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Molecular Docking Studies of Phytochemicals Against RNA-dependent RNA Polymerase of Mucormycosis

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Abstract: Background: Mucormycosis, also referred to as black fungus, is a fatal Angio invasive fungal infection caused by a colony of molds known as mucoromycetes. *Rhizopus oryzae* is a major fungus that is responsible for almost 70% of the total mucormycosis cases. RNA-dependent RNA polymerase (RdRp), is a crucial enzyme in the RNA polymerization mechanism in various species, including *R. oryzae*. In the past, inhibiting this enzyme has been found to be a viable technique for eradicating viral infections. This research aims to identify efficacious bioactive compounds by screening antifungal phytochemicals against the RNA-dependent RNA polymerase (RdRp) protein using a bioinformatic approach to develop an effective treatment for mucormycosis. Methods: The antifungal activity of various phytochemicals against the RNA-dependent RNA polymerase (RdRp) protein was studied using in silico screening. Using the Swiss ADME online server, phytochemicals with proven antifungal properties were assessed to predict the pharmacokinetic aspects and drug-like nature. Furthermore, Molecular docking and toxicity analysis was performed using PyRx and ProTox webserver tools respectively. Results: Among the 1000 antifungal phytochemicals chosen to be evaluated against RdRp, 209 molecules were shortlisted for further studies. The binding affinity scores revealed that Dregamine (-11.1 kcal/mol), Alantolactone (-9.5), Isoalantolactone (-9.5) and Solasodine (-9.5) exhibited the lowest energy value, indicating a strong binding affinity against RdRp. Conclusion: Eventually, the most promising analogues can be further synthesized and evaluated to confirm their actual antifungal activity, allowing them to be used as potent bioactive compounds in the treatment of mucormycosis.

Keywords: Mucormycosis, In Silico Screening, Phytochemicals, RNA Dependent RNA Polymerase (RdRp) Protein, Antifungal

1. Introduction

Mucormycosis, formerly known as “zygomycosis”, is a fungal infection and a rare illness that generally appears after the appearance of natural catastrophes such as tsunamis and hurricanes [1]. Mucormycosis, also referred to as black fungus, is a fatal angioinvasive fungal infection generally caused by a colony of molds known as mucoromycetes [2]. Mucormycosis is more common in cancer patients and people with diabetes with uncontrolled blood sugar levels [3-5]. Mucormycosis is a disease that also affects people who have had organ transplants [3, 4]. The rate of fatality exceeds 40%, and it varies based on the ailments involved (reaching up to 100% in haematological malignancies) [6]. The fungus belonging to Mucorales order causes mucormycosis, with *Rhizopus oryzae* accounting for 70% of all recorded infections [7].

RNA-dependent RNA polymerase (RdRp), is a critical enzyme in the RNA polymerisation mechanism in various species, including *R. oryzae*. In the past, inhibiting this enzyme has been found to be a viable technique for eradicating viral infections. The RNA-dependent RNA

polymerase (RdRp), also termed nsp12, is an important component of the virus replication and transcription complex that controls viral RNA replication and transcription. With the help of other cofactors, nsp7 and nsp8, Nsp12 has high polymerase activity, but it has low or no catalytic activity on its own. As a result, the most basic component required for virus RNA replication in *R. oryzae* is described as nsp12-nsp7-nsp8 [8]. The catalytic core of the RdRp protein is structured like a human right hand, with distinct palm, fingers, and thumb motifs. Its molecular weight ranges between 240 to 450 kDa [9]. RdRp plays an integral part in the life cycle of a pathogen, and since RdRp's active site is the most conserved and available region, inhibiting viral replication by targeting this region could be a successful treatment strategy.

