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The Effectiveness of Antibiofilm Synergy of Parijoto Leaf Extract (*Medinilla speciosa*) and Gentamicin on *Methicillin-Resistant Staphylococcus aureus* (MRSA) from Diabetic Ulcers

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ABSTRACT

Diabetic foot ulcers (DFU) are chronic complications of diabetes that are generally accompanied by bacterial infections. *Methicillin-Resistant Staphylococcus aureus* (MRSA) is one of the most dominant pathogenic bacteria isolated from DFU wounds. The significant number of MRSA bacteria and the formation of bacterial biofilms result in prolonged wound treatment and high amputation rates. Several medicinal plants in the Muria Mountains have the potential to be alternative antibiofilms supporting gentamicin antibiotic treatment, one of which is the parijoto plant *(Medinilla speciosa*). This study aims to evaluate the effectiveness of the combination of parijoto leaf extract with gentamicin in inhibiting the growth of MRSA biofilms isolated from DFU. Parijoto leaves were extracted using the UAE (*Ultrasound-Assisted Extraction*) technique, followed by phytochemical tests and antibiofilm inhibition tests using the microdilution method. The results of phytochemical identification showed that there were saponin, flavonoid, tannin, alkaloid, quinone, and phenol compounds. The combination of gentamicin and parijoto leaf extract with several variations obtained the highest percentage of biofilm inhibition, namely 95%. The IC50 value in the antibiofilm inhibition test of the combination of parijoto leaf extract and gentamicin was 6.90 µg/mL. The chemical bioactive content in parijoto leaf extract can synergize as a supporting treatment with gentamicin to accelerate the treatment of diabetic ulcer wound infections. Further research needs to be carried out to explore and further study to determine the QS (*Quorum Sensing*) mechanism against MRSA (*Methicillin-Resistant Staphylococcus aureus*) bacteria by bioactive compounds in parijoto leaf extract and invivo test.

Keyword: Parijoto leaf extract; MRSA; Antibiofilm activity; Synergistic effect

INTRODUCTION

Diabetes mellitus is a significant global health problem, with prevalence increasing annually[1]. It is estimated that by 2035, approximately 592 million people will be affected [1–3].The annual increase in the diabetic population causes significant social, economic, and health problems [2]. A common complication of diabetes is diabetic foot ulcers (DFU), which can affect 15% of individuals with this condition throughout the patient's life [4,5]. Chronic DFUs lead to severe infections, prolonged treatment, and even amputation [6]. DFUs are made more difficult by bacterial infections [7]. *Staphulococcus aureus* is the most frequently found bacterium isolated from diabetic foot ulcer patients, accounting for 46.1% [8,9]. Among the isolated S. aureus strains, 42.2% are methicillin-resistant [8]. *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria can cause numerous infections due to their ability to inhibit the immune response [10,11]. Previous studies have shown that MRSA infections can lead to amputations in diabetic patients [5,8] .

Based on these studies, the high prevalence of MRSA in DFUs indicates increased antibiotic use among diabetic patients [8]. Previous research has shown that bacteria isolated from DFUs, including MRSA, produce biofilms and exhibit resistance to commonly used antibiotics. Biofilms are the primary cause of many chronic infections in DFUs and allow multidrug-resistant strains to recur and lead to treatment failure [12–14]. Biofilms are organized collections of bacteria encased in an extracellular matrix produced by the bacteria themselves, facilitating attachment to surfaces produced by pathogenic bacteria, thus developing antibiotic resistance [5,15] . The body's natural immune system and antibiotic responses to these bacteria are hampered by the antibiotic-resistant MRSA biofilms isolated from DFUs [8,13,16] . Most antibiotics currently used for therapy do not target biofilm activity. As a result, the majority of MRSA strains have not yet developed resistance to compounds targeting biofilm mechanisms. Therefore, this type of therapy has ample opportunity for further development [17] . Several compounds are known to inhibit one of these mechanisms. However, antibiofilm and anti-*Quorum Sensing* (QS)

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agents are typically expensive to manufacture and require specialized, difficult-to-obtain ingredients, such as 2-benzene dicarboxylic acid, which must be synthesized [18,19] .

Traditional medicine using plants has been practiced for thousands of years. Several studies have shown that a number of plants possess antibacterial properties and are used to treat various infectious diseases affecting humans. In this study, the parijoto plant (Medinilla speciosa) was selected based on its history of medicinal use, namely its antidiabetic [20] , antifungal [21] , and antibacterial properties against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Salmonella typhi*, and *Shigella dysenteriae* [16,22–24] . Parijoto leaves have a sour, bitter, and refreshing taste. Parijoto leaves contain cardenolin compounds, from the flavonoid, saponin, and tannin groups [25,26] . This plant compound has the potential to be an antibacterial and antibiofilm agent when combined with gentamicin in MRSA bacteria from UKD. In addition, several studies have shown that various natural compounds can inhibit the QS process and biofilm. The use of monotherapy antibiotics such as gentamicin is not effective enough because bacterial resistance to these drugs is increasing [27–30] . However, research on antibiotic therapy and natural extracts that target biofilms is still limited. Meanwhile, it is necessary to fight biofilms so that they can kill pathogenic bacteria, especially for patients suffering from UKD infections caused by chronic diabetes mellitus [5,10,31] . Herbal plants such as parijoto leaves have antibacterial and antibiofilm properties because they contain active ingredients such as flavonoids, tannins, and saponins. By combining gentamicin and parijoto leaf extract, it is hoped that the two will work together to improve antibacterial performance while reducing the growth of MRSA bacterial biofilms from UKD.

