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International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

In Silico Evaluation of Selected Phytochemicals Against Bacterial Protein Targets

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DOI : https://doi.org/10.55248/gengpi.07.1026.34075

ABSTRACT :

This study presents an in silico assessment of selected plant-derived phytochemicals against bacterial protein targets associated with surface localization, transport, and virulence. Protein structures were retrieved from the Protein Data Bank (PDB), UniProt, AlphaFold, and SWISS-MODEL, while candidate phytochemicals were obtained from the IMPPAT database. OmpF (UniProt ID: P13794) was obtained as an AlphaFold Monomer v2.0 predicted structure, whereas the ABC transporter substrate-binding protein (Q2G2D8) and enterotoxin family protein (Q2FXX5) were modelled using SWISS-MODEL. Kaempferol, Curcumin, and Quercetin were evaluated by molecular docking against the three protein targets using CB-Dock2. The most favourable predicted interaction was observed between quercetin and enterotoxin, with a Vina docking score of −7.2 kcal/mol. These findings indicate that the selected phytochemicals show computationally predicted interactions with bacterial protein targets and support their prioritization for subsequent experimental investigation. Because docking scores are computational estimates, in vitro and in vivo validation is required before biological or therapeutic conclusions can be established.

Keywords: *phytochemicals; molecular docking; quercetin; kaempferol; curcumin; OmpF; ABC transporter; enterotoxin*

Introduction

Phytochemicals are structurally diverse, naturally occurring compounds produced by plants and are broadly represented by classes including alkaloids, flavonoids, phenolic compounds, terpenoids, tannins and saponins. They have attracted considerable interest because of their ability to display biological effects through interactions with cellular membranes, enzymes, receptors and other molecular targets. Bacteria are unicellular prokaryotic microorganisms whose cell-envelope composition forms the basis of their classification into Gram-positive and Gram-negative groups. Gram-positive bacteria are characterized by a thick, multilayered peptidoglycan cell wall containing teichoic acids and lacking an outer membrane, with surface-associated proteins contributing to adhesion, colonization, nutrient acquisition and virulence. Representative surface proteins include protein A of *Staphylococcus aureus*, M protein of *Streptococcus pyogenes*, pneumococcal surface protein A (PspA), internalins and S-layer proteins. In contrast, Gram-negative bacteria possess an inner cytoplasmic membrane which is a comparatively thin peptidoglycan layer, and an extra outer membrane containing lipopolysaccharide and diverse outer-membrane proteins. These proteins contribute to membrane permeability, transport, structural stability, host interaction, and virulence, with representative targets including outer membrane protein A (OmpA), outer membrane porin F (OmpF), outer membrane porin C (OmpC), OmpX, and TolC.

Surface localization and functional importance of bacterial proteins make them relevant molecular targets for investigating the potential antibacterial activity of plant-derived compounds. Interactions between phytochemicals and bacterial proteins can be investigated using in silico and bioinformatic approaches, which enable the structural characterization and computational evaluation of ligand–protein interactions before experimental validation. Protein sequence and functional information can be obtained from UniProt (Universal Protein Resource), while experimentally determined three-dimensional structures are available through PDB (Protein Data Bank). Information regarding medicinal plants and their phytochemical constituents and associated therapeutic properties can be found through IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics). Molecular docking gives a computational framework for evaluating the potential binding of phytochemicals to selected bacterial protein targets by estimating binding affinity and identifying favourable ligand-binding orientations. Such approaches can be used to prioritize phytochemical–protein interactions for subsequent experimental investigation, although predicted docking scores represent computational estimates and cannot independently establish antibacterial efficacy.

This study focuses on the structural characterization of selected bacterial surface-associated and virulence-related proteins and their potential interactions with plant-derived phytochemicals. Protein structures were identified and retrieved from the PDB, while protein sequence and functional information were obtained through UniProt. Candidate phytochemicals and their associated information were investigated using the IMPPAT database and subsequently evaluated

as potential ligands for the selected bacterial protein targets. Molecular docking was performed to examine the binding orientations and predicted affinities of the phytochemical–protein complexes, with the resulting docking scores compared to identify interactions with more favourable predicted binding. Among the interactions evaluated, quercetin demonstrated the most negative docking score with enterotoxin, with a predicted binding score of −7.2 kcal/mol which represents the most favourable predicted interaction obtained in the docking analysis. These findings provide a computational assessment of potential interactions between plant-derived phytochemicals and bacterial protein targets and may assist in prioritizing compounds and protein targets for subsequent experimental investigation.

