Vol. 4 No. 4 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

EXPLORING THE ANTIMICROBIAL POTENTIAL OF 3,5-DI-O-METHYL-8-PRENYLAFZELECHIN-4B-OL: AN IN-SILICO APPROACH

Imran Rabbani

Department of Pharmacy, Kohat University of Science and Technology, Kohat Pakistan

Zaib un Nisa

Department of Zoology, GC Woman University, Sialkot Pakistan

Tahseen Ismail

Department of Microbiology, Mirpur University of Science and Technology, AJK Pakistan

Izhar Ullah

Department of Pharmacy, Univrsity of Poonch Rawalakot, AJK Pakistan

izharpharma@upr.edu.pk

Author Details

Keywords:

Received on 30Oct, 2025

Accepted on 18 Dec, 2025

Published on 31 Dec, 2025

Corresponding E-mails & Authors*:

Izhar Ullah

izharpharma@upr.edu.pk

Abstract

The increasing threat of antimicrobial resistance has become a global health concern necessitating to explore novel antimicrobial compounds. As a part of efforts to curb antimicrobial resistance, the study is designed to investigate the antimicrobial potential of flavonoid compound 3,5-Di-O-Methyl-8-Prenylafzelechin-4β-ol (DMP) by in-silico approach. To access antimicrobial potential of DMP, molecular docking study of the compound was performed using Autodock Vina to determine its binding affinity and interaction with microbial DNA gyrase used as target protein. Pharmacokinetic and toxicity properties of the compound were evaluated by pkCSM while drug likeness was confirmed by online webtool SwissADME. The binding affinity of the compound was found to

be -7.1 kcal/mol against DNA gyrase of *Staphylococcus aureus*, indicating its potential to interfere with microbial growth. The ADMET profile of DMP has shown acceptable pharmacokinetic characteristics with reasonable intestinal absorption, moderate solubility, low CNS permeability, no CYP inhibition and low toxicity. The compound has shown compliance to Lipinski rule of five revealing its potential as a drug candidate. These results revealed the antimicrobial potential of DMP and its suitability for further experimental validation. This study also emphasizes the in-silico methods as cost-effective and efficient tools for initial drug evaluation. Further validation by experimental methods is required to confirm the antimicrobial nature of the compound.

INTRODUCTION

Flavonoids are the secondary metabolites commonly found in plants with diversified biological activities, including antibacterial, antiviral, antioxidant, anticancer, and anti-inflammatory properties. They are synthesized by the phenylpropanoid pathway. They exhibit antimicrobial properties by targeting bacterial cell wall and inhibiting nucleic acids synthesis (Roy et al., 2022; Górniak et al., 2019). The ability of flavonoids to bind to bacterial enzymes involved in DNA synthesis like DNA gyrase, make them suitable to be used as antimicrobial agent (Guseva et al., 2024; Sengupta et al., 2024). In addition, some studies reported the antimicrobial activity of flavonoids when they were used in combination with antibiotics and in some circumstances, reverted bacterial resistance to specific antibiotic (Donadio et al., 2021). A number of flavonoids like catechins, quercetin and kaempferol are reported for their antimicrobial activity against multidrug resistant pathogens because of their ability to bypass resistance mechanisms (Nikule et al., 2024). Traditionally used medicinal plants containing flavonoids with antimicrobial properties were commonly used. For example, *Acalypha indica* and *Terminalia catappa* have been investigated for their flavonoid content and antimicrobial activity (Zahidin et al., 2017).

The drug design mainly depends upon identification and targeting of specific microbial enzymes (Payne et al., 2007). The key protein targets for the compound DMP include enzymes crucial for survival of microorganisms like gyrase, dihydrofolate reductase, transglycosylase etc. These targets are frequently used in molecular docking studies for prediction of potential drug candidate's efficacy (Fernández-Cabal et al., 2025). The selection of target protein for an

antibiotic is not only related to its efficacy and specificity but also its ability to evade microbial resistance. By targeting common and less common microbial pathways, scientists are developing antibiotics that retain their effect against resistant pathogens (Silver 2007).

In silico studies have transformed drug discovery by presenting time saving and cost-effective substitutions to conventional experimental techniques (Lionta et al., 2014). Molecular docking, pharmacokinetic analysis (absorption, distribution, metabolism, and excretion), toxicity studies, and druglikness predictions are obligatory tools used in in-silico analysis (Daina et al., 2017). Docking studies are performed by tools like Autodock Vina to study the interactions of ligands with target proteins, giving information about binding affinity and mechanism of action of compounds (Trott and Olson 2010). Online webtools like SwissADME and pkCSM are commonly used for prediction of ADMET properties of compounds, thus further improving candidate selection and confirming only biologically active compounds go on to experimental phases (Chakraborty et al., 2024).

