Journal of
Food and Pharmaceutical Sciences

Original Article

In Vitro Anti-Bacterial Activity and Network Pharmacology Analysis of Aqueous Extract of *Marsdenia Tinctoria* R.Br. Leaves against *Staphylococcus aureus*

Anna Choirunnisa[1]*, Ari Sri Windyaswari[2], Guntur Berlian[3], Andrian Rafliana Supriatnaputra[1], and Suryani Suryani[1]

[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
[2]Department of Biology Pharmacy, Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
[3]Yayasan Buana Varman Semesta, The Varman Institute, West Bandung, Bandung, Indonesia
*Corresponding author: Anna Choirunnisa | Email: anna.choirunisa@lecture.unjani.ac.id

Received: 19 December 2025; Revised: 17 June 2026; Accepted: 21 July 2026; Published: 24 August 2026

Abstract: *Staphylococcus aureus* is a major Gram-positive microorganism implicated in a wide range of skin infections. The increasing prevalence of antimicrobial resistance among *S. aureus* emphasizes the need for the development of novel therapeutic interventions. This study evaluated the antibacterial efficacy and analysis the molecular mechanism of *Marsdenia tinctoria* R.Br. (Tarum Areuy) leaf aqueous extract against *S. aureus* through integrated in vitro and network pharmacology analyzed. The aqueous extract was prepared by infusion, and its metabolite profile was characterized using UPLC-HRMS. Antibacterial activity was quantitatively investigated using the agar well diffusion method against *S. aureus* ATCC 6538. Network pharmacology identified ten secondary metabolites with antibacterial potential, seven of which exhibited pharmacokinetic properties that met the established bioavailability criteria (BOILED-Egg method). Protein-protein interaction (PPI) analysis indicated that fifteen key proteins interact with the seven compounds, with quercetin identified as the major bioactive compound activating specific bacterial pathways (SAV0081 and pkrC) in *S. aureus*. The highest antibacterial effect was observed at an 8% extract concentration with an inhibition zone of 17.03 ± 1.78 mm. Overall, these results confirm that the aqueous extract of *M. tinctoria* leaves contains potential metabolites as antibacterials that could be further developed as therapeutic agents for the treatment of *S. aureus* infections.

Keywords: antibacterial activity; *Marsdenia tinctoria* R.Br; network pharmacology; *Staphylococcus aureus*

1. INTRODUCTION

Infections caused by *Staphylococcus aureus* represent a significant worldwide health threat, especially due to the increasing prevalence of antibiotic resistance, notably Methicillin-Resistant *S. aureus* (MRSA) strains. These bacteria induce a range of diseases, from mild skin infections to severe conditions such as pneumonia, endocarditis, and sepsis. The emergence of multidrug-resistant organisms requires the development of novel antibacterial agents derived from natural origins [1]–[3]. Reports of resistance of *S. aureus* to various antibiotics persistently arise in diverse locations and at varying intervals. In 2014, a study was performed on the sensitivity patterns of *S. aureus* at Dr. Soeardji Tirtonegoro General Hospital in Klaten. It was determined that 64.8% of isolates exhibited resistance to tetracycline antibiotics, 53.7% to erythromycin, and 40.7% to cloxacillin antibiotics [4].

An example of a natural plant reported to have antibacterial effects is Tarum areuy (*Marsdenia tinctoria*). *M. tinctoria* is a plant with deep cultural significance in Sundanese society. This plant has been traditionally cultivated in West Java and has been used for centuries, not only as a natural coloring agent in ancient textile traditions but also in traditional medicine [5]. This plant is one of the Marsdeniae genus of the Apocynaceae family, recognized for its production of chemicals commonly utilized in herbal medicine. The predominant natural substances described from the Marsdeniae

genus are steroids, alkaloids, and steroid glycosides [6]. Secondary metabolites derived from medicinal plants are recognized for their ability to restrict bacterial growth through several methods, including disruption of the cell membrane, inhibition of protein synthesis, and suppression of nucleic acid production [7]. However, the systemic mechanism of action of these compounds still needs further exploration, especially in the context of the multi-target interactions typical of natural compounds.

Based on this, this study aims to determine the content of metabolite compounds found in *M. tinctoria* plants that have potential antibacterial activity and predict the interaction of secondary metabolite compounds with bacterial target proteins through network pharmacology against the gram-positive model bacterium *S. aureus*. Network pharmacology provides a systems biology approach to map compound-target-pathway interactions systematically. To assess antibacterial activity as a validation method, we employed the agar well diffusion method—a well-established standard assay in microbiology. This method measures the zone of inhibition.

2. MATERIALS AND METHODS

2.1. Plant Collection

The leaves of *M. tinctoria* were cultivated from Pataruman-Indigo Experimental Station, Sukabumi, West Java, Indonesia and the determination number was conducted at the School of Life Sciences and Technology, Institut Teknologi Bandung (SITH-ITB) with certificate number 1028/IT1.C11.2/TA.00/2025).

2.2. Methods

2.2.1. Sample Preparation

The leaves are washed, cut, dried, and then powdered. The extract is obtained through infusion using an aqueous solvent. The method uses a drug to solvent (water) ratio of 1:10 on a weight per volume basis (w/v). The extract is dried using freeze-drying. The water extract of *M. tinctoria* leaves gave a yield of 27.4 % w/w.