Material and Method

Retrieval of Protein Structures from PDB Database

The 3D structures of the selected bacterial protein targets were retrieved from the PDB, and their corresponding identification codes were recorded for further analysis. The selected protein targets included OmpF, ABC protein and enterotoxin.

Protein Structure Modelling Using SWISS-MODEL

When suitable experimentally determined protein structures were unavailable in the PDB database, three-dimensional protein modelling was performed using SWISS-MODEL. SWISS-MODEL is a web-based platform that generates three-dimensional protein structures based on suitable structural templates. In the present study, the structures of ABC protein (UniProt ID: Q2G2D8) and enterotoxin (UniProt ID: Q2FXX5) were generated using SWISS-MODEL due to the unavailability of suitable experimentally determined structures for the selected sequences. The resulting structures were subsequently used as protein targets for molecular docking analysis.

Retrieval of Phytochemicals from IMPPAT Database

The selected phytochemicals were retrieved from the Indian IMPPAT database. The molecular structures of the selected phytochemicals were obtained and prepared as ligand molecules for subsequent molecular docking analysis. The selected phytochemicals were used to investigate their potential interactions with the bacterial protein targets obtained from PDB or modelled using SWISS-MODEL.

Retrieval of OmpF Structure from AlphaFold

The 3D structure of OmpF (UniProt ID: P13794) was obtained as an AlphaFold Monomer v2.0 predicted structure. The predicted structure was used as the protein model for subsequent preparation and molecular docking analysis.

Result

Outer membrane porin F

The Outer membrane porin F (OmpF) protein, corresponding to UniProt ID P13794, was obtained as an AlphaFold Monomer v2.0 predicted structure. The predicted three-dimensional structure was visualized using RasMol.

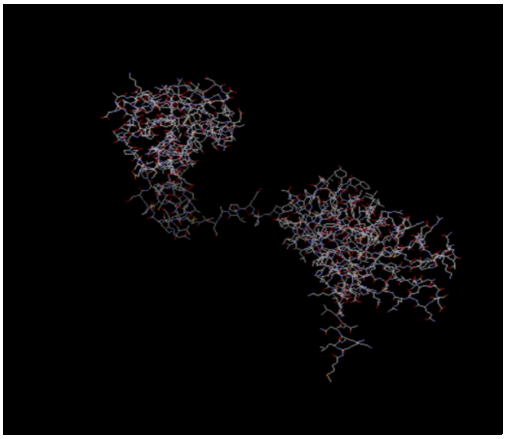


Figure 1. **Predicted three-dimensional structure of OmpF (UniProt ID: P13794) visualized using RasMol**

Enterotoxin Family Protein

The Enterotoxin Family Protein, corresponding to UniProt ID Q2FXX5, was obtained using the SWISS MODEL. The predicted three-dimensional structure was visualized using RasMol.

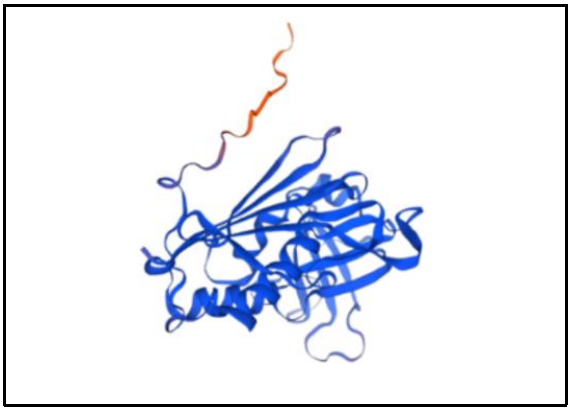


Figure 2. **SWISS-MODEL predicted three-dimensional structure of the enterotoxin family protein (UniProt ID: Q2FXX5).**

