Stifar-IPCHS: Stifar International Proceedings in Pharmaceutical, Clinical, and Health Sciences

E-ISSN: xxxx-xxxx
P-ISSN: xxxx-xxxx

Research Article

Inhibition of *Staphylococcus aureus* Biofilm by Ethanol Extract of *Nasturtium officinale*

Ramadhaniza Edelweis Puspatricia[1], Christina Astutiningsih[2], Endang Dwi Wulansari[3*]

[1,2] Undergraduate Program in Pharmacy, Stifar Yayasan Pharmasi Semarang, Indonesia
[3] Master's Program in Pharmacy, Stifar Yayasan Pharmasi Semarang, Indonesia
* Corresponding Author : dewi.ung@gmail.com

Abstract. The *Staphylococcus aureus* biofilm formation can increase antibiotic resistance in chronic diseases including wound infections. In a biofilm state, susceptibility to antibiotics is reduced, making it more difficult to eradicate bacteria. Watercress (*Nasturtium officinale*) is known to contain compounds that have the potential to be antibacterial and antibiotic agents. This study aims to determine the inhibition activity of the formation and degradation of *Staphylococcus aureus* biofilm from watercress leaf ethanol extract with concentrations of 1%, 5%, and 25%, as well as to determine the content of antibiofilm compounds in the extract. *Nasturtium officinale* ethanol extract is obtained by remaceration. Antibiofilm testing was performed with *Microtiter Plate Biofilm Assay,* and ciprofloxacin was used as a positive control. The results of the one-way anava test showed differences in activity both in inhibition of formation and degradation of Staphylococcus aureus biofilm. The extract with a concentration of 25% provided the greatest activity, which was 63.00% inhibition of biofilm formation and 52.77% biofilm degradation. Flavonoid compounds, phenolics, tannins, saponins, and steroids in the extract have the potential to provide activity.

Keywords: Biofilm Assay; Extract; Microtiter Plate; Watercress Leaves.

Received: April 19, 2026
Revised: April 28, 2026
Accepted: May 10, 2026
Published: May 16, 2026
Curr. Ver.: May 16, 2026

1. Introduction

Biofilms are an organized population of bacteria, in which their cells are attached to a biotic or abiotic surface and are lined by an extracellular matrix. Extracellular *matrix or Extracellular Polymeric Substance* (EPS) functions as an adhesive and facilitates the survival of biofilms in hostile and even extreme environments [1]. Currently, it is estimated that 80% of infections are caused by biofilms. Therefore, biofilms are considered to be a major factor in persistent chronic infectious diseases [2]. *Staphylococcus aureus* bacteria have the ability to form biofilms. This is due to proteins that facilitate adhesion or attachment to the host surface tissue [3]. The biofilm that forms is followed by an increased population density of bacteria, encouraging the bacteria to produce signaling molecules that induce drug resistance mechanisms. Resistant bacteria can reduce the level of antibiotic efficiency, so it can affect the management of treatment.

The use of antibiotics can be minimized by utilizing bioactive compounds contained in natural ingredients [4]. Watercress (*Nasturtium officinale*) is reported to have benefits not only as a functional food, but also shows benefits as an antioxidant[5], antifungal [6], antibacterial [6], [7], anticancer[8]. Watercress contains compounds such as flavonoids, phenolics, steroids, tannins, and saponins [9] which are reported to have the potential to provide biological activity.

Research by Hakim et al., [10] on the polyphenol compound test of watercress ethanol extract against the antibacterial activity *of Pseudomonas aeruginosa* and *Staphylococcus aureus*, reported that the content of the resulting polyphenol compounds functioned as antibacterial [11]. The results proved that at the highest concentration of watercress ethanol extract of 1 mg/mL, it produced an inhibition in *Staphylococcus aureus* bacteria of 8.98 mm.

Research on the antibiofilm activity of watercress leaf ethanol extract on *Staphylococcus aureus* bacteria has never been reported. Therefore, this study aims to determine the antibiofilm activity of watercress leaf ethanol extract in *Staphylococcus aureus bacteria*. As well as to find out the content of compounds that have the potential as antibiotics in watercress leaf ethanol extract.