According to WHO (World Health Organization), antimicrobial resistance has become major worldwide threat to human health (WHO 2014). Due to overuse of common antibiotics, traditional methods for eliminating pathogens are no longer effective due to emerging resistance to multiple antibiotics. This condition necessitates the urgency for hunting new antimicrobial metabolites to fight against resistant stains (Herina et al., 2024). Increasing number of scientists believe that natural compounds, specifically flavonoids, can be effective alternatives of manufactured antibiotics (Cushnie 2005).

Flavonoid compound 3,5-Di-O-Methyl-8-Prenylafzelechin-4β-ol has not been studied for antimicrobial activity. The present study investigates the antimicrobial potential of DMP by in silico methods. There are no studies reporting its specific biologically activity, especially antimicrobial activity. The unavailability of prior pharmacological investigation provides a prospect to evaluate its potential interactions with target proteins of microorganisms using computation techniques. The structural characteristics of the compound, such as its methylation and prenylation, are hypothesized to augment its biological activity, making it a striking candidate for further study. The use of computational techniques in this study highlights their significance in modern-day drug design. By utilising these techniques, scientists can effectively

recognize compounds, reducing dependence on expensive and time taking laboratory experiments.

Materials and methods

Antimicrobial activity prediction

The antimicrobial activity of 3,5-Di-O-Methyl-8-Prenylafzelechin-4β-ol was predicted by using online webserver PASSonline and Way2drug (Ali et al., 2019). PASSonline prediction analysis is based on relating molecular formula and chemical structure of the compound to its biological activity by employing MNA (multilevel neighbours of atoms) (Gulzar et al., 2019; Ramos et al. 2020). For this purpose, SMILES of the compound was retrieved from Pubchem and used as input into way2drug webtool.

Ligand preparation

The 3D structure of the compound DMP was obtained from Pubchem in SDF format. Then SDF format was converted to PDB format using Pymol, in order to use it in Autodock tools.

Selection of target protein

The selection of target DNA enzyme was based on literature mining, because of its important role in microbial growth and as potential target of known antibiotics (Maxwell and Lawson 2003). The search focus was on those bacterial proteins with their high-resolution 3D structures availability in Protein Data Bank (PDB).

Retrieval and preparation of target protein

The high resolution (2.30 Å) crystal structure of DNA gyrase (PDBID: 3G7B) of *Staphylococcus aureus* was downloaded from PDB database. The DNA gyrase was then prepared for docking by removing water and ligand molecules from 3D structure of the enzyme in Biovia discovery studio. Polar hydrogens were also added to protein (Lawal et al., 2020).

Binding site prediction

The Prankweb and CB-Dock2 web services were used to identify the amino acids involved in the development of active pockets. Both are web-based tools for identifying the architecture and site pockets within proteins, as well as for sorting out the amino alkanoic acid residues found in the proteins' active pocket (Jendele 2019). Prior to docking, lining the grid box requires site determination.

Molecular docking

Molecular docking studies were performed using the ligand DMP against bacterial protein, DNA gyrase, commonly associated with drug resistance. Docking simulations were carried out using AutoDock Vina software (Trott & Olson, 2010), which employs a semi-empirical free energy force field and the Lamarckian Genetic Algorithm (LGA) to provide reliable receptor-ligand interaction data. The grid was manually adjusted to align with the active binding sites of each protein structure. The binding energy of the compound to the receptor was analysed, with lower binding energy indicating a stronger interaction between the ligand and the target receptor.

Protein-ligand interactions

The docked complex of DMP with gyrase was visualized and evaluated for 2D interactions involving hydrophobic interactions and hydrogen bonding between the ligand and the protein in Discovery studio. The interacting amino acid residues of the target protein were observed and noted (Verma et al., 2020).

Drug likeness calculations

Drug likeness properties of the compound DMP was accessed using webtool SwissADME. This webtool is freely available and used for filtering in silico ADME approach (Lagorce et al., 2008). For this purpose, the SMILES representation of the compound was retrieved from the PubChem database and input into the SwissADME for computational prediction of drug likeness of the compound. The compound was analysed diagrammatically for its oral bioavailability and its

removal from central nervous system by p-glycoprotein was shown in BOILED-EGG diagram. The compound drug likeness was studied by Lipinski's rule of five.

ADME screening

Early ADMET property prediction of compounds has shown to be highly helpful in the drug discovery and development process. An online web server pkCSM (Pires et al., 2015) was used to predict water solubility, human intestinal absorption (%), skin permeability (logKp), BBB permeability (logBB), CYP2D6 substrate, CYP1A2 inhibitor, total renal clearance (logmL/min/kg), and Renal OCT2 substrate of the compound DMP.

Toxicity prediction

Additionally, the toxicological profile of the substances was predicted using pkCSM (Pires et al., 2015). Various parameters, including AMES toxicity, human maximum tolerated dose, oral rat acute toxicity (LD50), oral rat chronic toxicity (LOAEL), hepatotoxicity, skin sensitivity, and minnow toxicity, were assessed for the compound.

