

BREAKING THE BIOFILM: *Pouzolzia zeylanica* vs. MULTIDRUG-RESISTANT *Candida auris*

Maitrayee Dutta Swargiary* and Pekir Ete

Department of Bio-Sciences, Assam Don Bosco University, Tapesia Gardens, Sonapur 782402, India

*For correspondence. (maitrayee.swargiary@dbuniversity.ac.in)

Abstract: *Candida auris* is a multidrug-resistant fungal pathogen that poses serious global health challenges due to high mortality and rapid spread. Its resistance to conventional antifungals necessitates the search for novel therapies. In this study, an *in silico* drug design approach was used to evaluate bioactive compounds from *Pouzolzia zeylanica* (L.) Benn, a medicinal plant with diverse therapeutic properties. The target enzyme 14-alpha-demethylase, critical for ergosterol biosynthesis, was modelled using Swiss Model, Phyre2, and GalaxyWeb servers, with refinement and validation. Ligands were screened with SwissADME and Drulito for drug-likeness, followed by docking in AutoDock Vina. Compounds showed strong binding affinities of -8.2 and -8.4 kcal/mol, suggesting potential antifungal activity that warrants further experimental validation.

Keywords: *Pouzolzia zeylanica* (L.) Benn; ergosterol; *in silico*; Autodock vina; Homology modeling

1. Introduction:

Candida auris is a newly-emerging multidrug-resistant (MDR) ascomycetous yeast that has emerged as a major threat to global health due to its rapid spread and persistent lifestyle in hospitals. *C. auris* was first isolated in 2009, from the external ear canal of an admitted patient in Japan [1,2], and then from chronic otitis media patients in South Korea [3], where earlier bloodstream isolates were retrospectively identified in 1996, in South Korea and in 2008, in Pakistan. Since its appearance, this opportunistic pathogen is present in more than 40 countries, with invasive clinical situations, especially in immunocompromised patients, and is linked to high morbidity and mortality rates between 30% and 60% [4,5]. *C. auris* is of clinical interest due to its special features: the ability to adhere to surfaces, to form biofilms, to survive on substrates for weeks, and to spread nosocomial bacteria [6,7]. Clinically, *C. auris* colonizes skin and can travel in health care facilities, leading to hospital outbreaks difficult to manage because the organism is resistant to commonly used disinfectants and has no effective decolonization strategy. Furthermore, clonal transmission within and between healthcare institutions has been documented in areas such as India, South Africa and Korea [8].

Treating *C. auris* is extremely limited because it has developed resistance to several classes of antifungal drugs, including azoles, echinocandins and polyenes such as amphotericin B [9,10]. To complicate things even further, some strains are resistant to all three major classes. Moreover, the presence of biofilms aggravates drug resistance, with biofilms increasing tolerance to antifungal agents and overcoming host immune defense [11]. In *C. auris*, the formation of a biofilm has been linked to the upregulation of efflux pump genes such as MDR1 and CDR1 (Candida Drug Resistance 1) and the creation of an extracellular matrix of polysaccharides, proteins and extracellular DNA [12,8]. These features highlight the critical need for new antifungal agent that are effective

against *C. auris* biofilms. While biofilm formation in *Candida* species is frequently linked to the upregulation of efflux pump genes like MDR1 and CDR1, which contribute to multidrug resistance, this study emphasizes 14- α -demethylase (CYP51) due to its pivotal role in ergosterol biosynthesis, a pathway crucial for maintaining fungal cell membrane integrity. Inhibiting CYP51 disrupts membrane function and viability, establishing it as a validated antifungal target. Furthermore, CYP51 inhibitors have demonstrated efficacy against biofilm-associated fungal cells, indicating a broader therapeutic potential beyond merely modulating efflux pumps [13].

Due to increasing resistance to chemical drugs, there is a general interest in the search for plant-derived bioactive compounds with anti-fungal activity. *Pouzolzia zeylanica* (L.) Benn., a medicinal plant belonging to Urticaceae family, has been widely used in China, India and Southeast Asia for the treatment of inflammatory and infectious diseases [14,15]. Extracts of *P. zeylanica* show known pharmacological activities of anti-inflammatory, antibacterial, antioxidant, and anti-snake venom and are a rich source of bioactive constituents including alkaloids, glycosides, phenolics, and flavonoids [16].

In this study, molecular docking approach by AutoDock Vina was used to evaluate the inhibitory potential of selected bioactive compounds of *P. zeylanica* against a biofilm-related important target protein of *Candida auris*. These results provide useful information on the potential antifungal mechanism of action of these compounds and justify further studies on phytotherapeutic strategies for the treatment of multidrug-resistant fungal infections.

2. Materials and methods:

2.1. Selection of Plant and Organism

The plant *Pouzolzia zeylanica* (L.) Benn and the fungal pathogen *Candida auris* were chosen through literature survey for the investigation.

2.2. Retrieval and Modeling of Target Protein

The amino acid sequence of 14- α -demethylase (CYP51) was retrieved from UniProt database (<https://www.uniprot.org/>) and modelled using Swiss Model (<https://swissmodel.expasy.org>), Phyre2, and GalaxyWeb. Swiss Model selected SMTL ID: 5tz.1 (73.63% identity, GMQE 0.82, QMEAN 0.83). Three distinct protein models—Swiss-Model, Phyre2, and GalaxyWeb, substantially enhances the dependability and transparency of molecular docking studies, so three predicted protein models were used. GalaxyWeb refinement provided the best structure with MolProbity 1.593 and RMSD 0.9858. Validation was done with SAVES v6.0 Ramachandran plot analysis.

2.3. Protein Preparation

BIOVIA Discovery Studio (<https://3ds.com/discovery-studio-visualizer-download>) was used to prepare the protein by adding polar hydrogens, charges, and removing unwanted ligands and residues.

2.4. Binding Site Prediction

PrankWeb (<https://prankweb.cz>) predicted the active binding sites of the target protein to assist in receptor-ligand interaction analysis.

2.5. Retrieval and Screening of Ligands

Bioactive compounds from *P. zeylanica* were collected from PubChem (<https://pubchem.ncbi.nlm.nih.gov>) in SDF format. Screening was performed with SwissADME (<https://www.swissadme.ch/>) and Drulito to check ADMET properties and oral drug-likeness, following Lipinski's Rule of 5.

2.6. Ligand Preparation

Ligands were structurally optimized by adding polar hydrogens, charges, and saved as PDBQT files for docking.

2.7. Molecular Docking and Interaction Analysis

Docking was conducted in AutoDock Vina. Binding affinities were measured by Gibbs free energy scores. PyMOL and Discovery Studio visualized hydrogen bonds, hydrophobic interactions, and distances to identify the most stable ligand conformations.

3. Results:

3.1. Protein validation

After retrieving the 3D structure protein model for 14-alpha-demethylase from three different homology modelling sites, protein validation analysis showed "pass" in SAVES v6.0.0 and verified by the VERIFY3D tool (Table 1 and Figure 1)

Table 1: Validation analysis of the 3D structure of three model

Swiss model protein	95 per cent of the residues have an averaged 3D-1D score ≥ 0.2	Ramachandran plot show 90 per cent of the residues in the allowed region figure 1(a)
Phyre2 model protein	82.25 per cent of the residues have averaged 3D-1D score ≥ 0.2	Ramachandran plot show 90.9 per cent of the residues in the allowed region Figure 1(b)
Galaxyweb model protein	93.70 per cent of the residues have averaged 3D-1D score ≥ 0.2	Ramachandran plot show 93.1 per cent of the residues in the allowed region Figure 1(c)

