Pharmacology and Herbal Medicine Journal, 2026, **3**, 75–80
DOI: 10.20884/1.phmj.2026.1.3.19653
Research Paper

PHARMACOLOGY AND HERBAL MEDICINE JOURNAL
PHMJ

Effectiveness of Bay Leaf Ethanol Extract (*Syzygium polyanthum (Wight) Walp.)* in Inhibiting The Growth of *Staphylococcus aureus:* Study in Vitro

Zulfiya Rohmah[*1], Kanti Ratnaningrum[2], Ratna Sulistyorini[3]

[1]*Faculty of Dentistry, Muhammadiyah University of Semarang, Central Java, Indonesia,*
[2]*Deepartment of Tropical Medicine, Faculty of Medicine, Muhammadiyah University of Semarang, Indonesia*
[3]*Department of Oral Biology, Faculty of Dentistry, Muhammadiyah University of Semarang, Indonesia*
**Corresponding author. E-mail: zulfiarahma08@gmail.com*

Abstract

Background: Normal flora in the oral cavity mostly consists of commensal bacteria, but under certain conditions, these bacteria can develop into pathogens and cause infections, one of which is *Staphylococcus aureus.* These bacteria can cause various diseases in the oral cavity such as abscesses, stomatitis, and gingivitis, especially when the immune system is weak. Treatment of infections caused by *Staphylococcus aureus* often requires the use of antibiotics, but improper use can trigger antibiotic resistance. Bay leaves *(Syzygium polyanthum w)* contain antibacterial activity in secondary metabolite compounds, namely essential oils, saponins, tannins, and alkaloids. **Objectives:** This study aims to determine the effect of 70% ethanol extract of bay leaves *(Syzygium polyanthum w)* in inhibiting the growth of *Staphylococcus aureus.* **Methods:** This study was an experimental laboratory study with a post-test only control group design. This study was divided into 5 treatment groups consisting of 3 treatment groups and 2 control groups. *S.aureus* was grown on Muller Hinton Agar media. Each treatment group was given bay leaf ethanol extract with concentrations of 50%, 75%, and 100% and a control group consisting of a positive control using chlorhexidine and a negative control group using sterile aquadest. All petri dishes were put into an incubator for 24 hours at 37^{o}C, then their diameters were measured. Data analysis using the *Kruskal-Wallis* non-parametric test and continued with the *Mann Whitney-U* posthoc test. **Key findings:** The results showed an increase in the concentration of the inhibition zone for each extract concentration. The average diameter of the positive control inhibition zone was 22.20 mm; 100% concentration 15.10 mm; 75% concentration 13.85 mm; 50% concentration 11.40 mm, and negative control 0.00 mm. The *Kruskal-Wallis* test results showed significant differences. The *Mann Whitney-U* posthoc test showed significant differences for all groups except the negative control. **Conclusions:** Bay leaf extract *(Syzygium polyanthum W.)* can inhibit the growth of *Staphylococcus aureus* at concentrations of 50%, 75%, and 100%.

Keywords: Antibacterial, Bay leaves, Inhibition zone, *Staphylococcus aureus*

Introduction

The oral cavity reflects the health status of the human body, as it serves as the primary gateway for food required to support maximum growth and development. Frequently, various types of lesions occur in the oral cavity caused by several factors, including bacterial infections [1]. The oral cavity contains a population of microorganisms known as normal flora [2]. Bacteria that are initially commensal can develop into pathogens when the immune system is weak or when predisposing factors are present. *Staphylococcus aureus* is one of the bacteria commonly found in the oral cavity, with counts reaching ten to one thousand colonies per milliliter of saliva [3-4].

Staphylococcus aureus is a Gram-positive coccus bacterium that can become pathogenic if mucosal abrasion occurs, causing abscesses, stomatitis, gingivitis, and root canal infections. Common symptoms of diseases caused by this bacterium include necrosis, inflammation, and the development of abscesses [5]. Abscesses themselves occur due to bacterial infection accompanied by immune system disorders. *Staphylococcus aureus* is one of the primary bacteria causing oral cavity abscesses, with an increased incidence in dental abscesses from 0.7% to 15% [6].