Results

Antimicrobial activity prediction

The results of The PASSonline web server and platform Way2Drug, indicated that the compound has antibacterial potential as shown by its Pa values and confidence score. The results are given in Table 1.

Table 1. Probability of antibacterial activity of the compound against bacterial strains predicted by PASSonline and Way2Drug

Predictive probabilities	Value	Against
Confidence score	0.5156	Resistant Staphylococcus simulans
	0.4353	Staphylococcus sciuri
	0.4054	Staphylococcus simulans

Pa (antibacterial)	0.455	NA

Binding site analysis

Binding site pocket in DNA gyrase (3G7B) was determined using Prankweb and CB-Dock2 webservers. The binding site with highest score 5.13 and probability value 0.247 obtained from Prankweb was selected for docking with the ligand. The results of the Prankweb and CB-Dock2 are shown in Figure 1.

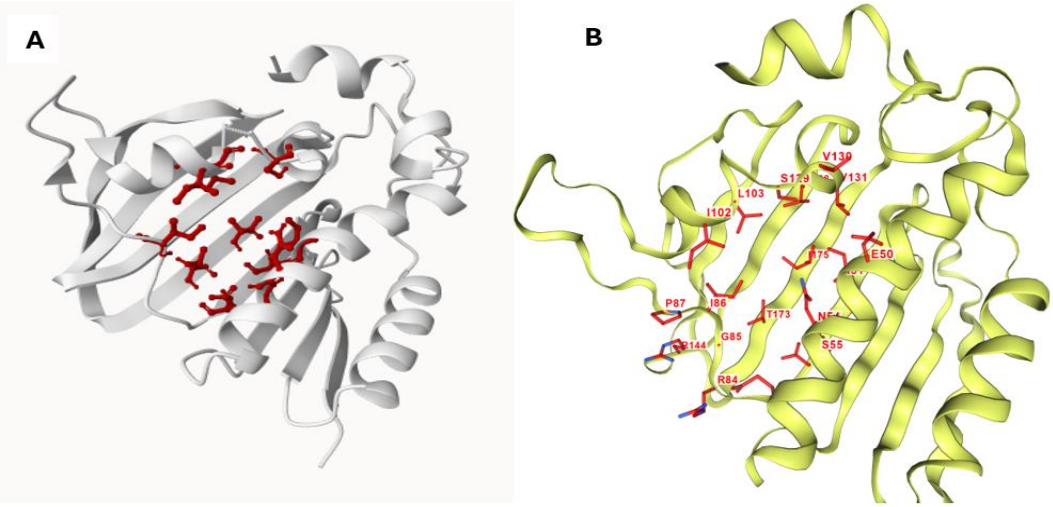

Figure 1. The binding site of target protein DNA gyrase (PDB ID: 3G7B) having highest probability is shown in A (from Prankweb) and B (from CB-Dock2). Red colour indicates the amino acid residues of binding site.

The binding site analysis indicated a number of amino acids residues within the binding site that would be involved in binding with ligands. These amino acids, as determined by CB-Dock2, are given in Table 2.

Table 2. Amino acid residues of binding site of DNA gyrase (3G7B)

Webtool	Amino acid residues
CB-Dock2	ILE51, ASN54, SER55, ASP57, GLU58, VAL79, ASP81, GLY83, ASP81, ARG84, GLY85, ILE86, PRO87, GLN91, ALA98, ILE102,

	LEU103, SER128, SER129, VAL130, VAL131, ASN132, LEU138, ARG144, THR173, ILE175

Molecular docking

For the docking study, the crystal structure of the DNA gyrase (PDB id: 3G7B) of *Staphylococcus aureus* was used. We used flavonoid compound DMP for the docking purpose. The compound has shown good docking score of -7.1 kcal/mol by using Autodock vina. Compared to standard inhibitor ciprofloxacin, we have found that the compound binds with the target with best binding energy of -7.1 kcal/mol shown in Table 3.

Table 3. Binding affinity of compound with DNA gyrase

	DMP	**Ciprofloxacin**
Binding affinity (kcal/mol)	-7.1	-6.5
Contacts residues	ILE86, ILE51, PRO87	ILE102, ILE86, ILE51
Hydrogen bonds	0	1
Alkyl bonds	2	3
van der Waal bonds	1	0

