



Exploring the *in silico* studies of the endophyte fungus *Phoma herbarum* against mur enzymes of *Staphylococcus aureus* – a computational approach

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Abstract

Endophyte fungus *Phoma herbarum* (NFCCI 4035) was previously isolated and identified from healthy leaf tissue of *Meriandra bengalensis* Benth plant from Manipur. We recently established the antibacterial activity of ethyl acetate extracts of endophyte fungus *P. herbarum* *in vitro* and identified some potentially active components using GC-MS. The current study aims to perform molecular docking of the discovered *P. herbarum* compounds against Mur enzymes (Mur A, Mur C, Mur D, and Mur F) of *Staphylococcus aureus* using Auto Dock Tools 1.5.6. SwissADME online tool was used to calculate the drug-likeness characteristics. According to the findings, only 14 of 23 bioactive compounds evaluated from *P. herbarum* extract adhered to Lipinski's Rule of Five (LRO5) and had binding affinity ranged between -3.5 to -6.5 (kcal/mol) with at least one of the four targeted Mur enzymes. When compared to all other bioactive compounds that interact with target Mur enzymes, 2-Palmitoylglycerol molecule demonstrated highest binding affinity (-6.5 kcal/mol) with Mur F ligase. However, (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine has shown excellent binding affinity for all selected target (Mur A, Mur C, Mur D, and Mur F) enzymes. Also, this study confirmed that the bioactive compounds can be developed into more potent inhibitors targeting *S. aureus*-related infections.

Keywords Endophytic fungi · Bioactive chemicals · Mur enzymes · Molecular docking · Drug-likeness

Introduction

Endophytes act as biological archives for unique natural chemicals, opening up new avenues for medication development. In the hunt for novel chemical entities, plant-associated microorganisms, including bacteria and fungi that inhabit the internal tissues of all plant species, are becoming more and more important candidates for bioprospecting

(Staniek et al. 2008; Tiwari et al. 2021). Endophytic fungus has been linked to the discovery of numerous significant bioactive compounds (Mousa and Raizada 2013). *Penicillium* and other endophytic fungal species have received more attention than other microbial species ever since the discovery of penicillin. They have strong track record of producing a wide range of chemical compounds that fall into several classes, such as alkaloids, antibiotics, hormones, mycotoxins, etc. (Mousa and Raizada 2013; Frisvad et al. 2013). These species are notable for the presence of particular mycotoxins such as patulin, citrinin, ochratoxin, etc., as well as the large number of pharmacologically significant chemicals that have so far been described from them (Tiwari et al. 2021). The rise of resistance to antibiotics by pathogenic species such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, etc. has become increasingly common and poses a serious threat to public health. In particular, infections related to *Staphylococcus aureus* (*S. aureus*) pathogens have not only become more prevalent but have also started developing resistance

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to available antimicrobial agents (Liu and Breukink 2016; Laddomada et al. 2016). Several existing antibiotics currently used in treating *S. aureus* infections are known to frequently target the following pathways: cell wall synthesis, nucleic acid synthesis, protein synthesis, folate synthesis, etc. This repeated exposure to antibiotics against similar pathways has eventually resulted in the production of mutant bacteria (Freiberg et al. 2006). Mehjabeen et al. (2013) have identified several key enzymes of *S. aureus* that are poorly exploited as potential drug targets. The identified targets are found to represent diverse classes of metabolic proteins as well as membrane-bound proteins. In this study, the proteins of Mur ligase family were examined, which include UDP-N-acetylglucosamine-1-carboxyvinyltransferase (MurA), UDP-N-acetylmuramate-L-alanine ligase (MurC), UDP-N-acetylmuramoyl-L-alanyl-D-glutamatesynthetase (MurD), and UDP-N-acetylmuramoyl-tripeptide-D-alanyl-D-alanine ligase (Mur F). Four proteins are known to play a crucial role in catalyzing the synthesis of peptidoglycan precursor (UDP-MurNAc-pentapeptide), which is the first step in peptidoglycan synthesis (Mol et al. 2003; Hrast et al. 2014; Liu and Breukink 2016; Jha et al. 2020; Gaur and Bera 2022).

It has been well documented that the gram-negative bacterial cell wall is composed of peptidoglycan, which acts as a protective barrier of cells. Their main functions include maintaining the integrity and shape of the cell along with participating in cell growth and cell division (Liu and Breukink 2016). They also serve as anchors by protecting various components of the cell envelope (Vollmer et al. 2008). The peptidoglycan structure is made up of trans-peptide-linked linear glycan chains with alternating N-acetylglucosamine and N-acetylmuramic acid residues (Yashodeep et al. 2021). Given their importance in maintaining the integrity of the cell wall, any disruption in the peptidoglycan synthesis will eventually lead to cell lysis, which, in turn, affects their survival (Vollmer et al. 2008). Due to their unique nature, the enzymes that participate in peptidoglycan biosynthesis have become attractive and selective drug targets for identifying potent drugs against *S. aureus* (Gaur and Bera 2022). Their biosynthesis involves several steps that occur in three different stages (cytoplasm, membrane-associated, and periplasm) (Liu and Breukink 2016). With beginning in the cytoplasm, peptidoglycan biosynthesis initiates with the conversion of uridine diphosphate-N-acetylmuramyl-pentapeptide (UDP-MurNAc-pp) from uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) by the enzymes of Mur pathway (Laddomada et al. 2016). MurA transferase, one of the enzymes of Mur pathway, catalyzes the first step of transferring enol pyruvate from phosphoenolpyruvate to UDP-GlcNAc, forming enolpyruvyl UDP-GlcNAc (Hrast et al. 2014; Funes Chabán et al. 2021). The sequential

addition of peptides is carried out by four ATP-dependent ligases, including MurC, MurD, MurE, and MurF (Hrast et al. 2014; Jha et al. 2020). These enzymes catalyze the addition of L-Ala, D-Glu, meso-diaminopimelic acid (or L-Lys), and D-Ala-D-Ala to UDP-N acetylmuramic acid (UDP-MurNAc) (Mihalovits et al. 2019; Jha et al. 2020).