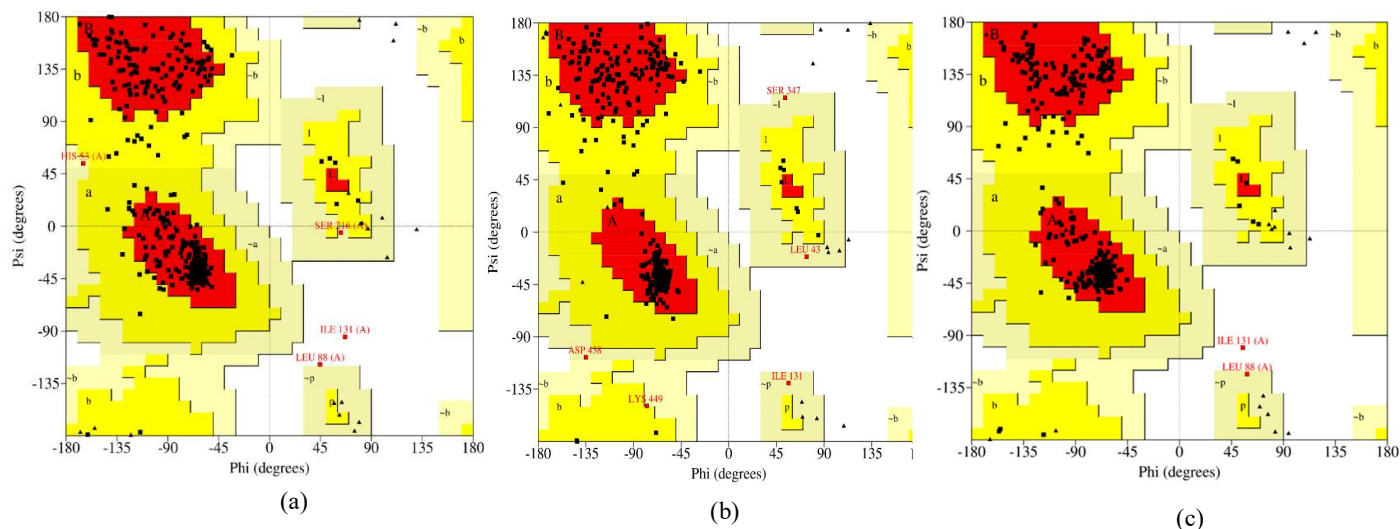
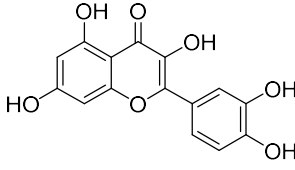
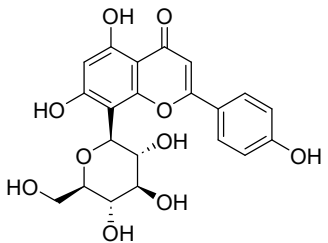


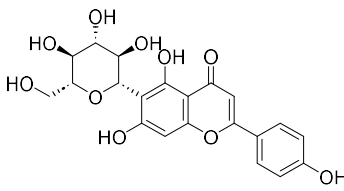
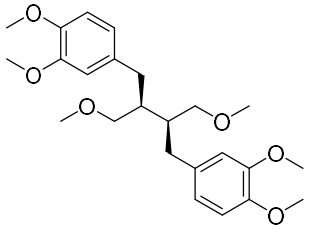
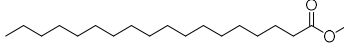
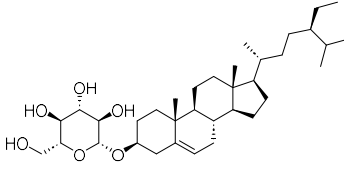
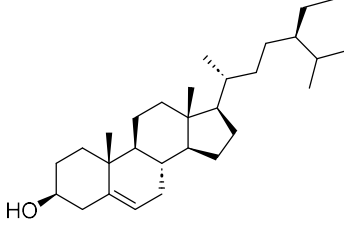
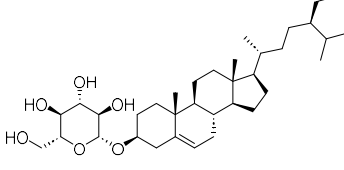
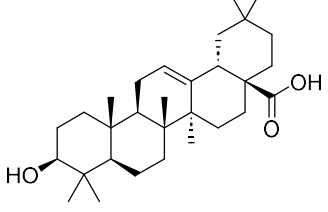
Fig. 1 (a, b, c) Shows the Ramachandran plot of the three-model protein structure

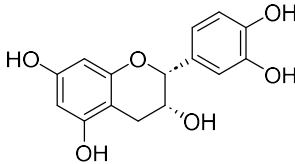
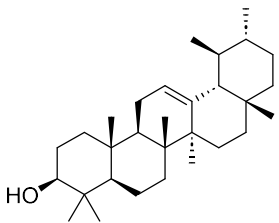
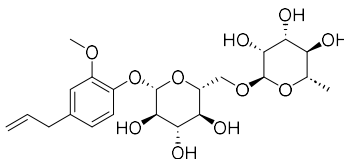
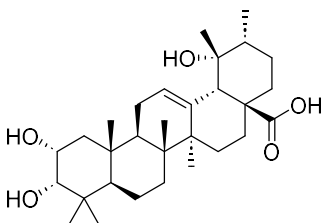
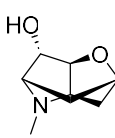
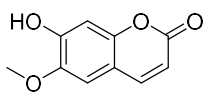
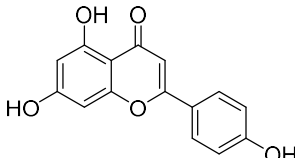
3.2. Ligand retrieval and ADMET properties

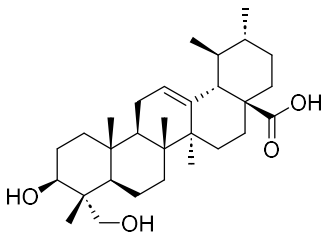
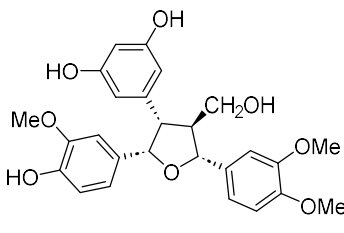
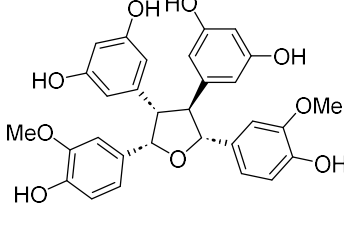
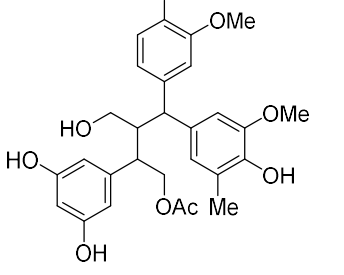
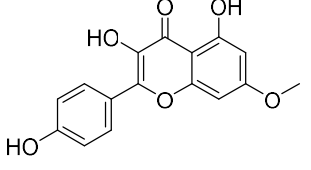
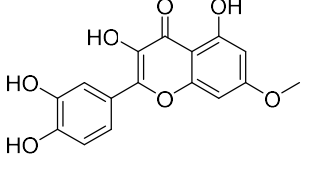
Phytochemicals of *Pouzolzia zeylanica* (L) Benn, which has antibacterial, antifungal, antioxidant, antiallergic and cytotoxic properties were retrieved (Table 2). Out of 40 compounds, 18 ligands passed the ADMET screening (Table 3).