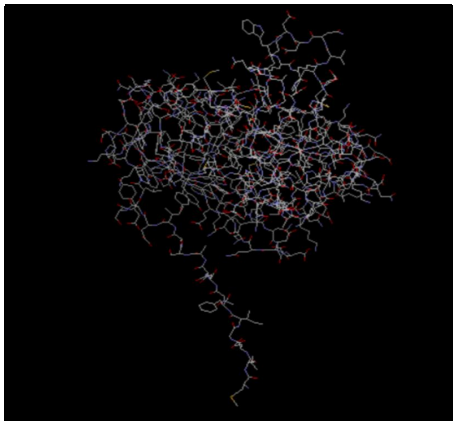


Figure 3. **RasMol visualization of the predicted enterotoxin family protein structure.**

ABC transporter substrate-binding protein, putative

The ABC transporter substrate-binding protein, putative, corresponding to UniProt ID Q2G2D8, was obtained using SWISS MODEL. The predicted three-dimensional structure was visualized using RasMol.

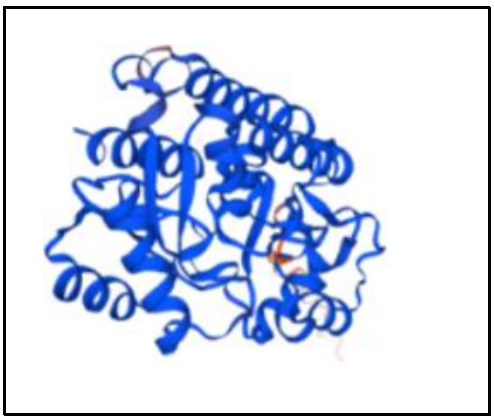


Figure 4. SWISS-MODEL predicted three-dimensional structure of the ABC transporter substrate-binding protein (UniProt ID: Q2G2D8).

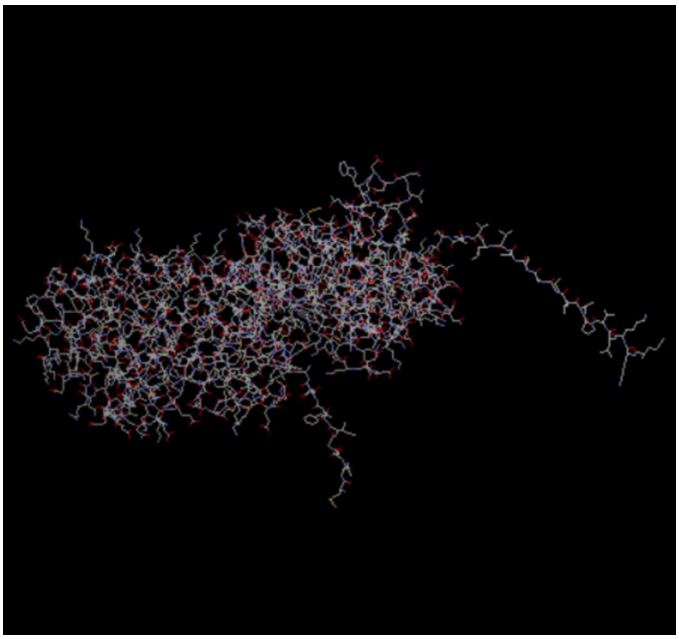


Figure 5. RasMol visualization of the predicted ABC transporter substrate-binding protein structure.

Kaemferol

Kaempferol- IMPPAT Phytochemical identifier: IMPHY004388.

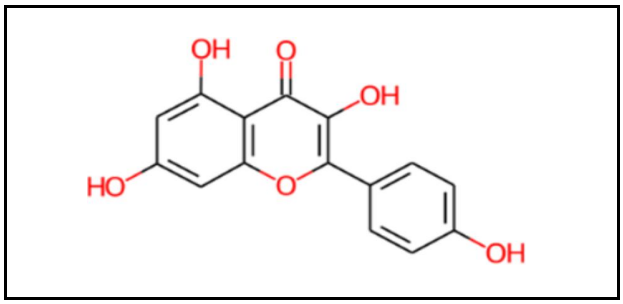


Figure 6. Molecular structure of kaempferol retrieved from the IMPPAT database.

Kaempferol is a tetrahydroxyflavone in which the four hydroxy groups are located at positions 3, 5, 7 and 4'. Acting as an antioxidant by reducing oxidative stress, it is currently under consideration as a possible cancer treatment.

Curcumin

Curcumin- IMPPAT Phytochemical identifier: IMPHY007574.

