Molecular Docking of Phytoconstituents from *Aristolochia Bracteolata* for the Effective Treatment of Bacterial and Fungal Infections

[1]L. Kiruthiga, [1]D. Suvedha, [1]R. Amertha, [1]K. Kandhan, [2]S. Padmapriya*, [3]A.N. Rajalakshmi

[1]Master of Pharmacy Students, [2]Associate Professor, [3]Professor and Head, Department of Pharmaceutics, Mother Theresa Post Graduate and Research Institute of Health Science, A Govt. of Puducherry Institution, Puducherry, India - 605006.

[1]kiruthigaloges@gmail.com, [1]suvedhausharani@gmail.com, [1]amertha2210@gmail.com
[1]kandhan841@gmail.com, [2]sppriyaexam2020@gmail.com, [3]ocusertraji@gmail.com

Abstract:

In silico approach is a rapidly growing area that globally covers the development of techniques using software to capture, analyse and integrate biological and medical data from many diverse sources. *Aristolochia bracteolata* Lam belonging to the family Aristolochiaceae is a perennial herb, also known as 'worm killer' possess many phytoconstituents and many pharmacological activities. The present work has been carried out to investigate the anti-bacterial and anti-fungal potential of phytoconstituents of *Aristolochia bracteolata* through molecular docking using PyRx software. Based on the literature review, the phytoconstituents such as Aristolochic acid I, Aristolochic acid II, Aporphine, Aristolactam, Beta sitosterol, Flavonoids and Protoberberines have been selected individually as a ligand to perform molecular docking studies. Penicillin Binding Protein, 1,5 Anhydro D-fructose reductase, Dihydrofolate reductase and DNA dependent RNA polymerase were selected as target proteins for anti-bacterial activity and Sterol-14α demethylase, Succinate dehydrogenase and Dihydro folate reductase were selected as target proteins for the anti-fungal activity. The proteins were selected from the RCSB Protein Data Bank. The binding free energy and binding mode of the standard ligand in the allosteric site of the enzymes have been considered as the reference for validation by docking. The binding free energies for the standard ligands (Amphicillin, Ciprofloxacin, Sulfamethoxazole, Rifampicin, Ketoconazole, Fenfuram and Methotrexate) were -9.3, -7.6, -7.0, -8.1, -9.0, -6.9, -9.3 kcal/mol and for the phytoconstituents of *Aristolochia bracteolata* ranges from -9.5 to -8.2, -9.7 to -7.7, -10.1 to -7.6, -8.3 to -7.1 kcal/mol for the anti-bacterial activity and -8.4 to -7.3, -10.0 to -8.9, -9.9 to -

[2] *Dr. S. Padmapriya,
Associate Professor,
Corresponding Author,
Department of Pharmaceutics,
Mother Theresa Post Graduate and Research Institute of Health Sciences,
Puducherry, India.
Email: sppriyaexam2020@gmail.com

8.1 kcal/mol for the anti-fungal activity and the binding energy values were found to be equal with the binding energy values of standards. Based on the findings, the interactions of phytoconstituents have been identified and indicated that the phytoconstituents of *Aristolochia bracteolata* can be the potential candidates for the effective treatment of bacterial and fungal infections.

Keywords: *Aristolochia bracteolata*, phytoconstituents, binding energies, antibacterial and antifungal activity.

1. Introduction

Computer -aided methods for drug development have advanced as enhanced technologies that can be employed to analyse and also validate the potential phytoconstituent for their pharmacological activity (1). In recent years, Molecular Docking has become an essential aspect of in-silico drug development that predicts the binding affinity of ligands to receptor proteins at the atomic level (2).

Currently there has been a notable increase in research focusing on discovering, screening and exploring the therapeutic potential of the phytoconstituents through computational prediction models in predicting the pharmacokinetic, pharmacological, and toxicological behaviour (1).

Antimicrobial drugs are one of the key elements in reducing the global burden of infectious diseases (3). Studies exploring the antibacterial and antifungal effects of phytoconstituents have unveiled promising findings (4). An increase in the antimicrobial resistance's, a serious worldwide public health issue which necessitates the invention of novel molecules to combat bacteria and fungus infection. Therefore, finding new, effective antibacterial and antifungal agents have become more difficult and demanding for synthetic chemists today (3).

The plants namely *Uvaria scheffleri* (Annonaceae) (5), *Clematis burgensis* (Ranunculaceae) (5), *Euphorbia schimperiana* (Euphorbiaceae) (5), *Arum maculatum* (Araceae) (6), *Lawsonia inermis* (Lythraceae)(6), *foeniculum vulgare* (Apiaceae) (7), *zingiber officinale* (Zingiberaceae) (7), *Quercus coccifera* (Fagaceae) (8), *Ocimum gratissimum* (Lamiaceae) (8), *Curcuma longa* (Zingiberaceae) (8), *Dryopteris ramose* (Dryopteridaceae) (9), *Geranium wallichianum* (Geraniaceae) (10), etc having antimicrobial activity has been reported using molecular docking.

Aristolochia bracteolata (Family: Aristolochiaceae), is a perennial herb found in the upper Gangetic plain, the Western peninsula, Bengal, Gujarat and in the south of India. It is commonly called as "worm killer" (11). The phytochemical analysis of this plant has revealed the presence of alkaloids, triterpenoids, steroids, sterols, flavonoids, tannins, phenolic compounds and cardio glycosides such as aristolochic acids and esters, aristolactams,

aporphines, protoberberines, isoquinolines, benzyl isoquinolines, amides, lignans, biphenyl ethers, coumarins, tetralones, terpenoids, benzenoids and others. Currently this plant being used as antibiotics, antimalarial and aphrodisiac has been reported by traditional healers of Southwestern Nigeria (12). Literature review revealed that Phytoconstituents of *Aristolochia bracteolata* exhibits a wide range of antibacterial and antifungal effect, encompassing various bacteria and fungi. Not much of antibacterial and antifungal activity has been authenticated in the literature using docking studies. Hence, the present study focuses molecular docking techniques to explore the potential of phytoconstituents of *Aristolochia bracteolata* against various bacterial and fungal targets. Among phytoconstituents, seven were selected for this study such as Aristolochic acid I, Aristolochic acid II, Aporphine, Aristolactam, Beta sitosterol, Flavonoids and Protoberberines. Selected phytoconstituents were subjected to docking studies and compared against standard drug as ligand.

2. Materials and methods

2.1 Ligands preparation

The phytoconstituents of *Aristolochia bracteolata* such as Aristolochic acid I, Aristolochic acid II, Aporphine, Aristolactam, Beta sitosterol, Flavonoids, Protoberberines and anti-bacterial drugs such as Ampicillin, Ciprofloxacin, Sulfamethoxazole and Rifamycin and anti-fungal drugs such as Ketoconazole, Fenfuram and Methotrexate were selected as the ligand molecules. The selected ligand molecules were downloaded from the online PubChem database (https://pubchem.ncbi.nlm.nih.gov/) as 3D Structure in the format of SDF files. Some of the phytoconstituents were drawn using ChemSketch (ACD/ChemSketch FREEWARE) chemical drawing tool. The outputs of the structures from ChemSketch were saved in mol file (.mol) formats. The 2D structures were converted into 3D coordinates and geometries and converted into SDF files using Open Babel (OpenBabel2.3.1_Windows_Installer) (13).

