ORIGINAL RESEARCH REPORT

Antibacterial Activity Test of Green Betel Leaf Extract (*Piper betle* Linn.) against Methicillin-Sensitive *Staphylococcus aureus*

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ABSTRACT

Background: Asia is one of the regions with the highest prevalence rates of *Staphylococcus aureus* (S. *aureus*) infection. Green betel leaves (*Piper betle* Linn.) have been shown to possess antibacterial potential due to their active compound content. **Objective:** To investigate whether betel leaf extract affects methicillin-sensitive *Staphylococcus aureus* (MSSA). **Material and Method:** This research was a descriptive experimental study focusing on the antibacterial activity test of green betel leaf extract against MSSA. The MSSA bacteria used were bacterial preparations in the Microbiology Laboratory of the Faculty of Medicine, Universitas Airlangga. The green betel leaf extract was obtained in 2023 in Batu City, East Java, Indonesia. The antibacterial activity test technique used was the dilution test. The tool used in data processing was Microsoft Excel. **Results:** Germs were found in the dilution test, particularly in tubes with concentrations of 50 mg/mL. The study also highlighted that no growth of germs was found based on the results obtained for the increase of MSSA on agar plates at concentrations of 800 mg/mL, 600 mg/mL, 400 mg/mL, and 200 mg/mL. The study also found that the growth of MSSA germs was at 100 mg/mL and 50 mg/mL. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of green betel leaf extract against MSSA were 100 mg/mL and 200 mg/mL, respectively. **Conclusion:** The extract of green betel leaves, particularly cultivated in Batu City, East Java, Indonesia has been shown to possess antibacterial activity, which can inhibit and kill MSSA bacteria. Further research is needed on the antibacterial activity of green betel leaf extract against other bacterial species.

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Highlights

1. Green betel leaf extract, particularly cultivated in Batu City, can inhibit and kill MSSA.
2. Green betel leaf extract, particularly cultivated in Batu City, can be developed into a new alternative treatment as an effective antibiotic to kill MSSA bacteria.

BACKGROUND

Asia is one of the regions with the highest prevalence of S. *aureus* infections, reaching 70%. Meanwhile, the prevalence of S. *aureus* infections in Indonesia, particularly in Java and Bali, is 3.1% (Fitranda, et al., 2023). Approximately 45.3% of 567 patients in Surabaya, Malang, and Bali who suffered from skin and soft tissue infections were clinically diagnosed with S. *aureus* infection (Jieputra, et al., 2024). S. *aureus* is a human pathogenic bacterium that can cause dangerous diseases. *Staphylococcus aureus* possesses various virulence factors, including the ability to form biofilm, which can cause a wide range of infectious diseases (Setiabudy, et al., 2023). This bacterium is an agent that causes infections in humans, including skin and wound infections, pneumonia, meningitis, toxic shock syndrome, scalded skin syndrome, bacteremia, sepsis, and bone infections (Mahon & Lehman, 2001; Riedel, et al., 2019; Jannah, et al., 2021; Putri, et al., 2021). S. *aureus* can become resistant to oxacillin and related β-lactams by acquiring new penicillin-binding proteins that are enzymatically active but not bound and inactivated by the antibiotics (Murray, et al., 2015; Lake, et al., 2019).

The type of infection and the presence of drug-resistant strains of S. *aureus* determine the best treatment method. In general, first-line parenteral antibiotics for MSSA are nafcillin, oxacillin, and first-generation cephalosporins. This therapy has side effects in the form of neutropenia, interstitial nephritis, hepatitis, allergic reactions, including anaphylactic shock, urticaria, fever, joint swelling, and skin rashes (Katzung, 2017). This therapy also requires high costs, so an alternative treatment using natural ingredients is needed, one of which is using green betel leaves (*P. betle* L.), which is known for its properties, is easy to obtain, has an affordable price, and has low toxicity (Sadiah, et al., 2022).

Green betel leaves (*P. betle* L.) have been used as an ingredient for traditional medicine by Indonesians and Asian peoples for a long time. Green betel leaves (*P. betle* L.) are commonly used by people to treat and prevent various diseases such as itching, coughs, colds, and toothache (Hulu, et al., 2022). Green betel leaves (*P. betle* L.) have potential as an antibacterial along with their active compounds, such as essential oils, phenols, chavicol, flavonoids, alkaloids, saponins, tannins, and steroids (Sadiah, et al., 2022).