2. Method

This research uses a laboratory experimental research design (*true experiment*). The population of this study is *Staphylococcus aureus bacteria* and the sample of this study is in the form *of Staphylococcus aureus* biofilm grown in *microplates*. The samples were divided into 6 groups, namely the watercress leaf ethanol extract treatment group (1%, 5%, and 25%), positive control (*ciprofloxacin* 0.05%), negative control (DMSO), and growth control. Each sample group has 4 replications in one *microplate*.

Materials and tools

The ingredients used are watercress leaves, *Staphylococcus aureus bacteria*, H_2SO_4, CH_3COOH, $K_2Cr_2O_7$, Mg powder, concentrated HCl, amyl alcohol, Bouchardat reagent, Mayer reagent, Hager reagent, $FeCl_3$ 1%, gelatin 1%, NaCl 10%, anhydrous acetic acid, chloroform (MERCK), methanol (MERCK), ethyl acetate (MERCK), formic acid, glacial acetic acid, n-hexane (MERCK), *Mannitol Salt Agar* (MSA), *Nutrient Agar* (NA), *Brain Heart Infusion Broth* (BHIB), aquadest, 1% violet crystal solution, ethanol (MERCK), *ciprofloxacin*, and DMSO (MERCK). The research tools used are autoclaves, incubators, LAF, *multimode readers* (Sinergy HTX), *microplates*.

Extraction

The research began with the preparation of watercress leaf dry powder and then an extraction process was carried out using the re-maceration method for 3 days, using 70% ethanol solvent. The resulting filtrate is concentrated using a rotary evaporator and water bath at a temperature of 50°C. The thick extract will be obtained and then the yield will be calculated. The extracts obtained were subjected to ethanol-free testing and then continued with phytochemical and TLC screening tests. This test was carried out on several groups of compounds, including flavonoids, phenolics, tannins, saponins, steroids, triterpenoids, and alkaloids.

Antibofilm Activity Test

Activity testing begins with the optimization of biofilm formation time, using a bacterial suspension that has been standardized to the McFarland 1/2 standard. Each inlet on the microplate was filled with BHIB media and a bacterial suspension of 70 μL each, incubated with time variations of 24, 48, 72, and 96 hours at a temperature of 37°C. The incubated microplate is washed with aquadest three times. Each well is filled with 200 μL of 1% violet crystal, incubated at room temperature for 15 minutes. The microplate is washed again three times and allowed to dry. Then, each well was added 200 μL of 96% p.a ethanol and incubated for a duration of 15 minutes at room temperature. Microplate readings of OD values were carried out with a multimode reader device at a wavelength of 595 nm [12].

The Biofilm Inhibition Test

The test of inhibition of biofilm formation used microplates filled with BHIB media, bacterial suspension, and samples with concentrations of 1%, 5%, and 25% of 70 μL, respectively. Furthermore, the microplate is incubated at a temperature of 37°C during the optimal incubation time. After the incubation period, the microplate is washed using aquadest three times. To each well, 200 μL of 1% violet crystal is added, incubated for 15 minutes at room temperature. The microplate is washed again three times and allowed to dry. Then, each feed was added 200 μL of 96% p.a ethanol and incubated for 15 minutes at room temperature. Microplate readings of OD values were carried out using a multimode reader tool at a wavelength of 595 nm [12].

The Biofilm Degradation Test

The biofilm degradation test is carried out by forming a biofilm first on a *microplate*. The biofilm is formed by filling the *microplate* with BHIB media and a bacterial suspension of 70 μL. Furthermore, *the microplate* is incubated during the optimal incubation time at a temperature of 37°C. *The incubated microplate* is washed using aquadest. In each drain, samples were added with concentrations of 1%, 5%, and 25% of 70 μL, respectively.

In positive and negative controls, they were given the same treatment, then re-incubated for 90 minutes at a temperature of 37°C. It was followed in the same way in the test of inhibition of biofilm formation to determine biofilm destruction activity[12]. Based on the results of the OD value obtained, then the percentage of antibiofilm activity was calculated with the following equation [13].

$$\%\ \textbf{\textit{Antibiofilm Activity}} = \left(\mathbf{1} - \frac{(\textbf{\textit{Sample OD}} - \textbf{\textit{Sample blank OD}})}{(\textbf{\textit{Control OD}} - \textbf{\textit{Control blank OD}})}\right) \times 100\% \quad (1)$$

3. Results and Discussion

This study used 300.28 grams of watercress leaf simplicia powder and produced a thick extract of 89.9726 grams, resulting in a yield of 29.96%. Based on the research of Amanda and Raharjo [14] the extraction of watercress simplicia using 70% ethanol produced more extract yields than extraction using 96% ethanol [5]. In addition, simplicia sifted with a 30/40 mesh showed a medium degree of fineness, resulting in a wider surface contact with the filter fluid and more maximal compound extraction.