2.2 Proteins preparation

The process of optimizing the protein structure and getting it ready for precise docking simulations is known as protein preparation (14). Ideally, the target structure should be determined experimentally by either X-ray crystallography or less frequently nuclear magnetic resonance, which can be downloaded from PDB (15,16) and the organism selected should be relevant to the studies conducted. Based on the literature review, Crystal 3D structures of Penicillin Binding Protein (17) (PDB ID: 1QME), 1,5 Anhydro D-fructose reductase (18) (PDB ID: 2GLX), Dihydrofolate reductase (19) (PDB ID: 3FL8), DNA dependent RNA polymerase (20) (PDB ID: 4XAX) were used for anti-bacterial activity and for anti-fungal activity, Sterol-14α demethylase (21) (PDB ID: 3KHM), Succinate dehydrogenase (22) (PDB ID: 6KU6), Dihydrofolate reductase (19) (PDB ID: 1KLK) were selected and downloaded from RCSB Protein Data Bank (https://www.rcsb.org/) in the PDB format and processed by removing ligand and water molecules attached to these enzymes to

avoid interfering with the docking study. Finally, 3D structures of the target proteins were saved and used for virtual screening and docking studies (13).

2.3 Molecular docking and Visualization

The molecular docking studies were carried out for all the phytoconstituents of *A. bracteolata* and the standards with different protein receptors for anti-bacterial and anti-fungal activity using AutoDock Vina implicated in PyRx. The conformation with the lowest binding energy (kcal/mol) of the protein-ligand complex was chosen as the best docking pose. Discovery Studio (BIOVIA Discovery Studio 2021) was used to investigate the interactions between ligands and protein (13).

3. Results and discussion

The computer-aided drug discovery approaches (such as in silico virtual screening approach), are faster and less expensive than in vitro high-throughput screening, play a vital role in dealing with such a vast number of compounds and also to assist the drug discovery process and validate the potential phytoconstituents of the medicinal herb that contributes to pharmacological activity with low toxicity and minimal side effects (1). In the present study, the in silico molecular docking analysis was used to identify the phytoconstituents of *A. bracteolata* for the effective potential antibacterial and antifungal effects against the selected multiple target proteins of bacterial and fungal infections (13).

3.1 Molecular docking study

The primary purpose of molecular docking is to determine how the ligands (both phytoconstituents and standards) connect with the biological target in concern. The AutoDock Vina technique has been utilized to conduct docking research on the suggested compounds, which resulted in the expected conclusions. The phytoconstituents (Aristolochic acid I, Aristolochic acid II, Aporphine, Aristolactam, Beta-sitosterol, Flavonoids and Protoberberines) and standards (Ampicillin, Ciprofloxacin, Sulfamethoxazole, Rifampicin, Ketoconazole, Fenfuram and Methotrexate) were docked into the grid box that have been constructed to encapsulate the active surface of the protein. Figure 1 & 2 represents a summary of the binding energies of the phytoconstituents and the standards for anti-bacterial and anti-fungal activity respectively. In accordance with the results for antibacterial activity in figure 1, the binding energies of the seven phytoconstituents with Penicillin binding protein (1QME), 1,5 Anhydro D-fructose reductase (2GLX), Dihydrofolate reductase (3FL8) and DNA dependent RNA polymerase (4XAX) were ranged from -9.5 to -8.2, -9.7 to -7.7, -10.1 to -7.6 and -8.3 to -7.1 kcal/mol respectively. Similarly for antifungal activity in figure 2, the binding affinities of the seven phytoconstituents of *A. bracteolata* with Sterol-14α demethylase (3KHM), Succinate dehydrogenase (6KU6) and Dihydrofolate reductase (1KLK) were ranged from -8.4 to -7.3, -10.0 to -8.9 and -9.9 to -8.1 kcal/mol respectively, based on the particular ligand.

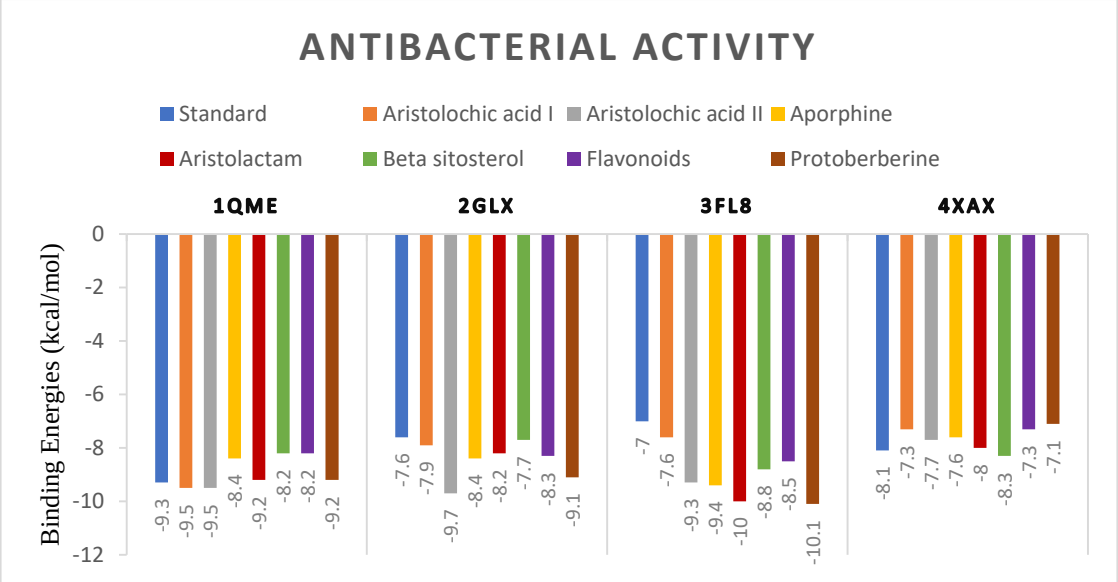

Figure 1. Vina score of molecular docking results of 7 phytoconstituents and standard for the four proteins against anti-bacterial activity.

