



Full Length Article

Exploring the synergistic therapeutic potential of *Morus alba* extract in tuberculosis: A computational analysis

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ARTICLE INFO

Keywords:

Natural compounds
Morus alba
 Antitubercular activity
 Docking
 Interaction study

ABSTRACT

Objectives: This study validates the synergistic impact hypothesis of *Morus alba* phytochemicals on *Mycobacterium* via molecular docking and simulation.

Methods: Phytochemicals (Petunidin-3-rutinoside, Beta-sitosterol, Ecdysterone, Quercetin-3'-glucoside, Quercitrin, Rutin, Scopolin) were tested against key metabolic proteins (MmpL3, DprE1, UgpABCE transporter, porins, OmpATb) of *Mycobacterium*. Additional proteins (CmaA2, oxidoreductase, FABH, Enoyl-ACP reductase, LpqN) were also included. Docked complexes were analyzed via MD trajectories (RMSD, RMSF, hydrogen bonds) over 100 ns.

Results: Binding affinity of phytochemicals with 1UUN (Beta-sitosterol: -9.12692 , Ecdysterone: -12.11162 kcal/mol) and 2KGS (Beta-sitosterol: -6.93) reflects the easy entry of phytochemicals. Phytochemicals interact within cells, inhibiting metabolic pathways and microbial growth. Beta-sitosterol (-7.1 kcal/mol) affects the mycolic acid transfer. Beta-sitosterol (-11.21 kcal/mol) and Ecdysterone (-9.15 kcal/mol) affect the ribose oxidase pathway. Both compounds also show affinity with UgpABCE transporter. During simulation studies, results showed that the average protein RMSD for the 6MNA-Beta-sitosterol, 6MNA-Scopolin, 4P8T-Beta-sitosterol, and 4P8T-Ecdysterone complexes were 1.87 \AA , 2.28 \AA , 2.34 \AA , and 2.61 \AA , respectively. The stability of each complex in dynamic states is ensured by low and steady RMSD variance. In addition, we have endeavored to prove the hypothesis of synergistic application by incorporating our latest findings into the data.

Conclusions: Concept of synergistic impact of phytochemicals can be a promising source of treatment after proper lab validation.

1. Introduction

Millions die annually from microbial infections, exacerbated by antibiotic ineffectiveness due to poor intracellular penetration and rising drug resistance (Tucker et al. 2021). Besides this, new resistant strains reduce medication efficacy or render them useless (Khameneh

et al. 2019). Effective antibiotics demand continuous investment and innovative approaches like combination therapy, yet their success hinges on understanding synergistic interactions between drugs (Sharma et al., 2019). However, combining drugs may not boost efficacy without knowing complementary mechanisms or synergistic interactions. Comprehensive microbial and host biology knowledge is

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<https://doi.org/10.1016/j.jksus.2024.103371>

Received 15 January 2024; Received in revised form 23 July 2024; Accepted 24 July 2024

Available online 26 July 2024

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crucial for targeting essential microbial components and resistance mechanisms (Srivastava et al., 2021; Kumar et al., 2021).

Molecular understanding guides effective drug combinations that minimize resistance (Manna and Shil, 2020; Róg et al., 2021; Berríos-Caro et al., 2021). Computational methods like molecular docking and MD simulations streamline drug discovery, predicting interactions and optimizing treatments cost-effectively. Analyzing *Morus alba*'s bioactive compounds computationally aims to uncover synergies against *Mycobacterium tuberculosis* (Mtb), offering promising candidates for experimental validation. This approach accelerates novel TB treatment strategies, emphasizing translational impact in pharmaceutical research.

2. Materials and methods

2.1. Hypothesis preparation

TB is primarily caused by Mtb, a Gram-positive, acid-fast bacterium known for its thick, lipid-rich cell envelope that contributes to drug resistance. Even when drugs penetrate this envelope, they can lose effectiveness due to enzymatic or structural modifications. Therefore, evaluating drug efficacy across multiple levels is crucial. This study focus on analyzing how selected metabolites interact with key proteins involved in various metabolic pathways of Mtb. Our objective was to assess the collective impact of these metabolites on Mtb's metabolism.

2.2. Selection of Mtb proteins as target

The present study evaluates targets within the mycobacterial cell using five proteins sourced from the RCSB PDB database: Mtb porins (PDB ID: 1UUN), OmpATb (PDB ID: 2KGS) Mycolic acid transporter protein MmpL3 (PDB ID: 7NVH), Ribose oxidase DprE1 (PDB ID: 4P8T), and UgpABCE transporter (PDB ID: 4MFI) (<https://www.rcsb.org/>).

2.3. Selection of ligands

From the previous investigation, 22 phytochemicals from *Morus alba* were screened against five target proteins of Mtb using *in-silico* tools. Out of these, seven phytochemicals exhibited promising activity against all five target proteins and were selected for further investigation.

2.4. Molecular docking studies

Molecular docking was conducted using Autodock, following the protocol described by Khan et al. (2023). Protein preparation included the removal of heteroatoms, addition of polar hydrogens, and Kollman charges, followed by saving in pdbqt format. Additionally, hydrogen atoms were added to enhance ligand stability in the selected protein structures. Grid coordinates were defined to specify the docking grid-box. Docking experiments were performed under default settings in normal mode, and binding energy predictions (ΔG -score) were recorded for each ligand against the respective proteins.

2.5. Molecular dynamics (MD) simulation

MD simulation studies over 100 ns, were conducted for the best docking poses of beta-sitosterol, scopolin, and ecdysterone complexes with the proteins LpqN (PDB ID: 6MNA) and DprE1 (PDB ID: 4P8T) using the Schrödinger Desmond 6.9.137 MD simulation program (version 2022–1). The simulations were performed on a Z4 HP workstation with Ubuntu 22.04.2 LTS, Intel Xeon W-2245 @ 3.90 GHz, 8 cores, CUDA 12, and NVIDIA RTX A4000 GPU. MD simulations utilized the OPLS3e force field and SPC solvation model. Simulation details and experimental protocols followed previously published methodologies (Puri et al. 2023; Shaw, 2020-3). Desmond's Simulation Interaction Diagram (SID) was employed to analyze ligand binding orientation and

stability based on MD trajectories (Desai et al. 2023).