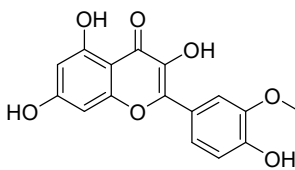
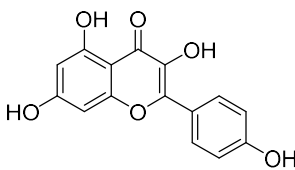
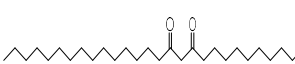
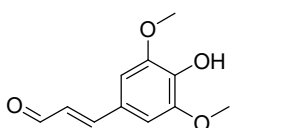
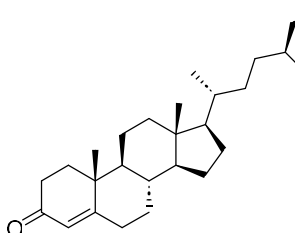
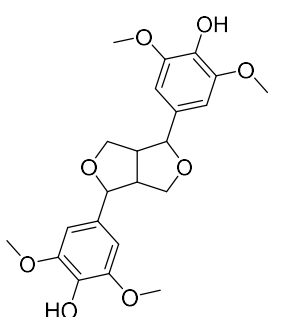
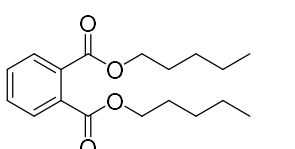
Table 2: Phytochemicals of *Pouzolzia zeylanica*

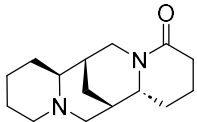
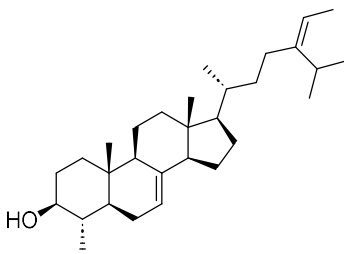
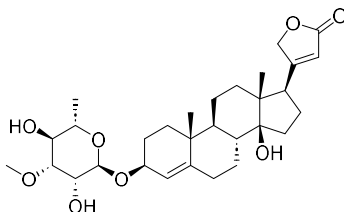
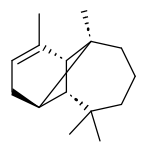
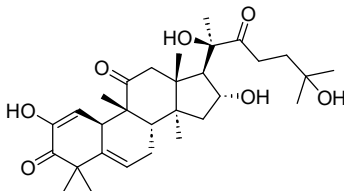
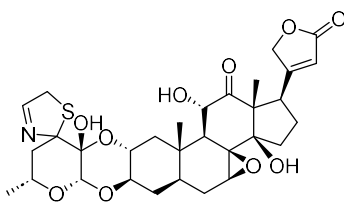
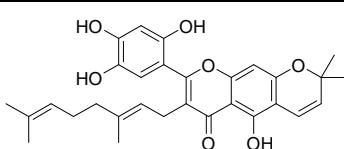
SL NO.	PubChem CID	Common Name	Structure
1	5280343	Quercetin	
2	5280441	Vitexin	

3	162350	Isovitexin	
4	358901	Phyllanthin	
5	8201	Methyl Stearate	
6	12309057	Beta-Sitosterol-3-O-Beta-D-Glucopyranoside	
7	222284	Beta-Sitosterol	
8	5742590	Daucosterol	
9	10494	Oleanolic Acid	

10	72276	Epicatechin	
11	73170	alpha-Amyrin	
12	15101911	Eugenyl beta-rutinoside	
13	73645	2alpha,3alpha,19alpha-Trihydroxyurs-12en-28-Oic	
14	261184	Scopoline	
15	5280460	Scopoletin	
16	5280443	Apigenin	

17	6918774	alpha- Hydroxyursolic	
18	132522240	Pouzolignan D	
19	132522241	Pouzolignan K	
20	132522243	Pouzolignan M	
21	5320946	Rhamnocitrin	
22	5281691	Rhamnetin	

23	5281654	Isorhamnetin	
24	5280863	Kaempferol	
25	441489	14,16-Hentriacontanedione	
26	5280802	Sinapaldehyde	
27	5484202	Stigmast-4-En-3-One	
28	100067	Syringaresinol	
29	21612477	Dipentylphthalate	

30	91471	Lupanine	
31	9548595	24-Ethylidenelophenol	
32	15558288	Acofrioside L	
33	520957	Alpha-Longipinene	
34	441820	Cucurbitacin L	
35	441862	Labriformin	
36	163184360	Artoindonesianin L	

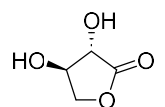
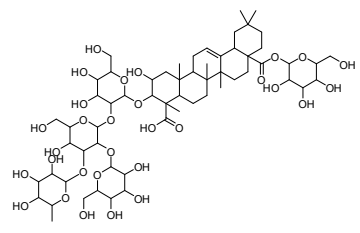
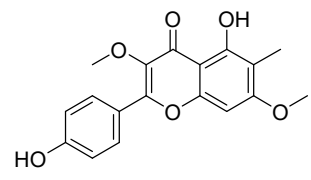
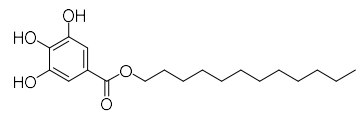
37	10351686	Threono-1,4-lactone	
38	14587733	Medicoside L	
39	14353449	8-Demethylatifolin	
40	14425	Lauryl gallate	

Table 3: ADMET Screening of the ligands from *Pouzolzia zeylanica* (L) Benn

Compound Name	Molecular weight (MW)	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Lipophilicity (LogP)	Lipinski violation	Result
Standard parameters	<500	<5	<10	<5	0	Accepted
Quercetin	302.0	5	7	0.524	0	Accepted
Phyllanthin	418.0	0	6	5.396	0	Accepted
Methyl Stearate	298.0	0	2	5.641	0	Accepted
Epicatechin	290.0	5	6	1.093	0	Accepted
Scopoline	155.0	1	2	1.256	0	Accepted

Scopoletin	192.0	1	4	0.978	0	Accepted
Apigenin	270.0	3	5	0.956	0	Accepted
Pouzolignan D	468.0	4	8	3.407	0	Accepted
Rhamnocitri n	300.0	3	6	1.349	0	Accepted
Rhamnetin	316.0	4	7	1.227	0	Accepted
Isorhamnetin	316.0	4	7	1.049	0	Accepted
Kaempferol	286.0	4	6	0.646	0	Accepted
Sinapaldehy de	208.0	1	4	1.550	0	Accepted
Syringaresin ol	418.0	2	8	3.658	0	Accepted
Dipentylphth alate	306.0	0	4	4.052	0	Accepted
Lupanine	248.0	0	1	2.997	0	Accepted
Threono-1,4- lactone	118.0	2	4	0.184	0	Accepted
8- Demethylati folin	328.0	2	6	2.143	0	Accepted

3.3. Protein ligand docking

Docking studies were performed on the binding pocket of three model proteins against 18 ADMET screen passed small molecules. The 18 ligands were docked with each protein model namely Swiss model build protein, Phyre2 build model protein, and GalaxyWeb build model protein, respectively and the best performance was displayed by Pouzolignan D in three model protein, which had a binding affinity of -8.2 kcal/mol, -8.4 kcal/mol, and -8.4 kcal/mol. The docking results of compounds shown in Table 4.