The use of antibiotics is a common therapy; however, misuse can lead to resistance. *Staphylococcus aureus* shows a high level of resistance to β-lactam antibiotics due to the production of the β-lactamase enzyme, and as many as 79.5% of its isolates are resistant to penicillin [7-8]. To reduce resistance, the utilization of herbal medicinal plants has become an alternative, as they are considered safe, have minimal side effects, and possess the potential to lower antibiotic resistance. Indonesia has high biodiversity with more than 30,000 types of plants, 90% of which have medicinal properties [9].

Spice plants are known to have antimicrobial effects. One of these is the bay leaf (*Syzygium polyanthum*), which can reduce the activity of bacteria such as *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and Methicillin-resistant *Staphylococcus aureus*. Bay leaves are easy to find, affordable, and commonly used in traditional medicine for conditions such as cholesterol, gastritis, diabetes, hypertension, and diarrhea [10]. Bay leaves contain bioactive compounds

Received: November 10, 2025. **Revised**: December 16, 2025. **Accepted**: 24 January 2026
 PHMJ 2026; 3:75-80; doi: 10.20884/1.phmj.2026.1.3.19653

PHARMACOLOGY AND HERBAL MEDICINE JOURNAL
PHMJ

such as phenolics, triterpenoids, steroids, tannins, alkaloids, and saponins, which play roles in biological activities such as anti-inflammatory, antioxidant, and other pharmacological activities [11]. Tannins and essential oils in bay leaves have potential as antibacterial, anti-inflammatory, and analgesic agents [10]. Furthermore, the ethanol extract of bay leaves is also known to have activity as an antifungal and antibacterial agent.

This study aims to determine the effectiveness of the ethanol extract of bay leaves in inhibiting the growth of *Staphylococcus aureus* in vitro and to measure the concentration that has the most significant influence on the inhibitory power against the bacterium.

Materials and Methods

This research is an experimental laboratory study using a post-test only control group design. The study is divided into 5 groups consisting of 3 treatment groups: P1 (bay leaf extract at 50% concentration), P2 (bay leaf extract at 75% concentration), P3 (bay leaf extract at 100% concentration), and 2 control groups: K1 as the positive control (chlorhexidine) and K2 as the negative control (sterile distilled water). Based on sample size calculations using the Federer formula, the experiment was repeated 5 times, resulting in a total of 25 samples. The samples used followed inclusion criteria for bay leaves, specifically young leaves with a dark green color. The research locations included the Biology Laboratory of FMIPA UNNES for the determination of the bay leaves, the Research Chemistry Laboratory of FMIPA UNNES for bay leaf extraction and phytochemical screening, and the Biomedical Laboratory of FK UNIMUS for the antibacterial activity testing. The antibacterial activity test method used was the well diffusion method.

Tools and Materials

Tools required for this research include: petri dishes, analytical scales, Erlenmeyer flasks, test tubes, an autoclave, an incubator, stirring rods, vernier calipers, a vacuum rotary evaporator, measuring cylinders, micropipettes, inoculation loops (ose), spreaders, cotton swabs, and Bunsen burners. Materials used include bay leaves obtained from the Purwareja Klampok area, Banjarnegara Regency, Central Java. *Staphylococcus aureus* bacterial isolates were obtained from the Biomedical Laboratory of the Faculty of Medicine, Universitas Muhammadiyah Semarang. Other materials include H_2SO_4, $BaCl_2$, ethanol, NaCl, spiritus, and Mueller Hinton Agar (MHA) media.

Determination of the Bay Leaf Plant

Identification of the plant aims to ensure the authenticity of the bay leaf samples based on macroscopic characteristics by comparing morphological features with existing literature. The identification process was conducted at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang.