3. Results and discussion

3.1. Background and hypothesis based on anti-mycobacterial activity of *M. alba*

The anti-inflammatory, liver and kidney-protective, hypotensive, diuretic, anti-cough, anticancer, and analgesic properties of *Morus alba* L. extract make it a compelling subject for study. Hence, Dried *M. alba* leaves were extracted with methanol, ethyl acetate, and chloroform (1:10 ratio) and tested for anti-tubercular activity using the Resazurin microtitre assay (REMA), as described in our previous study (Khan et al. 2023) and compared with the control drugs. Encouraging outcomes led to the hypothesis of synergistic effects of phytochemicals. The idea behind this approach is that diverse compounds can target multiple pathways in the pathogen, potentially preventing resistance development. We explored their potential for combination therapy, detailing our stepwise efforts below.

3.2. Target proteins

Porins and porin-like protein OmpATb were selected for study, as they facilitate hydrophilic nutrient passage across mycobacterial outer membranes. (Faller et al. 2004). The MmpL family facilitates lipid export from mycobacterial cytoplasm to periplasm (Melly and Purdy, 2019), another key focus of the study. The study also targets decaprenylphosphoryl-D-ribose oxidase and the UgpABCE transporter, enzymes crucial for mycobacterial arabinogalactan production (Suma et al. 2020). Additionally, five proteins from our previous study (Khan et al. 2023) were included to explore synergistic phytochemical effects: Mtb Mycolic Acid Cyclopropane Synthase CmaA2 (PDB ID: 3HEM), oxidoreductase (PDB ID: 4OTK), A246F mutant of beta-ketoacyl-acyl carrier protein synthase III (FABH) (PDB ID: 2QO0), Enoyl-ACP(CoA) reductase (PDB ID: 2AQ1), and LpqN involved in cell envelope biogenesis (PDB ID: 6MNA).

3.3. Target ligand interaction studies via docking approach

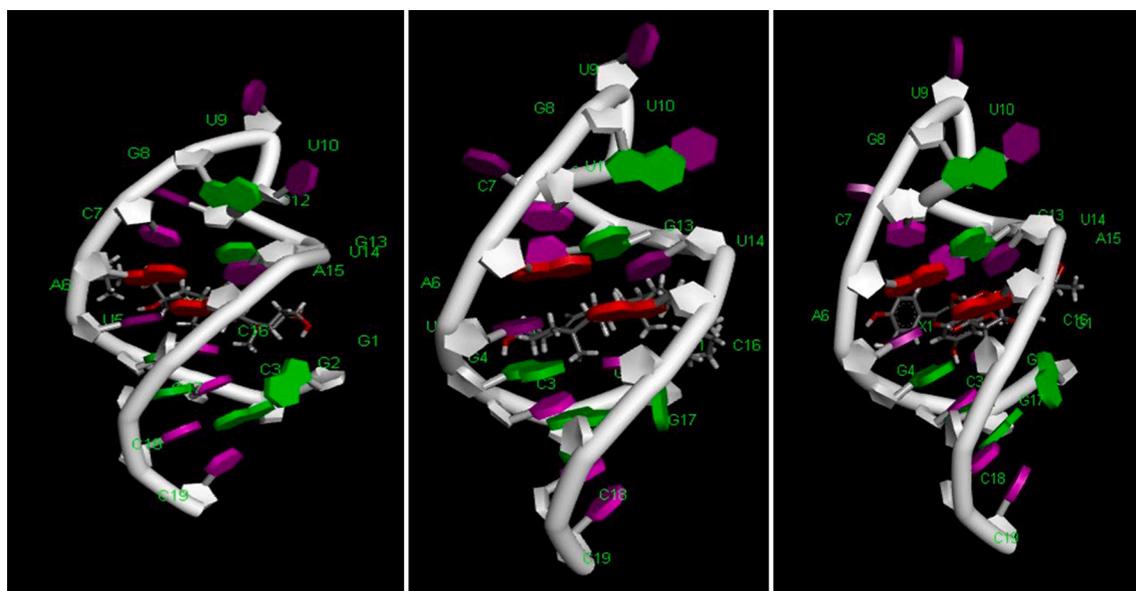
In our earlier work, MTT assay was used to confirm the antitubercular activity of *M. alba* extracts before *in-silico* investigations to evaluate their activity against target proteins. Here, we extended our analysis to explore the synergistic effect hypothesis by comparing the activity of the top seven compounds from the prior study against five more proteins and summarized results in Table 1. Selected compounds already have some bioactivity like sitosterol and rutin for antimicrobial, anticancer, anti-inflammatory, hepatoprotective, lipid-lowering effect, anxiolytic & sedative effects, analgesic, immunomodulatory; Ecdysterone increases protein synthesis in skeletal muscle (Khan et al. 2023; Babu, & Jayaraman, 2020).

3.4. Entry of phytochemicals into *Mycobacterium*

The docking results of *Mycobacterium* proteins (1UUN, 2KGS, 7NVH, 4P8T, 4MFI) and the phytochemicals of *Morus alba* are summarized in Table 1. The Mtb cell's thick, hydrophobic wall poses initial penetration challenges. Research indicates that porins may aid hydrophilic antibiotics' diffusion through this barrier, facilitating cell entry (Gygli et al., 2017). Similarly, OmpATb in Mtb, changes pH, blocking hydrophilic drug entry thus inhibiting OmpATb with specific phytochemicals can facilitate the entry of other phytochemicals (Kwofie et al., 2020). Docking results of the selected phytochemicals range from -8.06 (Scopolin) to -12.11 (Ecdysterone) for porin and -6.93 (Beta-sitosterol) to -1.65 (Petunidin-3-rutinoside) for OmpATb, suggesting high affinity of these phytochemicals for these surface proteins. According to hypothesis, inhibition of OmpATb with Beta-sitosterol can facilitate

Table 1
Interactions of phyto compounds with target protein in terms of Docking Energy (DG in Kcal/mole).