The condensed extract obtained is then tested for ethanol to ensure that the extract does not contain ethanol at all, as the ethanol residue left behind can affect the results of the antibiofilm activity test. The test results can be described in Table 1.

Table 1. Ethanol Free Test of Watercress Leaf Extract.

Reagents	Positive Results (based on Library)	Test Results	Remarks
glacial acetic acid + H_2SO_4 → heated	Construction ester	(-) Typical smell of the extract	Extracts do not contain ethanol
H_2SO_4 + $K_2Cr_2O_7$	Green/blue solution	(-) Blackish brown	Extracts do not contain ethanol

The identification of the compound content of watercress leaf ethanol extract was carried out by phytochemical screening test as a preliminary test and an affirmation test with Thin Layer Chromatography (TLC). The results of the study show that the extract contains flavonoid compounds, phenolics, tannins, saponins, and steroids presented in Table 2.

Table 2. Results of Phytochemical Screening and TLC Test of Watercress Leaf Extract.

Compound Groups	Test Results
Flavonoid	+
Phenolic	+
Tanin	+
Saponin	+
Steroid	+
Alkaloid	-

* (+) indicates the presence of compound content, (-) indicates the absence of compound content.

Figure. 1 presents a positive result of *Staphylococcus aureus* bacteria with colonies produced in the circular MSA media protruding having a golden yellow color. MSA media is highly selective against *Staphylococcus aureus bacteria* because it contains *mannitol* and phenol *red pH* indicators. The ability *of Staphylococcus aureus* bacteria to ferment *mannitol* will produce an acidic atmosphere, so that the pH of the medium will decrease and change the color of *the phenol red* indicator which was originally red to yellow [15].

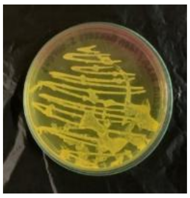


Figure 1. Identification Results of *Staphylococcus aureus Bacteria.*

The results of the optimization of biofilm formation time are shown in Figure. 2. Time optimization aims to determine the ideal time needed by *Staphylococcus aureus* bacteria to form a good biofilm. The highest result of *Staphylococcus aureus* bacterial biofilm formation is 72 hours, so that this time is the optimal time as an incubation time in testing antibiotic activity. In line with the research of Besan et al., [16] the optimal time for the formation of *Staphylococcus aureus* biofilm is at an incubation time of 72 hours [17]. The decrease in biofilm density on the fourth day is influenced by the stage of biofilm formation which enters the stage of dispersion or release from the biofilm, so that the number of biofilm colonies contained in *the microplate* well will be reduced [19].

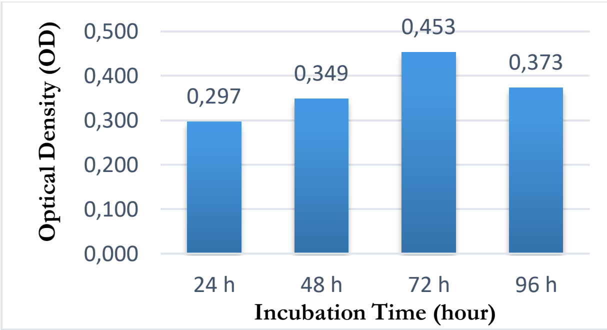
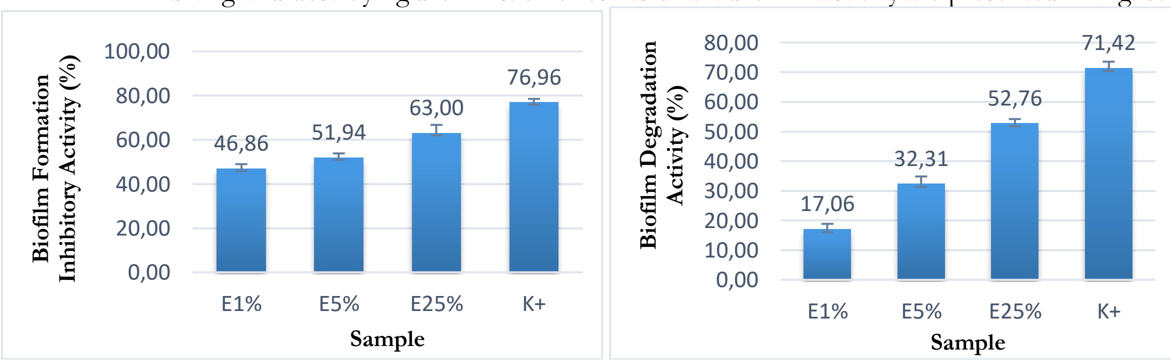


