Comparative Molecular Docking of Hyptis Verticillata Phytocompounds Against DNA Gyrase B and Multiple Bacterial Drug Targets

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INTRODUCTION

The rise of antimicrobial resistance (AMR) has become one of the most critical global health challenges, with multidrug-resistant bacteria severely reducing the efficacy of conventional antibiotics. According to the World Health Organization, resistant strains of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* pose significant therapeutic challenges due to multiple resistance mechanisms, including efflux pump activation, enzymatic degradation, and mutation of drug targets (WHO, 2020). These limitations highlight the urgent need for alternative therapeutic strategies and novel bioactive scaffolds.

Natural products, particularly phytochemicals, have historically served as abundant sources of antimicrobial agents, with more than half of approved antibiotics derived from natural origins (Newman & Cragg, 2020). Plants produce diverse secondary metabolites—such as terpenoids, alkaloids, phenolics, and lignans—that display significant antibacterial activity and structural diversity, making them promising candidates for antimicrobial drug discovery (Gibbons, 2004).

Hyptis verticillata Jacq., a medicinal plant from the Lamiaceae family, is traditionally used to manage infections, inflammation, wounds, and gastrointestinal disorders. Phytochemical studies have confirmed the presence of notable bioactive compounds including sesquiterpenoids such as aromadendr-1(10)-en-9-one and cadina-4,10(15)-dien-3-one (Delgado et al., 2005), monoterpenes like α-pinene and β-pinene (Wong et al., 1995), the phenolic monoterpenoid thymol (Saxena & Khosa, 1979), the lignan dehydropodophyllotoxin (Hernández et al., 1995), the triterpenoid oleanolic acid, and a pyrrolidinone derivative, (R)-5-hydroxypyrrolidin-2-one (Hernández et al., 1995; Bahado-Singh et al., 2018). Many of these compounds exhibit antibacterial, anti-inflammatory, antioxidant, and cytotoxic potentials, supporting their relevance in antimicrobial research.

One of the most validated bacterial drug targets is DNA gyrase B (GyrB), an ATP-dependent enzyme essential for DNA supercoiling, replication, transcription, and repair. Inhibitors targeting the ATP-binding pocket of GyrB—unlike fluoroquinolones that target GyrA—show promise against resistant strains and reduce the emergence of resistance (Maxwell & Lawson, 2003; Collin et al., 2011). Other bacterial protein targets, such as dihydrofolate reductase (DHFR), penicillin-binding proteins (PBPs), and enoyl-acyl carrier protein reductase (FabI), are equally critical for cell viability and have been successfully employed in computational drug discovery (Singh & Mishra, 2020). Molecular docking is an efficient computational tool used to predict ligand–protein interactions, enabling the identification of potential inhibitors and guiding rational drug development. Comparative docking, in particular, assesses the binding behavior of multiple compounds across different targets, facilitating the discovery of broad-spectrum or target-specific inhibitors. Given the growing antimicrobial resistance burden and the bioactive potential of *Hyptis verticillata*, this study employs comparative molecular

docking to evaluate eight confirmed phytochemicals—aromadendr-1(10)-en-9-one, cadina-4,10(15)-dien-3-one, α-pinene, β-pinene, thymol, dehydropodophyllotoxin, oleanolic acid, and (R)-5-hydroxypyrrolidin-2-one—against DNA gyrase B and additional bacterial drug targets. The findings aim to identify promising natural inhibitors that could contribute to the development of next-generation antibacterial agents.

METHODOLOGY

Ligand Selection and Preparation

Eight confirmed phytocompounds from *Hyptis verticillata*—aromadendr-1(10)-en-9-one, cadina-4,10(15)-dien-3-one, (R)-5-hydroxypyrrolidin-2-one, dehydropodophyllotoxin, oleanolic acid, α-pinene, β-pinene, and thymol—were selected as ligands for this study. Their 3D chemical structures were retrieved from the **PubChem database** (Kim et al., 2023). Each ligand was energy-minimized using the **MM2 force field in ChemDraw 3D**, and the minimized structures were converted to **PDBQT format using Open Babel** for docking. Partial charges were assigned via the Gasteiger method, and rotatable bonds were optimized to allow flexibility during docking (Morris et al., 2009).

Table 1: Phytocompounds Identified and Used for Docking

S/N	Compound Name	Molecular Formula
1	Aromadendr-1(10)-en-9-one	$C_{15}H_{22}O$
2	Cadina-4,10(15)-dien-3-one	$C_{15}H_{22}O$
3	Dehydropodophyllotoxin	$C_{22}H_{18}O_8$
4	Oleanolic Acid	$C_{30}H_{48}O_3$
5	Thymol	$C_{10}H_{14}O$
6	3a,4,5,6,7,7a-hexahydro-4,7-methanoindene	**$C_{10}H_{14}$**
7	4,7-methanon-1H-indene	**$C_{10}H_{14}$**
8	R-R,R-E-trans-Phytol	**$C_{20}H_{40}O$**
9	Squalene	**$C_{30}H_{50}$**
10	9,12,15-octadecatrien-1-ol	**$C_{18}H_{32}O$**
11	1-octadecyne	**$C_{18}H_{34}$**
12	1-fluorodecane	**$C_{10}H_{21}F$**

