

Article

Comparative Antibacterial Activity of Luteolin and Morin Against Gram-Positive Bacteria: *In Vitro* Evaluation and Molecular Docking Targeting Penicillin-Binding Protein 3**Majid Khan^{1,*}, Nauman Nazir¹, Jamal Shahzad¹, Muhammad Usman Amin^{1,2,*}, Najm Ur Rahman³, Ashraf Ullah Khan⁴**¹Faculty of Pharmaceutical Sciences, Abasyn University Peshawar, Peshawar, Khyber Pakhtunkhwa, Pakistan²Khyber Medical Institute of Medical Sciences, Kohat Khyber Pakhtunkhwa, Pakistan³Department of Pharmacy, Shaheed Benazir Bhutto University, Sheringal, Pakistan⁴Department of Pharmacy, City University, Peshawar, Pakistan

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Abstract

The rising prevalence of antibacterial resistance needs the exploration of novel antibacterial drugs from natural products. Flavonoids such as Morin and Luteolin possess various pharmacological applications, their antibacterial mechanism targeting Penicillin-Binding Protein 3 (PBP3) is not appropriately addressed. This study aimed to compare the antibacterial potential and mechanism of Morin and Luteolin targeting selected strains of gram-positive bacteria and their mechanism via interaction with PBP3 computationally, which is a key enzyme linked with the biosynthesis of bacterial cell wall. Antibacterial activity was investigated against *Streptococcus pneumoniae*, *Bacillus subtilis*, and *Staphylococcus aureus* harnessing methods such as Kirby-Bauer disc diffusion and broth dilution. Cefazolin selected a reference standard antibiotic. Besides, Molecular docking was conducted against PBP3 harnessing PyRx 0.8 to assess binding affinity and interaction behavior. Both flavonoids showed concentration dependent antibacterial activity. Morin revealed zones of inhibition of 12 ± 0.29 mm, 10 ± 0.29 mm, and 14 ± 0.46 mm against *S. pneumoniae*, *B. subtilis*, and *S. aureus*, respectively. In contrast, Luteolin showed inhibition zones of 10 ± 0.32 mm, 9 ± 0.24 mm, and 11 ± 0.35 mm. Luteolin revealed lower MIC values 256–512 $\mu\text{g/mL}$ than Morin 512 $\mu\text{g/mL}$ against *S. pneumoniae* and *S. aureus*, while both compounds showed an MIC of 512 $\mu\text{g/mL}$ against *B. subtilis*. Molecular docking showed favorable binding affinities for Cefazolin (-8.8 kcal/mol), followed by Luteolin (-8.1 kcal/mol), and Morin (-7.9 kcal/mol). Both flavonoids showed predicted interactions within the PBP3 active site region of the selected *P. aeruginosa* PBP3 structure. Morin and Luteolin revealed measurable antibacterial activity, and molecular docking suggested a potential interaction with PBP3 consistent with a possible interference with cell-wall synthesis mechanism computationally. Among the tested flavonoids, Luteolin showed superior antibacterial potency, supporting its potential as a promising natural antibacterial lead.

KeywordsLuteolin, Morin, Cefazolin, Antibacterial, Computational, *In vitro***Article History**

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1. Introduction

Flavonoids comprise a large group of polyphenolic compounds that are ubiquitously distributed in plants [1]. Flavonoids are a vast group of natural products with numerous phenolic chemical moieties and are abundantly seen in plants [2]. Preliminary, such compounds were isolated from oranges then a plethora of compounds were isolated from plants [1,3]. Using the phenyl propanoid route, they are produced [4]. Plants are known to produce flavonoids, which are hydroxylated phenolic compounds, in response to microbial infection [5]. Their actions are governed by structure. The structural class, level of hydroxylation, various substitutions and conjugations, and level of polymerization all affect the chemical makeup of flavonoids [6]. Chemically, flavonoids bear a 15-Carbons basic skeleton consisting of 2 benzene rings connected with heterocyclic ring they can be classified into numerous classes of flavones entails flavone, Luteolin, flavonols, quercetin, Morin and others [1,7]. The flavonoids investigated in the present study were Morin and Luteolin, and their chemical structures are shown in Figure 1.

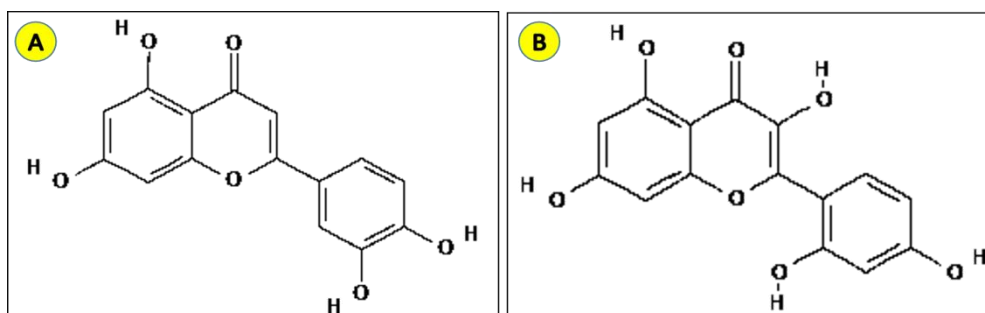


Figure 1. Chemical structures of (A) Luteolin and (B) Morin.

