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EXPLORING LULICONAZOLE AS A POTENT ANTIFUNGAL AGENT: MOLECULAR DOCKING FOR INTERACTION STUDIES IN THE DEVELOPMENT OF FORMULATION

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ABSTRACT

Continuous efforts are being made in pharmaceutical research to enhance the effectiveness of existing medications through the research and development of new drug delivery systems (DDS). Recent advancements in high-throughput screening have led to the development of lipophilic Active Pharmaceutical Ingredients (APIs). An example of a topical broad-spectrum antifungal agent is Luliconazole. Because of its poor water solubility, topical administration is limited, and topical absorption is restricted. The solubility of the drug in the stratum corneum's lipid phase also acts as a rate-limiting stage in penetration. Fungal infections impact the epidermis, dermis, and deeper layers of skin, necessitating treatment delivery tailored to target high drug concentrations at the epidermis and dermis layers. The present study involves interaction between the ideal candidates to optimize the Nano emulation. Interaction based on Docking provides Ricinoleic acid, a measured component of Caster oil, in addition to tween 80 and ethanol. The present study involves molecular docking studies that revealed an interesting binding profile.

Keywords: Drug delivery system, Antifungal, Docking, Nano-emulsion

1. INTRODUCTION

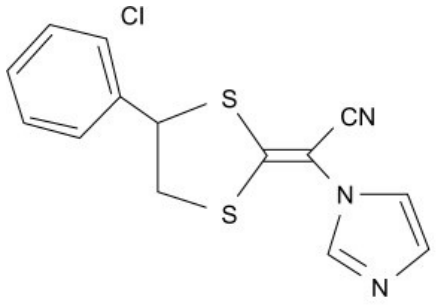
Fungal infections are increasingly common these days and contribute significantly to morbidity and death in humans. There are now five classes of medications, either

injectable or oral, available globally to treat fungal illnesses. These include azoles **[1-3]**, polyenes **[4]**, pyrimidine analogs **[5, 6]**, allylamines **[7]**, and echinocandins **[8, 9]**. Fungal infections represent a significant health issue and a significant contributor to illness **[10-12]**. Fungal infections can be classified as severe or noninvasive. Up to 20%–25% of people worldwide suffer from superficial fungal infections, which are linked to a worse quality of life, inability to carry out everyday tasks, and higher medical costs **[10]**. Progressive fungal infections typically develop because of several predisposing circumstances, such as in critically sick or immunocompromised individuals, as well as those with urinary catheters **[13]** and systems. Widespread fungal infections are a major cause of hospitalization and mortality **[14, 15]**. Candida genus yeasts cause candidosis. Candida most commonly causes superficial infections of the skin and mucous membranes, but it can also lead to deep invasive diseases such as meningitis, endocarditis, and septicemia. *Candida albicans* is the most commonly isolated genus member in cutaneous infections. Other members like *C. tropicalis, C. pseudotropicalis, C. parapsilosis, and C. krusei* are sporadic causes in humans **[16]**, particularly in immunocompromised and disseminated hosts. Oral thrush, also known as oral candidiasis, is a yeast infection of the oral mucosa. The majority of cutaneous candidosis cases happen in the skin folds or in areas that get unusually moist due to garment or medical dressing occlusion. Fingers that are often soiled with saliva and periorificial regions are particularly vulnerable. The two most prevalent manifestations are vulvovaginal candidiasis and Candida intertrigo **[17]**. It is demonstrated that the imidazole antifungal drug liconazole has strong action against a wide range of fungi, particularly dermatophytes. This supported by evidence review describes topical luliconazole's pharmacodynamics and its role in treating infections caused by fungi. In October 2013, a study was conducted in the English language medical literature using the terms "luliconazole" or "NND-502" across several databases.

