MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Abd Wahab *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 22 (2026) 1018–1029

RESEARCH ARTICLE

Natural Control of MRSA: Antibacterial and Virulence Gene Modulatory Effects of *Persicaria minor* Aqueous Extract

Noor Zarina Abd Wahab[a*], Muhammad Isma Aiman Ismail[a], Nazihah Zainal[a], Fong Si Wei[b], Sayed Mohd Saufi Fahmi Sayed Abdul Kadir[c], Mohd Hanif Abdullah[c]

[a]Faculty of Health Sciences, Universiti Sultan Zainal Abidin, 21300 Kuala Nerus, Terengganu, Malaysia; [b]Premier Integrated Labs, Hospital Pantai Kuala Lumpur, 59100 Kuala Lumpur, Malaysia; [c]Dtree Pharma Sdn Bhd, Lot 1, IKS Factory, Hulu Terengganu 21800, Malaysia

Abstract The emergence of antibiotic-resistant bacteria, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), poses a major public health challenge and highlights the urgent need for alternative antimicrobial agents. *Persicaria minor*, a medicinal plant widely used in traditional medicine, is known to contain diverse bioactive phytochemicals with potential antimicrobial properties. This study evaluated the phytochemical composition, antibacterial activity, and effects on gene expression of the aqueous extract of *P. minor* against clinical MRSA isolates. Qualitative phytochemical screening revealed the presence of saponins, steroids, tannins, flavonoids, phenols, and glycosides, whereas alkaloids were not detected. Antibacterial activity was assessed against eight clinical MRSA isolates and *Staphylococcus aureus* ATCC 25923 using disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. The extract produced inhibition zones ranging from 6.3 to 8.3 mm, with MIC values between 25 and 200 mg/mL and MBC values ranging from 50 to 200 mg/mL. An MBC/MIC ratio of 2 observed in most isolates suggested a bactericidal mode of action. Quantitative real-time PCR (qRT-PCR) analysis demonstrated significant upregulation of virulence-associated genes (*sea, seb, sec, tsst-1,* and *fnbA*) and the methicillin resistance gene (mecA) following exposure to sub-MIC concentrations of the extract. The highest expression was observed for sea (16.8-fold), followed by sec (6.8-fold), *seb* (6.3-fold), *mecA* (4.6-fold), *tsst-1* (3.8-fold), and *fnbA* (2.8-fold). These findings suggest that although *P. minor* aqueous extract exhibits measurable anti-MRSA activity, sub-inhibitory concentrations may induce bacterial stress-adaptation responses associated with virulence and resistance. Further studies are required to isolate the active compounds, investigate the molecular mechanisms involved, and evaluate the *in vivo* efficacy and safety of the extract before its development as a potential natural antimicrobial agent.

Keywords: medicinal plant, natural therapeutic agents, antimicrobial resistance bacteria, virulence genes, resistance genes.

***For correspondence:**
zarinawahab@unisza.edu.my

Received: 14 April 2026
Accepted: 10 July 2026

© Copyright Abd Wahab. This article is distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use and redistribution provided that the original author and source are credited.

Introduction

Antibacterial is defined as any substance that has the ability to either kill a specific type of bacteria or inhibit its ability to multiply and spread, ultimately preventing it from causing infections or disease, according to the [1]. These substances can be found in various forms, such as medications, plants

extract, chemicals, or even natural compounds. Antibiotics are vital for reducing bacterial growth and preventing the spread of bacterial illnesses in both clinical and daily settings. To effectively eliminate bacteria, antibacterial substances utilize several mechanisms of action. The first method is inhibiting bacterial cell wall synthesis, which will weaken the structural integrity of the bacteria, making it vulnerable and exposed to osmotic lysis. Furthermore, antibacterial drugs can enhance the permeability of the bacterial cell membrane, causing the outflow of important cellular components such as ions and proteins, finally resulting in bacterial death. Moreover, antibacterial substances interfere with vital cellular processes, including metabolic pathways, nucleic acid metabolism, and protein synthesis, which are essential for the bacteria's survival [2].

Moreover, the herbal plant is widely used as an antibacterial, as it contains many active components believed to have antimicrobial properties. The active components extracted from certain types of plants may have the capacity to provide some secondary metabolites of relatively complex structures that can perform antibacterial activities when introduced to the human body [3,4,5,6]. For example, *Psidium guajava* is a plant in the Myrtaceae family that is used to prevent and treat diarrhoea. An extract from this plant has shown powerful antibacterial activity against food-borne bacteria that cause diarrhoea such as *Pseudomonas* species, *Staphylococcus* species and *Shigella* species [7]. Similarly, *Persicaria minor* (locally known as kesum) is a medicinal and culinary herb in Southeast Asia that contains diverse bioactive compounds, including flavonoids, tannins, and essential oils. It has been reported to possess antimicrobial, antioxidant, and anti-inflammatory properties, which makes it a promising candidate for therapeutic applications. Many varieties of plant leaves possess biochemical components that are helpful and functional not only for antibacterial but also for antioxidant, anti-inflammatory, anti-venom, and anticancer capabilities. As a result, many researchers are interested in experimenting with herbal plants as medical alternatives, particularly in the pharmaceutical business, as they can potentially benefit the globe [8].

In a 2014 global assessment, the World Health Organization highlighted methicillin-resistant *Staphylococcus aureus* (MRSA) as one of seven priority infections of international concern, noting its association with higher mortality rates and a greater incidence of fatal septic shock compared with methicillin-susceptible *S. aureus* (MSSA) [9]. In Malaysia, the nationwide frequency rate of MRSA among *S. aureus* isolates taken from patients varied between 17.2% and 28.1%, with incidences ranging from 18% to 19.8% in 2016 and 2017, respectively, indicating that MRSA has develop into a significant public health involvement in Malaysia [10]. Moreover, in 2018, the Ministry of Health Malaysia reported a 23.5% and 19.4% prevalence of *S. aureus* and MRSA isolated from blood, respectively, across 41 hospitals. Between 2007 and 2016, there was an increase in the number of haemodialysis patients in Malaysia, leading to a higher prevalence of MRSA colonisation among these patients. This shows that MRSA as health-care-associated MRSA has increased in Malaysia because bacterium’s exposure during haemodialysis treatment [11]. Beyond hospitals, MRSA has become as a major source of community-associated MRSA, such as soft tissue and skin infections, demonstrating the growing dissemination of resistant strains in uncontrolled settings. MRSA epidemiology shifted dramatically when it was detected in persons who had no prior healthcare contact, leading to the introduction of CA-MRSA [12].