With the rapid advancement of technology, new algorithms and high-performance systems are viewed as a gateway to the computational biophysics area [10]. However, because the structure for *R. oryzae* has yet to be determined, RdRp homology modelling generates the *R. oryzae* RdRp 3D structural model. Molecular docking is an in-silico technique that has gained a lot of interest in the recent years [11]. Understanding the method of action of enzymes is made more accessible by emulating the behavior of biopolymers in binding the ligands. It also aids in the discovery of novel medication candidates that stop a target pathogenic protein from acting [12]. The objective of the present study is to predict promising inhibitors against Mucormycosis that will specifically bind to the RdRp protein. The analysis was performed using the PyRx tool. In vitro/in silico testing procedures can synthesize and analyse the most promising treatments. There are now relatively few effective medications available to treat the debilitating and life-threatening condition of mucormycosis, and the research presented here is an attempt to fulfil the urgent need to identify more potent analogues of phytochemicals.

2. Materials and Methods

2.1. Protein Retrieval

The sequence of *R. oryzae* RNA-dependent RNA polymerase (RdRp) (GenBank: BAH03542.1 region: RVT 1) was retrieved from NCBI server. The sequence was retrieved and utilized to construct a 3D protein model with the help of SWISS-MODEL.

2.2. Energy Minimization

The constructed protein structure was stabilized using the dock prep setup in discovery studio and Chimera software, respectively, prior to the docking studies. Dock preparation is a phase of optimization that corrects atomic and bond lengths, structure, and charge abnormalities. The protein's original inhibitors and water molecules were removed, and any missing hydrogen atoms were added [13]. Since the drawn chemical structures are not energetically beneficial, energy minimization is critical for establishing the right molecule arrangement.

2.3. Ligand Selection

Dr Duke's Phytochemical and Ethnobotanical databases were used to yield 1000 phytochemicals with specifically antifungal properties. The 2D structures of the 1000 phytochemicals were obtained from PubChem, along with their associated CIDs.

2.4. Pharmacokinetic and Pharmacological Properties Investigation

The Swiss ADME online tool was used to predict pharmacokinetic properties, namely ADME, bioavailability, drug-likeness, and the medicinal chemistry of ligands and hence evaluate them as potential lead molecules [2]. For the evaluation, this tool used the conventional SMILES string for each molecule. To detect drug-likeness, the technology estimates bioavailability radar based on six physicochemical properties: lipophilicity, size, polarity, solubility, versatility and saturation. The Swiss ADME web server visualized ADME profiles [14]. The molecules were filtered using a set of criteria, including 0 Lipinski violations, a molecular weight of 500 Da or fewer, strong lipophilicity (a Log P value of less than 5), hydrogen bond acceptors of less than 10, and H-bond donors of less than 5.

2.5. Prediction of Bioavailability and Toxicity of the Ligands

The drug-likeness criteria are linked to the water solubility and permeability characteristics, which define the initial stage of oral bioavailability. The bioavailability radar uses six physicochemical qualities to quickly assess a molecule's drug-likeness: saturation, lipophilicity, polarity, size, solubility, and flexibility. The molecules are intended to be orally accessible (low flexibility and polarity), less toxic, and absorption easily [15]. Chemical toxicity prediction is a critical stage in the pharmaceutical development process. Computational toxicity predictions not only make finding harmful doses in animals easier, but they also reduce the number of animal tests [16]. The toxicity of the specified phytochemicals was predicted using the ProTox website. To predict probable toxicities associated with the identified compounds, SMILES (Simplified Molecular-Input Line-Entry System) strings were used. The following factors were considered for predicting the toxicity: Toxicity class (oral/acute toxicity), estimated LD50, hepatotoxicity, carcinogenicity, immunotoxicity, and mutagenicity.

2.6. Molecular Docking

The PyRx tool was used to conduct docking analysis on the phytochemicals that were shortlisted. This was accomplished by uploading phytochemicals and receptor molecules in .pdb file format to the PyRx server and running the task. Once the protein was imported in PyRx, the ligands were added using the open babel widget to convert them into pdbqt format. Energy minimization was used to alter the

molecule to ligand_uff_e=194.360 after adding the ligands to the open babel. The _uff portion refers to the energy minimization force field, which is the universal force field by default. The energy of the minimized molecules is represented by the _E=194.360 portion [9].