According to earlier studies, green betel leaf extract (*P. betle* L.) from Makassar and Denpasar, when utilized appropriately, might suppress the growth of S. *aureus*. The extract's active ingredients included flavonoids, tannins, and phenols. However, there have been no investigations of the antibacterial properties of green betel leaves against MSSA, particularly the leaves cultivated in Batu City, East Java, Indonesia. Based on the problems above, it is necessary to examine whether green betel leaf extract, specifically planted in Batu City, affects MSSA (Alydrus & Khofifahl, 2022; Suyasa, et al., 2022).

OBJECTIVE

The objective of this research is to analyze the effect of green betel leaf extract, specifically planted in Batu City, on the growth of MSSA.

MATERIAL AND METHOD

This study was a true experimental research using posttest-only control group design. This research method used a dilution test on MSSA by administering betel leaf extract (*P. betle* L.) in various concentrations in vitro. This study employed the Federer Formula to determine the requirement of repetitions. Federer's formula is (t-1) (r-1) ≥ 15 where, t = number of treatment groups, t = number of repetitions. Therefore, if there are six treatments at concentrations of 800 mg/mL, 600 mg/mL, 400 mg/mL, 200 mg/mL, 100 mg/mL and 50 mg/mL, then the repetitions will be conducted 4 times. The study employed random sampling to take the samples. The bacteria were taken randomly from *Staphylococcus aureus* colonies growing on the media. The tool used in data processing was Microsoft Excel 2021 (Gates & Allen, 2021).

The materials utilized in this study included MSSA bacteria from the Department of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, and green betel leaves (*P. betle* L.), which were collected from Batu City in 2023. The study was conducted at the Microbiology Laboratory of the Faculty of Medicine at Universitas Airlangga, Surabaya. The study required 11 months to complete,

from July 2023 to May 2024. To ensure the suitability of the plants to be investigated, the green betel leaves were subsequently determined at the Batu Herbal Materia Medica Laboratory, UPT.

The betel leaf extract was produced using maceration method. The solvent used was 96% ethanol. After being cleaned, green betel leaves were dried at 40°C in an oven. Then, the leaves were kneaded and ground until it became powder. Following the weighing of 500 grams of betel leaf powder, the powder was soaked in 3.5 liters of 96% ethanol solvent for five days. The filtrate was removed for the filtration process. Stirring was done 12 times for 15 minutes. After 5 days, the betel leaf extract was filtered and squeezed using filter paper and a funnel to separate the filtrate from the dregs. The filtering results were then stored in a tightly closed new container. The remaining dregs were macerated with ethanol for two days and stirred occasionally. The extract was filtered, and the filtered results were combined with the filtrate from the first step. The solvent was removed using a rotary evaporator set to 55°C. Thereafter, it was concentrated at 55°C in a water bath. Then, 12 grams of a thick extract devoid of solvents and extracts used in further testing were obtained. Twenty-four-hour-old MSSA cultures were extracted as 0.1 mL of 0.5 McFarland (1.5 x 10^8 CFU/mL). Furthermore, they were placed in a test tube containing a liquid medium and incubated for 24 hours at 37°C in an O_2 incubator (Olla, 2019; Alydrus & Khofifahl, 2022).

The antibacterial activity test against MSSA used the dilution method to determine the MIC and MBC of betel leaf extract (*P. betle* L.). MIC is the smallest concentration of an antibiotic substance that can still inhibit the growth of germs. Meanwhile, MBC is the smallest concentration of an antibiotic substance that is capable of killing germs. The dilution test in this study required nine test tubes, including three control tubes and six test tubes. The first and second control tubes were negative controls, while the third control tube was the positive control. The first control tube contained two milliliters of sterile liquid medium. The second control tube contained one milliliter of sterile liquid medium and one milliliter of green betel leaf extract (*P. betle* L.). In addition, the third control tube contained one milliliter of sterile liquid medium and one milliliter of MSSA bacterial colonies.

