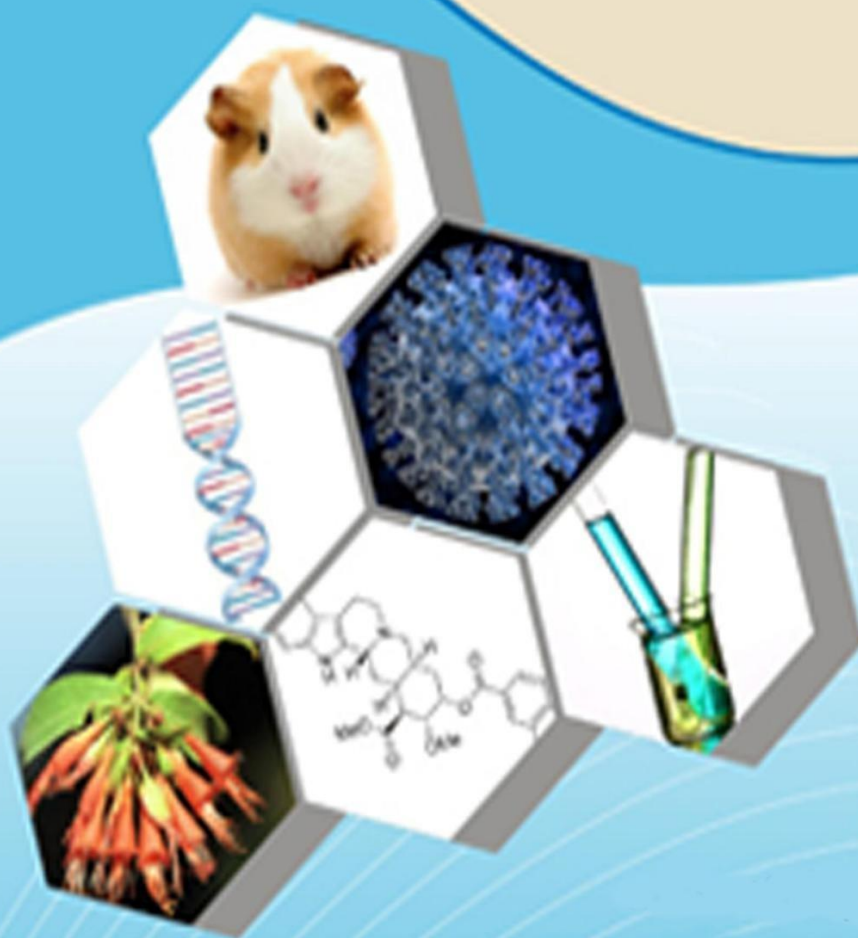




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Investigating *Tropheryma whipplei* murE protein inhibitors using pharmacoinformatic techniques that include formulation insights that improve solubility

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ABSTRACT:

Weight loss, diarrhea, joint pain, fever, and possibly neurological symptoms are just a few of the symptoms that make diagnosing *Tropheryma whipplei*, the causative agent of Whipple illness, difficult. Treatment is made much more difficult by its resistance to fluoroquinolones. Because *Tropheryma whipplei* cannot be cultivated on axenic medium, conventional techniques for assessing antibiotic susceptibility are unsuccessful. Since no drug targets from this bacterium have been investigated to far, we investigated possible drug targets inside its core genome in order to address this. A potential candidate that has both resistance and drug target features is murE, an enzyme that is resistant to macrolides. Three interesting candidates were found after screening more than 1,000 lead-like Ayurvedic compounds against the target enzyme UDP-N-acetylmuramyl-tripeptide synthetase: (1) Ergost-5-en-3-ol (3beta,24xi), (2) [6]-Gingerdiol 3-monoacetate, and (3) Valtrate. Consistent binding strength rankings were obtained using DiffDock and GNINA rescoring. Stable interactions with these chemicals were established by molecular dynamics simulations conducted over a period of 100 nanoseconds.

Low water solubility was found by ADMET analysis; however, solubility was enhanced by coupling with cyclodextrin SBE-β-CD. Valtrate had AMES toxicity, while none of the other chemicals shown hepatotoxic effects. Considering the positive

characteristics, we suggest more in vitro/in vivo research on [6]-Gingerdiol 3-monoacetate as well as scaffold hopping. Our research addresses the constraints caused by antibiotic resistance and provides possible strategies for preventing *T. whipplei* infections.

KEYWORDS

Tropheryma whipplei, Whipple's disease, Ayurveda, virtual screening, MD simulation

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1 Introduction

The rod-shaped Gram-positive bacterium *Tropheryma whipplei* belongs to the phylum Actinobacteria and order Actinomycetales (Liu, 2024). As a contributing factor to endocarditis and Whipple's disease, it is often present in drinking water, soil, and sewage (Marth, 2015; Marth et al., 2016). Infection with *Tropheryma whipplei* is more common among sewage workers in western nations and in children under seven in less sanitary nations (Keita et al., 2015). The uncommon but chronic Whipple's disease may impair the brain, eyes, skin,

joints, and heart, among other human organs. Diarrhea, weight loss, stomach discomfort, fever, exhaustion, joint pain, uveitis, skin disorders, and neurological abnormalities are among the many symptoms that this condition may present with (Marth, 2015). About 15% of pathogen infections are asymptomatic. This bacteria mostly affects males between the ages of 48 and 54 and has a greater percentage of infections in men (73%–87%) (Ruffer



et al., 2023). If not treated properly, it might be fatal even though the fatality rate is yet unclear (Albekairi et al., 2022).

T. whipplei often spreads via the oral-oral and oral-fecal channels (Shams et al., 2021). Since there is no known vector for these bacteria, humans are the only species that carry the illness. It can survive phagocytosis and proliferate in intestinal mucosa macrophages (Al Moussawi et al., 2010; Braubach et al., 2017). The bacterium may spread across the population because it can stay in a patient for a long time after they have developed an immune response to the first contact (Moos et al., 2025). Saliva samples had a much greater quantity of bacteria than stool samples, suggesting that the bacteria is a common gut bacterium (Dolmans et al., 2017). Because *T. whipplei* infections are rare and have complicated symptoms, detecting and treating them is still difficult despite improvements in diagnostic techniques and knowledge. A biopsy of the afflicted



tissue, usually the small intestine, is required for diagnosis (Anton-Vazquez et al., 2022). A definite diagnosis of Whipple's illness may now be made using a variety of methods, including immunohistochemical testing for several genes of interest on biopsy samples and polymerase chain reaction (PCR) (Baisden et al., 2002). In circumstances when the diagnosis cannot be confirmed by histology or in patients with unusual presentations, the PCR test has become an essential diagnostic tool for Whipple's illness (Boulos et al., 2004; Melas et al., 2021).

If Whipple's illness is not treated, it may be fatal. According to research, middle-aged men are consistently more likely to have CNS symptoms, diagnoses are delayed, and recurrence is more likely (Hujoel et al., 2019). Drugs with high absorption rates into the central nervous system (CNS) are essential to prevent neurological relapses, and antibiotic medication should be started as soon as possible because the majority of relapses occur in the CNS, which is also often involved in the disease (Renon et al., 2012; Bureš et al., 2013; Paddock et al., 2022). Because it may cross the blood-brain barrier, trimethoprim/sulfamethoxazole is often used as an antibiotic when the central nervous system is affected (El-Abassi et al., 2023). Despite their effectiveness, patients may acquire antibiotic resistance as a result of using such medications. Furthermore, recurrence or treatment failure

has been documented in individuals receiving treatment with trimethoprim/sulfamethoxazole (García-Álvarez and Oteo, 2021). There have also been reports of ceftriaxone or hydroxychloroquine with doxycycline treatment (Foteinogiannopoulou et al., 2021; Portillo et al., 2024). Antibiotic resistance and side effects, such as the Jarisch-Herxheimer reaction, are problems with antibiotic treatment, especially for patients on immunosuppressive medication or with central nervous system disorders that cause symptoms similar to those of systemic inflammatory response syndrome (Fong and Fong, 2020). Because Whipple's illness is directly linked to the intestine and the gut-brain axis, antibiotic-induced dysbiosis of the gut microbiota may also have serious consequences for patients. Examining other therapeutic modalities, such as plant chemicals, becomes essential in light of these ramifications. Plant-based compounds, such as flavonoids and polyphenols, have shown potential in the past for supporting gut health, lowering inflammation, and modifying the makeup of the gut microbiota (Direito et al., 2021; Fabbrini et al., 2022; Açar et al., 2023). For a long time, plant-based medications have been

used to cure illnesses and maintain health. Plant chemicals work in a number of ways, such as by promoting the development of beneficial bacteria (Santhiravel et al., 2022), modulating immunological responses (Alhazmi et al., 2021), and having antibacterial action against pathogens (Simonetti et al., 2020; Vaou et al., 2021). Furthermore, plant chemicals are less likely to produce side effects such as dysbiosis and to generate antibiotic resistance than antibiotics (Zhang L. et al., 2022; Vignesh et al., 2023). For patients with Whipple's illness, plant-based treatments provide a potentially safer and more sustainable therapy alternative by focusing on certain pathways implicated in disease development while maintaining the balance of the gut flora. Because the usage of medications and antibiotics has a number of negative consequences, the main goal of this study was to screen for lead-like ayurvedic substances that may suppress bacterial development.

