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Antibacterial Activity of *Planchonia valida* Against Methicillin-Resistant *Staphylococcus aureus* (MRSA): Phytochemical Profiling, LC-MS Analysis, and Molecular Docking Study

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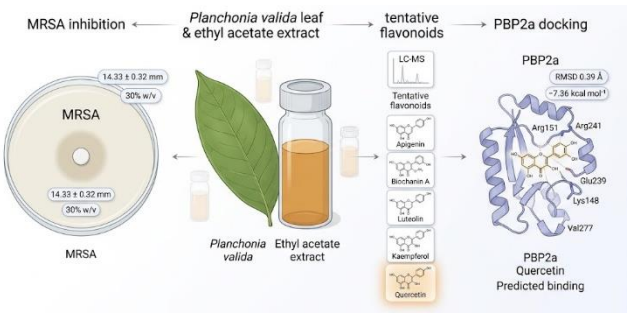
ABSTRACT



Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major antimicrobial-resistant pathogen, creating a need for alternative antibacterial agents from natural resources. This study aimed to evaluate the antibacterial activity of *Planchonia valida* leaf extracts against MRSA and investigate potential bioactive constituents associated with the observed activity through phytochemical profiling, LC–MS analysis, and molecular docking. Leaves of *P. valida* were extracted using n-hexane, ethyl acetate, and methanol, and antibacterial activity was evaluated using the disk diffusion method. The most active extract was further characterized through phytochemical screening and LC–MS analysis, while tentatively annotated metabolites were evaluated by molecular docking against penicillin-binding protein 2a (PBP2a). The ethyl acetate extract exhibited the strongest antibacterial activity, producing an inhibition zone of 14.33 ± 0.32 mm at 30% (w/v). Phytochemical screening indicated the presence of terpenoids, steroids, alkaloids, and flavonoids, with flavonoids showing the strongest qualitative response. LC–MS analysis tentatively annotated five flavonoid-related compounds, including apigenin, biochanin A, luteolin, kaempferol, and quercetin. Docking validation produced an RMSD value of 0.39 Å, and quercetin exhibited the most favorable predicted binding energy among the evaluated compounds (−7.36 kcal mol^{-1}). These findings suggest that *P. valida* leaf extract possesses antibacterial potential against MRSA, with flavonoid-related metabolites as promising candidates for further investigation.

Keywords: *Planchonia valida; MRSA; antibacterial activity; LC-MS; molecular docking.*

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is widely regarded as a pathogen of major clinical significance among multidrug-resistant organisms encountered worldwide [1],[2]. The increasing prevalence of MRSA infections has become a major public health concern due to limited therapeutic options, prolonged hospitalization, increased healthcare costs, and higher mortality rates. The remarkable ability of MRSA to develop resistance against multiple antibiotics has diminished the efficacy of conventional treatments [3]. It highlights the urgent need to discover new

antibacterial agents with alternative mechanisms of action.

The role of natural products in drug discovery has been well established over time, and they continue to represent a key resource for antimicrobial compound identification [4]-[6]. Plant-derived secondary metabolites, including flavonoids, phenolic acids, alkaloids, tannins, and terpenoids, have demonstrated significant antibacterial activities through diverse mechanisms [7]-[9], including damage to the bacterial cell membrane, suppression of cell wall formation, disturbance of nucleic acid production, and alteration of virulence-associated factors. Consequently, medicinal plants continue to attract considerable attention as promising reservoirs of novel bioactive compounds for combating antimicrobial resistance.

Planchonia valida (Blume) (Lecythidaceae), commonly known as putat, is a tropical medicinal plant widely distributed in wetland and swamp ecosystems of Indonesia [10],[11]. Previous phytochemical studies on *P. valida* have reported the presence of various bioactive secondary metabolites, including flavonoids, phenolic compounds, tannins, triterpenoids, and other polyphenolic constituents. These compounds are well known for their broad spectrum of biological activities [10]-[14]. Among these metabolites, flavonoids have attracted considerable attention because-of their ability to inhibit bacterial growth through multiple targets and mechanisms [15]-[18].

Recent advances in analytical chemistry and computational biology have provided powerful tools for accelerating natural product-based drug discovery [19],[20]. Liquid chromatography–mass spectrometry (LC-MS) enables rapid and sensitive characterization of complex phytochemical mixtures, facilitating the annotation of compounds that may be associated with biological activity [21],[22]. In parallel, molecular docking has emerged as a valuable computational approach for predicting the binding affinity and interaction patterns of phytochemicals with specific bacterial targets, thereby providing mechanistic insights that complement experimental findings [23]. The integration of in vitro antibacterial evaluation with LC-MS-based metabolite annotation and molecular docking analysis offers a preliminary strategy for identifying candidate bioactive compounds and generating hypotheses regarding their potential mechanisms of action [24].