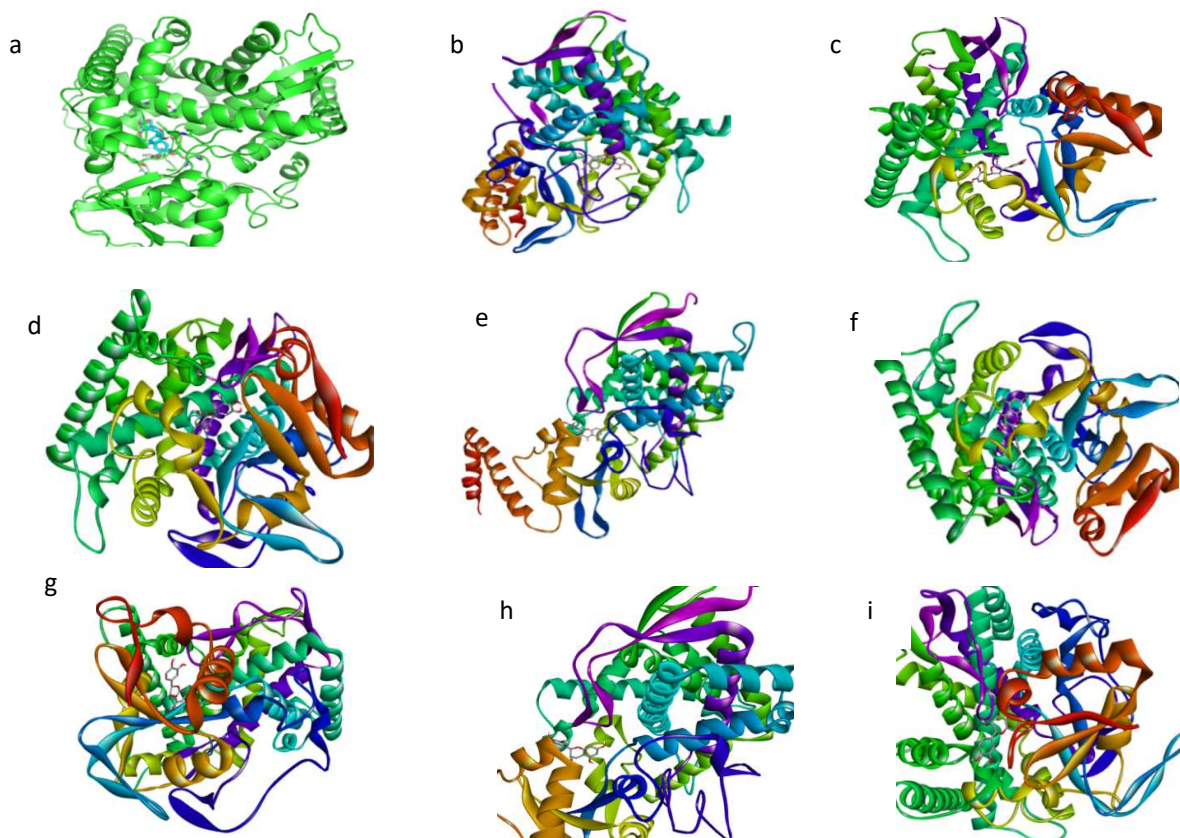
Table 4: Docking scores of 18 bioactive compounds against three protein models.

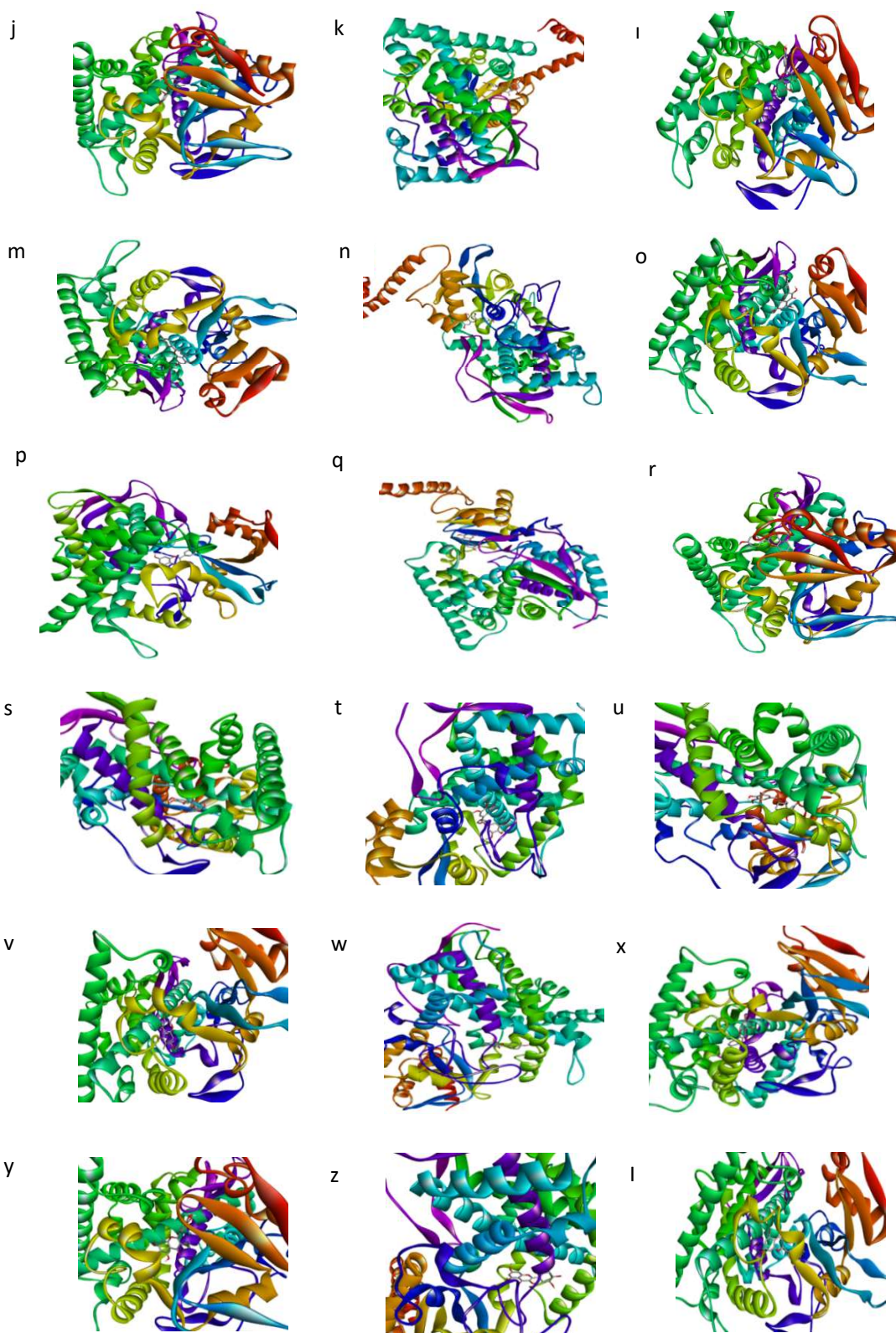
SL No.	LIGAND NAME	Swiss Model protein	Phyre2 Model Protein	GalaxyWeb Model Protein
		Docking scores		
1	Pouzolignan D	-8.2	-8.4	-8.4
2	Quercetin	-8.4	-7.7	-7.5
3	Epicatechin	-8.3	-8.0	-7.7
4	Isorhamnetin	-8.2	-7.4	-7.9
5	Kaempferol	-8.0	-7.6	-7.9
6	Apigenin	-8.0	-7.9	-7.8
7	Syringaresinol	-8.0	-7.4	-7.4
8	8-Demethylatifolin	-7.6	-7.8	-7.7
9	Rhamnetin	-7.6	-7.4	-7.7
10	Rhamnocitrin	-7.5	-7.7	-7.6
11	Lupanine	-7.2	-6.5	-7.2
12	Scopoletin	-6.5	-6.7	-6.4
13	Phyllanthin	-6.4	-6.4	-4.6
14	Dipentylphthalate	-6.0	-5.4	-6.0
15	Methyl Stearate	-6.0	-5.2	-5.2
16	Scopoline	-5.5	-5.3	-4.6
17	Sinapaldehyde	-5.5	-5.6	-5.8
18	Threono-1,4-lactone	-4.6	-3.9	-4.3

3.4. Interaction and visualization

After molecular docking process was completed, the protein ligand complexes formed against the target protein by the various bioactive compounds were analysed and visualised by using BIOVIA Discovery Studio software. For interaction analysis and visualization, compounds which showed lowest binding affinity of -8.4 kcal/mol to -6.5 kcal/mol in each protein models were selected. The protein ligand complexes of three model proteins with ligands were displayed in Figure 2. The 2D visualisation describes the various bonds formed between the receptor protein and ligands. Amino acids residues involved in Hydrogen bond are given in Table 5 and Figure 3. The sulfur interaction was also observed in some compounds with the model proteins which plays a crucial role in stabilizing the complex, and enhances the binding affinity and specificity of the ligands [17]. Pouzolignan D showed one Sulfur interaction with GalaxyWeb model protein amino acid i.e., CYS A:465; 4.7 Å. Syrgaresinol

exhibited one sulfur interaction with Swiss model protein amino acid i.e., CYS A:465; 5.9 Å, Rhamnocitrin displayed sulfur interaction with Phyre2 model protein amino acid i.e., CYS A:465; 5.3 Å, and Rhamnetin showed sulfur interaction with GalaxyWeb amino acid i.e., CYS A:465; 5.2 Å.