Not only that, from total *S. aureus* isolated in Malaysia, the MRSA rates show a significant decrease in years 2020 (14.9%), 2021 (7.0%), 2022 (6.0%), 2023 (5.5%) [13]. Although there is a decrease in MRSA isolated, but it does not eliminate the threat posed by MRSA to the vulnerable population because MRSA infection is still present in Malaysia. This is because MRSA infections are more difficult to treat than regular *S. aureus* infections because these bacterial strains are resistant to several types of antibiotics. The danger of MRSA infection is particularly high in situations such as hospitals, where invasive operations, lengthy hospital stays, contact sports, towel sharing, living in unclean conditions such as jail, and immunocompromised individuals are widespread. Furthermore, the rising incidence of community-acquired MRSA strains has increased the danger, making it a major issue in both clinical and community settings [14].

Currently, vancomycin is the therapy of last resort for the infection of MRSA. However, incidences of vancomycin-resistant MRSA strains have been recorded over the world, with the first discovered in the United States almost twenty years ago. For now, no cases of vancomycin resistance have been documented among MRSA isolates in Malaysia. Nonetheless, a vancomycin-resistant *S. aureus* (VRSA) strain, exhibiting a minimum inhibitory concentration (MIC) greater than 32 µg/mL, was isolated from a patient in neighbouring Indonesia. Therefore, it is anticipated that VRSA strains may eventually emerge in Malaysia [10]. Therefore, it is essential to find an alternative antimicrobial agent that is effective for MRSA. Antibacterial plant extracts are a good alternative since medicinal plants include bioactive chemicals such as tannins, coumarins, polyacetylenes, flavonoids, alkaloids, terpenoids, lectins, polypeptides, and phenolics, which serve as the foundation for antimicrobial research. This is because such substances can interact with and disrupt the components of microbial membranes, causing the

death of cells in Gram-positive as well as Gram-negative bacteria. Furthermore, these chemicals can interact with proteins, interrupting critical metabolic processes. Finally, plant extracts include phenolic chemicals that inhibit bacteria via a variety of methods, including enzyme inhibition, efflux pump inhibition and cell membrane contact [15].

In this study, the objective is to evaluate the antibacterial potential of *P. minor* aqueous extract against MRSA, with emphasis on its phytochemical composition and possible mechanisms of action. The novelty of this work lies in exploring *P. minor*, a locally available medicinal plant that remains underexplored for its anti-MRSA activity, thereby contributing new insights into plant-derived alternatives as potential candidates for combating resistant pathogens. In addition, this study bridges traditional medicinal knowledge with modern scientific validation by providing experimental evidence of antibacterial efficacy. Unlike most previous studies that have focused on well-established herbal remedies, this research highlights a less-studied species with the potential to yield unique bioactive compounds. Moreover, by investigating both the antibacterial effects and the possible molecular targets, this study sets the groundwork for future therapeutic development and supports the search for safer, cost-effective, and sustainable alternatives to conventional antibiotics in addressing multidrug-resistant infections.

Materials and Methods

Plant Collection and Extraction

Fresh leaves of *Persicaria minor* were collected from Kg Gong Tok Nasek, Kuala Terengganu, Malaysia (5.29049, 103.14958). The plant was taxonomically authenticated, and a voucher specimen was deposited at the UniSZA herbarium under the number UniSZA/A/000000044.

Freshly harvested leaves were rinsed under running tap water to eliminate soil particles and debris. The clean samples were air-dried at ambient temperature until all surface moisture had evaporated, followed by oven-drying at 60 °C for three days.

After complete drying, the leaves were chopped into smaller pieces and ground into a fine powder using an electric blender. The powdered plant material was then stored in airtight containers at room temperature [16].

The leaf powder was extracted by immersion in distilled water. The mixture obtained was subsequently passed through filter paper to separate and eliminate insoluble residues. The collected filtrate was then concentrated using a rotary evaporator, followed by freeze-drying. The dried extract was then kept at −20 °C for subsequent analyses.

Phytochemical Test

Qualitative phytochemical analysis of *P. minor* aqueous extract was performed using standard established methods to detect major secondary metabolites, namely saponins, steroids, alkaloids, tannins, flavonoids, phenolic compounds, and glycosides. All tests were carried out in triplicate to ensure the consistency and reproducibility of the findings. Saponins, steroids, alkaloids, tannins, flavonoids, phenolic compounds, and glycosides were screened according to [17–20] with slight modifications. Saponins were indicated by stable froth formation. Steroids were confirmed by a bluish-green colour in the Liebermann–Burchard test. Alkaloids produced cream and orange-red precipitates using Mayer's and Dragendorff's reagents. Tannins were identified by a blue-black or greenish-black colour using ferric chloride. Flavonoids were confirmed by an alkaline test showing a yellow colour that disappeared with acid. Phenols gave a deep blue or green colour with ferric chloride, while glycosides were confirmed by a reddish-brown ring formation after treatment with glacial acetic acid, ferric chloride, and concentrated sulfuric acid.

Bacterial Isolates and Culture Conditions

Eight clinical isolates of MRSA were obtained from Premier Integrated Labs Sdn. Bhd. A reference strain, *S. aureus* ATCC 25923, was also procured as a pure culture from the American Type Culture Collection (ATCC) and maintained in the culture collection of the UniSZA Microbiology Laboratory.

Antibacterial Assay

Disk Diffusion Test

The antibacterial activity of *P. minor* aqueous extract was assessed using the disk diffusion assay. Bacterial suspensions were adjusted to the 0.5 McFarland standard. Sterile 6 mm paper discs were impregnated with 20 µL of extract at concentrations of 200 to 3.125 mg/mL, then placed onto the

inoculated agar surface. Vancomycin (30 µg/mL) was used as the positive control, whereas sterile distilled water served as the negative control. The inoculated plates were incubated aerobically at 37 °C for 24 hours. Antibacterial activity was assessed by measuring the diameter of inhibition zones in millimeters [21].