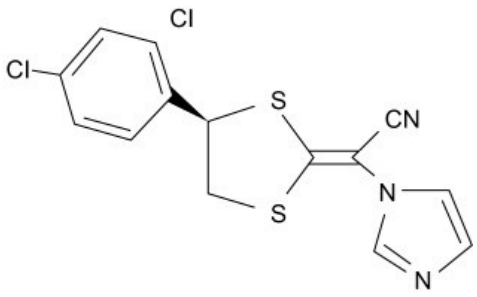
1.1 Chemistry and Pharmacokinetics

Luliconazole, also known as NND-502, is an imidazole anti-fungal first synthesized by Nihon Nohyaku Co Ltd (Osaka, Japan). It has a unique structure as the imidazole moiety is incorporated into the ketene dithioacetate structure. It is an optically related compound of lanoconazole, with a 2,4-dichlorophenyl group on the ketene dithioacetal structure. The chemical structure of luliconazole, ie, (−)-(*E*)-[4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-1-imidazolylacetonitrile, is shown in Fig 1 .Similar to lanoconazole, the S-enantiomer

is inactive, so luliconazole, being the active R-enantiomer, has more potent antifungal activity than lanoconazole. It has been reported to have strong in vitro antifungal activity against *Trichophyton spp.*, *C. albicans*, and *Aspergillus fumigatus* **[2, 11]**.



Lanoconazole



Luliconazole

Figure 1: (Luliconazole and Lanoconazole)

The US Food and Drug Administration authorized 1% Luliconazole cream in November 2013 for the medical management of interdigital tinea pedis, tinea cruris, and tinea corporis, among other conditions, in patients who are 18 years of age and older, which were brought on by the organisms *T. rubrum* and *E. floccosum*. The cream was initially authorized in Japan in 2005 for the care of tinea infections. For one week in cases of tinea corporis/cruris and two weeks in cases of tinea pedis, it is recommended to apply once regularly. The 1% cream received approval for Indian market distribution in June 2009. Luliconazole is effective in treating cutaneous dermatophyte infections in several clinical trials of which few are mentioned below **[2]**.

a. In a study focused on Tinea pedis, 489 patients participated, and the research design involved comparing the effectiveness of LLCZ 1% (applied once daily) for 2 weeks against BFZ 1% (applied once daily) for 4 weeks. The clinical outcomes were measured in terms of clinical efficacy, with LLCZ showing a rate of 91.5%, and BFZ exhibiting a rate of 91.7%. Microscopic examination revealed negative results for both treatments, with LLCZ at 76.1% and BFZ at 75.9%. Additionally, in terms of negative culture outcomes, LLCZ achieved 73%, while BFZ had a rate of 50%. The study followed a multicenter, randomized, single-blind, parallel, two-group comparison design.
b. For the study focusing on Tinea cruris, 256 participants were involved in a randomized, multicenter, double-blind, vehicle-controlled trial. The comparison was between LLCZ 1% (applied once

daily) and a vehicle for one week. The results indicated that LLCZ demonstrated superior outcomes. In terms of complete clearance, LLCZ achieved a rate of 21.2%, while the vehicle had a lower rate of 4.4%. When evaluating effective treatment, LLCZ showed a success rate of 43%, whereas the vehicle exhibited a rate of 18.7%. Furthermore, both microscopic examination and culture results were negative for a higher proportion of participants in the LLCZ group (78.2%) compared to the vehicle group (45.1%).

c. In a multicenter, randomized, open-label, comparative study focusing on cutaneous mycoses with 150 participants, the efficacy of various treatments was compared. Participants were subjected to a one-week treatment for tinea corporis/cruris and a two-week treatment for tinea pedis, using LLCZ, SER, AMO, TBF, and EBR (all applied once daily). The results indicated the effectiveness of these treatments in achieving efficacious outcomes. The success rates for efficacious treatment were as follows: SER 93.3%, LLCZ 86.6%, AMO 83.3%, TBF 80%, and EBR 73.3%. Unfortunately, specific details about mycologic outcomes (NA) were not provided in this study.

d. In a prospective, parallel study involving 60 participants with tinea corporis/cruris, the efficacy of TBF 1% was compared against LLCZ 1% over a 2-week treatment period. The evaluation criteria included a day 15 and day 30 composite score, both achieving a score of zero in both the TBF and LLCZ groups. Additionally, KOH microscopy results were consistently negative for all patients at day 15 (n=60) and remained negative for the majority at day 30 (n=51), indicating a favorable mycologic outcome for both treatments.