Cell wall synthesis inhibitors represent one of the earliest and most successful classes of antibacterial agents [8]. The discovery of penicillin by Alexander Fleming in 1928 revolutionized the treatment of bacterial infections and marked the beginning of the antibiotic era [9]. These agents exhibit their bactericidal mechanism by interfering the peptidoglycan synthesis, a crucial structural portion of the bacterial cell wall [10]. Moreover, the high emergence of bacterial resistance and the necessity for improvement of pharmacological features led to the development of semisynthetic β -lactam or cell wall synthesis inhibitor antibiotics, such as cephalosporins [11].

Cefazolin is a first-generation cephalosporin extensively utilized against Gram-positive bacterial pathogens [12-14]. Mechanistically, Cefazolin binds to Penicillin-binding proteins (PBPs), such as the transpeptidase enzymes liable for the cross-linking of peptidoglycan strands during the biosynthesis of bacterial cell wall. Inhibition of these enzymes prevents proper cell wall formation, resulting in structural instability, osmotic lysis, and ultimately bacterial cell death [15,16], as illustrated in Figure 2. Owing to its well-established mechanism of targeting PBPs, Cefazolin was employed in the present study as a reference antibacterial agent [1,17] to compare the *in vitro* and computational cell wall inhibitory potential of Morin and Luteolin.

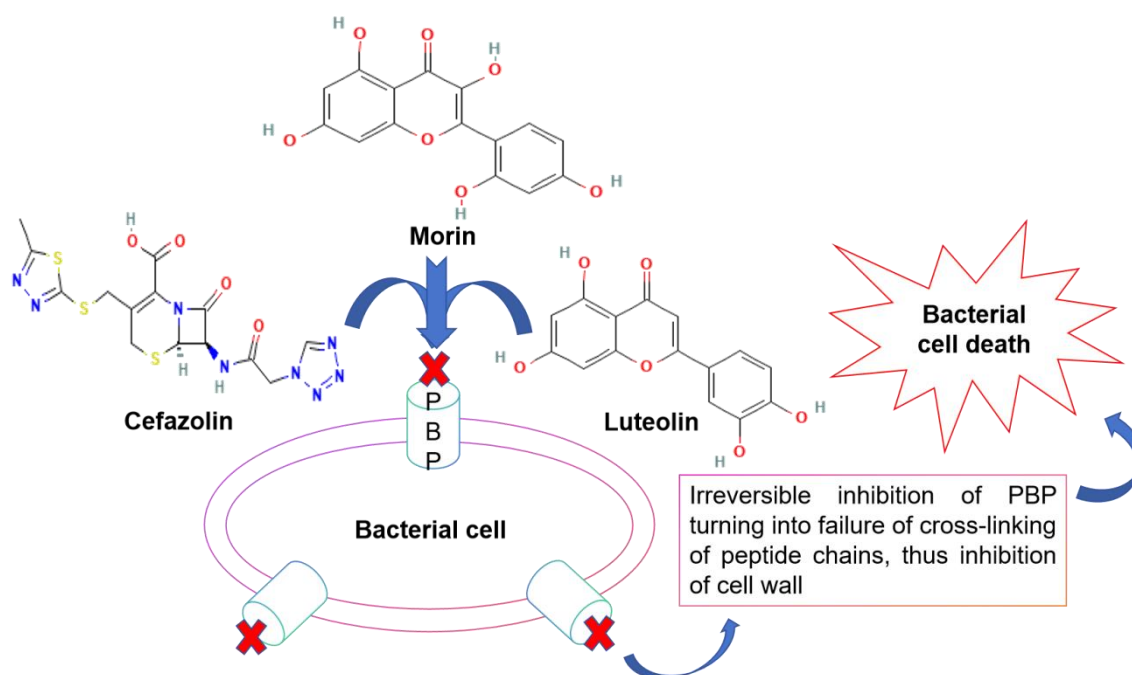


Figure 2. Comparative inhibition of penicillin binding protein and ultimately bacterial cell death harnessing Cefazolin, Luteolin and Morin.