In this context, it is noteworthy that all the enzymes selected for Mur (Mur A, Mur C, Mur D, and Mur F) pathway can be considered attractive drug targets for antibiotic discovery (Gaur and Bera 2022). A wide range of Mur ligase research has been conducted over the last decade, resulting in the discovery of a unique and diverse collection of inhibitors. All Mur ligases share a consistent method of action as well as a well-organized kinetic mechanism (Kotnik et al. 2007; Gaur and Bera 2022), contain a conserved ATP binding region with 22–26% identical sequences, and also have a P-loop with several glycine, glutamic acid, and histidine residues that regulate Mg²⁺ ions (Gaur and Bera 2022). Despite these commonalities, the configurational structure and arrangement of residues identified in the catalytic site of Mur ligases varies, i.e., MurD and Mur F, from various bacterial species such as *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium tuberculosis* etc. (Kotnik et al. 2007; Rani et al. 2018; Umamaheswari et al. 2010; Gaur and Bera 2022). Since, these enzymes are unique to prokaryotes and completely absent in mammals, including humans, targeting Mur enzymes could lead to the identification of potent drug candidates that can be used in treating *S. aureus* related infections (Kouidmi et al. 2014; Gaur and Bera 2022).

S. aureus and *E. coli* were widely reported for their alarming nosocomial effects due to their rapid multiplication and toxin production (Liu and Breukink 2016). Their antibiotic resistance mechanisms evolved rapidly. Thus, bacterial disease transmission is a major social issue. Screening for bioactive compounds is tedious, time-consuming, and expensive. Thus, computational molecular docking can overcome traditional methods' drawbacks (Vollmer et al. 2008; Gaur and Bera 2022). This method reduces the time needed to analyze and identify the most promising bioactive compound among many compounds (Funes Chabán et al. 2021). In drug development programs, this has been used to anticipate ligand-receptor binding modalities. *In silico* analysis can determine the bacterium's most likely compound binding site. The protein data bank model selection is the most crucial *in silico* study condition (Mehjabeen et al. 2013).

Materials and methods

Target protein and ligand preparation

X-ray Crystal structure data of a gram negative bacterial cell wall proteins, a peptididoglycan, Mur enzymes, viz. Mur A (PDB ID: 3ZH4) (Engel et al. 2013), Mur C (PDB ID: 1GQY) (Mol et al. 2003), Mur D (PDB ID: 2JFF) (Kotnik et al. 2007; Umamaheswari et al. 2010; Gaur and Bera 2022), and Mur F (PDB ID: 3ZM5) (Rani et al. 2018) were downloaded from RCSB PDB website (<https://www.rcsb.org/pdb>) (Supplementary File, Figure S1). Through a review of literature, the protein structural information was chosen, and these enzymes are attractive antibacterial targets since they are functionally important for bacterial survival and have no parallel in eukaryotic cells (Kouidmi et al. 2014).

The analyzed bioactive secondary metabolite compounds were previously isolated from *Phoma herbarum* (NFCCI 4035), an endophyte fungus, identified from the healthy fresh leaf segments of the bengle sage (*Meriandra bengalensis* Benth) plant from Manipur (Devi et al. 2022). The detailed descriptions of crude metabolite production, ethyl acetate-guided extraction, and the compounds identification by GC-MS (GC Clarus 500 Perkin Elmer) analysis was already disclosed in our prior study (Devi et al. 2022). 3D structures of the compounds were retrieved in SDF file format from PubChem database and then translated to pdb file format using BIOVIA Discovery Studio 2021 Client and served as ligands (Figure S2) (Ononamadu and Ibrahim 2021).

Drug likeness prediction

Lipinski's Rule of Five (LRO5) determines the consistency of orally active medicines drug-likeness (Lipinski, 2001). It predicts inclusion when $\text{ClogP} > 5.37$, $\text{MW} > 500$ g/mol, more than 10 acceptors, and more than five H-bond donors are present (Ononamadu and Ibrahim 2021). Swiss ADME predictor was utilized in this investigation to screen the bioactive chemicals and it provides information such as the number of rotatable bonds, hydrogen acceptors, and hydrogen donors. No LRO5 breaches were observed during molecular docking of the compounds (Daina et al. 2017).

Molecular docking

Auto Dock Tools 1.5.6 (Rath et al. 2021) was used for molecular docking analysis. The protein structure incorporated polar hydrogen atoms and Kollman charges. The resulting protein structure was saved in .pdbqt file format for further analysis. The number of genetic algorithm runs was set to ten, while the other docking analysis parameters

were kept at their default values. Based on Auto Dock 1.5.6, the optimal protein-ligand conformation was chosen and graded based on binding affinities. The identification of protein ligand interactions was determined using PLIP (Protein Ligand Interaction Profiler) online server. Post-docking analysis, i.e. visualization was performed using BIOVIA Discovery Studio 2021 Client (Ononamadu and Ibrahim 2021; Rath et al. 2021).