Emulsions can act as penetration enhancers in certain drug delivery systems. Emulsions are colloidal systems consisting of two immiscible liquids (such as oil and water) stabilized by an emulsifying agent **[3]**. They can be oil-in-water (O/W) or water-in-oil (W/O) emulsions, depending on the continuous phase.

1.2 ***Emulsions can enhance drug penetration through the following mechanisms:***

1) **Increased Solubility:** Emulsions can solubilize lipophilic drugs in the oil phase, making them more readily available for absorption through the skin. This is particularly beneficial for drugs with poor water solubility.
2) **Improved Partitioning**: The emulsion components can alter the partitioning of drugs between the vehicle and the skin, enhancing their penetration. The presence of both hydrophilic and

lipophilic phases in the emulsion can accommodate a wider range of drug molecules.

3) **Enhanced Skin Hydration**: Emulsions can have moisturizing properties, leading to increased skin hydration. Hydrated skin tends to have improved permeability, allowing for better drug penetration.
4) **Formation of Micelles**: Emulsifying agents in the emulsion can form micelles, which are small aggregates that can solubilize and carry hydrophobic drugs. These micelles can enhance the transport of lipophilic drugs through the stratum corneum, the outermost layer of the skin.
5) **Temporary Disruption of Skin Barrier:** Some emulsions may disrupt the stratum corneum temporarily, allowing drugs to penetrate more easily. However, this effect should be controlled to avoid irritation or damage to the skin barrier.

It is crucial to note that the efficiency of emulsions as penetration enhancers might vary depending on factors such as the exact emulsion formulation, the nature of the medicine, and the skin's properties. Formulation optimization and careful consideration of the desired therapeutic outcome are crucial in utilizing emulsions as penetration enhancers in drug delivery systems. Additionally, safety and skin compatibility should be thoroughly evaluated to ensure the well-being of the patient.

2. MATERIALS AND METHODS

2.1. Materials:

A free sample of Luliconazole was sent by Glenmark Pharmaceuticals, Mumbai, Tri ethyl amine and castor oil were purchased from Analab Fine Chemicals in Mumbai. While the distilled water was created in the lab, the tween 80 was bought from Analab Fine Chemicals, Ethanol Analab Fine Chemicals, and other analytical grade reagents.

2.2.Selection of oil, Surfactant, and co-surfactant:

Saturation solubility of the drug in various oils castor oil, oleic acid, olive oils surfactant tween 80, tween 20, cremophor RH40 and co-surfactant propylene glycol and ethanol was determined. Excess of luliconazole was added to 5 ml of each oil, surfactant, and co-surfactant. It was stirred for 48 hours on a magnetic stirrer at 500 rpm at room temperature. The solutions were then filtered through Whatman filter paper and scanned by UV spectrophotometer.

2.2.1 Construction of Pseudoternary Phase Diagram:

The development of a pseudo-ternary phase diagram using the water titration method was used to represent the nanoemulsion zone and provide component concentration ratios. S/Co-S and oil were combined in

multiple glass tubes at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, and 9:1. The aqueous phase was then injected drop-wise into each tube (at 26± 2°C), mixed for 3-5 minutes in a vortex, and then left to equilibrate after 30 minutes of magnetic stirring. Following the formation of equilibrium, the mixtures were defined in terms of phase clarity. A nanoemulsion was observed with a clear emulsion and good flowability. Ternary phase.com was used to construct *the ternary diagram* **[4-7]**.

From the pseudo-ternary phase diagram, suitable weight ratios (low, middle, and high) for the oil and S/Co-S were chosen, and formulas were proposed based on the nanoemulsion (NE). NE region in the diagram. O/w nanoemulsion was expected to form by these weight ratios due to a comparatively lower oil ratio than water.

The physical state and clarity were observed in these NE systems **[16-20]**. **Table 1** shows various formulations of the compositions for construction of phase diagram.