Ligands that were transformed into pdbqt format were selected under the vina wizard tab, and the vina space's maximize button was clicked, which initiated Auto dock vina and further docked each ligand. The vina wizard uses a stochastic gradient optimization method to estimate the binding affinities between ligands and receptors. PyRx advances to the Analyze Results page when virtual screening is done, where the results of the virtual screening computation are displayed. The results of binding affinity were downloaded and saved in a CSV file. To study potential receptor-ligand interactions, the best pose with the lowest binding energy score was chosen and assessed in Discovery studios 2021. The docked complex structure output formats were utilised to visualize the 2D interaction of the docked complexes using Biovia Discovery Studio Visualizer 2021.

3. Results and Discussion

Recent observations have shown that individuals with immense invulnerable health situations as a result of Covid-19 have diabetes or unmanageable sugar are infected with a disease caused by "black fungus". Antifungal drugs might also be employed as fungal infection inhibitors by targeting RNA Dependent RNA Polymerase (RdRp) [13]. As a result, targeting RdRp might provide a novel active antifungal strategy for treating black fungus. This work aims to evaluate the therapeutic potential of a key enzyme i.e RNA-dependent RNA polymerase (RdRp), found in the process of RNA polymerization in various species, which also includes R. oryzae.

The high incidence of errors during replication is the primary explanation for the function of RNA-dependent RNA polymerases (RdRps) in viral development. This is related to the absence of activity of a proofreading exonuclease. Some variations are chosen under the stresses exerted by host defense systems and other environmental variables due to the greater mutation rates in the offspring virus population. Furthermore, RdRp's strand swapping during replication allows for recombination, which allows for gene rearrangement or the acquisition of novel genes from other viruses or hosts [17].

3.1. Evaluation of Pharmacokinetic and Pharmacological Properties

The drug-likeness of the compound was determined using Lipinski's rule of five. It predicts drug absorption or penetration based on hydrogen donor-acceptor bonds, molecular weight, and lipophilicity [18]. One of the most cost-effective ways to save money during drug development is to predict pharmacokinetic qualities and ADME (absorption, distribution, metabolism, and excretion) aspects of therapeutic drugs beforehand. Apart from that, it primarily aids in the reduction of possible risk during clinical trials by providing critical information for determining if a chemical is suitable for further clinical testing [2]. For improved selectivity and drug-like physiological qualities, the molecule's properties must follow the rule. The molecule should have a molecular weight <500 Da, Log P<5, i.e., high lipophilicity, H bond acceptors <10, and H bond donors <5 [19]. When these conditions are breached, the interaction between the drug molecule and the lipophilic cell membrane is violated. Smaller, more lipophilic molecules have higher permeability. Passive diffusion is also favored when the drug molecule has a little positive charge because it provides a better impact on the cell membrane [20]. SWISS-ADME (http://www.swissadme.ch) was used to assess better ADME and other pharmacokinetic properties of the shortlisted compounds. It was revealed that among the 1000 antifungal phytochemicals chosen to be evaluated against RdRp by molecular docking, 209 compounds were potential lead molecules. The pharmacokinetic calculations suggest that antifungal phytochemicals evaluated in the present study were found to be biologically safe and can be utilized as therapeutics against Mucormycosis. More research is needed, however, to determine the specific mechanism behind the interaction between these phytochemicals and receptor proteins.

3.2. Bioavailability Radar and Toxicity Prediction

Following the ADME study, 209 compounds were chosen for further study. Bioavailability Radar is accessible for a swift assessment of drug-likeness. Bioavailability radar, an expository tool that considers six physicochemical parameters to determine the drug-likeness of a substance, was used for improved elucidation and comprehensive study. The six parameters assessed are polarity, lipophilicity, size, flexibility, solubility, and saturation. The solubility property of the drug is essential for absorption since it must be readily soluble in water to deliver an appropriate number of dynamic components in small dosages [21]. Low aqueous solubility translates to low saturation solubility, leading to poor bioavailability. A drug's lipophilicity must be high enough to interact with the lipid membrane [22]. On each axis, a physicochemical range is indicated as a pink region in which the molecule's radar plot must fall completely to be regarded to have potential drug-likeness [21]. Because they fitted the suggested characteristics, 79 molecules out of the 206 under study were revealed to be orally bioavailable.

