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Antimicrobial Potential of Selected Phytochemicals from *Hygrophila schulli*; Computational Insights

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Abstract

The escalating global concern over antibiotic resistance has led to an intensified exploration of alternative therapeutic strategies, including the utilization of plant-derived secondary metabolites. In this in-silico study, we investigated the structural inhibition of bacterial DNA Topoisomerase IV complex by major secondary metabolites extracted from the medicinal plant *Hygrophila schulli*. The plant is renowned for its rich phytochemical composition, possessing bioactive compounds with diverse pharmacological properties. Using computational approaches, we conducted molecular docking simulations to explore the binding affinities and interactions between the identified secondary metabolites from *Hygrophila schulli* and the target bacterial DNA Topoisomerase IV complex. Our results unveil promising interactions, suggesting a potential inhibitory effect on the targeted protein. Furthermore, molecular dynamics simulations were employed to examine the dynamic behavior of the ligand-protein complexes, providing insights into the stability and conformational changes over time. This in-silico exploration contributes valuable information to the understanding of the molecular interactions between plant-derived secondary metabolites and Bacterial DNA Topoisomerase IV, laying the groundwork for future experimental validations. The findings from this study may pave the way for the development of novel antimicrobial agents derived from natural sources, offering a sustainable and effective approach in the ongoing battle against antibiotic-resistant bacterial infections.

Keywords: DNA Topoisomerase; *Hygrophila schulli*; Lupeol; Molecular docking; Physicochemical.

INTRODUCTION

Natural materials, including parts of plants, animals, and microbes, have been used in medicine to heal illnesses since ancient times. Fossil evidence suggests that humans have been using plants as medicine for at least 60,000 years (Yuan et al., 2016). Plants generate phytochemicals, which are constitutive metabolites essential to their survival and well-being. These chemical constituents regulate pollination, fertilization, growth, and the rhizosphere environment in addition to shielding plants from rivals, diseases, and predators (Molyneux et al., 2007). These chemical constituents, which are thought to be antibiotic, antifungal, and antiviral, shield plants from infections. They are also essential UV-absorbing chemical components that shield leaves from serious light-induced damage (Bourgaud et al., 2001).

Sri Lanka is regarded as a hotspot for biodiversity with a high level of endemism. Because of this, the flora that thrive on this small island have special physical and ecological characteristics. Several medical systems, including traditional folk medicine, Ayurveda, Unani, and Siddha, have enhanced the use of herbs as therapeutic agents in Sri Lanka (Nissanka et al., 2023). One such plant, native to Sri Lanka, is *Hygrophila schulli*, which is widely utilized to treat a wide range of medical ailments in both traditional and ayurvedic systems. It is referred to as "Neeramulliya" or "Niramulli" in Sri Lanka (Hewawasam et al., 2003). The entire *H. schulli* plant, as well as its roots, leaves, flowers, and seeds, are employed in treatment (Shoma et al., 2018). *H. schulli* leaves and young stems are widely used in Ayurveda medicine to treat a variety of conditions, including hepatic diseases, microbial infections like gonorrhea and urinary tract infections, cough, joint pains, bacterial infections, rheumatism, and dysentery (Sethiya et al., 2018). Previous research in a rodent model demonstrates the strong anti-nociceptive and anti-inflammatory properties of *H. schulli* seed extract. Additionally, *H. schulli* leaf and root extracts showed antibacterial activity against a range of bacterial strains (Kannur et al., 2012), and an ethanolic extract of the seed was reported to exhibit antioxidant qualities against a variety of free radicals (Chandran et al., 2013). *H. sp.* leaves and young stems are widely used in Ayurveda medicine to treat a variety of conditions, including hepatic diseases, microbial infections like gonorrhea and urinary tract infections, cough, joint pains, bacterial infections, rheumatism, and dysentery (Sethiya et al., 2018).

DNA topoisomerases, also known as topoisomerases, are enzymes that mediate modifications to the topological state of DNA, transforming DNA into knotted and unknotted forms, relaxed and supercoiled forms, and linked and unlinked species (McKie et al., 2021). DNA's double helix structure is entangled, which can cause topological problems. Overwinding of the DNA duplex can occur during transcription and replication, for example. This torsion would eventually prevent the DNA or RNA polymerases engaged in these activities from moving further along the DNA helix if left unaltered. Replication-related DNA tangling or linking presents a second topological problem. Links between replicated DNA will prevent cells from dividing if they remain unbroken. These kinds of topological issues are avoided and resolved by the DNA topoisomerases (Sutormin et al., 2021). Gyrase and Topoisomerase IV are two type II topoisomerases that are involved in facilitating DNA replication and timely DNA segregation in *Escherichia coli*. These two enzymes function by causing a temporary double strand break in a single molecule, guiding a second DNA duplex through the cut, and then closing the cut again (Schoeffler & Berger, 2008).