2 Materials and methods

2.1 Data retrieval and pan-genomics

The BV-BRC database provided the *T. whipplei* genomes that were available (Olson et al., 2023). Each component's genome size and gene makeup were determined. Chaudhari et al. (2016) using BPGA to examine *T. whipplei*'s pan-genome. Using protein (.faa) or genbank format (.gbk) files, it makes it easier to compare genomes of the same species. The clustering method USEARCH (70% similarity cut-off) was used. Iterative pan-genome profiling (19 iterations, equal to the number of genomes) was conducted using the clustered output to create a tab-delimited binary gene presence-absence matrix (pan-matrix). This allowed for the identification of the core (genes present in all strains), dispensable (genes present in some strains), and accessory genome (genes unique to individual strains) (Basharat and Meshal, 2023). To find substantially overrepresented gene categories or pathways within the pan-genome components, functional enrichment analysis was performed (Sancho et al., 2022). This included using statistical tests to determine which categories or pathways were enriched in each component of the genome and annotating gene clusters with functional categories (e.g., COG, KEGG). Finding functional diversity and adaptive characteristics among *T. whipplei* strains was the goal. The functional diversity and possible adaptive characteristics of *T. whipplei* strains were revealed by this investigation. In order to determine the evolutionary links between the strains, a phylogenetic tree was also built using orthologous gene clusters from the core and pan-genomes. To evaluate genetic relatedness and



evolutionary divergence within the species, the neighbor-joining technique was used, and the resultant tree was displayed and analyzed (Inglin et al., 2018).

2.2 Drug target identification and structure analysis

The core genome consisting of genes conserved across all strains was used for this purpose. Subtractive genomics was performed using installed BLAST to identify potential drug targets by comparing the genome of the target organism with the human genome, essential gene databases and beneficial microbiota of the gut (Uddin and Saeed, 2014; Khan et al., 2022). Aim was to identify genes present in the target organism but absent or significantly different in non-pathogenic counterparts. These unique or significantly different genes are potential drug targets specific to the pathogen (Gupta et al., 2017; Gupta et al., 2019). The final set of subtracted sequences were analyzed based significance of the protein role in pathogenicity or survival, and druggability. Among these, *murE* (coding for UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-- 2,6-diaminopimelate ligase) was selected for downstream analysis (Kumar et al., 2023). This particular protein contributes to peptidoglycan biosynthesis, which is crucial for cell wall formation, shape and integrity maintenance, thereby influencing bacterial survival (Amera et al., 2019). MurE selectively incorporates meso-diaminopimelic acid (m-A2p.m.) in most Gram-negative bacteria and Bacilli, whereas Gram-positive bacteria typically use L-lysine (Hervin et al., 2023). Its function requires ATP to activate the carboxyl group of the peptide stem through acyl-phosphate formation, facilitating nucleophilic attack by the amino group of m-A2pm. This reaction results in the production of ADP and inorganic phosphate as byproducts. *murE* is often encoded in conserved operons, such as the *dcw* cluster, and can form chimeric complexes, like *murE-murF* in *Bordetella pertussis*, to enhance pathway efficiency (Gaur and Bera, 2022; Shirakawa et al., 2023). Active site of this enzyme enforces strict substrate specificity, ensuring proper amino acid incorporation and any errors in this

process can lead to bacterial cell lysis. Notably, *murE* is absent in mammalian cells, making it an attractive and validated target for novel antibiotics (Gaur and Bera, 2022). Inhibitors that disrupt its activity could provide an effective strategy for combating drug-resistant pathogens (El Zoeiby et al., 2003). Consequently, targeting Mur enzymes for inhibition presents a promising avenue for the development of new antibacterial agents (Amera et al., 2020; Cazzaniga et al., 2021; Koatale et al., 2024). Structure of the selected target (UDP-N-acetylmuramyl-tripeptide synthetase, also known as MurE synthetase) was obtained from AlphaFold

(Accession: AF-Q83HJ9-F1-v4) (Jumper et al., 2021). The metrics, such as per-residue model confidence score (pLDDT), and predicted aligned error (PAE) were evaluated to measure consistency with experimental data (Guo et al., 2022). This

assessment helped to assess the reliability and accuracy of the predicted structures.

2.3 Virtual screening

The BV-BRC database provided the *T. whipplei* genomes that were available (Olson et al., 2023). Each component's genome size and gene makeup were determined. Chaudhari et al. (2016) using BPGA to examine *T. whipplei*'s pan-genome. Using protein (.faa) or genbank format (.gbk) files, it makes it easier to compare genomes of the same species. The clustering method USEARCH (70% similarity cut-off) was used. Iterative pan-genome profiling (19 iterations, equal to the number of genomes) was conducted using the clustered output to create a tab-delimited binary gene presence-absence matrix (pan-matrix). This allowed for the identification of the core (genes present in all strains), dispensable (genes present in some strains), and accessory genome (genes unique to individual strains) (Basharat and Meshal, 2023). To find substantially overrepresented gene categories or pathways within the pan-genome components, functional enrichment analysis was performed (Sancho et al., 2022). This included using statistical tests to determine which categories or pathways were enriched in each component of the genome and annotating gene clusters with functional categories (e.g., COG, KEGG). Finding functional diversity and adaptive characteristics among *T. whipplei* strains was the goal. The functional diversity and possible adaptive characteristics of *T.*



whipplei strains were revealed by this investigation. In order to determine the evolutionary links between the strains, a phylogenetic tree was also built using orthologous gene clusters from the core and pan-genomes. To evaluate genetic relatedness and evolutionary divergence within the species, the neighbor-joining technique was used, and the resultant tree was displayed and analyzed (Inglin et al., 2018). used to identify macromolecular targets in humans. Hit Dexter is trained on PubChem data and focus on generating readouts from purified proteins or peptide data, under target-based assay category. This assay is trained on data obtained by biochemical or biophysical methods to measure the binding affinity or enzymatic activity of compounds against isolated target proteins. Conversely, cell-based assay, utilize living cell data to generate readouts. These assays are trained on data to measure specific protein-compound interactions within cellular environments, often employing fluorescence-based techniques or other molecular probes. Additionally, an extended selection of cell-based assay data, encompassing tissue-based and organism- based assays was also employed. These assays provide broader insights into compound effects, including nonspecific interactions such as cytotoxicity or organism-level responses.

2.4 Absorption, distribution, metabolism, excretion and toxicity (ADMET)

ADMET profiling was done using pKCSM (URL:<https://biosig.lab.uq.edu.au/pkcsim/>; retrieved 30 March 2024) (Pires et al., 2015) and SwissADME (URL: <http://www.swissadme.ch/>; retrieved 30 March 2024) (Daina et al., 2017). pKCSM uses machine learning algorithms trained on experimental data to estimate key properties related to pharmacokinetics and toxicity. SwissADME employs a combination of rule-based and machine learning methods to estimate various ADMET properties (Kumar et al., 2021). Estimations for several key properties, including physicochemical descriptors, drug-likeness, lipophilicity, distribution, absorption, excretion, metabolism, and toxicity-related parameters were obtained. This was to identify any potential issues or concerns associated with ligands that could hinder their development into drugs (Wu et al., 2020). For molecules with low water solubility and consequently poor oral bioavailability, cyclodextrin

complexation was evaluated as a strategy to enhance their solubility using Formulation AI (<https://formulationai.computpharm.org/>; accessed 10 January 2025). While other solubility-enhancing methods exist, cyclodextrins were specifically chosen in this study due to their safety, efficacy, ease of formulation, and established regulatory acceptance. Their ability to enhance solubility while maintaining drug stability and minimizing toxicity makes them a compelling option compared to lipid-based carriers, prodrugs, or nanocarriers. SMILE format of priority ligands was fed and temperature was kept 310K, while pH was kept 7. Eight

cyclodextrins were evaluated, including α -cyclodextrin (α -CD), β -cyclodextrin (β -CD), γ -cyclodextrin (γ -CD), 2-hydroxypropyl- β - cyclodextrin (HP- β -CD), methyl- β -cyclodextrin (M- β -CD), hydroxypropyl- γ -cyclodextrin (HP- γ -CD), sulfobutylether- β -cyclodextrin (SBE- β -CD), and randomly methylated- β - cyclodextrin (RM- β -CD). These cyclodextrins differ in their cavity size, substitution patterns, and solubility-enhancing properties. Specifically, α -CD has a smaller cavity and is typically used for smaller molecules (Sandilya et al., 2020), β -CD is commonly studied and utilized, providing moderate solubility enhancement, while γ -CD has a larger cavity suitable for larger molecules (Zhang et al., 2007). The modified cyclodextrins such as HP- β -CD, M- β -CD, SBE- β -CD and RM- β -CD offer improved solubility and stability profiles compared to their native counterparts. The energy values of complexation were noted and analyzed further.