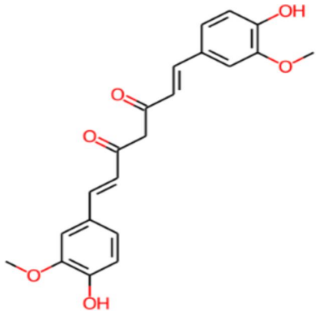


Figure 7. Molecular structure of curcumin retrieved from the IMPPAT database.

Curcumin is a beta-diketone that is methane in which two of the hydrogens are substituted by feruloyl groups. A natural dyestuff found in the root of *Curcuma longa*.

Quercetin

Quercetin- IMPPAT Phytochemical identifier: IMPHY004619.

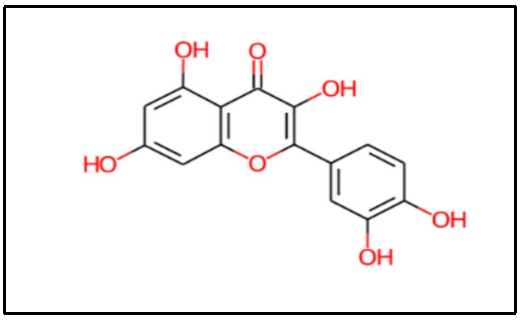


Figure 8. Molecular structure of quercetin retrieved from the IMPPAT database.

Quercetin is a pentahydroxyflavone having the five hydroxy groups placed at the 3-, 3'-, 4'-, 5-and 7-positions. It is one of the most abundant flavonoids in edible vegetables, fruit, and wine.

Docking of Phytochemicals with Enterotoxin

Enterotoxin and Kaempferol

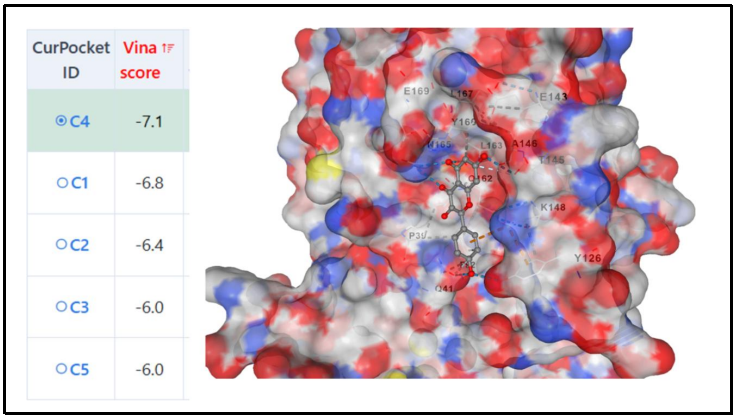


Figure 9. Docking interaction between enterotoxin and kaempferol.

Enterotoxin and Curcumin

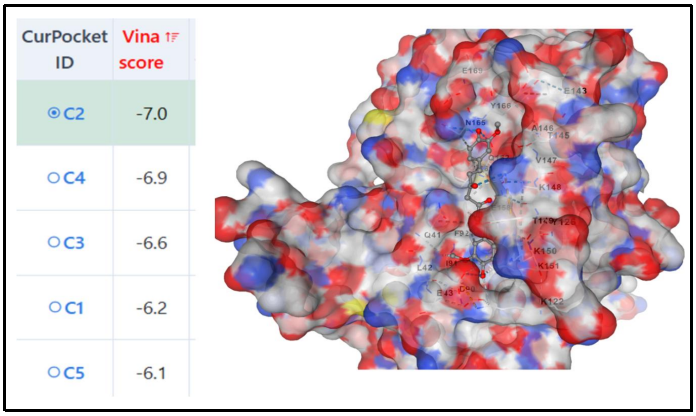


Figure 10. Docking interaction between enterotoxin and curcumin. Enterotoxin and Quercetin