MATERIAL AND METHOD

This form of research utilized an experimental design that was substantiated by a literature review. The experimental group's results were validated by a positive control group, and the testing was conducted in triplicate to ensure the reliability of the results. This investigation has been assigned the ethical clearance number 346/Z-7/KEPK/UMKU/IV/2025.

This study was conducted in July 2025 at the Microbiology Laboratory of Muhammadiyah University of Kudus and Gajah Mada University. The study sample used a combination of parijoto leaf extract and gentamicin on *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria. Parijoto leaves were collected from a local farm in Mount Muria, Kudus, Indonesia. This study used *Methicillin-Resistant Staphylococcus aureus* (MRSA) isolated from the foot ulcers of diabetic patients as the sample. A combination of parijoto leaf extract and gentamicin was prepared and tested for antibacterial and antibiofilm activity against *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria isolated from the foot ulcers of diabetic patients.

Fresh parijoto leaves were collected from the tree, washed with clean water, and wiped with a clean cloth. They were dried using a freeze dryer at 60°C for 15 hours. Then the results were ground using a grinder and filtered twice using 80 mesh filter paper. Storage was carried out in a closed and dry container. Parijoto leaf extract was made by the UAE (*Ultrasound-Assisted Extraction*) method using 70% ethanol solvent with a ratio of 1:10 (w/v) measured using an analytical balance. The ethanol extract solution was ultrasonicated for 60 minutes. Next, it was concentrated by evaporating ethanol through a rotary evaporator at a temperature of 40 °C, 200 mBar at a speed of 60 rpm for 120 minutes, then put into an oven to remove residual solvent until the final weight was reached.

Saponin, flavonoid, tannin, steroid, alkaloid, quinone, and phenolic tests were used to identify the chemical compounds in parijoto leaf extract. Saponin compound identification was carried out by taking 1 mL of parijoto leaf extract and adding 10 mL of hot water and then cooling it. Then the tube was shaken vigorously for 10 seconds. If a stable foam formed after adding one drop of 2N HCl solution, then the sample was positive and contained saponins [32] . Flavonoid examination, namely adding 100 mL of parijoto leaf extract to the tube, then heating for 5 minutes and filtering with filter paper, then pipetting 1 mL and adding 0.1 grams of Mg powder and 5 drops of concentrated HCl. The solution will turn orange-yellow to red if the sample is positive. Tannin identification examination, namely adding a few drops of iron (III) chloride solution to the tube, if the solution turns blue or blackish green then it is positive for tannin in the extract [33] . To test for steroids and terpenoids in parijoto leaf extract, three milliliters of chloroform, two milliliters of concentrated H_2SO_4, and two milliliters of anhydrous acetic acid are added to the test tube. In a blue or green solution, the sample is positive for steroids in a brownish red solution, the sample is positive for terpenoids [34] . By adding 5 mL of 25% ammonia and 20 mL of chloroform, the alkaloids in parijoto leaf extract are identified in a beaker. Afterward, one milliliter was added to one test tube, and two drops of Mayer's solution and two drops of Dragendroff's solution were added to another test tube. For the sample with Mayer's reagent, a white precipitate formed, and for the Dragendroff's reagent, a brownish-orange precipitate formed, indicating a positive result [35] . Quinone identification in parijoto leaf extract was carried out by taking the extract and homogenizing it with 5% H_2O_2 and 0.5 mol potassium hydroxide, then heating it for 10 minutes. After filtering, benzene and acetic acid were added to the mixture. After the benzene layer was removed, ammonia was added. A positive sample containing quinone was indicated by a colorless

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solution adhering to the benzene layer [36] . The phenolic test involved adding parijoto leaf extract to the test tube, followed by FeCl3, which turned the color blue or green [34] .

The test technique used was microdilution to inhibit the growth of MRSA biofilms. Preparation of MRSA bacterial suspension in *Brain Heart Infusion Broth* (BHIB) media based on MacFarland V standard (15x108 CFU/mL). Then, the concentration of parijoto leaf extract was made into concentrations of 100, 80, 60 and 40% with DMSO solution diluent and combined with 100 uL of gentamicin. A micropipette was used to insert the sample into a 96-well polystyrene flat-bottom microplate with a total volume of 100 uL. One test well and one blank well were used for testing. The test well consisted of a mixture of extract with gentamicin and 10% v/v bacterial suspension, and the blank well consisted of distilled water without additional bacterial suspension. The 96-well polystyrene flat-bottom microplate was covered with parafilm and then incubated for 24 hours at 37 °C. After leaving the incubator, the test solution was discarded and washed three times with running water. After drying, 125 µL of 1% crystal violet was pipetted into a 96-well polystyrene flat-bottom microplate and incubated at room temperature for 15 °C. The readings were read using a Benchmark Biorad Microplate reader (USA) at the UGM parasitology laboratory (37). The IC50 inhibitory power is the concentration of parijoto leaf extract that is still able to stop biofilm formation by 50%. The % inhibition formula is calculated as follows:

$$\frac{(\text{OD sampel} - \text{OD Blanko sampel})}{(\text{OD } \textit{vehicle} - \text{OD Blanko } \textit{vehicle})} \text{ X } 100$$

Next, the IC50 value was calculated using the probit method. This method was used to evaluate the best concentration for the 50% inhibitory concentration of the sample (Inhibitory concentration). The effect of concentration on the sample was evaluated using the probit method [37] .