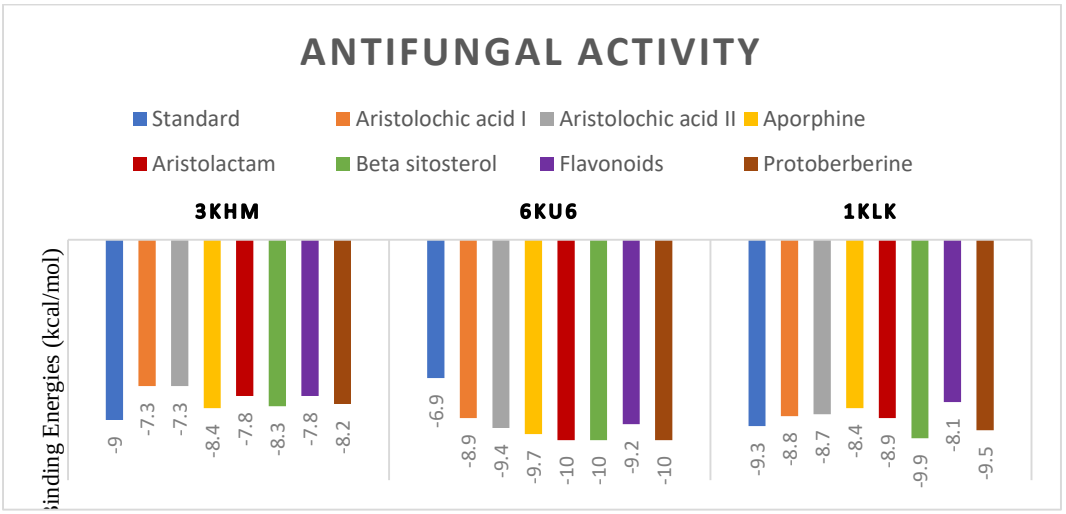

Figure 2. Vina score of molecular docking results of 7 phytoconstituents and standard for the three proteins against anti-fungal activity.

Based on the above findings, it was observed that all the seven phytoconstituents bind more effectively with the target proteins when compared with the standards (Ampicillin -9.3 kcal/mol, Ciprofloxacin -7.9 kcal/mol, Sulfamethoxazole -7.0 kcal/mol, Rifamycin -8.1 kcal/mol, Ketoconazole -9.0 kcal/mol, Fenfuram -6.9 kcal/mol and Methotrexate -9.3 kcal/mol). In the BIOVIA Discovery Studio Visualizer, the binding interaction concerning the active site of the target protein including amino acid residues have been observed.

3.1.1 Antibacterial activity

Analysis of the interaction of Penicillin binding protein (PDB: 1QME) with Ampicillin (std) and seven Phytoconstituents of *A. bracteolata*:

Penicillin-binding protein (PBP) in bacteria is responsible for the construction and maintenance of the bacterial cell wall (23). Penicillin-binding proteins (PBPs) are bacterial proteins (mainly transpeptidases and trans-glycosylases) that are essential in the synthesis of the peptidoglycan that forms cell walls of bacteria. For over 70 years in the modern system of medicine, β-lactam antibiotics are successfully used (24) and they target Penicillin-binding proteins (PBPs) (17). Hence, in this study, Penicillin-binding protein has been chosen as target protein to study the anti-bacterial effect of *A. bracteolata* and Ampicillin as standard ligand.

In the molecular docking study, the binding energies of standard (Ampicillin) and the phytoconstituents ranged from -9.3 kcal/mol and -9.5 to -8.2 kcal/mol respectively against PBP (1QME) as shown in the table 1. Among the selected seven phytoconstituents, Aristolochic acid I & II exhibited the least binding energy scores and hence identified as the more potent inhibitors than the Ampicillin and the other phytoconstituents.

In addition, the interactions of Penicillin binding protein with Ampicillin, Aristolochic acid I & II revealed that Ampicillin showed six conventional hydrogen bonds whereas Aristolochic acid I &II established nine & six conventional hydrogen bonds respectively with amino acid residues (table 1 & figure 10) assumed to boost its binding affinities as already discussed by Degfie *et.al* (25).

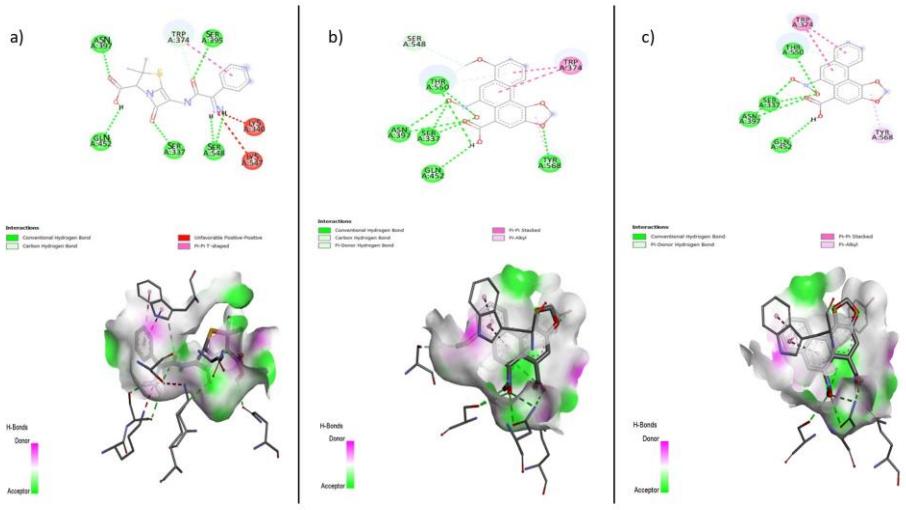

Figure 3. The 2D (Top) and 3D (Bottom) binding interactions of Ampicillin (a) and Aristolochic acid I (b) & II (c) from *A. bracteolata* against protein Penicillin Binding Protein.

Table 1. Summary of Molecular Docking studies – Seven phytoconstituents of *A. bracteolata* and standard with 1QME.

S.No.	Standard & Phytoconstituents	Binding affinity	No. of hydrogen bonds	Amino acid residues	Conventional hydrogen bond distance (A°)
1	Ampicillin (Std)	-9.3	6	SER A:337 SER A:395 ASN A:397 GLN A:452 SER A:548	2.99 2.93 2.84 2.48 2.17, 2.42
2	Aristolochic acid I	-9.5	9	SER A:337 ASN A:397 GLN A:452 THR A:550 TYR A:568	2.07, 3.03 3.08, 3.24, 3.33 2.77 2.74, 3.26 3.09
3	Aristolochic acid II	-9.5	6	SER A:337 ASN A:397 GLN A:452 THR A:550	2.91 3.18, 3.28, 3.30 2.57 3.30
4	Aporphine	-8.4	0	-	-
5	Aristolactam	-9.2	3	SER A:337 SER A:395 ASN A:397	2.53 2.16 3.12
6	Beta sitosterol	-8.2	1	GLN A:452	2.62
7	Flavonoids	-8.2	2	SER A:337	2.84, 3.14
8	Protoberberine	-9.2	0	-	-

Analysis of the interaction of 1,5-anhydro D-fructose reductase (PDB: 2GLX) with Ciprofloxacin (std) and seven Phytoconstituents of *A. bracteolata*:

1,5-Anhydro-D-fructose (AF) is a monosaccharide existing in bacteria, fungi, algae and plants. AF has antioxidant, antimicrobial and anti-inflammatory effects, whereas it is neither mutagenic to bacteria and mammalian cells nor toxic (18). In the modern system of medicine, fluoroquinone class of antibiotics are successfully used and they targeted 1,5-Anhydro-D-fructose reductase. Hence, in this study 1,5-Anhydro-D-fructose reductase has been chosen as target protein to study the antibacterial effect of *A. bracteolata* and Ciprofloxacin as standard ligand.