Vol. 4 No. 4 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

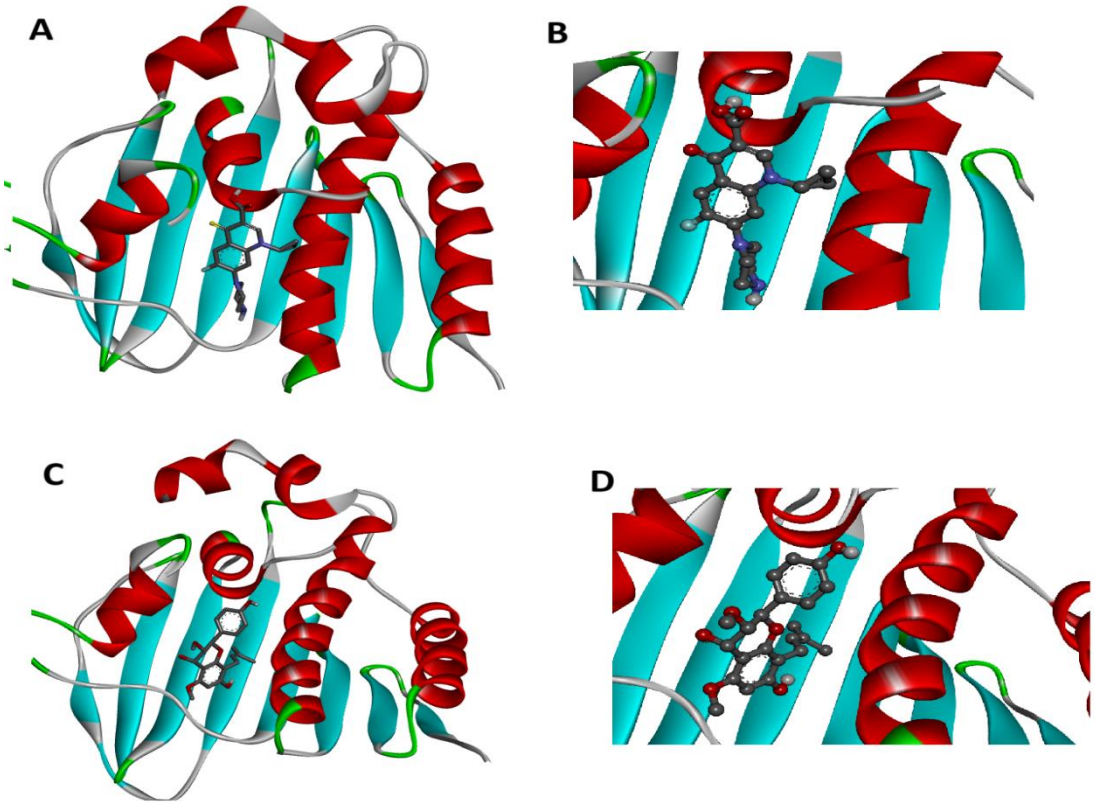

Figure 2. Molecular docking study of compound ciprofloxacin (A and B) and DMP (C and D) with DNA gyrase.

Protein-ligand interactions

Hydrophobic interactions, hydrogen bonding, and electrostatic forces are key factors in protein-ligand interactions, playing a crucial role in determining the ligand's binding affinity to proteins. Multiple bound contacts between the molecule and multiple site residues were observed. Two alkyl bond interactions between the molecule and the residues of the DNA gyrase protein (Ile51 and Ile86) were observed. Figure 3 illustrates the numerous types of van der Waals and hydrophobic interactions that the chemical has with distinct DNA gyrase protein residues.