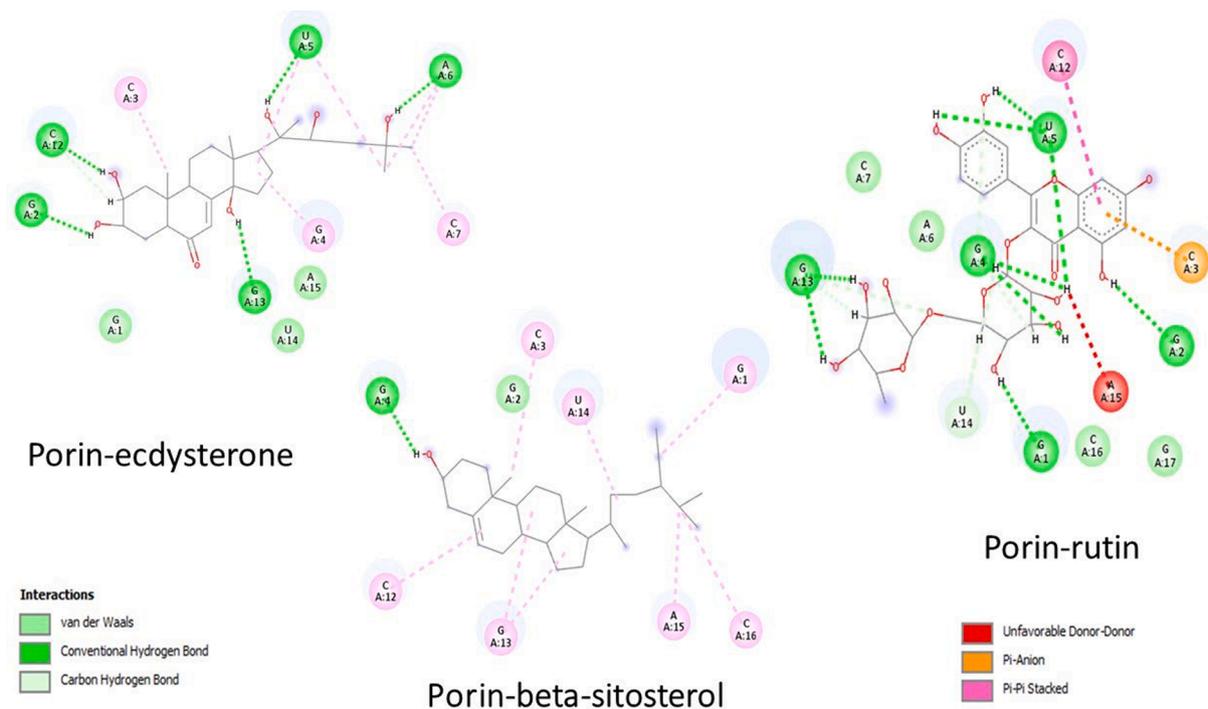
S. No.	Ligand Phyto compounds		study executed docking energy (Kcal/mole)					Protein targets literature (Khan et al., 2023) docking energy (Kcal/mole)				
	Name	ID	1UUN	2KGS	7NVH	4P8T	4MFI	3HEM	4OTK	2Q00	2AQI	6MNA
1.	Petunidin-3-rutinoside	101949843	-8.070	-1.65	-6.9	-7.81	-4.8	-8.2	-6.9	-9.0	-8.3	-7.8
2.	Beta-sitosterol	222284	-9.126	-6.93	-7.1	-11.21	-9.51	-9.0	-6.9	-7.4	-8.7	-6.7
3.	Ecdysterone	5459840	-12.111	-3.76	21	-9.15	-8.0	-7.5	-7.1	-8.2	-8.7	-6.3
4.	Quercetin-3'-glucoside	25203368	-8.250	-4.61	-6.6	-8.34	-6.86	-6.7	-7.6	-7.6	-7.6	-6.4
5.	Quercitrin	5280459	-8.308	-5.15	-6.9	-7.45	-6.92	-7.6	-7.6	-7.9	-7.4	-7.4
6.	Rutin	5280805	-9.832	-4.04	35.6	-7.05	-6.67	-7.8	-7.5	-9.1	-9.3	-6.9
7.	Scopolin	439,514	-8.068	-4.88	-2.7	-3.11	-6.63	-8.7	-6.9	-7.7	-7.4	-6.2



Porin-ecdysterone

Porin-beta-sitosterol

Porin-rutin



Porin-ecdysterone

Porin-rutin

Porin-beta-sitosterol

Fig. 1. A & Fig. 1b: Interaction of ecdysterone, beta-sitosterol and rutin with porins.

the entry of other phytochemicals showcasing a synergistic effect. The interactions of ecdysterone, beta-sitosterol and rutin with porins are summarised in Fig. 1A and 1B suggesting the significant role of covalent interactions in the attachment of these phytochemicals to the porin protein.

Hydrophobic compounds can pass through bacterial membranes by dissolving in the lipid bilayer, but hydrophilic compounds require protein channels like porins (MspA in *M. smegmatis*). Mtb uses porins like OmpATb in its outer membrane to facilitate hydrophilic solute entry (Niederweis, 2003). OmpATb is crucial for Mtb's survival in acidic environments. Kwofie et al. (2020) identified natural products that inhibit OmpATb, potentially disrupting Mtb's nutrient uptake and survival, offering new avenues for tuberculosis treatment development.

3.5. Action on different metabolic pathways

After entering, phytochemicals target vital biosynthetic pathways, crucial for the pathogen's structural and functional integrity. The absence of targeting proteins/pathways in the host or gut microbes will minimize the side effects (Maurya et al. 2022). The cell wall of Mtb, absent in humans and distinct from normal gut flora, is a prime target for drugs like isoniazid and ethionamide (Bolla, 2020).

3.6. Cell envelope biogenesis

The study also emphasizes Mycobacterial membrane protein large-3 (MmpL3; PDB ID: 7NVH), crucial for transporting precursors (trehalose monomycolates) in cell-envelope biogenesis. The docking results indicate that only Beta-sitosterol (−7.1) Petunidin-3-rutinoside (−6.9), Quercitrin (−6.9) and Quercetin-3'-glucoside (−6.6) exhibit a good affinity towards this protein, showcasing their potential to inhibit Mycobacterial cell envelope biogenesis. Fig. 2 summarizes the interaction of

these phytochemicals with MmpL3, highlighting the significant role of covalent interactions. MmpL3 facilitates the transport of TMM across the inner membrane and has been a critical target for various preclinical agents (Bolla, 2020).