In Molecular docking study, the binding energies of standard (Ciprofloxacin) and the phytoconstituents ranged from -7.6 kcal/mol and -9.7 to -7.7 kcal/mol respectively against 1,5-Anhydro-D-fructose reductase (2GLX) as shown in the table 2. Among the selected seven

phytoconstituents, Aristolochic acid II exhibited the least binding energy scores and has been identified as the most potent inhibitor than the Ciprofloxacin and the other phytoconstituents.

In addition, the interaction of 1,5-anhydro D-fructose reductase with Ciprofloxacin and Aristolochic acid II revealed that Ciprofloxacin showed no conventional hydrogen bonds whereas Aristolochic acid II established five conventional hydrogen bonds with amino acid residues (table 2 & figure 11) assumed to boost its binding affinities.

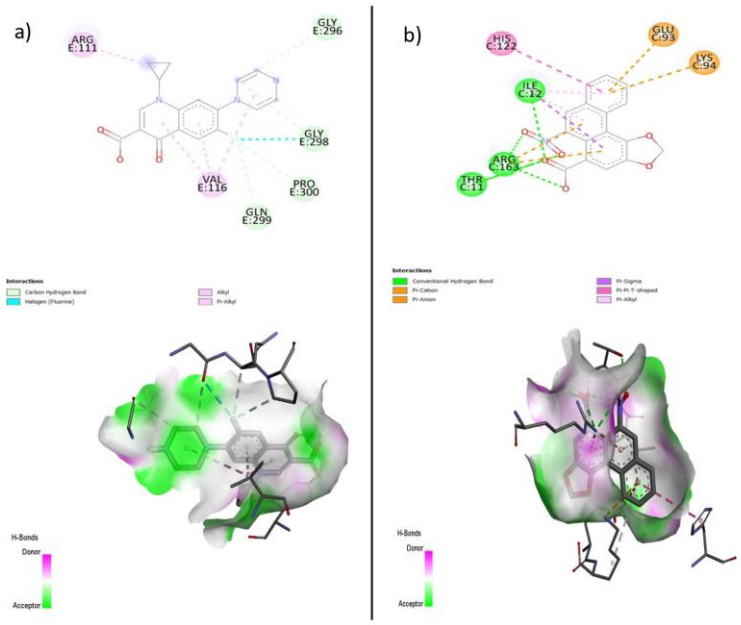

Figure 4. The 2D (Top) and 3D (Bottom) binding interactions of Ciprofloxacin (a) and Aristolochic acid II (b) from *A. bracteolata* against protein 1,5 Anhydro D-fructose reductase.

Table 2. Summary of Molecular Docking studies – Seven phytoconstituents of *A. bracteolata* and standard with 2GLX.

S.No.	Standard & Phytoconstituents	Binding affinity	No. of hydrogen bonds	Amino acid residues	Conventional hydrogen bond distance (A°)
1	Ciprofloxacin (Std)	-7.6	0	-	-
2	Aristolochic acid I	-7.9	4	ALA F:9 THR F:34 ARG F:38	2.97 2.99, 3.07 2.95
3	Aristolochic acid II	-9.7	5	THR C:11 ILE C:12 ARG C:163	2.70, 2.88 3.31 3.16, 3.29
4	Aporphine	-8.4	0	-	-

5	Aristolactam	-8.2	1	HIS C:180	2.65
6	Beta sitosterol	-7.7	0	-	-
7	Flavonoids	-8.3	4	LYS C:94 ARG C:163 GLN C:258 TYR C:283	3.19 3.31 3.11 3.04
8	Protoberberine	-9.1	0	-	-

Analysis of the interaction of Dihydrofolate reductase (PDB: 3FL8) with Sulfamethoxazole (std) and seven Phytoconstituents of *A. bracteolata*:

Dihydrofolate reductase (DHFR) is an important enzyme required to maintain bacterial growth. DHFR is an enzyme involved in the folic acid pathway, which reduces dihydrofolate to tetrahydrofolate, thereby promoting thymidylate biosynthesis, improving DNA translation, RNA transcription and protein replication and controlling cell proliferation. Inhibitors targeting DHFR may lead to bacterial death and be used to treat infection indicating that DHFR is a promising target for the treatment of bacterial infections (26). In the modern system of medicine, non-classical antifolates are successfully used and they targeted Dihydrofolate reductase (27). Hence in this study, Dihydrofolate reductase has been chosen as target protein to study the antibacterial effect of *A. bracteolata* and Sulfamethoxazole as standard ligand.

In Molecular docking, the binding energies of standard (Sulfamethoxazole) and the phytoconstituents ranged from -7.0 kcal/mol and -10.1 to -7.6 kcal/mol respectively against Dihydrofolate reductase (3FL8) as shown in the table 3. Among the selected seven phytoconstituents, Protoberberine exhibited the least binding energy scores and has been identified as the most potent inhibitor than the Sulfamethoxazole and the other phytoconstituents.

In addition, the interactions of Dihydrofolate reductase with Sulfamethoxazole and Protoberberine revealed that Sulfamethoxazole showed one conventional hydrogen bond whereas Protoberberine established two conventional hydrogen bonds with amino acid residues (table 3 & figure 12) assumed to boost its binding affinities.

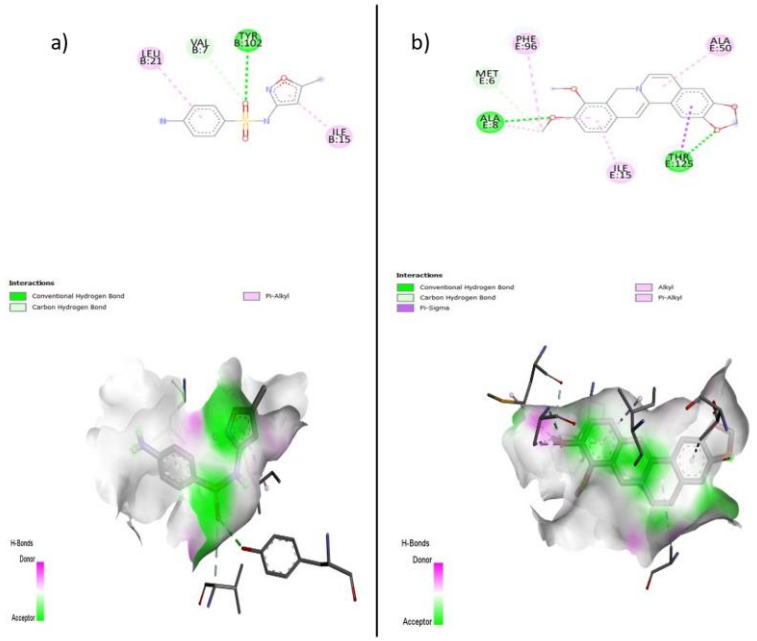

Figure 5. The 2D (Top) and 3D (Bottom) binding interactions of Sulfamethoxazole (a) and Protoberberine (b) from *A. bracteolata* against protein Dihydrofolate reductase.

Table 3. Summary of Molecular Docking studies – Seven phytoconstituents of *A. bracteolata* and standard with 3FL8.