2.2.2 Preparation of Nanoemulsion:

Table 1: Composition of Castor Oil, Smix and Water for preparation of Pseudoternary Phase Diagram

Formulation	Castor oil (ml)	Smix (ml)	Water(ml)
A1	1	9	1
A2	2	8	1
A3	3	7	0.5
A4	4	6	0.4
A5	5	5	0.5
A6	6	4	0.5
A7	7	3	0.7
A8	8	2	1
A9	9	1	0.8

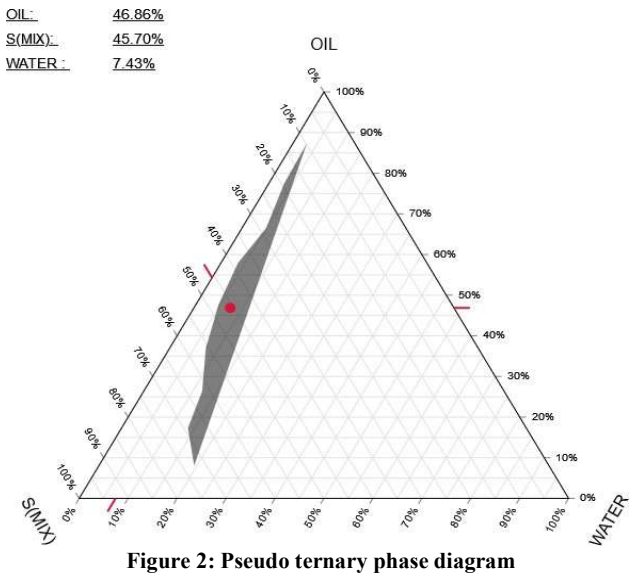


Figure 2: Pseudo ternary phase diagram

A pseudo ternary phase diagram **Figure 2** was designed for the optimization of concentration for oil, surfactant, and co-surfactant and to identify the NE zone in the absence of Luliconazole **[16, 18]**. The ratio of 1:2 for Smix was selected for the construction of the ternary diagram. The NE region is indicated by the shaded area **(Figure 2)**. From the pseudo-ternary phase diagram, different concentrations of oil, Smix and water were utilized to cover the maximum possible number of formulations from the NE region. The increasing concentration of oil might show a significant change in particle size. The particle size may increase upon further increase in oil contents. According to the criteria, the oil concentration should be such that it can completely solubilize the single dose of drug. For each NE formula, optimum concentration of S/Co-S mixture and water was selected for each percentage of selected oil. For the incorporation of Luliconazole in the selected oil, 1% drug was considered as the required dose for incorporation.

2.3.Docking analysis with binding energy and Interacting residue

2.3.1. Selection of Ligands

A variety of the ligands were obtained from the PubChem drug databases and used using AutoDock V4.2 software to dock them against Secreted aspartyl proteinase-5 (SAP5) (PDB ID: 2QZX). The ligands that are currently available for the proteins under investigation were selected via a comprehensive analysis of the literature. The current ligand amounts consist of 03, which are selected to enhance senses. The ligands that were retrieved from the Pubchem database were ricinoleic acid, ethanol, tween 80, and lindanazole.

2.3.2. Ligand Preparation

The inhibitors' three-dimensional structures were retrieved and stored in SDF format together with their corresponding PubChem CID. In addition, ligand preparations proceeded by utilizing Pymol software to convert the three-dimensional structures of all ligands from SDF to PDB format. Metals were also extracted from the ligand's structure using the Pymol program in order to conduct a suitable docking research. The generated ligands were stored for further docking research in PDB format.

2.3.3. Protein preparation

With accession number 2QZX, the protein target of *Candida albicans*, secreted aspartyl proteinase-5 (SAP5), was obtained from the Protein Data Bank (PDB; https://www.rscb.org). Using BIOVIA Discovery Studio Visualizer, the protein file (.pdb) was created by eliminating the water and the 3D structure's existing ligands. (http://www.discover.3ds.com).

aspartyl proteinase-5 (SAP5). PDB ID: 2QZX **(Table 2)**.