2.5 Molecular dynamics (MD) simulation

The GROMACS MD simulation package (<https://www.gromacs.org/>) (Abraham et al., 2015) version 2021 was utilized to investigate stability of prioritized complexes. Protein and ligand, were prepared by adding missing atoms and assigning OPLS-AA force field parameters to the biomolecules (Beckstein et al., 2014). The system was solvated with water TIP3P, and in order to balance the overall charge, counter ions were added. To relax the initial structure and alleviate steric clashes, an energy-minimization process was carried out. During the energy minimization, position restraints were applied to the biomolecules to prevent excessive



perturbations. Subsequently, a stepwise equilibration process was carried out to adjust the system gradually to the desired conditions. This process involved solvent equilibration, temperature equilibration, and pressure equilibration steps. Position restraints were also applied during equilibration to maintain the bimolecular structure. Once equilibrated, the production run was initiated for data collection. MD simulations were performed for 100 ns. Trajectory data obtained from the production run were analyzed to extract valuable insights into the system. Structural properties such as secondary structure content, radius of gyration, and root mean square deviation (RMSD) were computed. Dynamic properties like hydrogen bonding and root mean square fluctuations (RMSF) were also computed. Time-resolved principal component analysis (PCA) was performed on hydrogen bond and RMSD data extracted from GROMACS. xvg files, specifically complex RMSD and total hydrogen bonds. Feature values were sampled at defined time intervals and compiled into a matrix, followed by Z-score standardization. PCA was conducted using the scikit-learn library, retaining the first two principal components (PC1 and PC2) to capture the major variance in structural dynamics

over time. Complex-specific trajectories were visualized in PC space using matplotlib and seaborn, enabling interpretation of temporal conformational shifts. The resulting plots were used to assess whether the systems remained within a confined conformational space, indicating structural stability throughout the simulation.

2.6 Physiologically based pharmacokinetic (PBPK) profiling

PBPK profiling was conducted using the GastroPlus software v9.8.3 (Romero et al., 2020; Costa et al., 2024). The study focused on evaluating the pharmacokinetics of top compounds in three distinct categories of 50 people population each: normal, renally impaired, and cirrhotic. The anatomical and physiological parameters specific to the study group were incorporated into the models. These parameters included organ volumes, blood flow rates, tissue permeabilities, and enzyme activity levels (Alzamami et al., 2024). Molecular weight and lipophilicity of the compounds was also integrated. Once the models were established, simulations were performed to predict the pharmacokinetic behavior.

TABLE 1 Summary of core, accessory, and unique genes across analyzed *Tropheryma whipplei* strains.

Genome no.	Strain name	No. of core genes	No. of accessory genes	No. of unique genes	No. of exclusively absent genes
1	Art1	749	155	2	2
2	Art29	749	157	4	0
3	Bcu26	749	154	3	2
4	Dig7	749	147	4	1
5	Dig9	749	153	5	0
6	Dig10	749	163	6	1
7	Dig15	749	155	6	0
8	DigADP25	749	167	5	0
9	DigMusc17	749	177	2	1
10	Endo27	749	152	6	0
11	Endo32	749	163	3	0
12	Neuro1	749	154	7	0



13	Neuro14	749	180	51	13
14	Neuro20	749	174	10	0
15	Pneumo30	749	149	10	0
16	Sali28	749	174	9	1
17	Slow2	749	133	10	2
18	TW0827	749	161	47	5
19	Twist	749	193	27	5

simulations took into account factors such as oral absorption, distribution within various tissues, and metabolism by specific enzymes, and elimination via renal and hepatic pathways (Miller et al., 2019; Soliman et al., 2022). A comparative analysis of drug exposure and disposition among the different groups was done.

3 Results

Tropheryma whipplei has a 46% GC content and is the sole pathogen currently identified with a short genome (927 kb) (Bentley et al., 2003). The bacterium shows little variability, with different strains of *T. whipplei* showing 99% similarity (Bulut et al., 2022).

3.1 Pan-genome analysis

To understand the genetic composition, we conducted pan- genome analysis. The number of core genes was 749. Number of accessory genes ranged between 133-180 (Table 1). The number of gene families generally increased as more strains were included in the analysis, indicating the presence of some strain-specific genes (Figure 1A). Strain Neuro14 had the highest number of unique genes, with 51 unique genes. This indicates a significant divergence from other strains, as these genes are specific to Strain Neuro14 and not found in any other strain. However, this may also mean



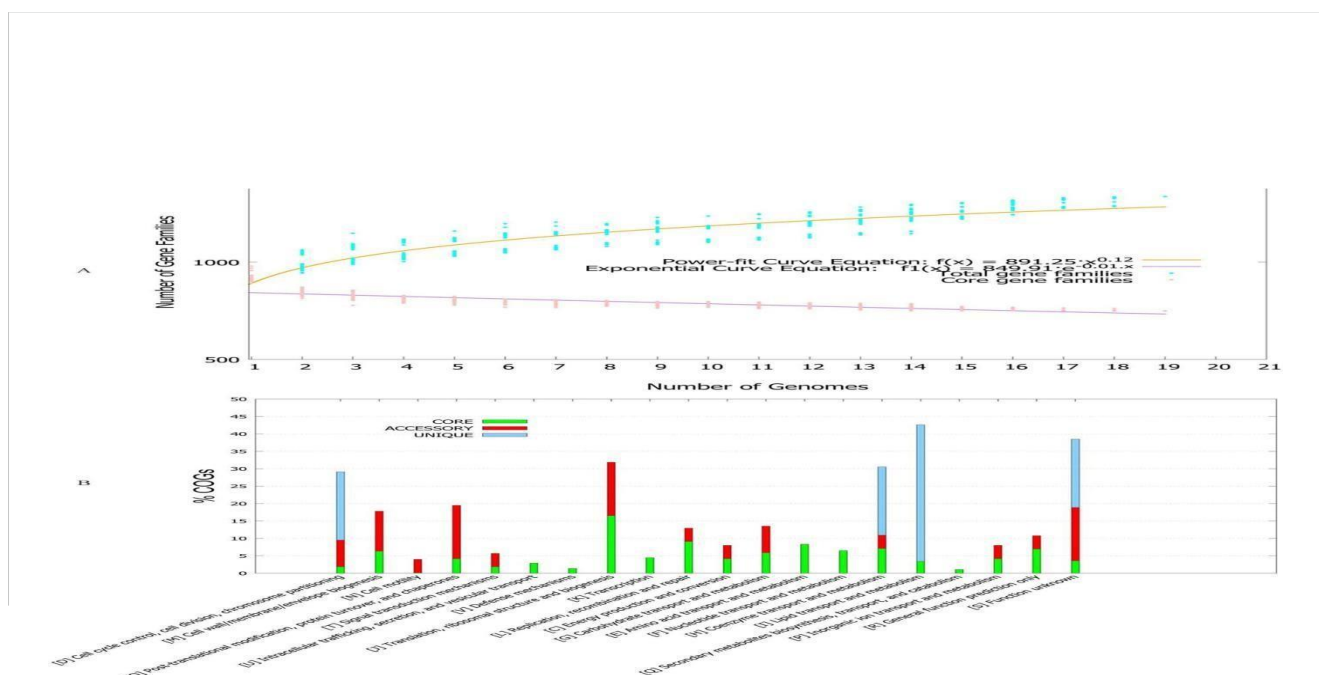
contaminated genome assembly. It also had largest number of missing genes, meaning these genes may have been lost during evolution or may be specific to certain serovars.

The power fit and exponential curve analysis indicate that as the number of genomes increases, the number of core gene families decreases. The negative exponent indicates that the curve is downward-sloping, suggesting a decreasing rate of change in core gene families with each additional genome. The rate of decrease is controlled by the exponent, with a higher value indicating a faster decay. Over time, the curve approaches an asymptote, representing a stabilization or saturation point where the number of core gene families stabilizes. Hence, the power-fit curve suggests a decreasing rate of change in core gene families with each additional genome, while the exponential curve suggests an initial rapid decay followed by stabilization.

Clusters of Orthologous Groups of proteins (COGs) were also

FIGURE 1

(A) Pan-genome accumulation curve of *Tropheryma whipplei* based on analysis of multiple genomes. The curve is modeled using an exponential regression equation, illustrating the growth of the pan-genome as more genomes are added. The x-axis represents the number of genomes included in the analysis, while the y-axis shows the cumulative number of gene families (orthologous groups). The shape of the curve provides insights into whether the species has an open or closed pan-genome, reflecting genomic diversity and gene acquisition potential. (B) Functional classification of genes within different genome fractions, expressed as percentages based on Clusters of Orthologous Groups (COG) categories. Genome fractions include the core genome (genes present in all strains), accessory genome (genes present in some but not all strains), and unique genome (strain-specific genes). Each bar represents the relative abundance of genes associated with specific COG functional categories, such as metabolism, cellular processes, information storage, and poorly characterized functions. This distribution reveals how functional roles are partitioned across conserved and variable regions of the genome.



and pan-genes revealed a diverse tree structure, with clusters positioned differently depending on gene allocation (Supplementary Figure S1). This variability underscores the dynamic nature of gene presence and absence across strains. Over time, certain strains may lose specific genes present in others, contributing to distortions in the depicted evolutionary relationships within the tree. While core genes represent a conserved set shared among all strains, they may not fully capture the intricate evolutionary history of a species. Conversely, pan-



genes provide a more comprehensive view by encompassing genes present in at least one strain within the population. However, pan-genes can also be heavily influenced by horizontally transferred gene events, introducing complexities into the evolutionary interpretation.

