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Antifungal Potential of Seabuckthorn (*Hippophae Rhamnoides L.*) Against *Rhizopus Azygosporus*: An *in-silico* Approach to Combat Mucormycosis

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ABSTRACT

Rhizopus species are opportunistic fungal pathogens from the Mucorales order, commonly found in soil, decaying organic matter, and indoor environments. While generally harmless, they can cause life-threatening infections in immunocompromised individuals, leading to mucormycosis. During the COVID-19 pandemic, mucormycosis cases surged, particularly in India, due to immune suppression caused by diabetes (present in 66.1 % of cases) and widespread corticosteroid use (80.3 % of cases). Standard treatments include antifungal agents like Amphotericin B, Posaconazole, and Isavuconazole, along with surgical debridement. Mucorales exhibit resistance to many antifungals due to their unique cell wall composition, making azoles like itraconazole and voriconazole ineffective, while echinocandins show minimal activity. Seabuckthorn (*Hippophae rhamnoides L.*), rich in bioactive compounds, exhibits antioxidant, anti-inflammatory, and antimicrobial properties, making it a promising candidate against *Rhizopus* infections. This study employs molecular docking using iGEM Dock to evaluate the binding affinity of Seabuckthorn bioactive compounds against ergosterol biosynthesis in *Rhizopus azygosporus*, a major causative agent of mucormycosis. ADMET analysis is performed to assess the pharmacokinetic profiles of the compounds, ensuring their suitability for therapeutic applications. The present study identifies futuristic novel drug targets and efficient antifungal agents to effectively address mucormycosis.

Keywords: Antifungal; Ergosterol; Mucormycosis; Rhizopus; Seabuckthorn

1. INTRODUCTION

Mucormycosis is a rapidly progressing fungal infection marked by its ability to invade blood vessels, caused by environmental moulds from the order *Mucorales*[1]. While these fungi are commonly found in the environment, they usually become pathogenic only in individuals with weakened immune systems. In such cases, they can cause severe infections marked by tissue damage and vascular invasion. The disease can present in various forms-such as rhino cerebral, pulmonary, cutaneous, gastrointestinal, or even widespread-based on how fungus enters the body and the patient's immune condition[2].

Among the various genera within *Mucorales*, *Rhizopus* species especially *Rhizopus arrhizus* and *Rhizopus microsporus* are identified as the most prevalent causes of mucormycosis worldwide[3]. These fungi possess natural resistance to several antifungal agents, largely due to their distinct cell wall composition and adaptive stress response systems, making treatment more challenging. Standard treatment protocols typically involve the use of high-dose Amphotericin B, Posaconazole, or Isavuconazole combined with aggressive surgical debridement[4]. However, therapeutic success remains limited, particularly due to delayed diagnosis, extensive angioinvasion, and the intrinsic antifungal resistance exhibited by *Mucorales* fungi[5].

The global burden of mucormycosis witnessed an alarming escalation during the COVID-19 pandemic, notably in India, where mucormycosis incidence rates were significantly higher than in developed nations[2]. A synergistic interaction between COVID-19-associated immune dysregulation, widespread corticosteroid therapy, and a high background prevalence of uncontrolled diabetes mellitus contributed to the so-called "epidemic within a pandemic"[4]. Studies report that Diabetes mellitus, particularly in the state of ketoacidosis, was present in a large proportion of mucormycosis cases during the pandemic[3]. Moreover, the rampant use of immunosuppressive therapies and the presence of comorbidities such as chronic kidney disease further predisposed individuals to severe mucormycosis.[4]

Despite significant advances in antifungal therapeutics, the treatment of mucormycosis remains challenging due to multiple factors including drug toxicity, the poor bioavailability in infected tissues, and emergence of drug-resistant strains[1]. Azoles such as Itraconazole and

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Voriconazole, which are effective against *Aspergillus* spp., exhibit minimal to no activity against *Mucorales* due to the fungal ability to circumvent ergosterol-targeted inhibition pathways[5]. Consequently, it is imperative to investigate alternative antifungal approaches without delay, including the identification of natural bioactive compounds with potent antifungal properties.

Seabuckthorn (*Hippophae rhamnoides L.*), a hardy deciduous shrub native to the cold areas of Europe and Asia, has garnered considerable scientific attention due to its rich phytochemical profile comprising flavonoids, phenolics, carotenoids, and essential fatty acids[6]. Traditionally acclaimed for its antioxidant, anti-inflammatory, and antimicrobial properties, Seabuckthorn has been employed in ethnomedicine for centuries[7]. Recent studies have illuminated its potential role in combating microbial infections, including antifungal effects against *Candida albicans* and *Aspergillus niger*, though its efficacy against pathogenic molds such as *Rhizopus* species remains underexplored[8].

In light of the urgent demand for novel antifungal treatments and the rich bioactive potential of *Hippophae rhamnoides,* this study explores the antifungal properties of compounds derived from Seabuckthorn against *Rhizopus azygosporus,* a key causative agent of mucormycosis. Adopting an integrated approach that combines molecular docking, ADMET analysis, the research aims to identify and characterise novel natural compounds capable of inhibiting critical fungal enzymes involved in ergosterol biosynthesis[9]. This work aspires to contribute to the future development of more effective antifungal therapies.

This research not only addresses the pressing global healthcare challenge posed by mucormycosis but also highlights the untapped potential of natural phytochemicals in antifungal drug discovery. The outcomes of this investigation could thus pave the way for safer, effective, and resistance-evading therapeutic alternatives in the management of invasive fungal infections.

2. MATERIALS AND METHODS

2.1 Selection and Retrieval of Ligands

Bioactive compounds from *H. rhamanoides* L. (Seabuckthorn) were identified through extensive literature review and database screening. The 2D structures of selected phytochemicals were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The 37 bioactive compounds were selected based on their known antimicrobial or antioxidant potential, which could theoretically contribute to antifungal activity against *Rhizopus* species (Table 1a). Besides, known antibiotics that are inhibitors of fungal cell membrane were also tested for molecular docking (Table 2b). Non-antifungal drugs like antivirals, RTI agents, ACE inhibitors, blood thinners, and iron chelators were included to explore their potential supportive roles in mucormycosis management. These includes Deferoxamine, Deferasirox as Iron Chelator; Antiviral drugs- Baloxavir marboxil, Danoprevir, Sofosbuvir, ACE Inhibitor-Fosinopril, Quinapril; Telmisartan as Angiotensin Receptor Blocker-, and Sulfamethoxazole as Cardiac glycosides and drugs used against Respiratory tract Infection (Table 3c).

2.2 Retrieval of Target Proteins

Key fungal proteins implicated in the ergosterol biosynthesis pathway of *R. azygosporus* were selected as molecular targets due to their critical role in maintaining fungal cell membrane integrity (Table 2). Three-dimensional (3D) structures of these proteins were retrieved in .pdb format from the Protein Data Bank (PDB) RCSB PDB: Homepage[10]. The physicochemical and functional characterization of these proteins was performed through Expasy's Prot-param server[11]. The 3D-protein homology models were generated from the Swiss model interactive workspace[12]. The protein homology models were validated with the Ramachandran plot using the Swiss model interactive workspace. The stereochemical quality of the protein structures was assessed by PROCHECK which evaluated the presence of conserved sequences and related geometry of proteins[13].