Despite the growing evidence of the antimicrobial potential of flavonoids, the comparative antibacterial efficacy and molecular mechanism of Morin and Luteolin against clinically relevant bacterial pathogens remain inadequately explored. Although the individual antibacterial properties of Luteolin and Morin, as well as docking interactions of various flavonoids with different PBPs, have been reported previously, to the best of our knowledge no earlier study has directly compared these two structurally related flavonoids side-by-side using a combined *in vitro* and computational framework targeting Penicillin-Binding Protein 3 (PBP3). Morin and Luteolin were selected because both share a common flavonoid backbone but differ in their hydroxylation pattern, offering a useful structure-activity contrast, while Cefazolin, a PBP-targeting cephalosporin, provided a clinically relevant benchmark. Therefore, the present study aimed to evaluate and compare the *in vitro* antibacterial activity of Morin and Luteolin and elucidate their potential mechanism of action through molecular docking against PBP3, using Cefazolin as a reference standard.

2. Materials and Methods

The methodology of the current research study is schematically presented in Figure 3.

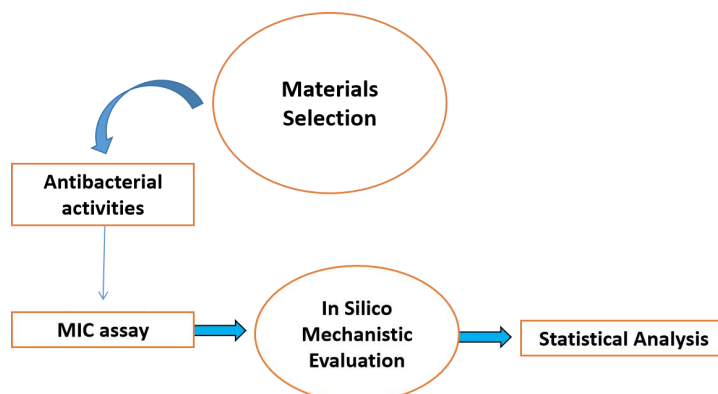


Figure 3. Schematic view of research methodology.

2.1 Materials

Flavonoids such as Morin, and Luteolin, standard bacterial strains such as *Streptococcus pneumoniae* (*S. pneumoniae*) NCTC 7466, *Bacillus subtilis* (*B. subtilis*) NCTC 8236, and *Staphylococcus aureus* (*S. aureus*) NCTC 6571. Various solvents such as chloroform, ethyl acetate, n-hexane, and n-butanol, were used. Additionally, Media particularly Nutrient agar (NA), Nutrient broth (NB), and Sabouraud dextrose agar (SDA). Standard antibacterial Cefazolin was procured from Sigma-Aldrich [1]. Morin and Luteolin were purchased from Macklin China Biochemical Industry with purity $\geq 98\%$ and molecular weight 302.24 g/mol and 286.24 g/mol respectively.

2.2 Methods

2.2.1 Antibacterial Sensitivity Assay

The flavonoids such as (Morin and Luteolin) were tested for their sensitivities against selected bacterial strains used in the study by Kirby disc diffusion method [18]. The flavonoids were dissolved in ethanol and then applied on the blank disc at different through micropipette. The bacterial strains were inoculated in 10 mL NB in test tubes and subjected to incubation at 37°C for 24 hours. Bacterial lawns were prepared on NA plates. The discs applied with the various concentrations of Morin and Luteolin (50, 100, 200, 300, 400, 500, 600, 800 and 1000 $\mu\text{g/mL}$) on the NA plates before incubation. The zone of inhibition (ZOI) around each disc was measured in millimetres (mm) using a digital caliper following the incubation period, with each assay performed in triplicate and the mean $\pm$ SD recorded. The flavonoids solutions were prepared according to their solubility in ethanol. Only that concentration of the compounds was considered at which maximum inhibitory zone was observed [1]. Bacterial suspensions were standardized to a 0.5 McFarland turbidity standard ($\sim 1.5 \times 10^8$ CFU/mL) and used to prepare uniform lawns on NA plates using sterile cotton swabs. Sterile blank paper discs (6 mm diameter) were impregnated with 20 μL of each flavonoid solution at the specified concentrations. An ethanol-only disc (20 μL of pure ethanol without any test compound) was included on every plate as a negative solvent control, alongside the Cefazolin positive reference control.

2.2.2 Minimum Inhibitory Concentration Assay

Minimum inhibitory concentration (MIC) assay was conducted using the macro-broth dilution method. The flavonoids (Morin & Luteolin) were used at different concentrations ranging from 08 to 1024 $\mu\text{g/mL}$. The bacterial cultures were multiplied, inoculated to sterile test tubes. Afterward, incubated at 37°C all-night. The MIC of the test drugs were investigated for the absence of any visual growth in test tubes [18]. The macro-broth dilution assay followed the general methodological framework of CLSI document M07, governing standardized antimicrobial susceptibility testing. A deliberate modification was made to the culture medium. NB was used for all isolates to maintain uniform, reproducible

growth conditions across strains and minimize inter-strain variability. It is acknowledged that CLSI M07 mandates cation-adjusted Mueller-Hinton Broth, with lysed horse blood supplementation required for fastidious organisms such as *S. pneumoniae*. NB was nonetheless employed here, reflecting practical constraints at this preliminary screening stage. Results should therefore be interpreted with due caution, pending confirmation under CLSI-standardized conditions. Bacterial suspensions were adjusted to a 0.5 McFarland standard and diluted in NB to a final inoculum concentration of approximately 5×10^5 CFU/mL per tube. A sterility control (NB and solvent only, no bacteria) and a growth control (NB and bacterial inoculum, no test compound) were included in every assay.