The first test tubes were filled with 1.6 mL of green betel leaf extract and 0.4 distilled water (800 mg/mL). The second tubes were filled with 0.6 mL of green betel leaf extract and 0.4 distilled water (600 mg/mL). The third to sixth tubes were filled with 1 mL sterile liquid medium. Then, 1 mL of the solution from the first test tube was taken and added to the third tube, and so on until the sixth tube. One mL of the sixth tube solution was discarded, so that the volume of solution for each test tube was 1 mL with different concentrations, 800 mg/mL, 600 mg/mL, 400 mg/mL, 200 mg/mL, 100 mg/mL and 50 mg/mL. Then, in the first to sixth test tubes, 1 mL of a colony of S. *aureus* bacteria was added so that the final volume of all tubes was 2 mL. MIC was determined by identifying the smallest concentration of green betel leaf extract (*P. betle* L.) in the tube that was still able to inhibit bacterial growth. The clear MIC tubes, from which the specimens had been taken, were then cultured on an Agar plate so that the MBC could be determined.

RESULT

The extraction of green betel leaves using ethanol produced 50 mL of liquid extract. The results obtained from the MIC test of green betel leaf extract against MSSA were the tubes with concentrations of 800 mg/mL, 600 mg/mL, 400 mg/mL, 200 mg/mL, and 100 mg/mL. The clear appearance indicated the absence of germ growth. On the other hand, the cloudy appearance of the tube at a concentration of 50 mg/mL implied the growth of germs, as demonstrated in Figure 1.

The MIC of the green betel leaves extract, particularly those cultivated in Batu City, against MSSA was 100 mg/mL, as all concentrations possessed the same minimal inhibitory concentration of 100 mg/mL. Table 1 demonstrates a summary of all the findings.

Figure 1. Test tube results in MSSA bacterial dilution test after 24 hours incubation.

Table 1. Results of MIC test on MSSA (mg/mL).

	800	600	400	200	100	50
I	-	-	-	-	-	+
II	-	-	-	-	-	+
III	-	-	-	-	-	+
IV	-	-	-	-	-	+

+ = Cloudy tube (presence of bacterial growth)
-= Clear tube (absence of bacterial growth)

Subsequent measurement of the MIC revealed that the tube fluid with a concentration ranging from 800 mg/mL to 50 mg/mL was cultivated on an agar plate. Furthermore, the authors conducted the observations to measure the MIC after incubation for 24 hours. The results obtained for the growth of MSSA demonstrated that at concentrations of 800 mg/mL, 600 mg/mL, 400 mg/mL, and 200 mg/mL, there was no growth of MSSA on the agar plates. Meanwhile, the MSSA growth was determined at concentrations of 100 mg/mL and 50 mg/mL, as shown in Figure 2.

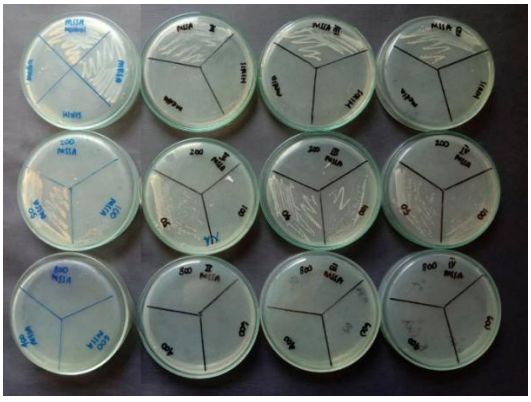

Figure 2. Agar culture results of the entire MSSA bacterial dilution test tubes.

The first, third, and fourth repetitions provided MSSA MBC results of 200 mg/mL, and the second repetition provided MSSA MBC results of 100 mg/mL. These findings indicated that the MBC value of the green betel leaf extract against MSSA in this investigation was 200 mg/mL, as demonstrated in Table 2.

Table 2. Results of MBC test on MSSA (mg/mL).

	800	600	400	200	100	50
I	-	-	-	-	+	+
II	-	-	-	-	-	+
III	-	-	-	-	+	+
IV	-	-	-	-	+	+

+ = Agar plate was overgrown with bacteria (presence of bacteria)
- = Agar plate was not overgrown with bacteria (absence of bacteria)

DISCUSSION

In the green betel leaf extraction process, the authors used ethanol as a solvent because it is a widely used extraction solvent for active ingredients that are antibacterial from within cells. Ethanol is a universal solvent that can extract polar or semi-polar compounds, non-toxic, able to mix with water, and requires less heat for concentrations. Therefore, the active ingredients used in this research could be extracted properly (Rahmawatiani, et al., 2020).

