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Original Article

Harnessing *Saussurea lappa* for Anti-leishmanial, Anti-toxoplasmic, and Anti-bacterial Activity: Molecular and Computational Insights

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ABSTRACT

Objectives: *Saussurea lappa* Clark has shown preliminary promise in various biological contexts. This study investigates the antileishmanial, antitoxoplasmic, and antibacterial activities of methanolic extracts from *S. lappa* roots, focusing on key active components, Dehydrocostuslactone (DHL) and Dihydrodehydrocostus Lactone (DDHL). **Materials and Methods:** Bioassays were conducted to assess the extract's efficacy against *Leishmania major, Toxoplasma gondii, Staphylococcus aureus,* and *Klebsiella pneumoniae*, with cytotoxicity tested on Vero cells using the MTT assay. Molecular docking analyses explored the binding interactions of DHL and DDHL with critical targets, such as pteridine reductase and squalene synthase in *L. major*; (acyl-carrier-protein) S-malonyltransferase and DNA gyrase in *S. pyogenes*; and carbapenemase and DNA gyrase in *K. pneumoniae*. **Results:** The extract exhibited moderate antileishmanial activity against Leishmania major promastigotes (IC_{50}: 14.7 µg/mL, SI: 1.2) and amastigotes (IC_{50}: 17.2 µg/mL, SI: 1.18), with lower potency compared to Amphotericin B. However, its high toxicity to host macrophages (CC_{50}: 20.3 µg/mL) limits further potential. It showed poor antitoxoplasmal activity (IC_{50}: 14.7 µg/mL, SI: 0.394) and was toxic to Vero cells (CC_{50}: 5.8 µg/mL). Antibacterial assays revealed concentration-dependent inhibition zones against *Streptococcus pyogenes* (24±1.7 mm at 250 µg/mL) and *Klebsiella pneumoniae* (37±1.7 mm at 250 µg/mL). Molecular docking showed strong interactions of DHL with Pteridine reductase (-7.1 kcal/mol) and DDHL with Carbapenemase (-7.9 kcal/mol). Both compounds exhibited favorable ADMET profiles, including high oral absorption, suggesting potential as therapeutic agents for further investigation and active compound isolation. **Conclusion:** These findings highlight the potential of *S. lappa* as a therapeutic agent and underscore the need for additional preclinical research to further validate and develop its antimicrobial applications.

Keywords: *Saussurea lappa*, Antimcrobial, Molecular docking, *Leishmania* major, ADMET.

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INTRODUCTION

There are 300 Saussurea species in the world. Of them, *Saussurea lappa* (Asteraceae) is a typical perennial plant found across the Himalayan area on a worldwide scale.[1] Research on the chemical components of *S. lappa* dates back to the 1950s. Numerous chemicals have been isolated up to this point. Terpenes make up the majority of its active ingredients, however it also contains favonoids, alkaloids, and anthraquinones.[1-3] Terpenes found in plants include dihydrocostunolide, 12-methoxydihydrocostunolide, costunolide, Dehydrocostuslactone (DHL), Dihydrodehydrocostus Lactone (DDHL), dehydrocostus lactone, and dihydrocostunolide, which are mostly anticancer and anti-inflammatory.[4] Costus oil is the oil that is derived from the roots of *S. lappa* and is used

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to make hair oil and luxury fragrances.[4] Pale yellow to brownish in hue, costus oil is also reputed to provide therapeutic benefits for leprosy.[5] Of all the species in the genus Saussurea, *S. lappa* is one of the most commercially viable. It is commonly used in many indigenous medical systems across the world to treat a wide range of conditions, including inflammation, dyspepsia, diarrhea, vomiting, and tenesmus. It is also recommended for tenesmus, irregular menstruation, and stomach discomfort. The Tibetan medical system, Ayurvedic medicine, and traditional Chinese medicine have all extensively recorded the plant's medicinal qualities. *S. lappa* was included as one of the primary constituents in 71 formulations out of 175 formulations in the Handbook of Traditional Tibetan Drugs. The roots of *S. lappa* are used as an antiseptic and to treat bronchial asthma. They have a strong, sweet, fragrant smell and flavor. It has also been stated that preparations derived from this species can treat a number of illnesses and ailments, such as headaches, hysteria, fever, cough, paralysis, asthma, deafness, and tridosha.[1-4,6-9]

Despite the widespread medical applications and proven pharmacological effectiveness, several biological characteristics of Costus roots still require further evaluation.[4] Prior investigations relied on preliminary examinations.[4,9] Furthermore, there is a lack of study in this specific area, as no prior studies have been conducted on the efficacy of this plant in treating parasite disorders. It is worth noting that the roots of this plant are found in many regions around the globe.[7] Furthermore, the computational analysis of the active components of this plant has not been conducted to elucidate its mode of action. Therefore, the objective of this study is to investigate the antileishmanial, antitoxoplasma, and antibacterial activities of methanolic extracts from *Saussurea lappa* Clark roots. By addressing existing research gaps, this research aims to explore the plant's potential in treating parasite disorders, an area lacking prior investigation. Additionally, the study seeks to computationally analyze the active components of *S. lappa* [Dehydrocostuslactone (DHL) and Dihydrodehydrocostus Lactone (DDHL)] to understand their molecular mechanisms of action.

MATERIALS AND METHODS

Sample and Plant Extraction

A sample of *S. lappa* roots was acquired from an exclusive vendor in the Jazan, Saudi Arabia, local market. The sample was finely milled to a powder with care. The powdered sample, around 100 g was extracted using pure methonol. For five days, there was constant shaking and a maceration with 500 mL of methanol at room temperature during the extraction procedure.[10] In preparation for future biological research, the extract was kept in glass containers that were sealed and kept at 4°C.

Anti-toxplasmal and Anti-leishmanial activities

Promastigotes of *Leishmania major*, isolated from a Saudi patient, were maintained in Schneider's Drosophila medium supplemented with 10% FBS at 26°C, with weekly transfers and cryopreservation in liquid nitrogen at concentrations of 3×10^6 parasite/mL. Virulent *L. major* parasites were propagated in female BALB/c mice through hind footpad injections. Amastigotes were isolated after 8 weeks and converted to promastigotes for infection studies. The evaluation of the activity against amastigotes in macrophages, culturing them in phenol red-free RPMI 1640 medium with 10% FBS, and incubating with *L. major* promastigotes. For *Toxoplasma gondii*, tachyzoites of the RH strain were cultured in Vero cells using RPMI 1640 medium with 10% FBS in a 5% CO_2 atmosphere at 37°C. Assays for both pathogens included treating cells with test extract, calculating inhibition from infection indexes, and determining EC_{50} values from three independent experiments. Cytotoxicity assessments were conducted using MTT assays on Vero cells, with IC_{50} values derived from multiple experiments.[11,12]