2.3 Ligand Preparation

The retrieved SDF-formatted ligand structures were converted into SMILES notation using an online SMILES Translator and Structure File Generator tool (Online SMILES Translator and Structure File - Chempedia - LookChem). This step facilitated the standardisation of chemical structures for further processing. Subsequently, the SMILES files were converted back into three-dimensional .pdb format using Open Babel (version 2.4.1), an open-source chemical toolbox, ensuring compatibility with docking software (http://openbabel.org/wiki/Main_Page)

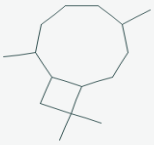
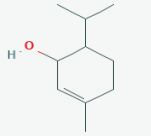
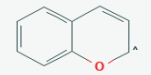
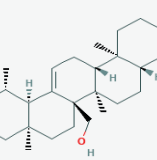
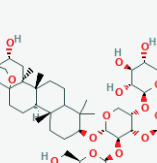
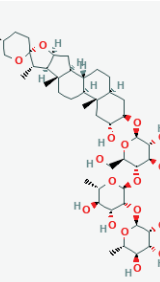
2.4 Molecular Docking Studies

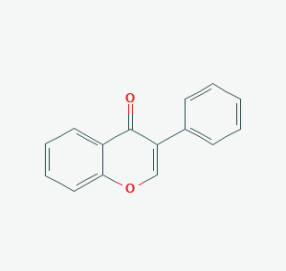
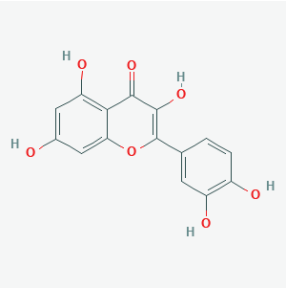
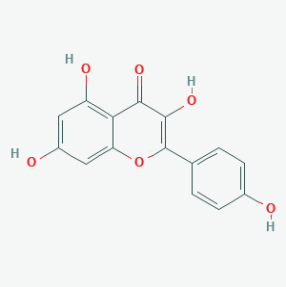
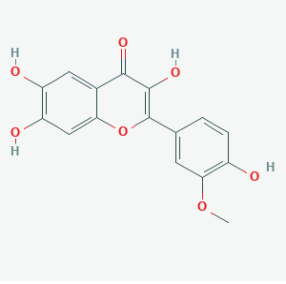
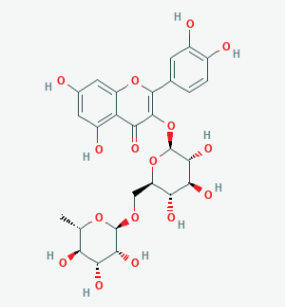
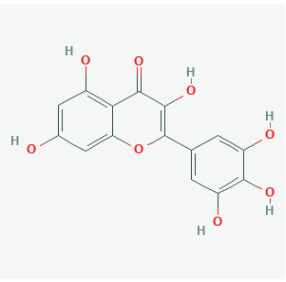
Molecular docking simulations were performed using iGEMDOCK software (version 2.1), a flexible and user-friendly tool specifically designed for structure-based drug discovery[14]. Docking experiments were conducted to evaluate the binding interactions between Seabuckthorn-derived ligands and the fungal target proteins. The scoring function integrated electrostatic, steric, and hydrogen bonding contributions to compute total binding energy, thus identifying the best possible ligand-protein complexes.

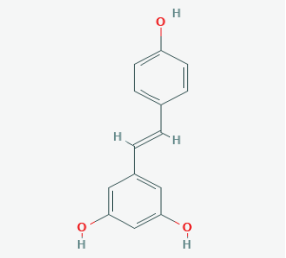
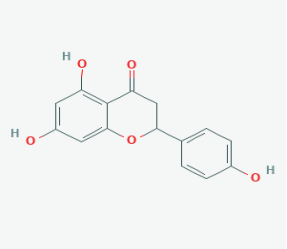
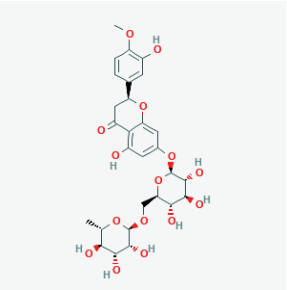
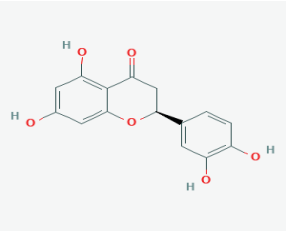
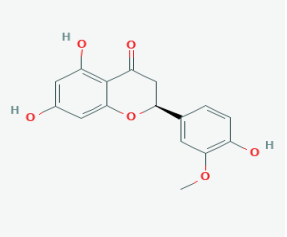
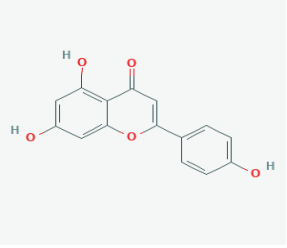
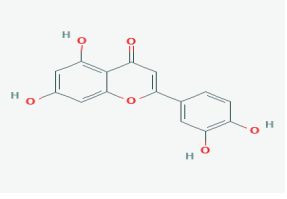
2.5 ADMET Analysis

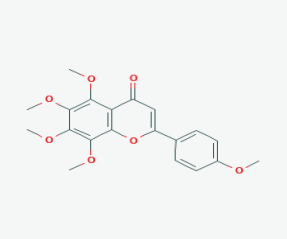
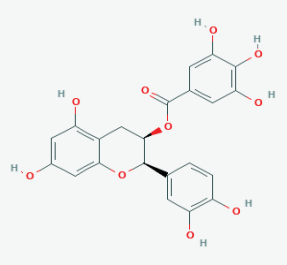
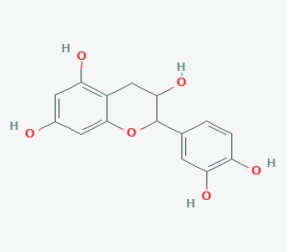
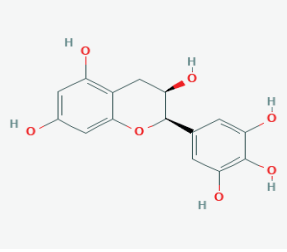
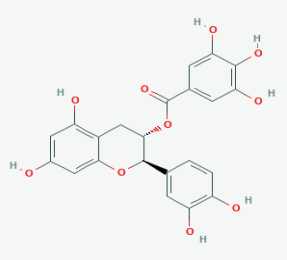
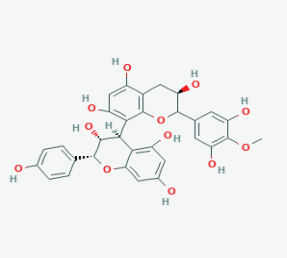
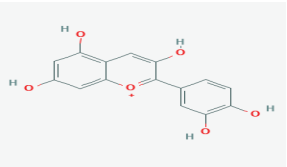
The assessment of drug-likeness and pharmacokinetic behaviour of the selected compounds, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling was conducted using ADMETlab 2.0 (https://admetlab3.scbdd.com). Critical parameters such as gastrointestinal absorption, blood-brain barrier permeability, cytochrome P450 inhibition, and potential toxicity were evaluated to ensure the pharmacological viability of the candidate molecule.