2.2.2. UHPLC–HRMS Analysis

The secondary metabolite profile of Aqueous Extract of *Marsdenia tinctoria* R.Br. Leaves (AEML) was determined using UHPLC–HRMS. This analysis was carried out in a Thermo Scientific™ Vanquish™ UHPLC Binary Pump system (Germany) coupled to a Thermo Scientific™ Q Exactive™ hybrid quadrupole Orbitrap™ high resolution mass spectrometer (Germany). The columns used are Thermo Scientific™ Accucore™ Phenyl Hexyl column (100 mm × 2.1 mm i.d., 2.6 μm) maintained at 40 °C with an injection volume of 3 μL. The mobile phase comprised solvent A (0.1% formic acid in water, MS grade) and solvent B (0.1% formic acid in acetonitrile, MS grade), with a flow rate of 0.3 mL/min applied in gradient elution. The gradient profile started at 5% B at 0 min, ramped linearly to 90% B by 16 min, maintained 90% B for 4 min, then decreased back to 5% B and re-equilibrated to 25 min.

High-resolution mass spectrometry was performed in full MS/dd-MS² mode with positive electrospray ionization (ESI+) using XCalibur 4.4 software. Nitrogen served as sheath gas (32 AU), auxiliary gas (8 AU), and sweep gas (4 AU). The spray voltage was 3.30 kV, the capillary temperature was 320 °C, and the auxiliary gas heater temperature was 30 °C. Data were collected over an m/z range of 66.7–1000, with a resolving power of 70,000 for full MS and 17,500 for dd-MS².

2.2.3. Phytochemical Components of Aqueous Extract of *Marsdenia tinctoria* R.Br. Leaves Screening

Phytochemical components of AEML were collected from UHPLC–HRMS Analysis, especially the secondary metabolite. The data was then organized by entering an Excel spreadsheet containing information associated with compound identity, which included the compound name, PubChem CID, Canonical SMILES, and other essential data.

ADME analysis of phytochemical components was conducted using SwissADME (http://www.swissadme.ch/index.php), with SMILES notations sourced from PubChem

(https://pubchem.ncbi.nlm.nih.gov/). Screening was performed based on three key criteria: high gastrointestinal absorption, no Lipinski rule violations, and a bioavailability score ≥ 0.55 [8].

2.2.4 Protein Target Prediction

Data collection for target proteins in *S. aureus* was performed by accessing the GeneCards database (https://www.genecards.org/) with the keyword "Antibacterial for *Staphylococcus aureus*" [9]. Prediction of proteins with the potential to interact with secondary metabolite compounds found in the AEML, performed by SwissTargetPrediction (http://www.swisstargetprediction.ch/), the criteria of probability ≥ 0 [8]. We merged protein binding from the two databases using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/). Protein intersections were analyzed for interactions between target genes by uploading relevant target genes for antibacterial activity to the STRING (https://cn.string-db.org/). Interactions were configured with parameters restricted to "Homo sapiens" and a confidence score threshold set to the highest level (>0.900). Protein-protein interaction data were generated and clustered using the CytoCluster tool in Cytoscape v.3.8.2 (https://cytoscape.org/) based on the top 15 targets determined by Maximal Clique Centrality (MCC) [10]. The STITCH database (http://stitch.embl.de) predicted metabolite-target interactions on *S. aureus*.

2.2.5 Antibacterial Activity Test by Well Diffusion Method

Staphylococcus aureus ATCC 6538 was inoculated by the spread plate method on MHA media and then incubated for 24 hours at 37 °C. *Staphylococcus aureus* was suspended in a tube containing 5 mL of a Mueller Hinton Broth (MHB) to reach an absorbance of 0.08-0.13, measured by UV-Vis spectrophotometer at 625 nm (equivalent to 0.5 McFarland standard) [11].

The serial concentrations of 4%, 6%, 8%, and 10% of AEML were dissolved in WFI. 1 mL of bacterial suspension was inserted into a petri dish containing NA media. The petri dish media were perforated using a 9 mm diameter tool to create four wells. As a positive control, tetracycline HCl 0.5% was used. 50 µL of the sample solution were put into the well. After that, the petri dish was incubated at 37 °C for 24 hours, and the clear zone formed was measured by their zone of inhibition diameter.

3. RESULTS AND DISCUSSION

This study focused on the potential antibacterial activity of Aqueous Extract of *Marsdenia tinctoria* R.Br. Leaves (AEML) whose interactions were predicted against target proteins of the Gram-positive bacterium *S. aureus*. These bacteria are widespread in the community and cause skin infections.

3.1. Extract Compounds

According to UHPLC-HRMS data, AEML was identified 82 compounds. These compounds were identified as major compounds based on the Similarity Index (range of 80-100%) using the MzCloud database, an annotation mass deviation of -5 to 5 g/ml. Then, a correlation analysis was performed between the phytochemical composition and the potential pharmacological activities of the aqueous extract of *M. tinctoria*. (Figure 1).

Figure 1 indicates that the aqueous extract of *M. tinctoria* leaves exhibits a broad pharmacological spectrum, with phenolic-related compounds (likely flavonoids), terpenoids, and alkaloids contributing predominantly to antioxidant, antimicrobial, and anti-inflammatory activities. Antimicrobial, anti-inflammatory, antioxidant, and antiviral effects appear as the major pharmacological potentials, while antidiabetic, anticonvulsant, and antitumor activities are present to a lesser extent. Most activities are associated with recurring contributions from flavonoids, alkaloids, and terpenoids, suggesting multitarget, synergistic interactions rather than single-compound effects and supporting the use of this extract as a phytopharmaceutical candidate for mild infections, inflammation, and antioxidant therapy, with additional prospects in antidiabetic, antitumor, and neuroprotective applications.