Table 2: Docking analysis with binding energy and Interacting residue

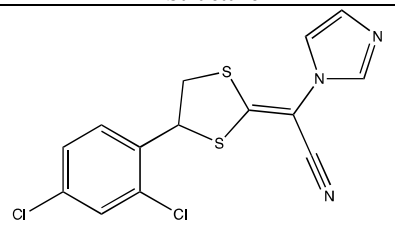
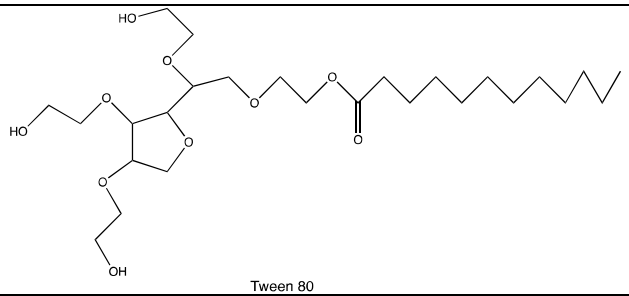
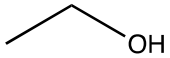
Proteins Name	Ligand Name	Binding Energy (kcal/mol)	No. of H Bonds	Interacting residue	Final Intermolecular Energy (kcal/mol)	vdW + Hbond + desolv Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Torsional Free Energy (kcal/mol)
2QZX	Luliconazole	-5.52	0	ALA119; ILE30; ASP86; ARG120; TYR84; GLY85; ILE305; ASP218; LYS193	-6.13	-6.15	+0.02	+0.60
	Tween 80	-4.79	0	ASP218; TYR225; ASP86	-13.33	-13.37	+0.05	+8.65
	Ethanol	-2.27	2 H1-2.04A^0 H2-LEU19 4A^0	THR33 (H1); LEU194(H2)	-2.57	-2.465	-0.12	+0.30
	Ricinoleic acid	-2.85	4 H1-3.08 H2-2.11 H3-3.23 H4-2.12	ARG297(H1); ALA11(H2); GLN282(H3); LEU280(H4); ALA162	-7.44	-6.43	-1.01	+5.07

The compounds were examined by application of Lipinski's Rule of Five, which declares of the molecule should not exceed more than 500 Daltons, that it can comprise a maximum of hydrogen bonds (05), hydrogen bond acceptors (10), rotatable bonds (10) and should be the number should not exceed more than above numbers. This is shown in **Table 3**.

Table 3: Lipinski's Rule information

Sr. No.	Name of Co-former	Mol Weight(g/mol)	XLogP3	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Rotatable Bond
1	Luliconazole	354.3 g/mol	4	0	4	2
2	Tween 80	522.7 g/mol	2.5	3	10	26
3	Ethanol	46.07 g/mol	-0.1	1	1	0
4	Ricinoleic acid	298.5 g/mol	5.7	2	3	15

Table 4: 2D Structure of compounds

Sr. No.	Ligand Name	2D Structure
1	Luliconazole	 Luliconazole
2	Tween 80	 Tween 80
3	Ethanol	 Ethanol
4	Ricinoleic acid	 Ricinoleic acid

3. RESULTS:

3.1.Molecular docking

A crucial part of computer-assisted drug discovery is molecular docking. It produces the proper interaction between the molecules and aids in the prediction of the intermolecular framework that forms between a protein and ligand. Using the built empirical free energy function and the Lamarckian Genetic Algorithm (LGA), the AutoDock 4.2.6 program carried out the docking process. AutoGrid was used to calculate the grid maps. A 40 x 48 x 58 grid map with 1.000 Å grid-point spacing was used in each docking. From the docking search, the best conformation with the lowest docked energy was selected. Pymol, UCSF Chimera, and Accelrys Discovery Studio Visualizer tools were used to evaluate the interactions of complicated protein-ligand conformations, including hydrogen bonds and bond lengths. **Table 4** shows the Structure of compounds involved in this research.