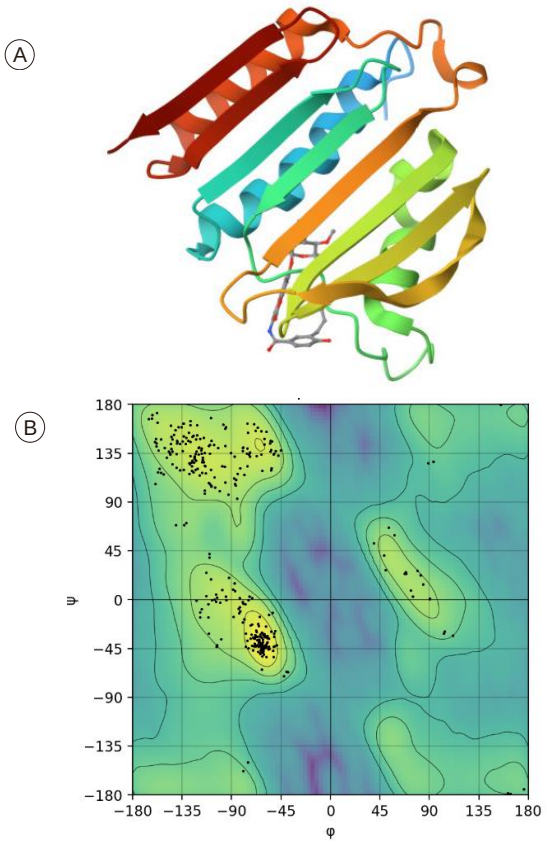


Figure 1. (**A**). - Crystal structure of Escherichia coli Topoisomerase IV ParE 24kDa subunit; RCSB PDB - 1S14: Crystal structure of Escherichia coli Topoisomerase IV ParE 24kDa subunit (**B**). Ramachandran plot of Topoisomerase IV ParE.

Antibacterial drugs based on quinolones target DNA gyrase and topoisomerase IV. The most potent and versatile oral antibacterial medications now being used in clinical settings are quinolones. Medications like ciprofloxacin are frequently used to treat a range of Gram-negative bacterial infections, such as infections of the gastrointestinal tract, bones, and joints (Gentry & Osheroff, 2013).

MATERIALS AND METHODS

Data Collection and Ligand Preparation

The approach commenced with a comprehensive literature survey aimed at identifying optimal secondary metabolites for study. Upon selection, the 3D chemical structures of these chosen secondary molecules were procured from the PubChem database, and the data were acquired in .sdf format. To ensure compatibility with physiological conditions, the retrieved chemical structures were imported into Avogadro v.1.2.0 software. Subsequently, adjustments were made to achieve the appropriate protonation states, and energy minimization was performed leveraging the universal force field, utilizing a conjugate gradient algorithm for 500 steps. For compatibility with downstream processes and analyses, the prepared ligands transformed and were saved in the .pdbqt format. The OpenBabelGUI tool played a pivotal role in facilitating file format conversion, ensuring seamless integration of the ligands into the subsequent stages of our research. This meticulous process of data collection and ligand preparation lays the foundation for robust molecular analyses, allowing for accurate and reliable investigations into the interactions and behaviors of the selected secondary metabolites.

Protein preparations

Crystal structure of *Escherichia coli* Topoisomerase IV ParE 24kDa subunit – PDB ID-1S14 were obtained from protein data bank in .pdb format. Protein preparation was done using Autodocktools version 1.5.7. The water molecules and heteroatoms were deleted. Missing atoms were repaired. Polar hydrogens and Kollman charges were added to the protein. Molecular Docking The grid parameters and map files were created using Autogrid 4.2. to perform the blind Docking. (Genetic algorithm parameters settings: number of genetic algorithm (GA) runs: 50, population size: 300, the maximum number of evaluations: 25 000 000 (medium), and maximum number of generations: 27 000). Molecular Docking was performed using Autodock 4.2, and results were generated in .dlg format (output – Lamarckian GA-4.2) (Lawan & Tharakee, 2023).

Interaction Visualization and Analysis Docking

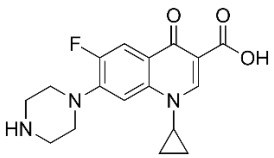
Results were analyzed using AutoDockTools version 1.5.6 to examine the binding energies and Inhibition constants. Binding interactions with the ligand were visualized using the Discovery Studio 2021 client. Protein-Ligand interaction profiler (PLIP - Welcome (tu-dresden.de)) and Proteins.plus (Zentrum für Bioinformatik: Universität Hamburg-Proteins Plus Server) web servers were also used to further analyze the binding residues.

In silico Physicochemical and Pharmacokinetic Parameter Prediction

Veber's rules (Veber et al., 2002) and Lipinski's rule of five (Lipinski et al., 2001) were applied to evaluate drug-likeness using the swissADME server (SwissADME). Secondary metabolites given in Table 1 were analyzed for their physicochemical properties, and the results are epitomized in Table 2. The ADMET characteristics of the Secondary metabolites were studied using the pkCSM server (pkCSM (uq.edu.au)) to understand their pharmacokinetic Parameters, and the results are given in Table 3

RESULTS AND DISCUSSION

Table 1. Calculated binding energies and inhibition constants of ciprofloxacin-a and selected phytochemicals.