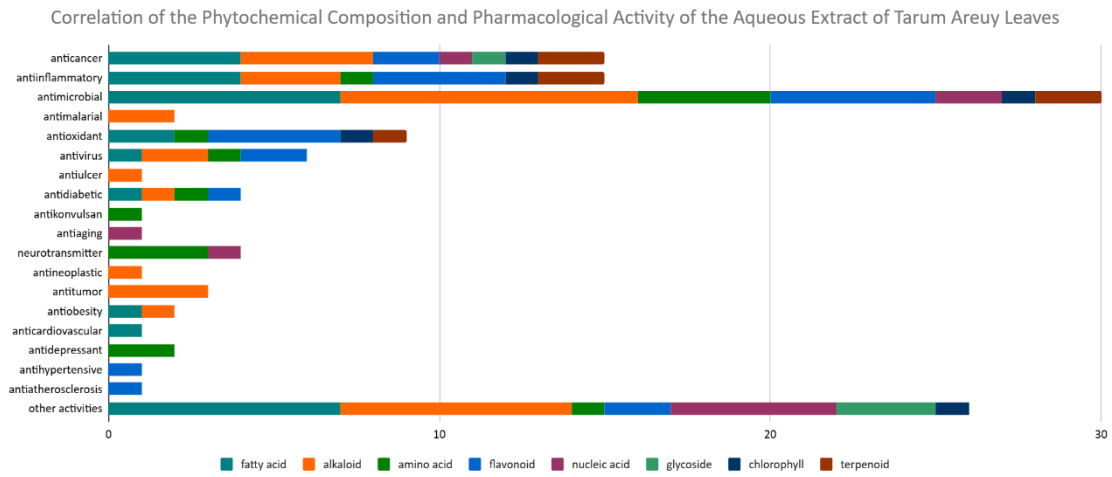


Figure 1. Pharmacology Potential in AEML

Subsequently, antibacterial compound activity was determined using databases such as PubChem, Google Scholar, Elsevier, ScienceDirect, ResearchGate, and reputable national journals. Ten secondary metabolites with potential antibacterial activity were collected and are presented in Table 1.

Table 1. Antibacterial Compounds In AEML

No	Name	Formula	Molecular Weight	RT (min)	Group
1	Glycoperine	$C_{19}H_{21}NO_8$	391.12667	5.712	Alkaloids
2	Methyl nigakinone	$C_{16}H_{12}N_2O_3$	280.08525	7.307	
3	Navenone A	$C_{15}H_{15}NO$	225.11522	10.441	
4	Terminaline	$C_{23}H_{41}NO_2$	363.31233	15.608	
5	1-(14-methylhexadecanoyl)pyrrolidine	$C_{21}H_{41}NO$	323.3181	16.682	
6	Quercetin	$C_{15}H_{10}O_7$	302.04186	5.432	Flavonoid
7	Rutin	$C_{27}H_{30}O_{16}$	610.15266	5.519	Flavonoid
8	2-Decylfuran	$C_{14}H_{24}O$	208.18256	10.808	Terpenoid
9	Echinenone	$C_{40}H_{54}O$	550.41753	17.296	Terpenoid
10	Pheophorbide A	$C_{35}H_{36}N_4O_5$	592.26805	14.802	Plant Pigment

Table 1 shows that AEML contains ten secondary metabolites belonging to four main groups: alkaloids, flavonoids, terpenoids, and chlorophyll derivatives. This is in line with the phytochemical profiles of Marsdenia species and the Apocynaceae family, which are known for their antibacterial properties. Flavonoids, including quercetin and rutin, match the flavone identified in Borneo *M. tinctoria* [12]. The alkaloid profiles (glycoperine, methyl nigakinone) are similar to retronecine-type alkaloids that were found through HPLC-MS/MS in Apocynaceae chemotaxonomy, including *M. tinctoria* [6]. Terpenoids and chlorophyll derivatives such as pheophorbide A, a common degradation product in green leaf extracts with photodynamic antibacterial potential, further validate the UHPLC-HRMS findings [13].

3.2. Network Pharmacology

The network pharmacology protocol consisted of six primary steps: (1) ADME analysis of phytochemical components of Aqueous Extract of *Marsdenia tinctoria* R.Br. Leaves; (2) Identification of probable biological targets of the phytoconstituents of AEML; (3) Identification of known targets related to *S. aureus*; (4) Determination of potential therapeutic targets of AEML by intersecting the protein targets of *S. aureus*; (5) Construction of protein–protein interaction networks; (6) Construction of a Compound–Target–Pathway Network.

3.2.1. Prediction of Oral Bioavailability Using the BOILED-Egg Model (SwissADME)

The analysis used the SwissADME platform and the BOILED-Egg method to assess the bioavailability of the compounds. Bioavailability is a critical parameter in pharmacokinetics, indicating the quantity and rate that a drug compound is absorbed by the body. Additionally, the SwissADME platform allows for the examination of other parameters such as compliance with Lipinski's rules, GI absorption, BBB permeability, and others.