Results

Identified *Phoma herbarum* bioactive compounds

The bioactive compounds were previously identified (Devi et al. 2022) in the active fractions of *Phoma herbarum* (NFCCI 4035) ethyl acetate extracts that were used for this study. *P. herbarum* derived bioactive compounds were such as E-11,13-Tetradecadien-1-ol; 2-Trifluoroacetoxytridecane; Palmitic Acid; Diethylene glycol monostearate; 1-Fluorodecane; 3-Heptafluorobutyroxytetradecane; Pentafluoropropionic acid, tetradecyl ester; 3-Acetoxytridecane; 9,12-Octadecadienoic acid (Z,Z)-, mixt. with (Z)-9-octadecenoic acid; Z-8-Methyl-9-tetradecenoic acid; 4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester); Methyl 10-bromoundecanoate; Erucic acid; 1-(4-Bromobutyl)-2-piperidinone; 2-Palmitoylglycerol; 1,1,1-Trifluoroheptadecan-2-one; 4-Tetradecanol; 4-Acetoxy-1-decene; (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine; 9-Octadecenal; 3-Decyn-2-ol; 17-Cyclohexyltritriacontane; and Sulfurous acid, dodecyl 2-propyl ester (Devi et al. 2022).

Prediction of drug-likeness

Swiss ADME online server was used to forecast the ligands physicochemical properties (Table 1). The ligands were filtered and screened using Lipinski's Rule of Five. After applying the rule, 23 possible active bioactive compounds were still present among the initial set of ligands. Nine of the 23 bioactive chemicals did not adhere to Lipinski's Rule. All of the remaining bioactive substances (14), however, adhered to Lipinski's Rule of Five. Following the screening procedure, 14 substances were chosen for additional docking research (Table 1). The compounds were (1) E-11,13-Tetradecadien-1-ol; (2) 1-Fluorodecane; (3) 3-Acetoxytridecane; (4) Z-8-Methyl-9-tetradecenoic acid; (5) 4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester); (6) Methyl 10-bromoundecanoate; (7) Erucic acid; (8) 1-(4-Bromobutyl)-2-piperidinone; (9) 2-Palmitoylglycerol; (10) 4-Tetradecanol; (11) 4-Acetoxy-1-decene; (12) (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]

Table 1 Drug likeness properties (Lipinski's rule) of bioactive compounds derived from *Phoma herbarum* using Swiss-ADME server

Bioactive Compounds	MW	LogP	HBA	HBD	NRB	Drug likeness (Violation)
E-11,13-Tetradecadien-1-ol	210.36	3.70	1	1	11	Yes, 0
2-Trifluoroacetoxytridecane	296.37	4.32	5	0	13	Yes, 1
Palmitic Acid	256.42	4.19	2	1	14	Yes, 1
Diethylene glycol monostearate	372.6	5.46	2	1	14	Yes, 1
1-Fluorodecane	160.27	4.06	1	0	8	Yes, 0
3-Heptafluorobutyroxytetradecane	410.4	7.63	9	0	16	Yes, 1
Pentafluoropropionic acid, tetradecyl ester	360.40	5.02	7	0	16	Yes, 1
3-Acetoxytridecane	242.40	3.94	2	0	12	Yes, 0
9,12-Octadecadienoic acid (Z,Z)-, mixt. with (Z)-9-octadecenoic acid	562.9	7.01	4	2	29	No, 2
Z-8-Methyl-9-tetradecenoic acid	240.38	3.84	2	1	11	Yes, 0
4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	172.16	0.36	4	0	3	Yes, 0
Methyl 10-bromoundecanoate	279.21	3.56	2	0	10	Yes, 0
Erucic acid	338.6	3.70	2	1	19	Yes, 0
1-(4-Bromobutyl)-2-piperidinone	234.13	1.93	1	0	4	Yes, 0
2-Palmitoylglycerol	330.50	3.18	4	2	18	Yes, 0
1,1,1-Trifluoroheptadecan-2-one	308.42	4.91	4	0	15	Yes, 1
4-Tetradecanol	214.39	3.95	1	1	11	Yes, 0
4-Acetoxy-1-decene	198.30	3.04	2	0	9	Yes, 0
(1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine	153.26	2.45	1	1	0	Yes, 0
9-Octadecenal	266.46	4.68	1	0	15	Yes, 1
3-Decyn-2-ol	154.25	2.70	1	1	4	Yes, 0
17-Cyclohexyltrtriacontane	547.04	10.69	0	0	31	No, 2
Sulfurous acid, dodecyl 2-propyl ester	292.5	3.47	3	0	14	Yes, 0

MW- Molecular Weight; LogP- Octanol-Water Partition Coefficient; HBA - Hydrogen Bond Acceptors; HBD- Hydrogen Bond Donors; NRB - Number of Rotatable Bonds

Table 2 Molecular docking analysis of *P. herbarum* bioactive compounds showing estimated binding affinity and interacting residues in the binding sites of Mur enzymes-Mur A (3ZH4), Mur C (1GQY), Mur D (2JFF) and Mur F (3ZM5) of best three compounds

Target Proteins	Ligand molecules	Binding affinity	Hydrogen bonds	Hydrogen bond-interacting amino acid residues
Mur A (3ZH4)	Z-8-Methyl-9-tetradecenoic acid	-5.1	1	ASN
	4-Acetoxy-1-decene	-5.1	2	VAL, GLY
	(1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine	-4.9	3	LYS, ASN, ASP
Mur C (1GQY)	(1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine	-5.7	2	ARG, TYR
	Erucic acid	-5.6	1	GLU
	3-Acetoxytridecane	-5.6		
Mur D (2JFF)	(1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine	-5.5		LEU
	Z-8-Methyl-9-tetradecenoic acid	-5.0	2	ASP, ASN
	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	-5.0	2	HIS, THR
	2-Palmitoylglycerol	-5.0	3	LYS, THR, ASN
Mur F (3ZM5)	2-Palmitoylglycerol	-6.5	3	SER, LEU, ARG
	(1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine	-6.1	2	ASN
	E-11,13-Tetradecadien-1-ol	-5.8	1	ARG

heptan-3-amine; 13) 3-Decyn-2-ol; 14) Sulfurous acid, dodecyl 2-propyl ester.