S.No.	Standard & Phytoconstituents	Binding affinity	No. of hydrogen bonds	Amino acid residues	Conventional hydrogen bond distance (A°)
1	Sulfamethoxazole (Std)	-7.0	1	TYR B:102	2.95
2	Aristolochic acid I	-7.6	1	GLN G:30	3.29
3	Aristolochic acid II	-9.3	4	MET G:6 ALA G:8 PHE G:96 TYR G:102	3.06 2.85 2.48 1.88
4	Aporphine	-9.4	0	-	-
5	Aristolactam	-10	1	ASN B:47	2.62
6	Beta sitosterol	-8.8	1	ASP B:103	2.99
7	Flavonoids	-8.5	1	ASN B:47	3.18
8	Protoberberine	-10.1	2	ALA E:8 THR E:125	3.05 2.90

Analysis of the interaction of DNA dependent RNA polymerase (PDB: 4XAX) with Rifamycin and seven Phytoconstituents of *A. bracteolata*:

DNA-dependent RNA polymerase (RNAP) is the key enzyme of gene expression and a target of gene regulation. It is responsible for the synthesis of all RNAs in the cell using ribonucleoside triphosphates (NTPs) substrates (28). In the modern system of medicine, Ansamycin antibiotics are successfully used and they targeted DNA-dependent RNA polymerase. Hence in this study, DNA-dependent RNA polymerase has been chosen as target protein to study anti-bacterial effect of *A. bracteolata* and Rifamycin as standard ligand.

In Molecular docking study, the binding energies of standard (Rifamycin) and the phytoconstituents ranged from -8.1 kcal/mol and -8.3 to -7.1 kcal/mol respectively against DNA-dependent RNA polymerase (4XAX) as shown in the table 4. Among the selected seven phytoconstituents, Beta sitosterol exhibited the least binding energy scores and has been identified as the most potent inhibitor than the Rifamycin and the other phytoconstituents.

In addition, the interactions of DNA dependent RNA polymerase with Rifamycin and Beta sitosterol revealed that Rifamycin showed four conventional hydrogen bonds whereas Beta sitosterol established two conventional hydrogen bonds with amino acid residues (table 4 & figure 13) assumed to boost its binding affinities.

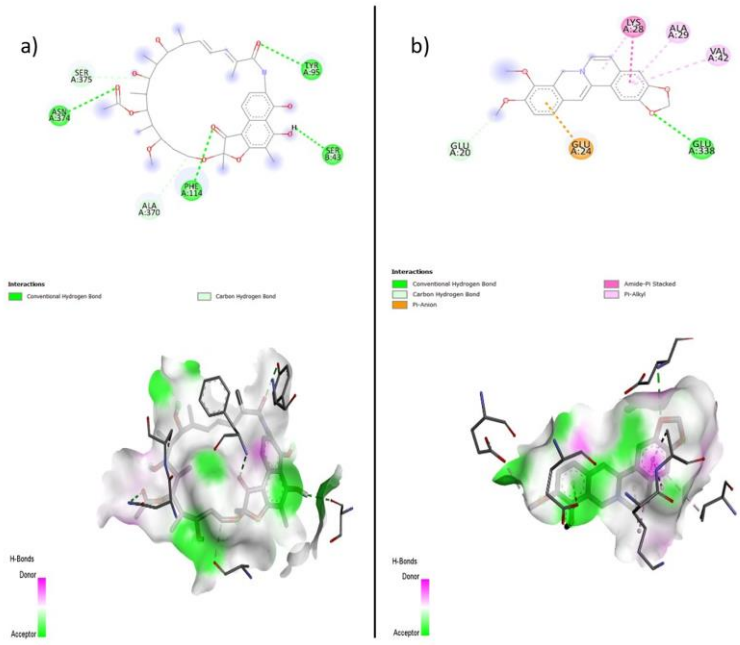

Figure 6. The 2D (Top) and 3D (Bottom) binding interactions of Rifamycin (a) and Beta sitosterol (b) from *A. bracteolata* against protein DNA dependent RNA polymerase.

Table 4. Summary of Molecular Docking studies – Seven phytoconstituents of *A. bracteolata* and standard with 4XAX.

S.No.	Standard & Phytoconstituents	Binding affinity	No. of hydrogen bonds	Amino acid residues	Conventional hydrogen bond distance (A°)
1	Rifamycin (Std)	-8.1	4	THR A:95 PHE A:114 ASN A:374 SER B:43	3.27 3.15 3.36 2.52
2	Aristolochic acid I	-7.3	2	TYR A:90 GLU A:123	2.92 2.10
3	Aristolochic acid II	-7.7	5	GLN A:91 GLU A:379 ARG A:383	2.82, 3.07 2.45, 3.06 3.25
4	Aporphine	-7.6	0	-	-
5	Aristolactam	-8.0	1	LEU A:383	2.77
6	Beta sitosterol	-8.3	2	ILE A:73 ASP A:75	2.38 2.12
7	Flavonoids	-7.3	0	-	-
8	Protoberberine	-7.1	1	GLU A:338	3.29

3.1.2 Antifungal activity:

Analysis of the interaction of Sterol-14α demethylase (PDB: 3KHM) with Ketoconazole and seven Phytoconstituents of *A. bracteolata*:

The enzyme 14-α demethylase (CYP51), located in the fungal endoplasmic reticulum, plays a crucial role in the ergosterol biosynthetic pathway. It catalyses the C-14 demethylation of lanosterol into 4,4-dimethylcholesta-8,14,24-trienol which is pivotal for ergosterol biosynthesis. Accumulated lanosterol is converted into other toxic sterols. The accumulation of toxic sterols and the absence of ergosterol results in the destabilization and disruption of the fungal plasma membrane (29). In the modern system of medicine, azole antifungal agents are successfully used and they targeted Sterol-14α demethylase. Hence in this study, Sterol-14α demethylase has been chosen as target protein to study the antifungal effect of *A. bracteolata* and Ketoconazole as standard ligand.

In Molecular docking study, the binding energies of standard (Ketoconazole) and the phytoconstituents ranged from -9.0 kcal/mol and -8.4 to -7.3 kcal/mol respectively against Sterol-14α demethylase (3KHM) as shown in the table 5. Among the selected seven phytoconstituents, Aporphine exhibited the least binding energy scores and has been identified as the most potent inhibitor than the Ketoconazole and the other phytoconstituents.

In addition, the interaction of Sterol-14α demethylase with Ketoconazole and Aporphine revealed that both were found to form no conventional hydrogen bond with amino acid residues (table 5 & figure 14).

