Manfish: Jurnal Ilmiah Perikanan dan Peternakan
Volume 3 Nomor 1 Tahun 2025
e-ISSN :3046-5052; p-ISSN: 3046-5338, Hal 11-17
DOI: https://doi.org/10.62951/manfish.v3i1.105
Available online at: https://journal.asrihindo.or.id/index.php/Manfish

Antibacterial Activity of Milkwood Bark Ethanol Extract (*Alstonia Scholaris* (L.) R. Br.) against *Staphylococcus Aureus* and *Escherichia Coli*

Sitti Khairul Bariyyah[1*], Muhammad Munawar Khalil[2], Rani Dewi Pratiwi[3], Elsye Gunawan[4]
[1]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Yapis Papua, Jayapura, Indonesia
[2-4]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Cenderawasih, Jayapura, Indonesia

Alamat: Jl. Dr. Sam Ratulangi No.11, Trikora, Kec. Jayapura Utara, Kota Jayapura, Papua 99113
*Author correspondence: rheea.tayang@gmail.com**

Abstract*. Milkwood bark (Alstonia scholaris (L.) R. Br.) is one of the medicinal plants of the genus Alstonia. This plant is traditionally used by the community as a medicine for diarrhea, diabetes, malaria, hemorrhoids, and antihypertensives. This plant contains secondary metabolites that have the potential as antibacterial such as alkaloids, flavonoids, saponins and tannins. The purpose of this study was to determine the inhibition of antibacterial activity and the most effective concentration in inhibiting Staphylococcus aureus and Escherichia coli. The sample used in this study was milkwood bark, the sample was extracted by maceration method using 96% ethanol as solvent. The antibacterial testing method used in this study was disc diffusion. The study used a completely randomized design with 9 treatments, namely 1000 ppm, 750 ppm, 500 ppm, 250 ppm, 100 ppm, 50 ppm, 10 ppm, Ciprofloxacin (positive control), sterile distilled water (negative control). The results of the antibacterial activity test at a concentration of 500 ppm showed that milkwood bark extract could inhibit the growth of S. aureus by 8.22 mm and at a concentration of 250 ppm showed that milkwood bark extract could inhibit the growth of E. coli by 8.75 mm. From the test results, it can be concluded that milkwood bark (A. scholaris (L.) R. Br.) has antibacterial activity that has moderate potential in inhibiting S. aureus and E. coli.*

Keywords*: Alstonia sp, Ciprofloxacin, Disc diffusion, Secondary Metabolites*

1. INTRODUCTION

The incidence of infectious diseases has continued to increase in recent years (Suoth *et al*., 2019). The use of antibacterial drugs for the treatment of infectious diseases caused by bacteria has been widely used, but the problem faced now is the occurrence of side effects for users, such as diarrhea, allergies, to other toxic hazards, as well as high consumption and high maintenance costs indicate the need for research. to develop new antibacterials, especially from natural ingredient (Sutrisno, 2014).

Milkwood (*A. scholaris* (L.) R. Br.) is one of the most common plants in Indonesia. This plant is used as a treatment for diseases such as diarrhea, diabetes, and hemorrhoids. Almost all parts of the milkwood plant are used in medicine, one of which is the bark. Milkwood bark is used as a sentry drug, tonic, colon disorders, beriberi, malaria, and antihypertensive drugs by drinking boiled water (Wiart, 2006; Zuraida *et al*., 2010). In Papua, the milkwood bark soaked water is often used by local communities as a cure for malaria and

Received: Desember 13, 2024; Revised: Januari 19, 2025; Accepted: Februari 01, 2025; Online Available: Februari 04, 2025

fever, while the Depapre community uses milkwood bark as a medicine for diarrhea (Chrystomo *et al*., 2016).