Molecular docking study

Molecular docking determines protein-ligand binding energy, an important metric. This shows protein and

ligand-receptor docking affinity. Affinity and docking increase with decreasing binding energy. Table 2 shows the docking scores/interaction for the best three compounds against *S. aureus* bacterial cell wall Mur enzymes (Mur A transferase, Mur C ligase, Mur D synthetase, and Mur F ligase). In detail, Mur A transferase (3ZH4) enzyme inhibition was investigated by docking of selected ligands (Table 2

and S1). During the docking process, close attention was paid to the ligands interactions and orientations. Among the ligands tested, Z-8-Methyl-9-tetradecenoic acid exhibited highest binding energy of -5.1 kcal/mol, followed by 4-Acetoxy-1-decene (-5.1 kcal/mol) and (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine (-4.9 kcal/mol). However, Z-8-Methyl-9-tetradecenoic acid, after its interaction with Mur A, docked at ASN23 site (Fig. 1, a and b). In a manner analogous, 4-Acetoxy-1-decene, binds to Mur A at the VAL165 and GLY166 sites (Figs. 1 and 2a and b). Last but not least, (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]

heptan-3-amine was shown to have an interaction with Mur A at LYS22, ASN23, and ASP306 site (Figs. 1 and 3a and b). It demonstrated a larger number of hydrogen bonds, which is indicative of improved inhibitory characteristics and possible effectiveness against Mur A enzyme target. In Mur C ligase (1GQY) enzyme (Table 2), maximum binding energy was found in (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine (-5.7 kcal/mol), followed by 3-Acetoxytridecane (-5.6 kcal/mol) and Erucic acid (-5.6 kcal/mol). ARG16 and TYR223 amino acid residues were involved in the interaction of (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]

Fig. 1 Molecular interaction of protein target Mur A enzyme (3ZH4) with 3D and 2D structures of (1a and 1b) Z-8-Methyl-9-tetradecenoic acid, (2a and 2b) 4-Acetoxy-1-decene and (3a and 3b) (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine

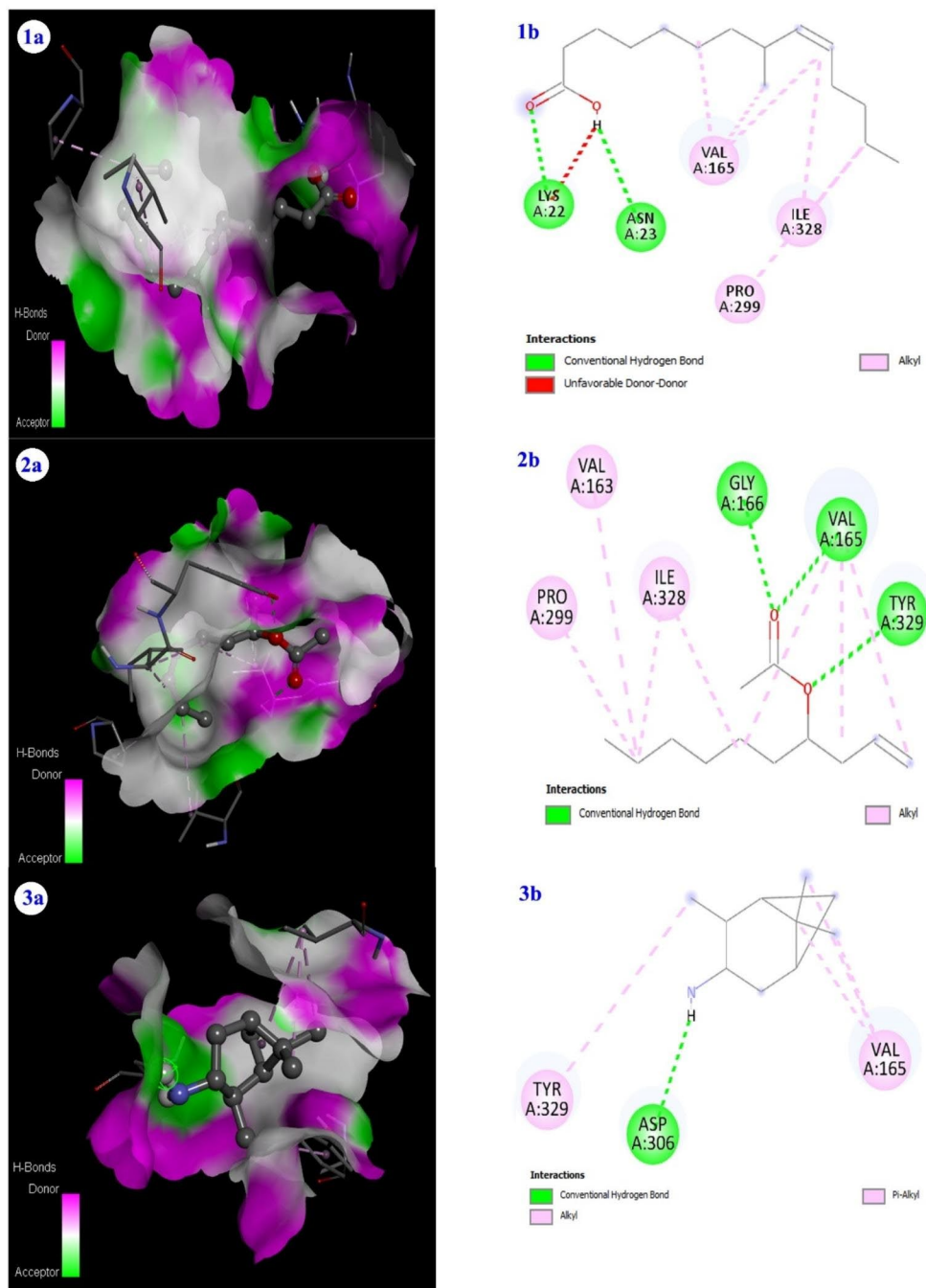
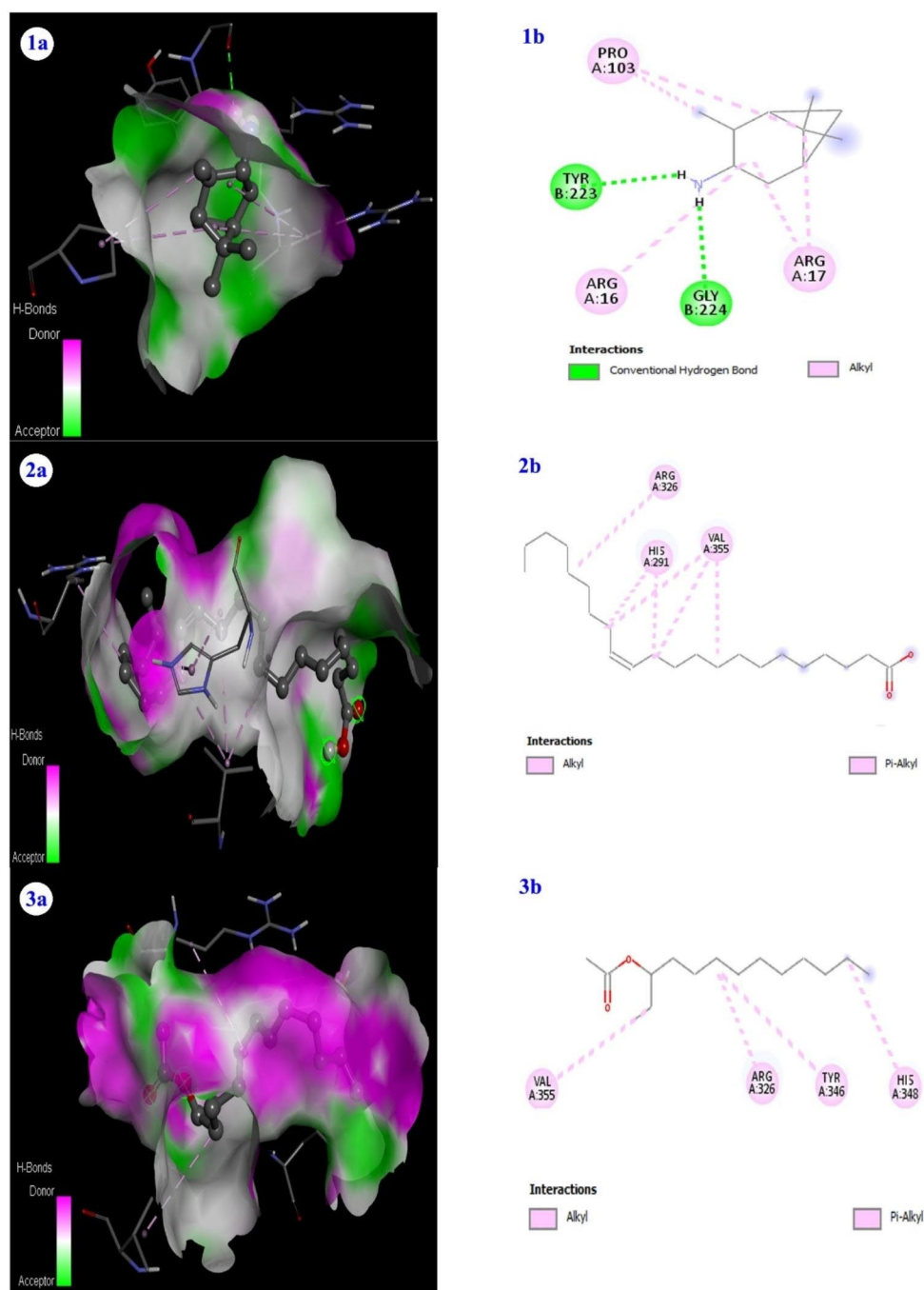