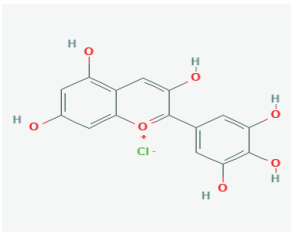
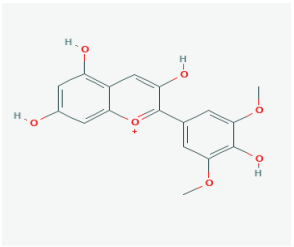
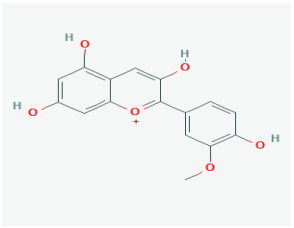
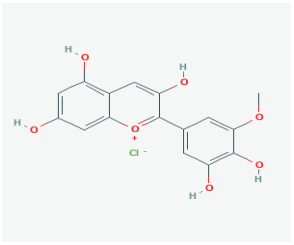
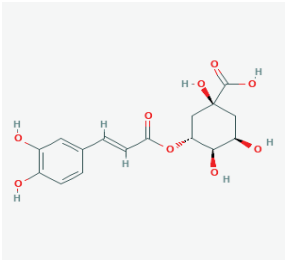
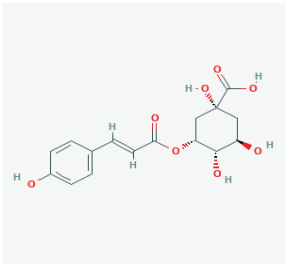
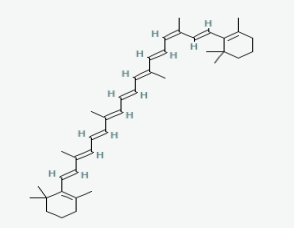
Table 1(a). Phytochemical constituents from *Hippophae rhamnoides L.* (Sea buckthorn) used as ligands for molecular docking

S.No.	Ligands	Pubchem ID	Smiles code	2-D structre
1.	Sesquiterpenes	177131	CC1CCCC(C2C(CC1)C(C2)(C)C)C	
2.	Monoterpenes	10282	CC1=CC(C(CC1)C(C)C)O	
3.	Chromene (2H-1-benzopyran-2-yl) radical	13382570	C1=Cc2c(O[CH]1)cccc2	
4.	Triterpenes (Obtusol)	15895316	OCC12CCC3(C(C1=CCC1C2(C)CCC2C1(C)CCC(C2(C)C)O)C(C)C(CC3)C)C	
5.	Saponins	198016	OCC1OC(OC2C(OCC(C2OC2OC(CO)C(C(C2O)O)O)OC2OCC(C(C2O)O)O)OC2CCC3(C(C2(C)C)CCC2(C3CCC34C2(C)CC(C2(C4CC(C)(C=O)CC2)CO3)O)C)C)C(C(C1O)O)OC1OC(CO)C(C(C1O)O)O	
6.	Tuberoside (Steroidal Saponin)	102019173	OCC1OC(OC2CC3CCC4C(C3(CC2O)C)CCC2(C4CC3C2C(C2(O3)CCC(CO2)CO)C)C)C(C(C1OC1OC(C)C(C(C1OC1OC(C)C(C(C1	

7.	Isoflavone	72304	O=c1c(coc2c1cccc2)c1ccccc1	
8.	Quercetin	5280343	Oc1cc(O)c2c(c1)oc(c(c2=O)O)c1ccc(c(c1)O)O	
9.	Kaempferol	5280863	Oc1ccc(cc1)c1oc2cc(O)cc(c2c(=O)c1O)O	
10.	Isohamnetin	15817847	COc1cc(ccc1O)c1oc2cc(O)c(cc2c(=O)c1O)O	
11.	Rutin	5280805	Oc1cc(O)c2c(c1)oc(c(c2=O)OC1OC(COC2OC(C)C(C(C2O)O)O)C(C(C1O)O)O)c1ccc(c(c1)O)O	
12.	Myricetin	5281672	Oc1cc(O)c2c(c1)oc(c(c2=O)O)c1cc(O)c(c(c1)O)O	

13.	Resveratrol	445154	Oc1ccc(cc1)C=Cc1cc(O)cc(c1)O	
14.	Naringenin	932	Oc1ccc(cc1)C1CC(=O)c2c(O1)cc(cc2O)O	
15.	Hesperidin	10621	COc1ccc(cc1O)C1CC(=O)c2c(O1)cc(cc2O)OC1OC(COC2OC(C)C(C(C2O)O)O)C(C(C1O)O)O	
16.	Eriodictyol	440735	Oc1cc2OC(CC(=O)c2c(c1)O)c1ccc(c(c1)O)O	
17.	Homoeriodictyol	73635	COc1cc(ccc1O)C1CC(=O)c2c(O1)cc(cc2O)O	
18.	Apigenin	5280443	Oc1ccc(cc1)c1cc(=O)c2c(o1)cc(cc2O)O	
19.	Luteolin	5280445	Oc1cc(O)c2c(c1)oc(cc2=O)c1ccc(c(c1)O)O	

20.	Tangeretin	68077	COc1ccc(cc1)c1cc(=O)c2c(o1)c(OC)c(c(c2OC)OC)OC	
21.	L- Epicatechin gallate	107905	Oc1cc(O)c2c(c1)OC(C(C2)OC(=O)c1cc(O)c(c(c1)O)O)c1ccc(c(c1)O)O	
22.	Catechin	1203	Oc1cc2OC(c3ccc(c(c3)O)O)C(Cc2c(c1)O)O	
23.	L-Epigallocatechin	72277	Oc1cc2OC(c3cc(O)c(c(c3)O)O)C(Cc2c(c1)O)O	
24.	Catechin 3'-O-gallate	5276454	Oc1cc(O)c2c(c1)OC(C(C2)OC(=O)c1cc(O)c(c(c1)O)O)c1ccc(c(c1)O)O	
25.	Proanthocyanidins	108065	COc1c(O)cc(cc1O)C1Oc2c(CC1O)c(O)cc(c2C1C(O)C(Oc2c1c(O)cc(c2)O)c1ccc(cc1)O)O	
26.	Cyanidin	128861	Oc1cc(O)c2c(c1)[o+]c(c(c2)O)c1ccc(c(c1)O)O	

27.	Delphinidin	68245	Oc1cc(O)c2c(c1)[o+]c(c(c2)O)c1cc(O)c(c(c1)O)O.[Cl-]	
28.	Malvidin	159287	COc1cc(cc(c1O)OC)c1[o+]c2cc(O)cc(c2cc1O)O	
29.	Peonidin	441773	COc1cc(ccc1O)c1[o+]c2cc(O)cc(c2cc1O)O	
30.	Petunidin	73386	COc1cc(cc(c1O)O)c1[o+]c2cc(O)cc(c2cc1O)O.[Cl-]	
31.	Chlorogenic acid	1794427	O=C(OC1CC(O)(CC(C1O)O)C(=O)O)C=Cc1ccc(c(c1)O)O	
32.	Coumaroylquinic acid	9945785	O=C(OC1CC(O)(CC(C1O)O)C(=O)O)C=Cc1ccc(cc1)O	
33.	Beta carotene	9828626	CC1=C(C(CCC1)(C)C)/C=C/C(=C/C=C/C(=C/C=C/C=C(\C)/C=C/C=C(/C)\C=C\C2=C(CCCC2(C)C)C)/C)/C	