Minimum Inhibitory Concentration (MIC)

MIC of *P. minor* aqueous extract was determined using the broth microdilution method in 96-well microplates. Two-fold serial dilutions (200-3.125 mg/mL) were prepared in MHB (100 µL/well) and mixed with an equal volume of bacterial suspension (1 × 10^6 CFU/mL) of *S. aureus* ATCC 25923 and MRSA isolates. Controls included vancomycin (positive), sterile water (negative), and appropriate viability and sterility controls. Plates were incubated at 37 °C for 24 h, followed by addition of 50 µL MTT and incubation for 2 h. Growth inhibition was indicated by yellow colour, while purple indicated bacterial growth [22].

Minimum Bactericidal Concentration (MBC)

To determine the MBC, 50 µL from MIC wells with no visible growth was transferred into 150 µL fresh Mueller-Hinton broth and incubated at 37 °C for 24 hours. Then, 10 µL was streaked onto nutrient agar and incubated for another 24 hours [22].

Gene Expression Analysis

The MRSA isolate exhibiting the highest susceptibility to *P. minor* aqueous extract in the antibacterial susceptibility test was selected for subsequent gene expression analysis. Two bacterial suspensions (100 µL each) were prepared from this strain and adjusted to the 0.5 McFarland standard in sterile Eppendorf tubes. One suspension was designated as the untreated control, while the second was exposed to 100 µL of sub-inhibitory concentration (12.5 mg/mL) of the aqueous extract. Both preparations were incubated overnight at 37 °C.

Following incubation, cultures were diluted 1:50 in Mueller–Hinton broth (MHB). Thereafter, 100 µL from each (treated and untreated) culture was transferred into labeled centrifuge tubes, followed by the addition of 4.9 mL fresh MHB. The cultures were further incubated at 37 °C for 5 hours to support bacterial growth. Subsequently, 1.8 mL aliquots were collected and centrifuged at 16,000 × g for 3 minutes, after which the supernatant was discarded and the cell pellets were retained.

Total RNA was extracted using the PrimeWay Total RNA Extraction Kit (1st Base, Singapore). RNA purity and concentration were assessed spectrophotometrically. Gene expression was then analyzed by qRT-PCR using a Toyobo PCR kit (Toyobo, Japan) on an Applied Biosystems system (Thermo Fisher Scientific, USA). The expression of *sea, seb*, *sec*, *tsst-1*, *fnbA*, *mecA*, and *nuc* genes was quantified, with *nuc* used as the housekeeping control for normalization [23] (Table 1).

Following amplification, cycle threshold (Ct) values were obtained, and relative gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method [24]. A fold change greater than 1.0 was interpreted as upregulation, values below 1.0 as downregulation, and values between 0.9 and 1.1 were considered not significantly different [25].

Table 1. List of primer for MRSA and housekeeping gene

Genes	Sequence
sea	F 5'-AGCTTGTATGTATGGTGGTGT-3 R 5'-ACGTCTTGCTTGAAGATCCA-3'
seb	F 5'-AGGACACTAAGTTAGGGAAT-3' R 5'-CTCAGTTACACCACCATACA-3
sec	F 5'-TGTAGGTAAAGTTACAGGTGGT-3' R 5'-TGTCTAGTTCTTGAGCTGTTAC-3'
tsst-1	F 5'-ATGGCAGCATCAGCTTGATA-3' R 5'- TTTCCAATAACCACCCGTTT-3'
fnbA	F 5'-CACAACCAGCAAATATAG-3' R 5'-CTG TGTGGTAATCAATGT-3'
mecA	F 5'AAAATCGATGGTAAAGGTTGGC-3' R 5'AGTTCTGCAGTACCGGATTTGC-3'
nuc	F 5'-GCGATTGATGGTGATACGGTI-3' R 5'AGCCAAGCCTTGACGAACTAAAGC-3'

Statistical Analysis

All qRT-PCR experiments were performed in three independent biological replicates. Relative gene expression levels were calculated using the 2^−ΔΔCt method and are presented as mean ± standard deviation (SD). Statistical comparisons between the untreated control and the *P. minor* aqueous extract-treated group for each gene were performed using a two-tailed unpaired Student's t-test in GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). A p-value < 0.05 was considered statistically significant. Since each gene represented an independent predefined biological endpoint and only a single comparison (treated versus untreated) was performed for each gene, no correction for multiple comparisons was applied.

Results and Discussion

Chemical composition of *P. minor* aqueous extract

The qualitative phytochemical screening of *P. minor* aqueous extract demonstrated the presence of saponins, steroids, tannins, flavonoids, phenols, and glycosides, whereas alkaloids were not detected (Table 2). The detection of multiple bioactive secondary metabolites suggests that *P. minor* possesses a broad spectrum of phytochemical constituents that may act synergistically to exert antibacterial effects against MRSA.

Table 2. Qualitative phytochemical screening results for *P. minor* aqueous extract

Phytochemical	Result	Interpretation
Saponin	+	Present
Steroid	+	Present
Alkaloid	-	Not detected
Tannin	+	Present
Flavonoid	+	Present
Phenol	+	Present
Glycosides	+	Present

Saponins are well-recognized for their ability to disrupt bacterial cell membranes through interaction with membrane sterols, resulting in pore formation, leakage of intracellular contents, and eventual cell death [26]. Such membrane-destabilizing effects are particularly relevant for MRSA, which relies on robust cell wall integrity to withstand environmental stress and antibiotic pressure. Steroids have been reported to alter membrane permeability and suppress bacterial efflux pumps, thereby enhancing intracellular accumulation of antibacterial agents [27]. Their presence in *P. minor* extract may contribute to potentiating the bactericidal action of other phytochemicals, further strengthening its anti-MRSA activity.