RESULTS

Parijoto leaves were picked from a garden located on Mount Muria, Kudus Regency, amounting to 6 kg. Next, the fresh leaves were sorted and separated from the branches, washed thoroughly and wiped with a clean cloth. Next, the parijoto leaves were put into a drying device (*food dehydrator*) at a temperature of 60 ° C for 15 hours, then put into the FGD-Z1000 Grinder (China) and made into a fine powder of 610 g, then the parijoto leaf extract was made using the UAE (*Ultrasound-Assisted Extraction*) method, namely by inserting the ultrasonicator into an ethanol solution added with parijoto leaf simplicia powder, the ultrasonicator process for 60 minutes. The UAE extraction results were filtered, then evaporated with a rotary evaporator to become a thick extract, then in a Waterbath, and a thick extract of 600 g was produced. Pure thick extract obtained from the UAE technique, then a test was carried out to identify the chemical compounds contained in the 70% ethanol extract of parijoto leaves, the results of which are shown in Table 1 below.

Figure 1. Parijoto leaves (*Medinilla speciosa*).

Table 1. Results of phytochemical tests on 70% ethanol extract of parijoto leaves (*Medinilla speciosa*).

Compound Group	Interpretion	Description
Saponin	+	There is foam in more than a minute
Flavonoid	+	The color of the sample changes to brownish red.
Tanin	+	There is a change in color from blackish green
Steroid	-	No color change
Alkaloid	+	There is a reddish orange color change
Quinon	+	There is a change in the color of the sample to red
Fenol	+	The sample color changes to black

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*Interpretation: Positive (+): Contains chemical compounds; Negative (-): Does not contain chemical compounds

Based on the results of phytochemical identification tests, 70% ethanol extract of parijoto leaves contains chemical compounds including saponins, flavonoids, tannins, alkaloids, quinones, and phenols. This is in line with the results of a previous study by Anas *et al.* (2019), which stated that parijoto leaves contain cardenolin, a compound of the flavonoid, saponin, and tannin groups, giving the leaves a sour, bitter, and fresh taste [25,38] . Other studies using quantitative testing also found that 95% ethanol extract of parijoto leaves contained phenolics of 56.62 mg QE/g and flavonoids of 56.62 mg QE/g [39] .

Methicillin-Resistant Staphylococcus aureus (MRSA) bacteria samples were isolated from diabetic foot ulcer patients at the Blora district diabetes wound care clinic, then inserted into the transport media and bacterial identification was carried out on *Blood Agar Plate* (BAP) media, catalase and oxidase tests and tests using the Vitek 2 tool, then purification was carried out, planted on *Brain Heart Infusion* (BHI) media and gram staining was carried out as in Figure 2.

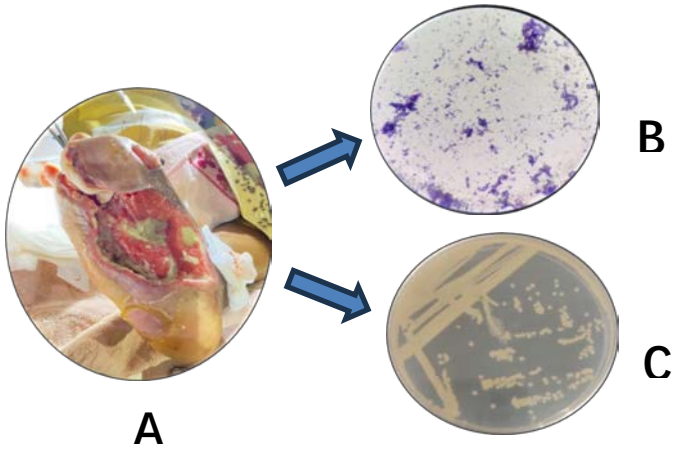

Figure 2. Schematic of isolation of *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria from diabetic foot ulcer wounds. A. Diabetic foot ulcer wounds; B. Gram staining; C. Pure colonies of *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria on *Brain Infusion Heart* (BHI) media.

The biofilm growth inhibition activity of a combination of gentamicin and several variations of parijoto leaf extract was tested, namely, 100, 80, 60, 40%, and without any extract mixture. The technique used was microdilution with 1% crystal violet staining, then read on a microplate reader with a wavelength of 595 nm. The absorbance results were calculated using the formula for % biofilm inhibition and the results obtained are in Figure 3.

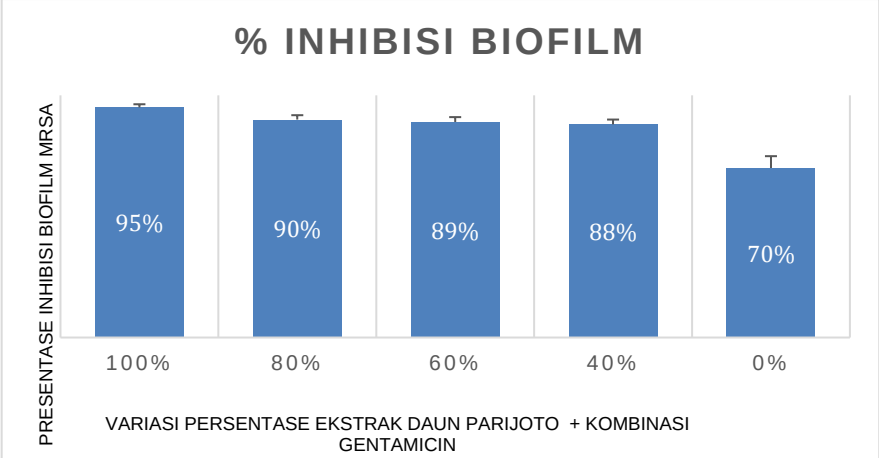

Figure 3. Percentage of inhibition of *Methicillin-Resistant Staphylococcus aureus* (MRSA) biofilm growth by a combination of gentamicin and parijoto leaf extract (*Medinilla speciosa*).