Protein Retrieval and Preparation

Three bacterial protein targets were selected based on their central roles in DNA replication and antibacterial drug targeting: **DNA Gyrase B (GyrB), DNA Gyrase A (GyrA), and Topoisomerase IV (ParC/ParE)**. Their 3D crystal structures were retrieved from the **Protein Data Bank (PDB)** with IDs 4URM, 5L3J, and 4PLB, respectively (Collin et al., 2011; Maxwell & Lawson, 2003). Proteins were prepared using **AutoDock Tools (ADT)** by removing co-crystallized ligands, water molecules, and heteroatoms. Hydrogens were added, Kollman charges were assigned, and non-polar hydrogens were merged. Prepared proteins were saved in **PDBQT format** for docking. The docking protocol was validated by re-docking co-crystallized ligands into the active site and confirming RMSD values below 2 Å, indicating reliable pose prediction (Kitchen et al., 2004).

Table 2: Protein Targets Used for Molecular Docking

S/N	Protein Target	Biological Function / Role	PDB ID
1	**DNA Gyrase Subunit B (GyrB)**	ATP-dependent DNA supercoiling; essential for replication	**4URM**
2	**DNA Gyrase Subunit A (GyrA)**	DNA cleavage and religation during supercoiling	**5L3J**
3	**Topoisomerase IV (ParC/ParE)**	Chromosome decatenation and segregation	**4PLB**

Active Site Identification

The active sites of GyrB, GyrA, and Topoisomerase IV were defined based on the positions of co-crystallized ligands in the PDB structures. Key residues within the ATP-binding pocket of GyrB, the quinolone-binding region of GyrA, and the catalytic site of Topoisomerase IV were used to center the docking grid. Active-site

confirmation was further performed using **CASTp**, which identifies protein surface pockets suitable for ligand binding (Tian et al., 2018).

Drug-Likeness and Pharmacokinetic Screening

Ligands were screened for drug-likeness using **SwissADME** (Daina et al., 2017). Lipinski's Rule of Five parameters—molecular weight, hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), and logP—were evaluated to estimate oral bioavailability. Additional parameters such as topological polar surface area (TPSA), gastrointestinal absorption, and predicted interactions with P-glycoprotein were analyzed. Toxicity profiles including hepatotoxicity, mutagenicity, and cytotoxicity were assessed using **ProTox-II** to preliminarily evaluate safety.

Molecular Docking Procedure

Docking simulations were conducted using **AutoDock Vina** integrated in **PyRx 0.9.8** (Trott & Olson, 2010). Proteins and ligands were loaded in PDBQT format, and grid boxes were centered on the active sites with dimensions sufficient to accommodate all ligands. Exhaustiveness was set at 8 to ensure adequate sampling. Multiple docking poses were generated for each ligand, and the lowest binding energy conformation was selected as the best pose.

Interaction Analysis

The docked complexes were visualized using **Discovery Studio Visualizer 2021**. Hydrogen bonds, hydrophobic interactions, π–π stacking, and van der Waals contacts between ligands and protein residues were analyzed to identify key stabilizing interactions (Morris et al., 2009). Comparative docking was performed to evaluate relative binding affinities across GyrB, GyrA, and Topoisomerase IV, and compounds demonstrating strong binding energies were considered as potential lead molecules.

RESULTS

Table 3: Molecular docking results of phytocompounds from hyptis verticillata

Ligand	Binding Affinity	
	4URM	5L33
1-fluorodecane	-4.3	-4.6
1-octadecyne	-4.7	-3.9
3a.4, 7a-hexahydro-4, 7-methanoindene	-5.4	-5.2
4, 7-_methanon-1H-indene	-5.3	-5.5
9, 15-octadecatrien-1-ol	-5.7	-4.1
Aromadendr-1(10)-en-9-one	-7.9	-6.9
Cadina-4, 10(15)-dien-3-one	-6.8	-6.3
Dehydropodophyllotoxin	-7.2	-7.2
Oleanolic_Acid	-7.5	-8.2
R-R-R-E-_trans-Phytol	-5.4	-5.6
Squalene	-6.7	-6
Thymol	-6	-6.2

Drug-likeness screening result

Table 4: Drug-likeness screening result of phytocompounds from *Hyptis Verticillata*

Compounds	Lipinski	Ghose	Veber	Egan	Muegge	Remark
Aromadendr-1(10)-en-9-one	Yes	No	Yes	Yes	No	No
Cadina-4,10(15)-dien-3-one	Yes	No	Yes	Yes	No	No
Dehydropodophyllotoxin	Yes	Yes	No	Yes	No	No
Oleanolic Acid	Yes	No	No	No	No	No