Staphylococcus aureus possesses various virulence factors that can cause a wide range of infectious diseases. *Staphylococcus aureus* produces the catalase enzyme. This enzyme breaks down hydrogen peroxide, a host defense mechanism, and enables intracellular survival. Coagulase is a surface protein of *Staphylococcus aureus* that produces fibrin from clotting factors that cause clotting. Toxins and extracellular substances include hemolysins that can destroy erythrocytes, leucocidin, which can cause skin necrosis, exfoliative toxins, and enterotoxins B and C, which can spread systemic inflammatory responses. These virulence factors can cause various clinical syndromes, including the development of abscesses (Cheung, et al., 2021).

Green betel leaves have antibacterial activity against MSSA bacteria because they contain various antibacterial active ingredients, including alkaloids, phenols, flavonoids, essential oils, chavicol, saponins, tannins, and steroids. These diverse compounds play a role in inhibiting cell wall synthesis, disrupting cell membrane permeability and cell metabolism, damaging nucleic acids, and inhibiting cell protein synthesis, which enable the bacterial cells to die. These compounds have antimicrobial, antioxidant, and anti-inflammatory effects (Sadiah, et al., 2022).

Other research demonstrate that green betel leaf extract is able to inhibit MSSA bacteria in various concentrations. Research by Tagrida & Benjakul (2022) using ethanol extract showed that the MIC of green betel leaves on MSSA was 3.12 mg/mL. According to the study, the encapsulation with liposomes can improve stability, and the antibacterial activity of green betel leaf extract that was placed inside liposomes was higher than that of the unencapsulated extract. Furthermore, the encapsulated green betel leaf extract exhibited poor triphenyl-2H tetrazolium chloride (TTC) dehydrogenase activity and a larger inhibitory zone. Lao et al. (2023) showed that the MIC of green betel leaves on MSSA is 2.5 mg/mL, and this can be interpreted as having a weak antimicrobial effect, while a study by Leesombun et al. (2023) revealed that the MIC of green betel leaves on MSSA is 1.024 mg/mL. Saeloh & Visutthi (2021) found that the MIC of green betel leaves on MSSA is 0.62 mg/mL. The study used plants from Northeast Thailand and used the broth microdilution method. Betel leaf extract can inhibit biofilm formation and support the eradication of biophiles in S. *aureus*. Another research demonstrated that the MIC of green betel leaves on MSSA is 0.25 mg/mL. According to this study, green betel leaf extract can prevent the development and growth of Staphylococcus biofilms that cause mastitis in cows by removing 54–86% of the viability of the biofilm that forms on the isolate (Sungkatavat, et al., 2023). Valle (2021) discovered that mice treated with green betel leaf extract cream experienced faster wound contraction and a significantly shorter time to re-epithelialization compared to the mice treated with mupirocin antibacterial cream and the mice treated with a cream base devoid of active ingredients.

Green betel leaf extract can kill MSSA bacteria in various concentrations. Research by Lao et al. (2023) showed that the MBC results of green betel leaves on MSSA are 5 mg/mL. Tagrida & Benjakul (2022) concluded that the MBC results of green betel leaves on MSSA are 3.12 mg/mL, while a research

by Saeloh & Visutthi (2021) showed that the MBC results of green betel leaves on MSSA are 0.62 mg/mL, and Sungkatavat et al. (2023) demonstrated that the MBC of green betel leaves on MSSA is 0.25 mg/mL. The ethanol extract of green betel leaf and red betel leaf comprises antibacterial activity against S. *aureus* with MICs of 6.25% and 100%, respectively. Ethanol extract of green betel leaf with concentrations of 100% and 50% can inhibit biofilm formation by 40%. Moreover, the ethanol extract of red betel leaf at a concentration of 6.25% can inhibit biofilm formation by 20%. Therefore, this study concluded that both the ethanol extract of green betel leaf and the ethanol extract of red betel leaf have the potential to be antibiofilm against S. *aureus* (Asnani, et al., 2022). A study by Jamil, et al., (2021) revealed that the entire betel leaf extract fractions demonstrated excellent antibacterial activity. However, the ethyl acetate fraction had the maximum activity when compared to the n-hexane and ethanol fractions. The betel leaf extract's ethyl acetate fraction includes anthraquinones, polyphenols, and terpenoids. The betel leaf extract's n-hexane fraction includes anthraquinones, polyphenols, flavonoids, terpenoids, and alkaloids. The ethanol fraction of betel leaf extract includes anthraquinones, polyphenols, terpenoids, and alkaloids (Jamil, et al., 2021). Situmorang (2024) conducted a study by measuring the levels of Neutrophil-Lymphocyte Ratio as an anti-inflammatory biomarker of green betel leaf extract on male Wistar rats infected with S. *aureus*. The author discovered that when male Wistar rats were treated with green betel leaf extract, there was a notable change in their neutrophil-lymphocyte ratio levels. In comparison to doses of 300 mg/KgBW, 500 mg/KgBW, positive control, and negative control, a dose of 1000 mg/KgBW of green betel leaf extract was successful in lowering the levels of Neutrophil-Lymphocyte Ratio in male Wistar rats infected with S. *aureus*. Based on this study, it can be said that green betel leaves have the potential to be an anti-inflammatory agent (Situmorang, 2024).