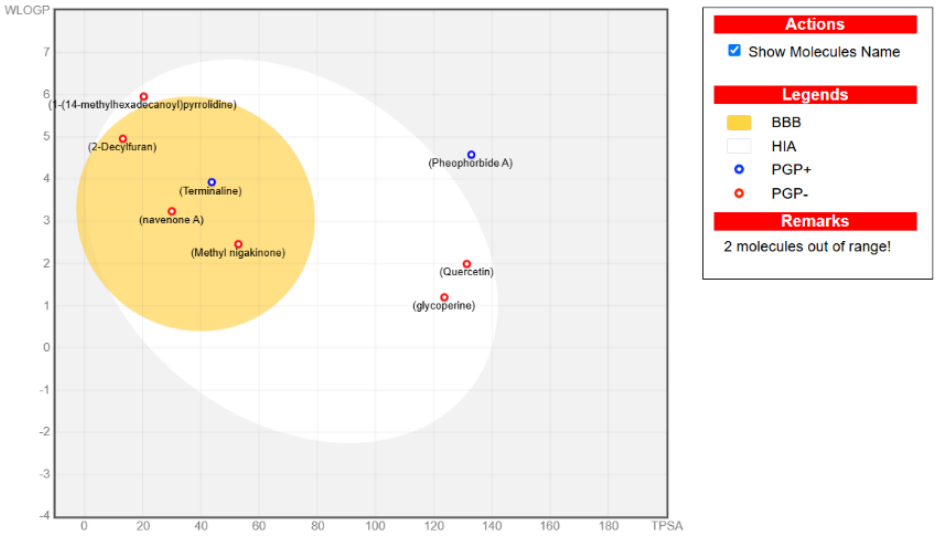


Figure 2. Visualization of predicted bioavailability and blood-brain barrier penetration of *Marsdenia tinctoria* secondary metabolite compounds using the BOILED-Egg method. BBB: Blood-Brain Barrier; HIA: Human Intestinal Absorption; PGP: P-glycoprotein Substrate.

Table 2. Prediction of bioavailability parameters using the BOILED-Egg method

Compound	GI Absorption	BBB	Lipinski of Rules
Glycoperine	High	No	Yes
1-(14-methylhexadecanoyl)pyrrolidine	High	No	Yes
Methyl nigakinone	High	Yes	Yes
Navenone A	High	Yes	Yes
Terminaline	High	Yes	Yes
Quercetin	High	No	Yes
Rutin	Low	No	No
2-Decylfuran	High	Yes	Yes
Echinenone	Low	No	Yes
Pheophorbide A	Low	No	Yes

Description:
BBB: Blood-Brain Barrier
GI Absorption: Gastrointestinal Absorption

The white area is most likely for passive absorption by the digestive tract, and the yellow area (yolk) is most likely for brain penetration. The yellow and white areas are not mutually exclusive. Additionally, The points will be colored blue if identified as P-gp substrates (PGP+) and red if identified as P-gp non-substrates (PGP−). Based on the analysis using the BOILED-Egg method, as illustrated in Table 2 and Figure 2, it was revealed that 3 out of 10 compounds fell outside the diagram and did not meet the specified parameters because they have poor pharmacokinetic profiles. These compounds are Rutin, Echinenone and Pheophorbide A. Rutin showed low GI absorption and fails Lipinski's rules due to its hydrophilic glycoside structure compared to quercetin, requiring enzymatic hydrolysis to quercetin aglycone for absorption but exhibiting poor passive diffusion through cell membranes [14]. Echinenone displayed low GI absorption due to its large lipophilic carotenoid scaffold limiting aqueous solubility and passive diffusion [15]. Pheophorbide A has low gastrointestinal absorption, has problems passing P-glycoprotein, and cannot penetrate the blood-brain barrier from its polar porphyrin structure, restricting oral bioavailability despite photodynamic potential [15], [16].

Therefore, only 7 compounds were passed on to the next stage because they had high GI absorption and followed Lipinski's Rule (MW <500 Da, optimal logP, H-bond ≤5/10), which meant that they could be absorbed well orally (>90%). Glycoperine and 1-(14-methylhexadecanoyl) pyrrolidine do not cross the BBB, which can be an advantage by reducing the risk of neurotoxicity while maintaining therapeutic plasma concentrations. Methyl nigakinone, Navenone A, Terminaline, and 2-Decylfuran can cross the BBB, potentially leading to systemic/CNS infections. High bioavailability ensures plasma concentrations exceed the MIC and reduces resistance thru multiple targets (membranes, biofilms) [15], [17].

3.2.2. Identification of *S. aureus* targets of AEML

A total of 555 protein *S. aureus* targets were obtained from the GeneCards database, 266 secondary metabolites of AEML were obtained from SwissTargetPrediction, and 51 intersecting targets between AEML and *S. aureus* were identified by merging the targets of phytochemical components with the targets of *S. aureus*. The convergence of targets was considered as the potential targets of AEML against *S. aureus*. (Figure 3).