Tannins, another major constituent, exert antimicrobial effects by precipitating microbial proteins, inactivating essential enzymes, and interfering with bacterial adhesion and biofilm formation [28]. Since MRSA is known for its capacity to form biofilms that protect against antibiotic treatment, the tannin content in *P. minor* could be particularly effective in disrupting this resistance mechanism. Flavonoids are widely recognized for their potent antibacterial properties, acting through inhibition of nucleic acid synthesis, disruption of energy metabolism, and interference with quorum sensing pathways [29]. In MRSA, flavonoids have been shown to impair biofilm development and attenuate virulence factors, thereby reducing pathogenicity [30].

Phenolic compounds contribute to antibacterial activity through multiple mechanisms, including membrane destabilization, oxidative stress induction, and chelation of metal ions essential for bacterial enzymes [31]. Their broad reactivity supports a synergistic effect with flavonoids and tannins in targeting MRSA. Glycosides, upon hydrolysis, can release aglycone moieties with known antimicrobial

potential. These compounds may inhibit bacterial enzymes or disrupt metabolic pathways critical for bacterial survival [32]. The combined presence of these phytoconstituents indicates that *P. minor* aqueous extract does not rely on a single mechanism of action but rather exerts a multifaceted antibacterial effect. This synergy between phytochemicals could explain the significant inhibition observed against MRSA in this study, highlighting the potential of *P. minor* as a promising natural source of anti-MRSA agents.

In vitro anti-MRSA activity of *P. minor* aqueous extract

The antibacterial activity of *P. minor* aqueous extract was assessed using the disc diffusion method, and the results are presented in Table 3. The extract exhibited concentration-dependent inhibition against MRSA clinical isolates and the reference strain *S. aureus* ATCC 25923. At the highest tested concentration (200 mg/mL), the extract produced inhibition zones ranging between 7.0–8.3 mm across MRSA isolates and 7.7 mm for the reference strain. In contrast, no substantial inhibition was observed at concentrations ≤50 mg/mL, where inhibition zones were limited to 6 mm, which corresponds to the disc diameter and thus indicates no real inhibitory effect. The negative control (distilled water) showed no inhibition, whereas the positive control vancomycin (30 µg/mL) consistently produced larger zones of 14.7–19.3 mm, confirming assay validity and highlighting the superior activity of the standard antibiotic.

Table 3. Zone of inhibition of disc diffusion method for *P. minor* against tested MRSA isolates

Bacterial isolates	Zone of Inhibition (mm)								
	P. minor concentration (mg/mL)							Vancomycin (30 µg/mL, positive control)	Distilled water (negative control)
	200	100	50	25	12.5	6.25	3.125		
MRSA Strain 1	7.3±0.03	6±0.00	6±0.00	6±0.00	6±0.00	6±0.00	6±0.00	16.3±0.00	6±0.00
MRSA Strain 2	8±0.50	7.3±0.50	6.3±0.50	6±0.00	6±0.00	6±0.00	6±0.00	16±0.00	6±0.00
MRSA Strain 3	7±0.00	6±0.00	6±0.00	6±0.00	6±0.00	6±0.00	6±0.00	17±0.00	6±0.00
MRSA Strain 4	7±0.00	6.3±0.05	6±0.00	6±0.00	6±0.00	6±0.00	6±0.00	14.7±0.00	6±0.00
MRSA Strain 5	7±0.20	6±0.00	6±0.00	6±0.00	6±0.00	6±0.00	6±0.00	16.3±0.00	6±0.00
MRSA Strain 6	8.3±0.05	6.7±0.05	6±0.00	6±0.00	6±0.00	6±0.00	6±0.00	15±0.00	6±0.00
MRSA Strain 7	7.3±0.00	7±0.05	6.7±0.05	6±0.00	6±0.00	6±0.00	6±0.00	19.3±0.00	6±0.00
MRSA Strain 8	7.7±0.50	7.3±0.03	6±0.00	6±0.00	6±0.00	6±0.00	6±0.00	16.3±0.00	6±0.00
S. aureus (ATCC 25923)	7.7±0.20	7±0.05	6±0.00	6±0.00	6±0.00	6±0.00	6±0.00	17.3±0.00	6±0.00

*Tests are performed in triplicates

Although the inhibitory zones of *P. minor* were smaller than vancomycin, the extract demonstrated reproducible antibacterial activity at higher concentrations against all tested MRSA isolates, which are notoriously resistant to β-lactam antibiotics. This suggests that the phytochemicals present in the extract, particularly flavonoids, tannins, phenolics, and saponins, are likely responsible for the observed antibacterial effects. Previous studies have reported that flavonoids and tannins interfere with bacterial cell wall synthesis, protein function, and biofilm formation, while saponins and steroids destabilize bacterial membranes, collectively contributing to the inhibition of MRSA growth [33].

The modest inhibition zones (7–8 mm) of *P. minor* aqueous extract, compared to vancomycin, indicate that while the extract may not serve as a direct replacement for conventional antibiotics, it possesses notable antibacterial potential when used at higher concentrations. Importantly, the consistent inhibitory effects across all MRSA isolates tested suggest broad-spectrum activity, rather than activity

limited to specific isolates. This reproducibility strengthens the argument for *P. minor* as a valuable candidate for further development.

Another critical observation is the higher susceptibility of the non-resistant *S. aureus* ATCC 25923 strain compared to MRSA isolates, as reflected in slightly larger inhibition zones at 200 mg/mL (7.7 mm vs. 7.0–8.3 mm for MRSA). This aligns with the expectation that resistant isolates generally exhibit reduced susceptibility to plant-derived antimicrobials due to altered membrane permeability, efflux pumps, and biofilm formation. Nonetheless, the extract maintained inhibitory action against MRSA, which is significant considering the pathogen's multidrug-resistant profile.

The antibacterial activity of *P. minor* aqueous extract could be explained by synergistic interactions among its phytochemical constituents. For instance, tannins and flavonoids can inhibit essential bacterial enzymes, while phenolic compounds generate oxidative stress that compromises bacterial survival. The presence of saponins and steroids may enhance cell permeability, thereby facilitating the penetration of other active compounds. Such synergy is crucial when targeting MRSA, a pathogen with multiple resistance mechanisms [34,35].