Figure 2. Results of Optimization of *Staphylococcus aureus Biofilm Formation Time.*

Testing of the activity of inhibiting the formation of biofilm was carried out to determine the ability of watercress leaf extract to inhibit the formation of *Staphylococcus aureus bacterial biofilm* at the stage of attachment and growth of bacterial cells. Meanwhile, the biofilm destruction activity test aims to determine the ability of watercress leaf extract to destroy biofilms that have been formed. The increase in concentration is directly proportional to the increase in the number of compounds, so that the activity produced is also greater in inhibiting and destroying biofilms. The results of antibiofilm activity are presented in Fig. 3.



(a) **(b)**

Figure 3. (a) Biofilm Formation Inhibition Activity Test, **(b)** Biofilm Degradation Activity Test of Watercress Leaf Extract

According to Famuyide et al. [20],the activity of antibiotics is good and effective if the percentage produced ≥ 50% and for the percentage of 0-49% can be said to be low [20]. Therefore, the results of the antibiofilm activity test from watercress leaf extract against *Staphylococcus aureus* bacteria were good at a concentration of 25%.

The content of active compounds in watercress leaf extract that can play a role in inhibiting the formation of biofilms is flavonoid and tannin compounds with an action mechanism that inhibits the work of *the Polysaccharide Intercellular Adhesion* (PIA) gene which is the main component of EPS, PIA plays a role as the adhesion of bacterial cells to a surface, so that there will be a decrease in bacterial adhesion and the formation of biofilms will be inhibited. In addition, phenolic compounds have hydroxyl groups that can denature proteins. Altered protein structures can affect bacterial cell metabolism and lead to bacterial death. So that the number of bacteria in a medium will decrease and inhibit the process of biofilm formation [21].

In biofilm destruction activity tests, steroid compounds can degrade the integrity of cell membranes so that lysosomes leak and degrade bacterial cell components [22]. Saponin compounds that interact with bacterial biofilms will cause bacteria to break down or lysis due to their ability to increase the permeability of cell membranes. The higher permeability of the cell membrane will make it easier for a compound to penetrate into the cell, so that the survival of bacterial biofilms will be disrupted [23].

4. Conclusions

Ethanol extract of watercress leaves has antibiofilm activity against *Staphylococcus aureus bacteria*. This is influenced by the content of compounds that have the potential to be antibiotics in watercress leaf ethanol extract, namely flavonoids, phenolics, tannins, saponins, and steroids. It is necessary to conduct in-depth follow-up research related to the visualization of biofilms formed using *Scanning Electron Microscopy* (SEM). Research on the antibiofilm activity of watercress leaf extract against other biofilm-forming bacteria is needed to complete the activity data. The development into topical preparations of antiseptics and disinfectants from watercress leaf extract has also become interesting to do.

Author Contributions: Conceptualization: EDW and REP; Methodology: EDW and CA; Validation: REP, CA and EDW.; Formal analysis: REP; Investigation: REP; Resources: REP; Data curation: REP.; Writing—original draft preparation: REP; Writing—review and editing: REP, CA, and EDW; Visualization: REP and EDW; Supervision: CA and EDW; Project administration: REP; Funding acquisition: REP"

Funding: This research received no external funding

Data Availability Statement: Data unavailable due to privacy or ethical restrictions.

Acknowledgements: Expression of gratitude to Stifar laboratory of Stifar Yayasan Pharmasi Semarang for the research facilities used.

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] D. E. Moormeier and K. W. Bayles, "Staphylococcus aureus Biofilm: A Complex Developmental Organism," *Mol. Microbiol.*, vol. 104 (3), no. 3, pp. 365–376, 2017, doi: 10.1111/mmi.13634.