Previous studies have reported the antibacterial activity of ethanol extracts of *P. valida* against the Gram-negative *bacterium Yersinia enterocolitica* [25]. However, the antibacterial potential of *P. valida* against MRSA has not been investigated. Furthermore, no study has yet integrated an antibacterial bioassay, phytochemical screening, LC-MS-based metabolite annotation, and molecular docking analysis targeting the MRSA resistance protein PBP2a in *P. valida* extracts. Therefore, the present study aimed to evaluate the antibacterial activity of *P. valida* leaf extracts against MRSA, characterize the phytochemical constituents of the most active extract, tentatively annotate its flavonoid metabolites by LC-MS, and explore their predicted interactions with PBP2a through molecular docking, providing an initial, integrated dataset intended

as a preliminary basis for future confirmatory and mechanistic investigation.

METHODS

1. Materials

Fresh leaves of *P. valida* were collected from Sungai Sariak, Padang Pariaman, West Sumatra, Indonesia (0°35′16.8″ S, 100°13′15.6″ E) in January 2026. Methanol, ethyl acetate, and n-hexane (technical grade) were used as extraction solvents. As technical-grade solvents were used, each solvent was redistilled before use to remove non-volatile residues. Dimethyl sulfoxide (DMSO, Merck, Darmstadt, Germany) was used for sample preparation. A rotary evaporator was used to remove any remaining solvent under reduced pressure. MRSA, obtained from the Laboratory of Microbiology, Dr. M. Djamil General Hospital, Padang, Indonesia, was used for antibacterial testing. Mueller–Hinton Agar (MHA; HiMedia Laboratories Pvt. Ltd., India) was used for antibacterial assays. Vancomycin (30 µg/disc, Oxoid, UK) was used as the positive control.

Phytochemical screening reagents included Dragendorff's reagent for alkaloids, magnesium powder and concentrated hydrochloric acid for flavonoids, Liebermann–Burchard reagent for steroids and terpenoids, and other analytical-grade chemicals. LC-MS measurements were carried out on a Shimadzu LC system coupled to a triple quadrupole mass spectrometer, using a Shim-Pack FC-ODS column (2.0 × 150 mm, 3 µm) for chromatographic separation. Methanol, acetonitrile, formic acid, and water used for LC–MS analysis were of LC–MS grade (Merck, Darmstadt, Germany). Molecular docking studies were conducted using the tentatively annotated flavonoid metabolites identified by LC–MS and the MRSA target protein obtained from the Protein Data Bank (PDB).

2. Procedures

a. Sample Preparation and Extraction

Fresh leaves of *P. valida* were thoroughly washed, cut into smaller segments, and oven-dried at 40 °C until a constant weight was achieved. Constant weight was defined as a difference of less than 0.1 g between two consecutive weighings performed. The dried material was subsequently pulverized into a fine powder before extraction. Three independent aliquots of 50 g powdered sample were each subjected separately to maceration using *n*-hexane, ethyl acetate, or methanol, at a fixed sample-to-solvent ratio of 1:10 (500 mL of solvent per cycle). Each extraction step was carried out for 24 h at room temperature with periodic stirring every 12 h. To ensure exhaustive recovery, the process was repeated for an exact total of three extraction cycles per solvent fraction. The combined filtrates from each respective solvent were concentrated under reduced pressure via rotary evaporation at 50 °C. This yielded crude extracts with final masses of 2.7 g for *n*-hexane (5.4% yield), 3.3 g for ethyl acetate (6.6% yield), and 2.1 g for methanol (4.2% yield).

b. Antibacterial Activity of Crude Extracts

The antibacterial activity of three crude extracts against MRSA was evaluated using a modified disk diffusion assay [26],[27]. A bacterial suspension of MRSA was prepared by picking isolated colonies from an 18–24 h culture on Mueller–Hinton Agar and

suspending them in sterile 0.85% saline. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard (equivalent to approximately 1.5 × 108 CFU/mL), verified using a spectrophotometer at 625 nm. The standardized suspension was used for inoculation within 15 minutes of preparation to prevent changes in cell density. The suspension was then uniformly spread onto Mueller–Hinton Agar (MHA) plates using a sterile cotton swab (spread plate technique), and plates were allowed to dry for 5 minutes before disk placement. Five sterile paper disks were then placed on the surface of the inoculated agar. Sterile blank paper disks (6 mm) were placed on the surface of Mueller–Hinton Agar plates (100 mm) poured to a uniform agar depth of 4 mm, positioned at least 24 mm apart (center-to-center) and at least 15 mm from the plate edge to minimize overlapping diffusion zones.

The first disk was impregnated with vancomycin (30 µg/disc) and served as the positive control, while the second disk received 10% dimethyl sulfoxide (DMSO) as the negative control. The remaining three disks were loaded with the test samples (10%, 20%, and 30% (%w/v)) dissolved in 10% DMSO. Given a disk-loading volume of 10 µL, the 10%, 20%, and 30% (w/v) extract solutions correspond to actual crude extract masses of 1000 µg, 2000 µg, and 3000 µg per disk, respectively. Disks were allowed to air-dry for 5 minutes under aseptic conditions before incubation. The plates were subsequently incubated at 37 °C for 24 h. Antibacterial activity was determined by the formation of a clear inhibition zone surrounding the disks. Inhibition zone diameters, including the disk diameter, were measured using a digital caliper. Each zone was measured along two orthogonal diameters, and the average of the two readings was recorded as the zone diameter for that replicate. All assays were performed in three technical replicates derived from the same bacterial inoculum and extract batch, plated in parallel on the same day. Inhibition zone diameters were expressed as mean ± standard deviation (SD) of these three technical replicates.

c. Phytochemical Screening

The most active extract underwent preliminary phytochemical screening to identify the major classes of secondary metabolites, including terpenoids, steroids, alkaloids, and flavonoids, using previously reported methods [28]–[30]. All tests were performed in triplicate (n = 3). Solvent alone (without extract) served as the negative control for each test.