3.7. Arabinogalactan biosynthesis

DprE1 (Decaprenylphosphoryl-β-D-ribose 2'-epimerase), crucial for mycobacterial cell-wall synthesis, is a significant target. DprE1, a flavoprotein, catalyzes the oxidation of decaprenylphosphoryl-D-ribose (DPR) to decaprenylphosphoryl-2-ketoribose (DPX), which subsequently reduced to decaprenylphosphoryl arabinose (DPA) by enzyme, DprE2. Arabinosyltransferase converts DPA into arabinogalactan and lipoarabinomannan, key components of Mtb cell-wall (Mali et al., 2022). Out of seven, two phytochemicals- Beta-sitosterol and Ecdysterone with docking energy −11.21 and −9.15 Kcal/mole, respectively show their affinity to bind with DprE1 enzyme (4P8T) (Table 1) indicating their potential to inhibit the normal functioning of DprE1. In both cases, PHE and GLN residues of the active site form conventional hydrogen bonds (Fig. 3). Mali et al. (2022) reported that non-covalent inhibitors (2-carboxyquinoxalines) possess 2-carboxylate moiety crucial for hydrogen-bond formations with the sidechain of Lysine-418 and the hydroxyl group of Tyrosine-60. In the current work, tyrosine and leucine are also found to form hydrogen bonds with Petunidin-3-rutinoside, indicating a similar inhibition mechanism as for 2-carboxyquinoxalines.

3.8. Peptidoglycan biosynthesis

The absence of peptidoglycan biosynthesis in humans makes it a promising target to inhibit Mtb (Isa and Mohammed 2021). This study focuses on the final steps of the UDP-N-acetylmuramic acid formation, catalyzed by UDP-N-acetylenolpyruvoylglucosamine reductase (MurB).

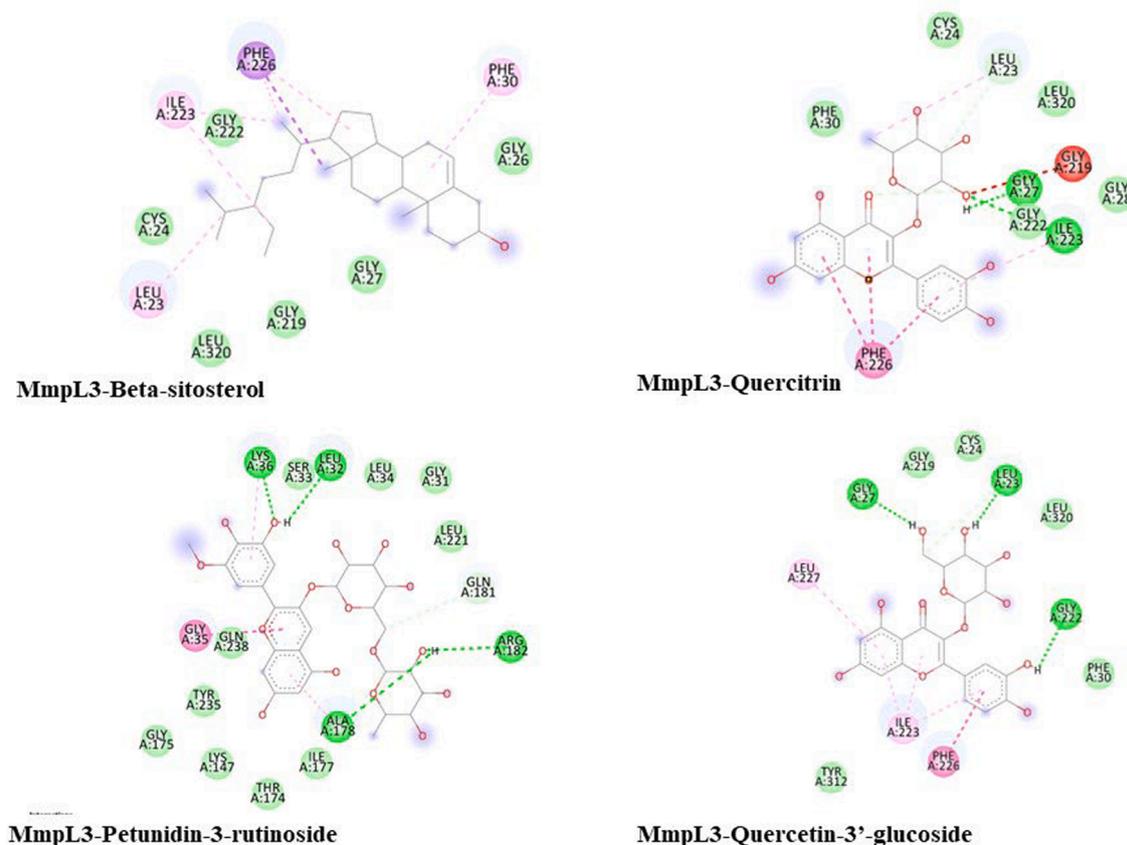


Fig. 2. Interaction of beta-sitosterol, Petunidin-3-rutinoside, quercitrin and Quercetin-3'-glucoside with MmpL3.

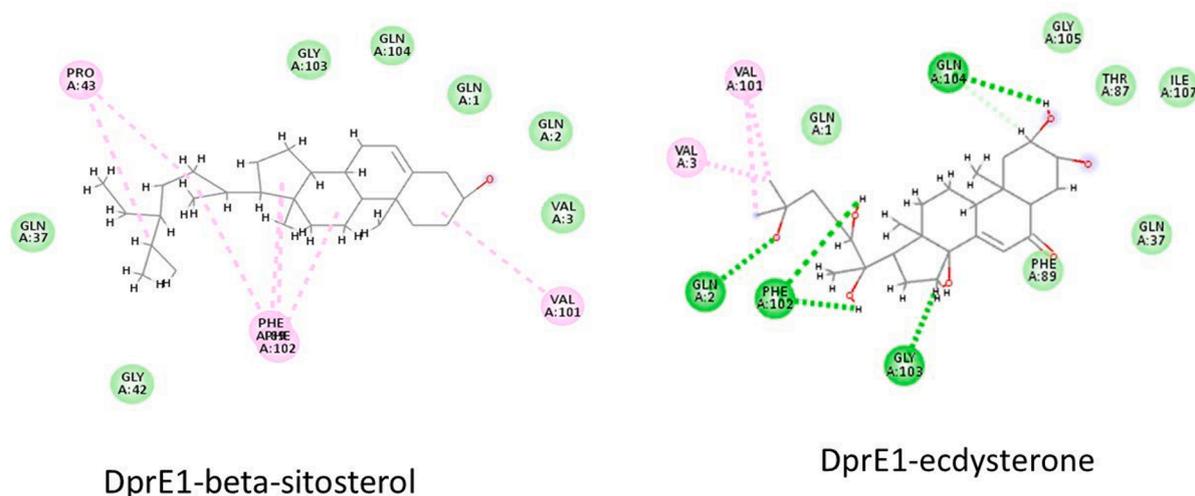


Fig. 3. Interaction of beta-sitosterol and ecdysterone with DprE1.