2.2.3 Molecular Docking

Initially, the chemical structures of the receptors 3OCN (<https://doi.org/10.2210/pdb3OCN/pdb>) was downloaded from RCSB-PDB (<https://www.rcsb.org/search>). Then cleaned the structures from water molecules and ligands with the help of BIOVIA Discovery Studio (<https://www.3ds.com/products/biovia/discovery-studio/visualization>). Furthermore, the 3-D structure SDV files of Cefazolin, Morin and Luteolin downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) [19,20]. Afterward the drugs were docked with protein receptors 3OCN in PyRx 0.8 version (<https://pyrx.sourceforge.io/>) [21,22]. The binding energies at the receptor sites were noted and compared for Cefazolin with Morin and Luteolin. The binding energies at the receptor sites were noted and compared for Cefazolin with Morin and Luteolin. The grid box was centred at X: 4.77, Y: 2.85, Z: -15.81 for Cefazolin; X: 2.59, Y: 1.89, Z: -17.54 for Luteolin; and X: 2.57, Y: 1.32, Z: -17.06 for Morin, using a uniform search volume of $22.5 \times 22.5 \times 22.5$ Å for all three ligands, an exhaustiveness setting of 8, and 9 generated poses per ligand. Protein preparation involved removal of water molecules, addition of polar hydrogens, and assignment of Kollman charges. To validate the docking protocol, the co-crystallized ligand (Ceftazidime) was redocked into the PBP3 active site, giving a root-mean-square deviation (RMSD) of 1.65 Å relative to its native pose, which is well in the acceptable threshold (< 2.0 Å), thereby confirming the reliability and accuracy of the docking protocol.

2.3 Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5. One-way analysis of variance (ANOVA) was performed to compare the mean ZOI values among the three treatment groups (Cefazolin, Morin, and Luteolin). A post-hoc analysis using Tukey's multiple comparisons test was applied to determine pairwise differences. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Luteolin Antibacterial Activities

The values of ZOI were compared among Luteolin and the Cefazolin against all the standard bacterial strains. The ZOI values reveal the potential of inhibition of bacterial growth, with higher values showing better inhibition.

Luteolin exhibited measurable antibacterial activity against the tested organisms, though consistently lower than that of Cefazolin, with the most pronounced effect observed against *S. aureus*, where a ZOI of 11 ± 0.35 mm was recorded at 400 µg/mL, corresponding to 54% inhibition; against *S. pneumoniae*, Luteolin produced a ZOI of 10 ± 0.32 mm (400 µg/mL, 43.5% inhibition) compared to 23 ± 0.56 mm for Cefazolin, while against *B. subtilis*, Luteolin yielded a ZOI of 9 ± 0.24 mm (500 µg/mL) versus 18.5 ± 0.62 mm for Cefazolin, as summarized in Table 1. Collectively indicating that although Luteolin possesses inherent antibacterial potential, its efficacy remains modest relative to the standard antibiotic across all three bacterial strains.

Table 1. Comparative zone of inhibition of Luteolin and standard drug antibacterial activity.

Bacterial Strains	Zone of Inhibition (mm)	
	Luteolin	Standard Drug
<i>Streptococcus pneumoniae</i>	10 ± 0.32	23 ± 0.56
<i>Bacillus subtilis</i>	9 ± 0.24	18.5 ± 0.62
<i>Staphylococcus aureus</i>	11 ± 0.35	20.5 ± 0.37

3.2 Morin Antibacterial Activities

Similarly, the values of ZOI were compared among Morin and the Cefazolin standard drug against all the standard bacterial strains. The ZOI values reveal the potential of inhibition of bacterial growth, with higher values showing better inhibition.

The efficacy of Morin against *S. pneumoniae* was evaluated. It was found that the ZOI of Morin was 12 ± 0.29 mm at 600 µg/mL and percentage inhibition was 52%. The ZOI of Cefazolin was 23 ± 0.56 mm, and inhibition was 65%. Morin shows ZOI and percentage inhibition in comparison to the Cefazolin.