Reference Compound	Binding Energy / kcal/mol	Ki-Inhibition constant/ µM
 Ciprofloxacin	-4.21	816.89

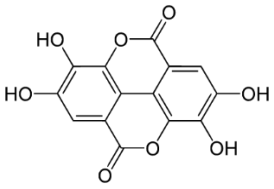
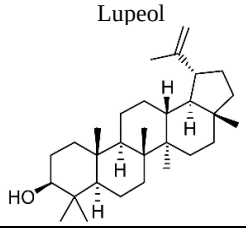
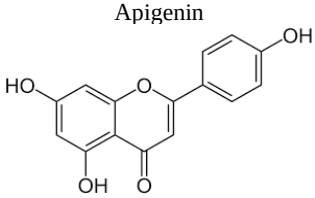
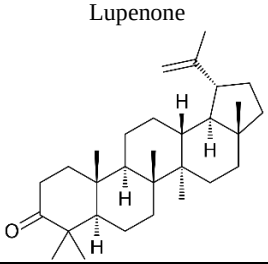
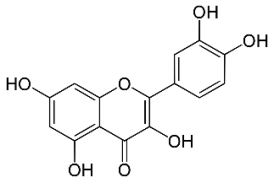
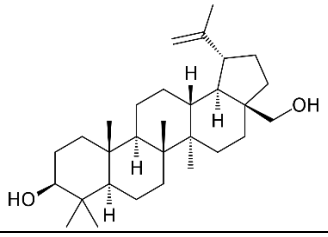
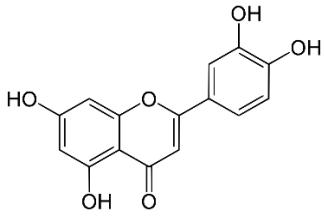
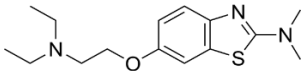
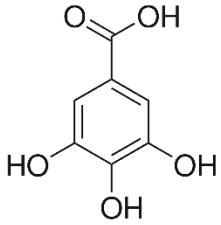
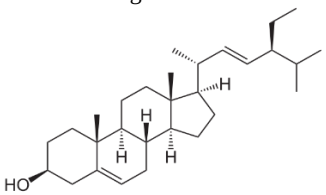
Compound	Binding Energy / kcal/mol	Ki-Inhibition constant/ µM	Compound	Binding Energy / kcal/mol	Ki-Inhibition constant/ µM
Ellagic acid 	-3.57	2430	Lupeol 	-6.05	37.27
Apigenin 	-4.65	393.37	Lupenone 	-6.15	31.03
Quercetin 	-4.07	1040	Betulin 	-5.40	109.28

Table 1. *Cont.*

Compound	Binding Energy / kcal/mol	Ki-Inhibition constant/ µM	Compound	Binding Energy / kcal/mol	Ki-Inhibition constant/ µM
Luteolin 	-4.58	435.83	Asterol 	-4.40	593.30
Gallic acid 	-3.81	1620	Stigmasterol 	-5.23	145.80