Vol. 4 No. 4 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

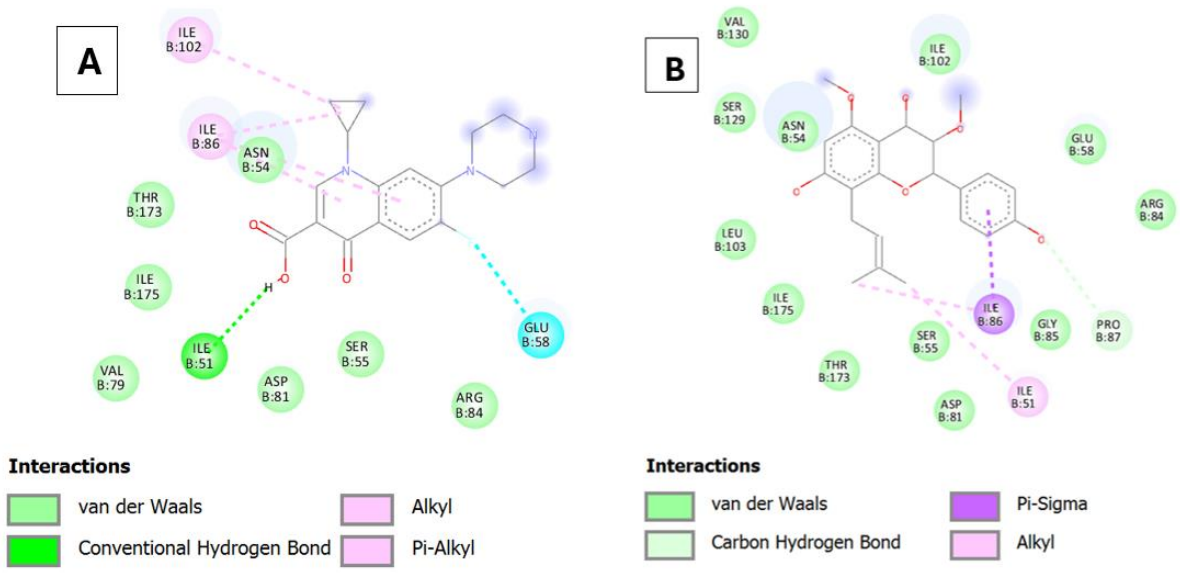

Figure 3. Interactions of ciprofloxacin (A) and DMP (B) with DNA gyrase as visualized in Discovery studio.

Drug likeness

The Lipinski Rule of Five evaluates the compound's drug-likeness based on key physicochemical properties. The compound adhered to all four rules, indicating a high likelihood of good oral bioavailability. The parameters are as follows: molecular weight (MW) < 500 Da, lipophilicity (LogP) < 5, hydrogen bond donors (HBD) ≤ 5, and hydrogen bond acceptors (HBA) ≤ 10. No violations were observed as shown in Table 4, suggesting favourable pharmacokinetics.

Table 4. Evaluation of 3,5-Di-O-Methyl-8-Prenylafzelechin-4β-ol by Lipinski Rule of Five

Compound	Mol. Wt	No. H-bond acceptors	No. H-bond donors	Log $P_{o/w}$ (MLOGP)	Follow Lipinski's RO5
DMP	386.44 g/mol	6	3	1.53	Yes; 0 violations
Ciprofloxacin	331.34	5	2	1.28	Yes;

	g/mol				0 violations

SwissADME bioavailability radar is used to demonstrate the physicochemical properties of the compound. Figure 4 represents the physicochemical profile of DMP in terms of six parameters, which includes LIPO (lipophilicity), INSATU (in-saturation), INSOLU (solubility), POLAR (polarity), SIZE (molecular size), and FLEX (flexibility). The centre pink area shows the ideal range of oral bioavailability. All six proprieties of the compound were found within range, indicating its favourable oral bioavailability.

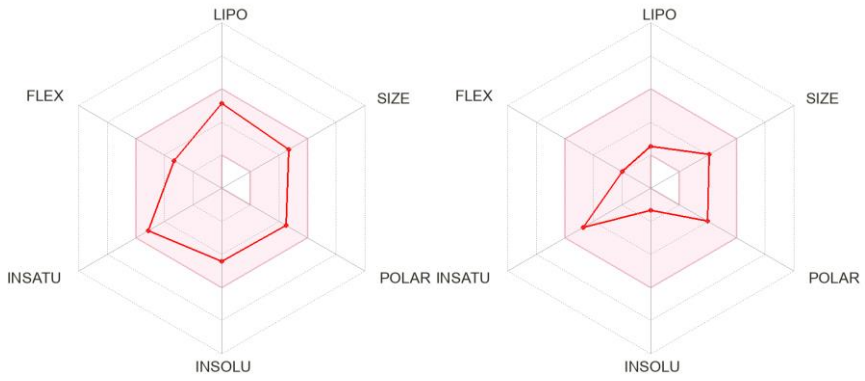

Figure 4. Physicochemical profile of the compound DMP as per SwissADME: The pink area indicates optimal oral bioavailability.

The SwissADME BOILED-Egg graph as shown in Figure 5 evaluates the compound's potential for gastrointestinal absorption (HIA) and blood-brain barrier (BBB) penetration. The compound appears in the white region, indicating high probability for intestinal absorption but limited BBB penetration, making it suitable for oral administration with low CNS effects.

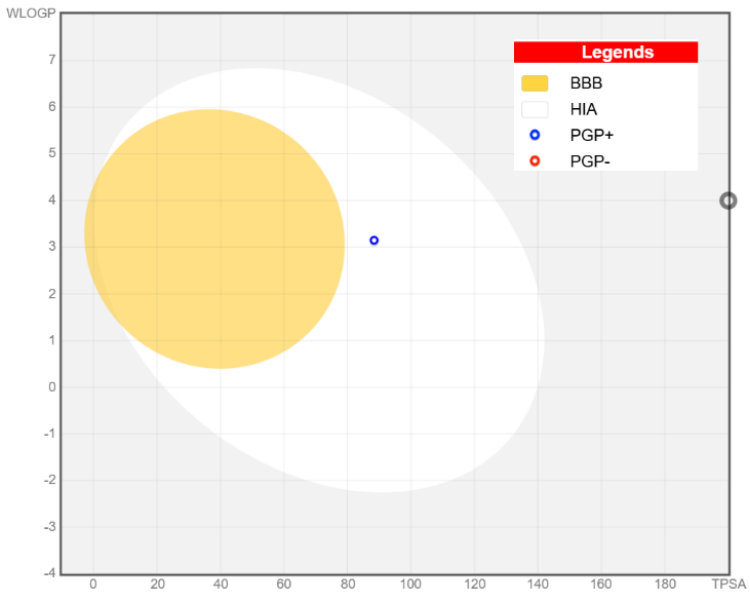

Figure 5. BOILED-Egg diagram of the compound DMP. The compound is in the white zone, meaning good oral absorption but low BBB penetration.