Secondary metabolites contained in the bark of the milkwood include acubins/iridoids, alkaloids, coumarins, flavonoids, leucoanthocyanins phlobatannis, reducing sugars, simple phenolics, steroids, saponins, and tannins that can be used as antimalarials and antibacterials (Khyade & Vaikos, 2009; Gunawan & Simaremare, 2016). Based on the antibacterial activity test of the n-hexane extract of the bark of *A. scholaris* (L.) R. Br. against human pathogenic bacteria *E. bacteria, Shigella dysentery, Enterobacter cloacae, Serratia marcescens*. The test results showed that the prevention of bacterial growth was 85.7%, 95.6%, 89.3%, and 94.4%, respectively. In addition, Pankti *et al*., (2012) stated that the antibacterial activity of the total alkaloid extract from the bark was able to fight two types of gram-positive bacteria and 4 types of gram-negative bacteria using the agar diffusion method.

Based on the research literature above, the researcher is interested in conducting further research by testing the antibacterial activity of the ethanol extract of the Milkwood Bark (*A. scholaris* (L.) R. Br.) against *S. aureus* and *E. coli*.

2. MATERIALS AND METHODS

Preparation of Milkwood Bark Extract

Milkwood bark samples were obtained from the Depapre area, Sentani, Papua. It is separated from the outer bark, washed, cut into small pieces, dried and mashed using a blender. The milkwood bark extraction process is carried out by the maceration method. A total of 500 grams of dried milkwood bark powder was mixed with 1.5 liters of 96% ethanol solvent which was stored for 3 x 24 hours. The maceration results are filtered and then concentrated with a rotary evaporator.

Phytochemical Screening of Milkwood Bark Extract

Phytochemical screening was carried out on milkwood bark extract by examining the groups of alkaloids, flavonoids, saponins, tannins and triterpenoids using the standard method by Bariyyah *et al*., (2022).

Bacterial Preparation

The test bacteria used consisted of two types of bacteria, namely *S. aureus* and *E. coli.* Bacteria that have been incubated are taken 1 or 2 oses, then suspended in 10 mL of sterile Physiological NaCl water in a test tube until the turbidity is the same as the MC Farland standard (10^8 CFU.mL^{-1}). MC Farland turbidity standard was prepared by adding 0.5 mL of 1% BaCl solution with 9.5 mL of 1% H_2SO_4.

Antibacterial Assay

Testing the antibacterial inhibitory activity of milkwood bark extract was carried out using the disc diffusion method. Each extract was made in various concentrations of 10, 50, 100, 250, 500, 750, 1000 ppm. The NA media (*Nutrient agar*) is heated first until it melts and then cooled to 40℃. The NA solution was then poured into a petri dish and each was mixed with 0.1 mL of a bacterial solution of *S. aureus* and *E. coli* then homogenized and allowed to solidify. Paper discs with a diameter of 5 mm were immersed in milkwood bark extract and positive control. The paper disc is then placed on the surface of the media with the help of sterile tweezers and then pressed. The media was then incubated at 37℃ for 1 × 24 hours until a zone of inhibition appeared which was indicated by the presence of a clear zone on the agar medium. Furthermore, the number of colonies in the petri dish was calculated using a colony counter. The antibacterial activity test was carried out in duplicate and repeated 3 times. The zone of inhibition was measured using a caliper to determine bacterial activity.

3. RESULTS AND DISCUSSION

Extraction

The results of the study (Table 1), samples of milk bark (*A. scholaris* (L.) R. Br) obtained from the Depapre area, Jayapura Regency as much as 5 kg. Milkwood bark is separated from the outer bark, then washed, cut into small pieces, and dried in an oven at 40 - 50°C to obtain 500 grams of dried simplicia. Then the results of the milk bark extract obtained were 59.4 grams with a yield value of 11.88%.

Table 1. Extraction Results of Milkwood Bark (*Alstonia scholari* (L.) R. Br)

Sample	Simplicia Weight (grams)	Solution Volume (mL)	Extract Weight (grams)	Yield (%)
Milkwood Bark (*Alstonia scholaris* (L.) R. Br)	500	1500	59.4	11.88

The choice of ethanol as a solvent in this study is because ethanol is selective, volatile and easily mixed with water, able to attract compounds such as alkaloids, flavonoids, saponins, tannins, triterpenoids, and steroids.

Extracts were made using the maceration method in which 500 grams of milk bark simplicia powder was weighed and soaked in 1500 mL of 96% ethanol for 3x24 hours with one stirring with the aim of ensuring that all surfaces of the powder could be in contact with

the solvent, so that the active substances could be dissolved. Then filtered using filter paper. The filtered filtrate was then evaporated using a rotary evaporator to obtain a thick extract.