Anti-bacterial Activity

Human pathogenic bacterial strains *Staphylococcus aureus* and *Klebsiella pneumoniae* (ATCC700603) were used in the study. Every bacterial culture used in this study was regularly maintained in the College of Pharmacy's microbiology lab by subculturing regularly at predetermined intervals. The cultures were standardized using the gradient dilution method using nutrient broth, utilizing the serial dilution approach from 10-1 to 10-7. To evaluate the viability of the bacterial cultures, the colony-forming unit in 1 mL (CFU/mL) was calculated. The procedure for carrying out the antimicrobial susceptibility test was previously mentioned.[12] To summarize, Muller Hinton agar plates were ready before the antibacterial investigation. First, the stock culture was used to create bacterial subcultures. The subcultures were employed for antibacterial investigations after a 24-hr incubation period. The agar well diffusion method was applied to the samples and the standard antibiotics. Different bacterial species were used for each individual injection. A sterile cotton swab was dipped into the standardized (CFU/mL) culture to equally disseminate the culture on the MH agar plate and streaked. The petri dish was rotated during the process. Sample analytes were then administered after the plates had been let to dry for roughly 10 min. Holes on the inoculated MH agar plates were punched using a standard sterile stainless-steel borer to carry out the agar well diffusion procedure. After 24 hr of incubation at 37°C, the antibacterial spectrum was evaluated by looking for the formation of inhibitory zones on the plates. To assess the bactericidal capability of ordinary Streptomycin (10 mcg/disc), the Kirby Bauer method was employed.[13] For 24 hr, the plates were incubated at 37°C. After a 24-hr incubation period, inhibitory zones formed around the discs to measure the antibacterial spectrum.

Molecular Docking

The target assignment and dataset retrieval

The targets for the described microorganisms such as *Leishmania major* (Pteridine reductase and Squalene synthase), *Streptococcus pyogenes* ((acyl-carrier-protein) S-malonyltransferase and DNA gyrase), and *Klebsiella pneumoniae* (Carbapenemase and DNA gyrase) were defined initially. The three-dimensional protein structure (3D) of the defined targets, including Pteridine reductase (PDB ID: 6RXC)[14] and Carbapenemase (PDB ID: 2OV5),[15] were obtained from the Protein Data Bank (PDB). The other targets, such as Squalene synthase (UniProt ID: Q25308; Model confidence (MC): 87.25%), DNA gyrase (UniProt ID: A0A660A3N4; MC: 85.94%), DNA gyrase (UniProt ID: A0A377VTL4; MC: 85.5%), were retrieved from the cutting-edge and highly anticipated AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/; Accessed on: 20 June 2024) with high model confidence.

Protein Structure modeling and its validation

The structure of (acyl-carrier-protein) S-malonyltransferase for *Streptococcus pyogenes* was unavailable in the PDB and alpha fold databases. In this case, the FASTA-formatted sequence was obtained from the UniProt Database (UniProt ID: A0A377VTL4), and the structure was modeled using ColabFold v1.5.5, which leverages AlphaFold2 runs based on the predicted local distance difference test ranges from 0-100.[16] The highly accurate modeled structure was then validated using the Ramachandran Plot (RM)[17] available in the SAVES v6.0 server (https://saves.mbi.ucla.edu/; Accessed on 21 June 2024), further enhancing our confidence in the accuracy of our methods.

Active site prediction

The active or ligand binding sites were meticulously predicted based on a robust machine learning method for the retrieved and modeled protein structures. This was achieved by implementing the PrankWeb server (https://prankweb.cz/; Accessed on 22 June 2024), a tool known for its accuracy and reliability in such predictions.[18]

Preparation of protein structures and ligands

The protein structures Pteridine reductase (PDB ID: 6RXC) and Carbapenemase (PDB ID: 2OV5) retrieved from the PDB were processed by implementing Discovery Studio Visualizer v19.1.0.18287 (www.accelerys.com), which removes water molecules and their associated ligands. The isolated compounds Dehydrocostuslactone (DHL) and Dihydrodehydrocostus lactone (DDHL) compounds were downloaded from the PubChem compound database in SDF format with provided 3D atomic coordinates.[19] Then, the SDF format was converted into PDB by implementing Open Bable. Then, the compounds were energy minimized using the Universal Force Field and the conjugate gradients optimization algorithm with 200 steps.

Molecular docking and Visualization

The molecular docking process was initiated for the defined targets, such as Pteridine reductase, Squalene synthase, (acyl-carrier-protein) S-malonyltransferase, Carbapenemase and DNA gyrase (UniProt ID: A0A660A3N4 and A0A377VTL4) with DHL and DDHL, by using Autodock Vina, which offers an integrated scoring algorithm and is integrated into PyRx software.[20]

The Autodock vina uses a new scoring mechanism,

$$C = \sum_{i<j} f_{titj}(r_{ij}),$$

C- Sum of intermolecular and intramolecular distance; Σ - Over all of the pairs of atoms; f_{titj} - Symmetric set of interaction functions; r_{ij} - Interatomic distance.

The prepared targets and ligands were converted into PDBQT format. The PyRx virtual screening tool identified the interacted active site residues of the targeted biomarkers and the grid box properties were set as for Pteridine reductase (size_x=29.92 Å; size_y=40.9 Å; size_z=35.90 Å), Squalene synthase (size_x=51.20 Å; size_y=42.40 Å; size_z=36.95 Å), (acyl-carrier-protein) S-malonyltransferase (size_x=33.27 Å; size_y=28.57 Å; size_z=30.86 Å), Carbapenemase (size_x=27.63 Å; size_y=27.33 Å; size_z=31.76 Å), DNA gyrase (UniProt ID: A0A660A3N4) (size_x=30.59 Å; size_y=28.03 Å; size_z=30.74 Å), and DNA gyrase (UniProt ID: A0A377VTL4) (size_x=31.25 Å; size_y=32.98 Å; size_z=29.51 Å).

After that, the docking propensity and interactions between the ligands DHL and DDHL with the molecularly docked targets were analyzed and then visualized in 2D and 3D format by importing the docked complex into Discovery Studio Visualizer v19.1.0.1828 (Dassault Systèmes BIOVIA, Rue Marcel Dassault, Vélizy-Villacoublay-78140, France (www.accelerys.com; Accessed on 23 June 2024). The 2D diagram was also obtained for the interacted targets with the DHL and DDHL using LigPlot+v2.2.8,[21] providing a comprehensive view of the docking and interactions.

ADMET Properties Prediction

The ADMET properties for the DHL and DDHL were identified using the QikProp tool in the Schrodinger (Schrödinger, LLC, NY, USA).

RESULTS

Anti-parasitic activity

Table 1 indicates a moderate activity of the extract against the promastigotes of *L. major in vitro* with IC_{50} 14.7 µg/mL, however

the toxicity against the host cell macrophages is more higher with CC_{50} 20.3 and SI 1.2. Further investigation for active compounds isolation and identification is recommended which can be more promising.

Table 2 indicates the extract is toxic to Vero cell line with CC_{50} 5.8 µg/mL which is lower than the activity against *T. gondii* parasites, so no further studies can be done for the extract as antitoxoplasmal.

Antibacterial Activity

Table 3 and Figure 1 presents the antibacterial activities of *S. lappa* against various pathogenic microorganisms at different concentrations (in µg/mL). The Table includes two bacterial test organisms: *S. pyogenes* and *K. pneumoniae*. For each pathogenic microorganism, the mean Zone of Inhibition (ZOI) in millimeters is listed. The antibacterial activities are demonstrated at concentrations ranging from 10 µg/mL to 250 µg/mL. For *S. pyogenes*, the mean ZOI values were 24±1.7 mm, 22±2.4 mm, 18±1.2 mm, 7±0.28 mm, and 4±0.66 mm at the respective concentrations. In the case of *K. pneumoniae*, the ZOI values were 37±1.7 mm, 32±1.2 mm, 26±0.49 mm, 16±0.59 mm, and 8±0.36 mm. These results, derived from three parallel experiments, reveal the varying degrees of antibacterial efficacy of *S. lappa* against these pathogenic microorganisms in a concentration-dependent manner. Further investigation for active compounds isolation and identification is recommended which can be more promising.