3.2 Drug target selection and 3D model study

Core proteome data was subjected to CD-HIT analysis, and a total of 749 sequences from the core proteome were obtained. Among these, 422 sequences were found to be common to the CEG, while 423 sequences were common to the DEG, and 409 sequences were found to be common to both CEG and DEG categories. Upon further analysis, 147 sequences were left after subtracting sequences similar to the human proteome. Subsequently, upon subtraction of these sequences from the gut beneficial microbiota proteome, 37 sequences remained. Within these, six were identified as virulence factors, indicating potential roles in pathogenicity. Only 11 were found to be similar to drug targets listed in the DrugBank database proteins (Supplementary Table S1). Among these, *murE* was selected as a drug target screening Ayurvedic products. *murE* is an enzyme that plays a crucial role in the biosynthesis of bacterial cell-wall peptidoglycan. Specifically, it catalyzes the addition of an amino acid to the nucleotide precursor UDP-N-acetylmuramoyl-L-alanyl-D-glutamate (UMAG). This enzymatic reaction is essential for the construction and maintenance of the bacterial cell wall, which provides structural support and protection to the cell. Hence, it could be used as a potential target for antibacterial agents due to its essential role in for bacterial survival.

3D structure of the *murE* gene product (UDP-N-acetylmuramyl-tripeptide synthetase) was obtained from AlphaFold

(<https://alphafold.com/entry/Q83HJ9>; retrieved 28 February 2024). Most of the structure has a very high pLDDT >90, depicting good quality (Supplementary Figure S2A). It was also evaluated by assess module of Swiss Model and showed 96.45% residues as Ramachandran favored and only 1.18% as outliers (Supplementary Figure S2B). PAE was low (Supplementary Figure S2C). This is a metric used to assess the accuracy

of residue alignments in a protein structure prediction. It measures the expected positional error (in Angstroms) between aligned residues in the predicted model and the corresponding residues in the true structure. A lower PAE value indicated a higher accuracy of residue alignment, meaning that the predicted model closely matched the true structure. It is particularly useful for assessing inter-domain accuracy, as it helps identify regions of the protein structure where the alignment may be less reliable.

3.3 Virtual screening

Autodock revealed top three interactors with lowest energy of -9.1, -8.6 and -7.9 kcal/mol as Ergost-5-en-3-ol, (3beta,24xi) (IUPAC name:

(3S,8S,10R,13R,17R)-17-[(2R,5S)-5,6-dimethylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta [a]phenanthren-3-ol), [6]-Gingerdiol 3-monoacetate (IUPAC name: [(3R,5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-yl] acetate) and Valtrate (IUPAC name: [(1S,6S,7R,7aS)-4-(acetyloxymethyl)-1-(3-methylbutanoyloxy)spiro [6,7a-dihydro-1H-cyclopenta [c]pyran-7,2'-oxirane]-6-yl] 3-methylbutanoate) (Figure 2). Ergost-5-en-3-ol, (3beta,24xi), with a molecular weight of 400.7 g/mol, made 19 interactions, with four hydrophobic (Ile152, Ala156, Cys162, Tyr482) and seven hydrophilic (Lys128, Glu158, Arg174, Arg352, Asp479, Asp480, Pro481) residue interactions. [6]-Gingerdiol 3-monoacetate, with a molecular weight of

338.4 g/mol, made 20 interactions with four hydrophobic (Ala313, Phe314, Met353, Ala374) and seven hydrophilic (Asn126, Lys128, His217, Arg352, Asp367, His368, His370) residues. This compound has been reported from the rhizome of the *Zingiber officinale* (Tao et al., 2008). Its anti-allergic potential has been noted



(Chen et al., 2009).

Valtrate, with a molecular weight of 422.5 g/mol, made 18 interactions, with four hydrophobic (Ile152, Phe314, Met353, Ala374) and nine hydrophilic (Asn126, Lys128, Glu216, His217, Arg352, Asp367, His368, His370, Asp479) residues (Figure 3). Valtrate belongs to iridoids monoterpenoids and is reported to be isolated from *Valeriana alliariifolia*, *Valeriana microphylla*, *Valeriana jatamansi*, *Valeriana vaginata*, *Valeriana sorbifolia*, *V. jatamansi*, *Valeriana officinalis* and *Valeriana pseudofficinalis* (source LOTUS database, <https://lotus.naturalproducts.net/>;

retrieved on 30 March 2024). It has shown good IC₅₀ against pancreatic cell lines PANC-1 (IC₅₀ = 23.7 ± 1.3 µM), BxPC-3 (IC₅₀ = 20.14 ± 1.2 µM), AsPC-1 (IC₅₀ = 10.67 ± 0.9 µM), Hs 766t (IC₅₀ = 24.12 ± 1.2 µM), and Capan-2 cells (IC₅₀ = 13.45 ± 0.92 µM)

for 24 h treatment (Chen et al., 2021). It accomplished anti-pancreatic cancer effect by inhibiting Stat3 signaling. It also exhibits apoptosis of breast cancer cells and their migration (Tian et al., 2020). In Glioblastoma, analysis of PDGFRA protein and downstream mediators have revealed that Valtrate effectively

inhibits PDGFRA/MEK/ERK signaling pathways (Liu et al., 2023). It also reduces tumor volume in tumor-bearing nude mice and improves survival outcomes.

The results obtained from the machine learning (ML)-based methods demonstrated a strong correlation with the AutoDock scores, confirming the consistency and reliability of the computational predictions (Table 2). Ergost-5-en-3-ol (3beta,24xi) demonstrated the strongest interaction with the target protein, as indicated by its combined Vinardo score and CNN affinity. [6]-Gingerdiol 3-monoacetate exhibited moderate binding affinity, with a DiffDock confidence score of -2.08, suggesting slightly greater uncertainty in its binding compared to Ergost-5-en-3-ol. However, its Vinardo score and CNN affinity score still indicated a reasonable strength of interaction. Valtrate showed the weakest binding affinity among the three ligands, with a DiffDock confidence score of -1.41, indicating moderate confidence in its binding. The Vinardo score, along with a lower CNN score and

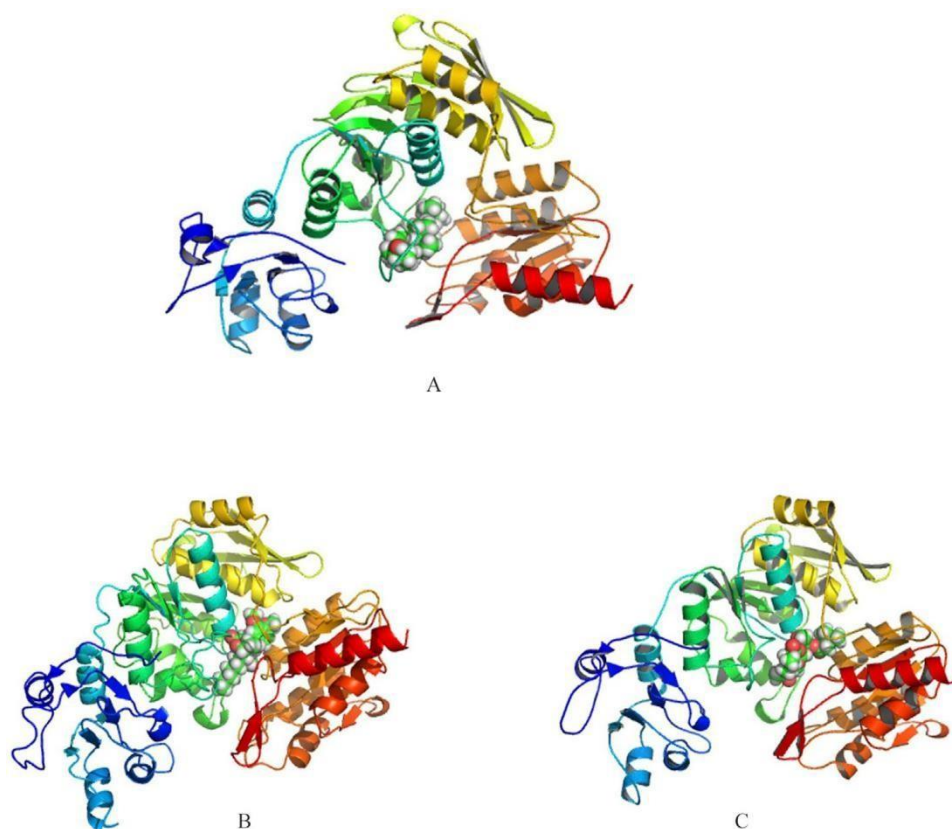


FIGURE 2

3D representations of the binding interactions between the predicted structure of UDP-N-acetylmuramyl-tripeptide synthetase and selected phytochemicals (A) Ergost-5-en-3-ol, (3beta,24xi), (B) [6]-Gingerdiol 3-monoacetate and (C) Valtrate. Phytochemicals are depicted in space-filling representation, while the protein is shown as a ribbon structure to illustrate the overall fold and ligand-binding orientation.