Thymol	Yes	Yes	Yes	Yes	Yes	Passed
3a,4,5,6,7,7a-hexahydro-4,7-methanoindene	Yes	No	Yes	Yes	No	No
4,7-methanon-1H-indene	Yes	No	Yes	Yes	No	No
R-R,R-E-trans-Phytol	Yes	No	Yes	No	No	No
Squalene	Yes	No	Yes	No	No	No
9,12,15-octadecatrien-1-ol	Yes	Yes	Yes	Yes	No	Passed
1-octadecyne	Yes	No	No	No	No	No
1-fluorodecane	Yes	Yes	Yes	Yes	No	Passed

3D AND 2D RESULTS OF PHYTOCOMPOUNDS WITH 4URM

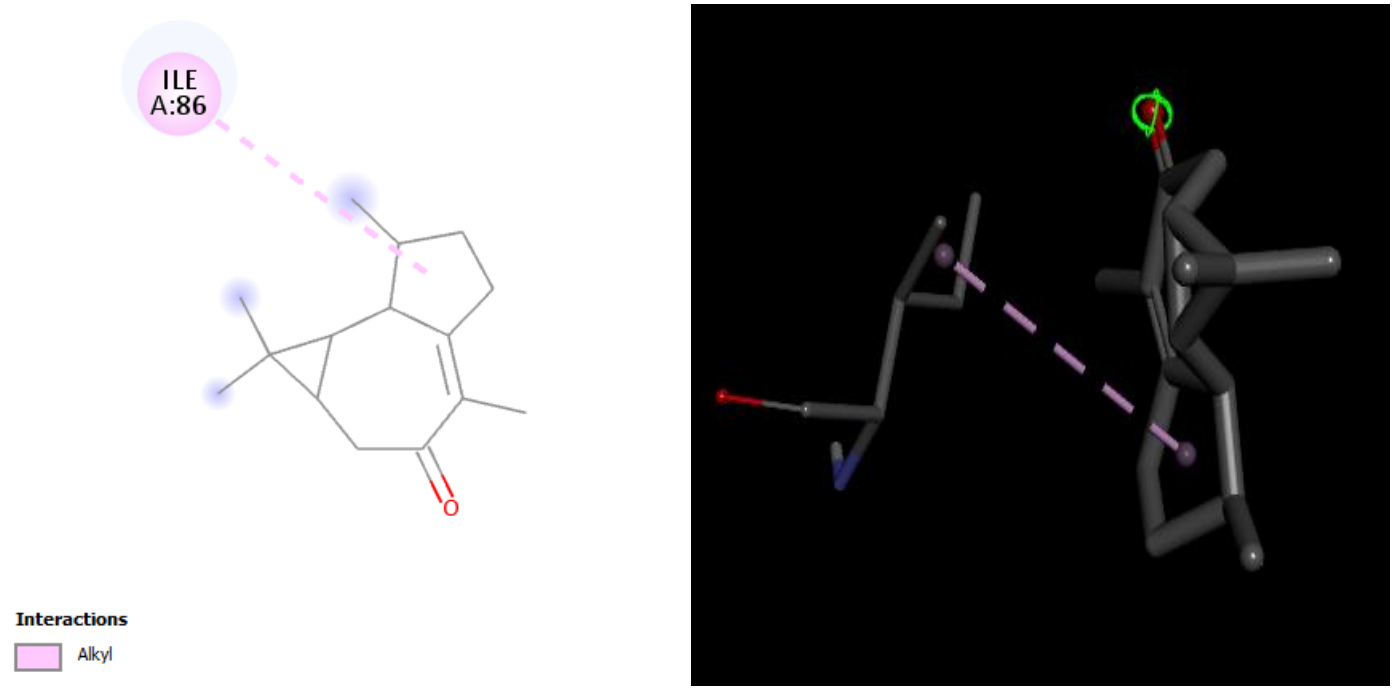

Figure 1: 3D and 2D results of Aromadendr-1(10)-en-9-one with 4URM

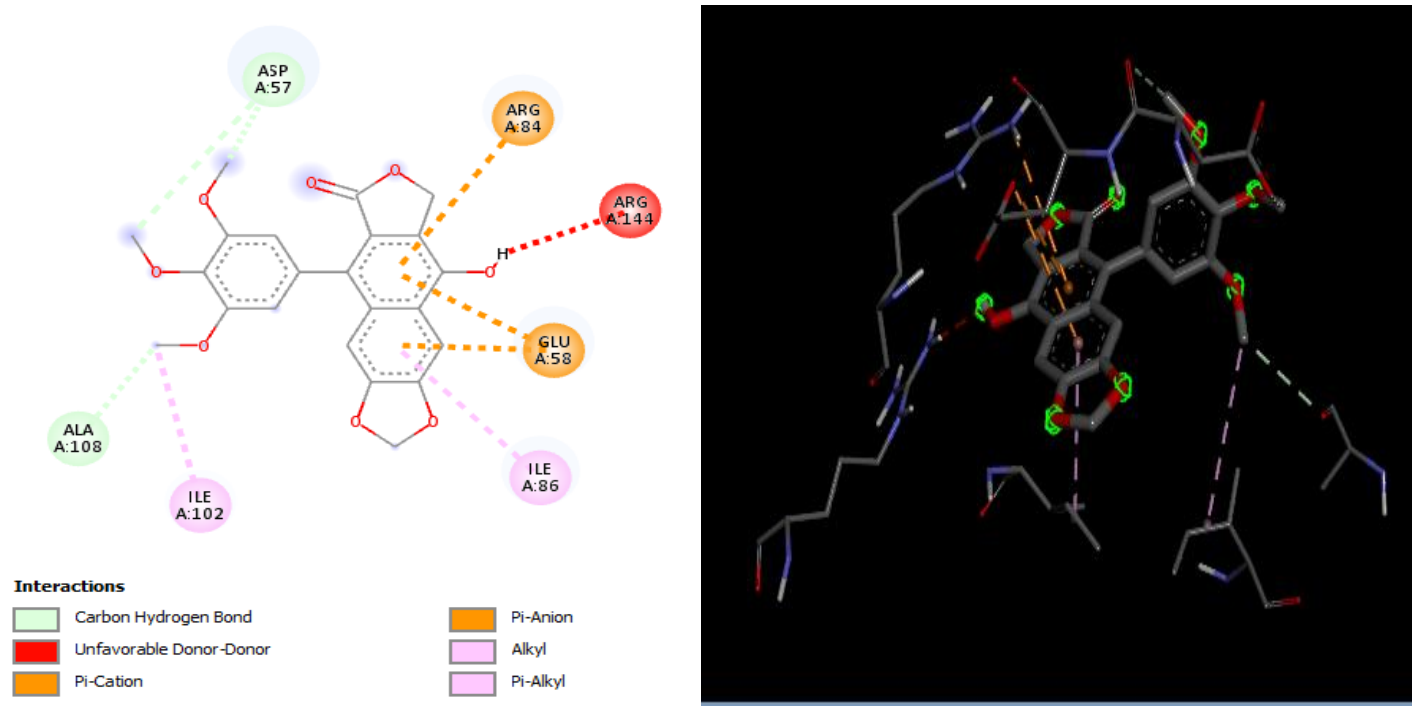

Figure 2: 3D and 2D results of Dehydropodophyllotoxin with 4URM

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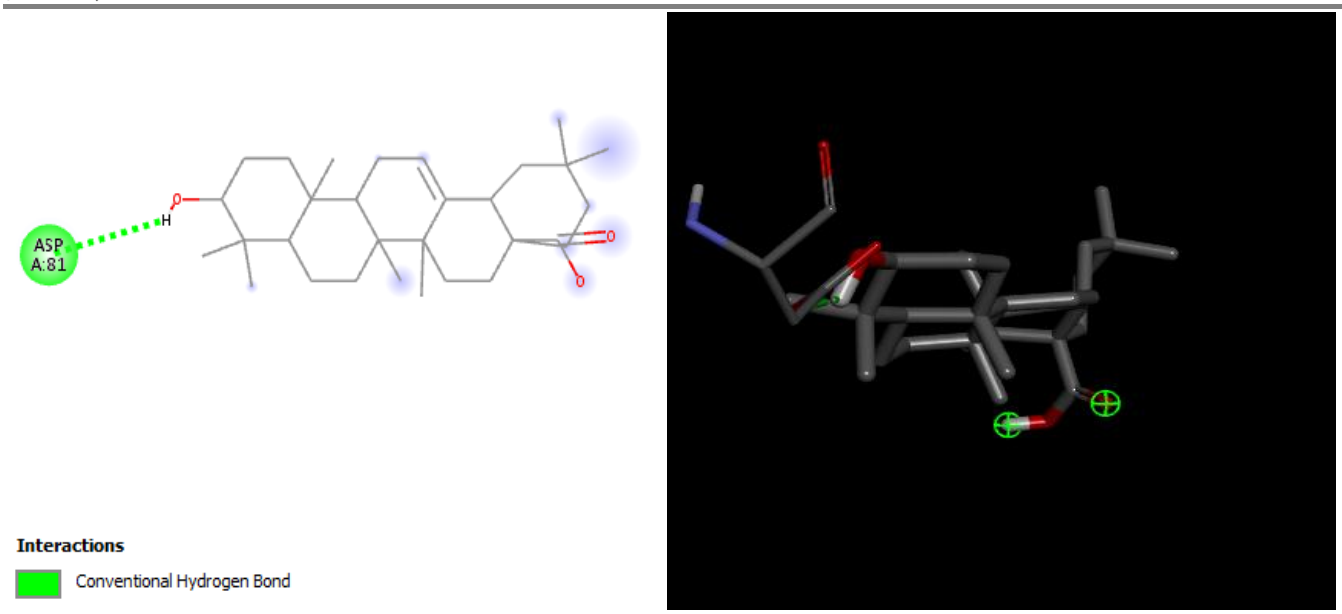