Molecular docking

The structure was modeled and validated for the (acyl-carrier-protein) S-malonyltransferase (UniProt ID: A0A377VTL4). The results revealed that the quality of the modeled structure was very high based on the pLDDT score (96.7). However, the model was further validated using the RM plot. The RM plot revealed that 92.8% of residues were scattered in the most favored region. The results showed that the modeled structure is very high quality and can be taken into further molecular docking studies (Supplementary File: Figure S1A-C). The molecular docking analysis was performed for the targets with the predicted active sites Pteridine reductase, Squalene synthase, (acyl-carrier-protein) S-malonyltransferase, Carbapenemase and DNA gyrase (UniProt ID: A0A660A3N4 and A0A377VTL4) with DHL and DDHL. Table 4 lists the resulting binding affinity, RMSD, and its interacting residues with the type of bonds.

Leishmania major

Pteridine reductase

The DHL formed three hydrogen bonds with the residues LEU18, GLY19, and ASN109 and alkyl bonds with ARG17, LEU18, MET179, and PRO224. The van der Walls interactions were also formed with the residues SER111, VAL180, TYR194, and LYE198. The binding affinity of the DHL with the Pteridine reductase was observed as -7.1 kcal/mol, and the RMSD was identified as 1.984 (Table 4 and Figure 2A, B). Less likely, the DDHL formed one hydrogen bond with the residue TYR194 and other bonds such as alkyl and pi-alkyl with the residues LEU18AL, PHE113AL, PRO224PAL and the van der Walls interaction with the residues

Table 1: Antileishmanial activity of extract in concentration of µg/mL against *Leishmania major* amastigotes and promastigotes *in vitro*.

sample	CC_{50} for macrophages	IC_{50} for *L. major* promastigotes	IC_{50} for *L. major* amastigotes	SI value for promastigotes	SI value for amastigotes
Extract	20.3	24.8	17.2	0.818	1.18
Amphotericin B	8.1	0.8	0.5	10.1	16.2

SI=Selectivity index calculated by dividing CC_{50} over IC_{50}.

Table 2: Antitoxplasmal activity of the extract in concentration of µg/mL against Toxoplasma gondii *in vitro*.

Sample	CC_{50} for Vero cell	IC_{50} for *T. gondii*	SI values
Extract	5.8	14.7	0.394
Atovaquone	9.5	0.07	136

SI=Selectivity index calculated by dividing CC_{50} over IC_{50}.

Table 3: Antibacterial activities of *S. lappa* against bacterial test organisms.

	Concentrations µg/mL				
Pathogenic microorganism	250	100	50	25	10
	Mean zone of inhibition (ZOI) in "mm"				
S. pyrogens	24±1.7	22±2.4	18±1.2	7±0.28	4±0.66
K. pneumoniae	37±1.7	32±1.2	26±0.49	16±0.59	8±0.36

Values are mean±SD of three parallel experiments.

ARG17VW, SER111VW, MET179VW, VAL180VW, ASP181VW, LEU188VW, LYS198VW were formed. The binding affinity and the RMSD were predicted for the Pteridine reductase with the DDHL is -7.0 kcal/mol and 1.982 (Table 4 and Figures 2C, D).

Squalene synthase

The DHL interacted with the residues ALA169, VAL172, LEU204, PHE45, PRO, VAL48, PHE283, GLY173, LEU176, GLY201, ASN208, and CYS284 via pi-alkyl, alkyl, pi-sigma, and van der Walls interactions, with the binding affinity of -8.1 kcal/mol and the RMSD of 1.811Å (Table 4 and Figures 2E, F). However, no hydrogen bond is formed between the DHL and squalene synthase. Meanwhile, the DDHL interacted with the residue ASN208 via hydrogen bond with the binding affinity of -7.0 kcal/mol and the RMSD of 2.736Å. Also, the DDHL interacted with the residues such as LEU68, VAL172, PHE45, TYR65, ARG69, ASP72, MET143, LEU204, GLN205 via pi-alkyl, alkyl, van der Walls interactions (Table 4 and Figures 2G, H).

Additionally, the interaction ability of DHL and DDHL was verified for the antibacterial activity against the defined targets of *Streptococcus pyogenes* and *Klebsiella pneumoniae*.

Streptococcus pyogenes

(acyl-carrier-protein) S-malonyltransferase

The (acyl-carrier-protein) S-malonyl transferase residue ASN158 interacted with the DHL via hydrogen bond. Likely, other residues such as LEU89PAL, MET130PAL, LEU192PAL, PHE198PAL, VAL277PAL, PHE281PAL, VAL194PAL, GLN11VW, GLY12VW, SER90VW, ASN160VW, GLN164VW, VAL166VW, HIS199VW interacted via pi-alkyl and van der Walls interactions with the binding affinity of -7.1 kcal/mol and the RMSD of 2.043Å (Table 4 and Figures 2I, J), while compared with the DHL. The DDHL interacted well with the (acyl-carrier-protein) S-malonyl transferase residues such as SER90, ASN158, and HIS199 via hydrogen bonds with the binding affinity of -6.5 kcal/mol and the RMSD of 1.927Å (Table 1 and Figures 2K, L).

DNA gyrase

The DHL interacted with the DNA gyrase residue ARG199 via hydrogen bond and with other residues, including HIS47, TRP50, ILE37, GLU43, GLY44 via pi-alkyl, alkyl, and van der Walls interactions (Table 4 and Figures 2M, N). The DDHL likely interacted with the residue HIS47 via a hydrogen bond, with a binding affinity of -6.9 kcal/mol and an RMSD of 2.025Å. Also,

Table 4: The table detailed the list of defined targets and their docking results (binding affinity kcal/mol), RMSD (Å), interacted residues, and type of bonds) with the isolated DHL and DDHL compound.