Terpenoids: The extract solution (0.4 mg/mL) was mixed with chloroform (0.5 mL) and concentrated sulfuric acid (H2SO4, 0.5 mL), added dropwise along the tube wall. The mixture was allowed to stand for 3 minutes at room temperature. The appearance of a reddish-brown color at the interface indicated the presence of terpenoids.

Steroids: The extract solution (0.4 mg/mL) was treated with chloroform (0.5 mL), acetic anhydride (1.0 mL), and concentrated H2SO4 (0.5 mL), added carefully along the tube wall, and observed after 3 minutes. The

development of a green color indicated the presence of steroids.

Alkaloids: The upper (chloroform) layer obtained from the steroid test was divided into three portions and treated separately with Dragendorff's, Mayer's, and Wagner's reagents (3 drops each). The formation of a brown precipitate (Dragendorff's), a white/cream precipitate (Mayer's), or an orange-brown precipitate (Wagner's) within 3 minutes indicated the presence of alkaloids.

Flavonoids: The extract (0.4 mg/mL) was dissolved in methanol (1 mL), followed by the addition of magnesium powder and concentrated hydrochloric acid (HCl, 3 drops). The development of a red or purple color within 3 minutes indicated the presence of flavonoids.

Each test was scored as (–) no color/precipitate change observed (negative), (+) a faint color/precipitate change observed (positive), or (++) a distinct, more intensely colored reaction observed consistently across all three replicates (strong positive).

d. LC-MS Analysis

10 mg of the dried sample was dissolved in 1 mL of HPLC-grade 80% methanol, vortexed for 2 min, filtered through a 0.20 µm nylon syringe filter, and transferred into an HPLC vial. LC-MS analysis was performed using a triple quadrupole mass spectrometer (Shimadzu) coupled with an LC system, equipped with a Shim-Pack FC-ODS column (2.0 × 150 mm, 3 µm). The column temperature was maintained at 40 oC. The mobile phase consisted of methanol, acetonitrile, and water (40:15:45, v/v), with 0.1% formic acid added to all three components to improve peak shape and retention reproducibility, delivered at a flow rate of 0.5 mL/min. The injection volume was set at 10 µL. Elution was carried out over 60 min under isocratic conditions.

An electrospray ionization (ESI) source operated in positive ion mode was employed for mass spectrometric detection, under the conditions described below: nebulizing gas flow 3.0 L/min, drying gas flow 10.0 L/min, desolvation line temperature 250 0C, and heat block temperature 400 0C. Full-scan spectra were acquired over an m/z range of 67–1000. Data acquisition and processing were performed using LabSolutions software (Shimadzu). Compound annotation was based on comparison of retention time and accurate precursor ion m/z with database records. Diagnostic MS/MS fragmentation data were not acquired in this analysis, as the method employed only full-scan acquisition.

e. *In Silico* Study

Docking simulations were performed using AutoDock 4.2.5.1 and AutoGrid 4.2.5, following the previously established protocol [31]. The three-dimensional X-ray crystallographic structure of Penicillin-Binding Protein 2a (PBP2a) from MRSA (PDB ID: 4CJN) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org). The structure contains two protein chains (A and B) and several non-protein entities. The co-crystallized beta-muramic acid (PDB ligand code: MUR) was used as the native ligand for redocking-based validation. Chain B was selected for docking based on preliminary redocking trials, which yielded a

lower RMSD than chain A. No missing atoms or alternate conformations were present in chain B within the modeled binding region. The receptor and native ligand were extracted from the PBP2a chain B structure using BIOVIA Discovery Studio and saved in PDB format. Crystallographic water molecules were removed because none were identified as directly mediating ligand binding at the allosteric site. The cadmium ion and chloride ions, which were identified as crystallization additives rather than catalytically or structurally essential cofactors, were also removed. Receptor preparation was performed using AutoDockTools 1.5.7 by adding polar hydrogens and assigning Kollman charges.

The docking grid was generated using AutoGrid4 with dimensions of 30 × 30 × 30 grid points and a spacing of 0.375 Å, corresponding to physical grid dimensions of 11.25 × 11.25 × 11.25 Å. The grid box was centered on chain B at the coordinates X = −1.759, Y = −7.470, and Z = −58.114, and was visually inspected to ensure that it covered the entire allosteric binding pocket. For electrostatic calculations, the default distance-dependent dielectric function implemented in AutoDock4 was used. Docking was performed using the Lamarckian Genetic Algorithm (LGA) with a population size of 150, a maximum of 2,500,000 energy evaluations, and a maximum of 27,000 generations. The mutation and crossover rates were set at 0.02 and 0.80, respectively. Each ligand was subjected to 100 GA-LS docking runs using the built-in seed generation method in AutoDock (seed pid time), resulting in two random seed values of 4036 and 1769408307 for the reported redocking run. The resulting conformations were clustered based on ligand heavy-atom RMSD using a tolerance of 2.0 Å. The best pose for each ligand was defined as the most energetically favorable conformation within the most populated cluster [32].