Although the inhibition zones observed are relatively modest compared to antibiotics, the results support the notion that *P. minor* may serve as a complementary antimicrobial agent, especially in formulations that combine plant-derived compounds with existing antibiotics to achieve synergistic effects. Previous studies have demonstrated that phytochemicals such as flavonoids and tannins can potentiate the activity of β-lactam and glycopeptide antibiotics against resistant *Staphylococcus aureus* isolates [36,37]. This raises the possibility that *P. minor* could be exploited in combination therapies aimed at restoring the efficacy of conventional antibiotics.

The disc diffusion results confirm that *P. minor* aqueous extract exhibits significant antibacterial activity against both MRSA clinical isolates and *S. aureus* ATCC 25923, with effects most pronounced at higher concentrations. The observed activity is attributable to the presence of multiple bioactive secondary metabolites, acting through different antibacterial mechanisms. While less potent than vancomycin, the consistent inhibition across resistant strains underscores the potential of *P. minor* as a promising natural source of anti-MRSA compounds.

Dose-Dependent Antibacterial Activity of *P. minor* Aqueous Extract: MIC and MBC Analysis

The antibacterial potential of *P. minor* aqueous extract was further evaluated using MIC and MBC assays, which provide quantitative measures of growth inhibition and bacterial killing, respectively. The MIC results revealed that the extract was able to inhibit the growth of all tested MRSA isolates and S. aureus ATCC 25923, with MIC values ranging from 25–200 mg/mL (Table 4). Specifically, MRSA2, MRSA7, and MRSA8 exhibited the highest susceptibility with MIC values of 25 mg/mL, while MRSA strain 6 was the least susceptible with an MIC of 200 mg/mL. The reference strain *S. aureus* ATCC 25923 had an intermediate MIC of 50 mg/mL, consistent with its non-resistant phenotype.

Table 4. Value of *P. minor* against MRSA isolates

Bacteria isolates	MIC (mg/mL)
MRSA1	50
MRSA2	25
MRSA3	50
MRSA4	50
MRSA5	100
MRSA6	200
MRSA7	25
MRSA8	25
S. aureus (ATCC 25923)	50

*Tests are performed in triplicates

These MIC results corroborate the disc diffusion findings, confirming that the antibacterial activity of *P. minor* aqueous extract is concentration-dependent. The variation in susceptibility among MRSA isolates likely reflects intrinsic differences in resistance mechanisms, such as cell wall thickness, biofilm formation, and efflux pump activity, which may reduce the intracellular accumulation of bioactive phytochemicals. Despite these variations, all tested MRSA isolates were inhibited at achievable extract concentrations, highlighting the broad-spectrum inhibitory potential of *P. minor*.

The MBC assay further demonstrated the bactericidal capability of *P. minor* aqueous extract. MBC values ranged from 50 to 200 mg/mL, with the MBC/MIC ratio ≤2 for all MRSA isolates (Table 5). According to established criteria, an MBC/MIC ratio ≤4 is indicative of bactericidal activity [38]. Therefore, these results confirm that *P. minor* aqueous extract is not only capable of inhibiting bacterial growth but can also effectively kill MRSA and *S. aureus* at sufficiently high concentrations. Notably, MRSA2, MRSA7, and MRSA8 showed an MBC of 50 mg/mL and an MBC/MIC ratio of 2, indicating that the bactericidal effect occurs at concentrations only twofold higher than the MIC, which is promising from a therapeutic perspective.

Table 5. MBC value and MBC/MIC ratio of *P. minor* against MRSA isolates

Bacteria isolates	MBC (mg/mL)	MBC/MIC ratio
MRSA1	100	2
MRSA2	50	2
MRSA3	100	2
MRSA4	100	2
MRSA5	200	2
MRSA6	200	1
MRSA7	50	2
MRSA8	50	2
S. aureus (ATCC 25923)	100	2

*Tests are performed in triplicates

The observed antibacterial and bactericidal activities of *P. minor* aqueous extract can be attributed to the synergistic action of multiple phytochemicals present in the extract, including flavonoids, tannins, phenols, saponins, steroids, and glycosides. Flavonoids and phenolic compounds are known to inhibit nucleic acid synthesis, interfere with energy metabolism, and generate oxidative stress in bacterial cells. Tannins can precipitate proteins and disrupt bacterial enzymes, thereby impairing essential metabolic functions, while saponins and steroids enhance cell membrane permeability, facilitating the entry of other antimicrobial compounds and potentiating bactericidal effects [39,40].

Glycosides may release active aglycones that inhibit enzyme activity and compromise bacterial viability. Collectively, the MBC/MIC data, together with the disc diffusion and MIC results, suggest that *P. minor* aqueous extract exhibits a multifaceted antibacterial action against MRSA, functioning through both growth inhibition and bacterial killing. Such dual activity is particularly advantageous in combating multidrug-resistant pathogens, where bactericidal agents are preferred over bacteriostatic compounds to reduce the risk of persistent infections and further resistance development [41]. Overall, these findings demonstrate that *P. minor* aqueous extract possesses significant anti-MRSA potential, acting as a bactericidal agent with broad activity across both resistant and non-resistant *S. aureus*.

Gene Expression Analysis

The antibacterial susceptibility assay identified MRSA7 as the most susceptible isolate to *P. minor* aqueous extract and was therefore selected for gene expression analysis. Quantitative real-time PCR (qRT-PCR) was performed to determine the expression levels of selected virulence and resistance genes, namely *sea*, *seb*, *sec*, *tsst-1*, *fnbA*, and *mecA*, following treatment with sub-minimum inhibitory concentration (sub-MIC) levels of the extract. Relative gene expression was calculated using the 2^-ΔΔCt method, with the *nuc* gene serving as the internal reference.

As shown in Figure 1, exposure of MRSA7 to sub-MIC concentrations of *P. minor* aqueous extract resulted in increased expression of all genes examined compared with the untreated control. Among the virulence genes, *sea* exhibited the highest level of induction (16.8-fold), followed by *sec* (6.8-fold) and *seb* (6.3-fold). Moderate upregulation was observed for *mecA* (4.6-fold) and *tsst-1* (3.8-fold), while *fnbA* showed the lowest increase in expression (2.8-fold). These findings suggest that sublethal exposure to *P. minor* extract triggers transcriptional responses associated with virulence and antimicrobial resistance in MRSA [42].