[2] Q. Peng, X. Tang, W. Dong, N. Sun, and W. Yuan, "A Review of Biofilm Formation of Staphylococcus aureus and Its Regulation Mechanism," *Antibiotics*, vol. 12 (12), no. 12, pp. 1–21, 2023, doi: 10.3390/antibiotics12010012.

[3] M. Kashi, M. Noei, Z. Chegini, and A. Shariati, "Natural Compounds in The Fight Against Staphylococcus aureus Biofilms: A Review of Antibiofilm Strategies," *Front. Pharmacol.*, vol. 15, pp. 1–25, 2024, doi: 10.3389/fphar.2024.1491363.

[4] M. Kashi, M. Noei, Z. Chegini, and A. Shariati, "Natural compounds in the fight against Staphylococcus aureus biofilms: a review of antibiofilm strategies," *Front. Pharmacol.*, vol. 15, p. 1491363, 2024, doi: 10.3389/fphar.2024.1491363.

[5] K. T. Amanda and S. J. Raharjo, "Antioxidant Potency of Combination Extract of Water-Ethanol in Selada Air (Nasturtium officinale R. Br) Simplisia," *PHARMADEMICA : Jurnal Kefarmasian dan Gizi*, vol. 1, no. 2, pp. 40–46, Mar. 2022, doi: 10.54445/pharmademica.v1i2.13.

[6] J. Agr, S.-T. Bili, and L. Ercan, "Investigation of antibacterial and antifungal efficacy of zinc and silver nanoparticles synthesized from Nasturtium officinale," *dergipark.org.tr*, vol. 29, no. 3, pp. 788–799, 2023, doi: 10.15832/ankutbd.1163132.

[7] M. Tabesh, M. E. Sh, M. Etemadi, F. Naddaf, F. Heidari, and J. Alizargar, "The Antibacterial Activity of Nasturtium Officinale Extract on Common Oral Pathogenic Bacteria," *Niger. J. Clin. Pract.*, vol. 25, no. 9, pp. 1466–1475, Sep. 2022, doi: 10.4103/njcp.njcp_1887_21.

[8] H. Feng *et al.*, "Exploring the Anti-Cancer Properties of Nasturtium officinale L. via the HOTAIR/miR-124/Notch1 Pathway in Rat Hepatocellular Carcinoma: An Investigation Using Biochemical, Molecular, Immunohistochemical, and Histopathological Methods.," *International Journal of Morphology*, vol. 42, no. 5, pp. 1361–1372, 2024.

[9] H. S. Faizy *et al.*, "Phytochemicals Analysis in Watercress (Nasturtium Officinale) Plant Extracts," *E&ES*, vol. 761, no. 1, p. 012042, May 2021, doi: 10.1088/1755-1315/761/1/012042.

[10] S. K. Hakim, S. Sudirman, M. Janna, G. D. Nugroho, and D. I. Sari, "Aktivitas Antibakteri Pseudomonas aeruginosa dan Staphylococcus aureus Dari Ekstrak Etanol Selada Air (Nasturtium officinale)," *J. Pengolah. Has. Perikan. Indones.*, vol. 27 (4), no. 4, pp. 319–326, 2024, doi: 10.17844/jphpi.v27i4.50254.

[11] S. K. Hakim, S. Sudirman, M. Janna, G. D. Nugroho, and D. I. Sari, "Antibacterial Activity (Pseudomonas aeruginosa and Staphylococcus aureus) of Ethanol Extract from Watercress (Nasturtium officinale)," *J. Pengolah. Has. Perikan. Indones.*, vol. 27, no. 4, pp. 319–326, 2024, doi: 10.17844/jphpi.v27i4.50254.

[12] C. H. B. Tobi, O. Saptarini, and I. Rahmawati, "Aktivitas Antibiofilm Ekstrak dan Fraksi-Fraksi Biji Pinang (Areca catechu L.) Terhadap Staphylococcus aureus ATCC 25923," *JPSCR: Journal of Pharmaceutical Science and Clinical Research*, vol. 7, no. 1, p. 56, Mar. 2022, doi: 10.20961/jpscr.v7i1.43698.