In silico drug or lead molecule screening offers several benefits over in vitro or in vivo techniques regarding time and resources. During drug development, the most essential concern is still safety, which includes a variety of dangers and potential side effects that must be explored during preclinical and clinical testing [23]. To minimize large financial losses later in the drug development phase, the toxicity of the lead compounds must also be investigated early in the process [24]. PROTOX -II webserver was used for the evaluation of toxicities present in the following shortlisted molecules.

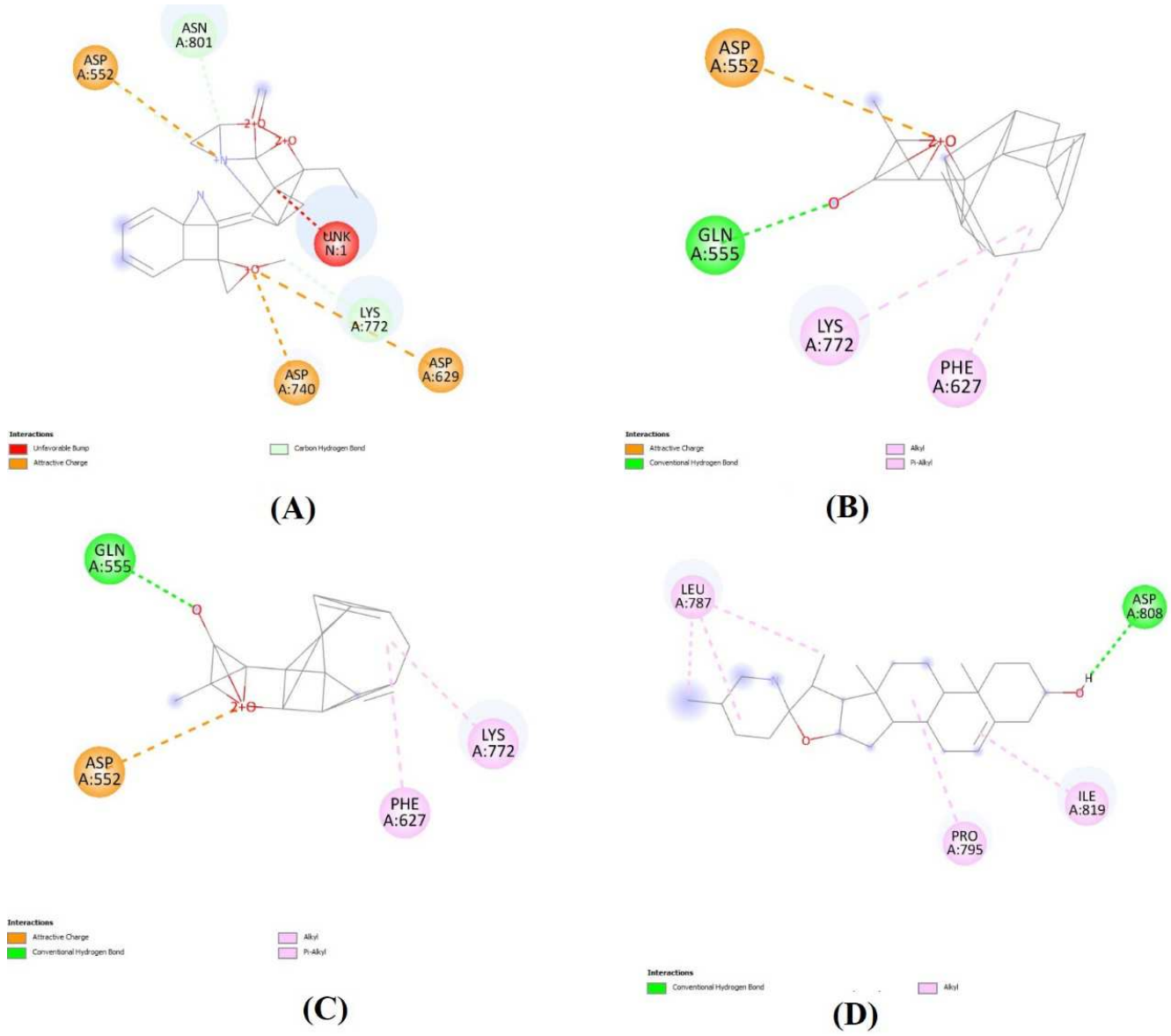

Figure 1. *2D Interactions of Mucormycosis protein with: (A) Dregamine (B) Alantolactone (C) Isoalantolactone (D) Solasodine.*

3.3. Molecular Docking

Antifungal phytochemicals have been demonstrated in several studies to work by binding to particular components of the fungal plasma membrane, metabolic pathways, and even cell wall components [25]. So, the binding energy for each of the 1000 phytochemicals was calculated. Following this, Tanshinone was shown to have the most conventional hydrogen bonds out of all 1000 phytochemicals studied. During docking studies, all the phytochemicals had significant binding energies, indicating that antifungal phytochemicals can effectively cure black fungus. As a result, additional study into whether it has antifungal capabilities against black fungus is needed. The larger the negative value of the docking score, the better is the docking affinity and ligand docking. The docking affinity and ligand docking are better when the negative value of the docking score is more significant. Taking into consideration the best five ligands, the binding affinity ranged from -11.8 to -9.8.