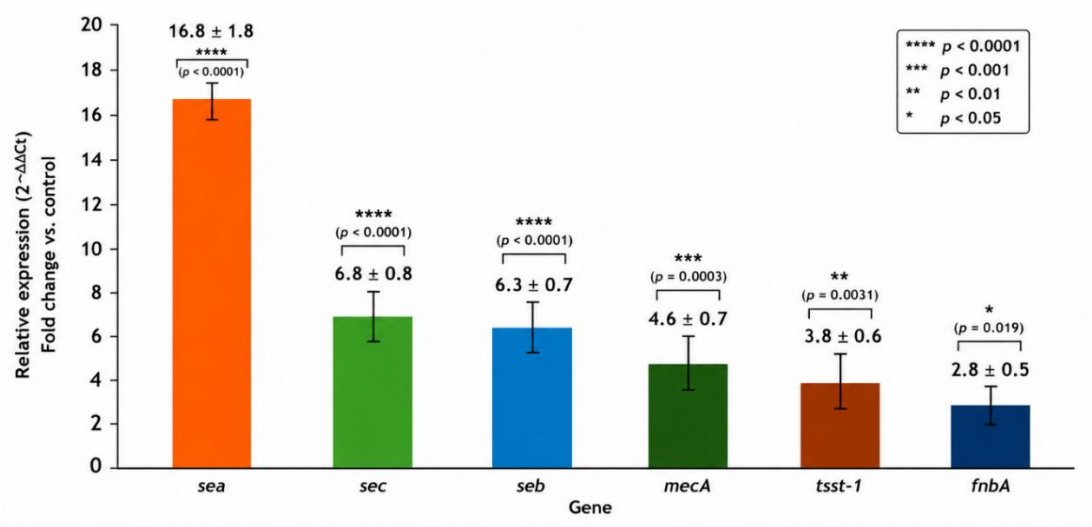


Figure 1. Relative expression of virulence (*sea*, *sec*, *seb*, *tsst-1*, and *fnbA*) and resistance (*mecA*) genes in MRSA7 following treatment with sub-MIC concentrations of *P. minor* aqueous extract. Relative expression levels were calculated using the 2^−ΔΔCt method. Data are presented as mean ± SD from three independent biological experiments. Statistical comparisons between treated and untreated control groups for each gene were performed using a two-tailed unpaired Student's t-test. Statistical significance was defined as $p < 0.05$ ($p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$).

The present study demonstrated that treatment of MRSA7 with sub-MIC concentrations of *P. minor* aqueous extract resulted in significant upregulation of the virulence genes *sea*, *seb*, *sec*, *tsst-1*, and *fnbA*, as well as the methicillin resistance determinant *mecA*. Although *P. minor* exhibited antibacterial activity against MRSA in the susceptibility assays, exposure to sub-inhibitory concentrations appeared to induce a stress-adaptive response that enhanced transcription of several pathogenicity-associated genes.

Sub-inhibitory concentrations of antimicrobial agents are increasingly recognized for their ability to modulate bacterial gene expression. Rather than killing bacterial cells directly, sub-MIC exposure can activate global stress response pathways that promote survival under adverse environmental conditions. Such responses often include increased expression of virulence factors, adhesins, biofilm-associated genes, and antimicrobial resistance determinants. Similar observations have been reported for several plant-derived antimicrobials and conventional antibiotics, where bacterial exposure to sublethal concentrations triggered adaptive transcriptional changes that enhanced persistence and survival [43].

Among the genes examined, *sea* exhibited the greatest induction, with expression increasing approximately 16.8-fold following treatment. The *sea* gene encodes staphylococcal enterotoxin A (SEA), one of the most prevalent and clinically significant superantigens produced by *S. aureus*. SEA is frequently associated with food poisoning outbreaks and contributes to excessive T-cell activation and cytokine release. The marked increase in *sea* expression suggests that MRSA may respond to extract-induced stress by activating toxin production pathways as a protective survival strategy [44].

The comparatively lower but still substantial upregulation of *seb* and *sec* further supports activation of enterotoxin-associated regulatory networks.

The simultaneous induction of *sea*, *seb*, and *sec* may be associated with activation of global regulatory systems such as the accessory gene regulator (Agr) quorum-sensing system, staphylococcal accessory regulator A (SarA), and alternative sigma factor B (SigB). These regulatory pathways coordinate bacterial adaptation to environmental stress and are known to influence expression of multiple exotoxin genes [45]. Exposure to phytochemicals present in *P. minor*, including flavonoids, tannins, and phenolic compounds, may create cellular stress conditions that stimulate these regulatory networks.

Similarly, the observed increase in *tsst-1* expression suggests activation of pathways involved in toxin-mediated virulence [46]. The *tsst-1* gene encodes toxic shock syndrome toxin-1 (TSST-1), a potent superantigen responsible for toxic shock syndrome. Upregulation of *tsst-1* following treatment may reflect an adaptive response that enhances bacterial fitness under stressful conditions. However, it is important to note that increased mRNA expression does not necessarily translate directly into increased protein production, as toxin synthesis is also influenced by post-transcriptional and environmental regulatory mechanisms [47].

The upregulation of *fnbA* further indicates a potential enhancement of adhesion and colonization capabilities. Fibronectin-binding protein A plays a crucial role in attachment to host tissues, invasion of epithelial cells, and biofilm formation. Increased *fnbA* expression has been reported in bacteria exposed to environmental stressors and may represent an attempt by MRSA to strengthen host interactions and establish protective biofilm communities. Such adaptations could contribute to bacterial persistence despite antimicrobial challenge [48].