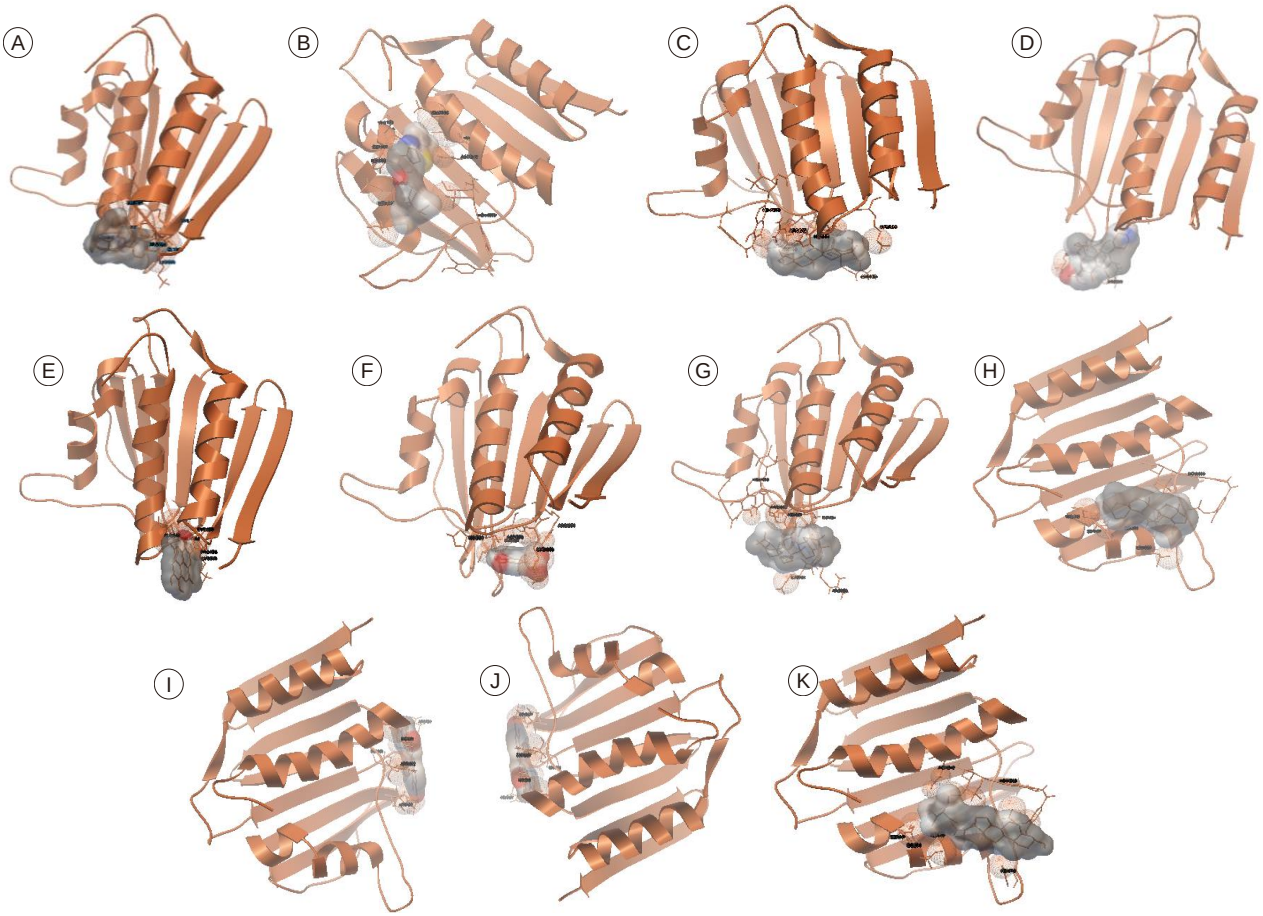


Figure 2. 3D visualization of the ligand-protein complexes, (**A**). Apigenin (**B**). Asterol (**C**). Butilin (**D**). Ciprofloxacin (**E**). Ellagic acid (**F**). Gallic acid (**G**). Lupenone (**H**). Lupeol (**I**). Quercetin (**J**). Luteolin (**K**). Stigmasterol obtained from Discovery Studio 2021 client.