[13] H. Ulya, E. U. Ulfa, and P. Endah, "Uji Aktivitas Antibakteri dan Antibiofilm Minyak Atsiri Rimpang Bengle (Zingiber purpureum Roscoe) terhadap Bakteri Staphylococcus epidermidis," *e-Jurnal Pustaka Kesehatan*, vol. 3, no. 2, pp. 267–271, May 2015

[14] K. T. Amanda and S. J. Raharjo, "Potensi Antioksidan Ekstrak Kombinasi Air-Etanol Pada Simplisia Selada Air (Nasturtium officinale R. Br)," *PHARMADEMICA : Jurnal Kefarmasian dan Gizi*, vol. 1 (2), no. 2, pp. 40–46, 2022, doi: 10.54445/pharmademica.v1i2.13.

[15] R. Khaerunnisa, I. Kurniati, D. Nurhayati, and A. Dermawan, "Pemanfaatan Air Rebusan Umbi Kuning dan Ungu Sebagai Media Alternatif Pertumbuhan Escherichia coli dan Staphylococcus aureus," *JURNAL RISET KESEHATAN POLTEKKES DEPKES BANDUNG*, vol. 11, no. 1, pp. 269–276, Jul. 2019, doi: 10.34011/juriskesbdg.v11i1.753.

[16] E. J. Besan, I. Rahmawati, and O. Saptarini, "Aktivitas Antibiofilm Ekstrak dan Fraksi-Fraksi Bunga Telang (Clitoria ternatea L.) terhadap Staphylococcus aureus," *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*, vol. 20 (1), no. 1, pp. 1–11, 2023, doi: 10.30595/pharmacy.v0i0.14437.

[17] E. J. Besan, I. Rahmawati, and O. Saptarini, "Aktivitas Antibiofilm Ekstrak dan Fraksi-Fraksi Bunga Telang (Clitoria ternatea L.) terhadap Staphylococcus aureus," *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*, vol. 20, no. 1, pp. 1–11, Aug. 2023, doi: 10.30595/pharmacy.v0i0.14437.

[18] D. E. Moormeier and K. W. Bayles, "Staphylococcus aureus biofilm: a complex developmental organism," *Mol. Microbiol.*, vol. 104, no. 3, pp. 365–376, May 2017, doi: 10.1111/mmi.13634.

[19] I. M. Famuyide, A. O. Aro, F. O. Fasina, J. N. Eloff, and L. J. McGaw, "Antibacterial and Antibiofilm Activity of Acetone Leaf Extracts of Nine Under-Investigated South African Eugenia and Syzygium (Myrtaceae) Species and Their Selectivity Indices," *BMC Complement. Altern. Med.*, vol. 19 (1), no. 1, pp. 1–13, 2019, doi: 10.1186/s12906-019-2547-z.

[20] I. M. Famuyide, A. O. Aro, F. O. Fasina, J. N. Eloff, and L. J. McGaw, "Antibacterial and antibiofilm activity of acetone leaf extracts of nine under-investigated south African Eugenia and Syzygium (Myrtaceae) species and their selectivity indices," *BMC Complement. Altern. Med.*, vol. 19, no. 1, Jun. 2019, doi: 10.1186/s12906-019-2547-z.

[21] E. Kinig, S. Falah, and N. Nurhidayat, "The In Vitro Antibiofilm Activity of Water Leaf Extract of Papaya (Carica papaya L.) against Pseudomonas aeruginosa," *Current Biochemistry*, vol. 2, no. 3, pp. 150–163, Mar. 2015, doi: 10.29244/cb.11.1.2.

[22] R. Sari, M. Muhani, I. Fajriaty, R. Sari, M. Muhani, and I. Fajriaty, "Uji Aktivitas Antibakteri Ekstrak Etanol Daun Gaharu (Aquilaria microcarpa Baill.) Terhadap Bakteri Staphylococcus aureus dan Proteus mirabilis," *Pharmaceutical Sciences and Research*, vol. 4, no. 3, p. 4, Dec. 2017, doi: 10.7454/psr.v4i3.3756.

[23] H. Herman, T. Sunarni, and O. Saptarini, "Uji Aktivitas Antibakteri dan Antibiofilm Fraksi Ekstrak Daun Gamal (Gliricidia Sepium (Jacq Walp)) Terhadap Staphylococcus Aureus ATCC 25923," *Jurnal Mandala Pharmacon Indonesia*, vol. 10, no. 1, pp. 314–327, Jun. 2024, doi: 10.35311/jmpi.v10i1.484.