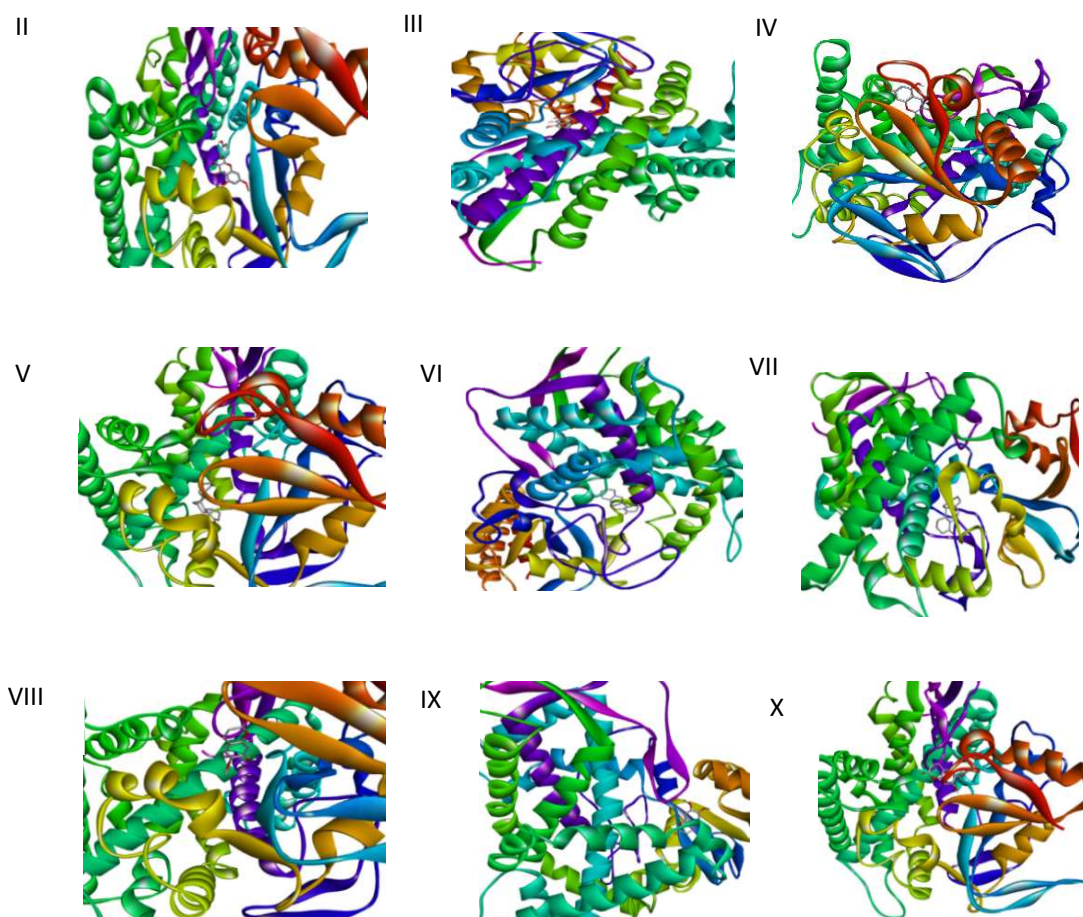


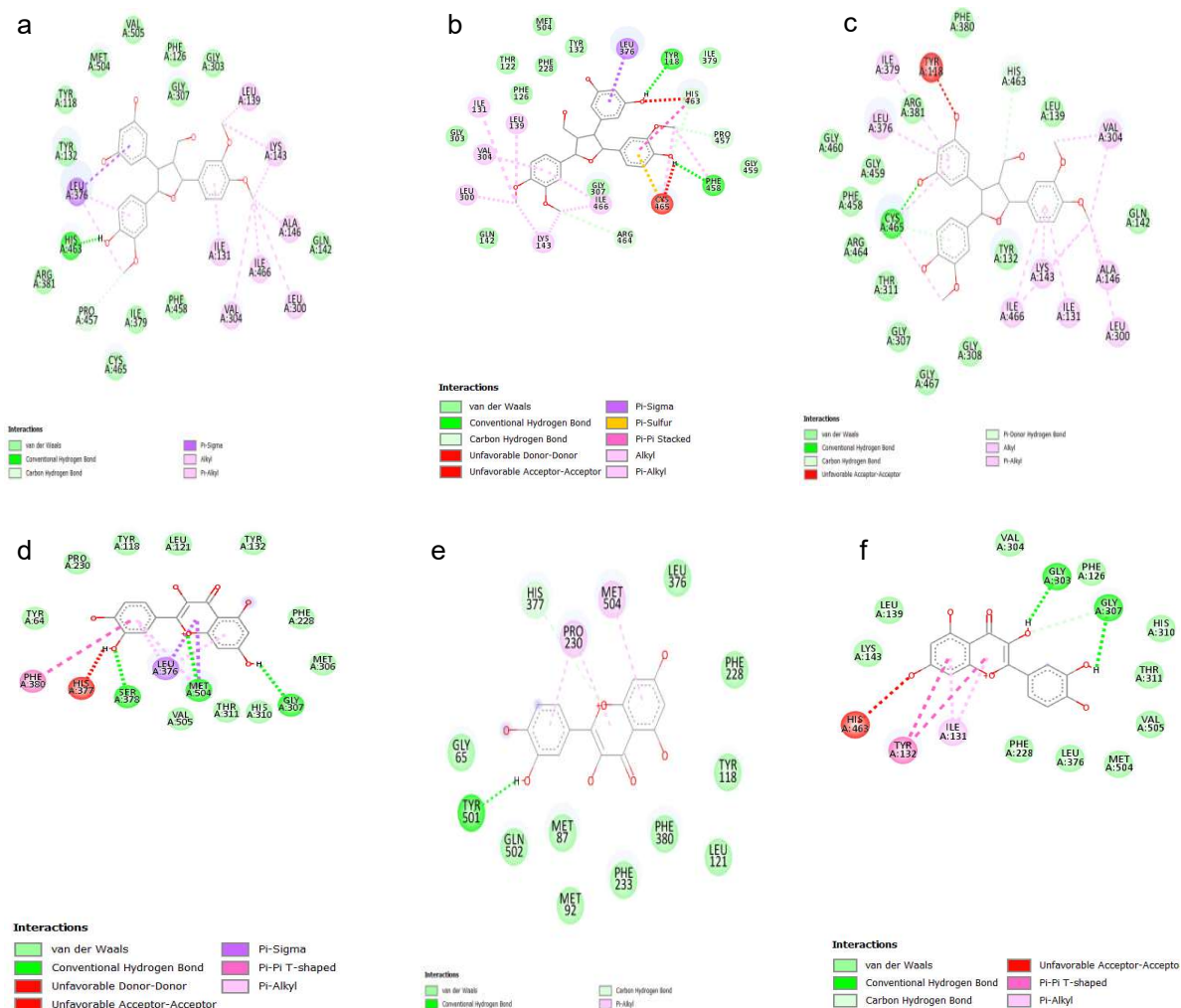
Fig. 2 Molecular docking representations of twelve phytochemical complexes—Pouzoligan D (a–c), quercetin (d–f), epicatechin (g–i), isorhamnetin (j–l), kaempferol (m–o), apigenin (p–r), syringaresinol (s–u), 8-Demethylatifolin (v–x), rhamnetin (y–I), rhamnocitrin (II–IV), lupanine (V–VII), and scopoletin (VIII–X)—in complex with Swiss-Model, Phyre2, and GalaxyWeb protein structures.