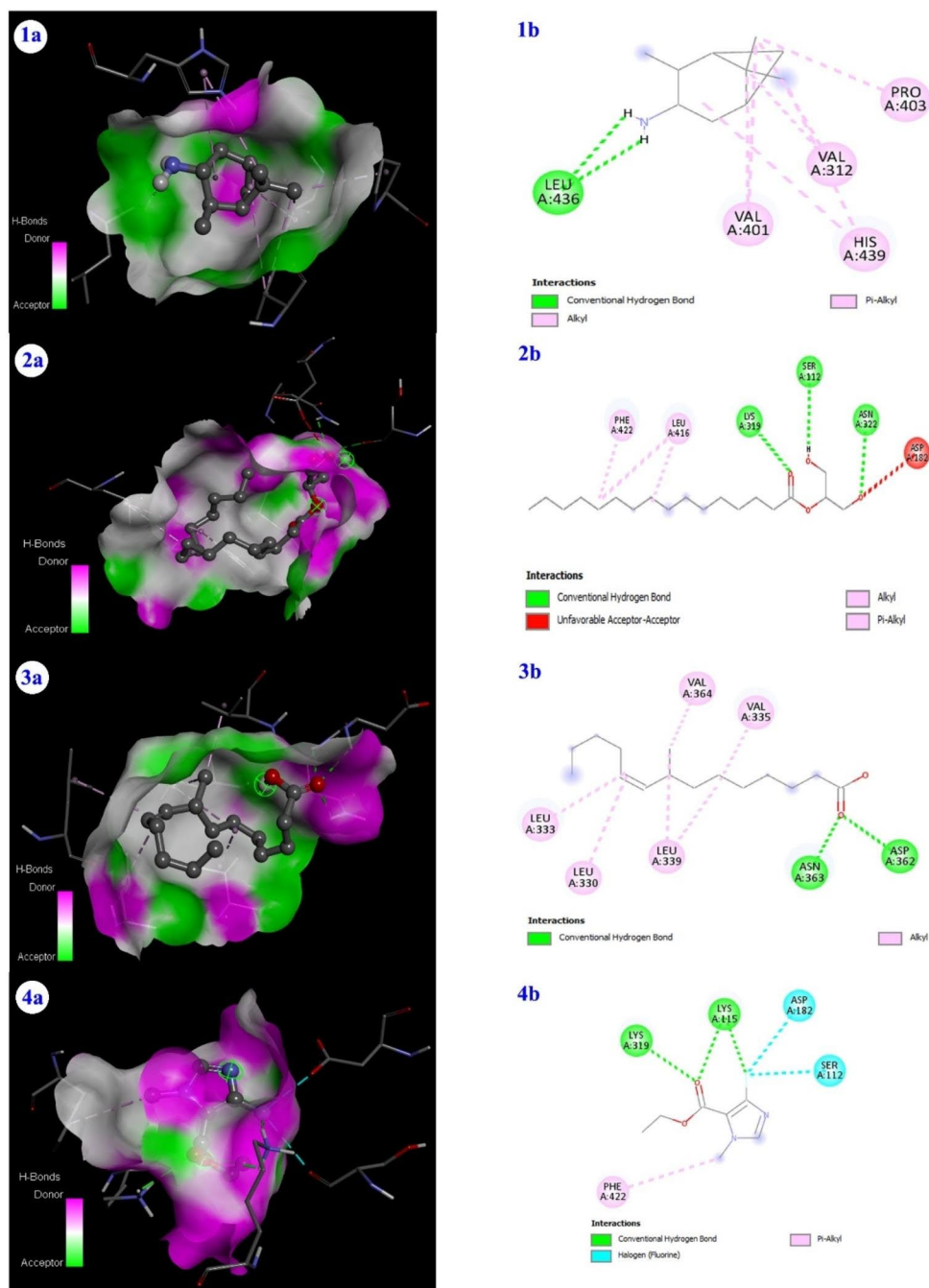
Fig. 2 Molecular interaction of protein target Mur C enzyme (1GQY) with 3D and 2D structures of (1a and 1b) (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine, (2a and 2b) 3-Acetoxytridecane and (3a and 3b) Erucic acid



heptan-3-amine with Mur C enzyme (Table S2). Figs. 2 and a and b depict the amino acid residues involved in the creation of hydrogen bonds. 3-Acetoxytridecane, on the other hand, showed no hydrogen bond interaction (Fig. 2, 2a and b). Furthermore, at GLU195, Erucic acid interacted with Mur C (Figs. 2 and 3a and b). In case of Mur D synthetase/ligase (2JFF), among the ligands, (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine demonstrated the highest binding energy (-5.5 kcal/mol), followed by Z-8-Methyl-9-tetradecenoic acid (-5.0 kcal/mol),

4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester) (-5.0 kcal/mol), and 2-Palmitoylglycerol (-5.0 kcal/mol) (Table 2 and S3). In Mur D enzyme, (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine established a hydrogen bond with LEU436 (Figs. 3 and 1a and b 3), while Z-8-Methyl-9-tetradecenoic acid docked at ASP362 and ASN363 sites (Figs 3 and 2a and b 3). Furthermore, 4-fluoro-1-methyl-5-carboxylic acid, ethyl(ester) interacted with HIS183 and THR321 in Mur D enzyme (Figs. 3 and 3a and b), and 2-palmitoylglycerol interacted with LYS115,