Targeted microbial species	Protein targets	DHL				DDHL			
		Binding affinity (kcal/mol)	RMSD (Å)	H-bonds	Other types of bonds	Binding affinity (kcal/mol)	RMSD (Å)	H-bonds	Other types of bonds
Leishmania major	Pteridine reductase	-7.1	1.984	LEU18, GLY19, ASN109	ARG17^{AL}, LEU18^{AL}, MET179^{AL}, PRO224^{AL}, SER111^{VW}, VAL180^{VW}, TYR194^{VW}, LYE198^{VW}	-7.0	1.982	TYR194	LEU18^{AL}, PRO224^{PAL}, PHE113^{AL}, ARG17^{VW}, SER111^{VW}, MET179^{VW}, VAL180^{VW}, ASP181^{VW}, LEU188^{VW}, LYS198^{VW}
	Squalene synthase	-8.1	1.811	-	ALA169^{PAL}, VAL172^{PAL}, LEU204^{PAL}, PHE45^{PAL}, PROP^{AL}, VAL48^{AL}, PHE283^{PS}, GLY173^{VW}, LEU176^{VW}, GLY201^{VW}, ASN208^{VW}, CYS284^{VW}	-7.0	2.736	ASN208	LEU68^{PAL}, VAL172^{AL}, PHE45^{AL}, TYR65^{AL}, ARG69^{AL}, ASP72^{VW}, MET143^{VW}, LEU204^{VW}, GLN205^{VW}

Targeted microbial species	Protein targets	DHL				DDHL			
		Binding affinity (kcal/mol)	RMSD (Å)	H-bonds	Other types of bonds	Binding affinity (kcal/mol)	RMSD (Å)	H-bonds	Other types of bonds
Streptococcus pyogenes	(acyl-carrier-protein) S-malonyltransferase	-7.1	2.043	ASN158	LEU89^{PAL}, MET130^{PAL}, LEU192^{PAL}, PHE198^{PAL}, VAL277^{PAL}, PHE281^{PAL}, VAL194^{PAL}, GLN11^{VW}, GLY12^{VW}, SER90^{VW}, ASN160^{VW}, GLN164^{VW}, VAL166^{VW}, HIS199^{VW}	-6.5	1.927	SER90, ASN158, HIS199	LEU89^{PAL}, MET130^{PAL}, ASN160^{VW}, LEU192^{PAL}, VAL277^{PAL}, PHE281^{PAL}, GLN11^{VW}, GLY12^{VW}, VAL194^{VW}, PHE198^{VW}, VAL252^{VW}
	DNA gyrase	-6.3	2.842	ARG199	HIS47^{PAL}, TRP50^{AL}, ILE37^{VW}, GLU43^{VW}, GLY44^{VW}	-6.9	2.025	HIS47	TRP50^{PAL}, ILE284^{PAL}, LYS348^{PAL}, ILE37^{VW}, GLU43^{VW}, GLY44^{VW}, ASP54^{VW}, ARG199^{VW}, HIS285^{VW}, THR347^{VW}
Klebsiella pneumoniae	Carbapenemase	-6.9	1.956	SER70, LYS73, SER130	TRP105^{PS}, LEU167^{AL}, ASN132^{VW}, ASN170^{VW}, THR237^{VW}	-7.9	1.392	SER70	TRP105^{AL}, LEU167^{PAL}, LYS73^{VW}, SER130^{VW}, ASN132^{VW}, GLU166^{VW}, THR216^{VW}, THR235^{VW}, GLY236^{VW}, THR237^{VW}
	DNA gyrase	-7.4	1.935	GLU264, ARG480	PHE458^{AL}, PHE262^{VW}, GLN263^{VW}, ASP313^{VW}, GLU317^{VW}, GLN517^{VW}	-7.9	1.41	-	ALA381^{AL}, ALA385^{AL}, ARG480^{PAL}, ARG382^{PAL}, PHE458^{PAL}, PHE262^{VW}, GLU317^{VW}, ARG389^{VW}, ASP459^{VW}, GLY479^{VW}

Note: AL – Alkyl bond; PAL -Pi-alkyl bond; VW – van der Walls interactions.

the DDHL interacted with the residues such as TRP50, ILE284, LYS348, ILE37, GLU43, GLY44, ASP54, ARG199, HIS285, THR347 via pi-alkyl and van der Walls interactions (Table 4 and Figures 2O, P).

Klebsiella pneumoniae

Carbapenemase

The DHL interacted with Carbapenemase residues such as SER70, LYS73, and SER130 via hydrogen bond and with other residues such as TRP105, LEU167, ASN132, ASN170, and THR237 via pi-sigma, alkyl, and van der Walls interactions, with a binding affinity of -6.9 kcal/mol and an RMSD of 1.956Å (Table 4 and Figures 2Q, R). Less Likely, the DDHL compound interacted with the Carbapenemase residue SER70 via hydrogen bond and other residues such as TRP105, LEU167, LYS73, SER130, ASN132, GLU166, THR216, THR235, GLY236, THR237 via alkyl, pi-alky, and van der Walls interactions with the binding affinity of -7.9 kcal/mol and the RMSD of 1.392Å (Table 4 and Figures 2S, T).

DNA gyrase

The DHL further interacted well with the DNA gyrase residues, such as GLU264 and ARG480, via hydrogen bonds with a binding affinity of -7.4 kcal/mol and an RMSD of 1.935Å. The other residues, such as PHE458, PHE262, GLN263, ASP313, GLU317, and GLN517, via alkyl and van der Walls interactions (Table 4 and Figure S, T). Less similarly, the DDHL interacted with the residues ALA381, ALA385, ARG480, ARG382, PHE458, PHE262, GLU317, ARG389, ASP459, and GLY479 via alkyl, pi-alkyl, and van der Walls interactions with the binding affinity of -7.9 kcal/mol and the RMSd of 1.41Å (Table 4 and Figures 2U, V).

Overall, the compound DHL interacted well with the Pteridine reductase (*Leishmania major*), Carbapenemase, and DNA gyrase (*Klebsiella pneumoniae*) (Table 4 and Figure 2A-B, Q-R, U-V). Meanwhile, the DDHL interacted well with Squalene synthase (*Leishmania major*), (acyl-carrier-protein) S-malonyltransferase, and DNA gyrase (*Streptococcus pyogenes*) (Table 4 and Figures 2G-H, K-L, O-P).

LigPlot⁺ Interactions and ADMET analysis

The molecular interactions between the isolated compounds DHL and DDHL and their target proteins were analyzed using LigPlot+ v2.2.8 to assess hydrogen bonding and hydrophobic interactions. The results closely matched the 2D binding interaction profiles from Discovery Studio, except for Pteridine reductase, where variations were observed (Supplementary File 1, Figure S2). The ADMET analysis indicated that both compounds fall within acceptable pharmacokinetic and drug-likeness criteria. As shown in Table 5, both compounds exhibited no violations in the "Stars" category, molecular weights within the optimal range (DHL: 230.306, DDHL: 232.322 Da), and Surface Accessible Surface Area (SASA) values (DHL: 465.136, DDHL: 472.131) consistent with good molecular flexibility. No violations of Lipinski's Rule of Five (Ro5) were observed, reinforcing their suitability for oral drug development. The solubility profiles (QPlogS: -3.298 for DHL, -3.593 for DDHL) and predicted blood-brain barrier permeability (QPlogBB: -0.011 for DHL, 0.074 for DDHL) suggest favorable pharmacokinetic properties. Both compounds demonstrated high predicted human oral absorption (score=3), a critical factor for bioavailability. These results suggest that DHL and DDHL possess promising ADMET properties, making them viable candidates for further pharmacokinetic and therapeutic development. However, additional *in vivo* metabolism and toxicity studies are required to confirm their biological efficacy and safety.).