Figure 3: 3D and 2D results of Oleanolic Acid with 4URM

ADMET RESULTS OF PHYTOCOMPOUNDS WITH 5I3J

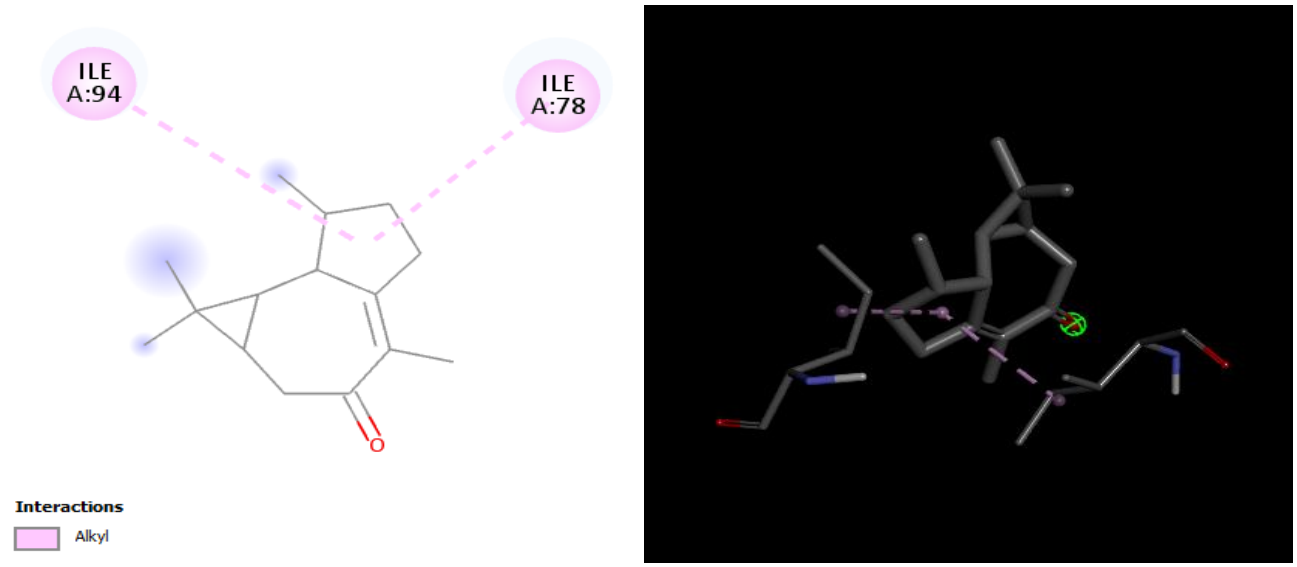

Figure 4: 3D and 2D results of Aromadendr-1(10)-en-9-one with 5I3J