Based on the data in Figure 3. shows that there is significant inhibition of MRSA biofilm. The highest level of biofilm inhibition is in the combination variation of gentamicin and parijoto leaf extract 100% as much as 95%. Furthermore, the percentage of biofilm inhibition is 90% by the combination variation of gentamicin and parijoto leaf extract 80%, decreasing to 89% in the variation of gentamicin and parujoto leaf extract 60%, and in the

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combination variation of gentamicin and parijoto leaf extract 40% by 88%, and the percentage of biofilm inhibition is obtained by 70% in the treatment of gentamicin without parijoto leaf extract. By increasing the dose of parijoto leaf extract, the biofilm inhibition function increases. After obtaining the percentage of biofilm inhibition, a probit analysis was carried out to determine the IC50 value and the result was 6.90 µg/mL.

The Shapiro-Wilk significance value (Sig.) was greater than 0.05 at all concentrations of the Combination of Gentamicin and leaf extract, with concentrations of 40, 60, 80, and 100%, and 0 (Gentamicin only/without extract), as determined by the results of the Shapiro-Wilk normality test. The range of the Sig. was 0.482 to 0.888. This implies that the data distribution is normally distributed. So, parametric tests were implemented to continue the analysis. The significance value (Sig.) in all calculation methods based on the mean (0.154), median (0.559), median with adjusted df (0.585), and trimmed mean (0.171) were all greater than 0.05, as demonstrated by the homogeneity of variance test using Levene's Test. This suggests that the variance between absorbance groups was not significantly different, and therefore, the One-way ANOVA parametric test was continued. The F value is 4.025, with a significance level (Sig.) of 0.034, as indicated by the ANOVA analysis results. Because the Sig. value is less than 0.05, it is possible to infer that the average absorbance of the various leaf extract concentration groups differs in a statistically significant manner. This implies that variations in absorbance are influenced by the concentration of leaf extract. The LSD post-hoc test demonstrated a significant difference between Gentamicin alone (0% parijoto leaf extract) and a combination concentration of gentamicin and extract of 40%, 60%, 80%, and 100%. Furthermore, there is a direct correlation between the concentration of extract and absorbance, whereby an increase in absorbance is directly proportional to an increase in the concentration of the parijoto leaf extract and gentamicin combination. Consequently, the effect can be enhanced by a higher concentration of the leaf extract combination, which is demonstrated by the absorbance measurement.

DISCUSSION

Diabetic foot ulcers (DFUs) are one of the most dangerous complications of diabetes mellitus, often leading to recurrent infections, poorly healing wounds, and, in the worst cases, amputation [29,40] . One of the most dominant bacterial species in DFU infections is MRSA, which has the ability to form biofilms and increase resistance to antibiotics [41] . Biofilms are a structural mode of bacterial growth, increasing bacterial survival, protecting organisms from antibiotics, and making infections difficult to treat [42] . Previous research has shown that 77.41% of S. aureus bacteria isolated from diabetic foot ulcers are resistant to methicillin [29] . The increasing prevalence of MRSA further complicates antibiotic treatment, necessitating the exploration of alternative treatments as a complement [42]. Treatment of MRSA infections in diabetic foot ulcers in hospitals using more than one type of antibiotic can result in higher treatment costs, and prolonged treatment can lead to a high risk of kidney complications [43,44] . Therefore, using a combination of treatments from natural plant ingredients with synergistic effects could be a potential treatment approach for MRSA bacterial infections in diabetic ulcers. The Muria Mountains are home to the numerous beneficial plants of the parijoto (Medinilla speciosa) plant. Tests have been conducted on the compound content of parijoto leaf extract, which contains phytochemicals, including saponins, flavonoids, tannins, alkaloids, quinones, and phenols. This finding aligns with previous research by Qudsiya *et al.* (2022), which found flavonoid levels of 56.62 mg QE/g and phenolics of 159.61 mg GAE/g in a 95% ethanol extract of parijoto leaves [39] . Other studies have also shown that parijoto leaves have antibacterial [38,45] , antidiarrheal [25] , and antibiofilm [26].

In this study, the average value test results for the most effective percentage of MRSA biofilm inhibition were variations of the combination of gentamicin and 100% parijoto leaf extract. And the IC_{50} value was 6.90 µg/mL. Parijoto leaves are rich in phytochemicals, one of which is flavonoids which have a bacterial antibiofilm effect by disrupting the process of MRSA bacterial biofilm formation and its membrane strength. Flavonoid aglycone compounds are lipophilic to organic solutions, so they are able to interfere with the process of bacterial cell and biofilm formation through their QS (*Quorum Sensing*) effect mechanism. As a result, there is a disruption in the intracellular response and increased membrane permeability [46–48] .Flavonoid compounds change the permeability and fluidity of bacterial membranes, which causes cytoplasmic leakage and cell lysis [49] . So this can increase the effectiveness and improve the efficacy of gentamicin antibiotic drugs by increasing intracellular drug absorption [50] .