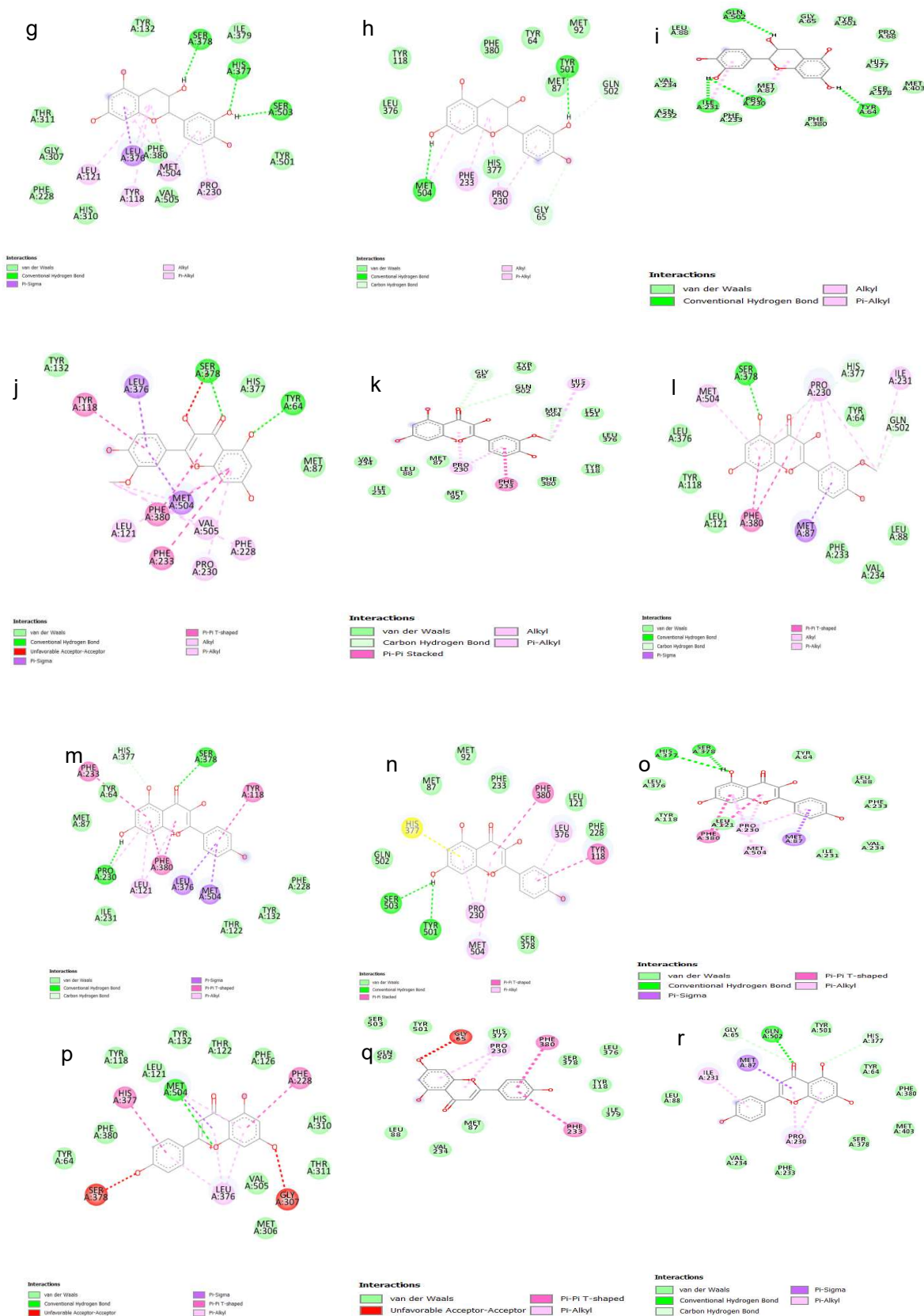
Table 5: Hydrogen bonds interacting with amino acid residues.

Name of the compound	Swiss Model protein		Phyre2 model protein		GalaxyWeb model protein	
	H-bond	Amino acid residues	H-bond	Amino acid residues	H-bond	Amino acid residues
Quercetin	3	GLY A:307(2.5 A°), MET A:504(3.1 A°), SER A:378(2.1 A°)	2	GLY A:307(2.6 A°), GLY A:303(2.3 A°)	1	TYR A:501(1.9 A°)

Pouzolignan D	1	HIS A: 463(2.24 A°)	1	CYS A:465	2	TYR A:465(2.2 A°), PHE A:458(3.0 A°)
Epicatechin	3	SER A:503(2.3 A°), SER A:378(2.1 A°), HIS A:377(2.4 A°)	2	MET A:504(2.6 A°), TYR A:501(2.43)	4	TYR A:64(2.3 A°), ILE A:231(2.2 A°), PRO A:230(2.8 A°), GLN A:502(2.5 A°)
Isorhamnetin	2	SER A:378(2.7 A°), TRY A:64	1	SER A:378(1.9 A°)	No H- bond	
Kaempferol	2	SER A:378(2.7 A°), PRO A:230(2.0 A°)	2	SER A:378(1.9 A°), HIS A:377(3.0 A°)	2	SER A:503(2.6 A°), TYR A:501(2.5 A°)
Apigenin	1	MET A:504(3.3 A°)	1	GLN A:502(2.9 A°)	No H- bond	
8-Demethylatifolin	1	TYR A:132(2.2 A°)	1	HIS A:463(2.4 A°)	No H- bond	
Syringaresinol	2	GLY A:467(2.03 A°), TYR A:13(2.18 A°)	No H- bond		1	THR A:311(3.04 A°)
Rhamnocitrin	No H- bond		No H- bond		2	TRY A:501(2.14 A°), GLN A:502(2.36 A°)

Metyl Sterate	2	HIS A:377(2.9 A°), TYR A:64(2.3 A°)	2	TYR A:501(2.6 A°), SER A:503(2.4 A°)	1	TYR A:64(2.6 A°)
Rhamnetin	3	SER A:377(2.6 A°), TYR A:118(2.6 A°), SER A:503(2.8 A°)	1	LEU A:139(2.75 A°)	2	ARG A:381(2.3 A°), CGS A:465(3.7 A°)