Interestingly, treatment with *P. minor* extract also resulted in a 4.6-fold increase in *mecA* expression. The *mecA* gene encodes penicillin-binding protein 2a (PBP2a), the primary determinant of methicillin resistance in MRSA. Increased expression of *mecA* suggests activation of cell wall stress response mechanisms, possibly triggered by bioactive compounds present in the extract. Previous studies have demonstrated that exposure to antimicrobial compounds can stimulate expression of resistance genes as part of a compensatory response aimed at preserving cell wall integrity and maintaining bacterial viability [49]. The observed induction of *mecA* therefore likely reflects an adaptive resistance response rather than a direct increase in methicillin resistance. In this context, MRSA may perceive the phytochemicals present in *P. minor* as environmental stressors, leading to activation of protective regulatory pathways that promote survival under unfavorable conditions. Similar stress-induced upregulation of resistance-associated genes has been reported following exposure to sub-inhibitory concentrations of antibiotics and plant-derived antimicrobial compounds. However, increased transcription of *mecA* does not necessarily correlate with increased PBP2a production or a corresponding increase in phenotypic methicillin resistance [50]. Further investigations involving protein expression analysis and antimicrobial susceptibility testing following extract exposure are required to determine whether the observed transcriptional changes translate into functional resistance. Nevertheless, the findings suggest that sub-MIC concentrations of *P. minor* extract may trigger bacterial adaptive responses that warrant consideration when evaluating its therapeutic application against MRSA.

The collective upregulation of virulence and resistance genes observed in this study may be explained by the use of sub-MIC concentrations of the extract. At concentrations below the inhibitory threshold, bacteria remain metabolically active and may perceive antimicrobial agents as environmental stress signals rather than lethal threats. Consequently, transcriptional regulators activate pathways associated with toxin production, adhesion, biofilm formation, and antimicrobial resistance to improve survival prospects. Similar stress-induced increases in virulence gene expression have been reported following exposure to sub-inhibitory concentrations of antibiotics such as β-lactams, fluoroquinolones, and macrolides [51].

These findings suggest that while *P. minor* aqueous extract possesses antibacterial activity against MRSA, exposure to sub-inhibitory concentrations may stimulate adaptive responses associated with pathogenicity and survival. Therefore, therapeutic applications of the extract should consider concentration-dependent effects to ensure that antimicrobial activity is achieved without inadvertently promoting virulence-associated gene expression. Further studies are warranted to determine whether the observed transcriptional changes translate into increased toxin production, biofilm formation, or resistance phenotypes at the protein and functional levels. Investigation of the regulatory pathways involved, particularly Agr, SarA, SigB, and SaeRS, would provide additional insight into the molecular mechanisms underlying MRSA adaptation to *P. minor* exposure [52].

Overall, the results demonstrate that sub-MIC concentrations of *P. minor* aqueous extract elicit significant transcriptional responses in MRSA, characterized by increased expression of major virulence and resistance genes. These findings highlight the complexity of bacterial responses to plant-derived antimicrobials and underscore the importance of evaluating both antibacterial efficacy and gene regulatory effects when assessing the therapeutic potential of natural products.

CONCLUSION

The *P. minor* aqueous extract shows clear potential as a source of natural anti-MRSA compounds, validating the search for plant-derived alternatives, but this potential is complicated by the simultaneous activation of bacterial virulence and resistance genes, which underscores the complex interplay between natural agents and microbial survival mechanisms, thereby highlighting the necessity for comprehensive molecular characterization beyond basic minimum inhibitory concentration assays; therefore, future research is essential and must prioritize time-kill kinetic analyses, synergistic assays with conventional antibiotics, isolation and characterization of individual bioactive compounds, and in vivo efficacy and safety evaluations to fully understand the plant's complex dual activity and pave the way for its safe and effective therapeutic development against MRSA.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

This work was financially supported by the Universiti Sultan Zainal Abidin and Dtree Pharma Sdn Bhd Matching Grant for the year 2024 (Grant Number: UniSZA/2023/PGP/04).

References

[1] National Cancer Institute, NCI Dictionary of Cancer Terms: Antibacterial, National Cancer Institute, Bethesda, 2024.
[2] B.J. Werth, Overview of antibacterial medications, in: MSD Manual Professional Edition, MSD Manuals, Kenilworth, 2024.
[3] A.K. Yahya, N.Z. Abd Wahab, N. Ibrahim, Malays. J. Chem. 26 (2024) 123–136.
[4] A.K. Yahya, N.Z. Abd Wahab, M. Abdul Ghani, Malays. J. Med. Health Sci. 20 (2024) 262–272.
[5] N. Zainal, N.Z. Abd Wahab, M. Abdul Ghani, Malays. J. Med. Health Sci. 20 (2024) 252–261.
[6] N.Z. Abd Wahab, N. Ibrahim, Molecules 27 (2022) 4566.
[7] H.A. Hemeg, I.M. Moussa, S. Ibrahim, T.M. Dawoud, J.H. Alhaji, A.S. Mubarak, S.A. Kabli, R.A. Alsubki, A.M. Tawfik, S.A. Marouf, Saudi J. Biol. Sci. 27 (2020) 3221–3227.
[8] M.F. Asanai, N. Haron, S.N. Camalxaman, E. Mohamed, A.S. Rambely, Healthscope 3 (2020) 44–50.
[9] S. SeyedAlinaghi, E. Mehraeen, P. Mirzapour, S. Yarmohammadi, S. Dehghani, S. Zare, S. Gholami, N. Attarian, A. Abiri, F. Farahani Rad, A. Tabari, S. Afroughi, A. Gholipour, M.M. Roozbahani, S. Jahanfar, Eur. J. Med. Res. 30 (2025) 525.
[10] M. Che, C.C. Yeo, S.M. Puah, K.H. Chua, C.H. Chew, Antibiotics 8 (2019) 128.
[11] K. Sukri, N. Mohamed, I. Isahak, A. Marwan, Malays. J. Med. Health Sci. 19 (2023) 2636–2636.