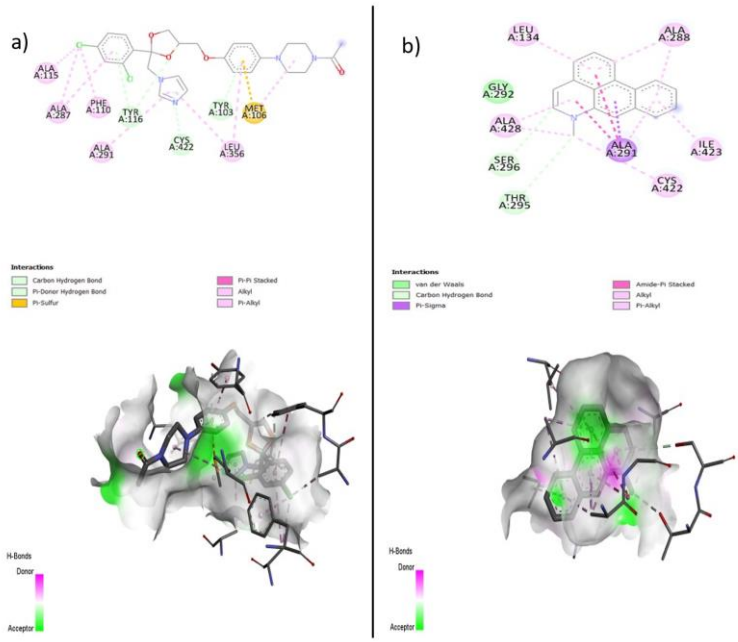

Figure 7. The 2D (Top) and 3D (Bottom) binding interactions of Ketoconazole (a) and Aporphine (b) from *A. bracteolata* against protein Sterol-14α demethylase.

Table 5. Summary of Molecular Docking studies – Seven phytoconstituents of *A. bracteolata* and standard with 3KHM.

S.No.	Standard & Phytoconstituents	Binding affinity	No. of hydrogen bonds	Amino acid residues	Conventional hydrogen bond distance (A°)
1	Ketoconazole (Std)	-9.0	0	-	-
2	Aristolochic acid I	-7.3	4	HIS A:306 ASN A:312 TRP A:400 VAL A:452	3.25 3.04 2.81 3.21
3	Aristolochic acid II	-7.3	5	GLU A:112 ARG A:233 HIS A:279	2.19 2.99, 3.25 2.78, 3.12
4	Aporphine	-8.4	0	-	-

5	Aristolactam	-7.8	1	LEU A:208	1.79
6	Beta sitosterol	-8.3	1	LEU A:208	1.95
7	Flavonoids	-7.8	0	-	-
8	Protoberberine	-8.2	1	GLY A:463	3.19

Analysis of the interaction of Succinate dehydrogenase (PDB: 6KU6) with Fenfuram and seven Phytoconstituents of *A. bracteolata*:

Succinate dehydrogenase (SDH), also called complex II, succinate-ubiquinone oxidoreductase, is the only enzyme complex that simultaneously participates in the tricarboxylic acid (TCA) cycle and electron transport chain, which plays an indispensable role in inner mitochondrial metabolism. SDH catalyses the oxidation of succinate to fumarate and transfers electrons to ubiquinone. Succinate dehydrogenase inhibitors (SDHIs) are a new class of fungicides, which can inhibit fungi electron transfer from succinate to ubiquinone and block energy metabolism, eventually lead to the death of pathogenic fungi. SDHIs, as a rapidly developing amide fungicides, have structurally-unique, high-efficiency, and environmentally-friendly fungicides (30). Hence in this study, Succinate dehydrogenase has been chosen as target protein to study the antifungal effect of *A. bracteolatea* and Fenfuram as standard ligand.

In Molecular docking study, the binding energies of standard (Fenfuram) and the phytoconstituents ranged from 6.9 kcal/mol and -10.0 to -8.9 kcal/mol respectively against (6KU6) as shown in the table 6. Among the selected seven phytoconstituents, Aristolactam, Protoberberine and Beta sitosterol were exhibited the least binding energies scores and have been identified as the most potent inhibitors than the Fenfuram as standard ligand.

In addition, the interaction of Succinate dehydrogenase with Fenfuram, Aristolactam, Protoberberine and beta sitosterol revealed that Fenfuram shown two conventional hydrogen bonds whereas Aristolactam Protoberberine and Beta sitosterol established two, one and no conventional hydrogen bonds with amino acid residues (table 6 & figure 15) assumed to boost its binding affinities.

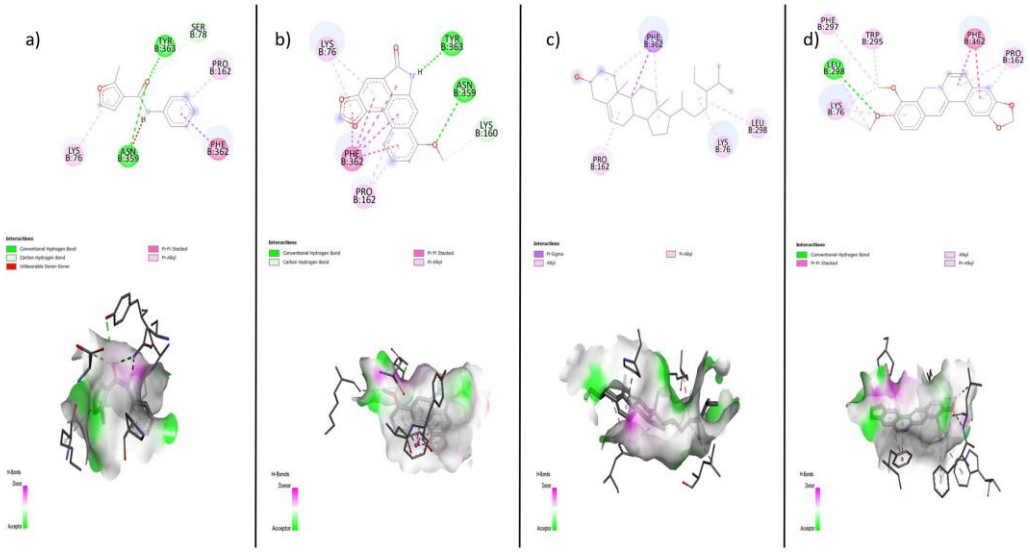

Figure 8. The 2D (Top) and 3D (Bottom) binding interactions of Fenfuram (a) and Aristolactam (b), Beta sitosterol (c) and Protoberberine (d) from A. bracteolata against protein Succinate dehydrogenase.

Table 6. Summary of Molecular Docking studies – Seven phytoconstituents of *A. bracteolata* and standard with 6KU6.

S.No.	Standard & Phytoconstituents	Binding affinity	No. of hydrogen bonds	Amino acid residues	Conventional hydrogen bond distance (A°)
1	Fenfuram (Std)	-6.9	2	ASN B:359 TYR B:363	3.25 3.16
2	Aristolochic acid I	-8.9	1	TYR H:363	3.17
3	Aristolochic acid II	-9.4	1	ASN H:359	3.11
4	Aporphine	-9.7	0	-	-
5	Aristolactam	-10.0	2	ASN B:359 TYR B:363	3.11 2.40
6	Beta sitosterol	-10.0	0	-	-
7	Flavonoids	-9.2	2	ASN B:359 TYR B:363	2.94 3.07
8	Protoberberine	-10.0	1	LEU B:298	3.21

Analysis of the interaction of Dihydrofolate reductase (PDB: 1KLK) with Methotrexate and seven Phytoconstituents of *A. bracteolata:*

Dihydrofolate reductase (DHFR) is a member of the reductase enzyme family, which is ubiquitously expressed in all organisms. Dihydrofolate reductase (DHFR) catalyses the

reduction of dihydrofolate to tetrahydrofolate (THF). THF is needed for the action of folate-dependent enzymes and is thus essential for DNA synthesis and methylation (31). In the modern system of medicine, non-classical antifolates are successfully used and they targeted Dihydrofolate reductase (27). Hence in this study, Dihydrofolate reductase has been chosen as target protein to study the antifungal effect of *A. bracteolata* and Methotrexate as standard ligand (32).