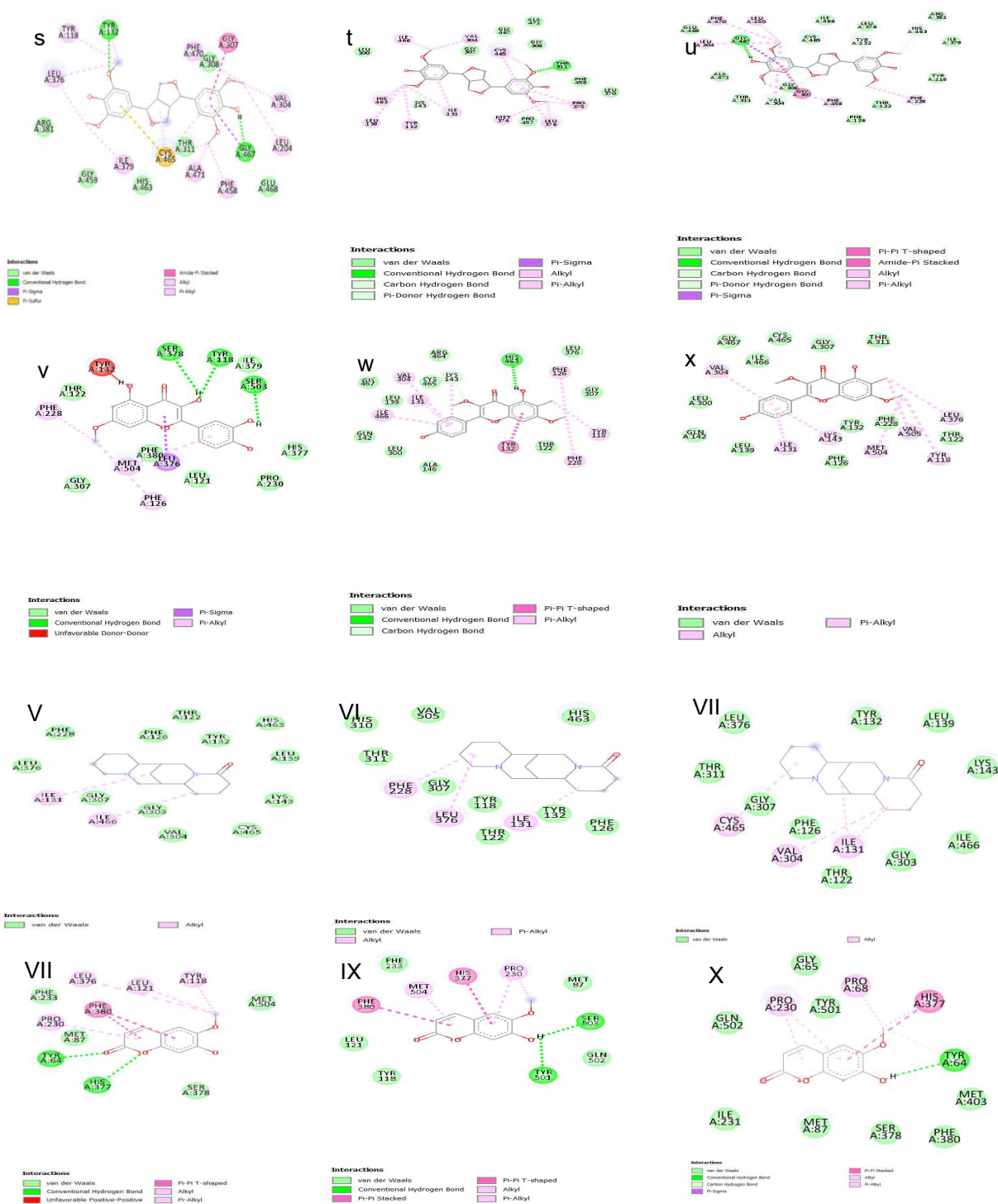


Fig. 3 2D visualizations of each compound generated using Swiss-Model, Phyre2, and GalaxyWeb: (a–c) Pouzoligan D; (d–f) Quercetin; (g–i) Epicatechin; (j–l) Isorhamnetin; (m–o) Kaempferol; (p–r) Apigenin; (s–u) Syringaresinol; (v–x) 8-Demethylatiafolin; (y–I) Rhamnetin; (II–IV) Rhamnocitrin; (V–VII) Lupanin; (VIII–X) Scopoletin

4. Discussion

In this study three different 3D structure protein was obtained through different homology modelling. After the ADMET screening process, 18 out of 40 compounds were obtained. These 18 compounds did not show violation for Lipinski's rule of 5. The ligand with the lowest binding affinity indicated a more stable binding and the formation of various interactive bonds and contacts served as the basis for determining the binding affinity between the ligands and receptor [18]. Although cysteine residues within proteins are relatively rare, covalent ligands that specifically target them have been highly valuable in drug discovery [19].

Pouzolignan D, a derivative of fused polyaromatic hydrocarbons, is hypothesized to contain a kinase-like ATP-binding region [20], which may disrupt disease signaling pathways, thus presenting itself as a promising pharmaceutical target. The hydrophobic pocket characteristic of Pouzolignan D is a common feature in fungal drug targets with hydrophobic active sites. Across all three modeled proteins, Pouzolignan D is predominantly encircled by LEU and ILE residues, with π -interactions from PHE and direct hydrogen bonding from amino acids HIS A: 463, TYR A: 118, and CYS A: 465. The compound engages through hydrophobic-aromatic interactions and is considered highly efficient [21,22]. The aromatic rings of Pouzolignan D interact with the amino acids LEU A: 376 via Pi-sigma and Pi-alkyl bonds, HIS A: 463 through a conventional hydrogen bond that stabilizes the ligand, and LEU A: 139, ILE A: 131, LYS A: 143, ALA A: 146, ILE A: 466, VAL A: 304, and LEU A: 300 through Pi-alkyl chains, creating a snug pocket with the Swiss-modeled protein and exhibiting a binding energy of -8.2 Kcal/mol. This demonstrates strong hydrophobic engagement, which could be further enhanced by incorporating H-bond donors/acceptors to improve affinity. The binding energies with the Phyre2-modeled protein are -8.4 Kcal/mol. The ligand interacts with amino acids ILE A: 131, LEU A: 139, VAL A: 304, LEU A: 300, LYS A: 143, ILE A: 466 via Pi-Pi stacked bonds, and GLY A: 307 through conventional H-bonding, LEU A: 376 by Pi-Sigma, and involves direct H-bonding with TYR A: 118 amino acid residues. HIS A: 463 is Pi-Pi stacked, and the aromatic rings show direct bonding with CYS 465 via Pi-sulfur. PHE A: 458 also directly bonds through conventional H-bonds, and PRO A: 457 exhibits van der Waals interaction. HIS A: 463 further displays unfavourable donor-donor and acceptor-acceptor properties with TYR A: 118 and the aromatic ring of the ligand and CYS A: 465 with PHE A: 458, indicating potential electrostatic repulsion may occur. However, CYS A: 465 has direct bonding via Pi-sulfur with the aromatic ring of the ligand, indicating stronger interaction strength, stabilizing the ligand pose, and making it more suitable as a lead inhibitor. The GalaxyWeb-modelled protein, with a binding energy of -8.4 Kcal/mol, demonstrates a well-distributed interaction network involving hydrogen bonds, π -interactions, and several hydrophobic contacts, supporting robust hydrophobic stacking with the ligand's aromatic rings, anchored by a few hydrogen bonds. Direct covalent hydrogen bond interactions are shown by CYS A: 465 with the aromatic rings of Pouzolignan D. ARG A: 381, LEU A: 376, ILEU A: 379, exhibit Pi-alkyl interactions, HIS A: 463 has direct interactions with conventional hydrogen bonding, and VAL A: 304, ILE A: 466, LYS A: 143, ILE A: 131, ALA A: 146, LEU A: 300 interact via Pi-alkyl side chains with an aromatic ring, indicating water exclusion, providing hydrophobic stabilization, and increasing the binding affinity.

Earlier studies confirmed that ethanolic extract of *Pouzolzia zeylanica* (L.) Benn had antifungal efficacy against *Blastomyces dermatitides*, *Aspergillus niger*, *Microsporum spp* & *Aspergillus nigerwa* [23]. Therefore,

investigating the active components responsible for its antifungal action is crucial, and since further work was suggested in this area to isolate natural compounds with antifungal activity, the molecular docking analysis, revealed that bioactive compounds of *Pouzolzia zeylanica* (L.) Benn had the potential antifungal activity against the target receptor. Out of the 18 phytochemicals, Pouzolignan D had shown the best lowest binding affinity of -8.2 kcal/mol, -8.4 kcal/mol, and -8.4 kcal/mol in each modelled protein. Additionally, Quercetin, Isorhamnetin, Epicatechin, Rhamnetin, Rhamnocitrin, Apigenin, Scopoletin, Syringaresinol, 8-Demethylatifolin, Lupanine, compounds also showed good interaction with the three-modelled protein showing low binding affinities which formed conventional hydrogen bonds with distance ranging from 1.0 Å - 3.5 Å. The presence of sulfur containing amino acids in Pouzolignan D, Syringaresinol, Rhamnocitrin and Rhamnetin indicates that, it may interrupt with microbial signalling and biofilm formation and suppress fungal growth by weakening the cell membrane structure and in turn cell membrane permeability [24,25]. Further, *Candida sp.* depends on quorum sensing and sulfur containing phytochemicals may be efficient inhibitors in providing an antifungal rationale by disrupting microbial communication, thereby preventing biofilm formation and controlling biofilm infections [26, 27].

5. Conclusion:

This *in silico* study highlights the therapeutic potential of bioactive compounds from *Pouzolzia zeylanica*, Pouzolignan D being the most active phytochemical. Molecular docking analysis demonstrated that this compound can effectively bind to and modulate the activity of target proteins, indicating strong inhibitory potential. Furthermore, pharmacokinetic and drug-likeness evaluations revealed favourable properties, including non-toxicity, acceptable water solubility, adherence to Lipinski's rule, and overall drug-like characteristics. Thus, the findings suggest that *Pouzolzia zeylanica* compounds may serve as promising candidates for further experimental validation in the development of safe and effective therapeutic agents.