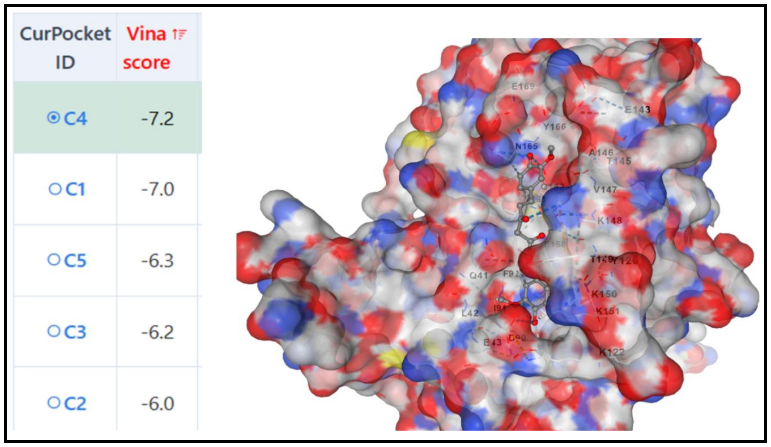


Figure 11. Docking interaction between enterotoxin and quercetin.

Docking of Phytochemicals with ABC Transporter

ABC transporter and Kaempferol

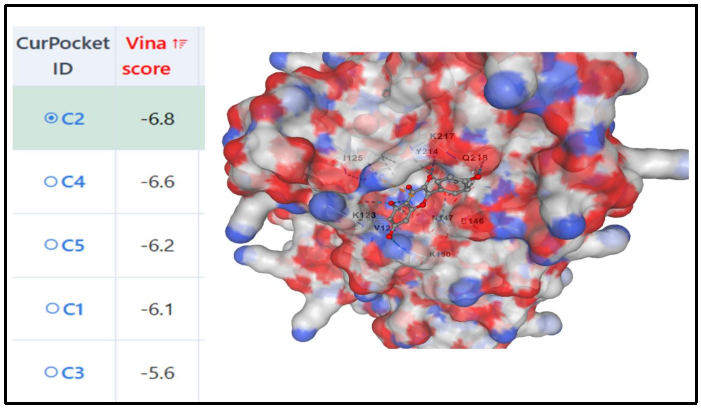


Figure 12. Docking interaction between the ABC transporter protein and kaempferol. ABC Transporter and Curcumin

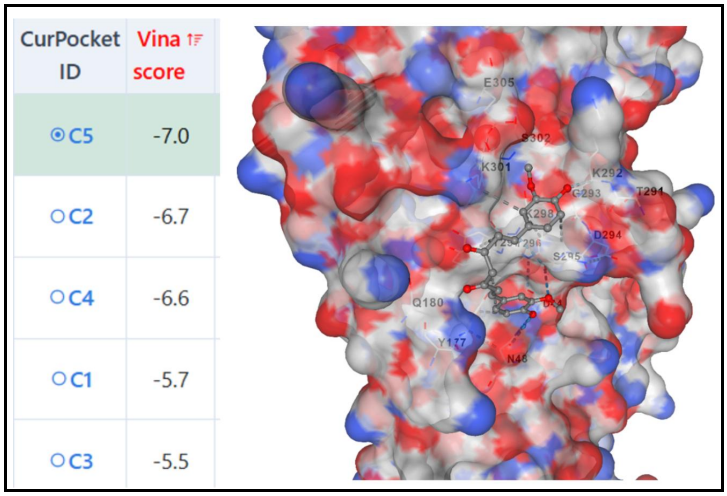


Figure 13. Docking interaction between the ABC transporter protein and curcumin.

ABC transporter and Quercetin

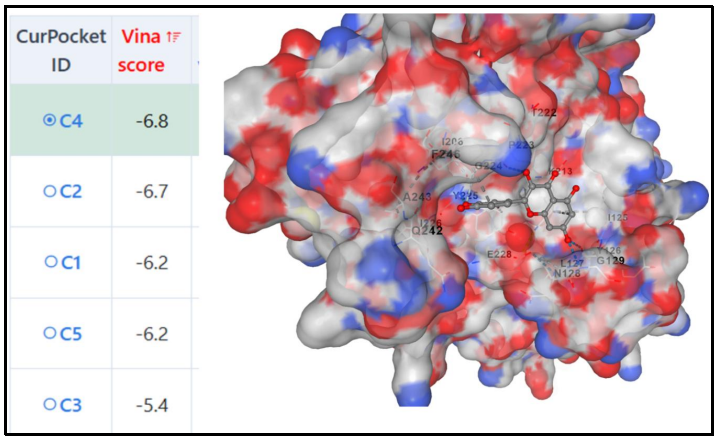


Figure 14. Docking interaction between the ABC transporter protein and quercetin.