Bay Leaf Extraction

The extraction of bay leaves utilized the maceration method: A total of 2 kg of bay leaves were washed under running water and then sun-dried for approximately seven days. Once dry, the leaves were crushed using a blender into a simplicia powder. A total of 250 grams of bay leaf simplicia powder was soaked in 70% ethanol with a solvent ratio of 1:10. On the first day, 1.2 liters of 70% ethanol were used, followed by 700 ml on the second day, and 600 ml on the third day in a single maceration process, ensuring all powder was perfectly submerged. This mixture was left for 3 times 24 hours with occasional stirring using a stirring rod or shaking. Afterward, the solution was filtered using filter paper, and this process was repeated three times. The filtrate or extraction results from the maceration were then collected and evaporated using a vacuum rotary evaporator at 70°C, producing 200 ml of a concentrated solution referred to as bay leaf extract (*Syzygium polyanthum* W.). To obtain various concentrations of the bay leaf extract, a dilution process was performed using distilled water as the solvent. The ethanol extract of the bay leaves was diluted into three concentrations: 50%, 75%, and 100%.

Chemical Content Testing for Phytochemical Screening

The phytochemical screening to identify the chemical content of the bay leaf extract was conducted through a series of qualitative tests. To identify flavonoids, 5 mL of distilled water was heated for 1 minute with 2 mg of concentrated simplicia extract, then filtered to collect the filtrate. This filtrate was then mixed with 0.1 gram of magnesium powder, 2 ml of an alcohol solution containing hydrochloric acid in a 1:1 ratio, and amyl alcohol solvent, followed by rapid stirring and separation; a positive reaction was indicated by a red or orange-yellow color in the amyl alcohol layer. For the tannin test, 10 mL of hot water was added to 2 mg of concentrated simplicia extract, boiled for 15 minutes, filtered, and then treated with 5 ml of 1% iron (III) chloride reagent, where a green or blue-black color signified a positive result.

The presence of essential oils was determined by pipetting one millilitre of the test solution and evaporating it over a porcelain dish to observe if the residue produced a characteristic odor. Saponin testing was carried out by weighing 1 gram of the 70% ethanol extract of bay leaves and mixing it with 20 ml of water, then stirring or shaking the test tube for 15 minutes, with the formation of stable foam indicating a positive result. Finally, the alkaloid test involved dissolving 2 ml of the 70% ethanol extract in 2 ml of 2% HCl, boiling the mixture for five minutes, and filtering it; the resulting filtrate was treated with two to three drops of Dragendorff's reagent, where the formation of an orange precipitate confirmed the presence of alkaloid compounds.

Preparation of Media

The process of making MHA media began by weighing 3.8 grams of the media and dissolving it in 100 ml of distilled water in a sterile 250 ml Erlenmeyer flask. The solution was heated until boiling, covered with gauze containing cotton, and then sterilized using an autoclave at 121°C for 15 minutes.

Pharmacology and Herbal Medicine Journal, 2026, **3**, 75–80
DOI: 10.20884/1.phmj.2026.1.3.19653
Research Paper

After sterilization, the media was cooled to a temperature of ±50°C, then poured (±20 ml) into petri dishes and allowed to solidify. The hardened media was then ready for testing.

Antibacterial Activity Test

The antibacterial test was performed by preparing petri dishes containing Mueller Hinton Agar (MHA). The *Staphylococcus aureus* bacterial suspension was taken using a sterile cotton swab and streaked in a zigzag pattern on the surface of the media, then left for 5–10 minutes to absorb. Wells were created in the media using a cork borer (6 mm diameter). Each well was then filled with 50 µl of bay leaf ethanol extract at concentrations of 50%, 75%, and 100%, chlorhexidine as the positive control, and sterile distilled water as the negative control using a micropipette. All dishes were incubated at 37°C for 24 hours.

After incubation, the inhibition zone was measured using vernier callipers with an accuracy of 0.02 mm. The measurement was performed using the well diffusion method on agar media. The inhibition zone is indicated by the formation of a clear area around the well due to the antibacterial activity of the tested extract. Measurements were taken in two directions: horizontally and vertically relative to the well. The width of the inhibition zone was calculated using the following formula:

$$L = [(D1 - D3) + (D2 - D3)] / 2$$

Where: L: Width of the inhibition zone (mm); D1: Horizontal diameter of the inhibition zone; D2: Vertical diameter of the inhibition zone; D3: Diameter of the well.