The findings of this study are consistent with the findings of (Kabotso, *et al.* 2024) research, which demonstrated that the combination of conventional antibiotics and rosemary essential oil can be used as a treatment for multidrug-resistant bacterial biofilms [51]. One of the phytochemical constituents in parijoto leaf extract, specifically phenolics, may contribute to the *quorum sensing* (QS) inhibition process in MRSA bacteria [39,52]. The QS phase is a critical stage in cellular communication involved in initiation, maturation, and biofilm development [53,54]. Due to the presence of phenolic compounds in parijoto leaf extract that inhibit the *quorum sensing* (QS) stage, the formation of extracellular structures is disrupted, leading to a reduction in virulence factor production [52]. Consequently, bacterial cells become more susceptible to antibiotics, particularly gentamicin.

This combined effect can be reached through several mechanisms of activity, specifically membrane disruption, quorum sensing inhibition, and interference with biofilm formation.Suppression of efflux pump

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activity, Consequently, combination therapy involving antibiotics and phytochemicals provides improved antibacterial effectiveness with lower concentrations, enhanced penetration into biofilms, suppression of resistance mechanisms, delayed development of antimicrobial resistance, and broad-spectrum synergistic capabilities [55–57]. In line with prior research, the combined application of antibiotics and natural compounds can augment their efficacy. Previous research indicated that the combination of thymol and carvacrol exhibited a pronounced effect against *Staphylococcus aureus* (MSSA). The inclusion of thymol, carvacrol, and chloramphenicol yielded even more pronounced results [58].

CONCLUSIONS AND SUGGESTIONS

The combination of gentamicin and parijoto leaf extract with several variations obtained the highest percentage of biofilm inhibition of 95% in the combination variation of gentamicin and parijoto leaf extract 100% and the lowest in the variation of gentamicin without a combination of parijoto leaf extract, namely 70%. The combination dose of gentamicin and parijoto leaf extract obtained an IC_{50} result of 6.90 µg/mL. The variations in leaf extract concentration exhibited a statistically significant difference, as indicated by the results of the One Way Annova test. The post-hoc LSD test demonstrated a substantial difference between Gentamicin alone and the combination of Parijoto leaf extract. In addition, a direct correlation was observed between the concentration of extract and absorbance. Specifically, an increase in absorbance was directly proportional to the concentration of the sinergy of the Parijoto leaf extract and gentamicin. Parijto leaf extract has various phytochemical contents, namely saponins, flavonoids, tannins, alkaloids, quinones, and phenols, so it can synergize as a supporting treatment with gentamicin to accelerate the treatment of diabetic ulcer wound infections. The molecular mechanism of QS (*Quorum sensing*) against MRSA (*Methicillin-resistant Staphylococcus aureus*) bacteria by bioactive compounds in parijoto leaf extract requires further exploration and study. This can be achieved by testing bioactive compounds quantitatively or using a metabolomic approach. Additionally, further testing is necessary in vivo with an experimental animal model infected with MRSA (*Methicillin-resistant Staphylococcus aureus*).

AUTHOR'S CONTRIBUTION STATEMENT

Arina Lis Sa'adah: Conceptualization,Methodology **Fariza Yulia Kartika Sari**: Project administration **Bintari Tri Sukoharjanti**: Writing- Reviewing and Editing **Sri Darmawati:** Reviewing

CONFLICTS OF INTEREST

No conflicts of interest are disclosed by the authors.

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REFERENCES

1. Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. *World J Diabetes*. 2015;6(6):850-867. doi:10.4239/wjd.v6.i6.850
2. Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045 : Results from the International Diabetes Federation Diabetes Atlas , 9 th edition. *Diabetes Res Clin Pract*. 2019;157:107843. doi:10.1016/j.diabres.2019.107843
3. Motala AA, Mbanya JC, Ramaiya K, Pirie FJ, Ekoru K. Type 2 diabetes mellitus in sub-Saharan Africa: challenges and opportunities. *Nat Rev Endocrinol*. 2022;18(4):219-229. doi:10.1038/s41574-021-00613-y
4. Armstrong DG, Boulton AJM, Bus SA. Diabetic Foot Ulcers and Their Recurrence. *N Engl J Med*. 2017;376(24):2367-2375. doi:10.1056/NEJMra1615439
5. Zhou S, Hu X, Wang Y, Fei W, Sheng Y, Que H. The Global Prevalence of *Methicillin-Resistant Staphylococcusa aureus* in Patients with Diabetic Foot Ulcers: A Systematic Review and Meta-Analysis. *Diabetes, Metab Syndr Obes*. 2024;17(January):563-574. doi:10.2147/DMSO.S446911
6. Raja JM, Maturana MA, Kayali S, Khouzam A, Efeovbokhan N. Diabetic foot ulcer: A comprehensive review of pathophysiology and management modalities. *World J Clin cases*. 2023;11(8):1684-1693. doi:10.12998/wjcc.v11.i8.1684

ISSN : 1907-8153 (Print)
e-ISSN : 2549-0567 (Online)