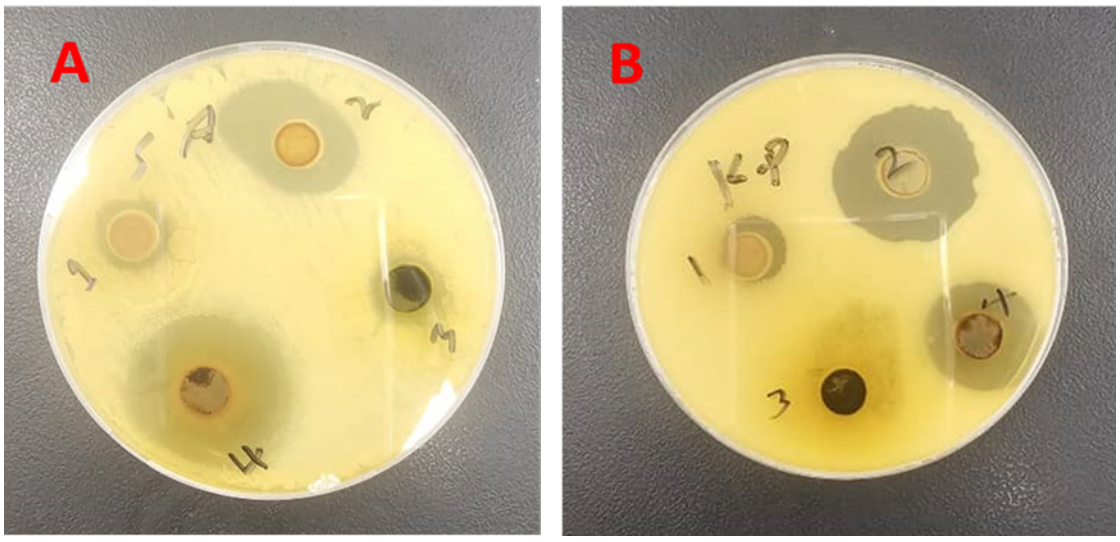


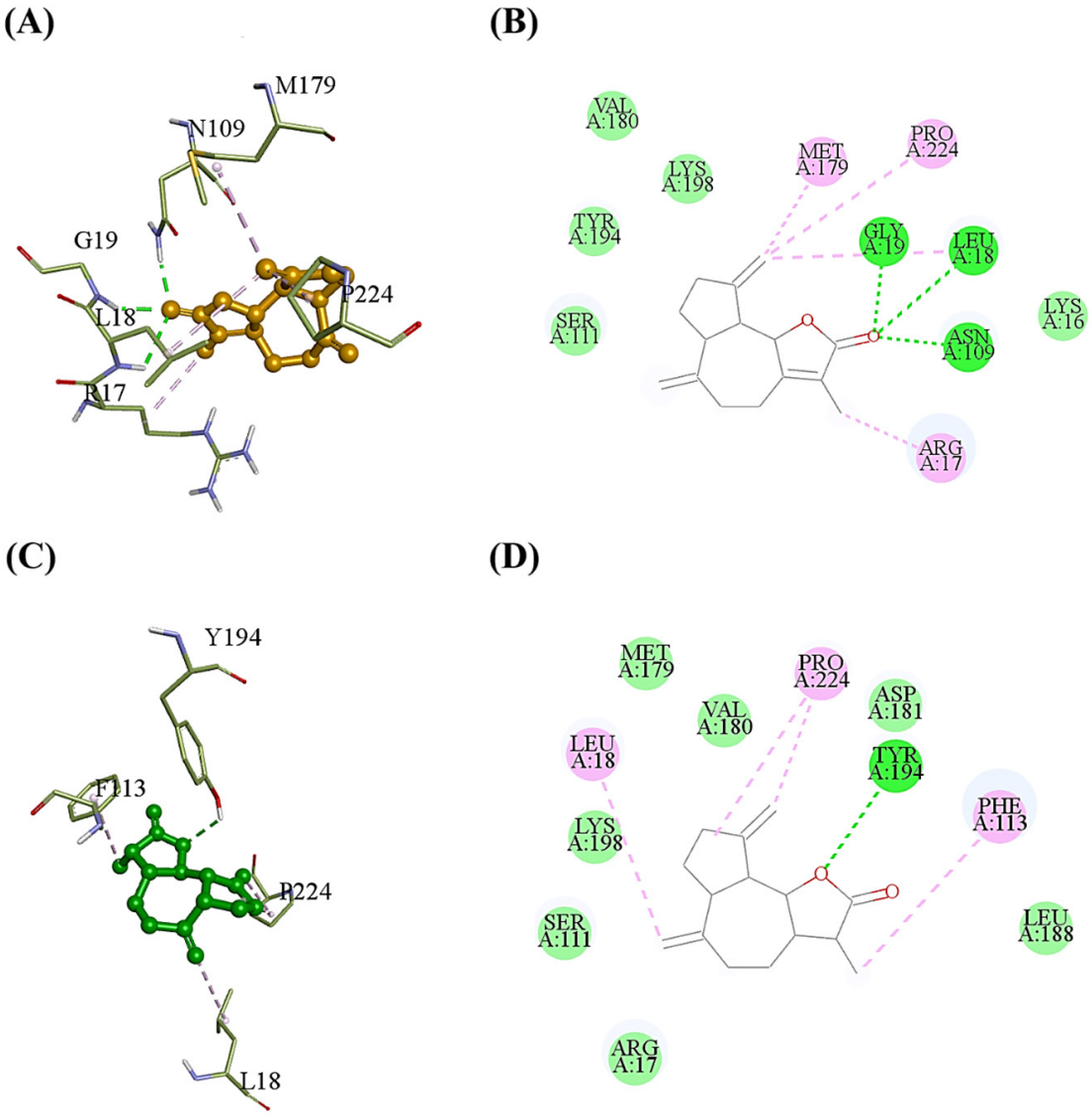
Figure 1: Antibacterial activities of *S. lappa* against *S. pyrogens* (A) and *K. pneumonia* (B).

Table 5: The predicted ADMET properties for the isolated DHL and DDHL.