In Molecular docking study, the binding energies of standard (Methotrexate) and the phytoconstituents ranged from -9.3 kcal/mol and -9.9 to -8.1 kcal/mol respectively against 1KLK as shown in the table 7. Among the selected seven phytoconstituents, Beta sitosterol exhibited the least binding energy scores and has been identified as the most potent inhibitors than the Methotrexate and the other phytoconstituents.

In addition, the interactions of Dihydrofolate reductase with Methotrexate and Beta sitosterol revealed that Methotrexate showed eight conventional hydrogen bonds whereas beta sitosterol established no conventional hydrogen bonds with amino acid residues (table 7 & figure 16) assumed to boost its binding affinities.

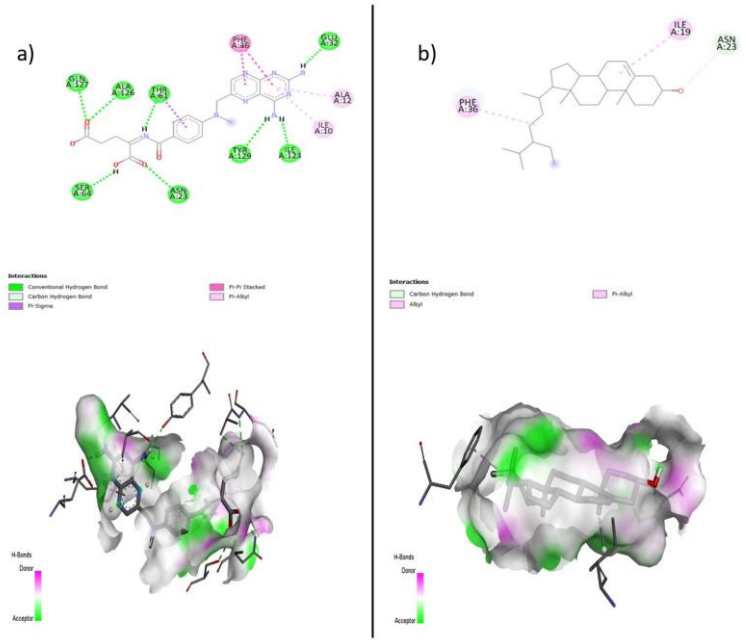

Figure 9. The 2D (Top) and 3D (Bottom) binding interactions of Methotrexate (a) and Beta sitosterol (b) from A. bracteolata against protein Dihydrofolate reductase.

Table 7: Summary of Molecular Docking studies – Seven phytoconstituents of *A. bracteolata* and standard with 1KLK.

S.No.	Standard & Phytoconstituents	Binding affinity	No. of hydrogen bonds	Amino acid residues	Conventional hydrogen bond distance (A°)
1	Methotrexate (Std)	-9.3	8	ASN A:23 GLU A:32 SER A:64 THR A:61 ILE A:123 ALA A:126 GLN A:127 TYR A:129	2.69 1.84 2.74 2.11 1.98 3.14 3.04 2.32
2	Aristolochic acid I	-8.8	5	ILE A:10 ALA A:12 TYR A:129	2.65 3.15, 3.29 1.85, 2.75
3	Aristolochic acid II	-8.7	4	ALA A:12 ILE A:123 TYR A:129	2.99 2.05 2.76, 2.83
4	Aporphine	-8.4	0	-	-
5	Aristolactam	-8.9	1	ALA A:12	3.19
6	Beta sitosterol	-9.9	0	-	-
7	Flavonoids	-8.1	1	ALA A:12	3.26
8	Protoberberine	-9.5	1	ASN A:23	3.07

4. Conclusion

Aristolochia bracteolata is a medicinal plant that possesses a treasure of active phytoconstituents of biological importance. The present study highlights the potential bacterial and fungal inhibitory action of phytoconstituents of *Aristolochia bracteolata such as* Aristolochic acid I, Aristolochic acid II, Aporphine, Aristolactam, Beta sitosterol, Flavonoids and Protoberberine against the selective target proteins by molecular docking study and hence considered as the promising candidates against microbial infections. Future studies, including in vitro and in vivo investigations are essential to fully explore the therapeutic efficacy, safety profiles and mechanism of action of these bioactive compounds. Ultimately, *Aristolochia bracteolata* could contribute to the development of novel, plant-based antimicrobial agents address the growing challenge of microbial resistance.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. All research and findings presented in this study are based solely on

scientific data and no financial or personal relationship have influenced the results or conclusions drawn.

Acknowledgement

Not applicable.

5. Reference

[1] Igwe OU, Ejezie MU, Otuokere IE. CHEMICAL COMPOSITION, ANTIMICROBIAL AND DOCKING STUDIES OF CHLOROFORM EXTRACTS OF Piliostigma thonningii LEAVES AND SEEDS. J Chem Soc Niger [Internet]. 2025 Feb 23 [cited 2025 Mar 19];50(1). Available from: https://journals.chemsociety.org.ng/index.php/jcsn/article/view/1046

[2] Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, et al. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep. 2023 Aug 17;13(1):13398.

[3] Theodore CE, Sivaiah G, Prasad SBB, Kumar KY, Raghu MS, Alharethy F, et al. Synthesis, antimicrobial activity and molecular docking studies of novel hydantoin derivatives as potential phospholipase A2 inhibitors. Chem Phys Impact. 2023 Dec;7:100319.

[4] Sarma SK, Dutta U, Das BK, Bharali A, Laloo D, Kalita JM, et al. Evaluation of Lakadong turmeric derived curcumin nanogel against resistant biofilms: In-silico, antibacterial and antibiofilm analysis. J Appl Pharm Sci [Internet]. 2024 [cited 2025 Mar 20]; Available from: https://japsonline.com/abstract.php?article_id=4332&sts=2

[5] Anza M, Endale M, Cardona L, Cortes D, Eswaramoorthy R, Zueco J, et al. Antimicrobial Activity, in silico Molecular Docking, ADMET and DFT Analysis of Secondary Metabolites from Roots of Three Ethiopian Medicinal Plants. Adv Appl Bioinforma Chem. 2021 Aug;Volume 14:117–32.

[6] Mustapha A, AlSharksi A, Eze U, Samaila R, Ukwah B, Anyiam A, et al. Phytochemical Composition, In Silico Molecular Docking Analysis and Antibacterial Activity of Lawsonia inermis Linn Leaves Extracts against Extended Spectrum Beta-Lactamases-Producing Strains of Klebsiella pneumoniae. BioMed. 2024 Aug 26;4(3):277–92.