7. Baig MS, Banu A, Zehravi M, et al. An Overview of Diabetic Foot Ulcers and Associated Problems with Special Emphasis on Treatments with Antimicrobials. *Life (Basel, Switzerland)*. 2022;12(7). doi:10.3390/life12071054
8. Kananizadeh P, Moghadam SO, Sadeghi Y, Foroushani AR, Adibi H, Pourmand MR. Molecular characteristics of *Methicillin-resistant Staphylococcus aureus* (MRSA) isolated from diabetic foot infection. *Iran J Pathol*. 2019;14(4):329-337. doi:10.30699/IJP.2019.101092.2035
9. Anvarinejad M, Pouladfar G, Japoni A, et al. Isolation and Antibiotic Susceptibility of the Microorganisms Isolated from Diabetic Foot Infections in Nemazee Hospital, Southern Iran. *J Pathog*. 2015;2015:1-7. doi:10.1155/2015/328796
10. Du F, Ma J, Gong H, et al. Microbial Infection and Antibiotic Susceptibility of Diabetic Foot Ulcer in China: Literature Review. *Front Endocrinol (Lausanne)*. 2022;13:881659. doi:10.3389/fendo.2022.881659
11. Cheung GYC, Bae JS, Otto M. Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*. 2021;12(1):547-569. doi:10.1080/21505594.2021.1878688
12. Khariunnisa R, Soleha TU, Ramadhian MR. Identifikasi dan Uji Resistensi *Staphylococcus aureus* pada Ulkus Diabetik di Instalasi Penyakit Dalam RSUD Dr. H. Abdul Moeloek Identification and Resistency Test of *Staphylococcus aureus* on Diabetic Ulcer in Internal Medicine Installation at Dr. *J Agromedicine Unila*. 2020;7:1-6.
13. Iyamba JML, Bassom VMHN, Lukukula CM, et al. Study of Biofilm Formation and Antibiotic Resistance Pattern of Bacteria Isolated from Diabetic Foot Ulcers in Hôpital de Référence Saint Joseph, Kinshasa, Democratic Republic of Congo. *Adv Microbiol*. Published online 2021. https://api.semanticscholar.org/CorpusID:236582449
14. Banu A, Noorul Hassan MM, Rajkumar J, Srinivasa S. Spectrum of bacteria associated with diabetic foot ulcer and biofilm formation: A prospective study. *Australas Med J*. 2015;8(9):280-285. doi:10.4066/AMJ.2015.2422
15. Papadopoulou V, Sidders AE, Lu KY, et al. Overcoming biological barriers to improve treatment of a *Staphylococcus aureus* wound infection. *Cell Chem Biol*. 2023;30(5):513-526.e5. doi:10.1016/j.chembiol.2023.04.009
16. Wahyuni RA, Putri IY, Jayadi EL, Prastiyanto ME. Antibacterial Activity Of Parijoto Fruit Extract (Medinilla Speciosa) On Bacteria Extended Spectrum Betalactamase (Esbl) Escherichia Coli And *Methicillin Resistant Staphylococcus aureus* (MRSA). *J Media Anal Kesehat*. 2019;10(2):106. doi:10.32382/mak.v10i2.1250
17. Bheemavarapu H, Mohammad A, Ahmad A, Patel I. Nosocomial Infections in India: The Unaddressed Lacunae! *J Pharm Pract Community Med*. 2018;4:43. doi:10.5530/jppcm.2018.2.11
18. Lu L, Zhao Y, Yi G, et al. Quinic acid: a potential antibiofilm agent against clinical resistant *Pseudomonas aeruginosa*. *Chin Med*. 2021;16. doi:10.1186/s13020-021-00481-8
19. Wan B, Zhu Y, Tao J, et al. Biological and Medical Applications of Materials and Interfaces Alginate-lyase Guided Silver Nanocomposites for Eradicating *Pseudomonas aeruginosa* from lung. Published online 2020. doi:10.1021/acsami.9b21815
20. Advistasari YD, Vifta RL. Uji Antidiabetes Ekstrak Etanol Buah Parijoto (*Medinilla speciosa B.*) dan Fraksinya. *Repos STIFAR*. 2019;0(0 SE-Articles). https://repository.stifar.ac.id/Repository/article/view/173
21. Astutik P, Yuswantina R, Vifta RL. Perbandingan Aktivitas Antifungi Ekstrak Etanol 70\% Dan 96\% Buah Parijoto (*Medinilla speciosa*) Terhadap *Candida albicans*. *J Holistics Heal Sci*. Published online 2021. https://api.semanticscholar.org/CorpusID:236750202
22. Sugiarti L, Pujiastuti E. Uji Aktivitas Antibakteri Ektrak Etanol Buah Parijoto (*Medinilla Speciosa Blume*) terhadap Bakteri *Staphylococcus aureus* dan *Escherichia Col*i. *Cendekia J Pharm*. 2017;1:25-33. doi:10.31596/cjp.v1i1.4
23. Milanda T, Lestari K, Tarina NTI. Antibacterial Activity of Parijoto (*Medinilla speciosa Blume*) Fruit Against Serratia marcescens and *Staphylococcus aureus*. *Indones J Pharm Sci Technol*. 2021;8(2):76. doi:10.24198/ijpst.v8i2.32166
24. Anastasia FF, Aziz IR, Oktaviola V, Iswara A. Antibacterial Activity Of Combination Cold Plasma And Parijoto (*Medinilla speciosa*) against *Staphylococcus aureus* and *Pseudomonas aeruginosa* on Diabetic Ulcer. *El-Hayah*. 2020;7(4):133-138. doi:10.18860/elha.v7i4.10313
25. Anas Y, Rakhmawati D, Fuadah L, Rahayu NC. Efek Antidiare Ekstrak Etanol Daun Parijoto (Medinilla speciosa Blume) Pada Mencit Jantan Galur Balb/C. *JIFFK J Ilmu Farm dan Farm Klin*. 2019;16(01):28. doi:10.31942/jiffk.v16i01.2926
26. Sa'adah AL, Kartikasari FY, Mundriyastutik Y, Hayati DN, Faizal IA. Potensi Ekstrak Daun Parijoto (*Medinilla speciosa*) Sebagai Penghambatan Biofilm *Methicillin-resistant Staphylococcus aureus* (MRSA). *J ILMU Kedokt DAN Kesehat*. 2025;12(10):2025.