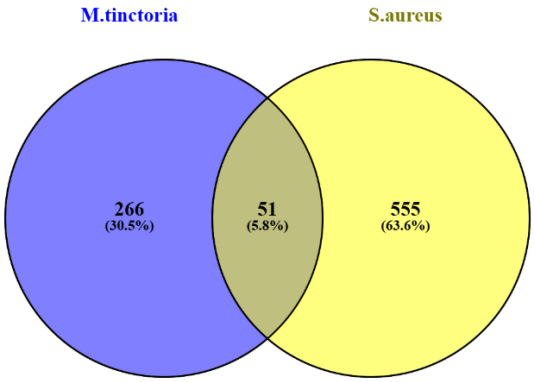


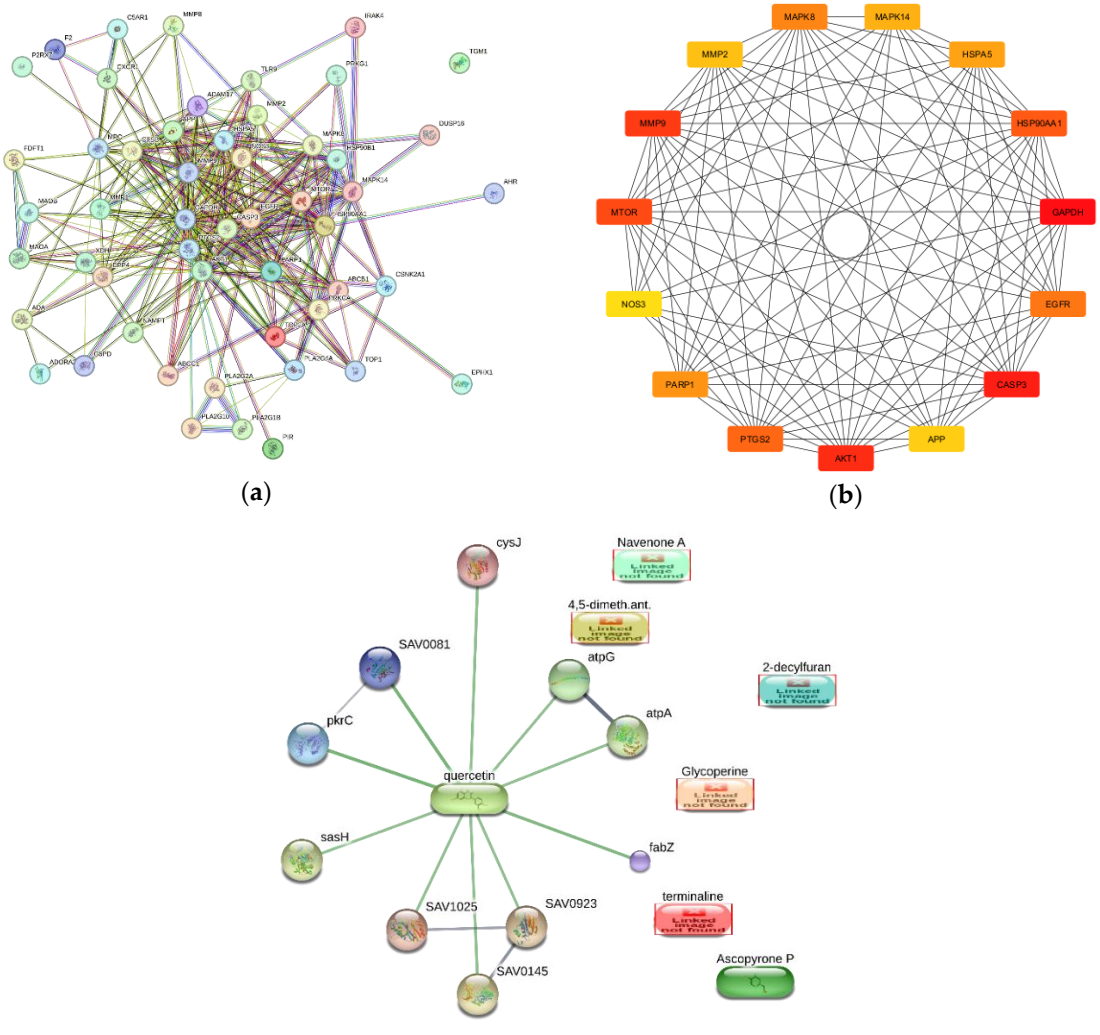
Figure 3. Venn diagram of the relationship between target compounds in *Marsdenia tinctoria* and target proteins in *Staphylococcus aureus* bacteria

3.2.3. Prediction of Protein-Protein Interaction

As can be seen in Figure 4a, 51 target proteins in *Staphylococcus aureus* that are predicted to interact with secondary metabolites compounds AEML were analyzed using String-DB. String-DB is a database with more than nine million proteins from various sources that can be used to predict interactions between proteins. This analysis was conducted to obtain selected target proteins with biological pathways related to these proteins. There are 52 nodes, 298 edges, and an expected number of edges of 120 for *S. aureus*. The number of nodes and edges obtained in the network illustrates the level of interaction activity. The resulting STRING network shows that the 52 proteins form a closely related functional group, not a random collection of proteins, because the number of observed

interactions (298 edges) is much greater than the number of interactions expected by chance (120 edges). This indicates that these proteins tend to be involved in the same biological pathways/processes [18], [19].

MCC analysis is assessed based on its centrality, which measures the extent to which a protein influences relationships within the MCC network. The aim of MCC is to identify components and target proteins that have better potential than previously collected proteins and are most likely to influence the antibacterial activity of AEML [20]. Next, the top 15 target proteins were determined based on MCC, which can be seen in Figure 4b. The 15 target proteins describe the targets of the antibacterial mechanism of action. They can be linked to antibacterial mechanisms of action through their roles in cellular immune pathways, inflammatory responses, and the inhibition of bacterial pathogens. These proteins are involved in processes such as bacterial glycolysis, phagocytosis, the production of antimicrobial radicals, and the regulation of immune signals that support bacterial clearance [21]–[26].