In another study, different quantities of green betel leaf extract were also found to inhibit Methicillin-Resistant *Staphylococcus aureus* (MRSA) germs. For instance, a particular study showed that the MIC of green betel leaves ethanol extract against MRSA is 0.62 mg/mL. Green betel leaves have a high antibacterial effect and have potential as an anti-biofilm for various infectious diseases caused by S. *aureus* (Saeloh & Visutthi, 2021). Meanwhile, Leesombun et al. (2023) revealed that the MIC of green betel leaves against MRSA is 0.256 mg/mL. Saeloh & Visutthi (2021) found that green betel leaf extract possesses the ability to kill MRSA bacteria in various concentrations. The outcomes of MBC for green betel leaves against MRSA are 0.62 mg/mL. Several bioactive compounds, such as piperine, diisooctyl phthalate, and hyroxcavicol contained in green betel leaf extract show strong binding affinity with the MRSA protein target. Thus, these compounds are effective in inhibiting the growth of MRSA (Yasir, et al., 2024). Phensri et al. (2022) discovered that the mean inhibitory concentration (MIC) of raw betel leaf extract against Staphylococcus strains was 252.78 mg/L. Moreover, the MICs of benzoyl peroxide and azelaic acid against Staphylococcus strains were 1342.70 and 963.49 mg/L, respectively. This demonstrates the superiority of betel leaf extract over benzoyl peroxide and azelaic acid. In the case of dogs with pyoderma caused by Staphylococcus strains, betel leaf extract can be administered as an alternate topical antibiotic (Phensri, et al., 2022). In an experiment on the growth of MRSA bacteria from diabetic patients' wounds, Raudah tested the antibacterial activity of green betel leaf extract. The findings showed that the extract could stop MRSA growth at a 20% concentration. Red betel leaf could also inhibit MRSA obtained from wounds of diabetes mellitus patients, starting at a concentration of 40%. Based on the Test of Between-Subjects Effect conducted by Raudah, it can be concluded that green betel leaf extract can inhibit the growth of MRSA bacteria obtained from diabetes mellitus wounds in vitro faster than red betel leaf extract (Raudah, et al., 2022).

The results of this study indicated that the MIC of MSSA was 100 mg/mL, and the MBC of MSSA was 200 mg/mL with a typical local plant of green betel leaf, particularly planted in Batu City. There are discrepancies in the results from several research outcomes mentioned previously, which might occur due to factors inherent to the green betel leaf plant. This phenomenon could be due to the composition of the active ingredients in green betel leaves, which is affected by several factors. The quality of the active ingredients contained in the green betel leaf medicinal plant can be affected by external and internal factors. External factors include the growing conditions of green betel plants, such as land conditions, temperature, climate, altitude, environmental pollution, pests, high ultraviolet intensity, heavy metal contamination, and humidity where the plants grow. In contrast, the internal factors consist of genetic quality and plant age. The higher the height at which plants grow, or the lower the temperature of the environment, the more the levels of essential oils in these plants will increase (Putri & Paramita, 2023).