Data Analysis

Data analysis was performed using the SPSS program. Data normality was analysed using the Shapiro-Wilk test. If the Shapiro-Wilk test resulted in a p-value < 0.05, the data was considered not normally distributed. Subsequently, Levene's test was used to test for homogeneity or equality of variance. Because the data was not normally distributed, analysis was conducted using the non-parametric Kruskal-Wallis test to determine significance. If significant differences were found, a Post-Hoc test was performed to identify which groups showed meaningful differences.

Results and Discussion

The determination results confirmed that the bay leaf samples used belong to the species *Syzygium polyanthum* (Wight) Walpers. Chemical content testing was performed qualitatively using various solutions. This testing is an initial step in research aimed at identifying the groups of compounds present in the plant under study. The results of the chemical content analysis of the bay leaf extract indicated that the extract contains secondary metabolites such as alkaloids, saponins, tannins, and essential oils, but does not contain flavonoids. The chemical content results in this study differ from previous research, where bay leaf extracts were found to contain alkaloids, tannins, saponins, flavonoids, and terpenoids [12-13]. These differences may be attributed to environmental conditions where the plants grew, the duration of maceration (previous studies yielding flavonoids used 1x24 hours, while this study used 3x24 hours), the temperature during evaporation using a rotary vacuum evaporator (previous studies used 50°C, while this study used 70°C), and the effectiveness of the solvent during the extraction process. The antibacterial activity testing of the bay leaf ethanol extract against *Staphylococcus aureus* was conducted by measuring the diameter of the inhibition zone. The presence of inhibitory activity against the growth of *Staphylococcus aureus* is indicated by a clear zone around the well, which is then measured. The results of the inhibition zone diameter measurements of the bay leaf ethanol extract against the growth of *Staphylococcus aureus* showed the largest result at a 100% concentration, as presented in Table 1.

The research results show that bay leaf extract at concentrations of 50%, 75%, and 100% is effective in inhibiting the growth of *Staphylococcus aureus* bacteria compared to the negative control group (sterile distilled water). Significant differences are visible in the inhibition zone diameters across the three concentration groups (50%, 75%, and 100%) when compared to the sterile distilled water control group. However, the effectiveness of these three concentrations is not yet equivalent to the positive control (chlorhexidine). A comparison between the three concentrations indicates that the higher the concentration of the bay leaf extract, the larger the diameter of the inhibition zone formed. This is consistent with previous theories stating that a higher extract concentration contains a greater amount of chemical compounds [14].

This study shows results consistent with previous research conducted by Masyita and Asfiria (2021), which used bay leaves with a different solvent, namely the ethyl acetate fraction [14]. The well diffusion results in that study showed that higher extract concentrations led to larger inhibition zone diameters, specifically 10.5 mm, 12.25 mm, and 16.58 mm at concentrations of 5%, 10%, and 20%, respectively [14]. Other research using the same material but a different antibacterial testing method, the disc diffusion test, also showed that bay leaf extract can inhibit the growth of *Staphylococcus aureus* with inhibition zone diameters of 8.94 mm, 9.55 mm, 10.51 mm, and 10.12 mm at extract concentrations of 25%, 50%, 75%, and 100% [12]. The results of this study align with those findings despite the absence of flavonoid compounds. Bay leaf extract remains significant in inhibiting *Staphylococcus aureus* without flavonoid content.

Essential oils can inhibit enzymes involved in energy production, slow down cell growth, and at high levels, can cause protein denaturation [15]. Tannins play a role in altering the permeability of bacterial cell membranes and preventing plasma coagulation in *Staphylococcus aureus*, characterized by cell wall shrinkage and disruption of bacterial cell permeability [14]. Saponins possess antibacterial activity by causing the leakage of proteins and enzymes from within the cell, which disrupts bacterial survival [16]. Alkaloids inhibit the formation of peptidoglycan in the bacterial cell wall, causing the cell wall to form sub optimally, which ultimately leads to bacterial death [14]. Based on these theories, it is evident that the essential oils, tannins, saponins, and alkaloids contained in the bay leaf extract in this study contribute to inhibiting *Staphylococcus aureus* bacteria.