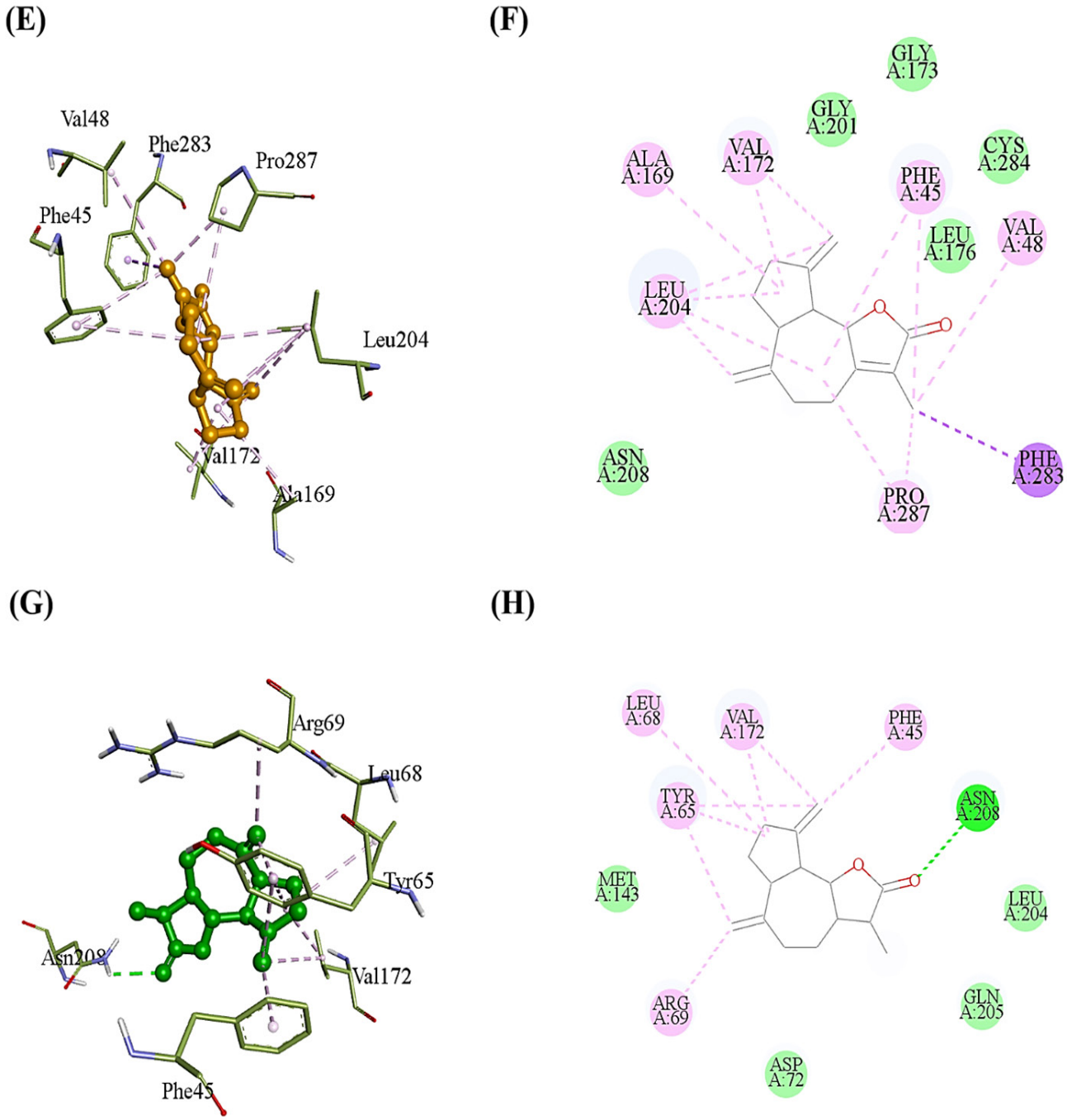
ADEMET Parameters	DHL	DDHL	Normal Range
Stars	0	0	0-5
Molecular Weight	230.306	232.322	130.0 - 725.0
SASA	465.136	472.131	300.0-1000.0
WPSA	0	0	0.0 – 175.0
Donor HB	0	0	0.0-6.0
AcceptHB	3	3	2.0-20.0
QPlogS	-3.298	-3.593	-6.5-0.5
QPlogBB	-0.011	0.074	-3.0-1.2
Human Oral Absorption	3	3	1 (low), 2 (medium), 3 (high)
Rule of Five	0	0	≤ 4

Leishmania major

Pteridine reductase (PDB ID: 6XRC)

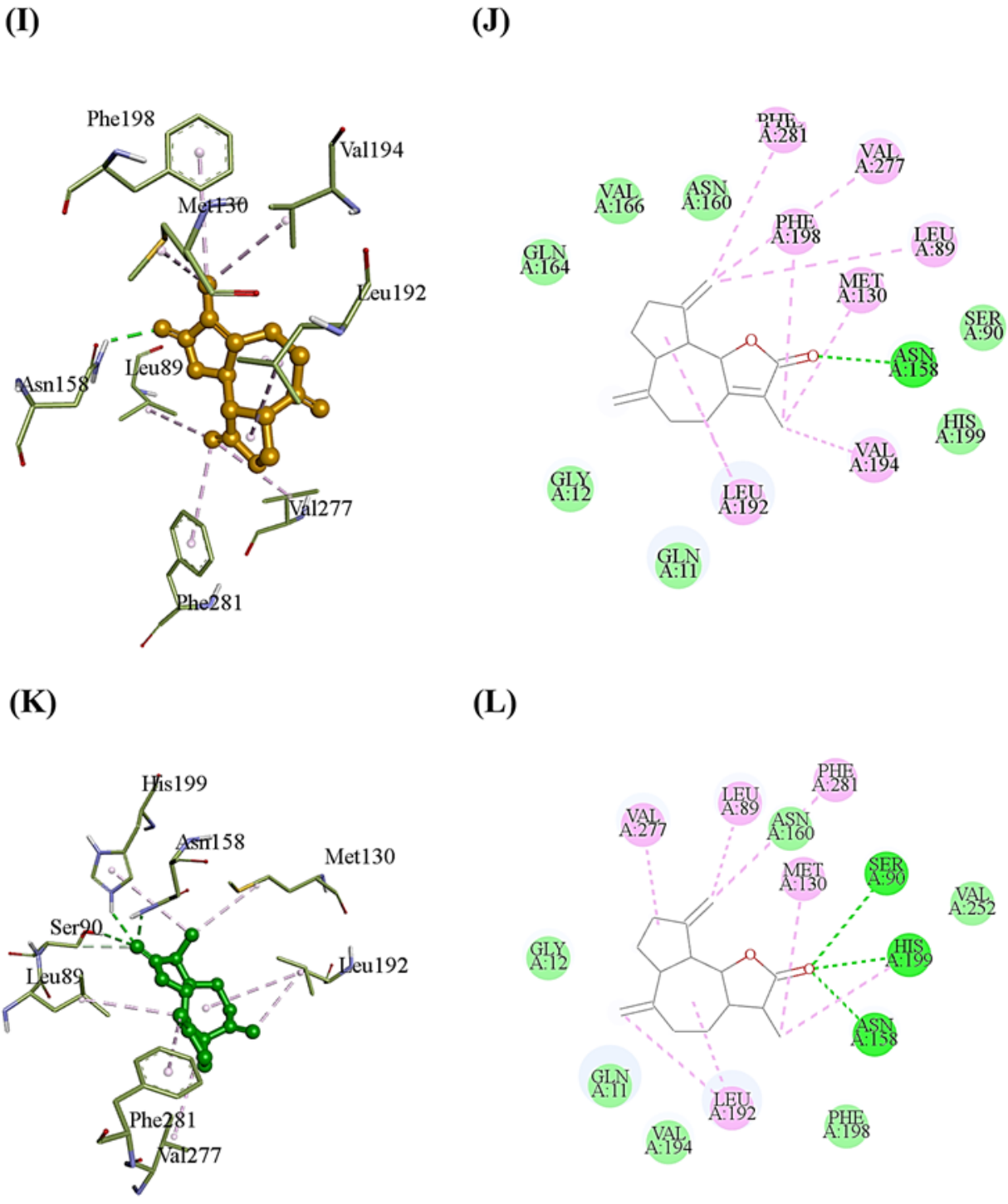


Squalene synthase (UniProt ID: Q25308)