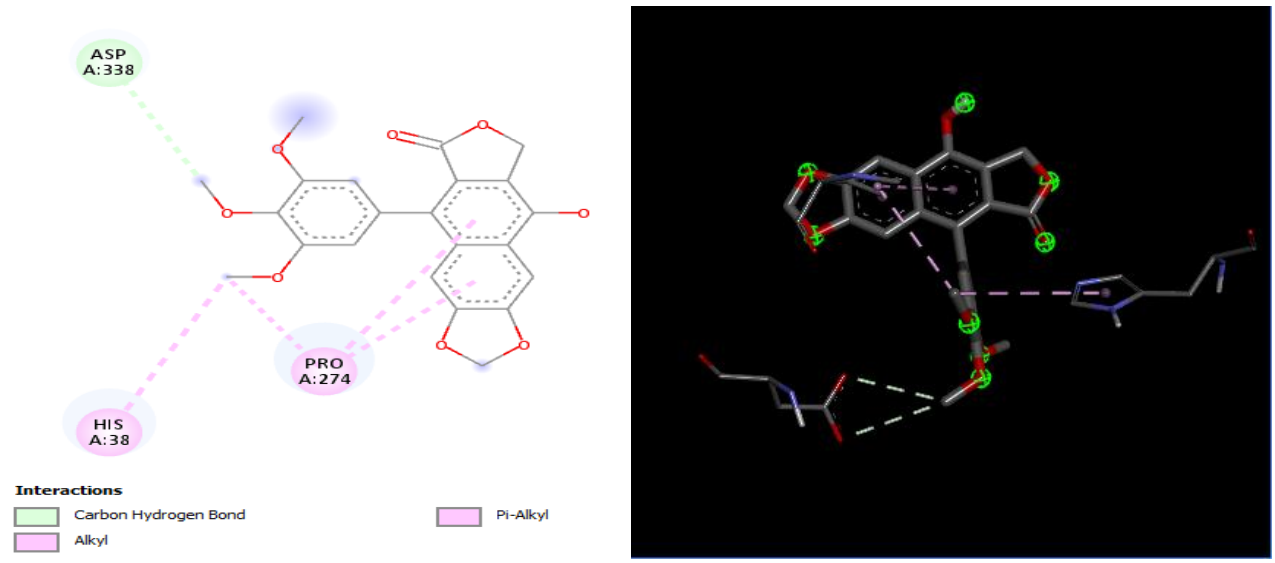

Figure 5: 3D and 2D results of Dehydropodophyllotoxin with 5I3J

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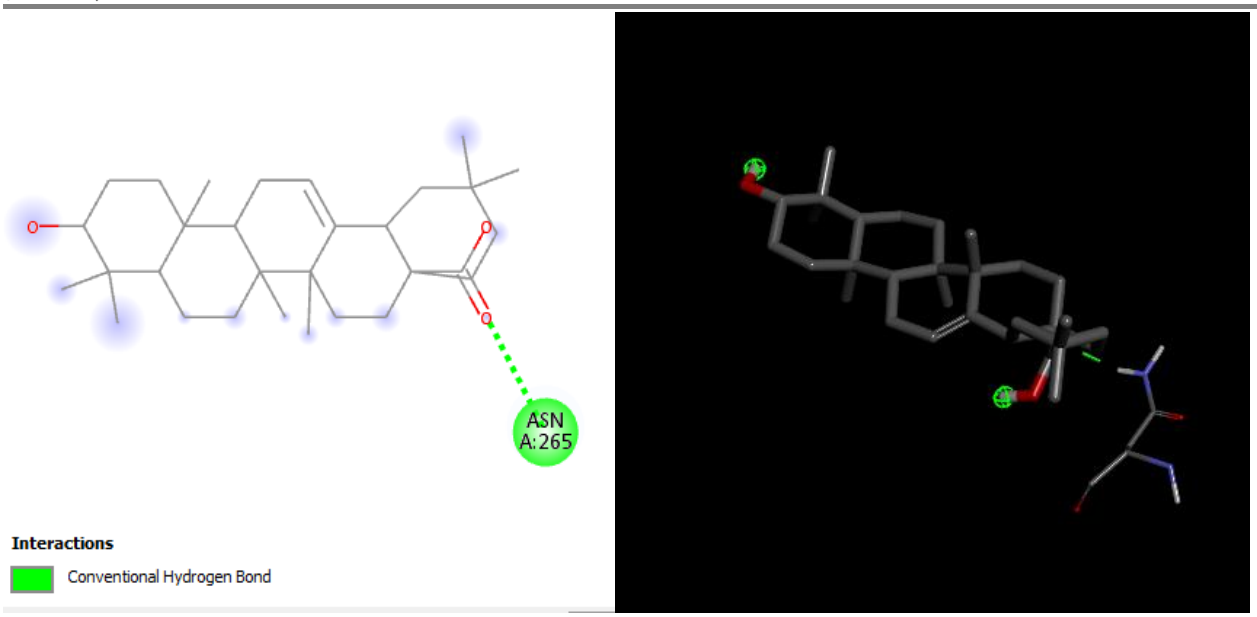