Docking of Phytochemicals with OmpF

OmpF and Kaempferol

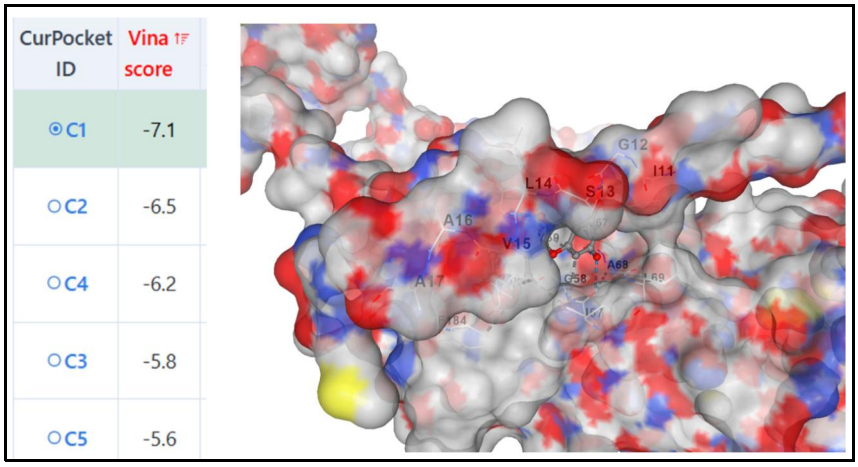


Figure 15. Docking interaction between OmpF and kaempferol.

OmpF and Curcumin

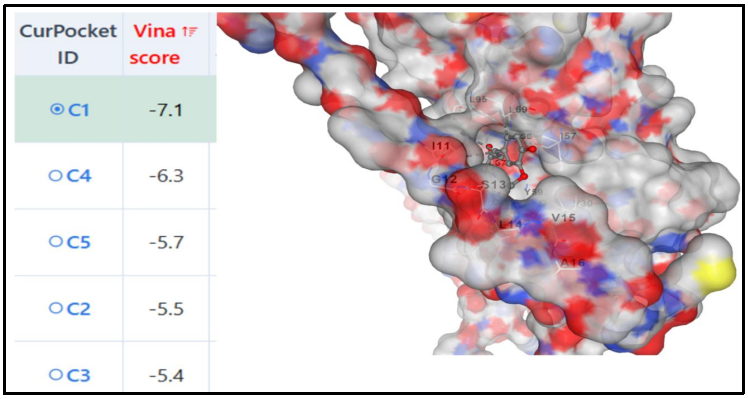


Figure 16. Docking interaction between OmpF and curcumin. OmpF and Quercetin

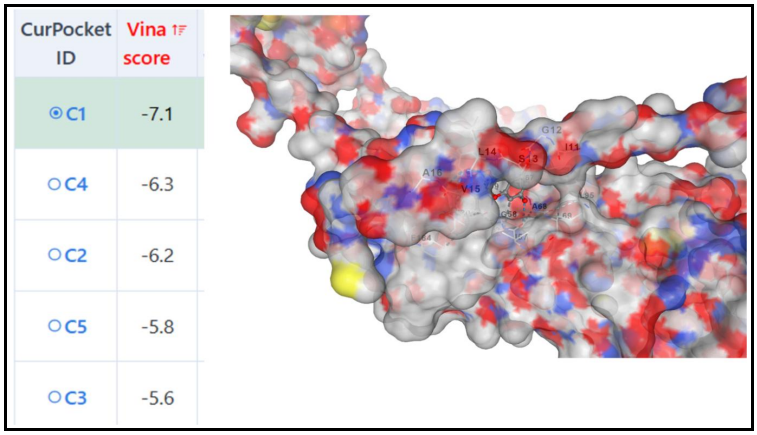


Figure 17. Docking interaction between OmpF and quercetin.

Comparative Analysis of Docking Scores and Binding Interactions

Protein target	Kaempferol	Curcumin	Quercetin
Enterotoxin	-7.1	-7.0	-7.2
ABC protein	-6.8	-7.0	-6.8
OmpF	-7.1	-7.1	-7.1

The most negative values are Enterotoxin and Quercetin, with a score of -7.2.