Degramine is a natural monoterpene indole alkaloid first found in wild frangipani [26]. It showed a binding energy of -11.8 kcal/mol which was the lowest of all. Hence, Dregamine can be considered a better molecule as compared to other ligands. It offers 3 types of interactions, Carbon-Hydrogen bond, unfavorable positive-positive bond, and attractive charge bond. LYS A:772 formed a carbon-hydrogen bond. UNK N:1 formed an unfavorable positive positive bond and ASP A:552, ASP A:740, ASP A:629 formed attractive charged bonds. Refer to Figure 1(A).

Alantolactone is a sesquiterpene lactone and occurs in the roots of Inula helenium and other Inula species. Its variety of in vitro biochemical properties include inducing apoptosis, suppressing STAT3 activation, and anti-inflammatory effects by inhibition of chemokine [27]. The binding energy that was

exhibited by Alantolactone was -9.5 kcal/mol. It has four types of interactions altogether: conventional hydrogen bond, alkyl, and pi-alkyl bonds, attractive charge. GLN A:555 is the residue involved in the formation of a conventional hydrogen bond. LYS A:772 and PHE A:627 formed alkyl and pialkyl bonds. ASP A:552 formed an attractive charge. Refer to Figure 1(B).

Isolantolactone is a sesquiterpene lactone that was discovered in various inula plants and was initially isolated from Inula helenium [28]. It is the most essential and significant bioactive component. It has hepatoprotective characteristics and is toxic to leukocytes in vitro cultures. It also exhibits anti-inflammatory, anticancer, and antifungal properties [29]. Isolantolactone has a binding energy of -9.5 kcal/mol. It comprises 4 types of interactions: a conventional hydrogen bond, an attractive charge, an alkyl, and a pi-alkyl bond. The residue involved in the formation of a conventional hydrogen bond was GLN A:555. Alkyl and pi-alkyl bonds were produced by LYS A:772 and PHE A:627. The attractive charge of ASP A:552 was formed. Refer to Figure 1(C).