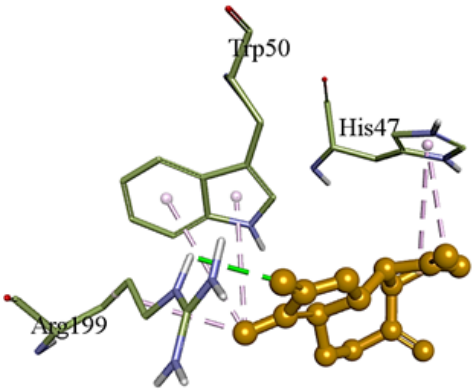
Streptococcus pyogenes

[acyl-carrier-protein] S-malonyltransferase (UniProt ID: A0AAE9U1B1)

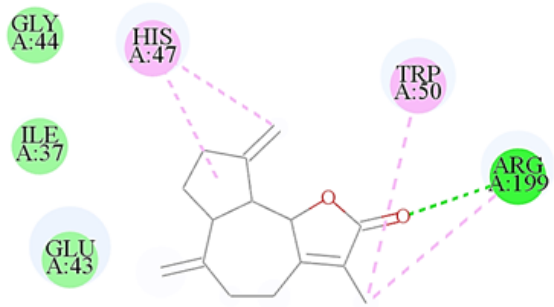


DNA gyrase

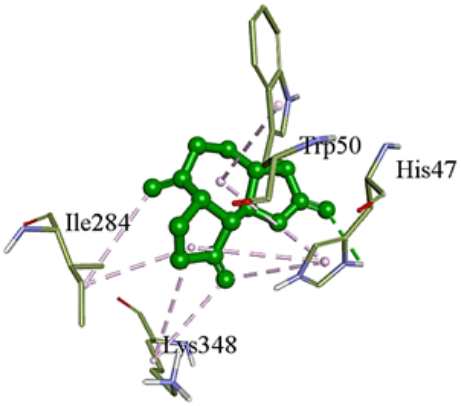
(M)



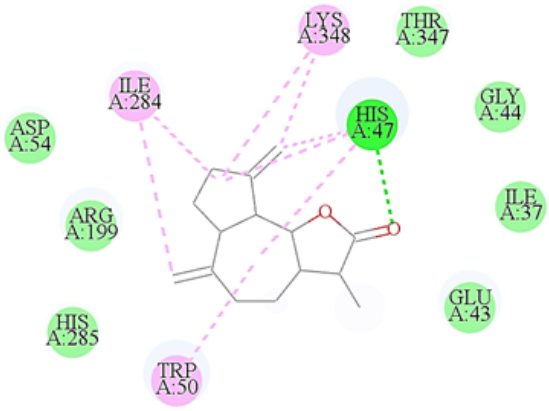
(N)



(O)



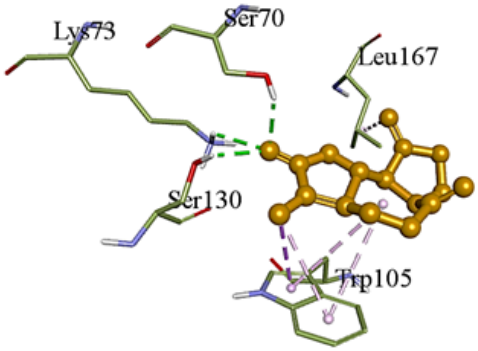
(P)



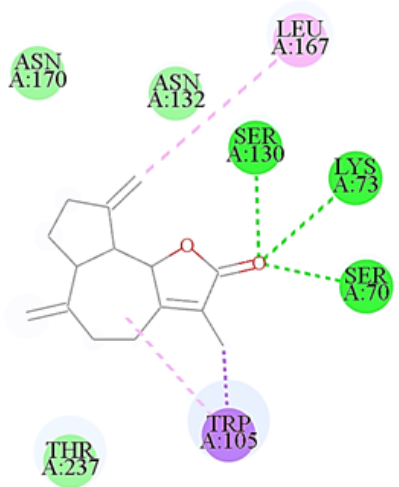
Klebsiella pneumoniae

Carbapenemase (PDB ID: 2OV5)

(Q)



(R)



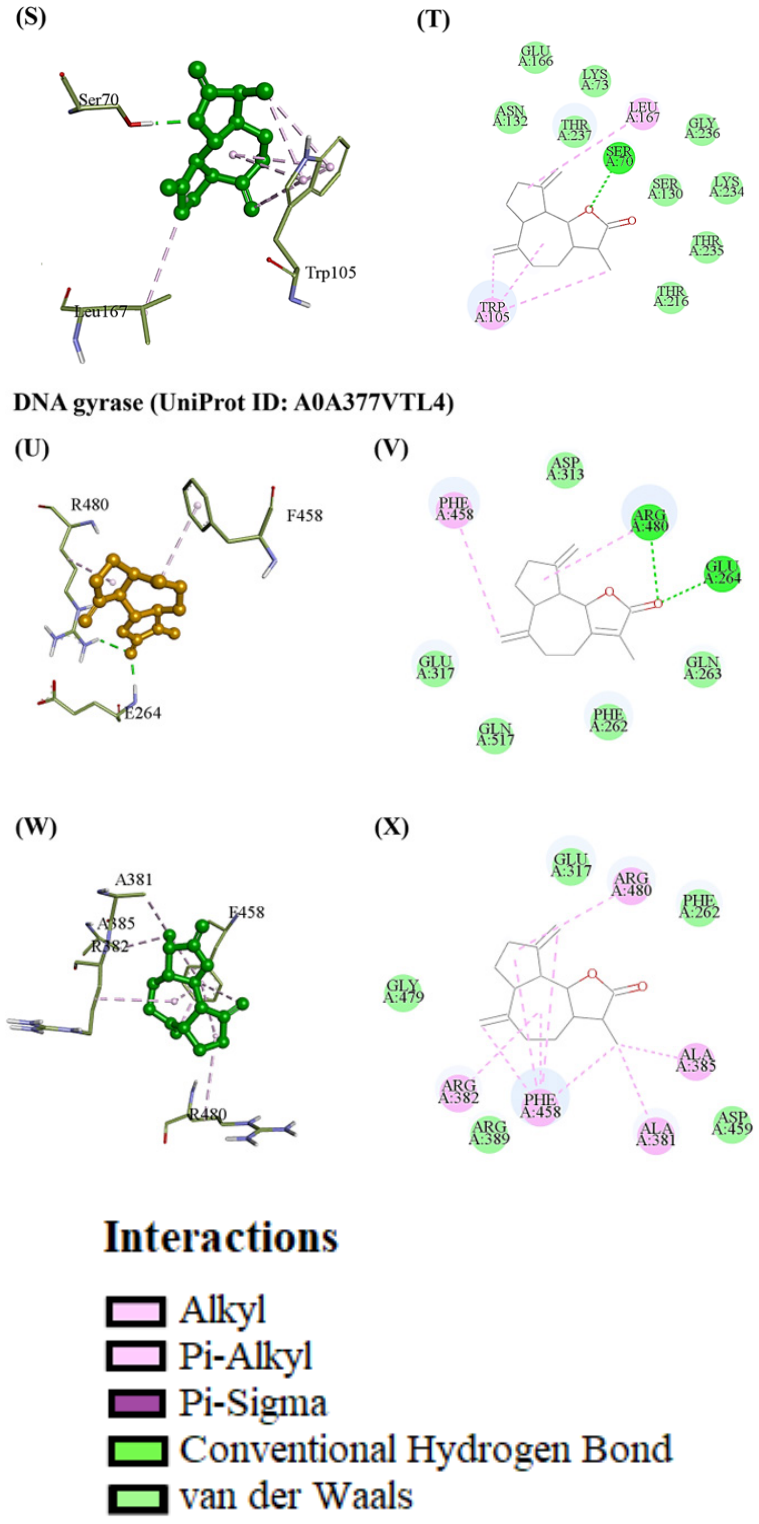


Figure 2: The docking pose and 2D interactions of the selected targets (A-D) Pteridine reductase, (E-H) Squalene synthase (*Leishmania major*), (I-L) (acyl-carrier-protein) S-malonyltransferase, (M-P) DNA gyrase (*Streptococcus pyogenes*), (Q-T) Carbapenemase, (U-X) DNA gyrase (*Klebsiella pneumoniae*) with DHL and DDHL based on the binding affinity, RMSD (≤3.0 Å), and number of hydrogen bonds interacted with the DHL and DDHL.