ADME properties

Since they define the qualities of an effective oral medication, pharmacokinetic properties (PKs) are considered significant in drug manufacture. One of the main factors influencing drug interactions, which can lead to medication toxicity and a decrease in pharmacological effect, is drug metabolism via CYP isoenzymes. The PKs for the compound are given in Table 5.

Table 5. Pharmacokinetic properties predicted by pkCSM for compound DMP.

Pharmacokinetic properties	pkCSM
Water solubility	-2.239 log mol/L
Intestinal absorption (human)	50.665 %
Skin Permeability	-2.795 log Kp
BBB permeability	-0.524 log BB
CNS permeability	-3.733 log PS
CYP2D6 substrate	No
CYP1A2 inhibitor	No
Total Clearance	0.972 log ml/min/kg
Renal OCT2 substrate	No

Toxicological properties

To predict toxicity properties, the compound was analyzed bt pkCSM web server as shown in Table 6. The results explained that the compound is not mutagenic as determined by AMES toxicity, non-hepatotoxic, and don't cause skin sensitisation. The max. tolerated dose of -0.102 log mg/kg/day is well below the acceptable limit. The compound was found to have low toxicity in rats, as determined by Oral Rat Chronic Toxicity (LOAEL) and Oral Rat Acute Toxicity (LD50). Minnow toxicity result indicates the compound non-toxic nature to aquatic organisms.

Table 6. Toxicological properties predicted for compound.

Toxicity	pkCSM
AMES toxicity	No
Max. tolerated dose (human)	-0.102 log mg/kg/day
Oral Rat Acute Toxicity (LD50)	2.134 mol/kg
Oral Rat Chronic Toxicity (LOAEL)	1.868 log mg/kg_bw/day
Hepatotoxicity	No
Skin Sensitisation	No
Minnow toxicity	1.481 log mM

Pakistan Journal of Medical & Cardiological Review
https://pakjmcr.com/index.php/1/about
Online ISSN 3007-2387
Print ISSN 3007-2379

Discussion

The present study employed in-silico methodologies to assess the antimicrobial potential of a flavonoid compound DMP against bacterial targets, particularly DNA gyrase. The results indicate a promising interaction between the compound and the bacterial enzyme, suggesting its potential role as an antimicrobial agent. Flavonoids have exhibited antimicrobial properties by different mechanisms like enzyme deactivation, membrane lysis, and generation of reactive oxygen species (Gorniak et al., 2019).

The antibacterial potential of the compound DMP was predicted by online webtools PASSonline and Way2Drug, with probability score (Pa) of 0.455, suggesting moderate confidence about its antimicrobial potential. The compound has shown antibacterial activity against resistant *Staphylococcus simulans* and *Staphylococcus sciuri*, which are common opportunistic pathogens. The confidence scores of these pathogens were found to be 0.5156 and 0.4353 respectively, recommending the compound potential to target resistant bacterial strains. These findings align with the previous studies demonstrating the enhanced antimicrobial activity of prenylated flavonoids by disrupting bacterial cell membranes (Cushine et al., 2011). To confirm these antimicrobial predictions, experimental validation is required and to ascertain the compound minimum inhibitory concentration.

Prankweb, an online tool, was used to identify binding pockets of enzyme DNA gyrase, which recognised binding pockets. The binding sites showing highest probability score of 0.247 and confidence level of 5.13 was selected for docking. The important amino acid residues at binding pocket were found to be PRO87, ILE51 and ILE86. These amino acids are significant for enzyme function and their interaction with ligand will indicate its strong inhibitory potential.

The molecular docking study indicated good binding affinity of -7.1 kcal/mol of 3,5-Di-O-Methyl-8-Prenylafzelechin-4β-ol against target gyrase (PDBID: 3G7B), which was found to be greater than ciprofloxacin (-6.5 kcal/mol) against same enzyme. This proposes that the compound has similar antimicrobial potential to the known antibiotic. DNA gyrase is a well-known target for many available antibiotics, which bind to active site of the enzyme disrupting DNA replication and hence producing bactericidal effect (Maxwell and Lawson, 2003). Upon visualization of interactions, it was found that compound DMP interacts with important residues

ILE51, ILE86, and PRO87. The amino acid residue ILE86 has shown interaction with both compound DMP and ciprofloxacin, further confirming its antimicrobial effect. Similarly DMP has shown hydrophobic interactions, mainly alkyl and van der Waal interactions with target protein. Hydrophobic interactions are important in binding of drug to receptor resulting in improved bioactivity of drug (Bikadi and Hazai, 2009).