The distinction in antibacterial sensitivity observed in previous research may be attributable to several factors, including the choice of solution, pH, storage time of green betel leaf extract, the extraction method, and the use of bacterial isolates in the research. The selection in the extraction process requires an appropriate solution, as the active compound taken is based on the solution used during extraction. Betel leaf extract, which has a longer storage time, may experience a decrease in quality due to a decline in antibacterial compounds. The method of the extraction process can influence the yield value, which is related to the success in extracting the amount of active compound. Gram-positive bacteria are more sensitive than Gram-negative bacteria because their cell walls are made up of only a few layers of peptidoglycan. This means that the compounds found in green betel leaf extract, such as alkaloids, phenols, flavonoids, essential oils, chavicol, saponins, tannins, and steroids, can easily denaturize their cells. On the outside of the peptidoglycan layer of Gram-negative bacteria, there are three wrapping polymers, namely lipoproteins, outer membranes, and lipopolysaccharides (Weinreb, 2024). The bacterial isolates and strains used may differ due to distinctions in research locations, which can influence the results of existing research. The limitation of this research lies in the fact that betel leaves consist of a wide range of varieties, but this research only examined green betel leaves. Based on the results obtained in this study, a concentration of 100 mg/mL of green betel leaf extract from Batu City inhibits the growth of MSSA bacteria. Additionally, a concentration of 200 mg/mL of green betel leaf extract annihilates MSSA bacteria. Therefore, this concentration can be developed into a new alternative treatment in the form of a safe and relevant herbal medicine with green betel leaf extract for treating various diseases caused by MSSA infections as an effective antibiotic in killing MSSA bacteria (Putri & Paramita, 2023).

Strengths and limitations

The strength of this study lies in its originality, as it examines the antibacterial effects of a particular green betel leaf extract cultivated in Batu City, on MSSA growth. A lack of variation in the dataset is one of the limitations of this study, as evidenced by the examination of a solitary isolate. Furthermore, there were also restrictions in the examination of the concentrations.

CONCLUSION

The green betel leaf extract (*P. betle* L.), specifically planted in Batu City, East Java, Indonesia, can inhibit and annihilate the MSSA bacteria. The extract can be developed as a new alternative treatment, such as an antibiotic that is effective in annihilating the growth of MSSA bacteria.

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Conflict of Interest

The authors declared there is no conflict of interest.

Ethic Consideration

This is an in vitro study.

Funding Disclosure

There was no funding for this study.

Author Contribution

SAZ, NW, and YS designed the study and wrote the manuscript. SAZ and NW were responsible for gathering information and conducting a review of the background literature. The results and discussion were under the supervision of NW and YS. The final manuscript was reviewed and approved by all authors.

Data Availability

Available

REFERENCES

Alydrus, N. L., & Khofifahl, N. 2022. Efektifitas antibakteri ekstrak daun sirih hijau (*Piper betle* L.) terhadap *Staphylococcus aureus*. Inhealth: Indonesian Health Journal, 1(1), 56–61. doi: 10.56314/inhealth.v1i1.23.

Asnani, A., Ruwielanisa, P., Hernayanti, & Anjarwati, D. U. 2022. The potency of ethanolic extracts of betel leaves as an antibiofilm against methicillin-resistant *Staphylococcus aureus*. AIP Conference Proceedings, 020021. doi: 10.1063/5.0103681.

Cheung, G. Y. C., Bae, J. S., & Otto, M. 2021. Pathogenicity and virulence of *Staphylococcus aureus*. Virulence, 12(1), 547–569. doi: 10.1080/21505594.2021.1878688.

Fitranda, M., Salasia, S. I. O., Sianipar, O., Dewananda, D. A., Arjana, A. Z., Aziz, F., Wasissa, M., Lestari, F. B., & Santosa, C. M. 2023. Methicillin-resistant *Staphylococcus aureus* isolates derived from humans and animals in Yogyakarta, Indonesia. Veterinary World, 239–245. doi: 10.14202/vetworld.2023.239-245.

Gates, B., & Allen, P. 2021. *Microsoft Excel*. Microsoft. https://office.microsoft.com/excel.

Hulu, L. C., Fau, A., & Sarumaha, M. 2022. Pemanfaatan daun sirih hijau (*Piper betle* L.) sebagai obat tradisional di Kecamatan Lahusa. TUNAS : Jurnal Pendidikan Biologi, *3*(1), 46–57. doi: 10.57094/tunas.v3i1.480.

Jamil, A. S., Rofida, S., Farida, D., Nur Syahida, D. R., & Nazah, T. H. 2021. Inhibitory activity of several extracts of *Piper betle* leaf against *S. aureus.* Pharmaciana, 11(2), 261. doi: 10.12928/pharmaciana.v11i2.16999.

Jannah, S. N., Arfijanto, M. V., Rusli, M., & Widodo, A. D. W. 2021. Sepsis: Antibiotic resistances of Gram-positive and Gram-negative bacteria in a tertiary care hospital. JUXTA: Jurnal Ilmiah Mahasiswa Kedokteran Universitas Airlangga, 12(1), 29. doi: 10.20473/juxta.V12I12021.29-37.