DISCUSSION

This study aims to investigate the antileishmanial, antitoxoplasma, and antibacterial activities of methanolic extracts from *S. lappa* roots, filling research gaps in parasite disorder treatments. Additionally, it will computationally analyze *S. lappa*'s active components to elucidate their molecular mechanisms of action. Previous research has examined *S. lappa*'s ability to combat *Clonorchis sinensis, Trypanosoma cruzi*, and some nematodal diseases. In previous studies,[6] the plant's decoction was administered orally to rabbits infected with *Clonorchis sinensis*, and it was shown to be somewhat effective. The effectiveness of *S. lappa* in children infected with the corresponding worms was also investigated.[6] At a concentration of 100 mg/mL, the methanolic extract of *S. lappa* was studied in axenic cultures containing *Trypanosoma cruzi* in the epimastigote form. The antiparasitic activity of *S. lappa* extract has been previously demonstrated.[4] The current study showed that *S. lappa* extract exhibited moderate activity against *L. major* promastigotes (IC_{50} 14.7 µg/mL), albeit with higher toxicity towards host cell macrophages. Further investigation for active compound isolation is recommended.

The extract of *S. lappa* had antibacterial activities against *S. pyogenes* and *K. pneumoniae* that varied depending on the dose used, suggesting its potential effectiveness. The findings of our study align with other published research, indicating that the methanol and ethanol extracts derived from *S. lappa* exhibited notable antibacterial efficacy.[22] The antibacterial effectiveness of essential oils derived from the plant roots was also shown to be high.[23]

These results suggested that the extract contains phytochemical compounds that are responsible for the antibacterial and antileishmanial actions. Terpenes, favonoids, alkaloids, and anthraquinones are its main active components.[4,23] Plant terpenes including dihydrocostunolide, 12-methoxydihydrocostunolide, costunolide, Dehydrocostuslactone (DHL), and Dihydrodehydrocostus Lactone (DDHL) are generally anticancer, antimicrobial and anti-inflammatory (2, 8). However, prior research has not yet examined the exact way in which these drugs work utilizing computational methods and proteins that are unique to these microorganisms. This work is distinctive since it performed a computer analysis of the interaction between two active chemicals, DHL and DDHL, in bacterial and leishmanial proteins.

Pteridine reductase 1 is a folate and pterin pathway enzyme unique for pathogenic protozoan parasite Leishmania. Likely, the first step of sterol biosynthesis is catalyzed by the enzyme Squalene synthase, which is essentially needed for the survival and growth of the *Leishmania* species.[24-26] (acyl-carrier-protein) S-malonyltransferase was determined as a novel target to inhibit *Streptococcus pyogenes*.[27] Meanwhile, the DNA gyrase is involved in the modulation of DNA topology and is crucial for the survival of bacterial cells.[28] Also, developing strategies to inhibit carbapenemase enzyme is crucial to combat multidrug-resistant *Klebsiella pneumoniae* infections.[29,30] Hence, the validated drug target, *Leishmania major* (Pteridine reductase and Squalene synthase), *Streptococcus pyogenes* ((acyl-carrier-protein) S-malonyltransferase and DNA gyrase), and *Klebsiella pneumoniae* (Carbapenemase and DNA gyrase), was focused on identifying the inhibitory effects of DHL and DDHL. The molecular docking analysis revealed that DHL and DDHL act well against the *Leishmania major*. Likely, the DDHL interacted better with the *Streptococcus pyogenes* than the DHL. Alternatively, the DHL interacted better with *Klebsiella pneumoniae* than the DHL. Overall, the isolated compounds DHL and DDHL interact with the *Leishmania major, Streptococcus pyogenes,* and *Klebsiella pneumoniae.* The predicted ADMET properties clearly stated that DHL and DDHL may act as drug-like compounds against the *Leishmania major* by hindering the drug-resistance mechanisms.

CONCLUSION

this study delved into the potent antiparasitic, antibacterial, and antibacterial activities of methanolic extracts from *S. lappa* roots. Notably, the research highlighted the efficacy of *S. lappa* against Leishmania major, Streptococcus pyogenes, and Klebsiella pneumoniae, shedding light on its potential as a treatment option for these infectious diseases. The active compounds within *S. lappa*, particularly terpenes like DHL and DDHL, were computationally analyzed, revealing their interactions with specific proteins unique to the targeted microorganisms. The study identified key drug targets in Leishmania major, Streptococcus pyogenes, and Klebsiella pneumoniae, emphasizing the inhibitory effects of DHL and DDHL. Molecular docking analyses illustrated the promising interactions of these compounds with the targeted pathogens, suggesting their potential as effective treatments. Moreover, the predicted ADMET properties indicated that DHL and DDHL could serve as valuable drug-like compounds in combating Leishmania major infections by impeding drug-resistance mechanisms. Overall, this research contributes significantly to understanding the molecular mechanisms underlying the therapeutic properties of *S. lappa*, paving the way for further exploration and development of novel treatments for parasitic and bacterial infections. Further investigations into the isolation and characterization of active compounds are recommended to advance the development of potential pharmaceutical interventions based on *S. lappa* extracts.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity; **CFU:** Colony-forming unit; **DDHL:** Dihydrodehydrocostus lactone; **DHL:** Dehydrocostuslactone; **FBS:** Fetal bovine serum; **PDB:** Protein Data Bank; **PDBQT:** Protein Data Bank, Partial Charge (Q), and Atom Type (T) format; **RMSD:** Root mean square deviation; **RPMI:** Roswell Park Memorial Institute; **ZOI:** Zone of inhibition.

AUTHORS' CONTRIBUTIONS

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

SUMMARY

This study explores the antimicrobial potential of *Saussurea lappa* root extracts, focusing on their antileishmanial, antitoxoplasmic, and antibacterial activities. Bioassays demonstrated moderate efficacy against *Leishmania major* but limited therapeutic potential due to host cell toxicity. Antibacterial screening revealed significant inhibition against *Streptococcus pyogenes* and *Klebsiella pneumoniae*. Molecular docking studies confirmed strong interactions of key bioactive compounds, Dehydrocostuslactone (DHL) and Dihydrodehydrocostus Lactone (DDHL), with essential microbial targets. ADMET analysis indicated favorable pharmacokinetics, highlighting the potential of *S. lappa* for further therapeutic development. These findings warrant additional preclinical research to refine its antimicrobial applications.

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