Acknowledgement: The authors acknowledge, Dr. Subhasish Roy, Assistant Professor (Selection), Department of Chemistry, Assam Don Bosco University, Tapesia Gardens, Sonapur, for his contribution in generating a few ChemDraw structures in this manuscript.

References:

- [1]. K. Satoh, K. Makimura, Y. Hasumi, Y. Nishiyama, K. Uchida, and H. Yamaguchi, "Candida auris sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital," *Microbiol. Immunol.*, vol. 53, no. 1, pp. 41–44, 2009.
- [2]. H. Du, J. Bing, T. Hu, C. L. Ennis, C. J. Nobile, and G. Huang, "Candida auris: epidemiology, biology, antifungal resistance, and virulence," *PLoS Pathog.*, vol. 16, no. 10, p. e1008921, 2020.
- [3]. W. G. Lee, J. H. Shin, Y. Uh, M. G. Kang, S. H. Kim, K. H. Park, *et al.*, "First three reported cases of nosocomial fungemia caused by Candida auris," *J. Clin. Microbiol.*, vol. 49, no. 9, pp. 3139–3142, 2011.

- [4]. I. Baldim, M. H. Paziani, P. H. G. Barião, M. R. Kress, and W. P. Oliveira, "Nanostructured lipid carriers loaded with essential oil as a strategy to combat the multidrug-resistant," *Pharmaceutics*, vol. 14, no. 1, 2022.
- [5]. T. Vila, A. S. Sultan, D. Montelongo-Jauregui, and M. A. Jabra-Rizk, "Candida auris: a fungus with identity crisis," *Pathog. Dis.*, vol. 78, no. 4, 2020.
- [6]. C. J. Clancy and M. H. Nguyen, "Emergence of Candida auris: an international call to arms," *Clin. Infect. Dis.*, vol. 64, no. 2, pp. 141–143, 2017.
- [7]. R. Kean, C. Delaney, L. Sherry, A. Borman, E. M. Johnson, M. D. Richardson, *et al.*, "Transcriptome assembly and profiling of Candida auris reveals novel insights into biofilm-mediated resistance," *mSphere*, vol. 3, no. 4, 2018.
- [8]. M. Gowri, B. Jayashree, J. Jeyakanthan, and E. K. Girija, "Sertraline as a promising antifungal agent: inhibition of growth and biofilm of Candida auris with special focus on the mechanism of action in vitro," *J. Appl. Microbiol.*, vol. 128, no. 2, pp. 426–437, 2020.
- [9]. S. Schelenz, F. Hagen, J. L. Rhodes, A. Abdolrasouli, A. Chowdhary, A. Hall, *et al.*, "First hospital outbreak of the globally emerging Candida auris in a European hospital," *Antimicrob. Resist. Infect. Control*, vol. 5, no. 1, 2016.
- [10]. V. Srivastava and A. Ahmad, "Abrogation of pathogenic attributes in drug resistant Candida auris strains by farnesol," *PLoS One*, vol. 15, no. 5, p. e0233102, 2020.
- [11]. M. V. Horton and J. E. Nett, "Candida auris infection and biofilm formation: going beyond the surface," *Curr. Clin. Microbiol. Rep.*, vol. 7, no. 3, pp. 51–56, 2020.
- [12]. L. Forgács, A. M. Borman, E. Prépost, Z. Tóth, G. Kardos, R. Kovács, *et al.*, "Comparison of in vivo pathogenicity of four Candida auris clades in a neutropenic bloodstream infection murine model," *Emerg. Microbes Infect.*, vol. 9, no. 1, pp. 1160–1169, 2020.
- [13]. Jabeen, S., Khan, M. U., Ejaz, H., Waqar, S., Farhana, A., Alruwaili, M., ... & Ali, Q., Identifying novel inhibitors against drug-resistant mutant CYP-51 Candida albicans: A computational study to combat fungal infections. *PLoS One*, 20(3), 2025
- [14]. X. M. Chen, Z. H. Li, S. H. Tao, Y. F. Chen, Z. H. Chen, and L. B. Guo, "Effect of FPZ, a total flavonoids ointment topical application from Pouzolzia zeylanica var. microphylla, on mice skin infections," *Rev. Bras. Farmacogn.*, vol. 28, no. 6, pp. 732–737, 2018.
- [15]. N. D. Tan, V. T. T. Le Quoc Viet, and N. M. Thuy, "Optimization of polyphenol, flavonoid and tannin extraction conditions from Pouzolzia zeylanica L. Benn using response surface methodology," *Can Tho Univ. J. Sci.*, vol. 5, pp. 122–131, 2017.
- [16]. L. Wang, D. Gao, Z. Xu, F. Yang, and Z. Xia, "Chemical constituents of Pouzolzia zeylanica with PPAR γ and PPAR β activities," *Chem. Nat. Compd.*, vol. 51, no. 6, pp. 1157–1159, 2015.
- [17]. A. Alisaac, In silico analysis of quorum sensing modulators: Insights into molecular docking and dynamics and potential therapeutic applications. *PLoS One*, 20(6), 2025
- [18]. K. C. Hsu, Y. F. Chen, and J. M. Yang, "Binding affinity analysis of protein-ligand complexes," in *Proc. 2nd Int. Conf. Bioinformatics and Biomedical Engineering*, 2008.

- [19]. G. Kim, R. J. Grams, and K.-L. Hsu, “Advancing covalent ligand and drug discovery beyond cysteine,” *Chemical Reviews*, 2025.
- [20]. J. L. Li, J. Y. Jiang, S. S. Gu, L. Zhang, Y. Sun, X. H. Wang, S.S. Jiang, W.Z. Zhu, H.W. Jiang and H. J. Zhang, Identification of lignans as ATP citrate lyase (ACLY) inhibitors from the roots of *Pouzolzia zeylanica*. *Natural Product Research*, 38(10), 1719-1726, 2024.
- [21]. R. Patil, S. Das, A. Stanley, L. Yadav, A. Sudhakar, and A. K. Varma, Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing. *PloS one*, 5(8), 2010.
- [22]. R. F. De Freitas, and M. Schapira, A systematic analysis of atomic protein–ligand interactions in the PDB. *Medchemcomm*, 8(10), 1970-1981, 2017
- [23]. D. Saha, S. Paul, and S. Chowdhury, “Antibacterial activity of ethanol extract of *Pouzolzia zeylanica* (L.) Benn.,” *Int. J. Pharm. Innov.*, vol. 2, no. 1, pp. 1–5, 2012.
- [24]. D. C. Bouttier-Figueroa, M. Cortez-Valadez, M. Flores-Acosta, and R.E. Robles-Zepeda, Synthesis of Metallic nanoparticles using plant’s natural extracts: synthesis mechanisms and applications. *Biotechnia*, 25(3), 125-139, 2023.
- [25]. G. Zara, M. Bou Zeidan, F. Fancello, M. L. Sanna, I. Mannazzu, M. Budroni, and S. Zara, The administration of L-cysteine and L-arginine inhibits biofilm formation in wild-type biofilm-forming yeast by modulating FLO11 gene expression. *Applied Microbiology and Biotechnology*, 103(18), 7675-7685, 2019.
- [26]. N. Li, J. Zhang, F. Yu, F. Ye, W. Tan, L. Hao, L. S. Li, J. Deng and X. Hu, Garlic-derived quorum sensing inhibitors: a novel strategy against fungal resistance. *Drug Design, Development and Therapy*, 6413-6426, 2024.
- [27]. M. S. A. Khan, M. M. Altaf, and M. Sajid, Insights of phyto-compounds as antipathogenic agents: controlling strategies for inhibiting biofilms and quorum sensing in *Candida albicans*. In *New Look to Phytomedicine*, pp. 367-389). Academic Press, 2019. *Phytomedicine*, pp. 367-389). Academic Press, 2019.