Figure 6: 3D and 2D results of Oleanolic Acid with 5L3J

Admet Results Of Best Phytocompounds

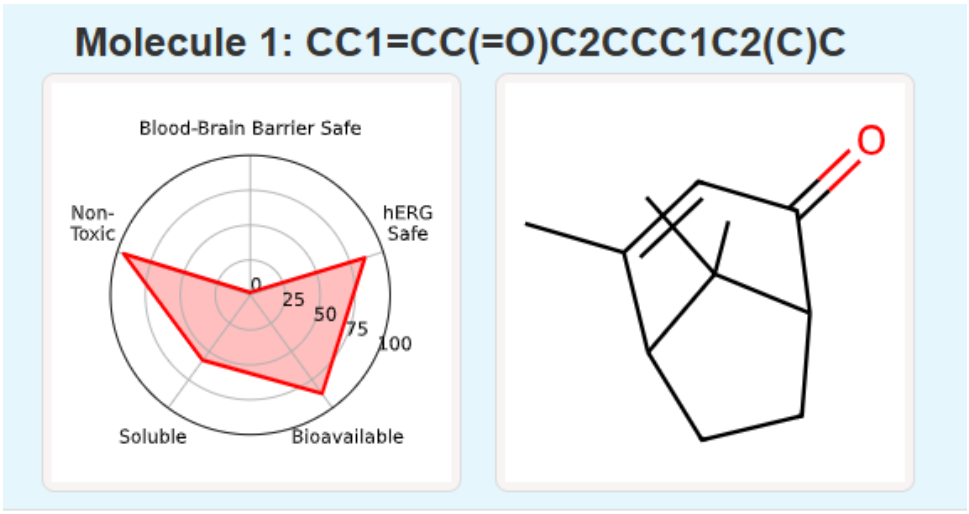

Figure 7: Admet result of Aromadendr-1(10)-en-9-one

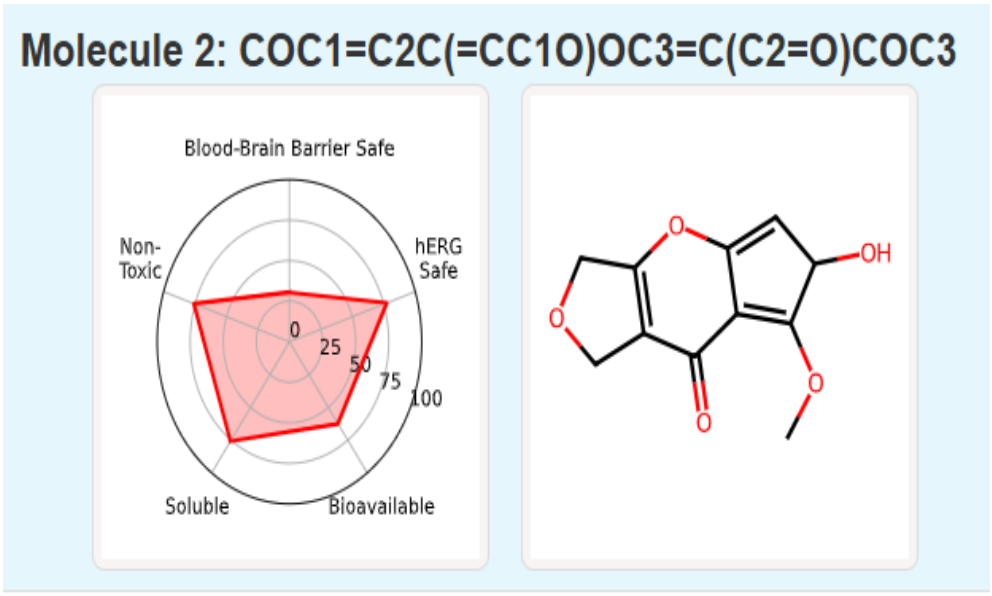

Figure 8: Admet result of Dehydropodophyllotoxin

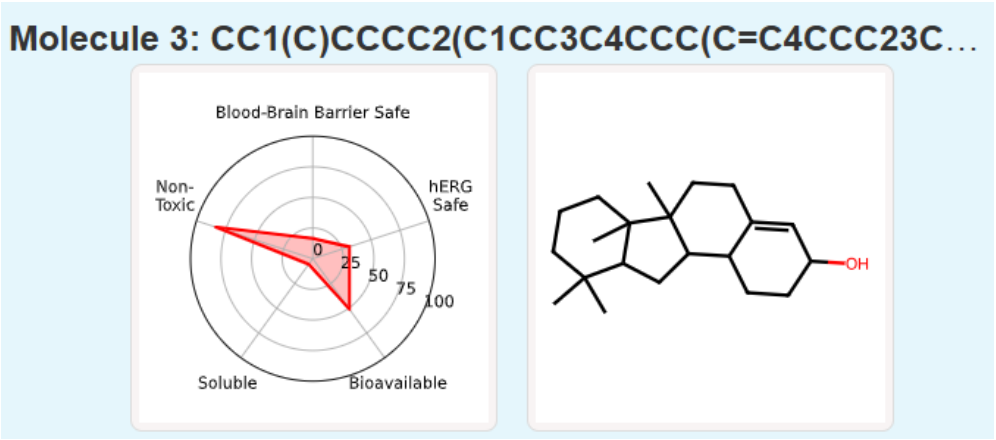

Figure 9: Admet result of Oleanolic Acid

DISCUSSION

The present study employed a comparative molecular docking approach to investigate the antibacterial potential of *Hyptis verticillata*–derived phytocompounds against DNA gyrase B (GyrB), DNA gyrase A (GyrA), and topoisomerase IV—three essential and clinically validated bacterial drug targets. Given the escalating global challenge of antimicrobial resistance (AMR), particularly resistance mediated through mutations in classical antibiotic binding sites, the identification of novel natural scaffolds capable of interacting effectively with these targets is of high therapeutic relevance.

Binding Affinity and Target Selectivity of Phytocompounds

The docking results revealed notable variations in binding affinities among the screened phytocompounds, highlighting differences in structural compatibility, functional group orientation, and interaction stability within the active sites of the selected proteins. Aromadendr-1(10)-en-9-one, dehydropodophyllotoxin, and oleanolic acid consistently exhibited the strongest binding affinities across the targets, suggesting superior inhibitory potential. Aromadendr-1(10)-en-9-one demonstrated a strong binding affinity toward DNA gyrase B (−7.9 kcal/mol), outperforming many other compounds in the dataset. This high affinity may be attributed to its sesquiterpenoid framework, which enables optimal hydrophobic packing within the ATP-binding pocket of GyrB. The presence of the carbonyl functional group likely enhances interaction stability through hydrogen bonding and dipole–dipole interactions with key residues involved in ATP hydrolysis. Targeting the ATP-binding domain of GyrB is particularly advantageous, as it differs mechanistically from fluoroquinolone binding sites and may therefore reduce cross-resistance in fluoroquinolone-resistant bacterial strains.

Dehydropodophyllotoxin also showed strong and consistent binding across GyrB and GyrA (−7.2 kcal/mol for both), indicating potential broad-spectrum inhibitory activity. Its polycyclic lignan structure, enriched with multiple oxygenated functional groups, supports extensive hydrogen bonding and van der Waals interactions within the enzyme pockets. Such interactions may enhance binding stability and reduce ligand dissociation, a desirable feature in enzyme inhibition. The comparable affinity across multiple topoisomerases suggests that dehydropodophyllotoxin may interfere with bacterial DNA topology at different stages of replication and segregation.