(**a**) (**b**)

(**c**)

Figure 4. Results figure of prediction of protein-protein interaction. (**a**) 51 protein-protein interaction networks are identified as potential targets of AEML in relation to *S. aureus*.; (**b**) Selection of 15 major protein targets using CytoHubba related to *S. aureus* based on the MCC approach; (**c**) Network of interactions between 15 major target proteins in *Staphylococcus aureus* and 7 (seven) compounds from *Marsdenia tinctoria* using the STITCH Platform Significant cluster of *S.aureus*-related targets

The results from CytoHubba are forwarded to STITCH. STITCH is used to determine how active compounds interact with target proteins. The following is an overview of the interaction network developed by STITCH between 7 active compounds in AEML and 15 target proteins in *S. aureus* (Figure 4c). Strong interactions are indicated by thick lines. The thicker the interaction line, the

stronger the relationship between the active compound and its target protein [27]. Green lines represent interactions between compounds and proteins. Meanwhile, gray lines represent interactions between proteins.

The STITCH analysis found that the interaction between 15 main target proteins in *S. aureus* and 7 active compounds in AEML results in the identification of quercetin as an active compound component. Quercetin interacts with several target proteins in S. aureus identified to have a line thickness or nodes exceeding 0.400, classified as high interactions, thereby playing an important role in antibacterial activity. Meanwhile, no interactions with target proteins were found for the other compounds.

The two target proteins with the highest interactions measured based on the thickness of the interaction line with quercetin were SAV0081 and pkrC in *S. aureus*. The antibacterial mechanism of pkrC or protein kinase is through the regulation of cell wall and virulence, where inhibition of phosphorylation can make bacteria more sensitive to β-lactam antibiotics [28]. Furthermore, SAV0081 shows similarity to serine/threonine kinases, which have autokinase activity on serine and threonine residues and are localized to the bacterial membrane. Its role is related to serine/threonine signal transduction, which influences cell wall metabolism and virulence [29].

Quercetin, a flavonoid compound, is known to have a broad spectrum of antibacterial activity, primarily by disrupting cell membrane integrity, damaging the cell wall, and inhibiting nucleic acid synthesis and enzymatic functions. This mechanism corresponds with the study's findings, wherein quercetin exhibited significant interactions with protein targets related to cell wall regulation, virulence, and antibiotic resistance [30].

3.3. In Vitro Antibacterial Activity

For the purpose of validation, in vitro antibacterial testing was performed on the AEML against *S. aureus* at four different concentrations using the agar well diffusion method. It is the most commonly used method for qualitative initial screening of antibacterial activity, and this study used it to confirm the presence or absence of *M. tinctoria* extract antibacterial activity. The agar diffusion method is a simple, fast and efficient method and thus is very suitable for studies that use antibacterial tests only as supporting data to validate the biological potential of a plant [31], [32]. The antimicrobial activity of AEML against *Staphylococcus aureus* can be seen from the presence of inhibition zones of various sizes around the agar well. The presence of AEML inhibition zones against *Staphylococcus aureus* bacteria can be seen in Table 3.

Table 3. Inhibition zones (mm ± S.E.M) of AEML against *S.aureus*

Plants	Concentration (%)	Inhibition zone (mm)
aqueous extract of M. tinctoria leaves	4	14.24 ±2.78
	6	16.01 ±0.95
	8	17.03 ±1.78
	10	14.90 ±0.62
Tetracycline HCl 0,5%		29.51 ±1.50

The result is our optimal concentration, showing its highest activity at 8%. In comparison, we evaluated tetracycline HCl 0.5%, a conventional antibiotic against *S. aureus*, which yielded an inhibitory zone of 29.51 ± 1.50 millimeters—1.7 times greater than our most effective plant extract result. The decrease of inhibition zone at concentration 10% compared to 8% can be caused by the limitation of agar diffusion method, where many factors other than concentration also affect the inhibition zone, therefore the relation between concentration and the area of inhibition zone is not always linear. If the concentration is too high, the extract solution viscosity will increase, which may inhibit the rate of the active compound diffusion into the agar matrix, so the diffusion range becomes more limited, and the measured inhibition zone becomes smaller [33], [34].

The experimental in vitro results confirm this prediction, as the extract reduced the growth of *S. aureus* with optimal efficacy, confirming that the active chemicals suggested by network pharmacology do elicit antibacterial activities against *S. aureus*.

3.4. Research limitations

The possible mechanism of bioactive compounds in AEML—Quercetin against *S. aureus* has been known in this study, that two pathways are targeted to control infection of *S. aureus.* However, this study still has limitations, the effectiveness, and the mechanism of Quercetin which have resulted from network pharmacology analysis, are still a predictions. Therefore, further studies are needed on other in vitro analyses such as SEM and TEM to help explain its mechanism of action at the cellular and cell wall level. The pharmacological effect of inhibiting the pathological process from *S. aureus* infection by the AEML compound also could be validated through in vivo analysis.

4. CONCLUSION

Protein-protein interaction (PPI) analysis indicated that fifteen key proteins interact with the seven compounds, with quercetin identified as the major bioactive compound activating specific bacterial pathways (SAV0081 and pkrC) in *S. aureus* with the prediction that the antibacterial mechanism occurs by the inhibition of cell wall synthesis. The highest antibacterial effect was observed at an 8% extract concentration with an inhibition zone of 17.03 ± 1.78 mm.