Following validation, five flavonoid derivatives tentatively annotated by LC–MS analysis apigenin (1), biochanin A (2), luteolin (3), kaempferol (4), and quercetin (5) were selected as docking ligands. The three-dimensional structures of the five annotated compounds were energy-minimized using the Merck Molecular Force Field (MMFF94) in Avogadro and subsequently processed in AutoDockTools 1.5.7. Gasteiger partial charges were assigned, non-polar hydrogens were merged, polar hydrogens were retained, rotatable bonds were defined based on each ligand's torsion tree, and protonation states were assigned according to the default settings appropriate for physiological pH (~7.4) before conversion to PDBQT format. Docking was then performed against the same allosteric site of chain B using the identical grid and LGA parameters established during redocking validation. Molecular interactions between each flavonoid ligand and PBP2a were subsequently analyzed using BIOVIA Discovery Studio, including hydrogen bonds, hydrophobic contacts, π-based interactions, and other non-covalent interactions that contribute to ligand stabilization within the allosteric pocket.

RESULTS AND DISCUSSION

1. Antibacterial Assay of Crude Extracts

The antibacterial activity of *P. valida* leaf extracts against MRSA was evaluated using the disk diffusion method, and the results (Table 1) demonstrated varying degrees of inhibitory activity depending on both the extraction solvent and extract concentration. DMSO at a concentration of 10% was used as the solvent system based on its ability to solubilize the crude extracts while remaining compatible with bacterial viability at the volume applied per disk (10 µL). The absence of an inhibition zone around the DMSO-only negative control disk confirmed that DMSO at this concentration did not exert a detectable antibacterial effect under the tested conditions. It should be noted, however, that this control does not rule out other potential contributing factors, such as residual extraction solvent, contaminants, or plate-position and inoculum variability. Extraction blanks were not included in the present study, and we recommend their inclusion in future work to more rigorously exclude solvent-derived effects and further substantiate that the observed antibacterial activity originates specifically from the plant-derived constituents of the extracts.

In general, a concentration-dependent increase in antibacterial activity was observed for all extracts. The n-hexane extract at 10% concentration (w/v) did not exhibit any inhibitory effect, whereas higher concentrations (20% and 30%, w/v) produced measurable inhibition zones. Similarly, both the ethyl acetate and methanol extracts showed progressively larger inhibition zones as the concentration increased from 10% to 30% (w/v).

Table 1. Inhibition zone diameters (mm) of crude extracts against MRSA at different concentrations, compared with positive and negative controls

Sample	Concentration/Dose	Mean Inhibition Zone (mm) ± SD	n
Vancomycin (positive control)	30 µg/disk	15.47 ± 0.31	3
DMSO (negative control)	10 µL	0	3
n-hexane extract	10% *w/v* (1 mg/disk)	0	3
	20% *w/v* (2 mg/disk)	7.30 ± 0.44	3
	30% *w/v* (3 mg/disk)	10.33 ± 0.15	3
Ethyl acetate extract	10% *w/v* (1 mg/disk)	7.70 ± 0.17	3
	20% *w/v* (2 mg/disk)	13.13 ± 0.06	3
	30% *w/v* (3 mg/disk)	14.33 ± 0.32	3
Methanol extract	10% *w/v* (1 mg/disk)	5.43 ± 0.21	3
	20% *w/v* (2 mg/disk)	7.67 ± 0.32	3
	30% *w/v* (3 mg/disk)	10.60 ± 0.26	3

Among the tested extracts, the ethyl acetate extract exhibited the strongest antibacterial activity against MRSA. At a concentration of 30% (3 mg/disk), the extract produced an inhibition zone of 14.33 ± 0.32 mm. For reference, vancomycin (30 µg/disk), used as the positive control, produced an inhibition zone of 15.47 ± 0.31 mm; given the approximately 100-fold difference in mass loaded per disk between the two, this value is reported for reference only and does not imply comparable antibacterial potency. The

methanol and *n*-hexane extract concentrations of 30% generated inhibition zones of 10.60 ± 0.26 mm and 10.33 ± 0.15 mm, respectively. The larger inhibition zone observed for the ethyl acetate extract may reflect differences in extraction yield, the relative concentration of antibacterially active constituents, or their solubility and diffusion behavior in agar. Minimum Inhibitory Concentration (MIC) determination normalized to extract mass would be required to conclude comparative extraction or potency efficiency across solvents. In addition, the consistently low SD values observed across treatments reflect measurement precision under the tested conditions; however, given the limited sample size (n = 3), these values should not be interpreted as a general demonstration of reproducibility, and further replication would be needed to more robustly establish the consistency of this effect.