ISSN : 1907-8153 (Print)
e-ISSN : 2549-0567 (Online)

27. Zhu M, Tse MW, Weller J, Chen J, Blainey PC. The future of antibiotics begins with discovering new combinations. *Ann N Y Acad Sci*. 2021;1496(1):82-96. doi:10.1111/nyas.14649
28. Septiani, Yulia KSF. Pengaruh Sinbiotik Terhadap Mikrobiota Saluran Cerna Pada Anak Stunting. *J Med Indones*. 2023;4(2):23-29.
29. Nazari M, Taheri M, Nouri F, Bahmanzadeh M, Alikhani MY. The antimicrobial and antibiofilm effects of gentamicin, imipenem, and fucoidan combinations against dual-species biofilms of *Staphylococcus aureus* and A*cinetobacter baumannii* isolated from diabetic foot ulcers. *Ann Clin Microbiol Antimicrob*. 2024;23(1). doi:10.1186/s12941-024-00760-w
30. Bonincontro G, Scuderi SA, Marino A, Simonetti G. Synergistic Effect of Plant Compounds in Combination with Conventional Antimicrobials against Biofilm of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida spp*. *Pharmaceuticals*. 2023;16(11). doi:10.3390/ph16111531
31. Li Z. Antibacterial Activity of Surfactin and Synergistic Effect with Conventional Antibiotics Against *Methicillin-Resistant Staphylococcus aureus* Isolated from Patients with Diabetic Foot Ulcers [Response to Letter]. *Diabetes, Metab Syndr Obes*. 2023;16(November):4233-4234. doi:10.2147/DMSO.S453962
32. Turangan ATM, Wewengkang DS, Yudistira A. Uji Aktivitas Antioksidan Ekstrak Etanol Kulit Batang Mahoni (*Swietenia mahagoni Jacq.*) Menggunakan Metode DPPH (1,1 diphenyl-2-picrylhydrazyl). *Pharmacon*. 2019;8(3):548. doi:10.35799/pha.8.2019.29329
33. Noer S, Pratiwi RD, Gresinta E. Penetapan Kadar Senyawa Fitokimia (Tanin, Saponin dan Flavonoid) sebagai Kuersetin Pada Ekstrak Daun Inggu (*Ruta angustifolia L.*). *J Eksakta*. 2018;18(1):19-29. doi:10.20885/eksakta.vol18.iss1.art3
34. Farida DO, Suyanto S. Skrining Fitokimia, Kandungan Flavonoid Total, Dan Aktivitas Antioksidan Ekstrak Etanol Tumbuhan *Selaginella doederleinii*. *J Kim Ris*. 2021;6(2):141-153.
35. Wigati EI, Pratiwi E, Nissa TF, Utami NF. Uji Karakteristik Fitokimia Dan Aktivitas Antioksidan Biji Kopi Robusta (*Coffea canephora Pierre*) Dari Bogor, Bandung Dan Garut Dengan Metode DPPH (1,1-diphenyl-2-picrylhydrazyl). *Fitofarmaka J Ilm Farm*. 2018;Vol. 8(1):59-66. doi:DOI : 10.33751/jf.v8i1.1172
36. Asmara AP. Uji Fitokimia Senyawa Metabolit Sekunder Dalam Ekstrak Metanol Bunga Turi Merah (*Sesbania grandiflora L. Pers*). *Al-Kimia*. 2017;5(1):48-59. doi:10.24252/al-kimia.v5i1.2856
37. Dewi ZY, Nur A, Hertriani T. Efek antibakteri dan penghambatan biofilm ekstrak sereh (Cymbopogon nardus L.) terhadap bakteri *Streptococcus mutans*. 2015;3:136-141.
38. Nafi'ah LN. REVIEW ARTICLE: Aktivitas Farmakologi Tanaman Parijoto (*Medinilla speciosa*). *J Ris Rumpun Ilmu Kesehat*. 2022;1(1):09-18. doi:10.55606/jurrikes.v1i1.172
39. Qudsiya A, Lutfiani D, Da'i M. Penetapan Kadar Flavonoid Dan Fenolik Ekstrak Etanol Tanaman Parijoto (*Medinilla speciosa*) Serta Aktivitas Sitotoksiknya Terhadap Sel Kanker Serviks Hela Determination Of Flavonoid And Phenolic Content Of Ethanol Extract Of Parijoto Plant (*Medinilla speciosa*) . *Usadha J Pharm*. 2022;1(4):505-520. https://jsr.lib.ums.ac.id/index.php/ujp
40. Hurlow JJ, Humphreys GJ, Bowling FL, McBain AJ. Diabetic foot infection: A critical complication. *Int Wound J*. 2018;15(5):814-821. doi:10.1111/iwj.12932
41. Pouget C, Dunyach-Remy C, Pantel A, Schuldiner S, Sotto A, Lavigne JP. Biofilms in Diabetic Foot Ulcers: Significance and Clinical Relevance. *Microorganisms*. 2020;8(10). doi:10.3390/microorganisms8101580
42. Afonso AC, Oliveira D, Saavedra MJ, Borges A, Simões M. Biofilms in Diabetic Foot Ulcers: Impact, Risk Factors and Control Strategies. *Int J Mol Sci*. 2021;22(15). doi:10.3390/ijms22158278
43. Roszell S, Jones C. Intravenous administration issues: a comparison of intravenous insertions and complications in vancomycin versus other antibiotics. *J Infus Nurs Off Publ Infus Nurses Soc*. 2010;33(2):112-118. doi:10.1097/NAN.0b013e3181cfcee4
44. Joseph WS, Kosinski MA, Rogers LC. Parenteral Vancomycin in the Treatment of MRSA-Associated Diabetic Foot Infections: An Unnecessary Risk. *Int J Low Extrem Wounds*. Published online October 2023:15347346231207552. doi:10.1177/15347346231207553
45. Fadel MN, Huda N, Hasriyani, Besan EJ, Ayuningsih S. Aktivitas Antibakteri Ekstrak Etanol Daun Parijoto (*Medinilla speciosa Blume*) Pada Sediaan Gel Hand Sanitizer. *IJF (Indonesia J Farm*. 2024;9:1-12.
46. Zhou H, Chen L, Ouyang K, Zhang Q, Wang W. Antibacterial activity and mechanism of flavonoids from Chimonanthus salicifolius S. Y. Hu. and its transcriptome analysis against *Staphylococcus aureus*. *Front Microbiol*. 2023;13(January). doi:10.3389/fmicb.2022.1103476
47. Naeem A, Ming Y, Pengyi H, et al. The fate of flavonoids after oral administration: a comprehensive overview of its bioavailability. *Crit Rev Food Sci Nutr*. 2021;62:1-18. doi:10.1080/10408398.2021.1898333
48. Chen X, Jia X, Yang S, et al. Optimization of ultrasonic-assisted extraction of flavonoids, polysaccharides, and eleutherosides from Acanthopanax senticosus using response surface methodology in development