3.4 Target promiscuity

All the compounds did not indicate promiscuity (Supplementary Figure S3), using both cell based and target based model. This implies that the compounds exhibited selectivity towards their intended targets without significantly interacting with other off-target molecules or pathways. Such specificity is desirable in drug development, as it minimizes the risk of unintended side effects and enhances the therapeutic efficacy of the compounds. The results obtained from Swiss TargetPrediction (Supplementary Figure S4) for the top 15 enzyme classes revealed distinctive patterns of interaction for Ergost-5-en-3-ol, (3beta,24xi), [6]-Gingerdiol 3-monoacetate, and Valtrate. Ergost-5-en-3-ol, (3beta,24xi) exhibited the highest fraction of interaction with cytochrome P450, suggesting a tendency for metabolic interactions (Jumpa-Ngern et al., 2022). Additionally, it demonstrated significant binding affinity towards LXR-alpha and

the androgen receptor, indicating potential involvement in lipid/carbohydrate metabolism (Mohammadi and Oshaghi, 2014) and hormonal regulation (Heilmann-Heimbach et al., 2020), respectively. On the other hand, [6]-Gingerdiol 3-monoacetate displayed affinity for kinases, GPCR-A class, and the nuclear receptor family, suggesting a potential role in signaling pathways and cellular regulation. Valtrate exhibited high affinity for proteases, kinases, and the GPCR A class family, suggesting its potential involvement in modulating various cellular signaling pathways and biological processes.

3.5 ADMET properties

ADMET properties were compared for the prioritized compounds (Table 3) and comparison highlighted potential differences between the studied compounds. Ergost-5-en-3-ol, (3beta,24xi) had the lowest water solubility, followed by Valtrate and [6]-Gingerdiol 3-monoacetate, potentially impacting its absorption. To improve absorption, cyclodextrins are a viable option. They



are widely recognized as safe excipients in pharmaceutical formulations, with approved applications in drug delivery. Unlike some lipid-based or nanocarrier formulations that may pose toxicity concerns, cyclodextrins generally have low

immunogenicity and toxicity, making them a preferred choice for improving drug solubility and stability (Nicolaescu et al., 2025). Hence, AI based cyclodextrin complexation was done for this

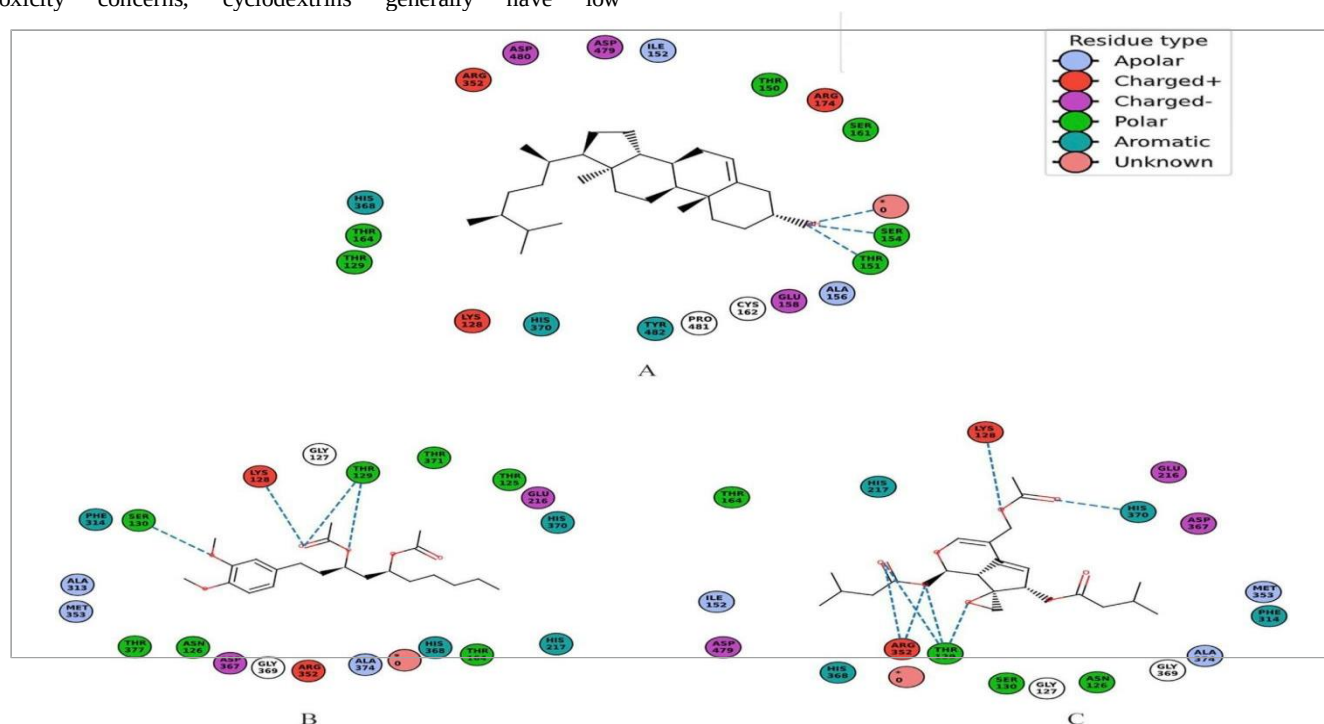


TABLE 2 Comparative binding affinity scores of candidate compounds docked with UDP-N-acetylmuramyl-tripeptide synthetase, using AutoDock, DiffDock, and CNN-based methods.

Ligand	AutoDock (Kcal/mol)	score	DiffDock confidence score	Vinardo score	CNN score	CNN affinity (Kcal/mol)
Ergost-5-en-3-ol, (3beta,24xi)	-9.1		-1.88	-6.465	0.403	6.243
[6]-Gingerdiol 3-monoacetate	-8.6		-2.08	-5.538	0.156	4.604
Valtrate	-7.9		-1.41	-4.689	0.089	4.87

TABLE 3 Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of prioritized inhibitors.

Property	Model name	Ergost-5-en-3-ol, (3beta,24xi)	[6]-Gingerdiol 3-monoacetate	Valtrate	Unit
Absorption	Water solubility	-7.068	-3.607	-4.459	Numeric (log mol/L)
	Caco2 permeability	1.223	0.965	0.968	Numeric (log Papp in 10 ⁻⁶ cm/s)



	Intestinal absorption (human)	94.543	89.858	98.778	Numeric Absorbed) (%)
	Skin Permeability	-2.86	-2.765	-2.838	Numeric (log Kp)
	P-glycoprotein substrate	No	No	No	Categorical (Yes/No)
	P-glycoprotein inhibitor I	Yes	Yes	Yes	Categorical (Yes/No)
	P-glycoprotein inhibitor II	Yes	No	Yes	Categorical (Yes/No)
Distribution	VDss (human)	0.427	0.27	-0.181	Numeric (log L/kg)
	Fraction unbound (human)	0	0.249	0.194	Numeric (Fu)
	BBB permeability	0.774	-0.466	-1.105	Numeric (log BB)
	CNS permeability	-1.758	-2.858	-2.928	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	No	No	Categorical (Yes/No)
	CYP3A4 substrate	Yes	Yes	Yes	Categorical (Yes/No)
	CYP1A2 inhibitor	No	No	No	Categorical (Yes/No)
	CYP2C19 inhibitor	No	Yes	No	Categorical (Yes/No)
	CYP2C9 inhibitor	No	No	No	Categorical (Yes/No)
	CYP2D6 inhibitor	No	No	No	Categorical (Yes/No)
	CYP3A4 inhibitor	No	No	No	Categorical (Yes/No)
Excretion	Total Clearance	0.572	1.353	0.724	Numeric (log mL/min/kg)
	Renal substrate OCT2	No	No	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	No	Yes	Categorical (Yes/No)
	Max. tolerated dose (human)	-0.458	0.787	0.178	Numeric (log mg/kg/day)
	hERG I inhibitor	No	No	No	Categorical (Yes/No)
	hERG II inhibitor	Yes	No	No	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	2.08	2.014	3.201	Numeric (mol/kg)



Oral Rat Chronic Toxicity (LOAEL)	0.892	2.03	1.38	Numeric (log mg/kg_bw/day)
Hepatotoxicity	No	No	No	Categorical (Yes/No)
Skin Sensitisation	No	No	No	Categorical (Yes/No)
<i>T.Pyiformis</i> toxicity	0.631	1.332	0.308	Numeric (log ug/L)
Minnow toxicity	-1.94	0.87	0.482	Numeric (log mM)

cyclodextrins such as DMCD and TMCD showed comparable but slightly weaker binding (Supplementary Table S2). Valtrate and Ergost-5-en-3-ol, (3beta,24xi) had a more consistent pattern across cyclodextrins, with SBE-β-CD and TMCD generally producing the most favorable results. Overall, SBE-β-CD appeared to be the most effective cyclodextrin for improving the solubility of these compounds.

All compounds had relatively low predicted Caco2 permeability (<1.5), suggesting moderate passive

diffusion across cell membranes. They also depicted good predicted intestinal absorption (>89%), where it was highest for Valtrate followed by ergost-5-en-3-ol, (3beta,24xi) and then [6]-Gingerdiol 3-monoacetate. Skin permeability appeared to be similar for all. All three were predicted to be P-gp inhibitors, potentially enhancing their own absorption and that of co-administered drugs. Valtrate had the highest predicted volume of distribution (Vdss), indicating wider distribution in the body. Valtrate and [6]-Gingerdiol 3-monoacetate had predicted protein

TABLE 4 Physiologically-Based Pharmacokinetic (PBPK) values for prioritized compounds. AUC = area under curve.