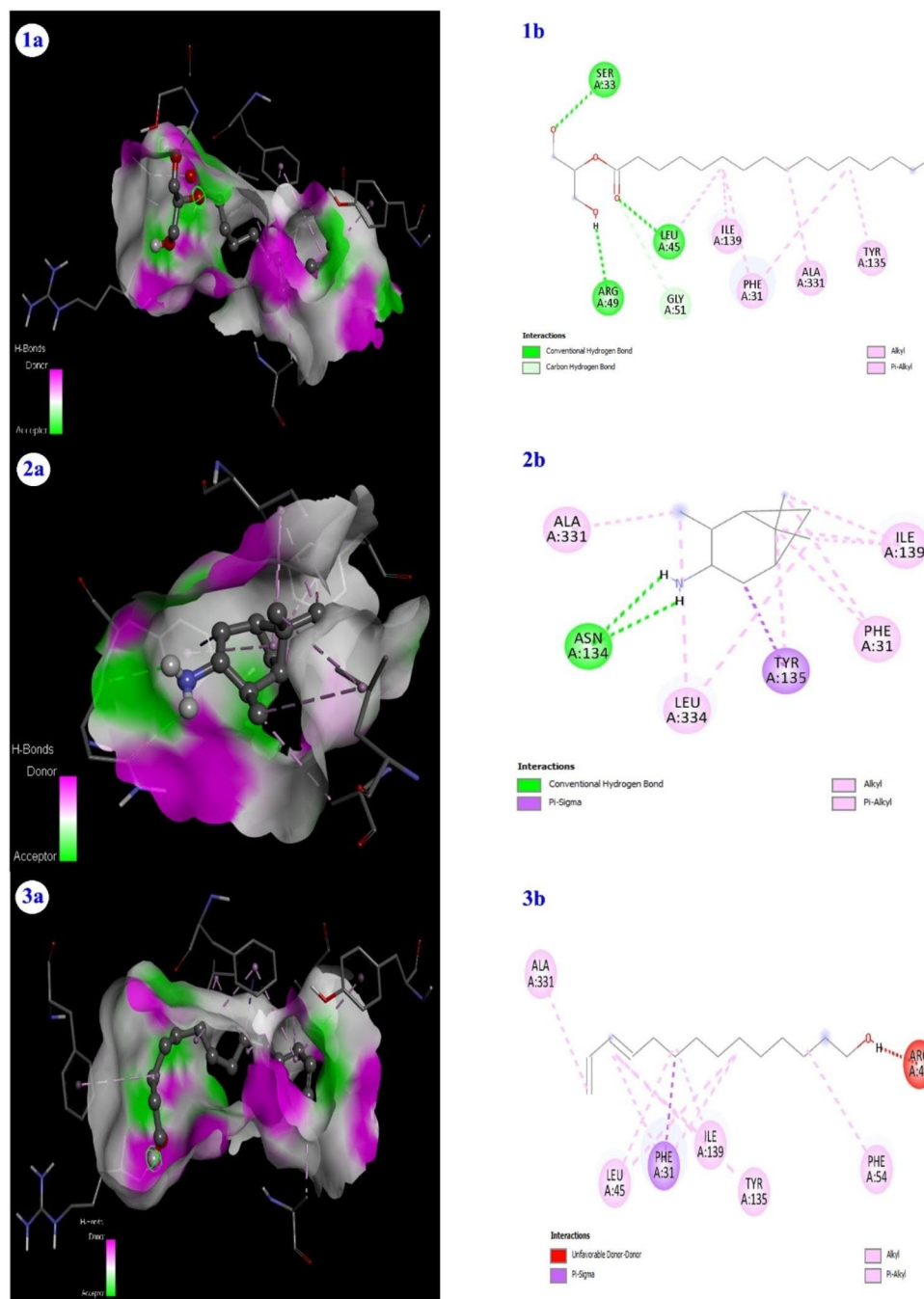
Fig. 3 Molecular interaction of protein target Mur D enzyme (2JFF) with 3D and 2D structures of (1a and 1b) (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine, (2a and 2b) Z-8-Methyl-9-tetradecenoic acid, (3a and 3b) 4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester) and (4a and 4b) 2-Palmitoylglycerol



THR321, and ASN322 in Mur D enzyme (Figs. 3 and 4a and b). Notably, 2-Palmitoylglycerol had a higher number of hydrogen bonds in the catalytic region than other chemicals, indicating improved inhibitory characteristics and possible effectiveness against Mur D enzyme. In Mur F ligase (3ZM5), results (Table 2 and S4) highlighted the best-performing ligands in terms of binding energies. The maximum binding energy was found in 2-Palmitoylglycerol (-6.5 kcal/mol), followed by (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine (-6.1 kcal/mol) and

E-11,13-Tetradecadien-1-ol (-5.8 kcal/mol). The docking of ligands, as well as their interactions and orientations, were carefully observed. At SER33, LEU45, and ARG49, 2-palmitoylglycerol interacted with Mur F enzyme (Figs. 4 and 1a and b 4). The interaction of (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine with target protein revealed two hydrogen bond interactions (Figs. 4 and 2a and b 4) where ASN134 and ASN326 are two examples. At ARG49, E-11,13-tetradecadien-1-ol interacted with Mur F enzyme (Figs. 4 and 3a and b 4). Finally, all bioactive

Fig. 4 Molecular interaction of protein target Mur F enzyme (3ZM5) with 3D and 2D structures of (1a and 1b) 2-Palmitoylglycerol, (2a and 2b) (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine and (3a and 3b) E-11,13-Tetradecadien-1-ol



substances demonstrated high affinity for the target Mur enzymes. When compared to all other bioactive compounds that interact with target enzymes, 2-Palmitoylglycerol molecule demonstrated the highest binding affinity with Mur F enzyme. However, (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine shown good binding affinity for all selected target enzymes (Mur A, Mur C, Mur D, and Mur F).

Discussion

The emergence of methicillin-resistant and methicillin-sensitive *S. aureus* (MRSA and MSSA) strains had become a global health challenge (Rani et al. 2018; Gaur and Bera 2022). Repeated exposure to the methicillin antibiotic has enabled these strains to develop resistance as well as become sensitive to any kind of medication (Freiberg et al. 2006). Most of the existing drugs currently used in treating *S. aureus* related infections often target only one

enzyme of this pathogen (Rani et al. 2018; Funes Chabán et al. 2021). As a result, identifying potent drug-like compounds capable of multi-target inhibition has gained prominence in drug-discovery efforts (Mehjabeen et al. 2013). Mur pathway enzymes can be considered potential targets for developing multi-target inhibitors. Mur enzymes constituting Mur A transferase, Mur C ligase, Mur D synthetase, and Mur F ligase are known to be structurally similar and possess many conserved amino acid residues (Mehjabeen et al. 2013; Gaur and Bera 2022). It is well known that the bacterial cell walls are made up of peptidoglycan layers consisting of glycan chains, which in turn are made up of repeating N-acetylglucosamine and N-acetylmuramic acid residues cross-linked by peptide chains (Vollmer et al. 2008). Extensive studies performed on these polymers have shown that they play a crucial role in maintaining the integrity of cell walls. They help in enduring osmotic pressure, maintaining the cell shape, and also providing a platform for anchoring the other cell wall components (Laddomada et al. 2016). Peptidoglycan biosynthetic pathway is initiated by the synthesis of most important precursor molecule, UDP-MurNAc-pp, which is required for the formation of peptidoglycan building blocks (Laddomada et al. 2016; Mihalovits et al. 2019; Yashodeep et al. 2021). Mur cascade enzymes (MurA-MurF) are known to be collectively involved in this peptidoglycan biosynthesis, making them attractive drug targets (Mehjabeen et al. 2013).