MurB is a Flavoprotein with a flavin adenine dinucleotide (FAD) binding structure, belonging to the superfamily of FAD-binding proteins (Murzin, 1996). Hence the peptidoglycan synthesis regulated by MurB is supported by the nutrient uptake facilitated by UgpABCE, highlighting a complex interplay between cell wall biosynthesis and nutrient acquisition in bacterial physiology (Soni et al., 2020). Ecdysterone with good binding affinity -9.51 kcal/mol with UgpABCE transporter (PDB ID: 4MFI) suggests indirect control on peptidoglycan biosynthesis.

3.9. Mycolic acid biosynthesis

Our previous research demonstrated the affinity of these compounds towards crucial proteins of Mycolic acid biosynthesis, including CmaA2 (3HEM: ΔG 6.7–9.0 kcal/mol), FaB (2Q00: ΔG 7.4–9.1 kcal/mol), and LpqN (6MNA: ΔG -6.2 to -7.8 kcal/mol). Petunidin-3-rutinoside, Beta-sitosterol, and Scopolin showed affinity for 3HEM, while Petunidin-3-rutinoside, Ecdysterone, and Rutin had affinity towards the 2Q00 protein. Additionally, these phytochemicals exhibited affinity towards 2AQ1 (ΔG -7.4 to -8.3 kcal/mol) enzyme having important role in FAS II cycle (Khan et al., 2023). β -sitosterol is well-documented for its antitubercular activity. Otari et al (2012) reported the antitubercular activity of ethanolic extract of plant *Vitex negundo* Linn containing betulinic acid, ursolic acid and β -sitosterol. Similarly, Sasikumar et al., (2018) reported the antimycobacterial potentials of quercetin and rutin.

3.10. MD trajectory analysis

The Glide docking protocol is flexible for small molecules but overlooks receptor structural dynamics in docking. Beta-sitosterol, Scopolin, and Ecdysterone were studied in complex with LpqN (6MNA), and DprE1 (4P8T) proteins to validate the docking results and assess their conformational stability. Each system underwent a 100 ns MD simulation, evaluating root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and hydrogen bond interaction using MD trajectories. The values of each trajectory analyzing parameter including maximum, minimum, and average values are summarised in Table 2.

The RMSD of alpha-carbon atoms helps to understand their stability (Kikiwo et al. 2022). Fig. 4A and 5A, for the 6MNA-Beta-sitosterol complex, the protein backbone's RMSD peaked at approximately 2.33 Å within the initial 24 ns, and stabilized around 2 Å for the remaining period. Similarly, Beta-sitosterol-4P8T complex exhibited stable RMSD values around 3.2 Å throughout the simulation. For 6MNA-Scopolin Complex, an initial rise in RMSD around 2.97 Å occurred within 32 ns, stabilizing with minor fluctuations. The alpha-carbon of 4P8T-Ecdysterone complex exhibited stable variations with RMSD of below 3 Å with

Table 2

The Minimum, maximum and average values of different parameters, RMSD, RMSF, RGyr, and Hydrogen Bonding of studied complexes.

	6MNA-Beta-sitosterol	6MNA-Scopolin	4P8T-Beta-sitosterol	4P8T-Ecdysterone
Root-mean-square deviation Å (RMSD)				
Minimum	1.01	1.20	0.96	0.92
Maximum	2.48	3.00	2.80	3.24
Average	1.87	2.28	2.34	2.61
Root-mean-square fluctuation Å (RMSF)				
Minimum	0.43	0.49	0.37	0.38
Maximum	3.89	5.69	4.77	6.33
Average	0.96	1.15	0.89	0.99
Radius of gyration Å (RGyr)				
Minimum	20.82	20.96	20.73	20.81
Maximum	21.36	21.33	21.23	21.09
Average	21.04	21.19	21.00	20.93
Hydrogen Bonding				
Minimum	1.00	1.00	1.00	1.00
Maximum	3.00	4.00	2.00	5.00
Average	0.66	1.19	1.13	1.96

some initial fluctuations upto 21 ns.

The initial RMSD rise may reflect structural changes or rearrangements in the protein backbone during phyto-compound interactions, possibly involving conformational adjustments or binding events. However, the subsequent RMSD stabilization indicates the complex settled into a relatively stable conformation, showing minor fluctuations in the simulation.

The 6MNA and 4P8T backbone complexes with phyto-compounds remained stable throughout the simulation, showing no significant deviations. The average protein backbone RMSD were 1.87 Å (6MNA-Beta-sitosterol), 2.28 Å (6MNA-Scopolin), 2.34 Å (4P8T-Beta-sitosterol), and 2.61 Å (4P8T-Ecdysterone). The maximum RMSD across all frames was 3.24 Å, indicating no extreme deviation during the MD simulation. The low average and RMSD support the stability of each complex in dynamic states (Fig. 4A and 5A).

Pandey et al. (2022) elucidated the role of amino acids in maintaining protein-ligand complexes during dynamic phases. The amino acid deviation of individual 6MNA and 4P8T proteins are shown in Fig. 4B and 5B. In the 6MNA protein complex, a maximum fluctuation of 3.89 Å and 3.321 Å is detected in Ser100 and Ala113, respectively. The visual analysis of MD simulation trajectories suggests that both Beta-sitosterol and Scopolin engaged in significant binding interactions with residues, Ala184, Ala203, Ala217, Ile121, Ile220, Leu201, Leu213, Met214, Ser154, Thr182, and Thr210 of the 6MNA protein. Residues