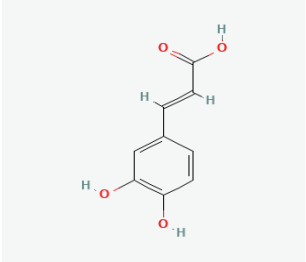
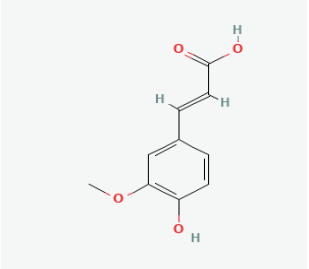
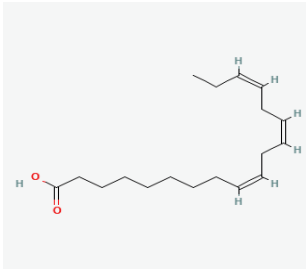
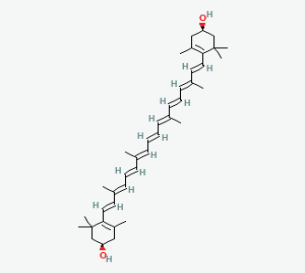
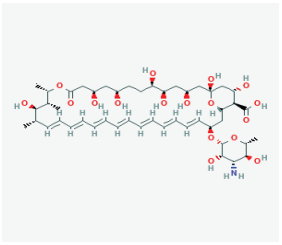
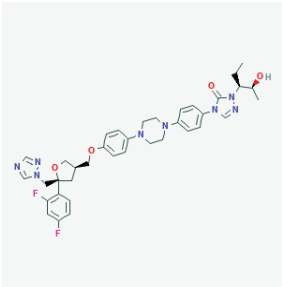
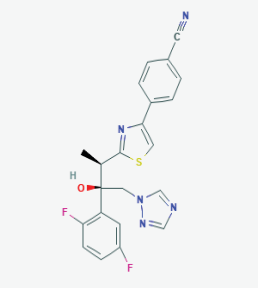
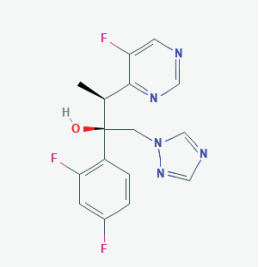
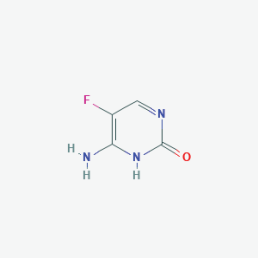
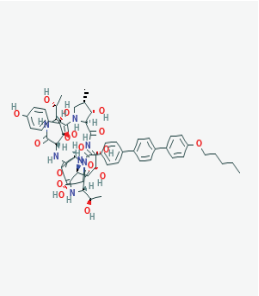
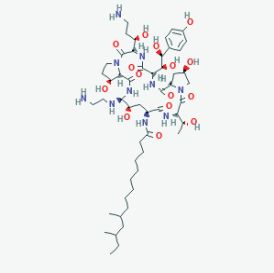
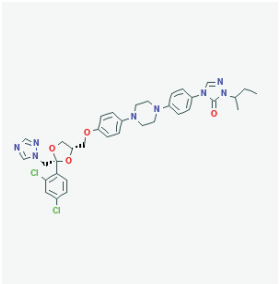
34.	Caffeic Acid	689043	C1=CC(=C(C=C1/C=C/C(=O)O)O)O	
35.	Ferulic Acid	445858	COC1=C(C=CC(=C1)/C=C/C(=O)O)O	
36.	α-Linolenic acid	5280934	CC/C=C\C/C=C\C/C=C\CCCCCCCC(=O)O	
37.	Zeaxanthin	5280899	CC1=C(C(C[C@@H](C1)O)(C)C)/C=C/C(=C/C=C/C(=C/C=C/C=C(/C=C/C=C(/C=C/C2=C(C[C@H](CC2(C)C)O)C)\C)\C)/C)/C	

Table 1(b). Standard antifungal drugs used as ligands for molecular docking

S.No.	Ligands	Pubchem ID	Smiles Code	2-D Structure
1.	Amphotericin B	5280965	OC1CCC(O)C(O)CC(O)CC2(O)CC(O)C(C(O2)CC(C=CC=CC=CC=CC=CC=CC(C(C(C(OC(=O)CC(C1)O)C)C)O)C)O)C)OC1OC(C)C(C(C1O)N)O)C(=O)O	
2.	Posaconazole	468595	CCC(n1ncn(c1=O)c1ccc(cc1)N1CCN(CC1)c1ccc(cc1)OCC1COC(C1)(Cn1cncn1)c1ccc(cc1F)F)C(O)C	

3.	Isavuconazole	6918485	N#Cc1ccc(cc1)c1csc(n1)C(C(c1cc(F)ccc1F)(Cn1cncn1)O)C	
4.	Voriconazole	71616	Fc1ccc(c(c1)F)C(C(c1ncncc1F)C)(Cn1cncn1)O	
5.	Flucytosine	3366	Nc1c(F)cnc(=O)[nH]1	
6.	Anidulafungin	166548	CCCCCOc1ccc(cc1)c1ccc(cc1)c1ccc(cc1)C(=O)NC1CC(O)C(O)NC(=O)C2C(O)C(CN2C(=O)C(NC(=O)C(NC(=O)C2N(C(=O)C(NC1=O)C(O)C)CC(C2)O)C(C(c1ccc(cc1)O)O)O)C(O)C)C	
7.	Caspofungin	2826718	NCCNC1NC(=O)C2C(O)CCN2C(=O)C(NC(=O)C(NC(=O)C2N(C(=O)C(NC(=O)C(CC1O)NC(=O)CCCCCCCCC(CC(CC)C)C)C(O)C)CC(C2)O)C(C(c1ccc(cc1)O)O)O)C(CCN)O	
8.	Itraconazole	55283	CCC(n1ncn(c1=O)c1ccc(cc1)N1CCN(CC1)c1ccc(cc1)OCC1COC(O1)(Cn1cncn1)c1ccc(cc1Cl)Cl)C	

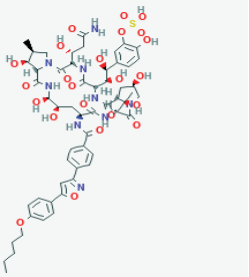
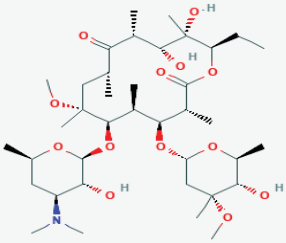
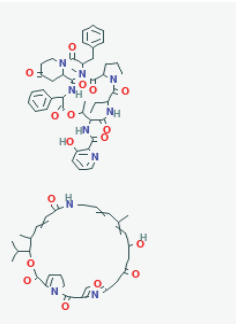
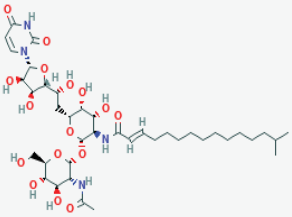
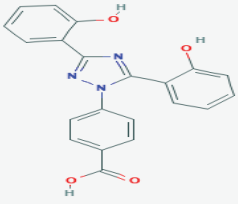
9.	Micafungin	477468	CCCCCOc1ccc(cc1)c1onc(c1)c1ccc(cc1)C(=O)NC1CC(O)C(O)NC(=O)C2C(O)C(CN2C(=O)C(NC(=O)C(NC(=O)C2N(C(=O)C(NC1=O)C(O)C)CC(C2)O)C(C(c1ccc(c(c1)OS(=O)(=O)O)O)O)O)C(CC(=O)N)O)C	
10.	Clarithromycin	84029	CCC1OC(=O)C(C)C(OC2CC(C)(OC)C(C(O2)C)O)C(C)C(OC2OC(C)CC(C2O)N(C)C)C(CC(C(=O)C(C(C1(C)O)O)C)C)(C)OC	
11.	Virginiamycin	73160420	O=C1NCC=CC(=CC(O)CC(=O)Cc2nc(C(=O)N3C(=CCC3)C(=O)OC(C(C=C1)C)C(C)C)co2)C.CCC1NC(=O)C(NC(=O)c2ncccc2O	
12.	Tunicamycin	56927836	OCC1OC(OC2OC(CC(C3OC(C(C3O)O)n3ccc(=O)[nH]c3=O)O)C(C(C2NC(=O)C=CCCCCCCCCCCC(C)C)O)O)C(C(C1O)O)NC(=O)C	

Table 1(c). Repurposed drug candidates with antiviral, RTI, ACE inhibition, and iron chelation properties

S.No.	Ligands	Pubchem ID	Smiles Code	2-D Structure
1.	Deferoxamine	2973	NCCCCCN(C(=O)CCC(=O)NCCCCCN(C(=O)CCC(=O)NCCCCCN(C(=O)C)O)O)O	
2.	Deferasirox	214348	OC(=O)c1ccc(cc1)n1nc(nc1c1ccccc1O)c1ccccc1O	