According to established interpretive criteria [33], inhibition zones greater than 12 mm were categorized as very strong, 8–12 mm as strong, 4–8 mm as moderate, and less than 4 mm as weak. Based on these results, the ethyl acetate extract at 20% and 30% concentrations displayed very strong antibacterial activity against MRSA, whereas the methanol and *n*-hexane extracts exhibited moderate activity at comparable concentrations. The pronounced antibacterial effect observed for the ethyl acetate extract highlights its potential as a promising source of anti-MRSA compounds. Given its superior performance compared with the other extracts, the ethyl acetate extract was selected for further chemical characterization to explore compounds potentially associated with its antibacterial activity.

2. Phytochemical Screening and Profiling of the Most Active Extract

Phytochemical screening of the ethyl acetate extract, which exhibited the highest antibacterial activity against MRSA, revealed the presence of terpenoids, steroids, and alkaloids, while flavonoids produced a stronger qualitative color reaction relative to the other metabolite classes tested (Table 2). It should be noted that this scoring (+, ++) was based solely on the visual intensity of the color reaction observed during qualitative phytochemical testing and does not represent a quantitatively validated measure of metabolite concentration. The occurrence of these secondary metabolites may contribute to the antibacterial activity observed in the disk diffusion assay. Previous studies have demonstrated that terpenoids, steroids, alkaloids, and flavonoids possess antibacterial properties through various mechanisms, including disruption of bacterial membrane integrity, interference with metabolic processes, and inhibition of essential cellular functions [7],[8].

Among the detected metabolites, flavonoids produced the most intense qualitative color reaction in the ethyl acetate extract during phytochemical screening. Flavonoids are widely recognized for their broad-spectrum antibacterial activity and have been reported to be effective against both susceptible and drug-resistant strains of S. aureus. Their antibacterial effects are commonly associated with membrane

disruption, inhibition of nucleic acid synthesis, enzyme inactivation, and interference with cell wall formation [15],[16]. The relatively high abundance of flavonoids in the most active extract, together with its strong inhibitory effect against MRSA, suggests that this class of compounds may play an important role in the antibacterial activity of *P. valida*. Therefore, the ethyl acetate extract was selected for further LC-MS analysis to tentatively annotate its flavonoid compounds and to provide preliminary insight into compounds potentially associated with its antibacterial activity against MRSA.

Table 2. Phytochemical screening of the ethyl acetate extract of *P. valida*

Secondary Metabolites	Results*
Terpenoids	+
Steroids	+
Alkaloids	+
Flavonoids	++

* (+) faint color/precipitate reaction observed; (++) distinct, more intensely colored reaction observed consistently across all three replicates (n = 3). No metabolite class tested negative under the conditions used.

LC-MS profiling of the ethyl acetate extract revealed a complex chemical composition characterized by multiple chromatographic peaks, indicating the presence of diverse secondary metabolites. Based on retention time and accurate precursor ion *m/z* comparison against reference database records, five flavonoid derivatives were tentatively annotated and selected for further molecular docking analysis: apigenin (**1**), biochanin A (**2**), luteolin (**3**), kaempferol (**4**), and quercetin (**5**) (Table 3 and Figure 1). Because the LC-MS method employed in this study used only full-scan acquisition, diagnostic MS/MS fragmentation data were not obtained. These annotations are therefore based solely on accurate mass and retention time comparison. They should be regarded as tentative candidates, pending confirmation by MS/MS fragmentation in future work. Notably, luteolin (**3**) and kaempferol (**4**) share the same molecular formula ($C_{15}H_{10}O_6$) and exact mass, and therefore could not be unambiguously distinguished based on precursor ion mass using the LC-MS method employed in this study. Both are reported as tentatively present in the extract pending further confirmation.

The selection of these tentatively annotated compounds was guided by several criteria: (i) consistency with the classes of secondary metabolites detected in phytochemical screening; (ii) a library match score above 95%; (iii) the presence of a clearly resolved, well-defined molecular structure; and (iv) exclusion of peaks associated with early elution times, known analytical artifacts, and siloxane contaminants. This systematic selection strategy minimized the inclusion of ambiguous signals and ensured that only chemically plausible metabolites, supported by consistent chromatographic and spectral evidence, were subjected to subsequent computational analysis.

Table 3. Tentatively annotated five flavonoid-related metabolites from the ethyl acetate extract of *P. valida* based on LC-MS

RT (min)	Compound	Formula	Exact Mass	Source Match Score (
9.365	Apigenin (**1**)	$C_{15}H_{10}O_5$	270.0528	95
10.011	Biochanin A (**2**)	$C_{16}H_{12}O_5$	284.0685	96
10.265	Luteolin (**3**)	$C_{15}H_{10}O_6$	286.0477	95
10.322	Kaempferol (**4**)	$C_{15}H_{10}O_6$	286.0477	95
11.427	Quercetin (**5**)	$C_{15}H_{10}O_7$	302.0427	97