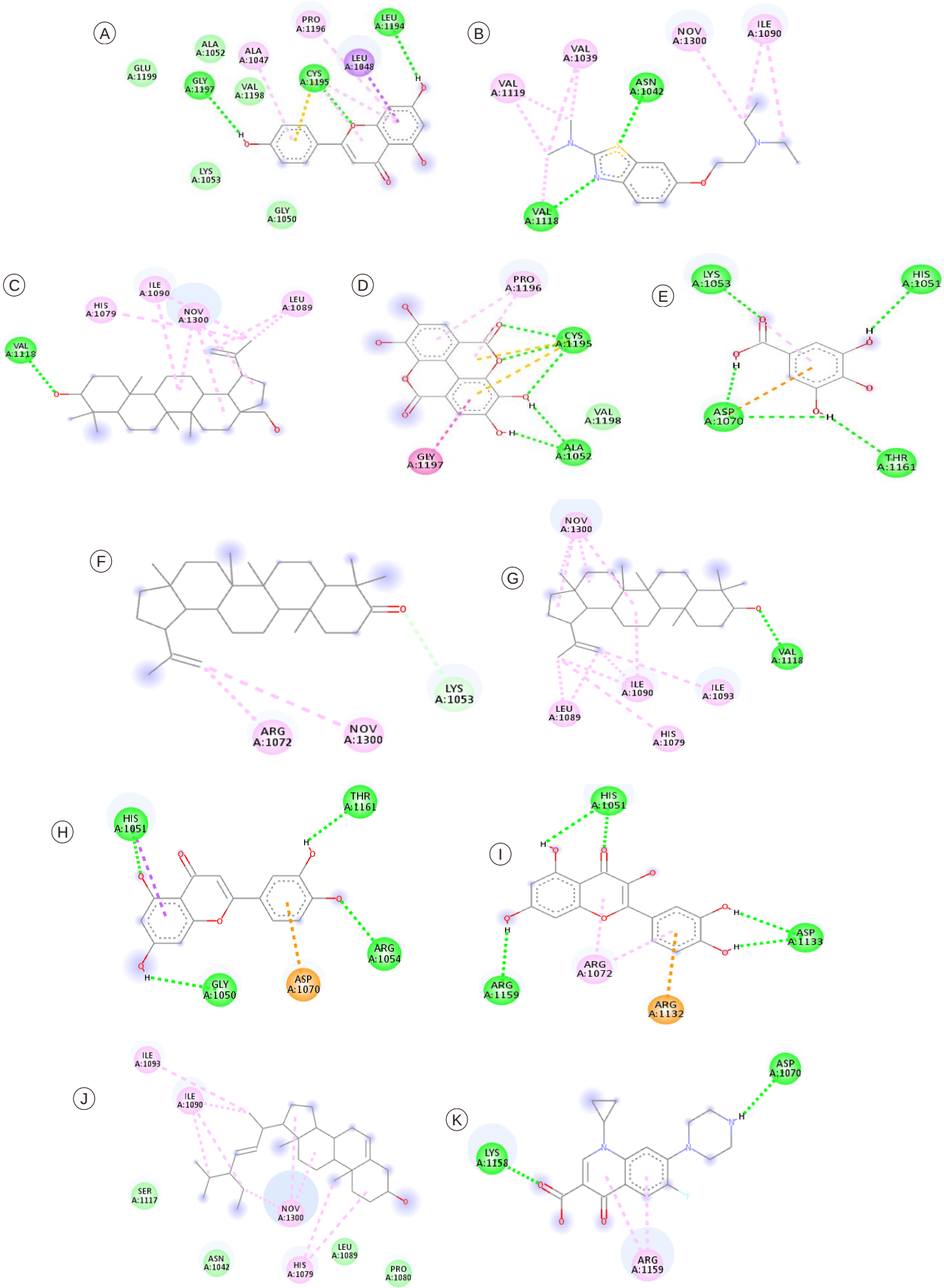


Figure 3. Diagram showing the amino acid residues that interact with ligand, (**A**). Apigenin (**B**). Asterol (**C**). Butilin (**D**). Ellagic acid (**E**). Gallic acid (**F**). Lupenone (**G**). Lupeol (**H**). Luteolin (**I**). Quercetin (**J**). Stigmasterol (**K**). Ciprofloxacin that generated using the Discovery Studio 2021 client.