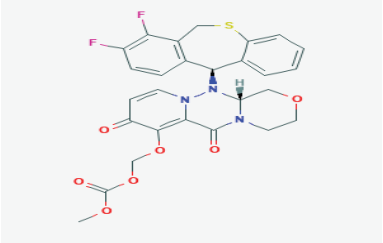
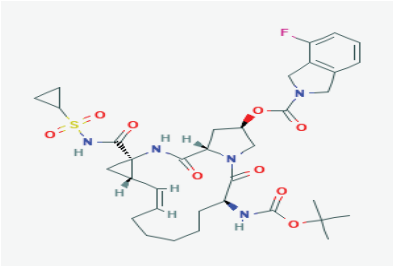
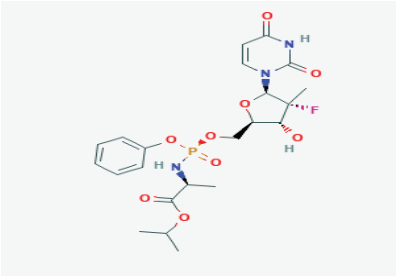
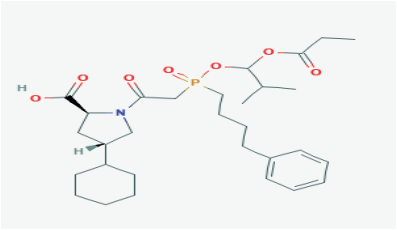
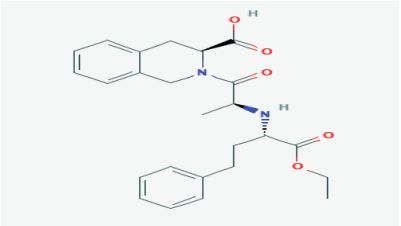
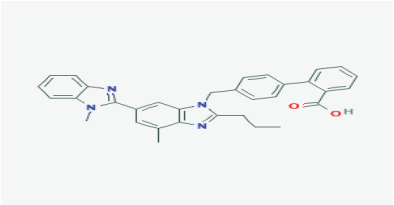
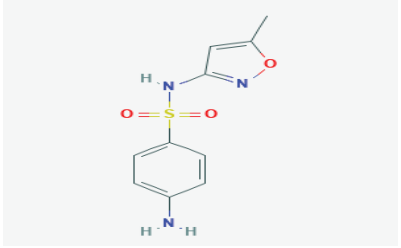
3.	Baloxavir marboxil	124081896	COC(=O)OCOc1c(=O)ccn2c1C(=O)N1CCOCC1N2C1c2ccccc2SCc2c1ccc(c2F)F	
4.	Danoprevir	11285588	O=C(OC(C)(C)C)NC1CCCCCC=CC2C(NC(=O)C3N(C1=O)CC(C3)OC(=O)N1Cc3c(C1)cccc3F)(C2)C(=O)NS(=O)(=O)C1CC1	
5.	Sofosbuvir	45375808	CC(OC(=O)C(NP(=O)(Oc1ccccc1)OCC1OC(C(C1O)(C)F)n1ccc(=O)[nH]c1=O)C)C	
6.	Fosinopril	55891	CCC(=O)OC(C(C)C)OP(=O)(CC(=O)N1CC(CC1C(=O)O)C1CCCCC1)CCCCc1ccccc1	
7.	Quinapril	54892	CCOC(=O)C(NC(C(=O)N1Cc2ccccc2CC1C(=O)O)C)CCc1ccccc1	
8.	Telmisartan	65999	CCCc1nc2c(n1Cc1ccc(cc1)c1ccccc1C(=O)O)cc(cc2C)c1nc2c(n1C)cccc2	
9.	Sulfamethoxazole Cardiac glycosides and drugs used against Respiratory tract Infection (RTI)	5329	Nc1ccc(cc1)S(=O)(=O)Nc1noc(c1)C	

Table 2. Quality assessment and structural properties of fungal sterol biosynthesis

S. No.	Gene	Protein	Length	Mol. Wt.	pI
1	ERG1	Squalene epoxidase	466	51970.34Da	7.63
2	ERG2	C-8 Sterol isomerase	227	25706.71Da	6.16
3	ERG3	C-5 Sterol desaturase	306	36487.09Da	6.87
4	ERG5	RNA polymerase C-22 sterol desaturase	724	84340.47Da	8.63
5	ERG6	Sterol 24 -C-methyltransferase	375	42182.71 Da	5.92
6	ERG7	Lanosterol synthase	730	83545.94 Da	5.83
7	ERG11	14 α -Sterol demethylase A	507	57,479.14 Da	6.71
8	ERG12	Mevalonate kinase	404	44,037.32 Da	5.51
9	ERG13	3-Hydroxy-3-methylglutaryl coenzyme A reductase	1102	119,395.95 Da	8.77
10	ERG20	Farnesyl Pyrophosphate Synthetase	352	40,708.88 Da	5.05
11	ERG24	C-14 Sterol reductase	438	50,298.60 Da	8.96
12	ERG25	C-4 Sterol methyl oxidase	293	35,165.57 Da	7.34
13	ERG26	Sterol-4-α-carboxylate3-dehydrogenase	347	38,275.76 Da	7.63

3. RESULTS

Physico-chemical and functional characterisations of 13 target ERG proteins were analysed using Expasy's Prot-param server. The homology modelling of differnt proteins was performed to determine the structural template. ERG 3, ERG 5, ERG 6 showed 00 % of sequence identity with a template in homology modelling (Table 3) and while ERG 1 protein depicted 97.64 % of sequence identity with template A0A1X0QR28.1.A (Table 3).

The analysis of the ERG protein structure provides supporting evidence that the predicted 3D structure of ERG is of good quality The structural stability of all 13 ERG proteins was depicted through Ramachandran phi-psi plot confirming the residues in the favourable region (Figure1; Table 3). The study revealed 99.0 % to 92.24 % of residues in the allowed region (dark green) and only 0.00 % to 1.73 % lay in the (i) disallowed region (light green).

Table 3. Protein validation and homology modelling estimation using the SWISS-MODEL interactive workspace.

S. No.	Protein	Template	Sequence identity (%)	Residues in favourable region (%)	Residues in unfavourable region (%)	C-β deviation	Mol probity score
1.	ERG1	A0A1X0QR28.1.A	97.64	98.06	0.00	1	0.60
2.	ERG2	A0A0A1NVG8.1.A	96.48	95.56	0.44	1	0.81
3.	ERG3	A0A367J0C8.1.A	100	97.37	0.00	0	0.76
4.	ERG5	A0A367JUG4.1.A	100	92.24	3.46	23	1.46
5.	ERG6	A0A1X0S4K6.1.A	100	98.66	0.27	2	0.84
6.	ERG7	A0A2G4SWW2.1.A	100	97.80	0.14	0	0.72
7.	ERG11	A0A2G4SNJ8.1.A	77.28	98.61	0.40	3	0.97
8.	ERG12	A0A367JTM1.1.A	97.68	99.00	0.00	0	0.81
9.	ERG13	A0A1X0RHY0.1.A	99.91	93.64	1.73	5	1.30
10.	ERG20	A0A068S1L5.1.A	84.38	98.86	0.00	0	0.57
11.	ERG24	A0A167JQB3.1.A	70.02	96.55	0.23	0	1.28
12.	ERG25	A0A1X0R3H9.1.A	96.59	98.28	0.34	0	0.70
13.	ERG26	A0A1X0RLI1.1.A	97.69	97.68	0.00	0	0.70