Pharmacology and Herbal Medicine Journal, 2026, **3**, 75–80
DOI: 10.20884/1.phmj.2026.1.3.19653
Research Paper

PHARMACOLOGY AND HERBAL MEDICINE JOURNAL
PHMJ

The inhibition zone diameters were further subjected to a Kruskal-Wallis statistical analysis to observe the differences in the mean inhibition zone diameters across the treatment groups. The results showed a significant difference with a p-value = 0.000. To determine which groups showed significant differences, a Post-Hoc Mann-Whitney U test was required. Based on Table 2, the Post-Hoc Mann-Whitney U test results above show that all significance values between treatment group pairs have a p-value < 0.05. This indicates that there is a statistically significant difference between each treatment group.

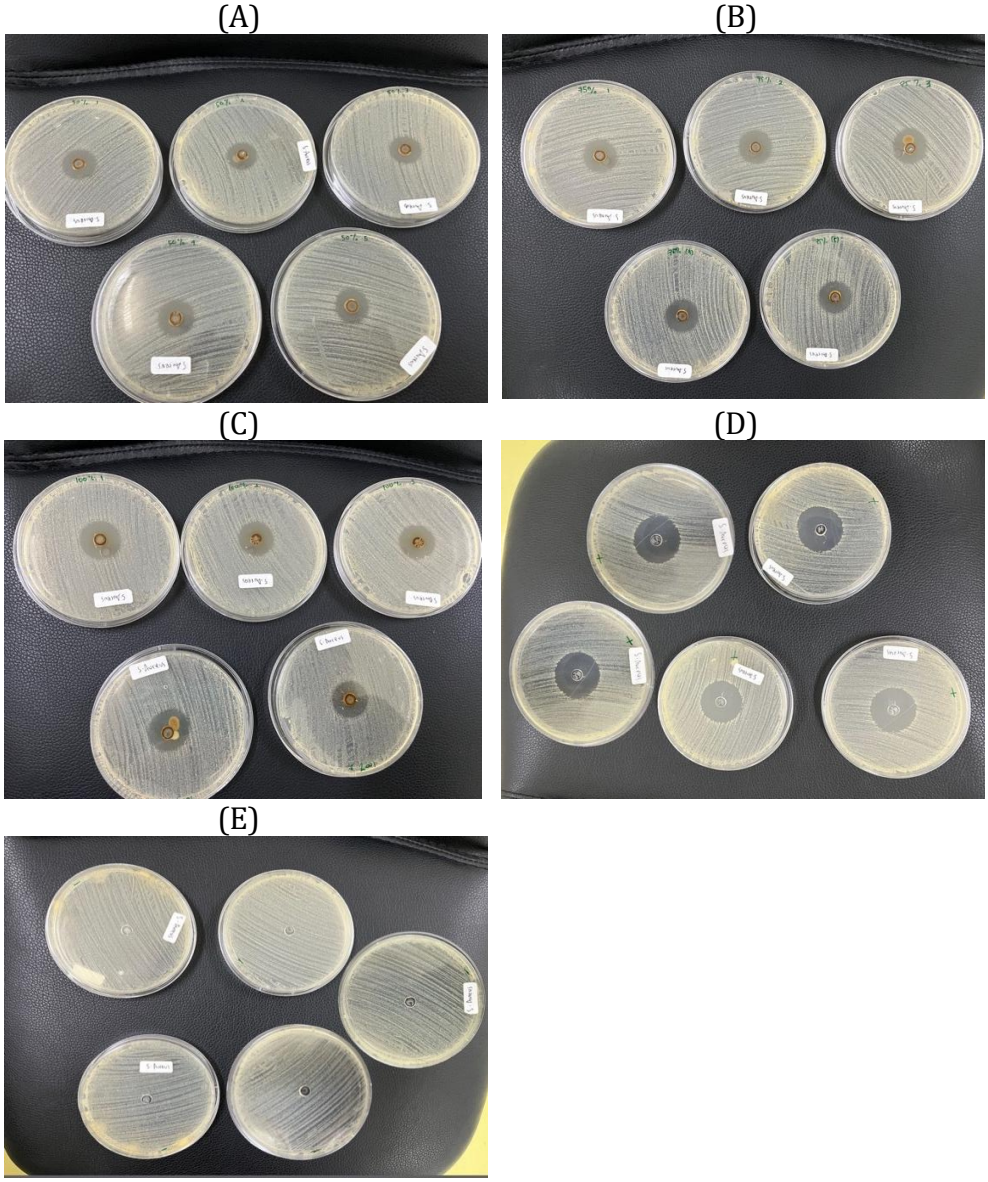

Figure 1. Bacterial inhibition zone of 50% bay leaf extract concentration (A), Bacterial inhibition zone of 75% bay leaf extract concentration (B), Bacterial inhibition zone of 100% bay leaf extract concentration (C), Bacterial inhibition zone of K+ (Chlorhexidine) (D), Bacterial inhibition zone of K- (Sterile Distilled Water) (E).

Table 1. Inhibition Zone Diameter Measurement Results

Group	Mean ± SD
K+ (Chlorhexidine)	22,20 ± 0.84
K- (Sterile Distilled Water)	0,00 ± 0.00
Bay leaf extract 50% concentration	11,40 ± 0.42
Bay leaf extract 75% concentration	13,85 ± 0.49
Bay leaf extract 100% concentration	15,10 ± 0.55

PHARMACOLOGY AND HERBAL MEDICINE JOURNAL
PHMJ

Table 2. Rat Serum Albumin Levels Across Various Treatment Groups

	K2	P1	P2	P3	K1
K2	-	0.005	0.005	0.005	0.005
P1	-	-	0.007	0.008	0.008
P2	-	-	-	0.008	0.008
P3	-	-	-	-	0.008
K1	-	-	-	-	-

Conclusion