The process that occurs when immersing simplicia using the maceration method is the diffusion process. The diffusion process is a process where the solvent will penetrate the cell wall that has been damaged during the manufacture of simplicia, then enter the cell cavity containing the active substance so that the active substance inside the cell will come out. The choice of maceration method as an extraction method is because it is good for compounds that are not heat resistant, relatively safe, also uses simple equipment and the process is relatively short and easy (Depkes, 2000).

Phytochemical Screening

Based on the results of phytochemical tests, it is known that the milkwood bark (*A. scholaris* (L.) R. Br) contains alkaloids, flavonoids, saponins and tannins. The results of phytochemical screening are shown in Table 2. This happened because the ethanol extract had the same level of polarity with the compounds obtained. Ethanol is a polar solvent that is widely used to extract the polar components of a natural material and is known as a universal solvent. Polar components of a natural substance in ethanol extract can be extracted by extraction techniques through a separation process. Triterpenoid compounds were not found in the observations because these compounds were nonpolar. This opinion is in accordance with Khyade & Vaikos, (2009); Gunawan & Simaremare, (2016) that these metabolites are contained in milkwood bark extract.

Table 2. The Phytochemical Analysis of Milkwood Bar Ethanol Extract

Metabolites	Results
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Triterpenoids	-

Information: + = Yes - = None

Antibacterial Assay

The antibacterial activity of milkwood bark extract (*A. scholaris* (L.) R. Br.) *against S. aureus* and *E. coli* was carried out using the disc diffusion method. Disc diffusion is a method of testing antibacterial activity by looking at the presence or absence of a clear area formed in the area of the paper disc. The results of the diameter of the inhibition zone from this study can be seen in Table 3.

Table 3. Test Results of Antibacterial Activity of Milkwood Bark Extract

Concentration Treatment	Average Inhibitory Zone Diameter (mm)	
	Staphylococcus aureus	***Escherichia coli***
10 ppm	7.40	7.54
50 ppm	7.58	7.90
100 ppm	7.72	8.08
250 ppm	8.04	8.73
500 ppm	8.22	8.85
750 ppm	8.58	9.42
1000 ppm	9.05	9.73
Control (+)	22.62	29.93
Control (-)	0	0

Description: C+ : ciprofloxacin 5 µg, C- : sterile distilled water

≤ 5 mm = weak, 5 - 10 mm = medium, 10 - 20 mm = strong (Greenwood & Slack, 1995)

The positive control used was Ciprofloxacin 5 g disc paper. Ciprofloxacin is an antibiotic that belongs to the second generation fluoroquinolone group. Ciprofloxacin is active against Gram-positive and Gram-negative bacteria that work by inhibiting DNA, topoisomerase II, topoisomerase IV, which are needed to separate bacterial DNA so that it inhibits cell division (Katzung *et al*., 2013). The negative control used as a comparison in this test is distilled water. Aquadest was chosen because it does not have antibacterial activity and was also used to dissolve the test sample.

The results in the table above show that the ethanolic extract of milk bark (*A. scholaris* (L.) R. Br.) has antibacterial activity against *S. aureus* and *E. coli*. The average diameter of the inhibition zone for the two test bacteria was seen in the range of 5 - 10 mm. From these results, the inhibitory power of the ethanol extract of milkwood bark against the two test bacteria was categorized as medium. However, the higher the concentration of the extract treatment, the greater the diameter of the inhibition zone, with the increase in the concentration of the extract, it means the greater the content of the active ingredient that functions as an antibacterial. According to Bariyyah *et al*., (2019), the higher the concentration of the antibacterial material used, the faster the bacteria will be killed.

The inhibition of milkwood bark (*A. scholaris* (L.) R. Br) on *E. coli* has a larger inhibition zone than *S. aureus*, this is probably due to the peptidoglycan layer that composes the cell wall of *S. aureus* which consists of structures that thick and stiff, while the peptidoglycan layer that composes the cell wall of *E. coli* is thin so that the cell wall is easily damaged. Due to the small and thin layer of peptidoglycan that composes the cell wall of *E. coli* which causes the cell wall to be more susceptible to damage when given antibacterial (Rastina *et al*., 2015).