Funding: This research was funded by Lembaga Penelitian dan Pengabdian Masyarakat (LPPM) Unjani.
Acknowledgments: The authors would like to express sincere gratitude to the Faculty of Pharmacy, Universitas Jenderal Achmad Yani, for the support provided throughout this study
Conflicts of interest: The authors declare no conflict of interest.

References

[1] L. Klorida Br Barus, Masfria, and D. Satria, "Antibacterial activity of Kemloko fruit ethanol extract (Phyllanthus emblica L .) against Staphylococcus aureus and MRSA bacteria," *Int. J. Basic Clin. Pharmacol.*, vol. 13, no. 4, pp. 439–443, 2024.

[2] D. Made Dwi Widyastrini, E. Cahyaningsih, and I. G. Agung Ayu Kusuma, "Aktivitas Antibakteri Ekstrak Tanaman Obat Terhadap Methicillin-Resistant Staphylococcus aureus (MRSA) Antibacterial Activity of Medicinal Plants Extract against Methicillin-Resistant Staphylococcus aureus (MRSA)," *USADHA J. Integr. Obat Tradis.*, vol. 1, no. 1, pp. 30–37, 2021.

[3] B. P. F. de A. Gomes and D. R. Herrera, "Etiologic role of root canal infection in apical periodontitis and its relationship with clinical symptomatology," *Braz. Oral Res.*, vol. 32, no. suppl 1, pp. 82–110, Oct. 2018, doi: 10.1590/1807-3107bor-2018.vol32.0069.

[4] A. Nuryah, N. Yuniarti, and I. Puspitasari, "Prevalensi dan Evaluasi Kesesuaian Penggunaan Antibiotik pada Pasien dengan Infeksi Methicillin Resistant Staphylococcus Aureus di RSUP Dr . Soeradji Tirtonegoro Klaten Prevalence and Evaluation of Suitability of Antibiotic use in Patients with Methicilli," *Maj. Farm.*, vol. 15, no. 2, pp. 123–129, 2019, doi: 10.22146/farmaseutik.v15i2.47911.

[5] G. T. Kusumawardhana, "Risalah Tarum Areuy," *Varman Institute, Pusat Kajian Sunda*, 2020. https://varmaninstitute.com/2020/03/2-risalah-tarum-areuy/

[6] L. A. Barny *et al.*, "Chemotaxonomic investigation of Apocynaceae for retronecine-type pyrrolizidine alkaloids using HPLC-MS/MS," *Phytochemistry*, vol. 185, p. 112662, May 2021, doi: 10.1016/j.phytochem.2021.112662.

[7] N. Purnamaningsih, K. Hadibah, and S. Atun, "Uji Aktivitas Antibakteri Ekstrak Temulawak (Curcuma xanthorrhiza) Terhadap Bakteri Escherichia coli ATCC 11229 dan Staphylococcus aureus ATCC 25923," *J. Penelit. Saintek*, vol. 22, no. 2, pp. 140–147, 2017.

[8] R. B. Indradi, D. Ayu Eka Pitaloka, and Suryani, "Network pharmacology to uncover potential anti-inflammatory and immunomodulatory constituents in Curcuma longa rhizome as complementary treatment in COVID-19," *Pharmacia*, vol. 69, no. 4, pp. 995–1003, 2022, doi: 10.3897/pharmacia.69.e89799.

[9] K. Wei, "Prediction of Quercetin's Therapeutic Potential Against Pathogen-Related Diseases Based on Network Pharmacology," *Adv. Eng. Technol. Res.*, vol. 14, pp. 931–939, 2025.

[10] A. Khoirunnisa, A. Shobrun Jamil, and M. A. Muchlisin, "Analisis Network Pharmacology Senyawa Metabolit Sekunder Tanaman Ciplukan (Physalis angulata L .) Pada Penyakit Kanker Paru-Paru," *Maj.*

Farm., vol. 20, no. 2, pp. 282–291, 2024, doi: 10.22146/farmaseutik.v20i2.96275.

[11] A. Choirunnisa and A. B. Sutjiatmo, "Pengaruh kombinasi ekstrak etanol herba cecendet (Physalis angulata l.) dengan beberapa antibiotik terhadap bakteri Staphylococcus aureus dan Klebsiella pneumonie," *Kartika J. Ilm. Farm.*, vol. 5, no. 2, p. 50, 2017, doi: 10.26874/kjif.v5i2.114.

[12] N. A. Mohd Nasuha and Y. M. Choo, "A new flavone from Malaysia Borneo Marsdenia tinctoria," *Nat. Prod. Res.*, vol. 30, no. 13, pp. 1532–1536, 2016, doi: 10.1080/14786419.2015.1118629.

[13] T. Fauziah and O. Suryani, "Pheophorbide as Natural Pigment and Applications," *J. Pendidik. Tambusai*, vol. 8, no. 2, pp. 30104–30111, 2024.

[14] C. Yang *et al.*, "Bioavailability and metabolic pharmacokinetics of rutin and quercetin in rats Bioavailability and Metabolic Pharmacokinetics of Rutin and Quercetin in Rats," *J. Food Drug Anal.*, vol. 13, no. 3, pp. 244–250, 2005.

[15] A. Daina, O. Michielin, and V. Zoete, "SwissADME : a free web tool to evaluate pharmacokinetics , drug-likeness and medicinal chemistry friendliness of small molecules," *Nat. Publ. Gr.*, vol. 7, pp. 1–13, 2017, doi: 10.1038/srep42717.