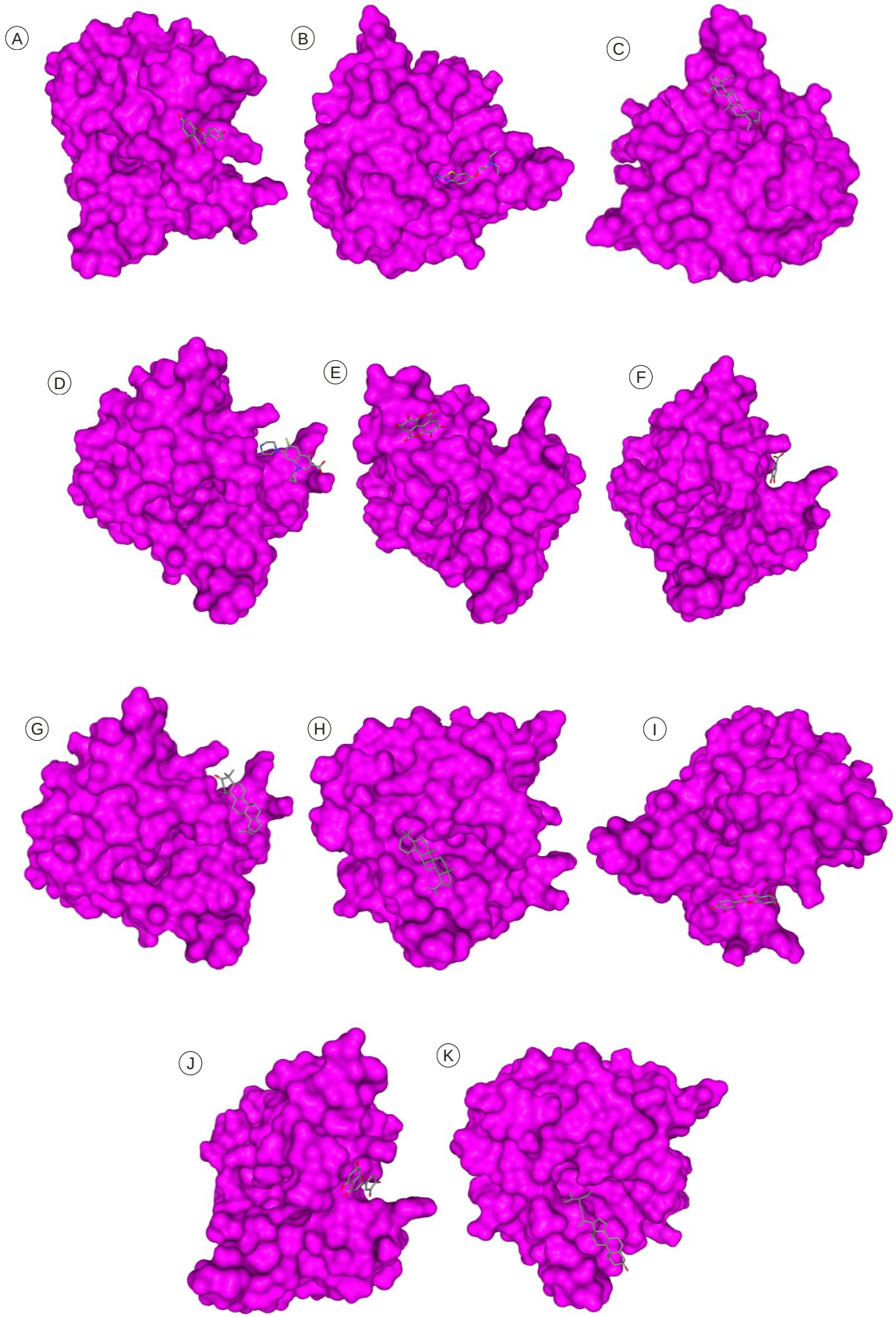


Figure 4. Diagram showing the binding pockets of amino acid residues that interact with ligand, (**A)**. Apigenin (**B)**. Asterol (**C)**. Butilin (**D)**. Ellagic acid €. Gallic acid (**F**). Lupenone (**G)**. Lupeol (**H)**. Luteolin (**I)**. Quercetin (**J**). Stigmasterol (**K)**. Ciprofloxacin that generated using the Discovery Studio 2021 client.

Table 2. Predicted Physicochemical properties of studied secondary meabolites; 1- Molecular weight (g/mol) 2- Num. heavy atoms 3- Num. rotatable bonds 4- Num. H-bond acceptors 5- Num. H-bond donors 6-Topological polar surface area(Å²) 7-Lipinski's rule of five 8- Veber's rule.

Compound	MW[1]	n-nha[2]	n-rot[3]	n-Hba[4]	n-Hbd[5]	TPSA[6]	Lpi[7]	Ver[8]
Ciprofloxacin	331.34	24	3	5	2	74.57	Yes	Yes
Ellagic acid	302.19	22	0	8	4	141.34	Yes	Yes
Apigenin	270.24	20	1	5	3	90.9	Yes	Yes
Quercetin	302.24	22	1	7	5	131.36	Yes	Yes
Luteolin	286.24	21	1	6	4	111.13	Yes	Yes
Gallic acid	170.12	12	1	5	4	97.99	Yes	Yes
Lupeol	426.72	31	1	1	1	135.14	Yes	Yes
Lupenone	424.7	31	1	1	0	134.18	Yes	Yes
Betulin	442.72	32	2	2	2	40.46	Yes	Yes
Asterol	293.43	9	7	3	0	56.84	Yes	Yes
Stigmasterol	412.69	30	5	1	1	20.23	Yes	Yes

Table 3. Predicted pharmacokinetic properties of studied secondary metabolites; 1-Water solubility (log mol/L) 2- $CaCO_3$ permeability 3-Human Intestinal absorption (% Absorbed) 4- Skin permeability (log Kp) 5-P-glycoprotein substrate 6 - P-glycoprotein I inhibitor 7-P-glycoprotein II 8-VDss-human (log L/kg) 9-Fraction unbound 10-BBB permeability (log BB) 11-CNS Permeability (log PS) 12 – CYP2D6 Substrate 13-CYP3A4 Substrate 14- CYP1A2 Inhibitor 15- CYP2C19 Inhibitor 16 – CYP2C9 inhibitor 17 -CYP2D6 inhibitor 18-CYP3A4 inhibitor 19-Total Clearance (log ml/min/kg) 20- Renal OCT2 substrate 21 -AMES toxicity 22 – Human max. tolerated dose (log mg/kg/day) 23- hERG I inhibitor 24- hERG II inhibitor 25- Oral Rat Acute Toxicity (LD50 mol/kg) 26- Oral rat Chronic Toxicity (LOAEL log mg/kg_bw/day) 27- hepatotoxicity 28-Skin sensitization 29- *T.Pyriformis* toxicity (log µg/L) 30- Minnow toxicity (log mM).

Compound	Adsorption				Distribution						
	WS[1]	$CaCO_3P$[2]	IB(Human)[3]	SP[4]	P-gly Sub[5]	P-gly I inb[6]	P-gly II inb[7]	VDss (human)[8]	FU (human)[9]	BBB P[10]	CNS P[11]
Ciprofloxacin	-2.897	0.492	96.466	-2.734	Yes	No	No	-0.17	0.648	-0.587	-2.999
Ellagic acid	-3.181	0.335	86.684	-2.735	Yes	No	No	0.375	0.083	-1.272	-3.533
Apigenin	-3.329	1.007	93.25	-2.735	Yes	No	No	0.822	0.147	-0.734	-2.061
Quercetin	-2.925	-0.229	77.207	-2.735	Yes	No	No	1.559	0.206	-1.098	-3.065
Luteolin	-3.094	0.096	81.13	-2.735	yes	No	No	1.153	0.168	-0.907	-2.251
Gallic acid	-2.56	-0.081	43.374	-2.735	No	No	No	-1.855	0.617	-1.102	-3.74
Lupeol	-5.861	1.226	95.782	-2.744	No	yes	yes	0	0	0.726	-1.714
Lupenone	-5.828	1.448	98.467	-2.567	No	yes	yes	-0.216	0	0.751	-1.568
Betulin	-5.446	1.191	94.539	-2.789	No	yes	yes	-0.177	0	-0.295	-2.03
Asterol	-2.855	1.655	93.417	-2.823	No	No	No	0.77	0.327	0.404	-2.512
Stigmasterol	-6.682	1.213	94.97	-2.783	No	yes	yes	0.178	0	0.771	-1.652