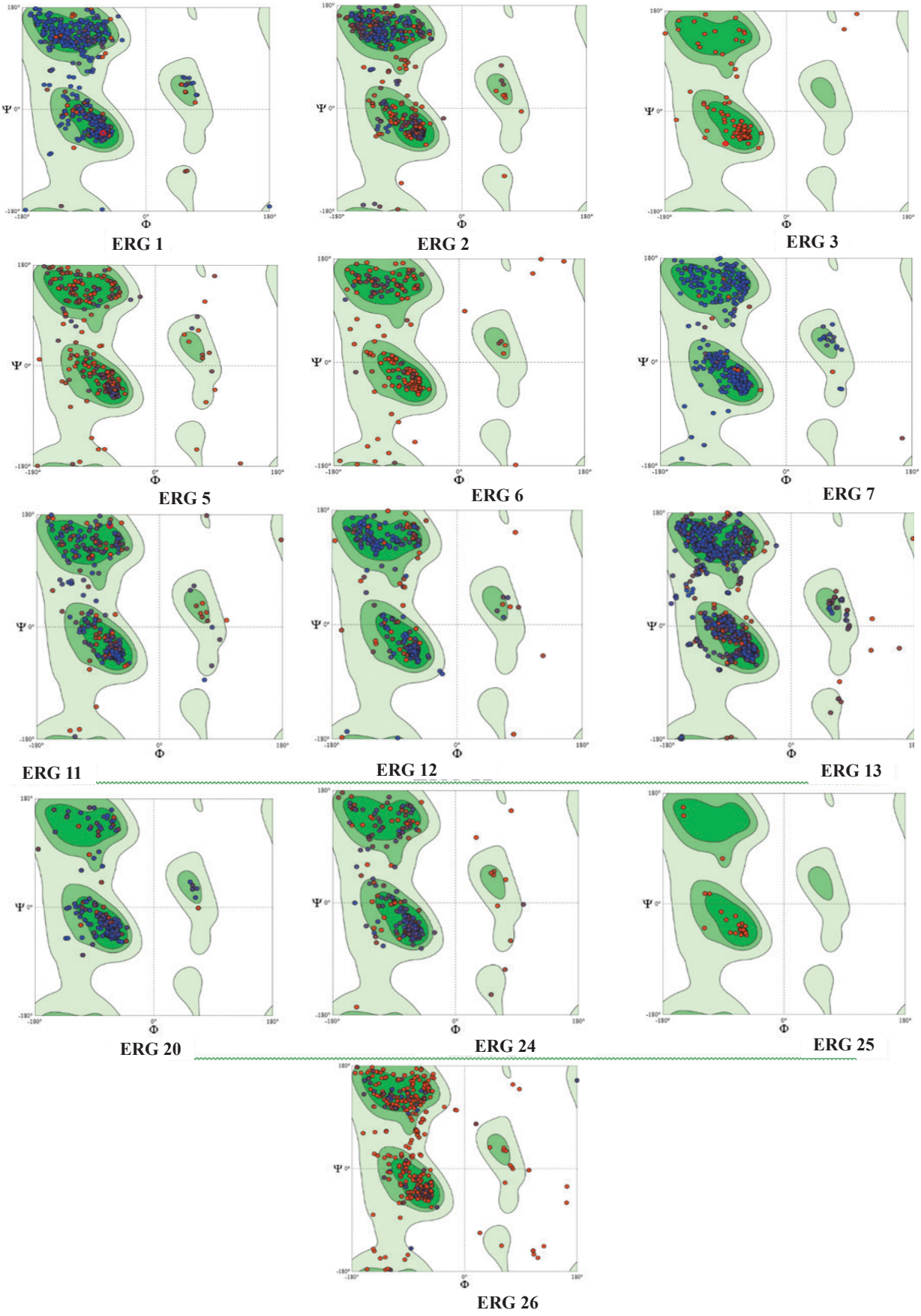


Figure 1. Ramachandran plot of target ERG proteins.

Among the 37 natural compounds derived from *H. rhamnoides*, Saponins exhibited a remarkably strong binding affinity with a docking score of -979.72 kcal/mol against the fungal target ERG5(C-22 desaturase) (Figure 2; Table 4). This high binding energy indicates a robust interaction with the fungal protein, highlighting saponins as promising candidates for antifungal drug development due to their potential efficacy and favourable safety profile[15].

Virginiamycin showed the highest binding affinity with a docking score of -1159.50 kcal/mol against the ERG5 target protein (Table 4b). However, despite their potent antifungal activity, the clinical use of such antibiotics is often limited by significant toxicity and adverse side effects, emphasising the need for safer alternatives[16].

Among the repurposed drug candidates, Danoprevir an antiviral drug displayed a least binding affinity with a docking score of -574.865kcal/mol against ERG5, suggesting significant molecular interaction and antifungal potential of Virginiamycin (Figure 2; Table 4c).

Table 4(a). Binding energies of *H. rhamanoides L.* bioactive compounds against key ergosterol biosynthesis (ERG) proteins in *R. azygosporus.*

S. No.	Ligands	Binding energy	Target ERG gene	Target ERG protein
1.	Sesquiterpenes	-177.471	ERG5	C-22 Sterol Desaturase
2.	Monoterpenes	-127.805	ERG5	C-22 Sterol Desaturase
3.	Chromene (2H-1-benzopyran-2-yl) radical	-116.014	ERG7	Lanosterol Synthase
4.	Obtusol	-373.928	ERG5	C-22 Sterol Desaturase
5.	Saponins	-979.72	ERG5	C-22 Sterol Desaturase
6.	Tuberoside	-725.927	ERG5	C-22 Sterol Desaturase
7.	Isoflavone	-196.767	ERG5	C-22 Sterol Desaturase
8.	Quercetin	-253.578	ERG5	C-22 Sterol Desaturase
9.	Kaempferol	-242.231	ERG7	Lanosterol Synthase
10.	Isohamnetin	-250.021	ERG6	Sterol 24-C Methyltransferase
11.	Rutin	-510.737	ERG7	Lanosterol Synthase
12.	Myricetin	-253.927	ERG6	Sterol 24-C Methyltransferase
13.	Resveratrol	-179.651	ERG2	C-8 Sterol Isomerase
14.	Naringenin	-230.429	ERG5	C-22 Sterol Desaturase
15.	Hesperidin	-495.754	ERG7	Lanosterol Synthase
16.	Eriodictyol	-242.24	ERG24	C-14 Sterol Reductase
17.	Homoeriodictyol	-253.517	ERG7	Lanosterol Synthase
18.	Apigenin	-122.165	ERG7	Lanosterol Synthase
19.	Luteolin	-242.341	ERG7	Lanosterol Synthase
20.	Tangeretin	-311.107	ERG5	C-22 Sterol Desaturase
21.	L- Epicatechin gallate	-227.579	ERG7	Lanosterol Synthase
22.	Chlorogenic acid	-270.568	ERG26	Sterol 4-Alpha Carboxylate3Dehydrogenase
23.	Coumaroylquinic acid	-280.957	ERG24	C-14 Sterol Reductase
24.	Catechins	-241.98	ERG7	Lanosterol Synthase
25.	L- Epigallocatechin	-255.921	ERG24	C-14 Sterol Reductase
26.	Catechin 3'-O-gallate	-371.689	ERG24	C-14 Sterol Reductase
27.	Proanthocyanidins	-495.497	ERG5	C-22 Sterol Desaturase
28.	Cyanidin	-242.31	ERG24	C-14 Sterol Reductase
29.	Delphinidin	-254.039	ERG5	C-22 Sterol Desaturase
30.	Malvidin	-276.573	ERG5	C-22 Sterol Desaturase
31.	Peonidin	-260.212	ERG6	Sterol 24-C Methyltransferase
32.	Petunidin	-277.2	ERG7	Lanosterol Synthase
33.	Beta carotene	-91.342	ERG11	14-Alpha Sterol Demethylase A
34.	Caffeic Acid	-75.646	ERG11	14-Alpha Sterol Demethylase A
35.	Ferulic Acid	-74.678	ERG11	14-Alpha Sterol Demethylase A
36.	α-Linolenic acid	-92.414	ERG9	C-5 Sterol Desaturase
37.	Zeaxanthin	-100.014	ERG7	Lanosterol Synthase

Table 4(b). Binding energies of antifungal drugs against key ergosterol biosynthesis (ERG) proteins in *R. azygosporus.*

S. No.	Ligands	Binding energy	Target ERG gene	Target ERG protein
1.	Amphotericin B	-744.073	ERG24	C-14 Sterol Reductase
2.	Posaconazole	-579.618	ERG5	C-22 Sterol Desaturase
3.	Isavuconazole	-359.964	ERG7	Lanosterol Synthase
4.	Voriconazole	-291.287	ERG5	C-22 Sterol Desaturase
5.	Flucytosine	-111.732	ERG5	C-22 Sterol Desaturase
6.	Anidulafungin	-870.322	ERG5	C-22 Sterol Desaturase
7.	Clarithromycin	-552.294	ERG7	Lanosterol Synthase
8.	Virginiamycin	-1159.5	ERG5	C-22 Sterol Desaturase
9.	Tunicamycin	-686.218	ERG5	C-22 Sterol Desaturase
10.	Caspofungin	-807.501	ERG7	Lanosterol Synthase
11.	Itraconazole	-533.916	ERG6	Sterol 24-C Methyltransferase
12.	Micafungin	-100.24	ERG6	Sterol 24-C Methyltransferase

Table 4(c). Binding energies of antivirals, RTI, ACE inhibition, and iron chelators against key ergosterol biosynthesis (ERG) proteins in *R. azygosporus*.