In contrast to the *B. subtilis*, the ZOI of Morin was 10 ± 0.29 mm at concentration of $500 \mu\text{g/mL}$ and percentage inhibition was 54%. The values for ZOI of standard drug Cefazolin was 18.5 ± 0.62 mm. Morin showed ZOI and percentage inhibition in comparison to the standard drug. Besides, the ZOI of Morin against *S. aureus* at concentration of $500 \mu\text{g/mL}$ was revealed as 14 ± 0.46 mm. Morin showed measured antibacterial activity against all three bacterial strains, with ZOI values of 12, 10 and 14 mm against *S. pneumoniae*, *B. subtilis*, and *S. aureus*, respectively, as summarized in Table 2.

Table 2. Comparative zone of inhibition of Morin against Cefazolin.

Bacterial Strains	ZOI (mm)	
	Morin	Standard Drug
<i>Streptococcus pneumoniae</i>	12 ± 0.29	23 ± 0.56
<i>Bacillus subtilis</i>	10 ± 0.29	18.5 ± 0.62
<i>Staphylococcus aureus</i>	14 ± 0.46	20.5 ± 0.37

The results clearly revealed that Luteolin generally showed a comparable antibacterial effects to the standard drug in most cases. Importantly, the ZOI of Luteolin were more pronounced but the difference was not statistically significant to Morin. The result has been statistically illustrated in Figure 4.

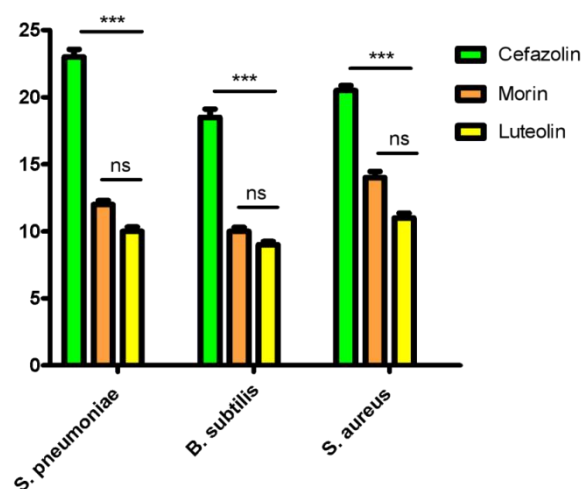


Figure 4. Comparative zones of inhibition of Cefazolin, Morin, and Luteolin against *S. pneumoniae*, *B. subtilis*, and *S. aureus*.

Note: Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. Cefazolin vs. Morin, $P = 0.0003$; Cefazolin vs. Luteolin, $P = 0.0001$; Morin vs. Luteolin, $P = 0.6421$ (ns). *** $P < 0.001$; ns, not significant.

3.3 Minimum Inhibitory Concentration (MIC)

The MIC values of Morin and Luteolin against the tested bacterial strains are presented in Table 3. Morin showed comparatively higher MIC values than Luteolin against entire bacterial strains tested except *B. subtilis*, for which both exhibited identical MIC value of $512 \mu\text{g/mL}$.

Table 3. MIC's of Luteolin and Morin against bacterial strains.

Bacterial Strains	MIC ($\mu\text{g/mL}$)	
	Luteolin	Morin
<i>Streptococcus pneumoniae</i>	256	512
<i>Bacillus subtilis</i>	512	512
<i>Staphylococcus aureus</i>	256	512

3.4 Molecular Docking

Molecular docking produced favorable predicted binding scores for the investigated ligands. The potential standard Cephalosporin antibiotic, such as Cefazolin, showed a premier docking score of -8.8 kcal/mol, providing a reference baseline docking score for comparison with the tested flavonoids. Notably, the natural plant-derived micro molecules successfully shown this competitive profile; Luteolin revealed a marginally more favourable binding affinity of -8.1 kcal/mol relative to Morin at -7.9 kcal/mol (a difference of only 0.2 kcal/mol, within the typical margin of docking score

variability, and therefore not indicative of a clear superiority in binding), as reflected in Figure 5, Figure 6 and Figure 7 respectively and such values are comprehensively compared in Table 4. Besides, visual spatial positioning and subsequent two-dimensional (2D) interaction analysis performed via PyRx 0.8 Screening Tool and BIOVIA Discovery Studio showed that all three ligands evidently converged into the actual same localized binding groove regional attachment by Cefazolin. The structural coordinate model for the primary bacterial receptor, PBP3, was retrieved from the RCSB PDB based on PDB ID: 3OCN, while the 3D structures of Cefazolin, Morin, and Luteolin were obtained from PubChem, consistent with the docking protocol described in the Methods section.