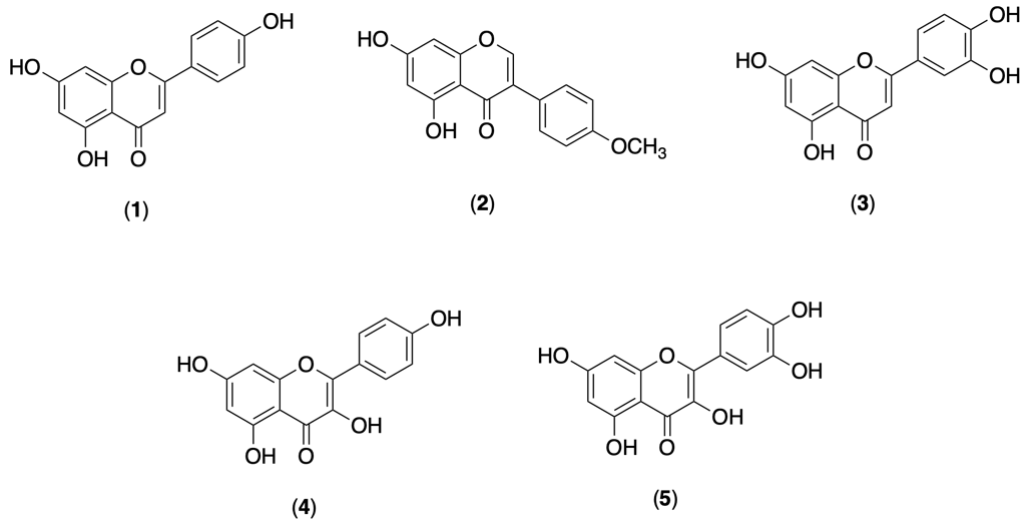

Figure 1. Structures of tentatively annotated five flavonoid-related metabolites in the ethyl acetate extract of *P. valida*

The identification of these flavonoids is particularly noteworthy because flavonoids represent one of the most extensively studied classes of plant-derived antibacterial agents. Their tentative annotation in the ethyl acetate extract is consistent with the phytochemical screening results, which indicated a stronger qualitative color reaction for flavonoids relative to the other metabolite classes tested. Previous studies have reported antibacterial activity of flavonoids against a range of Gram-positive pathogens, including MRSA, attributed to multiple proposed mechanisms such as disruption of cytoplasmic membrane integrity, inhibition of nucleic acid synthesis, interference with energy metabolism, and modulation of proteins involved in bacterial survival and virulence [7],[8].

The detection of several structurally distinct, tentatively annotated flavonoids in the ethyl acetate extract suggests that the observed antibacterial activity of the crude extract may be associated with the combined presence of these compound classes; however, it has not been established in this study that these specific compounds are the active constituents, nor whether their combined effect is additive, synergistic, antagonistic, or unrelated. Dedicated combination studies using isolated compounds would be required to characterize any interactive effects and to confirm their individual or combined

contribution to the antibacterial activity observed.

3. *In Silico* Evaluation

In this study, molecular docking was employed to predict and characterize the binding energy and interaction modes of five compounds tentatively annotated from *P. valida* leaf extract within the selected PBP2a binding site. Molecular docking is a computational approach used to predict the interaction between a protein and a ligand, providing insight into their probable binding modes and relative affinities [31]. This approach has been widely applied in drug discovery and molecular interaction studies. Docking simulation outputs are commonly assessed using the Docking Score (DS), where lower (more negative) values indicate a stronger predicted binding affinity between the ligand and the target [34]. Beyond scoring, docking outputs are further examined in terms of ligand–protein interactions, including through hydrogen bonding, π-cation, π-alkyl, and π-sigma interactions, which may contribute to stabilizing the predicted ligand orientation within the binding pocket.

To validate the docking protocol before screening the target ligands, a redocking procedure between the native ligand and receptor was performed as the initial step of the simulation. This redocking procedure was executed utilizing a grid box with dimensions of 30 x 30 x 30 Å and centered at the coordinates -1.759, -7.47, and -58.114. These grid box parameters and center coordinates were defined during the preparation phase via AutoDockTools 1.5.7 using the "center on ligand" feature. Redocking of beta-muramic acid into the allosteric site of chain B generated four conformational clusters from the 100 independent runs, indicating consistent convergence rather than a single isolated result. The most populated cluster contained 71 of 100 poses, with a mean binding energy of −5.41 kcal mol-1 and the lowest binding energy of −5.86 kcal mol-1. The representative pose from this cluster (Run 87) reproduced the crystallographic native ligand conformation with a heavy-atom RMSD of 0.39 Å. The RMSD visualization and two-dimensional interaction map between beta-muramic acid and residues are presented in Figure 2. Three hydrogen bonds were observed with residues Lys148, Arg151, and Arg241, all exhibiting bond lengths below 3.5 Å. These values successfully satisfied the criteria for hydrogen bonding, which require a spatial separation of less than 3.5 Å between the donor and acceptor atoms [35]. These findings confirm that the identified coordinates accurately map the receptor's binding pocket, thereby establishing a reliable reference for subsequent docking simulations of target molecules.