Bay leaf extract (*Syzygium polyanthum* W.) can inhibit the growth of *Staphylococcus aureus* bacteria at concentrations of 50%, 75%, and 100%. The antibacterial activity is influenced by the levels of secondary metabolite compounds present in the bay leaves (*Syzygium polyanthum* W.), the concentration of the extract, the extraction method, the type of bacteria, and the specific techniques employed. Several limitations in this study that may influence the results include the use of an extract containing the full range of secondary metabolite compounds from the bay leaves, which makes it impossible to identify the specific primary antibacterial agent responsible for inhibiting the growth of *Staphylococcus aureus*.

Supplementary Material

None

Author Contributions

ZR : Conceptualization, Methodology, Writing-Original Draft. **KR** : Data Curation, Formal Analysis, Visualization. **RS** : Supervision, Funding Acquisition, Writing- Review & Editing.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

Acknowledgement

The author expresses gratitude to the Biology Laboratory of FMIPA UNNES, the Research Chemistry Laboratory of FMIPA UNNES, and the Biomedical Laboratory of FK UNIMUS for their assistance in the implementation of this research.

References

[1] Yusran, A., & Wardana, U. A. (2020). Inhibition test of moringa seeds extract against the growth of *Staphylococcus aureus.* Makassar Dental Journal, 9(3), 202–204.

[2] Nasution M. (2019). Pengantar mikrobiologi. Medan: USU Press,2019:62,77.

[3] Almasyhuri, & Sundari D. (2019). Uji aktivitas anti septik ekstrak etanol daun sirih (*piper betle linn*) dalam obat kumur terhadap *Staphylococcus aureus* secara in vitro. J Kefarmasian Indonesia 9(1):1

[4] Astuti P, Meilawaty Z. (2018). Efek antibakteri pasta gigi yang mengandung *tea tree oil* terhadap bakteri *Staphylococcus aureus, Staphylococcus mutans dan S. viridans. Stomatognatic* (J.K.G Unej);10:121.

[5] Supriyatin, S., Solikhah, S., & al Sas, O. (2023). Daya Hambat Obat Kumur dengan Kandungan Povidone Iodine dan Metil Salisilat Terhadap Pertumbuhan Staphylococcus Aureus. Jurnal Multidisiplin Indonesia, *2*(1), 81–88.