Endpoint	Fa [%]	FD p [%]	F [%]	Cmax [ug/mL]	Tmax [h]	AUC(0-inf) [ng-h/mL]	AUC(0-t) [ng-h/mL]
Ergost-5-en-3-ol (3beta,24xi) in normal	3.3489	3.3486	3.3483	0.046	24	943.41	943.41
Ergost-5-en-3-ol (3beta,24xi) in cirrhosis	3.5286	3.5282	3.5282	0.0081	2.1424	504.41	66.041
Ergost-5-en-3-ol (3beta,24xi) in renally impaired	3.3726	3.3722	3.3722	0.0107	2.1952	273.99	85.388
[6]-Gingerdiol monoacetate in normal	3-97.65	97.65	97.65	0.2772	5.2776	22,180	3822.2
[6]-Gingerdiol monoacetate in cirrhosis	3-97.62	97.62	97.62	0.1937	5.5336	135,000	2929.9
[6]-Gingerdiol monoacetate in renally impaired	3-96.94	96.948	96.948	0.2958	5.6008	22,550	4198.5



Valtrate in normal	99.976	99.974	99.974	0.5825	2.3444	294,500	5389.5
Valtrate in cirrhosis	99.919	99.917	99.917	0.4641	2.3236	4,613,000	5091
Valtrate in renally impaired	99.982	99.98	99.98	0.5059	2.2768	289,800	4712.6

binding, potentially affecting their free drug concentration available for action.

All of the compounds had low predicted blood-brain barrier (BBB) permeability, suggesting limited brain penetration. None was predicted to be substrates for CYP2D6, a common drug metabolizing enzyme and all were predicted to be substrates for CYP3A4, another major drug metabolizing enzyme. This could impact their half-life and potentially lead to interactions with other CYP3A4 substrates. Valtrate had the highest predicted total clearance, suggesting faster elimination from the body. Only Valtrate was predicted to be a potential Ames mutagen, requiring further investigation. All had relatively high predicted oral rat acute toxicity (LD50) values, suggesting low acute toxicity.

3.6 PBPK profile

PBPK values of these compounds under different conditions (normal, cirrhosis, and renal impairment) were calculated (Table 4) and compared. Fraction of the dose absorbed (Fa) from the gastrointestinal tract was high (above 96%) for all compounds, suggesting good oral bioavailability for all compounds, regardless of the condition. Fraction of the dose delivered to the systemic circulation (FDp) after escaping first-pass metabolism was very similar to Fa, implying minimal first-pass metabolism for all compounds. Overall fraction of the dose available systemically was also identical to Fa and FDp due to the minimal first-pass effect. Ergost-5-en-3-ol showed a significant decrease in maximum concentration (Cmax) of the drug in the blood after administration under cirrhosis and renal impairment compared to normal, suggesting a potential impact of these conditions on its absorption or distribution. [6]-Gingerdiol 3-monoacetate showed some decrease in Cmax under cirrhosis but not under renal impairment. Valtrate had the highest Cmax in all conditions, and the impact of cirrhosis or renal impairment seemed minimal.

The analysis of pharmacokinetic parameters unveiled intriguing trends for Ergost-5-en-3-ol, [6]-Gingerdiol 3-monoacetate, and Valtrate across

different physiological conditions. Ergost-5-en-3-ol exhibited a notable decrease in Tmax under cirrhosis and renal impairment, suggesting unexpectedly faster absorption rates. This phenomenon contradicted the anticipated slower absorption due to compromised liver or kidney function, prompting the need for deeper investigation into its mechanisms. Conversely, [6]-Gingerdiol 3-monoacetate showed a modest increase in Tmax across all conditions compared to the normal state, indicating a tendency for slightly delayed absorption. Meanwhile, Valtrate consistently displayed the shortest Tmax under all conditions, implying rapid absorption independent of physiological status. The AUC(0-inf) (ng-h/mL) parameter represents the area under the concentration-time curve from time zero to infinity, providing a measure of total drug exposure. Ergost-5-en-3-ol exhibited a significant decrease in AUC(0-inf) under cirrhosis and renal impairment compared to normal conditions, aligning with the observed reduction in Cmax. Conversely, [6]-Gingerdiol 3-monoacetate displayed a slight decrease in AUC(0-inf) under cirrhosis but not under renal impairment. Valtrate consistently demonstrated the highest overall AUC(0-inf) across all conditions, indicating the greatest systemic exposure. Similarly, the AUC(0-t) (ng-h/mL) parameter, representing the area under the concentration-time curve from time zero to the last



measured time point, mirrored the trends observed for AUC(0-inf).

This analysis underscores the potential impact of liver and kidney function on the pharmacokinetics of these compounds. Ergost-5-en-3-ol demonstrates the most pronounced susceptibility to cirrhosis and renal impairment, as evidenced by reduced C_{max}

and AUC(0-inf) under these conditions. In contrast, [6]-Gingerdiol 3-monoacetate exhibits a moderate impact of cirrhosis, with slight decreases in both C_{max} and AUC(0-inf). Valtrate, on the other hand, appears to be minimally affected by cirrhosis or renal impairment, maintaining consistently high C_{max} and AUC(0-inf) across all conditions.

3.7 MD simulation

The RMSD, RMSF, and Rg values obtained from molecular dynamics simulations provide valuable insights into the structural stability and flexibility of the studied compounds and their

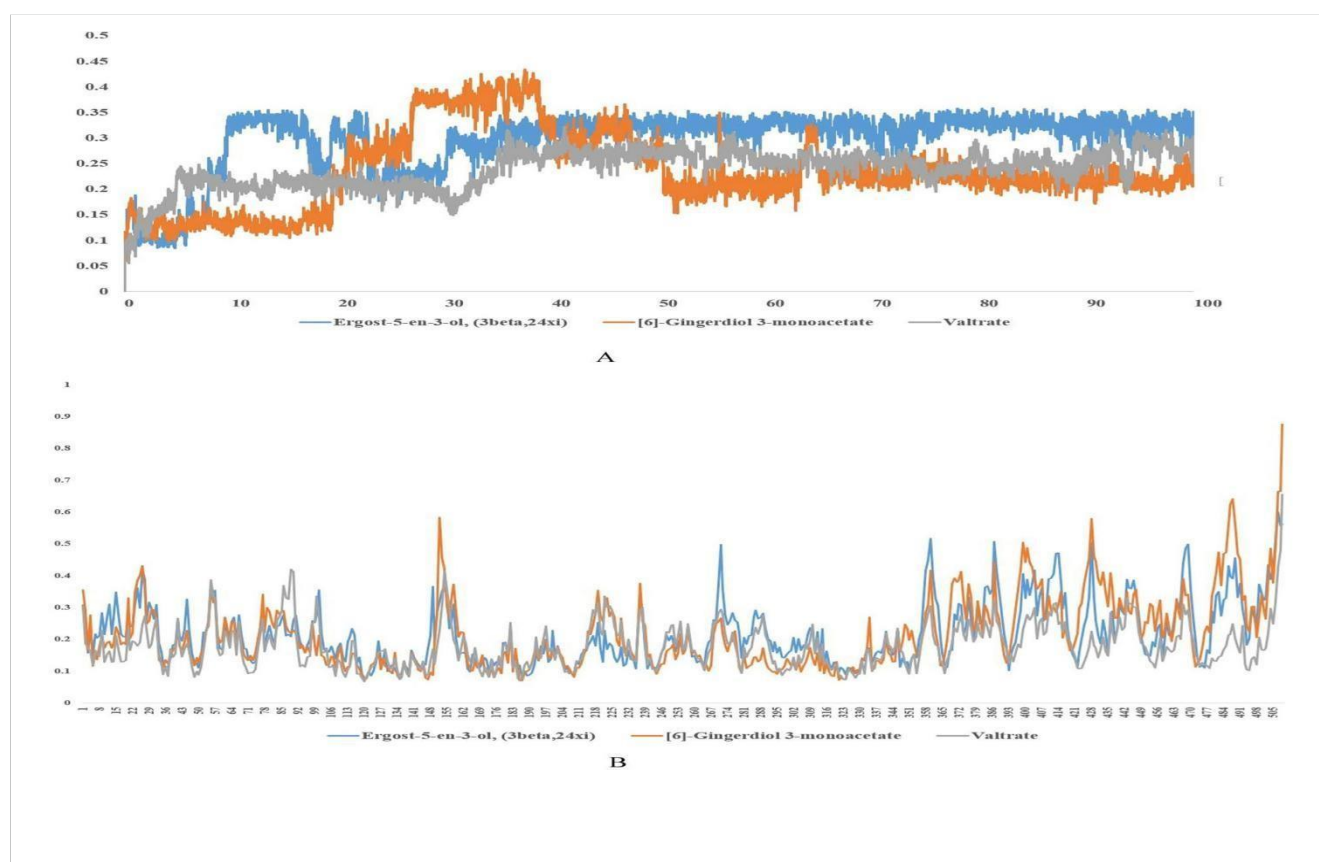


FIGURE 4

(A) Root Mean Square Deviation (RMSD) plot showing the structural stability of UDP-N-acetylmuramyl-tripeptide synthetase in complex with each ligand (Ergost-5-en-3-ol, [6]-Gingerdiol 3-monoacetate, and Valtrate) over a 100 ns molecular dynamics simulation. RMSD values are measured in nanometers (nm) and represent backbone displacement over time, indicating conformational stability and ligand binding persistence. (B) Root Mean Square Fluctuation (RMSF) plot of the enzyme residues during the 100 ns simulation for each ligand-bound complex. RMSF values (in nm) reflect the flexibility and local motion of amino acid residues, highlighting regions of structural rigidity or fluctuation upon ligand binding. Interactions with the UDP-N-acetylmuramyl-tripeptide synthetase protein. The compounds were able to maintain their structural integrity and form stable interactions with the