[12] A.S. Lee, H. de Lencastre, J. Garau, J. Kluytmans, S. Malhotra-Kumar, A. Peschel, S. Harbarth, Nat. Rev. Dis. Primers 4 (2018) 33.
[13] Institute for Medical Research, National Antibiotic Resistance Surveillance Report (NSAR) 2023, Ministry of Health Malaysia, Kuala Lumpur, 2023.
[14] A.A. Abebe, A.G. Birhanu, Infect. Drug Resist. 16 (2023) 7641–7662.
[15] P. Angelini, Antibiotics 13 (2024) 746.
[16] N.Z. Abd Wahab, A.H.A. Abd Rahman, J. Pure Appl. Microbiol. 16 (2022) 2568–2575.
[17] N.Z. Abd Wahab, A. Azizul, N. Ibrahim, Iran. J. Microbiol. 12 (2020) 460–465.
[18] S. Haida, K. Bakkouche, A.R. Kribii, A. Kribii, Biochem. Res. Int. 2021 (2021) 6669877.
[19] N. Kancherla, A. Dhakshinamoorthi, K. Chitra, R.B. Komaram, Maedica 14 (2019) 350–356.
[20] D.A. Okuna, C.I. Nyamwange, H. Nyandieka, Kabarak J. Res. Innov. 14 (2024) 230–241.
[21] .Z. Abd Wahab, S.N.A. Noor Azam, S.M.S.F. Sayed Abdul Kadir, M.H. Abdullah, Trop. J. Nat. Prod. Res. 9 (2025) 14–23.
[22] N.Z. Abd Wahab, M.N. Maizatul Najwa Malza, Y. Rukayadi, Hayati J. Biosci. 31 (2024) 903–915.
[23] N.Z. Abd Wahab, S.M.S.F. Sayed Abdul Kadir, K.S. Kamal Rul Azrul, N.A.N. Mohd Yuseri, A.K. Yahya, F. Si Wei, M.H. Abdullah, Bacteria 5 (2026) 13.
[24] K.J. Livak, T.D. Schmittgen, Methods 25 (2001) 402–408.
[25] X. Huang, Z. Zhou, X. Lin, Biostat. Bioinforma. Biomath. 3 (2013) 71–80.
[26] I.I. Tatli Cankaya, E.I. Somuncuoglu, Evid.-Based Complement. Altern. Med. 2021 (2021) 6814215.
[27] L. Zhang, X. Huang, Z. Zhou, X. Lin, Y. Wang, Pharmaceutics 16 (2024) 170.
[28] Q. Huang, X. Liu, G. Zhao, T. Hu, Anim. Nutr. 4 (2018) 137–150.
[29] J.B. do Nascimento, J.G.M. da Costa, Int. J. Microbiol. 2025 (2025) 9961121.
[30] N.F. Shamsudin, Q.U. Ahmed, S. Mahmood, S.A. Ali Shah, A. Khatib, S. Mukhtar, M.A. Alsharif, H. Parveen, Z.A. Zakaria, Molecules 27 (2022) 1149.
[31] A. Lobiuc, N.E. Pavăl, I.I. Mangalagiu, R. Gheorghiță, G.C. Teliban, D. Amăriucăi-Mantu, V. Stoleru, Molecules 28 (2023) 1114.
[32] K. Rachwał, K. Gustaw, Appl. Sci. 15 (2025) 6774.
[33] B. Khameneh, M. Iranshahy, B.S. Fazly Bazzaz, Antibiotics 10 (2021) 1044.
[34] Y. Liu, J. Zhu, Z. Liu, Y. Zhi, C. Mei, H. Wang, Int. J. Mol. Sci. 26 (2025) 2455.
[35] J. Huang, M. Zaynab, Y. Sharif, J. Khan, R. Al-Yahyai, M. Sadder, M. Ali, S.R. Alarab, S. Li, Toxicon 247 (2024) 107812.
[36] B.M. Kyaw, S. Arora, C.S. Lim, Braz. J. Microbiol. 43 (2012) 938–945.
[37] R.D. Wojtyczka, M. Kępa, D. Idzik, R. Kubina, A. Kabała-Dzik, A. Dziedzic, J. Stojko, R. Stojko, T.J. Wasik, Molecules 18 (2013) 9623–9640.
[38] D.B. Whyte, The minimum bactericidal concentration of antibiotics, BMG Labtech, Ortenberg, 2024.
[39] H. Zhou, L. Chen, K. Ouyang, Q. Zhang, W. Wang, Front. Microbiol. 13 (2022) 1103476.
[40] M. Takó, E.B. Kerekes, C. Zambrano, A. Kotogán, T. Papp, J. Krisch, C. Vágvölgyi, Antioxidants 9 (2020) 165.
[41] E.T. Jeong, S.K. Park, D.M. Jo, F. Khan, T.H. Choi, T.M. Yoon, Y.M. Kim, J. Microbiol. Biotechnol. 31 (2021) 1288–1294.
[42] E. Viedma, D. Pérez-Montarelo, J. Villa, I. Muñoz-Gallego, N. Larrosa, N. Fernández-Hidalgo, J. Gavaldà, B. Almirante, F. Chaves, BMC Infect. Dis. 18 (2018) 177.
[43] J. Chen, H. Zhou, J. Huang, R. Zhang, X. Rao, J. Adv. Res. 31 (2021) 165–175.
[44] J. Schelin, Y.B. Susilo, S. Johler, Toxins 9 (2017) 401.
[45] X. Wu, H. Wang, J. Xiong, G.X. Yang, Biofilm 7 (2024) 100175.
[46] R. Touaitia, N. Ibrahim, E. Abdullah Almuqri, N.S. Basher, T. Idres, A. Touati, Toxins 17 (2025) 323.
[47] G. Ménard, C. Silard, M. Suriray, A. Rouillon, Y. Augagneur, Int. J. Mol. Sci. 23 (2022) 7346.
[48] K. Evans, A.R. Horswill, Microbiol. Mol. Biol. Rev. 84 (2020) e00026-19.
[49] J.J. Evans, D.D. Bolz, Curr. Opin. Infect. Dis. 32 (2019) 217–222.
[50] L. Chen, Z. Wang, T. Xu, H. Ge, F. Zhou, X. Zhu, X. Li, D. Qu, C. Zheng, Y. Wu, K. Zhao, Front. Microbiol. 12 (2021) 727104.
[51] B.L. Bilyk, V.V. Panchal, M. Tinajero-Trejo, J.K. Hobbs, S.J. Foster, Microbiol. Mol. Biol. Rev. 86 (2022) e00159-21.
[52] C. Jenul, A.R. Horswill, Microbiol. Spectr. 7 (2019) GPP3-0031-2018.