa* Korth.) Sebagai Antibakteri *Propionibacterium acnes* ATCC 6919 Penyebab Jerawat. *Protobiont*, **12(2)**: 43-49.

Rahman, I.W., Arfani, N. and Tadoda, J.V. 2023. Deteksi Bakteri MRSA Methicillin-Resistant *Staphylococcus aureus* pada Sampel Darah Pasien Rawat Inap. *Jurnal Ilmu Alam dan Lingkungan*, **14(1)**: 48–54.

Ramamurthy, T. and Nair, G.B. 2014. Bacteria: Vibrio parahaemolyticus, *Encyclopedia of Food Safety*, **1**: 555–563.

Ranjbar, R. and Alam, M. 2024. Antimicrobial Resistance Collaborators 2022. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Evidence-Based Nursing*.

Risal, D. 2023. Isolasi dan Uji Toksisitas Senyawa Metabolit Sekunder Fraksi Kloroform pH 12 dari Kulit Batang Pulai Basung *Alstonia spatulata*. *Skripsi* [Preprint].

Sanam, M.U.E., Detha, A.I.R. and Rohi, N.K. 2022. Detection of antibacterial activity of lactic acid bacteria, isolated from Sumba mare's milk, against Bacillus cereus, Staphylococcus aureus, and *Escherichia coli*. *Journal of Advanced Veterinary and Animal Research*, **9(1)**: 53-58.

Utami, R., Winarsih, D., Fernando, A., Fadhli, H., Furi, M. and Susanti, E., 2020. Uji Aktivitas Antibakteri Ekstrak dan Fraksi dari Akar dan Batang Tumbuhan Sekunyit (*Fibraurea tinctoria* Lour). JFI, **12(2)**: 105-114.

Zhao, M.X., Cai, J., Yang, Y., Xu, J., Liu, W.Y., Akihisa, T., Li, W., Kikuchi, T., Feng, F. and Zhang, J. 2023. Traditional uses, chemical composition and pharmacological activities of *Alstonia* R. Br. (Apocynaceae): A review. *Arabian Journal of Chemistry*, **16(8)**: 104857.

Zuraida and Sulistiyani 2020. Screening phytochemical compound of Alstonia scholaris R.Br in different sites in Indonesia. *IOP Conference Series: Earth and Environmental Science*, **591(1)**: 012034.