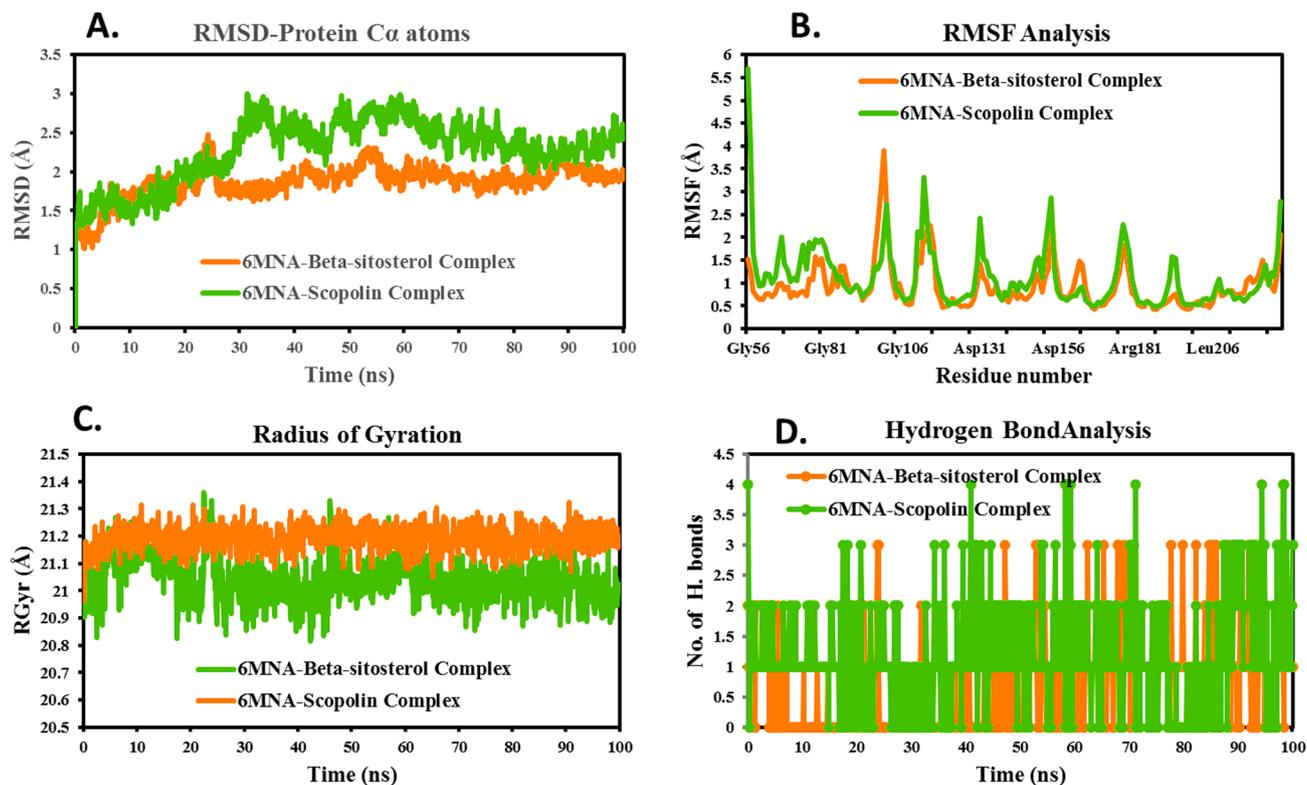


Fig. 4. MD simulation Trajectory analysis of ligand-6MNA complexes A. Time dependent RMSD plot; B. Individual amino acids RMSF plot; C. Time dependent Hydrogen bond analysis; D. Time dependent Radius of gyration plot.

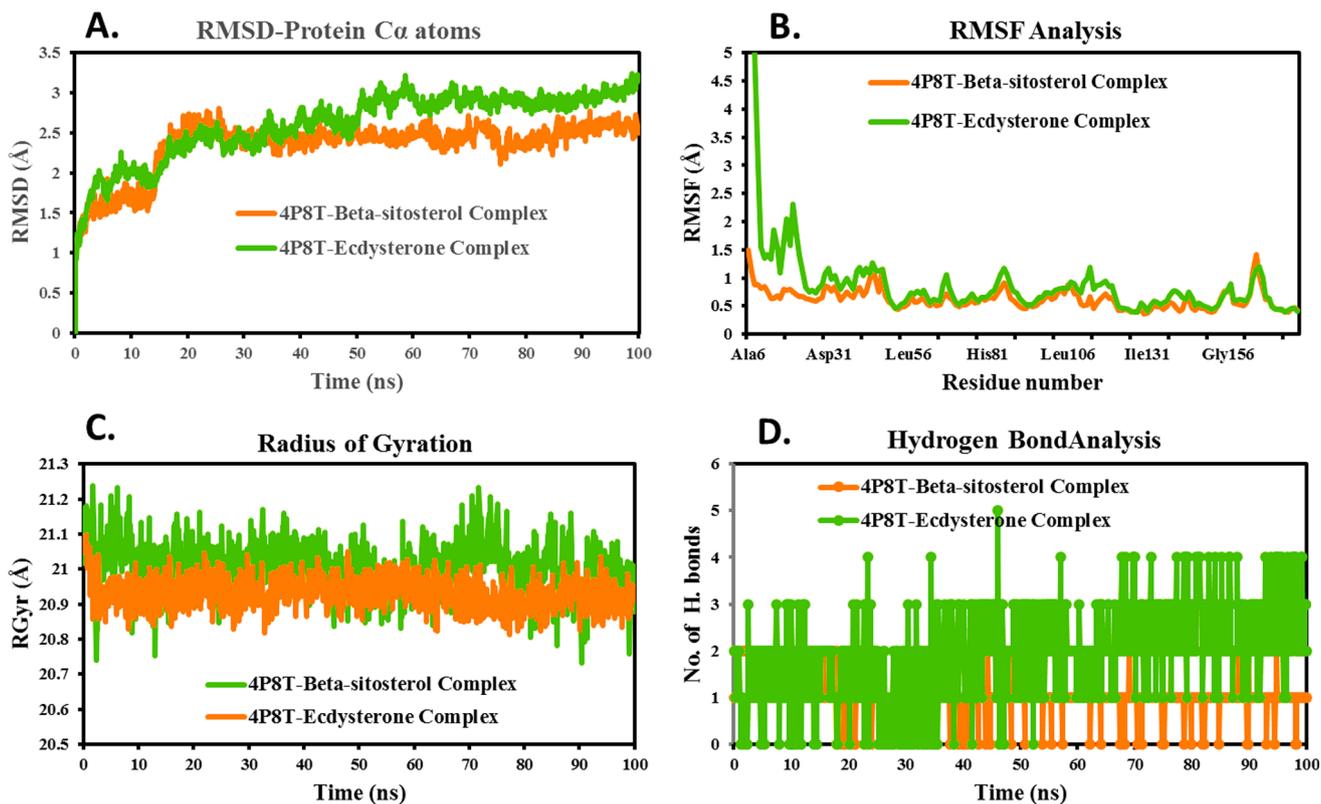


Fig. 5. MD simulation Trajectory analysis of ligand-4P8T complexes A. Time dependent RMSD plot; B. Individual amino acids RMSF plot; C. Time dependent Hydrogen bond analysis; D. Time dependent Radius of gyration plot.