Jieputra, A., Purwanta, M., Mustika, A., & Retnowati, W. 2024. In vitro antimicrobial activity test of *Zingiber officinale* var. *rubrum* rhizome extract against methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA). JUXTA: Jurnal Ilmiah Mahasiswa Kedokteran Universitas Airlangga, 15(1), 57–63. doi: 10.20473/juxta.V15I12024.57-63.

Katzung, B. G. 2017. *Basic and clinical pharmacology* (14th ed.). McGraw Hill Professional. Available at: https://books.google.co.id/books/about/Basic_and_Clinical_Pharmacology_14th_Edi.html?hl=id&id=-W5ADwAAQBAJ&redir_esc=y.

Lake, W. K., Hamid, I. S., Saputro, A. L., Plumeriastuti, H., Yustinasari, L. R., & Yunita, M. N. 2019. Uji aktivitas antibakteri dari ekstrak n-heksana dan kloroform daun sirsak (*Annona muricata* L.) terhadap pertumbuhan bakteri *Staphylococcus aureus* secara in vitro. Jurnal Medik Veteriner, 2(1), 60. doi: 10.20473/jmv.vol2.iss1.2019.60-65.

Lao, R. C. C., Yabes, A. M., Tobias-Altura, M., Panganiban, L. C. R., & Makalinao, I. R. 2023. In vitro antibacterial and antibiofilm activities of *Piper betle* L. ethanolic leaf extract on *Staphylococcus aureus* ATCC 29213. Acta Medica Philippina, 57(12), 53–60. doi: 10.47895/amp.vi0.6412.

Leesombun, A., Sungpradit, S., Bangphoomi, N., Thongjuy, O., Wechusdorn, J., Riengvirodkij, S., Wannawong, J., & Boonmasawai, S. 2023. Effects of *Piper betle* extracts against biofilm formation by methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs. Pharmaceuticals, 16(5), 741. doi: 10.3390/ph16050741.

Mahon, C. R., & Lehman, D. C. 2001. *Textbook of diagnostic microbiology* (3rd ed.). Laboratory Medicine, 32(3), 163–164. doi: 10.1309/U0MB-0P7R-RRWF-4BTH.

Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. 2015. *Medical microbiology* (8th ed.). Elsevier Health Sciences. doi: 10.1016/0166-0934(89)90139-0.

Olla, L. R. Y. 2019. Uji aktivitas antimikroba ekstrak daun sirih hijau (*Piper betle* L.) terhadap pertumbuhan bakteri *Staphylococcus aureus* [Undergraduate thesis, Politeknik Kesehatan Kemenkes Kupang]. Available at: http://repository.poltekeskupang.ac.id/id/eprint/785.

Phensri, P., Thummasema, K., Sukatta, U., Morand, S., & Pruksakorn, C. 2022. In vitro antimicrobial activity of *Piper betle* leaf extract and some topical agents against methicillin-resistant and methicillin-susceptible *Staphylococcus* strains from canine pyoderma. Animals, 12(22), 3203. doi: 10.3390/ani12223203.

Putri, N. I. C. A., Ramadhani, R., & Wasito, E. B. 2021. Gram-negative bacteria (*Escherichia coli*) win

against Gram-positive bacteria (*Staphylococcus aureus*) in the same media. Biomolecular and Health Science Journal, 4(2), 113. doi: 10.20473/bhsj.v4i2.30177.

Putri, N. L. P. T., & Paramita, N. L. P. V. 2023. Review aktivitas antibakteri ekstrak daun sirih hijau (*Piper betle* L.) metode difusi dan mikrodilusi. Journal Scientific of Mandalika (JSM), 4(2), 6–18. doi: 10.36312/10.36312/vol4iss2pp6-18.

Rahmawatiani, A., Mayasari, D., & Narsa, A. C. 2020. Kajian literatur: Aktivitas antibakteri ekstrak herba suruhan (*Peperomia pellucida* L.). *Proceeding of Mulawarman Pharmaceuticals Conferences, 12,* 117–124. doi: 10.25026/mpc.v12i1.401.