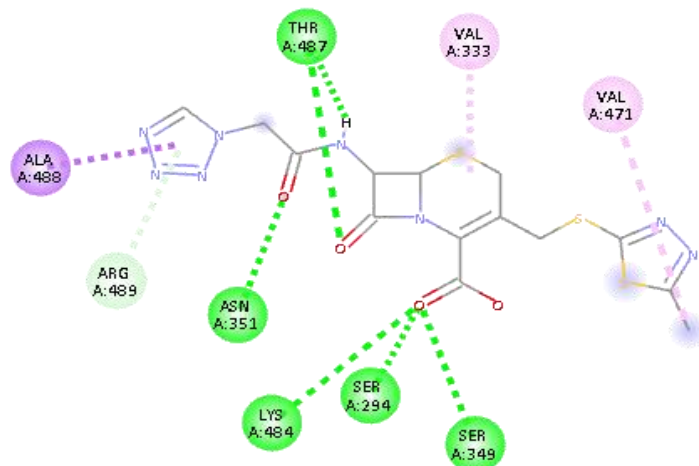


Figure 5. The 2D interaction of Cefazolin docked into the PBP3 and active site, showing hydrogen bonds and hydrophobic contacts with key catalytic residues.

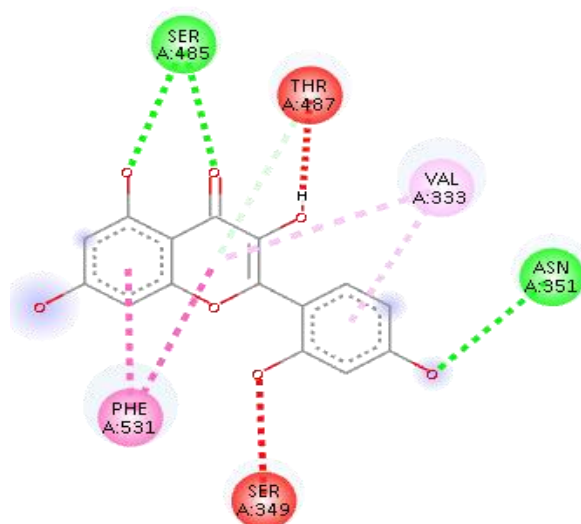


Figure 6. The 2D interaction of Luteolin docked into the PBP3 and active site, showing hydrogen bonds and hydrophobic contacts with key catalytic residues.

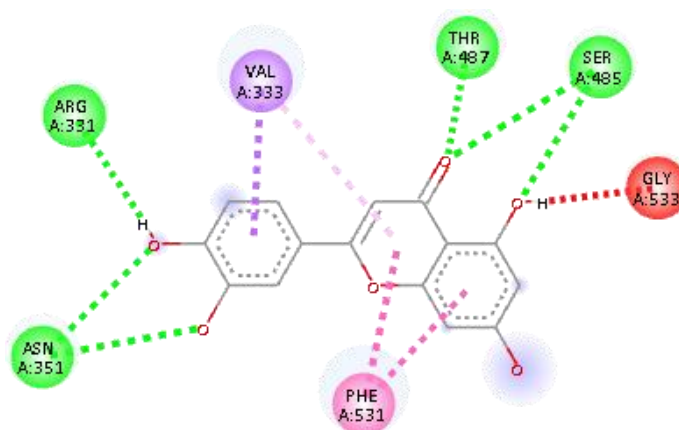


Figure 7. The 2D interaction of Morin docked into the PBP3 and active site, showing hydrogen bonds and hydrophobic contacts with key catalytic residues.

Table 4. Summary of ligands interactions with PBP3.

Ligands	Docking Score (kcal/mol)	Hydrogen Bonding Residues	Hydrophobic Interaction Residues
Cefazolin	-8.8	Ser294, Ser349, Asn351, Thr487, Lys484	Val333, Ala488
Luteolin	-8.1	Asn351, Arg331, Ser465, Thr487	Phe531, Val333
Morin	-7.9	Asn351, Ser465	Phe531, Val333

4. Discussion

According to the WHO 80% of the world developing population rely on plants for their routine ailments based on their safety, easy availability and last resort against Multidrug resistance [23,24]. Morin and Luteolin plants secondary metabolites [25], generally showed measurable antibacterial activity compared to Cefazolin across most bacteria strains such as *S. pneumoniae*, *B. subtilis*, and *S. aureus* were determined. Cefazolin is a standard drug consistently reveal the higher values for ZOI and percentage inhibition, showing their better effectiveness against these strains of bacteria.

According to Qian *et al.* 2020, Luteolin, a natural product occurred in different plants, has recently earned attention for its antibacterial properties against bacterial infections such as *S. aureus* and *L. monocytogenes*. Moreover, the possible mechanisms of Luteolin antibacterial action have not fully investigated. This study purposed to evaluate the antibacterial activities of Luteolin and its mechanism against both planktonic cells and biofilms of *S. aureus* and *L. monocytogenes*. The MIC and Minimum bactericidal concentration (MBC) of Luteolin in contrast to *S. aureus* and *L. monocytogenes* were investigated using the broth microdilution method. This research also investigated variations in cell membrane structure to investigate Luteolin mechanism of action [26]. Similarly, in our study molecular docking reveals Luteolin may intervene with the biosynthesis of bacterial cell wall via computational interaction with PBP3, furthermore it revealed lower MIC values than Morin against *S. pneumoniae*, and *S. aureus*, with comparable MIC values against *B. subtilis*.