with low RMSF values (0.43 Å and 1.95 Å) indicate stable Beta-sitosterol and Scopolin structures during simulation, suggesting higher protein stability. Amino acids of the 4P8T protein, including, Ala244, Asn385, Gln334, Gln336, Gly117, His132, Leu115, Leu317, Lys134, Lys418, Phe320, Pro116, Pro316, Ser228, Trp230, Trp296, Tyr297, Tyr303, Tyr314, and Val365, interact with Beta-sitosterol and Ecdysterone which have RMSF values less than 2.5 Å.

From Table 2, the average RMSF of 6MNA-Beta-sitosterol, 6MNA-Scopolin, 4P8T-Beta-sitosterol, and 4P8T-Ecdysterone complexes was found to be 0.96, 1.15, 0.89, 0.99 Å respectively. Loop regions distant from the binding pocket show higher fluctuations in RMSF. These residues lack binding interactions with the studied phyto-compounds, as indicated by their above-average values, explanation, and Fig. 4B and 5B. This suggests that during dynamic phases, all amino acids in the 6MNA and 4P8T proteins consistently and strongly bind the phyto-compounds.

Radius of gyration (RGyr) assesses structural compactness in complexes by measuring mass concentration distance from an axis, crucial for understanding macromolecules' 3D structure, folding behavior, and conformational changes during simulations. (Maurya et al. 2022; Patel et al. 2023). Large RGyr values correspond to more flexible conformations, while small RGyr values indicate more constrained conformations (Ayipo et al. 2022). The minimal Deviations for 6MNA (20.82–21.36 Å) and 4P8T (20.73–21.23 Å) complexes with phyto-molecules (Fig. 4C, 5C) suggest stable structures with limited conformational changes during simulations, indicating protein stability. RGyr deviation for 6MNA-Beta-sitosterol, 6MNA-Scopolin, 4P8T-Beta-sitosterol, and 4P8T-Ecdysterone, was 0.36, 0.54, 0.51, and 0.29 Å, respectively suggesting the firmly defined structure of proteins with few conformational changes.

In MD simulations, hydrogen bonds (HBs) ≤ 3 Å between ligands and proteins enhance complex stability (Tabti et al. 2023). Beta-sitosterol and Scopolin showed significant HB formation profiles over 100 ns, influencing binding affinity by establishing multiple specific interactions, as depicted in Fig. 4D and 5D.

4. Conclusion

In-silico investigation identified *Morus alba* phytocompounds (e.g., Petunidin-3-rutinoside, Beta-sitosterol) with strong binding affinity to Mtb targets: MmpL3 (7NVH), DprE1 (4P8T), UgpABCE transporter (4MFI), and OmpATb (2KGS). They also bind CmaA2 (3HEM), oxidoreductase (4OTK), FABH (2Q00), ACP(CoA) reductase (2AQ1), and LpqN (6MNA). This suggests their potential for combined therapy against Mtb, pending lab validation, highlighting the benefits of targeting multiple pathways for improved treatment outcomes.

Funding

This research has been funded by Scientific Research Deanship at university of Ha'il – Saudi Arabia through project number MDR-22020.