protein. RMSD of UDP-N-acetylmuramyl-tripeptide synthetase and Ergost-5-en-3-ol (3 β ,24xi) as well as [6]-Gingerdiol 3-monoacetate was less than or around 0.5 nm (or 5 Å) for the whole simulation period, while it was less than 0.4 nm (4 Å) for Valtrate (Figure 4). For Ergost-5-en-3-ol, (3 β ,24xi), it was on average less than 0.35 nm (3.5 Å) and increased upto 4 nm (40 Å) for [6]-Gingerdiol 3-monoacetate, from 20–30 ns but decreased sharply around 50 ns and remained less than 0.25 for rest of the simulation time. For Valtrate, it increased from 0.2–0.25 nm (2–2.5 Å) after 40 ns but stabilized

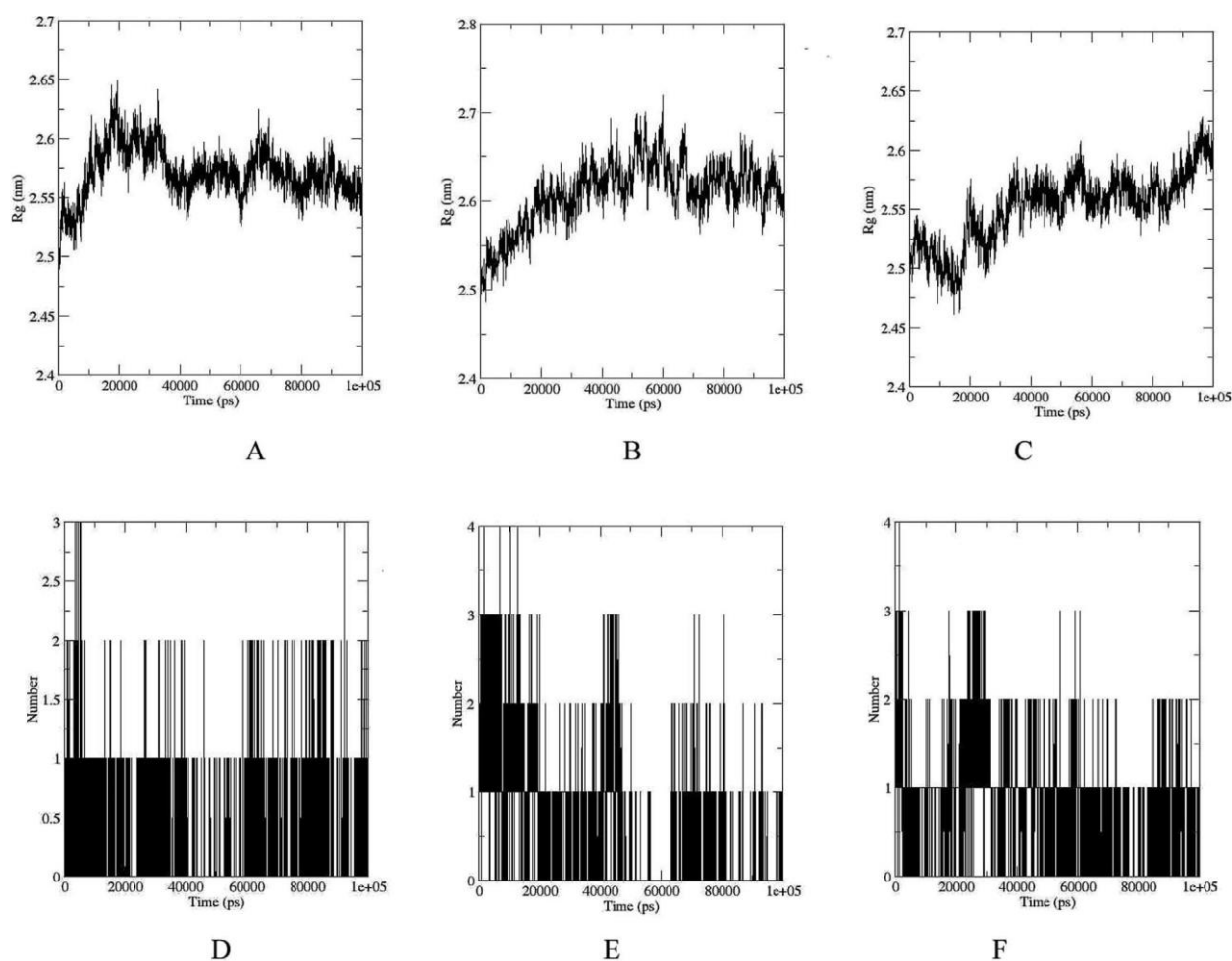


FIGURE 5

Rg plots of UDP-N-acetylmuramyl-tripeptide synthetase bound with (A) Ergost-5-en-3-ol, (3 β ,24xi) (B) [6]-Gingerdiol 3-monoacetate (C) Valtrate. Peaks indicate regions of higher mobility, whereas troughs represent structurally stable regions, often corresponding to secondary structure elements or ligand interaction sites. Time evolution of hydrogen bonds formed between the enzyme and each ligand during the simulation: (D) Ergost-5-en-3-ol, (3 β ,24xi) (E) [6]-Gingerdiol 3-monoacetate, and (F) Valtrate.



after 50 ns and remained less than 0.3 nm (3 Å) for rest of the time. RMSF was most stable and remained less than 0.25 nm (2.5 Å) for Valtrate, comparatively lower than other compounds. This indicates that it may exhibit less conformational variability and maintain a more rigid structure during interaction with the protein. Rg (Figures 5A–C) also remained less than 0.6 nm (6 Å) for all compounds. Molecules remained relatively compact throughout the simulation, indicating globular or tightly packed 3D structure. Such compactness is often advantageous as it enhances the ability of molecules to passively diffuse through cell membranes. This property is particularly desirable for drugs that need to efficiently reach their target sites within cells but factors like charge and hydrophobicity also play a role in membrane permeability, apart from size and shape.

The analysis of hydrogen bond interactions (Figures 5D–F) revealed that UDP-N-acetylmuramyl-tripeptide synthetase and Ergost-5-en-3-ol, (3beta,24xi) complex did not surpass three hydrogen bonds over the 100 ns simulation period. Moreover, the majority of the time, only two hydrogen bonds were observed. Similarly, for [6]-Gingerdiol 3-monoacetate and Valtrate,

the number of hydrogen bonds did not exceed four, with predominantly two bonds observed throughout most of the simulation time. These findings indicate that the compounds may not form extensive or strong hydrogen bonding networks with UDP-N-acetylmuramyl-tripeptide synthetase. Instead, other types of interactions, such as hydrophobic interactions or van der Waals forces, dominate the binding between the compounds and the protein.

PCA on hydrogen bond data (Supplementary Figure S5A) and trajectory RMSD (Supplementary Figure S5B) was also carried out, where hydrogen bond PCA exhibited a linear and scattered distribution along PC1 and PC2, indicative of temporal variability in bonding pattern. This variability reflects intrinsic conformational flexibility rather than structural instability, as no collapse or major disruption was observed. While hydrogen bonds are critical for molecular recognition and structural cohesion, their transient nature during MD simulations is well-documented and does not inherently imply instability. The observed fluctuations in hydrogen bond patterns are consistent with solvent-driven dynamics and conformational flexibility typical of stable

TABLE 5 Molecular Mechanics/Poisson–Boltzmann Surface Area (MM/PBSA) values of prioritized compounds over 100 ns. Components include van der Waals, electrostatic, polar solvation, and non-polar solvation contributions.

MM/PBSA energy values	Average MM/PBSA for ergost-5-en-3-ol, (3beta,24xi)	Standard deviation	Average MM/PBSA for [6]-Gingerdiol 3- monoacetate	Standard deviation	Average MM/ PBSA for Valtrate	Standard deviation
Complex	–31918.63	319.07	–32018.68	344.44	–31953.99	291.28
Receptor	–32012.72	317.60	–32056.34	340.43	–32019.67	288.81
Ligand	123.48	8.44	61.11	5.96	89.58	6.38
Delta (Complex - Receptor - Ligand)	–29.39	2.43	–23.45	4.18	–23.90	3.23

biomolecular complexes. Overall stability arises from the interplay of multiple non-covalent interactions, including hydrophobic contacts, van der Waals forces, salt bridges, and entropic effects, which together preserve the structural and energetic resilience of the assemblies. PCA of RMSD data revealed moderate dispersion with



overlapping data points across complexes, suggesting structural integrity was largely retained throughout the simulation.