Raudah, S., Wahid, R. S. A., & Huzaimah. 2022. Uji aktivitas antibakteri ekstrak daun sirih hijau (*Piper betle* L.) dan daun sirih merah (*Piper crocatum* Ruiz & Pav) terhadap bakteri pada luka diabetes mellitus secara in vitro. Conference on Innovation in Health, Accounting, and Management Sciences, 2, 59–69. doi: 10.31001/cihams.v2i.78

Riedel, S., Morse, S. A., Mietzner, T. A., & Miller, S. 2019. *Jawetz, Melnick & Adelberg's medical microbiology* (28th ed.). McGraw Hill Professional. Available at: https://books.google.co.id/books/about/Jawetz_Melnick_Adelbergs_Medical_Microbi.html?hl=id&id=1dSWDwAAQBAJ&redir_esc=y.

Sadiah, H. H., Cahyadi, A. I., & Windria, S. 2022. Kajian Daun Sirih Hijau (Piper betle L) Sebagai Antibakteri. Jurnal Sain Veteriner, 40(2), 128. doi: 10.22146/jsv.58745.

Saeloh, D., & Visutthi, M. 2021. Efficacy of Thai plant extracts for antibacterial and anti-biofilm activities against pathogenic bacteria. Antibiotics, 10(12), 1470. doi: 10.3390/antibiotics10121470.

Setiabudy, M., Masyeni, D. A. P. S., Indraningrat, A. A. G., Suryawan, K., & Adhiputra, I. K. A. I. 2023. Biofilm formation in *Staphylococcus aureus* and coagulase-negative *Staphylococcus*. Folia Medica Indonesiana, 59(3), 222–228. doi: 10.20473/fmi.v59i3.44598.

Situmorang, B. J. 2024. Pengaruh pemberian ekstrak daun sirih hijau (*Piper betle* L.) terhadap rasio neutrofil-limfosit pada tikus wistar (*Rattus novergicus*) jantan yang terinfeksi *Staphylococcus aureus* [Undergraduate thesis, Universitas HKBP Nommensen Medan]. UHN Repository. Available at: https://repository.uhn.ac.id/handle/123456789/10207.

Sungkatavat, P., Khongkhai, H., Kanchana, W., Saengsawarng, P., Sangkanu, S., Nissapatorn, V., Pereira, M. de L., Ontong, J. C., & Mitsuwan, W. 2023. *Piper betle* extract and its application in bovine teat dipping solution inhibit and eliminate biofilms in bovine mastitis-inducing staphylococci. Veterinary World, 2135–2142. doi: 10.14202/vetworld.2023.2135-2142.

Suyasa, I. B. O., Bekti, H. S., Rinawati, L. P., Laksmi, L. P., Wahyuni, P. D., Agustini, D. G. D., & Rakhmawati, A. 2022. Daya hambat ekstrak daun sirih dan daun legundi terhadap pertumbuhan bakteri *Staphylococcus aureus*. The Journal of Muhammadiyah Medical Laboratory Technologist, 5(1), 29–34. doi: 10.30651/jmlt.v5i1.11015.

Tagrida, M., & Benjakul, S. 2022. Liposomes loaded with betel leaf (*Piper betle* L.) extract: Antibacterial activity and preservative effect in combination with hurdle technologies on tilapia slices. Food Control, 138, 108999. doi: 10.1016/j.foodcont.2022.108999.

Valle, D. L. J. 2021. Antimicrobial efficacy and activity of ethanolic extract of *Piper betle* L. on *Staphylococcus aureus*-infected wound in mice and clinical isolates of multiple drug-resistant bacterial pathogens Tropical Biomedicine, 38(2), 134–142. doi: 10.47665/tb.38.2.049.

Weinreb, R. N. 2024. Review of antibacterial activity of green betel leaf extract (*Piper betle* L.) methods of diffusion and microdilution. Join: Journal of Social Science, 1(1), 1–18. doi: 10.59613/q6zxqm74.

Yasir, B., Mus, S., Rahimah, S., Tandiongan, R. M., Klara, K. P., Afrida, N., Nisaa, N. R. K., Risna, R., Jur, A. W., Alam, G., & Rohman, A. 2024. Antimicrobial profiling of *Piper betle* L. and *Piper nigrum* L. against methicillin-resistant *Staphylococcus aureus* (MRSA): Integrative analysis of bioactive compounds based on FT-IR, GC-MS, and molecular docking studies. Separations, 11(11), 322. doi: 10.3390/separations11110322.