[6] Poernomo, H., Ma'ruf, M. T., & Dewi, L. N. (2022). The Comparison Of Maceration And Soxhacletation Methods Effectiveness On Garlic Extract To Inhibit Staphylococus Aureus Growth. Interdental Jurnal Kedokteran Gigi (IJKG), 18(2), 86–92.

[7] Aziz, F., Fitriana, F., Setyorini, D. R., Putri, S. A., Maulina, T. R., Dewi, V. K., Prihanani, N. I., & Andityas, M. (2023). The Comparisons of Phenotypic and Genotypic Resistance of Staphylococcus aureus Isolates Against β-lactam and Tetracycline Antibiotics. *Jurnal Sain Veteriner*, *41*(3), 313.

[8] Nuryah, A., Yuniarti, N., & Puspitasari, I. (2019). Prevalensi dan evaluasi kesesuaian penggunaan antibiotik pada pasien dengan infeksi *Methicillin Resistant Staphylococcus aureus* di RSUP Dr. Soeradji Tirtonegoro Klaten. *Majalah Farmaseutik*, *15*(2), 123–129. DOI: 10.22146/farmaseutik. v15i2.47911.

[9] Katno, Pramono S. (2019). Tingkat Manfaat Dan Keamanan Tanaman Obat dan Obat Trasional. Balai Penelitian Obat Tawangmangu. Fakultas Farmasi Universitas Gajah Mada [pressrelease]. Yogyakarta: Fakultas Farmasi UGM.

[10] Nugrahani, T., Kilis, I. M., Karauwan, F. A., Sambou, C. N., & Lengkey, Y. K. (2020). Formulasi Sediaan Salep Ekstrak Daun Salam Syzygium polyanthum Sebagai Antibakteri Staphylococcus aureus. *Jurnal Biofarmasetikal Tropis*, (1), 46–53.

[11] Habibi, A., Firmansyah, R. A., dan Setyawati, S. (2018). Skrining fitokimia ekstrak n-heksan korteks batang salam (*Syzygium polyanthum*). *Indonesian Journal of Chemical Science*. 7(1), 1-4.

[12] Haerussana, A. N. E. M., Dwiastuti, W. P., & Sukowati, C. A. (2021). Antibacterial Activity of Salam (Syzygium polyanthum) Leaves 70%

Ethanolic Extract on *Staphylococcus aureus* and *Staphylococcus epidermidis. Journal of Tropical Pharmacy and Chemistry*, *5*(4), 375–380.

[13] Kusuma Bhakti, U., Sasmito, E., Santoso, J., & Kesehatan, F. I. (2024). Uji Aktivitas Antibakteri Ekstrak Daun Salam (*Syzygium Polyanthum (Wight.) Walp.)* Terhadap Bakteri *Staphylococcus Aureus* Dan *Pseudomonas Aeruginosa. Jurnal Kesehatan Republik Indonesia*, *1*(7).

[14] Masyita Dewi, L., & Asfiria Arlita, S. (2021). *Efek Antibakteri Fraksi Etil Asetat Daun Salam (Syzygium polianthum [Wight.] Walp.) Terhadap Bakteri Staphylococcus aureus*.

[15] Maramis, A. Y., Asri, M. T., Biologi, J., Matematika, F., Ilmu, D., Alam, P., & Surabaya, U. N. (2022). *Uji Aktivitas Antibakteri Hand Sanitizer Ekstrak Daun Salam (Syzygium polyanthum) Terhadap Pertumbuhan Bakteri Staphyloccocus epidermidis*

[16] Rumela Sirait, N., Sitohang, R., Bemby Sinaga, A., Maya Sari, R., Studi, P. S., Farmasi dan Ilmu Kesehatan, F., & Sari, U. (2022). Uji Aktivitas Ekstrak Etanol Daun Salam *(Syzygium Polyanthum)* Terhadap Pertumbuhan Bakteri *Methicilin-Resistant Staphylococcus Aureus* (Vol. 4, Issue 1).