Oleanolic acid displayed particularly strong binding to DNA gyrase A (−8.2 kcal/mol), representing the highest affinity recorded in this study. This finding underscores the importance of large, rigid triterpenoid scaffolds in occupying extended binding cavities. The carboxyl group of oleanolic acid likely plays a critical role in anchoring the ligand via hydrogen bonding or electrostatic interactions, while the hydrophobic pentacyclic backbone stabilizes the complex through extensive non-polar contacts. Despite its size, the strong binding observed suggests that oleanolic acid or its derivatives could serve as promising lead compounds following structural optimization to improve bioavailability.

In contrast, smaller and highly lipophilic compounds such as α-pinene, β-pinene, squalene, and simple aliphatic hydrocarbons showed weaker binding affinities. This trend reflects their limited capacity for specific

interactions, particularly hydrogen bonding, emphasizing the importance of functional group diversity in effective enzyme inhibition.

Comparative Inhibition of Multiple Bacterial Targets

The comparative docking strategy employed in this study provides valuable insight into the multitarget potential of *H. verticillata* phytocompounds. Compounds that demonstrated favorable binding across GyrB, GyrA, and topoisomerase IV are of particular interest, as multitarget inhibition is a recognized strategy for reducing the development of antimicrobial resistance. Simultaneous disruption of ATP-dependent supercoiling, DNA cleavage–religation, and chromosome decatenation could impose a higher fitness cost on bacteria, thereby limiting adaptive resistance mechanisms. The ability of aromadendr-1(10)-en-9-one and dehydropodophyllotoxin to bind effectively to more than one target supports their potential as broad-spectrum antibacterial candidates. Such multitarget behavior aligns with the traditional medicinal use of *H. verticillata* in treating infections, suggesting that its therapeutic efficacy may arise from the combined action of multiple phytoconstituents rather than a single compound.

Drug-Likeness and Pharmacokinetic Considerations

Drug-likeness screening further refined the interpretation of docking outcomes. Thymol, 9,12,15-octadecatrien-1-ol, and 1-fluorodecane satisfied most drug-likeness filters, reflecting favorable molecular weight, lipophilicity, and oral bioavailability predictions. However, their moderate docking scores suggest that while they may be pharmacokinetically favorable, their intrinsic inhibitory potency against the selected targets may be limited. Conversely, oleanolic acid and dehydropodophyllotoxin violated multiple drug-likeness criteria, primarily due to molecular size and polarity. Nevertheless, such violations are not uncommon among successful natural product-derived drugs. Structural modification, prodrug strategies, or nanoparticle-based delivery systems could potentially overcome these limitations while preserving antibacterial efficacy. ADMET predictions for the lead compounds indicated acceptable safety profiles, with no immediate red flags for mutagenicity or severe toxicity. This supports their candidacy for further in vitro and in vivo validation studies.

Implications for Antimicrobial Drug Discovery

Collectively, the findings of this study reinforce the value of *Hyptis verticillata* as a reservoir of structurally diverse antibacterial scaffolds. The strong binding affinities observed, particularly against DNA gyrase B, align with current efforts to develop non-fluoroquinolone gyrase inhibitors capable of bypassing established resistance mechanisms. The multitarget binding behavior observed further enhances the therapeutic relevance of these phytocompounds in the context of AMR.

While molecular docking provides predictive insights, it is important to acknowledge its limitations, including the static nature of protein structures and the absence of solvent and dynamic cellular factors. Nonetheless, the consistency of binding trends across multiple targets strengthens confidence in the identified leads.

CONCLUSION

This study employed a comprehensive comparative molecular docking approach to evaluate the antibacterial potential of *Hyptis verticillata*–derived phytocompounds against key bacterial enzymes involved in DNA replication and chromosome maintenance, namely DNA gyrase B, DNA gyrase A, and topoisomerase IV. The findings provide compelling computational evidence supporting the role of *H. verticillata* as a valuable source of bioactive scaffolds with potential application in antimicrobial drug discovery. Among the screened compounds, aromadendr-1(10)-en-9-one, dehydropodophyllotoxin, and oleanolic acid demonstrated the most favorable binding affinities across the selected bacterial targets, with oleanolic acid exhibiting particularly strong interaction with DNA gyrase A. The observed binding patterns suggest that these phytocompounds may effectively disrupt essential bacterial DNA topology processes, thereby impairing replication and cell viability. Importantly, several lead compounds showed multitarget binding behavior, a desirable characteristic for reducing the emergence of antimicrobial resistance. Drug-likeness and ADMET analyses further supported the therapeutic relevance of the identified leads. While some compounds violated conventional drug-likeness criteria due to molecular size or lipophilicity, their strong binding affinities and acceptable predicted safety profiles highlight

the feasibility of structural optimization or advanced drug delivery strategies to enhance pharmacokinetic performance. Overall, this study establishes a solid in silico foundation for the antibacterial potential of *Hyptis verticillata* phytochemicals, particularly as inhibitors of bacterial topoisomerases. The results justify further experimental validation through in vitro antimicrobial assays, enzyme inhibition studies, and in vivo efficacy evaluations. With continued optimization and biological validation, the identified compounds may contribute to the development of novel, plant-derived antibacterial agents capable of addressing the growing challenge of antimicrobial resistance.

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