Solasodine is a steroid alkaloid sapogenin and oxaspiro compound discovered in the solanum family. It's considered as an important precursor for the synthesis of complex steroid hormones. It tends to possess anticancer, anti-inflammatory, and antibacterial properties [30]. Solasodine has a binding energy of -9.5 Kcal/mol. It demonstrates two forms of interactions, conventional hydrogen bond and alkyl bond. LEU A:787, PRO A:795, and ILE A:819 produce alkyl bonds, whereas ASP A:808 aids in the formation of conventional hydrogen bonds. Refer to Figure 1(D).

Considering bioavailability radar (Figure 2) and toxicity profiles (Table 1), it can be inferred that the ligands listed above can be used to develop solutions for the treatment of Mucormycosis since they are non-toxic and have greater LD50 values when compared to other ligands. On the other hand, ligands such as Atractylenolide III; Emodin; Ginkgolides, ginkgolide B; Withaferin A are although bioavailable, cannot be considered since they are poisonous and hence inappropriate for oral intake. Dregamine is bioavailable and may be regarded as safe for the treatment of Mucormycosis; nevertheless, because it belongs to Class 5 toxicity and can be carcinogenic if taken in excess, the dose must be carefully monitored. To make these drugs as prospective lead compounds for therapeutic development, a thorough analysis is required to lower their toxicity.