Compound	Metabolism							Excretion	
	CYP2D6 sub[12]	CYP3A4 sub[13]	CYP1A2 inb[14]	CYP2C19 inb[15]	CYP2C9 inb[16]	CYP2D6 inb[17]	CYP3A4 inb[18]	TC[19]	R-OCT2 sub[20]
Ciprofloxacin	No	No	No	No	No	No	No	0.633	No
Ellagic acid	No	No	Yes	No	No	No	No	0.537	No
Apigenin	No	No	Yes	Yes	No	No	No	0.566	No
Quercetin	No	No	Yes	No	No	No	No	0.407	No
Luteolin	No	No	Yes	No	yes	No	No	0.495	No
Gallic acid	No	No	No	No	No	No	No	0.518	No
Lupeol	No	Yes	No	No	No	No	No	0.153	No
Lupenone	No	Yes	No	No	No	No	No	0.102	No
Betulin	No	Yes	No	No	No	No	No	0.236	No
Asterol	No	Yes	Yes	No	No	Yes	No	0.878	No
Stigmasterol	No	Yes	No	No	No	No	No	0.618	No

Compound	Toxicity									
	AMES t[21]	M.T.D (human)[22]	hERG I inb[23]	hERG II inb[24]	ORAT (LD50)[25]	ORCT (LOAEL)[26]	Hepatptox[27]	Skin S[28]	*T.Pyriformis* T[29]	Min T[30]
Ciprofloxacin	No	0.924	No	No	2.891	1.036	Yes	No	0.286	1.194
Ellagic acid	No	0.476	No	No	2.399	2.698	No	No	0.295	2.11
Apigenin	No	0.328	No	No	2.45	2.298	No	No	0.38	2.432
Quercetin	No	2.471	No	No	2.471	2.612	No	No	0.288	3.721
Luteolin	No	0.499	No	No	2.455	2.409	No	No	0.326	3.169
Gallic acid	No	0.7	No	No	2.218	3.06	NO	No	0.285	3.188
Lupeol	No	-0.502	No	yes	2.563	0.89	NO	No	0.316	-1.696
Lupenone	No	0.008	No	yes	2.556	0.831	NO	No	0.315	-1.777
Betulin	No	-0.794	No	yes	2.699	2.172	Yes	No	0.315	-1.118
Asterol	No	0.103	No	No	2.991	1.235	Yes	No	1.173	0.956
Stigmasterol	No	-0.664	No	yes	2.54	0.872	NO	No	0.433	-1.675

Discussion

In this study, ciprofloxacin was used as the reference compound, the known bacterial Topoisomerase IV inhibitor, to compare the binding energies and inhibition constants with the examined secondary metabolites. The concentration needed to cause half of the maximum inhibition is known as the inhibition constant (Ki), which serves as a measure of an inhibitor's potency. When comparing the binding energies of docked ligands and reference drugs, Apigenin (-4.65 kcal/mol), Luteolin (-4.58 kcal/mol), Asterol (-4.40 kcal/mol), Stigmasterol (-5.23 kcal/mol), Betulin (-5.40 kcal/mol), Lupeol (-6.05 kcal/mol), and Lupenone (-6.15 kcal/mol) exhibit even better binding affinity to the target protein than ciprofloxacin (- 4.21 kcal/mol). (Table 01) Also, those two compounds show lower Ki values than ciprofloxacin, indicating better potency towards the Topoisomerase IV inhibition. It was evident that the top-ranked phytochemical structures' hydroxyl groups play a substantial role in the establishment of potent hydrogen bonds with amino acid residues in the binding pockets. (Figure 02)

The in-silico analysis presented in the uploaded document explores the potential structural inhibition of bacterial DNA topoisomerase IV by major secondary metabolites extracted from the medicinal plant *Hygrophila schulli*. The study utilized computational approaches, including molecular docking simulations and molecular dynamics simulations, to investigate the binding affinities, interactions, and dynamic behavior of the identified secondary metabolites from *Hygrophila schulli* with the target bacterial DNA topoisomerase IV. The results of the molecular docking simulations revealed promising interactions, suggesting a potential inhibitory effect on the targeted protein. The study compared the binding energies and inhibition constants of the secondary metabolites with the reference compound, ciprofloxacin, a known bacterial DNA topoisomerase IV inhibitor. The findings indicated that several secondary metabolites, including Apigenin, Luteolin, Asterol, Stigmasterol, Betulin, Lupeol, and Lupenone, exhibited even better binding affinity and lower inhibition constants than ciprofloxacin, indicating their potential potency towards the inhibition of DNA topoisomerase IV.