MM/PBSA calculation (Table 5) showed that three compounds demonstrated negative binding free energy ($\Delta(\text{Complex} - \text{Receptor} - \text{Ligand})$) values, implying favorable interactions. Among them, Valtrate exhibited the most negative Δ value, indicating potentially stronger binding affinity compared to Ergost-5-en-3-ol and [6]-Gingerdiol 3-monoacetate. However, the differences in Δ values between the compounds were relatively small. Overall, the MM/PBSA results suggest that all three compounds have the capacity to bind to the receptor UDP-N-acetylmuramyl-tripeptide synthetase with favorable free energy.

4 Discussion

The prevalence of the bacteria *Tropheryma whipplei* seems to be higher than first thought. The symptoms seen by many individuals infected with this bacteria are not always pinpointed. But since the bacteria is so difficult to routinely cultivate, making a diagnosis of this illness is quite challenging (Dolmans et al., 2017). It is very meticulous and clusters cells together (Massetot et al., 2003). It presents clinically seldom but may be lethal if left untreated (Edouard et al., 2020). Its disease progression is biphasic, beginning with a prodromal phase marked by a wide range of symptoms associated with persistent nonspecific symptoms, such as arthralgia and arthritis (Marth et al., 2016). Weight loss, diarrhea, and other systemic indications are defined by the other stage, which depends on the area of involvement (Rocha et al., 2015). Penicillin, streptomycin, and tetracycline were the antibiotics of choice for a long time, however there were reports of a high recurrence rate after their use. Additionally, it has been observed that *T. whipplei* can resist the antibiotic fluoroquinolones (Lv et al., 2022). According to Zhou et al. (2022), one possible way to overcome resistance is to mix IFN- γ with antibiotics or doxycycline with hydroxychloroquine. To avoid recurrence, doxycycline should be used for life.

The variety and abundance of plant chemicals provides a treasure trove of unique chemical structures that may have medicinal uses (Lautie et al., 2020). Digoxin (*Digitalis purpurea*), aspirin (*Salix alba*) (Nica et al., 2020), and morphine (*Papaver somniferum*) (Shetge et al., 2020) are only a few examples of the many commonly used pharmaceuticals that have their origins in natural products. According to Berdigaliyev and Aljofan (2020), medicinal chemists may use natural products as a foundation to create more effective and selective derivatives, even if they aren't ideal drugs in and of themselves. For this reason, we tested over a thousand lead-like compounds against the Ayurvedic medicinal plant *T. whipplei*'s UDP-N-

acetylmuramyl-tripeptide synthetase. Traditional Indian treatment has relied heavily on Ayurvedic botanicals for thousands of years (Parveen et al., 2020). The medicinal qualities of Indian plants are highly esteemed and used for a wide range of purposes, including health promotion, illness prevention, and the treatment of different maladies (Prasathkumar et al., 2021). The adaptogenic qualities of Ashwagandha (*Withania somnifera*) aid the body in adjusting to stressful situations (Gupta et al., 2021). Wolfe et al. (2020), Jantan et al. (2021), and Zeng et al. (2021) all attest to turmeric's (*Curcuma longa*) anti-inflammatory and antioxidant properties, which make it highly sought-after. The antibacterial and immune-boosting qualities of tulsi (*Ocimum sanctum*) have earned it much respect (Rao et al., 2023).

The present investigation used structural docking to rank three compounds—Ergost-5-en-3-ol, [6]-Gingerdiol 3-monoacetate, and Valtrate—against a therapeutic target, UDP-N-acetylmuramyl-tripeptide synthetase, derived from the core genome of *T. whipplei*. Because they did not display any promiscuous behavior, these compounds demonstrated target selectivity. Treatment results and resistance development might be improved by using them alone or by investigating combination therapy options that use lead compounds' synergistic interactions or their combination with current antibiotics (Zhu et al., 2021). Although the drugs varied in a number of pharmacokinetic characteristics, they all had positive ADME profiles. Of the three chemicals tested, Ergost-5-en-3-ol (3 β ,24 α) had the lowest water solubility, which may limit its absorption in the digestive tract because of its poor dissolution. Valtrate and [6]-Gingerdiol 3-monoacetate were more soluble in water, which meant they were more absorbable. All of the substances demonstrated significant passive diffusion across cell membranes, as anticipated by their comparatively low Caco2 permeability. In spite of this, they showed excellent projected intestinal



absorption (>89%), which indicates effective absorption in the intestines. There was little acute toxicity as all of the compounds had quite high LD50 values for oral rat acute toxicity.

In studies conducted by Tian et al. (2020), Chen et al. (2021), and Liu et al. (2023), it was shown that valtrate inhibits the multiplication of cancer cells in different types of tumors. Additionally, it exhibited the greatest anticipated total clearance, suggesting quicker removal from the body, but it also showed signs of AMES toxicity. According to da Silva Dantas et al. (2020), the AMES test is a commonly used method for determining mutagenicity, which might mean that the substance could cause cancer in people. Because of this, its therapeutic potential and safety profile are major questions. This could make it harder to use Valtrate as a therapy, particularly for longer treatments or in cases where continuous exposure is required. To improve Valtrate's safety profile, we may look for strategies to reduce genotoxicity, such structural changes or combination therapy with protective drugs.

In all tested settings, including normal, cirrhosis, and renal impairment, the oral bioavailability of all drugs was good, with fractions of the dosage absorbed (Fa) from the gastrointestinal tract reaching 96%. In addition, there was negligible first-pass metabolism for all drugs, as shown by the percentage of the dosage that made it to the systemic circulation after escaping first-pass metabolism (FDp), which closely matched Fa. So, total systemic availability of the dosage was similar to Fa and FDp, demonstrating that first-pass metabolism had no effect on systemic exposure. Nevertheless, under various physiological circumstances, the compounds' maximum concentrations of the drug in the blood (Cmax) varied significantly. Cirrhosis and renal impairment were associated with a markedly lower Cmax for ergost-5-en-3-ol compared to healthy subjects, which may indicate changes in the kinetics of absorption or distribution. Cmax decreased somewhat with cirrhosis but not under renal impairment for [6]-Gingerdiol 3-monoacetate as well. Yet, valtrate showed the greatest Cmax in every condition except cirrhosis and renal impairment, where it had no effect. These results indicate that the lead compounds' pharmacokinetics were affected differently by distinct physiological states, with Valtrate showing the strongest distribution and absorption patterns. The lead compounds bound stably to UDP-N-acetylmuramyl-tripeptide synthetase, as validated by the MD simulation data. The protein-ligand combination was confirmed to be stable upon examination of intermolecular interactions, which

included hydrophobic contacts and hydrogen bonds. The binding free energy was further clarified and the findings of the MD simulations were further validated by the MM-PBSA analysis.

One promising avenue for the development of new antibacterial medicines is the inhibition of bacterial cell wall production, which has recently been the target of lead compounds with inhibitory action (Zhang F. et al., 2022). Studies using computer-aided drug design do have certain drawbacks, however. It has been suggested that the results produced by computational virtual screening, while useful for drug discovery (Bender and Cortés-Ciriano, 2021; Sadybekov and Katritch, 2023), may not necessarily correspond to the actual results. It is very uncommon for these *in silico* models to make assumptions about receptor conformations, solvation dynamics, and ligand flexibility that could lead to an oversimplification of biological complexity. Addressing these shortcomings requires more experimental confirmation by cytotoxicity profiling and *in vitro* enzyme inhibition studies.

verify that the lead compounds are safe, have the expected bioactivity, and binding affinity. Therapeutic potential evaluation in biological systems also requires *in vivo* investigations, which include pharmacokinetic and toxicity evaluations. Furthermore, other possible targets and treatment methods may have been overlooked due to the study's exclusive concentration on UDP-N-acetylmuramyl-tripeptide synthetase as a viable therapeutic approach for Whipple's illness. A more solid foundation for *T. whipplei* drug development might be a systems biology or multi-target strategy. There has to be further research on the structure-activity relationship and optimization of AMES toxicity with Valtrate since it makes one wonder about the drug's safety profile and if it is mutagenic. In conclusion, this study provides valuable information for medication development in the treatment of Whipple's illness, but its results may not be applicable to other infectious diseases or bacterial infections; more investigation is needed to determine the study's generalizability.

5 Conclusion

Specifically, *T. whipplei* is responsible for causing Whipple's illness. So yet, no natural products have been tested against it, as far as the authors are aware. The protein UDP-N-acetylmuramyl-tripeptide synthetase is crucial for the construction of cell walls, and this research tested lead-like ayurvedic chemicals against it. An inhibitor prioritization algorithm was



developed using a computational virtual screening approach. Both docking scores and molecular dynamics (MD) simulations verified that these chemicals interacted steadily. The solubility was enhanced by the discovery of cyclodextrin SBE- β -CD. The safety profiles of the lead compounds were generally positive, offering prospects for future optimization and development, even if AMES toxicity did occur with Valtrate. Because medicinal chemistry methods and structure-activity relationship

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studies may help with the discovery and synthesis of analogs with better therapeutic profiles, efforts might be made to optimize Valtrate such that its characteristics are enhanced. The safety profiles and inhibitory activity of the lead drugs should be confirmed via further, more thorough in vitro and in vivo validation.

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