Our results are further evidenced by the findings of Al-Oudah *et al.* 2025, who evaluated the antibacterial power of flavonoids isolated from *Origanum majorana* via integrated *in vitro* and computational approaches targeting β -lactamase and PBP. Their study revealed that flavonoids showed strong antibacterial effects and better binding affinities toward both targets, with reference to the standard drug Cefazolin in certain *in silico* studies. The authors further articulated that flavonoid–protein interactions were optimized via many hydrogen-bonding and hydrophobic interactions, contributing to increase inhibitory potential in contrast to bacterial resistance-linked proteins [27]. Similarly, in the current study, Morin and Luteolin showed moderate antibacterial activity and favorable binding toward PBP3, showing potential active-site interactions that may intervene with peptidoglycan cross-linking.

Furthermore, consistent with the residue-level data summarized in Table 4, all three ligands converged on a shared binding interface within the PBP3 active site, with Asn351 and Val333 emerging as conserved contact points across Cefazolin, Morin, and Luteolin. This convergence suggests that, despite differences in their chemical scaffolds, the two natural polyphenols may occupy a spatial position broadly comparable to that of Cefazolin, providing computational support for a possible PBP3-mediated mechanism underlying their observed antibacterial activity.

A notable differences in antibacterial effects between Luteolin and Morin may be due to subtle structural variations that variate their physicochemical properties and bondings with bacterial targets [28,29]. Both drugs belong to the flavonoid family and possess a common phenolic chemical moiety; however, variations in the positions of hydroxyl groups can substantially affect membrane permeability, protein-binding potential, and sum of biological activity [28,30]. Previous studies have showed that the antibacterial power of flavonoids is closely associated to the number of hydroxyl positions [31], which investigate their capacity to form hydrogen bonds with important bacterial enzymes and structural proteins. The superior antibacterial activity noted for Luteolin in contrast to the tested bacterial strains may therefore be linked to its increased ability to penetrate bacterial cell wall and develop a stable interactions with proteins associated to the biosynthesis of cell-wall.

Furthermore, the direct inhibition of PBP, flavonoids have been articulated to exert antibacterial effects via multiple complementary mechanisms [32,33]. These include disruption the integrity of cytoplasmic membrane, changes in membrane permeability, inhibition in synthesis of nucleic acid, interruption with energy metabolism, and alleviation of bacterial virulence factors. Such multitarget effects are particularly beneficial in the current era of antimicrobial resistance because it decreases the likelihood that bacteria will quickly develop resistance via single genetic mutation. The potential of flavonoids to concurrently modulate numerous cellular pathways differentiate them from many traditional antibiotics that commonly act on one primary molecular target [32].

Another important consideration is the potential of flavonoids to alleviate oxidative stress within bacterial cells [34–36]. Phenolic compounds can turn on intracellular reactive oxygen species or free radicals accumulation, turning in oxidative damage to macromolecules such as proteins, lipids, and nucleic acids [37]. Many investigations have showed that flavonoid-induced oxidative imbalance linked significantly to the inhibition of bacterial growth and cellular death [32,38].

Therefore, the antibacterial potential observed in the current study may arise not only from a potential interaction with PBP3 but also secondary oxidative mechanisms that overall impairment in bacterial cells survival.

The docking analysis conducted in this study provides additional computational support suggesting that these compounds may interact with PBP3. PBPs play pivotal role in the synthesis of peptidoglycan and maintenance of the integrity of bacterial cell-wall [15,39]. Inhibition of these proteins compromises cell-wall integrity, eventually leading to bacterial cells lysis [40,41]. The investigation of multiple hydrogen and hydrophobic bonds between the identified flavonoids and active-site residues of PBP3 suggests that these compounds may potentially interact with the catalytic site of the enzyme. Similar observations have been reported for other naturally occurring flavonoids, such as quercetin, apigenin, and kaempferol, which have revealed affinity toward bacterial proteins associated with the biosynthesis of cell-wall [32,42]. These results collectively support the growing evidence that plant-derived polyphenols represent promising scaffolds for the development of novel antibacterial agents.

The susceptibility of the Gram-positive strains evaluated in this study to Luteolin and Morin may be related to the relatively simple architecture of the Gram-positive cell envelope, which consists primarily of a thick peptidoglycan layer without an outer membrane, potentially facilitating flavonoid penetration to intracellular and cell-wall-associated targets [43,44].

Natural products continue to catch considerable interest as sources of new drugs development [43–45] such as antibacterial agents because of their structural diversity, favorable safety profiles, and historical utilization in traditional medicine [46]. Flavonoids are particularly attractive candidates due to their enrichment in medicinal plants and dietary supplements. Unlike many synthetic antibacterial compounds, flavonoids frequently show additional pharmacological properties, such as antioxidant, anti-inflammatory, anticancer, and immunomodulatory effects [1,47]. Such multifunctional features may provide therapeutic benefits, particularly in infectious diseases where inflammation contributes significantly to tissue damage and disease progression.