S. No.	Ligands	Binding energy	Target ERG gene	Target ERG protein
1.	Baloxavir marboxil	-442.128	ERG24	C-14 Sterol Reductase
2.	Danoprevir	-547.865	ERG26	Sterol 4-Alpha Carboxylate3Dehydrogenase
3.	Sofosbuvir	-425.9	ERG5	C-22 Sterol Desaturase
4.	Fosinopril	-435.559	ERG2	C-8 Sterol Isomerase
5.	Quinapril	-350.613	ERG5	C-22 Sterol Desaturase
6.	Telmisartan	-427.216	ERG5	C-22 Sterol Desaturase
7.	Sulfamethoxazole	-196.277	ERG5	C-22 Sterol Desaturase
8.	Deferoxamine	-413.16	ERG5	C-22 Sterol Desaturase
9.	Deferasirox	-318.051	ERG7	Lanosterol Synthase

The ADMET properties are absorption, distribution, metabolism, excretion, and toxicity. Further, solubility, dissolution, and permeability across the GI barrier are important factors for drug absorption[17]. Table 5 represents the findings of drug parameters assessed to determine the feasibility and stability of ligands.

4. DISCUSSION

The study depicted the promising potential of Seabuckthorn active constituents against *R. azygosporus*.

Among the phytochemicals tested, saponins emerged as the top candidate, showing a binding energy of -979.72 kcal/mol against the fungal target ERG5(C-22 desaturase). This strong interaction suggests that saponins may inhibit fungal growth by disrupting membrane biosynthesis, thus compromising fungal cell viability. The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties revealed the strengths and limitations of saponins from *H. rhamanoides* L. It has poor water solubility and a high molecular weight, which result in low absorption through the gastrointestinal tract, suggesting it is unsuitable for oral or systemic use[18]. However, saponin shows a favourable safety profile suggesting its non-toxic, non-mutagenic behaviour and non-interference with liver enzymes such as the cytochrome P450 family, making it a promising candidate for drug discovery[19]. The saponins showed inability to cross the blood-brain barrier, rendering their safe application against fungal infections. Given these properties, saponin may be best suited for non-oral delivery systems like topical creams, gels, or nano-formulations, which can improve local absorption and effectiveness without causing systemic side effects[20].

C-27 steroidal saponins have demonstrated potential antifungal activity against *Cryptococcus neoformans*, and *Aspergillus fumigatus*, suggesting the futuristic investigations on exploring the application of saponins against fungal diseases[21]. Likewise, previous study has also report antifungal activity of plant based active compounds viz., iscisoflavone C, 8-o-methylaverufin and Punicalagin against glucoamylase enzyme of *Rhizopus oryzae.*[22]

Virginiamycin, an antibiotic, exhibited the highest binding affinity among all tested compounds, with a docking score of -1159.50 kcal/mol against ERG5 (C-22 desaturase). However, despite this promising docking result, antibiotics like Virginiamycin are often associated with significant health risks, including toxicity, disruption of beneficial microbiota, and the potential for resistance development[23]. These adverse effects limit their suitability for long-term or preventive antifungal applications in humans.

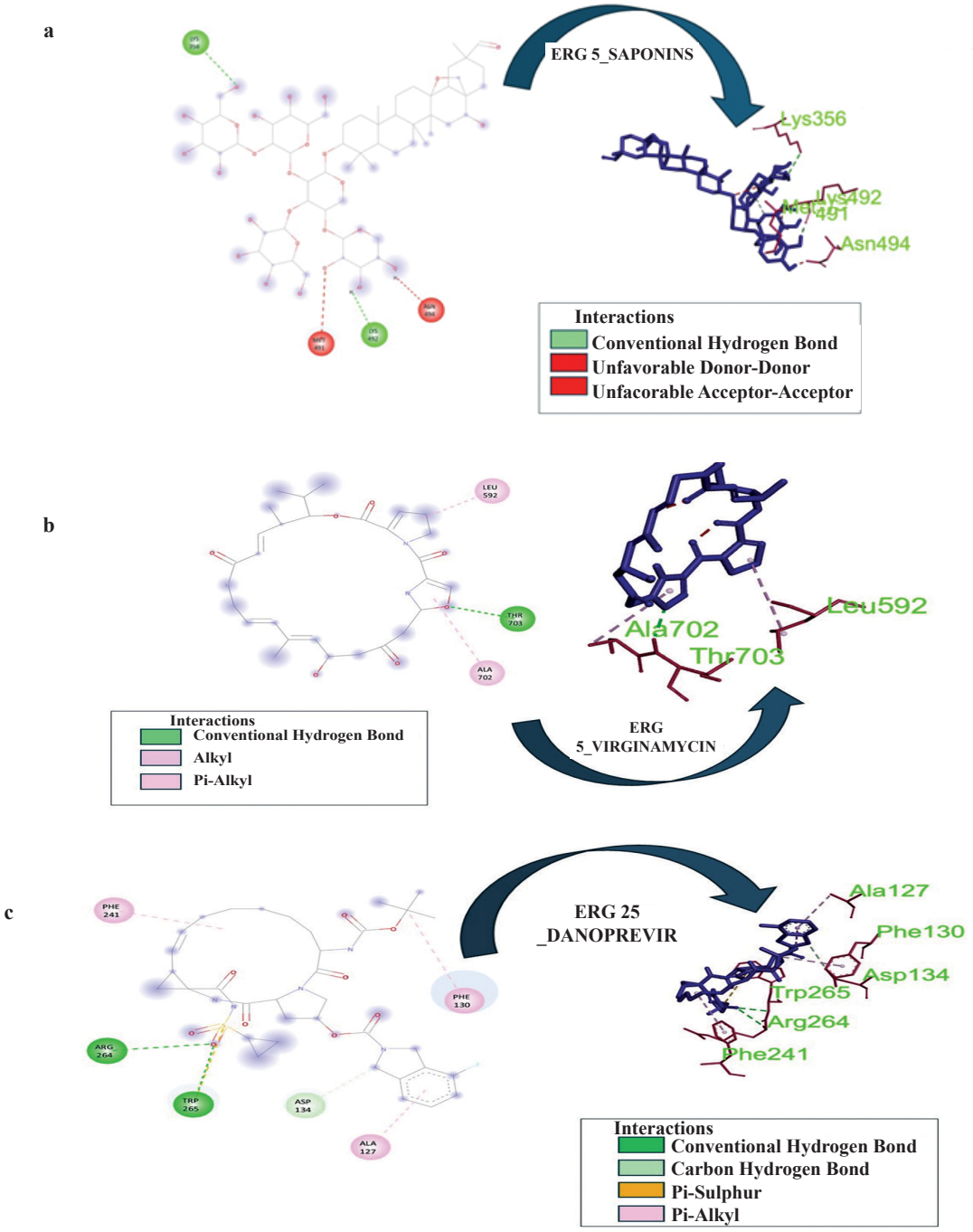


Figure 2. Molecular docking images of some efficient interaction between ERG protein and ligands: (a.) Saponin- Bioactive compound of *H. rhamanoides L.*, (b.) Virginiamycin - antifungal drug, (c.) Danoprevir – an antiviral drug.