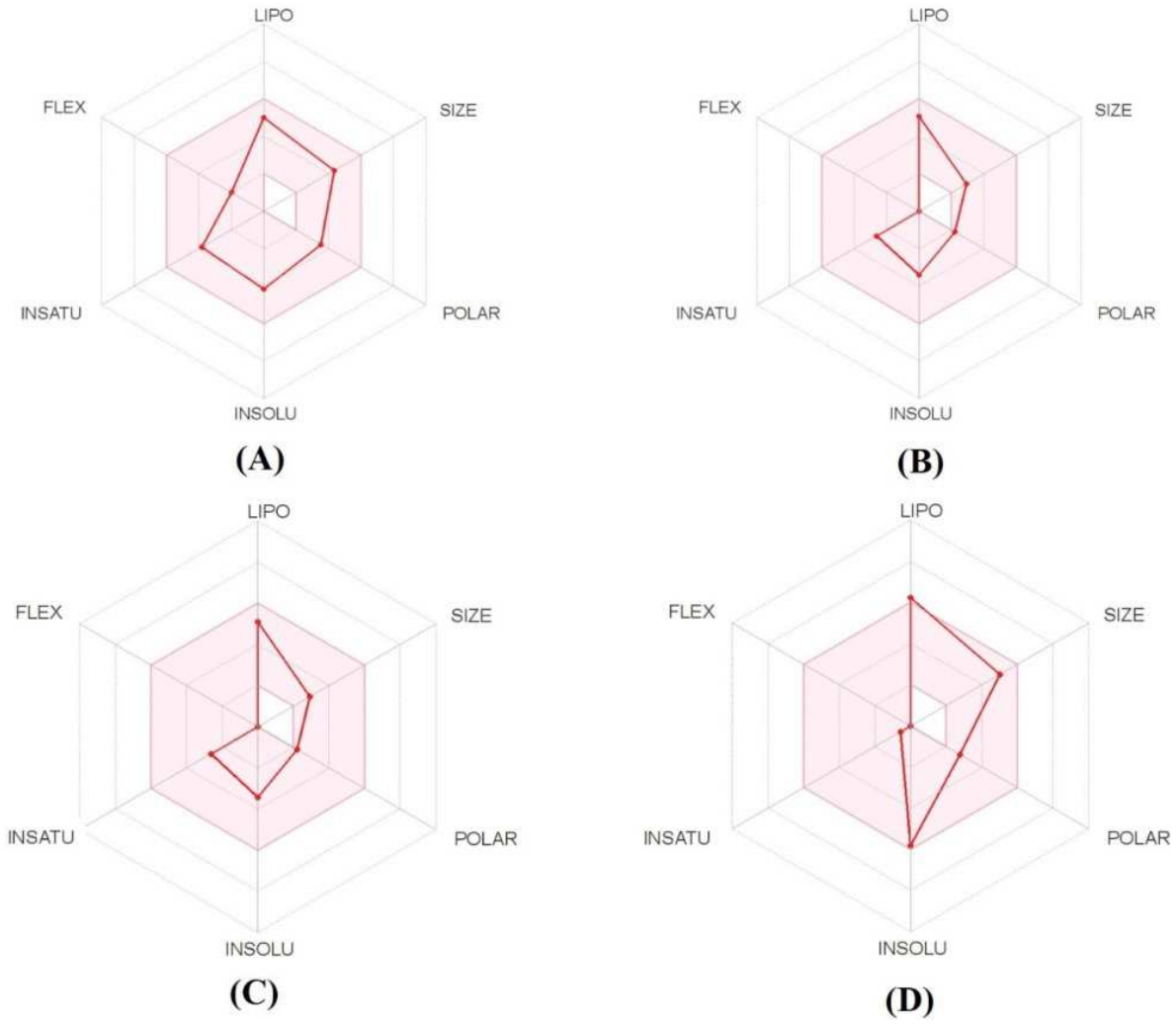

Figure 2. *Biavailablity radar of (A) Dregamine (B) Alantolactone (C) Isoalantolactone (D) Solasodine.*

Table 1. *Binding affinity and toxicity profile of the ligands.*

Name	Binding affinity	Predicted LD50 score (mg/kg)	Toxicity class	Hepatotoxicity
Dregamine	-11.1	2500mg/kg	5	Inactive
Alantolactone	-9.5	5000mg/kg	5	Inactive
Isoalantolactone	-9.5	3424mg/kg	5	Inactive
Solasodine	-9.5	28mg/kg	2	Inactive

Table 1. *Continued.*

Name	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Dregamine	Active	Inactive	Inactive	Inactive
Alantolactone	Active	Active	Inactive	Inactive
Isoalantolactone	Inactive	Active	Inactive	Active
Solasodine	Inactive	Active	Inactive	Inactive

4. Conclusion

Mucormycosis is a fungal illness that spreads quickly. It is caused due to a family of molds belonging to the Mucorales order. It is a life-threatening infection that affects people who have a weakened immune system (immune-compromised), such as those who have unregulated diabetes mellitus, decreased levels of neutrophils (a type of white blood cell that helps the body fight infection and heals itself), or whose immune systems have been suppressed by medications (immunosuppression) used to treat blood cancer, hematopoietic stem cell transplantation (hematological malignancy), or solid organ transplantation. Given the pressing demand for antifungal drugs and surgeries, in silico screening of chemicals that are bioactive can be an excellent method for reducing the time it takes to create new treatments. The current study underlines the efficacy of the in-silico technique in identifying potent inhibitor drugs for RNA Dependent RNA Polymerase.

Lipinski's rule of five was used to evaluate the 1000 ligands, which included examining their pharmacokinetic properties. This aided in identifying drug-like compounds among the long list of the various selected phytochemicals. The drug-likeness and toxicity of the discovered ligands were studied further in order to find the most promising candidates with high bioavailability and low toxicity. Dregamine, Alantolactone, Isoalantolactone and Solasodine exhibited the lowest energy value, indicating that they had a strong binding affinity for RNA Dependent RNA Polymerase. Dregamine was clearly the most promising ligand based on all the previous analyses. Dregamine was also shown to be safe when compared to other drugs, according to toxicological predictions. Molecular dynamics simulations of the protein model and experimental research on animal models would further validate these results, opening the way for the development of effective targeted therapeutics for Mucormycosis.

Abbreviations

kDa: kilodalton
NCBI: National Center for Biotechnology Information
LD50: Lethal Dose 50
RNA: Ribonucleic acid
RdRp: RNA-dependent RNA polymerase
SMILES: Simplified Molecular-Input Line-Entry System

Conflict of Interest

The authors declare that they have no competing interests.

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