Another promising feature of flavonoid-based treatment is their potential concoction or in combination with existing antibiotics. Numerous studies have showed synergistic interactions between flavonoids and conventional antibiotics. By increasing membrane permeability, inhibition of efflux pumps, or targeting bacterial mechanistic pathways, flavonoids can enhance the efficacy of standard antibiotics and potentially repair susceptibility in resistant bacterial isolates [33,48]. Consequently, future investigations should evaluate the combinational effects of Luteolin and Morin with clinically suitable antibiotics such as Ampicillin, Ceftriaxone, Ciprofloxacin, and Vancomycin. Such studies may show novel therapeutic modalities to combat multidrug-resistant infections.

In short, the findings of the current study contribute to the growing body of evidence proving the antibacterial potential of plant-derived flavonoids. The integrated *in vitro* and computational approaches used in this study aid complementary proof showing that Luteolin and Morin may interact with bacterial growth via interactions with PBP3 and associated biosynthesis of cell-wall processes. Continued investigation of these compounds could aid the development of alternative antibacterial therapies with the aim to address the burning issue of antimicrobial resistance.

5. Conclusion

Conclusively, the current study provides preliminary evidence that Morin and Luteolin possess antibacterial activity in contrast to three strains of gram-positive bacteria such activities were validated for potential via *in vitro* approaches and mechanism was investigated through *in silico* studies. The proposed target site for the antibacterial effects was PBP3, which was evaluated *in silico* for Morin and Luteolin. Luteolin showed comparatively higher *in vitro* antibacterial activity between the two flavonoids tested; however, as this conclusion is based solely on *in vitro* assays and computational docking, without enzyme inhibition assays, molecular dynamics simulations, toxicity evaluation, or animal studies, further comprehensive investigation is required before Luteolin could be considered a candidate for development into a novel antibacterial or adjunctive cell-wall-synthesis-inhibiting agent alongside existing antibiotics.

6. Limitations and Future Perspective

This research provides initial evidence of Morin and Luteolin antibacterial potential against Gram-positive bacteria and their mechanism targeting PBP-3. Such results must be further validated using enzyme inhibition assays, molecular dynamics simulations, and *in vivo* activities are required to further confirm their antibacterial potential and mechanism. As this study relied primarily on molecular docking to propose a PBP3-mediated mechanism, the mechanistic conclusions presented here should be regarded as preliminary; more comprehensive experimental evidence, such as direct PBP3 enzyme inhibition assays, is required before a definitive mechanism of action can be established. A further limitation of the computational framework employed in this study concerns its reliance on a single PBP3 crystal structure (PDB ID: 3OCN), derived from *Pseudomonas aeruginosa*, a Gram-negative bacterium. This stands in direct contrast to our *in vitro* antibacterial evaluation, conducted exclusively against three Gram-positive species: *S. pneumoniae*, *B. subtilis*, and *S. aureus*. Because Gram-negative and Gram-positive bacteria possess fundamentally distinct cell envelope architectures, their corresponding PBP enzymes may exhibit considerable divergence in active-site topology and ligand-binding affinity.

Extrapolating mechanistic inferences from a Gram-negative docking template to Gram-positive strains therefore constitutes a cross-phylum assumption that warrants caution. Although PBP active sites retain several conserved catalytic motifs across diverse bacterial phyla, unequivocal validation of these *in silico* findings will require future investigations employing species-matched PBP3 crystal structures or, in their absence, high-fidelity homology models constructed specifically for the Gram-positive organisms examined herein. The redocking RMSD value confirming the reliability of the docking protocol against the PBP3 active site is being finalized and it was 1.65 Å. Future work should also incorporate molecular dynamics simulations to better characterize complex stability, and *in vitro* cytotoxicity evaluation, to more comprehensively characterize the antibacterial potential of Luteolin and Morin.

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Ethics Statement

Not applicable.

Informed Consent

Not applicable.

Data Availability Statement

The entire data is included in the manuscript.

Author Contributions

Idea Generation & Conceptualization: Majid Khan, Muhammad Usman Amin, Nauman Nazir. Data analysis: Majid Khan, Najm Ur Rahman, Jamal Shahzad, Nauman Nazir, Writing and editing: Majid Khan, Jamal Shahzad, Muhammad Usman Amin, Proof reading and Corresponding: Majid Khan and Muhammad Usman Amin. Visualization: Majid Khan, Jamal Shahzad, & Ashraf Ullah Khan.

Conflict of Interest

None.

Generative AI Statement

No generative AI tools were used in the preparation, and writing of this manuscript.

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