In the present study, non-antifungal drugs like antivirals, RTI agents, ACE inhibitors, blood thinners, and iron chelators were also explored for their promising drug repurposing potential against mucormycosis management. These agents may help reduce inflammation, prevent blood clots, or limit iron availability essential for fungal growth[24-25]. Their inclusion supports a drug repurposing approach through molecular docking. Danoprevir, originally developed as an HCV NS3/4A protease inhibitor[26] was evaluated for antifungal activity against ERG26 (C-3 sterol dehydrogenase). It demonstrated a moderate binding affinity with ERG26, and a docking score of -547.87 kcal/mol. This interaction indicates a potential for disrupting fungal sterol metabolism, contributing to antifungal efficacy. ADMET profiling of Danoprevir showed moderate gastrointestinal absorption, high plasma protein binding, and favourable toxicity parameters, although it does inhibit the CYP3A4 liver enzyme. These properties suggest that Danoprevir could be used as a treatment for fungal infections throughout the body. However, understanding the mechanism of CYP3A4 inhibition, and thorough clinical management is required for its future application[27].

Table 5. ADMET analysis of *H. rhamanoides* L. bioactive compounds, antifungal drugs, and repurposed drug candidates

S. No.	Ligand name	Water solubility	GI absorption	BBB permeability	CYP450 inhibition	AMES toxicity
1.	Sesquiterpenes	Low	High	Yes	No	Non-toxic
2.	Monoterpenes	Low	High	Yes	No	Non-toxic
3.	Chromene (2H-1-benzopyran-2-yl) radical	Moderate	High	Yes	No	Non-toxic
4.	Obtusol	Very low	Low	Yes	No	Non-toxic
5.	Saponins	Very low	Low	No	No	Non-toxic
6.	Tuberoside	Very low	Low	No	No	Non-toxic
7.	Isoflavone	Low	High	Moderate	Weak inhibitor	Non-toxic
8.	Quercetin	Low	Low	No	Inhibits CYP1A2, 3A4	Non-toxic
9.	Kaempferol	Low	Moderate	No	Inhibits CYP1A2	Non-toxic
10.	Isohamnetin	Low	Moderate	No	Inhibits CYP1A2, 3A4	Non-toxic
11.	Rutin	Very low	Low	No	No	Non-toxic
12.	Myricetin	Low	Low	No	Inhibits CYP1A2	Non-toxic
13.	Resveratrol	Moderate	High	Yes	Weak CYP1A2 inhibitor	Non-toxic
14.	Naringenin	Low	Moderate	No	Inhibits CYP3A4	Non-toxic
15.	Hesperidin	Very low	Low	No	No	Non-toxic
16.	Eriodictyol	Low	Moderate	No	Weak CYP inhibition	Non-toxic
17.	Homoeriodictyol	Low	Moderate	No	Possible CYP inhibitor	Non-toxic
18.	Apigenin	Low	High	No	Inhibits CYP1A2	Non-toxic
19.	Luteolin	Moderate	High	No	Inhibits CYP1A2	Non-toxic
20.	Tangeretin	Low	High	No	Inhibits CYP3A4	Non-toxic
21.	L- Epicatechin gallate	Low	Moderate	No	Inhibits CYP3A4	Non-toxic
22.	Chlorogenic acid	Moderate	High	No	Weak CYP inhibition	Non-toxic
23.	Coumaroylquinic acid	Moderate	High	No	No data	Non-toxic
24.	Catechins	Moderate	High	No	Inhibits CYP3A4	Non-toxic
25.	L- Epigallocatechin	Moderate	High	No	Inhibits CYP3A4	Non-toxic
26.	Catechin 3'-O-gallate	Low	Moderate	No	Inhibits CYP3A4	Non-toxic
27.	Proanthocyanidins	Low	Low	No	No data	Non-toxic
28.	Cyanidin	Moderate	Moderate	No	No data	Non-toxic
29.	Delphinidin	Moderate	Moderate	No	No data	Non-toxic
30.	Malvidin	Moderate	Moderate	No	No data	Non-toxic
31.	Peonidin	Moderate	Moderate	No	No data	Non-toxic
32.	Petunidin	Moderate	Moderate	No	No data	Non-toxic
33.	Beta carotene	Very low	High (oral)	Yes (likely)	No significant inhibition	Non-toxic
34.	Caffeic Acid	Moderate	High	No	Weak CYP inhibition	Non-toxic
35.	Ferulic Acid	Moderate	High	No	Weak CYP inhibition	Non-toxic
36.	α-Linolenic acid	Very low	High	Yes (likely)	No	Non-toxic
37.	Zeaxanthin	Low	Moderate	Limited	No	Non-toxic
38.	Amphotericin B	Very low	Low	No	No	Non-toxic
39.	Posaconazole	Very low	Moderate	No	Strong CYP3A4 inhibitor	Non-toxic
40.	Isavuconazole	Low	High	Moderate	Inhibits CYP3A4	Non-toxic

41.	Voriconazole	Moderate	High	Yes	Inhibits CYP2C19, 2C9, 3A4	Non-toxic
42.	Flucytosine	High	High	No	No	Non-toxic
43.	Anidulafungin	Very low	Low (IV only)	No	No	Non-toxic
44.	Caspofungin	Very low	Low (IV only)	No	No	Non-toxic
45.	Itraconazole	Very low	Moderate	No	Strong CYP3A4 inhibitor	Non-toxic
46.	Micafungin	Very low	Low (IV only)	No	No	Non-toxic
47.	Baloxavir marboxil	Low	Moderate	No	No	Non-toxic
48.	Danoprevir	Low	Moderate	No	Inhibits CYP3A4	Non-toxic
49.	Sofosbuvir	Moderate	High	No	No	Non-toxic
50.	Fosinopril	Moderate	High	No	No	Non-toxic
51.	Quinapril	Moderate	High	No	No	Non-toxic
52.	Telmisartan	Low	Moderate	No	No	Non-toxic
53.	Sulfamethoxazole	Moderate	High	No	Weak CYP2C9 inhibitor	Toxic
54.	Clarithromycin	Low	High	No	Inhibits CYP3A4	Non-toxic
55.	Virginiamycin	Very low	Low	No	No	Non-toxic
56.	Tunicamycin	Very low	Low	No	No	Non-toxic
57.	Deferoxamine	High (very polar)	Low	No	No	Non-toxic
58.	Deferasirox	Low	High	No	Inhibits CYP1A2, 2C9	Non-toxic

Henceforth, based on the *in-silico* analyses of phytochemicals, antifungal and other drugs targeting proteins of ergosterol biosynthesis pathway in *R. azygosporus,* Saponins from Seabuckthorn, Virginiamycin (an antifungal drug) the Danoprevir (antiviral drug) are the recommended candidates to be further evaluated for their *in vitro* inhibitory potential against causative agents of mucormycosis. Determining their minimum inhibitory concentration, mechanism of action and subsequent clinical studies will be essential to validate the safety and therapeutic application of these compounds.

5. CONCLUSION

Saponins from *H. rhamanoides* emerged as the most promising natural antifungal candidate, showing the strongest binding affinity among the tested compounds. Although Virginiamycin exhibits a strong binding affinity, its known adverse effects on human health limit its clinical application. Danoprevir, originally an antiviral drug, also showed moderate antifungal potential and could be explored further for systemic treatment options. These findings support the continued investigation of both plant-based and repurposed compounds in the development of safer and more effective antifungal therapies.

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