Docking of bioactive compounds of *P. herbarum* (Devi et al. 2022) was performed against the target bacterial (*S. aureus*) cell wall proteins of Mur enzymes. Lipinski's Rule of Five (LRO5) mandates that a compound's weight be < 500 g/mol, lipophilicity (LogP) < 5, H-bond donors be 5 or < 5, and H-bond acceptors be 10 or < 10. These properties alter intestinal permeability and dissolve during oral bioavailability. Chemicals are not drugs if law violence surpasses two (Lipinski et al. 2001). Bioactive substances that fail LRO5 are harmful. ADME factors explain drug molecular pharmacokinetics (Ononamadu and Ibrahim 2021). The docking analysed compounds have lipophilicity (LogP) less than five: 2-Trifluoroacetoxytridecane, Palmitic Acid, Diethylene glycol monostearate, 3-Heptafluorobutyryoxytetradecane, Pentafluoropropionic acid tetradecyl ester, 1,1,1-Trifluoroheptadecan-2-one, and 9-Octadecenal. MW of 9,12-octadecadienoic acid (Z,Z)-, mix with (Z)-9-octadecenoic acid and 17-Cyclohexyltrtriacontane, is greater than 500 g/mol. Docking study was done utilising Lipinski's Rule-compliant molecules. Toxicities often outweigh ADME factors. Toxic effects break down medications in clinical trials (Rath et al. 2021; Ononamadu and Ibrahim 2021).

In silico modeling is used to analyse protein targets and tiny ligands (compounds) via molecular docking.

The ligand's protein target binding affinity determines the docking score or binding energy (Gaur and Bera 2022). The bioactive compound which exhibits the best binding affinity with Mur A (Z-8-Methyl-9-tetradecenoic acid with -5.1 kcal/mol), Mur C ((1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine with -5.7 kcal/mol), Mur D ((1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine with -5.5 kcal/mol) and Mur F (2-Palmitoylglycerol with -6.5 kcal/mol) have been mentioned. Thus, molecular docking investigations of bioactive chemicals with *S. aureus* Mur enzymes revealed that the compounds discovered in ethyl acetate extract of *P. herbarum* aid in the effective inhibition of oral *S. aureus* pathogenicity. Previous research has demonstrated that bioactive chemicals produced from plants and fungi block bacterial peptidoglycan synthesis, and Mur enzyme is thought to be one of the promising targets for the creation of novel antibacterial medicines (Mehjabeen et al. 2013; Gaur and Bera 2022). In addition, Rani et al. (2018) and Yashodeep et al. (2021) found that Mur enzymes were a vulnerability in the defenses of *Mycobacterium tuberculosis* and *Staphylococcus aureus*, and that they might be used as a target to suppress the production of their cell walls and populations.

Furthermore, Z-8-Methyl-9-tetradecenoic acid, discovered in *Psidium guajava* leaf, is anti-salmonella (Adetutu et al. 2021). (1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine, found in *Piper betle* extract, has Xanthine oxidase (XO) enzyme, which directly causes gout and indirectly causes cancer, diabetes, and metabolic syndromes (Chakravarthi et al. 2023). Hashmi et al. (2019) reported substantial molecular interaction between *Garcinia kola* seeds oil and the active site of human Epidermal growth factor Receptor (EGFR), indicating antibacterial, antioxidant, and anticancer properties. Srinivasan et al. (2023) found PPAA (porcine pancreatic alpha-amylase) and RIG (rat small intestine alpha-glucosidase) inhibition in *Phoenix pusilla* (Arecaceae) fruit methanol extracts. Suresh and Sureshkumar (2019) found *Calotropis gigantea* compounds, bicyclo [3.1.1] heptane,2,6,6-trimethyl-,[1R-(1.alpha.,2.beta.,5.alpha)] effective at degrading protein (4CN8) and antimicrofouling. Adnan et al. (2019) suggested that *Ophiorrhiza rugosa* var. prostrata extracted phytochemicals 2-Palmitoylglycerol showed a promising anti-bacterial effect on *Escherichia coli* (Gram-negative microorganism). Further study in this area may be conducted in order to reduce the in vitro evaluation of these enzymes. Furthermore, the production of these molecules was found to be feasible. As a result, this research will be useful in identifying novel antimicrobial drugs with Mur enzyme inhibitory activity.

Conclusion

Using *in silico* approaches, the current study explored possible antibacterial chemicals found in a prior publication of *P. herbarum* extract. The results supported the extract's antibacterial activity, with some of the discovered compounds demonstrating substantial binding affinity with *S. aureus* target Mur enzymes (Mur A, Mur C, Mur D, and Mur F). Furthermore, 14 bioactive molecules adhered to Lipinski's Rule of Five during drug-likeness screening. Mur A (Z-8-Methyl-9-tetradecenoic acid, -5.1 kcal/mol), Mur C and D ((1R,2R,3R,5 S)-2,6,6-Trimethylbicyclo[3.1.1]heptan-3-amine, -5.7 kcal/mol), and Mur F (2-Palmitoyl-glycerol, -6.5 kcal/mol) have the highest binding affinity. According to molecular docking studies, these compounds have the potential to be developed into more effective inhibitors of *Staphylococcus* infections generated by *S. aureus*.

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Declarations

Conflict of interest The authors have declared no conflicts of interest.

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