CRedit authorship contribution statement

Mahvish Khan: Writing – original draft. **Saif Khan:** Funding acquisition, Resources. **Freah L Alshammary:** Software. **Urvashi Goyal:** Writing – original draft, Methodology. **Vineeta Singh:** Supervision, Writing – review & editing, Conceptualization. **Iqrar Ahmad:** Validation. **Harun Patel:** Validation. **V.K. Gupta:** Writing – review & editing. **Shafiul Haque:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Ayipo, Y.O., Ahmad, I., Alananzeh, W., Lawal, A., Patel, H., Mordi, M.N., 2022. Computational modelling of potential Zn-sensitive non- β -lactam inhibitors of imipenemase-1 (IMP-1). *J. Biomol. Struct. Dyn.* 1–21. <https://doi.org/10.1080/07391102.2022.2153168>. Advance online publication.
- Babu, S., Jayaraman, S., 2020. An update on β -sitosterol: A potential herbal nutraceutical for diabetic management. *Biomed. Pharmacother.* 131, 110702.
- Berrios-Caro, E., Gifford, D.R., Galla, T., 2021. Competition delays multi-drug resistance evolution during combination therapy. *J. Theoret. Biol.* 509, 110524.
- Bolla, J.R., 2020. Targeting MmpL3 for anti-tuberculosis drug development. *Biochem. Soc. Trans.* 48 (4), 1463–1472.
- Faller, M., Niederweis, M., Schulz, G.E., 2004. The structure of a mycobacterial outer-membrane channel. *Sci.* 303 (5661), 1189–1192.
- Gygli, S.M., Borrell, S., Trauner, A., Gagneux, S., 2017. Antimicrobial resistance in *Mycobacterium tuberculosis*: mechanistic and evolutionary perspectives. *FEMS Microbiol. Rev.* 41 (3), 354–373.
- Isa, M.A., Mohammed, M.M., 2021. Molecular docking and dynamic simulation of UDP-N-acetylenolpyruvoylglucosamine reductase (MurB) obtained from *Mycobacterium tuberculosis* using in silico approach. *Netw. Model. Anal. Health Inform. Bioinform.* 10 (1), 40.
- Khameneh, B., Iranshahy, M., Soheili, V., Fazly Bazzaz, B.S., 2019. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrob. Resis. Infect. Cont.* 8 (1), 1–28.
- Khan, M., Khan, S., Alshammary, F.L., Zaidi, S., Singh, V., Ahmad, I., Haque, S., 2023. In silico analysis to identify potential antitubercular molecules in *Morus alba* through virtual screening and molecular dynamics simulations. *J. Biomol. Struct. Dyn.* 1–8.
- Kikiowo, B., Ahmad, I., Alade, A.A., Ijatuyi, T., Iwaloye, O., Patel, H.M., 2022. Molecular dynamics simulation and pharmacokinetics studies of ombuin and quercetin against human pancreatic α -amylase. *J. Biomol. Struct. Dyn.* 1–8. Advance online publication. [Doi: 10.1080/07391102.2022.2155699](https://doi.org/10.1080/07391102.2022.2155699).
- Kumar, B., Zaidi, S., Haque, S., Dasgupta, N., Hussain, A., Pramodh, S., Mishra, B.N., 2021. In silico studies reveal antiviral effects of traditional Indian spices on COVID-19. *Curr. Pharm. Des.* 27 (32), 3462–3475.
- Kwofie, S.K., Adobor, C., Quansah, E., Bentil, J., Ampadu, M., Miller III, W.A., Wilson, M. D., 2020. Molecular docking and dynamics simulations studies of OmpATb identifies four potential novel natural product-derived anti-*Mycobacterium tuberculosis* compounds. *Comput. Biol. Med.* 122, 103811.
- Mali, S.N., Pandey, A., Bhandare, R.R., Shaik, A.B., 2022. Identification of hydantoin based Decaprenylphosphoryl- β -D-Ribose Oxidase (DprE1) inhibitors as antimycobacterial agents using computational tools. *Sci. Rep.* 12 (1), 16368.
- Manna, M., Shil, A., 2020. A review on potential drug delivery system as a treatment of intercellular bacterial infection. *J. Pharmacovig. Drug Res.* 1 (2), 13–23.
- Maurya, S., Alhazmi, A., Vidarthi, A.S., Jain, A., Singh, V., Khan, F., Mishra, B.N., 2022. In-silico study reveals potential antitubercular drug targets unique to *Mycobacterium tuberculosis* H37Rv. *Minerva Biotechnol. Biomol.* 34 (2).
- Melly, G., and Purdy G. E., 2019. "MmpL Proteins in Physiology and Pathogenesis of *M. tuberculosis*." *Microorganisms* 7.3 (2019): 70.
- Murzin, A.G., 1996. Structural classification of proteins: new superfamilies. *Curr. Opin. Struct. Biol.* 6 (3), 386–394.
- Niederweis, M., 2003. Mycobacterial porins—new channel proteins in unique outer membranes. *Mol. Microbiol.* 49 (5), 1167–1177.
- Otari, K.V., Bichewar, O.G., Shete, R.V., Upasani, C.D., 2012. Effect of hydroalcoholic extract of *Vitex negundo* Linn. leaves on learning and memory in normal and cognitive deficit mice. *Asian Pac. J. Trop. Biomed.* 2 (1), S104–S111.
- Pandey, R., Dubey, I., Ahmad, I., Mahapatra, D.K., Patel, H., Kumar, P., 2022. In silico study of some dexamethasone analogs and derivatives against SARS-CoV-2 target: a cost-effective alternative to remdesivir for various COVID phases. *Curr. Chin. Sci.* 2 (4), 294–309.
- Patel, K. B., Rajani, D., Ahmad, I., Patel, H., Patel, H. D., Kumari, P., 2023. Chrysin based pyrimidine-piperazine hybrids: design, synthesis, in vitro antimicrobial and in silico *E. coli* topoisomerase II DNA gyrase efficacy. *Molecular Diversity*, 1-16.
- Puri, S., Ahmad, I., Patel, H., Kumar, K., Juvale, K., 2023. Evaluation of oxindole derivatives as a potential anticancer agent against breast carcinoma cells: In vitro, in silico, and molecular docking study. *Toxicol. In Vitro* 86, 105517. <https://doi.org/10.1016/j.tiv.2022.105517>.
- Róg, T., Gyrych, M., Bunker, A., 2021. Mechanistic understanding from molecular dynamics in pharmaceutical research 2: lipid membrane in drug design. *Pharmaceuticals* 14 (10), 1062.
- Sasikumar, K., Ghosh, A. R., Dusthacker, A., 2018. Antimycobacterial potentials of quercetin and rutin against *Mycobacterium tuberculosis* H37Rv. *J. Biotech.* 8, 1-6.
- Sharma, N., Singh, V., Pandey, A.K., Mishra, B.N., Kulsoom, M., Dasgupta, N., Haque, S., 2019. Preparation and evaluation of the ZnO NP-ampicillin/sulbactam nanoantibiotic: Optimization of formulation variables using RSM coupled GA method and antibacterial activities. *Biomolecules* 9 (12), 764.
- Shaw D. E. Research, Schrödinger Release (2020-3). Desmond molecular dynamics system. Maestro-Desmond interoperability tools.
- Soni, D.K., Dubey, S.K., Bhatnagar, R., 2020. ATP-binding cassette (ABC) import systems of *Mycobacterium tuberculosis*: target for drug and vaccine development. *Emerging Microbes Infect.* 9 (1), 207–220.
- Srivastava, R., Tripathi, S., Unni, S., Hussain, A., Haque, S., Dasgupta, N., Mishra, B.N., 2021. Silybin B and cyanidanol inhibit Mpro and spike protein of SARS-CoV-2: Evidence from in silico molecular docking studies. *Curr. Pharm. Des.* 27 (32), 3476–3489.
- Suma, K.B., Kumari, A., Shetty, D., Fernandes, E., Chethan, D.V., Jays, J., Murahari, M., 2020. Structure based pharmacophore modelling approach for the design of

- azaindole derivatives as DprE1 inhibitors for tuberculosis. *J. Mol. Graph. Modell.* 101, 107718.
- Tabti, K., Ahmad, I., Zafar, I., Sbai, A., Maghat, H., Bouachrine, M., Lakhlifi, T., 2023. Profiling the Structural determinants of pyrrolidine derivative as gelatinases (MMP-2 and MMP-9) inhibitors using in silico approaches. *Comput. Biol. Chem.* 107855 <https://doi.org/10.1016/j.compbiolchem.2023.107855>.
- Tucker, A.N., Carlson, T.J., Sarkar, A., 2021. Challenges in drug discovery for intracellular bacteria. *Pathogens* 10 (9), 1172.