4. CONCLUSIONS

Based on the results of research that has been conducted regarding the antibacterial activity of milkwood bark extract (*Alstonia scholaris* (L.) R. Br.), it can be concluded that: Milkwood bark extract (*Alstonia scholaris* (L.) R. Br.) has antibacterial activity medium potential against *S. aureus* and *E. coli*. The concentration of milkwood bark extract (*Alstonia scholaris* (L.) R. Br.) that was most effective in inhibiting the growth of *S. aureus* was 500 ppm and *E. coli* was 250 ppm.

REFERENCES

Bariyyah, S. K., Prajitno, A., & Yuniarti, A. (2019). Phytochemical screening and antimicrobial activity of roselle (*Hibiscus sabdariffa* L.) flower extract against *Aeromonas hydrophila*. *Journal of Experimental Life Science*, *9*(2), 65–69. https://jels.ub.ac.id/index.php/jels/article/view/306/298

Bariyyah, S. K., Tuhumury, R. A. N., Prayitno, Y., Saleh, S. M., & Achmad, M. I. (2022). Analysis of secondary metabolic compounds of purple leaf extract (*Graptophyllum pictum* (L.) Griff.) on *Aeromonas hydrophila*. *The Journal of Fisheries Development*, *5*, Januari. http://jurnal1.uniyap.ac.id/uyp/index.php/tjfd

Chrystomo, L. Y., Karim, A. K., Antari, N. N., Wona, Y., & Pongtiku, A. (2016). *Buku tumbuhan kerarifan lokal Papua/Papua traditional medicine herbs*. Nulisbuku Jendela Dunia.

Departemen Kesehatan Republik Indonesia. (2000). *Parameter standar umum ekstrak tumbuhan obat* (1st ed.). Depkes RI.

Greenwood, D., & Slack, R. (1995). *Medical microbiology*. ELBS.

Gunawan, E., & Simaremare, E. S. (2016). Formulasi sirup antimalaria ekstrak kulit batang kayu susu (*Alstonia scholaris* (L.) R. Br.). *Pharmacy*, *13*(1), 1–9.

Kalaria, P., Payal, G., Manodeep, C., & Jagadish, K. (2012). A phytopharmacological review of *Alstonia scholaris*: A panoramic herbal medicine. *International Journal of Research in Ayurveda and Pharmacy*, *3*(3). www.ijrap.net

Katzung, B. G., Masters, S. B., & Trevor, A. J. (2013). *Farmakologi dasar & klinik* (2nd ed.). EGC.

Khyade, R. B., & Vaikos, N. P. (2009). Phytochemical and antibacterial properties of leaves of *Alstonia scholaris*. *African Journal of Biotechnology*, *8*(22), 6434–6436. http://www.academicjournals.org/AJB

Rastina, R., Sudarwanto, M., & Wientarsih, I. (2015). Aktivitas antibakteri ekstrak etanol daun kari (*Murraya koenigii*) terhadap *Staphylococcus aureus, Escherichia coli*, dan *Pseudomonas* sp. *Jurnal Kedokteran Hewan - Indonesian Journal of Veterinary Sciences*, *9*. https://doi.org/10.21157/j.ked.hewan.v9i2.2842

Suoth, J. A. T., Sudewi, S., & Wewengkang, D. S. (2019). Analisis korelasi antara flavonoid total dengan aktivitas antibakteri ekstrak dan fraksi daun gedi hijau (*Abelmoschus manihot* L.). *Pharmacon*, *8*(3).

Sutrisno, J. (2014). Uji aktivitas antibakteri ekstrak etanol biji pinang (*Areca catechu* L.) terhadap *Staphylococcus aureus* secara in vitro. *Fakultas Kedokteran, Universitas Tanjungpura Pontianak*, 18.

Wiart, C. (2006). *Medicinal plants of Asia-Pacific: Drugs for the future*. World Scientific Publishing Co. Pte. Ltd.

Zuraida, R. E., & N, L. (2010). Prospek pulai (*Alstonia* sp) sebagai bahan baku industri obat antikolesterol. *Jurnal Kementerian Kehutanan*.