When considering the binding interactions of those seven ligands, ciprofloxacin shows only one Hydrogen bonding interaction with the protein (with Asp1070). But Apigenin and Luteolin show three (with Leu1048A, Leu1194A & Gly1197A) and six (with Gly1050A, His1051A, Arg1054A, Asn1160A, & Thr1161A) (Figure 3) hydrogen bonding interactions with the target protein, respectively. Lupenone shows the lowest (-6.15 kcal/mol) binding affinity towards the target protein and the highest inhibition constant (31.03µM) from the examined ligands. Although it is not much as (-)-catechin and Procyanidin-B2, phytochemicals Procyanidin-B3(-5.59 kcal/mol), B4(-5.60 kcal/mol), (-)-Epicatechin (-5.32 kcal/mol) and Quercetin (-5.36 kcal/mol) also exhibits good binding capabilities. Major types of binding interactions between the protein and the ligands are H-bonding and Hydrophobic interactions. Other than that, salt bridge Interactions also can be observed in one case which is gallic acid. Interestingly, in this case, salt bridge Interactions have occurred between the carboxylate groups in the ligands and the Lys1053A and Arg1054A amino acids. From the leading phytochemicals observed with the highest binding affinities, Gallic acid meets Lipinski's and Veber's rules, fulfilling strong physicochemical requirements (Table 4) as a potential drug.

Also, gallic acid shows a satisfactory percentage of absorption through the human intestinal tract. Another important characteristic is that all the screened phytochemicals from *Hygrophila schulli* plant extract don't show hepatotoxicity (Table 4), even though the well-known commercially available antibiotic ciprofloxacin shows some level of hepatotoxicity. In pharmacokinetics and toxicity analysis, it is categorized that values between -7 and -2 log mol/L as water "soluble "compounds. Interestingly all the compounds examined come under that category. Out of the phytochemicals from the *Hygrophila schulli* plant extract assessed, including the reference compound most of the chemicals acted as a substrate for Cytochromes P450 isozymes. However, predicted data revealed that Apigenin, Quercetin, Asterol, and Ellagic acid have the potential to inhibit CYP1A2 isozyme. This predicted data further revealed that none of the phytochemicals don't have the potential to inhibit CYP2D6 and CYP3A4 isozymes. Those predicted data revealed that Lupeol, Lupenone, Betulin, and Asterol have the potential to inhibit CYP3A4 isozyme. Predicted data revealed that Apigenin has the potential to inhibit CYP2C19 isozyme. Those predicted data revealed that Luteolin has the potential to inhibit CYP2C9 isozyme. Those predicted data revealed that Asterol has the potential to inhibit CYP2D6 isozyme.

Furthermore, the study analyzed the binding interactions of the top-ranked phytochemical structures with the target protein, highlighting the role of hydroxyl groups in establishing potent hydrogen bonds with amino acid residues in the binding pockets. The interactions were visualized and analyzed to understand the molecular mechanisms underlying the inhibitory effects of the secondary metabolites. In addition to the molecular docking simulations, the study also conducted in-silico physicochemical and pharmacokinetic parameter predictions for the studied secondary metabolites. The analysis evaluated drug-likeness, physicochemical properties, ADMET characteristics, and predicted pharmacokinetic parameters of the secondary metabolites, providing insights into their potential as antimicrobial agents derived from natural sources.

The comprehensive in-silico analysis presented in the document positions the secondary metabolites from *Hygrophila schulli* as promising candidates for further experimental validations as potential inhibitors of bacterial DNA topoisomerase IV. The findings contribute valuable information to the understanding of the molecular interactions between plant-derived secondary metabolites and bacterial DNA topoisomerase IV, laying the groundwork for the development of novel antimicrobial agents derived from natural sources. This in-silico exploration offers a sustainable and effective approach in the ongoing battle against antibiotic-resistant bacterial infections.

Overall, the study provides a strong foundation for future experimental investigations to validate the inhibitory effects of the identified secondary metabolites from *Hygrophila schulli* on bacterial DNA topoisomerase IV, potentially leading to the development of novel antimicrobial agents with diverse pharmacological properties.

CONCLUSIONS

Selected secondary metabolites found in *Hygrophila schulli* plant extract were tested in silico for their potential inhibitory action towards Topoisomerase IV enzyme, pharmacokinetic and Physicochemical properties, and compared with the commercial antibiotic, ciprofloxacin. Out of the eleven phytochemicals assessed, Lupeol and Lupenone indicated the best receptor inhibition capabilities. Comprehensive docking, pharmacokinetic, and physicochemical tests indicated that Lupenone would be a viable Topoisomerase IV inhibitor because it binds firmly to the binding pocket, inhibiting the native conformation of the protein. Hence, Lupenone is an exceptional candidate for further in vitro/in vivo studies.

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