The ADME analysis indicates that the compound adheres to Lipinski's Rule of Five, suggesting favourable drug-likeness properties. The compound has a molecular weight of 386.44 g/mol, logP of 1.53, and no violations of the rule, making it a potential oral drug candidate (Lipinski et al., 2012). Furthermore, the SwissADME bioavailability radar confirms that its physicochemical properties fall within the optimal range for oral bioavailability. The BOILED-Egg analysis supports this by demonstrating high absorption in GIT and low penetration in blood-brain barrier, which is advantageous in minimizing central nervous system-related side effects (Daina et al., 2017).

The in-silico toxicity prediction using pkCSM suggests that DMP exhibits no mutagenicity (negative AMES test), hepatotoxicity, or skin sensitization (Pires et al., 2015). Moreover, its maximum tolerated dose (-0.102 log mg/kg/day) is well below harmful thresholds, indicating a good safety profile. Compared to existing antibiotics, the compound demonstrates lower toxicity risks, which is crucial for its therapeutic viability. The absence of interaction with CYP enzymes like CYP2D6 and CYP1A2 further suggests a lower likelihood of drug-drug interactions, an essential factor in pharmacokinetic optimization (Ai et al., 2015).

The binding affinity of DMP surpasses that of ciprofloxacin, a widely used fluoroquinolone. This suggests that the compound could serve as an alternative treatment, particularly against ciprofloxacin-resistant bacterial strains. The ability to target DNA gyrase effectively while exhibiting minimal toxicity underscores its potential for further in-vitro and in-vivo studies.

Future prospects include the wet-lab experimental validation of the antimicrobial activity of compound DMP by microbiological assays like agar well diffusion and MIC assays in order to confirm the in-silico analysis predictions. Furthermore, to improve solubility and bioavailability, the compound needs modification, as flavonoid based drug candidates frequently need structural improvements for clinical application (Cushnie et al., 2011).

Conclusion

The study results have shown strong support towards antibacterial potential of the compound DMP. The high binding affinity (BA), drug-likeness properties and acceptable toxicity profile place it as favourable lead compound for developing new antibiotics. These results reveal the significance of in-silico approaches in enhancing drug discovery while decreasing experimental costs. Future study includes laboratory validation to confirm its antimicrobial potential.

REFERENCES

Ai, N., Fan, X. and Ekins, S., 2015. In silico methods for predicting drug–drug interactions with cytochrome P-450s, transporters and beyond. *Advanced drug delivery reviews*, *86*, pp.46-60.

Ali, S., Khan, F.I., Mohammad, T., Lan, D., Hassan, M.I. and Wang, Y., 2019. Identification and evaluation of inhibitors of lipase from Malassezia restricta using virtual high-throughput screening and molecular dynamics studies. *International journal of molecular sciences*, *20*(4), p.884.

Bikadi, Z. and Hazai, E., 2009. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *Journal of cheminformatics*, *1*, pp.1-16.

Chakraborty, A., Giri, S., Shah, A.D. and Adhikari, T., 2024. Phytochemistry and pharmacological potential of *Aloscasia macrorrhiza*: A comprehensive review. *Journal of Applied Pharmaceutical Research*, *12*(6), pp.73-87.

Cushnie, T.T. and Lamb, A.J., 2005. Antimicrobial activity of flavonoids. *International journal of antimicrobial agents*, *26*(5), pp.343-356.

Cushnie, T.T. and Lamb, A.J., 2011. Recent advances in understanding the antibacterial properties of flavonoids. International journal of antimicrobial agents, *38*(2), pp.99-107.

Daina, A., Michielin, O. and Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, *7*(1), p.42717.

Donadio, G., Mensitieri, F., Santoro, V., Parisi, V., Bellone, M.L., De Tommasi, N., Izzo, V. and Dal Piaz, F., 2021. Interactions with microbial proteins driving the antibacterial activity of flavonoids. *Pharmaceutics*, *13*(5), p.660.

Fernández-Cabal, J., Avilés-Betanzos, K.A., Cauich-Rodríguez, J.V., Ramírez-Sucre, M.O. and Rodríguez-Buenfil, I.M., 2025. Recent Developments in Citrus aurantium L.: An Overview of Bioactive Compounds, Extraction Techniques, and Technological Applications. *Processes*, *13*(1), p.120.

Górniak, I., Bartoszewski, R. and Króliczewski, J., 2019. Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry reviews*, *18*, pp.241-272.

Gulzar, M., Syed, S.B., Khan, F.I., Khan, P., Ali, S., Hasan, G.M., Taneja, P. and Hassan, M.I., 2019. Elucidation of interaction mechanism of ellagic acid to the integrin linked kinase. *International journal of biological macromolecules*, *122*, pp.1297-1304.