ISSN : 1907-8153 (Print)
e-ISSN : 2549-0567 (Online)

of health wine. *Lwt*. 2022;165(June):113725. doi:10.1016/j.lwt.2022.113725
49. Wang Z, Zheng Y, Hu Y, et al. Improvement of antibacterial activity of polysaccharides via chemical modification: A review. *Int J Biol Macromol*. 2024;269(Pt 2):132163. doi:10.1016/j.ijbiomac.2024.132163
50. Wang Z, Zhou X, Liang X, et al. Antioxidant and antibacterial activities of a polysaccharide produced by Chaetomium globosum CGMCC 6882. *Int J Biol Macromol*. 2023;233:123628. doi:https://doi.org/10.1016/j.ijbiomac.2023.123628
51. Kabotso DEK, Neglo D, Gaba SE, et al. In Vitro Evaluation of Rosemary Essential Oil: GC-MS Profiling, Antibacterial Synergy, and Biofilm Inhibition. *Pharmaceuticals*. 2024;17(12). doi:10.3390/ph17121653
52. Sa'adah AL, Kartikasari FY, Mundriyastutik Y, Defi Hayati N, Faizal IA. Potensi Ekstrak Daun Parijoto (*Medinilla speciosa)* Sebagai Penghambatan Biofilm *Methicillin-resistant Staphylococcus aureu*s (MRSA). J Ilmu Kedokt dan Kesehat [Internet]. 2025;12(10):2306–12. Available from: https://ejurnalmalahayati.ac.id/index.php/kesehatan/article/view/20935
53. Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, et al. Bacterial biofilm and associated infections. J Chinese Med Assoc [Internet]. 2018;81(1):7–11. Available from: https://doi.org/10.1016/j.jcma.2017.07.012
54. Singh S, Singh SK, Chowdhury I, Singh R. Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. Open Microbiol J. 2017;11(1):53–62.
55. Roy R, Das A, Ganguly D, Chakraborty P, Paul P, Das S, et al. Cuminaldehyde synergistically enhances the antimicrobial and antibiofilm potential of gentamicin: A direction towards an effective combination for the control of biofilm-linked threats of Staphylococcus aureus. Brazilian J Microbiol [publication Brazilian Soc Microbiol. 2025 Jun;56(2):1033–48.
56. Kungwani NA, Panda J, Mishra AK, Chavda N, Shukla S, Vikhe K, et al. Combating bacterial biofilms and related drug resistance: Role of phyto-derived adjuvant and nanomaterials. Microb Pathog. 2024 Oct;195:106874.
57. Zhou K, Shi M, Chen R, Zhang Y, Sheng Y, Tong C, et al. Natural phytochemical-based strategies for antibiofilm applications. Chinese Med (United Kingdom). 2025;20(1).
58. Nikolic I, Aleksic Sabo V, Gavric D, Knezevic P. Anti-Staphylococcus aureus Activity of Volatile Phytochemicals and Their Combinations with Conventional Antibiotics Against *Methicillin-Susceptible S. aureus* (MSSA) and *Methicillin-Resistant S. aureus* (MRSA) Strains. Antibiot (Basel, Switzerland). 2024 Oct;13(11).