Discussion

"Evaluation of broad-spectrum antibacterial efficacy of quercetin by molecular docking, molecular dynamics simulation and in vitro studies" investigated the interaction of quercetin with four bacterial virulence-related proteins: Sortase B, Toxic Shock Syndrome Toxin-1, AdeJ and LasR. The study combined molecular docking with molecular dynamics and *in vitro* antibacterial testing to evaluate the potential of quercetin as an antibacterial compound. In contrast, the present study evaluates quercetin and other selected phytochemicals against OmpF, ABC protein and enterotoxin using CB-Dock2 for molecular docking.

"Synergistic antibacterial activity of *Phyllanthus emblica* fruits and its phytocompounds with ampicillin" investigated phytocompounds including ellagic acid, quercetin and rutin against penicillin-binding protein 3 (PBP3) using molecular docking, molecular dynamics and experimental antibacterial analysis. Unlike this study, the present study focuses on different bacterial protein targets (OmpF, ABC protein and enterotoxin) and compares the predicted binding of selected phytochemicals using CB-Dock2. The most favourable interaction observed in the present analysis was quercetin with enterotoxin, with a docking score of −7.2 kcal/mol.

Conclusion

This in-silico study demonstrated that selected phytochemicals can interact with bacterial protein targets and showed differences in their predicted binding affinities. Among the phytochemicals evaluated quercetin demonstrated the most favourable predicted interaction with enterotoxin with a Vina score of −7.2 kcal/mol. The study demonstrates the usefulness of phytochemical-based molecular docking as an initial screening approach for identifying promising phytochemical–protein interactions and prioritizing compounds for further investigation. Such computational screening can contribute to future drug discovery and lead identification by narrowing down potential compounds before experimental testing. The identified phytochemical–protein interactions may also provide a basis for further clinical research including experimental validation of antibacterial activity, mechanism of action, and potential therapeutic applications. However, further *in vitro* and *in vivo* studies are required to establish the biological and clinical relevance of the predicted interactions.

REFERENCES

1. Majumdar, G., & Mandal, S. (2024). Evaluation of broad-spectrum antibacterial efficacy of quercetin by molecular docking, molecular dynamics simulation and in vitro studies. *Chemical Physics Impact*, *8*, 100501.
2. Saini, R., Kumar, V., Patel, C. N., Sourirajan, A., & Dev, K. (2024). Synergistic antibacterial activity of Phyllanthus emblica fruits and its phytocompounds with ampicillin: a computational and experimental study. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *397*(2), 857-871.
3. Mohanraj, K., Karthikeyan, B. S., Vivek-Ananth, R. P., Chand, R. B., Aparna, S. R., Mangalapandi, P., & Samal, A. (2018). IMPPAT: A curated database of I ndian M edicinal P lants, P hytochemistry A nd T herapeutics. *Scientific reports*, *8*(1), 4329.
4. Vivek-Ananth, R. P., Mohanraj, K., Sahoo, A. K., & Samal, A. (2023). IMPPAT 2.0: an enhanced and expanded phytochemical atlas of Indian medicinal plants. *ACS omega*, *8*(9), 8827.
5. Arnold, K., Bordoli, L., Kopp, J., & Schwede, T. (2006). The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics*, *22*(2), 195-201.
6. Bordoli, L., Kiefer, F., Arnold, K., Benkert, P., Battey, J., & Schwede, T. (2009). Protein structure homology modeling using SWISS-MODEL workspace. *Nature protocols*, *4*(1), 1-13.
7. Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., ... & Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Research*, *46*(W1), W296-W303.
8. Mendie, L. E., & Hemalatha, S. (2022). Molecular docking of phytochemicals targeting GFRs as therapeutic sites for cancer: an in silico study. *Applied biochemistry and biotechnology*, *194*(1), 215-231.
9. Terefe, E. M., & Ghosh, A. (2022). Molecular docking, validation, dynamics simulations, and pharmacokinetic prediction of phytochemicals isolated from Croton dichogamus against the HIV-1 reverse transcriptase. *Bioinformatics and biology insights*, *16*, 11779322221125605.
10. Wahyuni, D. K., Nuha, G. A., Atere, T. G., Kharisma, V. D., Tari, V. S., Rahmawati, C. T., ... & Purnobasuki, H. (2024). Antimicrobial potentials of Pandanus amaryllifolius Roxb.: Phytochemical profiling, antioxidant, and molecular docking studies. *Plos one*, *19*(8), e0305348.