Guseva, E.V., Khromova, N.Y., Karetkin, B.A., Artemiev, A.I., Demkin, K.M., Puzankova, J.M., Shakir, I.V. and Panfilov, V.I., 2024. Biological value of berry polyphenols and prospects for supercritical extraction application for their isolation: A review.

Herlina, T., Nishinarizki, V., Hardianto, A. and Latip, J.B., 2025. Review on antibacterial flavonoids from genus Erythrina: Structure-activity relationship and mode of action. *Heliyon*, *11*(1).

Jendele, L., Krivak, R., Skoda, P., Novotny, M. and Hoksza, D., 2019. PrankWeb: a web server for ligand binding site prediction and visualization. *Nucleic acids research*, *47*(W1), pp.W345-W349.

Lagorce, D., Sperandio, O., Galons, H., Miteva, M.A. and Villoutreix, B.O., 2008. FAF-Drugs2: free ADME/tox filtering tool to assist drug discovery and chemical biology projects. *BMC bioinformatics*, *9*, pp.1-9.

Lawal, M., Verma, A.K., Umar, I.A., Gadanya, A.M., Umar, B., Yahaya, N. and Auwal, B., 2020. Analysis of new potent anti-diabetic molecules from phytochemicals of Pistia strateotes with Sglt1 and G6pc proteins of Homo Sapiens for treatment of diabetes mellitus. An in silico approach. *Silico Approach IOSR JPBS*, *15*, pp.59-73.

Lionta, E., Spyrou, G., K Vassilatis, D. and Cournia, Z., 2014. Structure-based virtual screening for drug discovery: principles, applications and recent advances. *Current topics in medicinal chemistry*, *14*(16), pp.1923-1938.

Lipinski, C.A., Lombardo, F., Dominy, B.W. and Feeney, P.J., 2012. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced drug delivery reviews*, *64*, pp.4-17.

Maxwell, A. and Lawson, D.M., 2003. The ATP-binding site of type II topoisomerases as a target for antibacterial drugs. *Current topics in medicinal chemistry*, *3*(3), pp.283-303.

Nikule, H.A., Nikam, T.D., Borde, M.Y., Pawar, S.D., Shelke, D.B. and Nitnaware, K.M., 2024. Phytochemical and pharmacological insights into Salacia chinensis L.(Saptarangi): an underexplored important medicinal plant. *Discover Plants*, *1*(1), pp.1-26.

Payne, D.J., Gwynn, M.N., Holmes, D.J. and Pompliano, D.L., 2007. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nature reviews Drug discovery*, *6*(1), pp.29-40.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C. and Ferrin, T.E., 2004. UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, *25*(13), pp.1605-1612.

Pires, D.E., Blundell, T.L. and Ascher, D.B., 2015. pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of medicinal chemistry*, *58*(9), pp.4066-4072.

Ramos, R.S., Macêdo, W.J., Costa, J.S., da Silva, C.H.D.P., Rosa, J.M., da Cruz, J.N., de Oliveira, M.S., de Aguiar Andrade, E.H., e Silva, R.B., Souto, R.N. and Santos, C.B., 2020. Potential inhibitors of the enzyme acetylcholinesterase and juvenile hormone with insecticidal activity: Study of the binding mode via docking and molecular dynamics simulations. Journal of Biomolecular Structure and Dynamics, 38(16), pp.4687-4709.

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B.S., Babalghith, A.O., Alshahrani, M.Y., Islam, S. and Islam, M.R., 2022. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. *BioMed research international*, *2022*(1), p.5445291.

Sengupta, S., Biswas, S., Pradhan, A. and Bhattacharya, M., 2024. Brief review study on citrus fruits: Blessing of nature. *Journal homepage: www. ijbpr. in*, *12*(3), pp.01-10.

Silver, L.L., 2007. Multi-targeting by monotherapeutic antibacterials. *Nature Reviews Drug Discovery*, *6*(1), pp.41-55.

Trott, O. and Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry*, *31*(2), pp.455-461.

Verma, A. K., Bala, H. A., Muhammad, I. I., Muhammad, A., Kori, A. R., & Barik, M., 2020. Virtual screening, molecular docking, pharmacokinetic, physicochemical and medicinal properties of potential curcumin derivatives against SARS-CoV-2 main protease (Mpro). *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 8(4), 153–179.

World Health Organization, 2014. *Antimicrobial resistance: global report on surveillance.* World Health Organization.

Zahidin, N.S., Saidin, S., Zulkifli, R.M., Muhamad, I.I., Ya'akob, H. and Nur, H., 2017. A review of Acalypha indica L.(Euphorbiaceae) as traditional medicinal plant and its therapeutic potential. Journal of ethnopharmacology, 207, pp.146-173.