[16] S. Paul, A. Pallavi, and N. R. Gandasi, "Exploring the potential of pheophorbide A , a chlorophyll-derived compound in modulating GLUT for maintaining glucose homeostasis," *Front. Endocrinol. (Lausanne).*, no. March, pp. 1–13, 2024, doi: 10.3389/fendo.2024.1330058.

[17] V. Ivanović, M. Rančić, B. Arsić, and A. Pavlović, "Lipinski ' s rule of five , famous extensions and famous exceptions Lipinski rule of five," *Chem. Naissensis*, vol. 3, no. 1, pp. 171–177, 2020.

[18] D. Szklarczyk *et al.*, "STRING v11 : protein – protein association networks with increased coverage , supporting functional discovery in genome-wide experimental datasets," *Nucleic Acids Res.*, vol. 47, no. November 2018, pp. 607–613, 2019, doi: 10.1093/nar/gky1131.

[19] D. Szklarczyk *et al.*, "The STRING database in 2023 : protein – protein association networks and functional enrichment analyses for any sequenced genome of interest," *Nucleic Acids Res.*, vol. 51, no. November 2022, pp. 638–646, 2023.

[20] D. Hanif, M. Fath, M. A. Muchlisin, and A. S. Jamil, "Analisis Network Pharmacology Senyawa Metabolit Sekunder Tanaman Lengkuas (Alpinia galanga) pada Penyakit Kanker Network Pharmacology Analysis of Secondary Metabolite Compounds of Galangal Plant (Alpinia galanga) in Cancer Disease," vol. 9, no. 1, 2024, doi: 10.18860/jip.v9i1.27094.

[21] V. Winstel, O. Schneewind, and Dominique Missiakas, "Staphylococcus aureus Exploits the Host Apoptotic Pathway To Persist during Infection," *MBio*, vol. 10, no. 6, pp. 1–12, 2019.

[22] J. Purves, A. Cockayne, P. C. E. Moody, and J. A. Morrissey, "Comparison of the Regulation , Metabolic Functions , and Roles in Virulence of the Glyceraldehyde-3-Phosphate Dehydrogenase Homologues gapA and gapB in Staphylococcus aureus ᴑ," *Infect. IMMUNITY*, vol. 78, no. 12, pp. 5223–5232, 2010, doi: 10.1128/IAI.00762-10.

[23] C. J. Genito *et al.*, "mTOR signaling is required for phagocyte free radical production, GLUT1 expression, and control of Staphylococcus aureus infection," *MBio*, vol. 15, no. 6, 2024.

[24] J. Wang, Y. Huang, X. Wu, and Dongqing Li, "MicroRNA-939 ampli fi es Staphylococcus aureus-induced matrix metalloproteinase expression in atopic dermatitis," *Front. Immunol.*, vol. 15, pp. 1–10, 2024, doi: 10.3389/fimmu.2024.1354154.

[25] F. Xu *et al.*, "Akt1-Mediated Regulation of Macrophage Polarization in a Murine Model of Staphylococcus aureus Pulmonary Infection," *J. Infect. Dis.*, vol. 208, no. 3, pp. 528–538, 2013, doi: 10.1093/infdis/jit177.

[26] D. O. Schairer, J. S. Chouake, J. D. Nosanchuk, and A. J. Friedman, "The potential of nitric oxide releasing therapies as antimicrobial agents," *Virulence*, vol. 3, no. 3, pp. 271–279, 2012.

[27] M. R. Duhita, R. Susilowati, S. Q. Aini, and R. Annisa, "Synergistic Potential Of Nigella sativa L . and Trigonella foenum-graecum : Integrated Network Pharmacology for Diabetic Wound Healing," *Int. J. Appl. Pharm.*, vol. 16, no. 6, pp. 345–351, 2024.

[28] W. Zheng, X. Cai, S. Li, Z. Li, and E. Giraud, "Autophosphorylation Mechanism of the Ser / Thr Kinase Stk1 From Staphylococcus aureus," *Front. Microbiol.*, vol. 9, no. April, pp. 1–10, 2018, doi: 10.3389/fmicb.2018.00758.

[29] M. De, S. Dramsi, O. Dussurget, M. Nahori, E. Vaganay, and A. Cozzone, "Characterization of a Serine / Threonine Kinase Involved in Virulence of Staphylococcus aureus ᴑ," *J. Bacteriol.*, vol. 191, no. 13, pp. 4070–4081, 2009, doi: 10.1128/JB.01813-08.

[30] W. Qi, W. Qi, D. Xiong, and M. Long, "Quercetin : Its Antioxidant Mechanism, Antibacterial Properties and Potential Application in Prevention and Control of Toxipathy," *Molecules*, vol. 27, p. 6545, 2022.

[31] M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: A review," *J. Pharm. Anal.*, vol. 6, no. 2, pp. 71–79, 2016, doi: 10.1016/j.jpha.2015.11.005.

[32] B. Kowalska-Krochmal and R. Dudek-Wicher, "The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance," *Pathogens*, vol. 10, no. 2, p. 165, Feb. 2021, doi: 10.3390/pathogens10020165.

[33] J. N. Eloff, "Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results," *BMC Complement. Altern. Med.*, vol. 3, pp. 1–8, 2019.

[34] L. Hossain, L. Y. Lim, K. Hammer, D. Hettiarachchi, and C. Locher, "A Review of Commonly Used Methodologies for Assessing the Antibacterial Activity of Honey and Honey Products," *Antibiotics*, vol. 11, 2022, doi: https://doi.org/10.3390/antibiotics11070975.



© 2026 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).