[7] Salehzadeh J, Kheirjou M. Monitoring, antimicrobial activity evaluation, and molecular docking studies of chemical composition of various plant species to identify an inhibitory drug for Clostridium perfringens bacteria isolated from drinking water resources in Ardabil province. Results Chem. 2024 Aug;10:101721.

[8] Zouine N, Ghachtouli NE, Abed SE, Koraichi SI. A comprehensive review on medicinal plant extracts as antibacterial agents: Factors, mechanism insights and future prospects. Sci Afr. 2024 Dec;26:e02395.

[9] Khanum A, Bibi Y, Khan I, Mustafa G, Attia KA, Mohammed AA, et al. Molecular docking of bioactive compounds extracted and purified from selected medicinal plant species against covid-19 proteins and in vitro evaluation. Sci Rep. 2024 Feb 14;14(1):3736.

[10] Mir WR, Bhat BA, Rather MA, Muzamil S, Almilaibary A, Alkhanani M, et al. Molecular docking analysis and evaluation of the antimicrobial properties of the constituents of Geranium wallichianum D. Don ex Sweet from Kashmir Himalaya. Sci Rep. 2022 Jul 22;12(1):12547.

[11] Shirwaikar A, SOMASHEKAR A. Antiinflammatory activity and free radical scavenging studies of Aristolochia bracteolata Lam. Indian journal of pharmaceutical sciences. 2003;65(1):67-9.

[12] Nandhini DU, Rajasekar M, Venmathi T, Koopen AW. A review on worm killer: Aristolochia bracteolata. J Pharmacogn Phytochem. 2017;6(2):6-9.

[13] Gowtham HG, Murali M, Singh SB, Shivamallu C, Pradeep S, Shivakumar CS, et al. Phytoconstituents of Withania somnifera unveiled Ashwagandhanolide as a potential drug targeting breast cancer: Investigations through computational, molecular docking and conceptual DFT studies. Tiwari AK, editor. PLOS ONE. 2022 Oct 6;17(10):e0275432.

[14] Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, et al. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep. 2023 Aug 17;13(1):13398.

[15] Raval K, Ganatra T. Basics, types and applications of molecular docking: A review. IP Int J Compr Adv Pharmacol. 2022 Mar 28;7(1):12–6.

[16] Hernndez-Santoyo A, Yair A, Altuzar V, Vivanco-Cid H, Mendoza-Barrer C. Protein-Protein and Protein-Ligand Docking. In: Ogawa T, editor. Protein Engineering - Technology and Application [Internet]. InTech; 2013 [cited 2025 Apr 1]. Available from: http://www.intechopen.com/books/protein-engineering-technology-and-application/protein-protein-and-protein-ligand-docking

[17] Chauhan A, Sharma AD. Unlocking the therapeutic potential of phytocompounds from essential oils of Melaleuca alternifolia and Rosmarinus officinalis targeting bacterial penicillin-binding proteins by computational and experimental validation. Discov Chem. 2025 Mar 5;2(1):28.

[18] Hara A, Nishinaka T, Abe N, El-Kabbani O, Matsunaga T, Endo S. Dimeric dihydrodiol dehydrogenase is an efficient primate 1,5-anhydro-D-fructose reductase. Biochem Biophys Res Commun. 2020 Jun;526(3):728–32.

[19] Aljohny BO, Rauf A, Anwar Y, Naz S, Wadood A. Antibacterial, Antifungal, Antioxidant, and Docking Studies of Potential Dinaphthodiospyrols from *Diospyros lotus* Linn Roots. ACS Omega. 2021 Mar 2;6(8):5878–85.

[20] Bheemireddy S, Sowdhamini R, Srinivasan N. Computational analysis of the effect of a binding protein (RbpA) on the dynamics of Mycobacterium tuberculosis RNA polymerase assembly. De March M, editor. PLOS ONE. 2025 Jan 30;20(1):e0317187.

[21] Saqallah FG, Hamed WM, Talib WH, Dianita R, Wahab HA. Antimicrobial activity and molecular docking screening of bioactive components of Antirrhinum majus (snapdragon) aerial parts. Heliyon. 2022 Aug;8(8):e10391.

[22] Yang M, Lin G, Duan W, Li F, Cui Y, Man Y, et al. Synthesis, antifungal activity, and molecular simulation study of nootkatone-based thiazole-hydrazone compounds as novel succinate dehydrogenase inhibitor. Pest Manag Sci. 2025 Jan 21;ps.8669.

[23] Gordon E, Mouz N, Duée E, Dideberg O. The crystal structure of the penicillin-binding protein 2x from Streptococcus pneumoniae and its acyl-enzyme form: implication in drug resistance 1 1Edited by R. Huber. J Mol Biol. 2000 Jun;299(2):477–85.

[24] Lai CKC, Ng RWY, Leung SSY, Hui M, Ip M. Overcoming the rising incidence and evolving mechanisms of antibiotic resistance by novel drug delivery approaches – An overview. Adv Drug Deliv Rev. 2022 Feb;181:114078.

[25] Degfie T, Endale M, Tafese T, Dekebo A, Shenkute K. In vitro antibacterial, antioxidant activities, molecular docking, and ADMET analysis of phytochemicals from roots of Hydnora johannis. Appl Biol Chem. 2022 Dec;65(1):76.

[26] He J, Qiao W, An Q, Yang T, Luo Y. Dihydrofolate reductase inhibitors for use as antimicrobial agents. Eur J Med Chem. 2020 Jun;195:112268.

[27] Wróbel A, Arciszewska K, Maliszewski D, Drozdowska D. Trimethoprim and other nonclassical antifolates an excellent template for searching modifications of dihydrofolate reductase enzyme inhibitors. J Antibiot (Tokyo). 2020 Jan;73(1):5–27.

[28] Lee J, Borukhov S. Bacterial RNA Polymerase-DNA Interaction—The Driving Force of Gene Expression and the Target for Drug Action. Front Mol Biosci [Internet]. 2016 Nov 9 [cited 2025 Apr 3];3. Available from: http://journal.frontiersin.org/article/10.3389/fmolb.2016.00073/full

[29] Vanreppelen G, Nysten J, Baldewijns S, Sillen M, Donders G, Van Dijck P. Oteseconazole (VIVOJA) for prevention of recurrent vulvovaginal candidiasis. Trends Pharmacol Sci. 2023 Jan;44(1):64–5.

[30] Zhang H, Ye Z, Yang S, Zhang M, Xia Y, Liu Z, et al. Succinate dehydrogenase governs the sensitivity to SDHI fungicides and fruit pathogenicity of Colletotrichum gloeosporioides. J Agric Food Res. 2025 Mar;19:101587.

[31] Askari BS, Krajinovic M. Dihydrofolate reductase gene variations in susceptibility to disease and treatment outcomes. Curr Genomics. 2010 Dec;11(8):578–83.

[32] Yang J, Gao L, Yu P, Kosgey JC, Jia L, Fang Y, et al. In vitro synergy of azole antifungals and methotrexate against Candida albicans. Life Sci. 2019 Oct;235:116827.