Following the successful validation of the docking procedure, docking was carried out on the five LC-MS-annotated flavonoid compounds in the leaf extract of *P. valida* using the optimized parameters from the redocking analysis. Docking scores (DS) and protein-ligand interactions between each of the compounds and PBP2a are presented in Figure 3 and Table 4. Out of the five ligands used for the docking experiments, all showed lower DS than the native ligand, indicating better predicted accommodation of the ligands in the modeled allosteric pocket. Further

analysis of the interactions between the PBP2a allosteric site and the flavonoid ligands revealed the involvement of amino acids in the ligand binding process. The interactions occurred via various non-covalent interactions such as hydrogen bonding, hydrophobic interactions, and π-interactions. As summarized in Table 4, four residues, including Lys148, Arg151, Arg241, and Val277, were repeatedly involved in interactions across the native ligand and the five flavonoid complexes, suggesting that these residues represent frequently contacted positions within the modeled allosteric pocket rather than experimentally validated functional determinants.

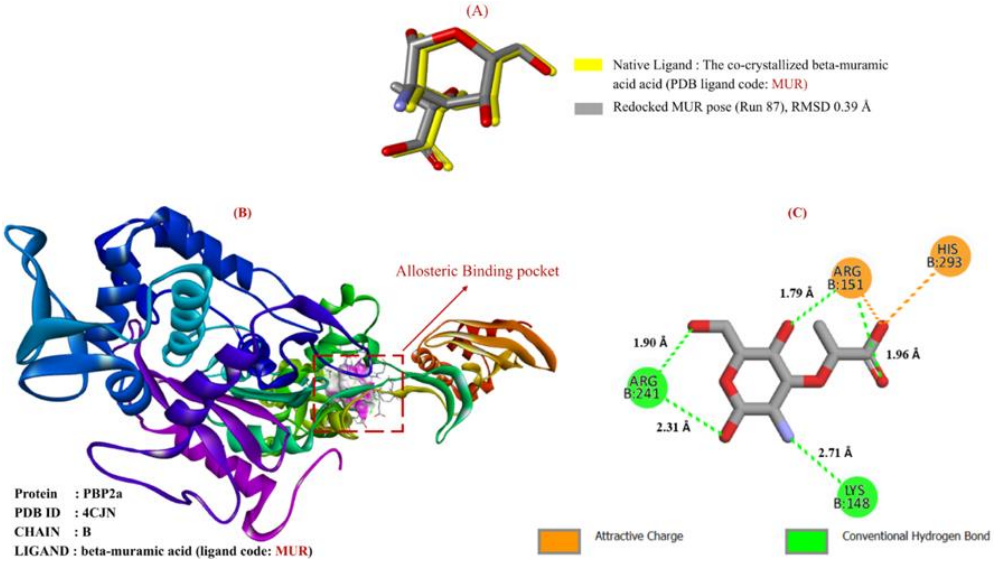

Figure 2. Redocking validation of beta-muramic acid (MUR) at the PBP2a allosteric binding site. (A) Superimposition of experimental crystallographic pose (yellow) and redocked pose (gray), showing RMSD = 0.39 Å. (B) Overall structure of PBP2a chain B showing the location of beta-muramic acid within the allosteric binding pocket (red box). (C) Two-dimensional interaction map showing hydrogen bonding and attractive charge interactions between beta-muramic acid and residues Lys148, Arg151, Arg241, and His293

Table 4. Molecular docking interactions of native ligand and selected compounds with PBP2a (PDB ID: 4CJN)

Compounds	Docking Score (kcal mol^{-1})	Amino Acid Residue	Type of Bond
Native ligand	−5.86	Lys 148, Arg 151, Arg 241	Hydrogen
Apigenin (1)	−6.76	Arg 241, Ser 149, Lys 148	Hydrogen
		Val 277	Pi-sigma
		Arg 151	Pi-alkyl
		His 293	Pi-cation
Biochanin A (2)	−6.14	Arg 151, Glu 239	Hydrogen
		Lys 148	Pi-cation
		Arg 241, Val 277, Val 256	Pi-alkyl
Luteolin (3)	−7.11	Arg 151, Arg 241, Glu 239, Val 277	Hydrogen
		Lys 148	Pi-cation
		Val 256, Val 277, Arg 151, Arg 241	Pi-alkyl
Kaempferol (4)	−6.47	Arg 241, Ser 149, Lys 148	Hydrogen
		His 293	Pi-cation
		Arg 151, Arg 241, Val 277	Pi-alkyl
		Val 277	Pi-sigma
Quercetin (5)	−7.36	Arg 151, Arg 241, Glu 239, Val 277	Hydrogen
		Lys 148	Pi-cation
		Val 256, Val 277, Arg 151, Arg 241	Pi-alkyl

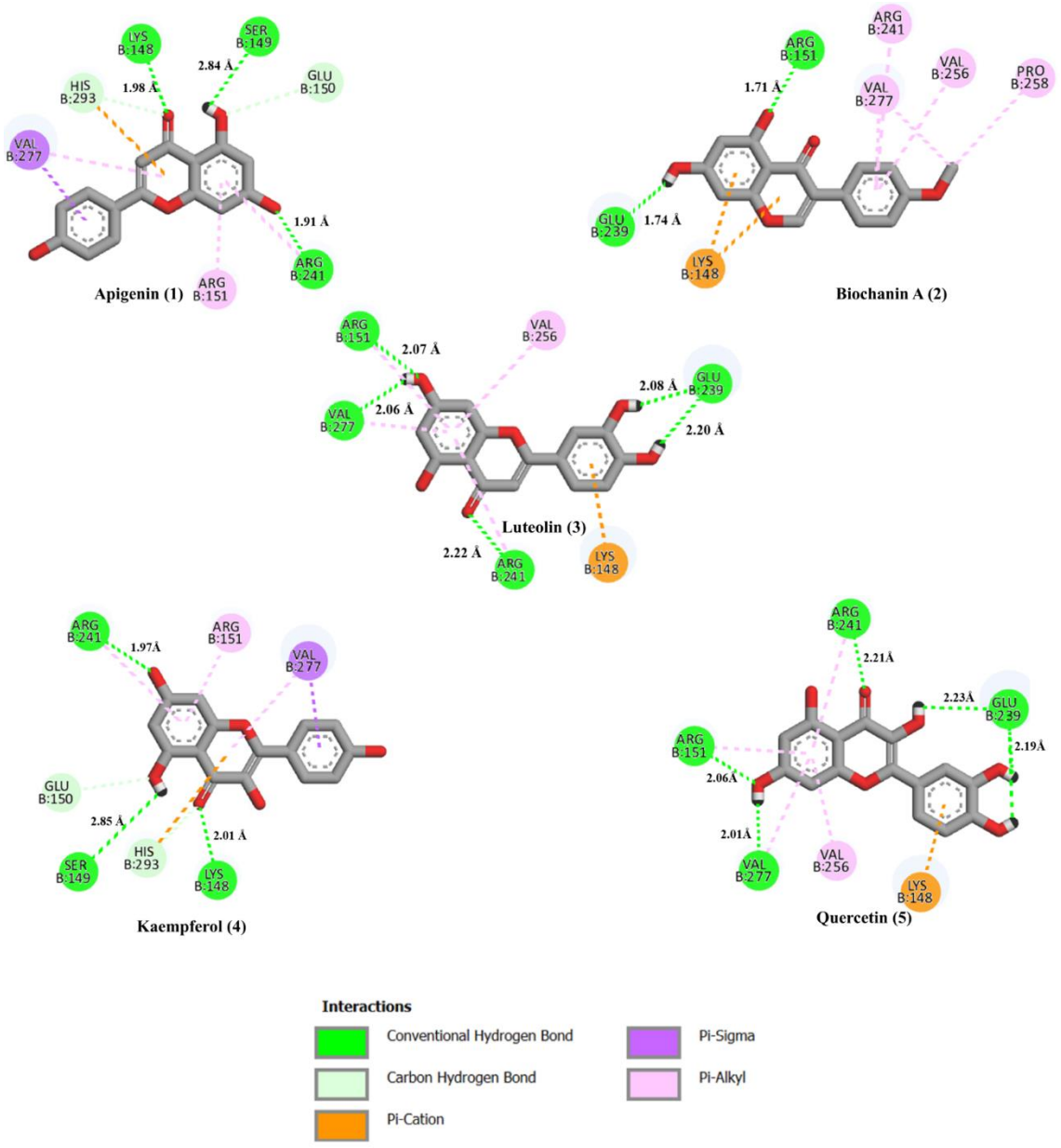

Figure 3. Two-dimensional representation of predicted molecular interactions between PBP2a (PDB ID: 4CJN) and LC-MS-annotated flavonoids: apigenin (**1**), biochanin A (**2**), luteolin (**3**), kaempferol (**4**), and quercetin (**5**). The maps depict hydrogen bonding, hydrophobic, and π-based interactions within the modeled non-covalent allosteric binding pocket of PBP2a, representing the predicted ligand-binding modes. Hydrogen-bond distances are indicated in the interaction maps.

Furthermore, other residues like Ser149, Glu239, Val256, and His293 were also shown to have ligand-specific interaction patterns, meaning that different flavonoid compounds have unique binding patterns inside the active site. The binding pattern of quercetin (**5**), which showed the most favorable predicted binding energy (-7.36 kcal mol^{-1}), consisted of hydrogen bonding interactions with Arg151, Arg241, Glu239, and Val277, along with a π-cation interaction between Lys148 and π-alkyl interactions with

Val256, Val277, Arg151, and Arg241. Similarly, luteolin (**3**) also had interactions with Arg151, Arg241, Glu239, and Val277, which may contribute to its favorable predicted interaction profile. Thus, the docking results suggest that the tentatively annotated compounds may be capable of forming a stable predicted complex with PBP2a, warranting further experimental investigation of PBP2a inhibition as a possible mechanism.

CONCLUSION

The present study provides preliminary evidence that crude ethyl acetate extract of *P. valida* leaves exhibits antibacterial activity against MRSA, with stronger activity than methanol and *n*-hexane extracts. Phytochemical screening and LC–MS profiling suggested the presence of flavonoid-related metabolites, with tentative annotations of apigenin, biochanin A, luteolin, kaempferol, and quercetin based on accurate mass and retention time comparisons. Molecular docking predicted favorable interactions of these flavonoids with PBP2a, particularly quercetin; however, these findings remain computational predictions and do not confirm direct inhibition or compound responsibility for antibacterial activity. Further studies should include MIC and MBC determination, bioassay-guided isolation, structural confirmation, evaluation of compound interactions, and experimental validation of PBP2a inhibition.

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