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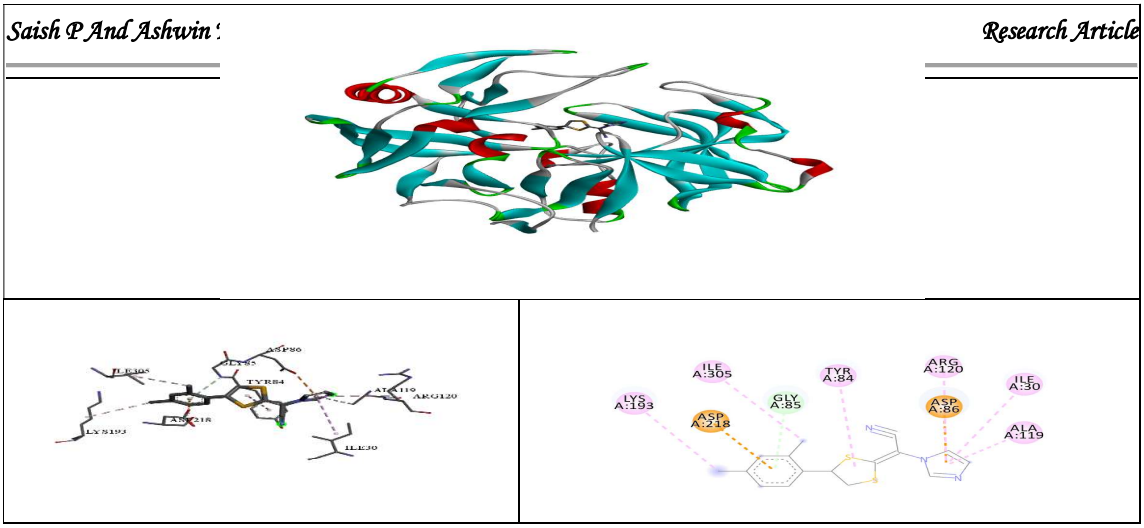


Figure 3: Molecular docking of Secreted aspartyl proteinase-5 (SAP5)1. PDB ID: 2QZX with Luliconazole shows 3D model of the interactions and the 2D interaction patterns and H-bond interaction

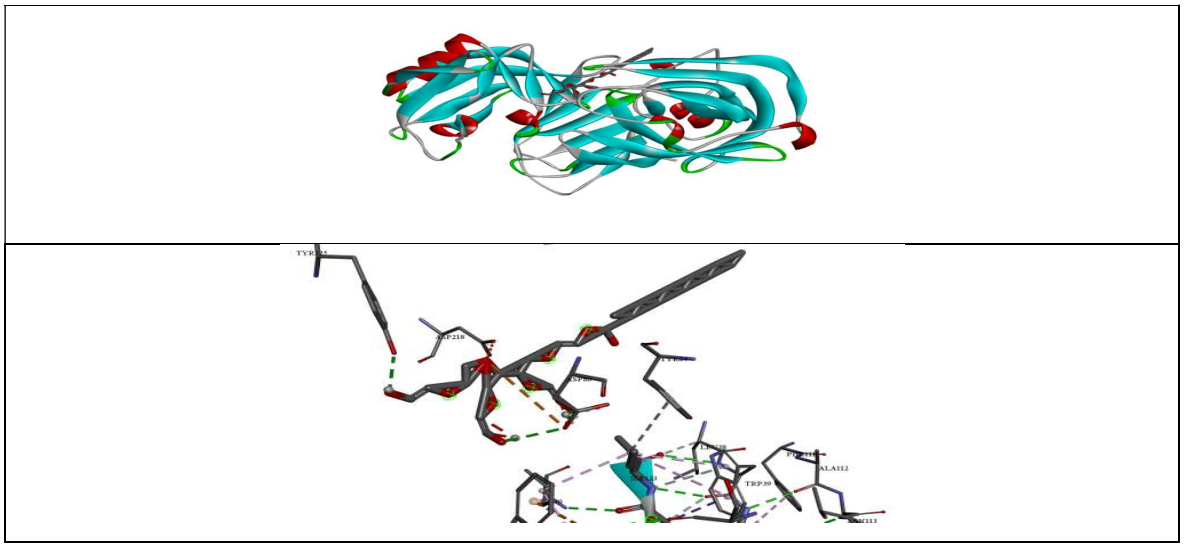
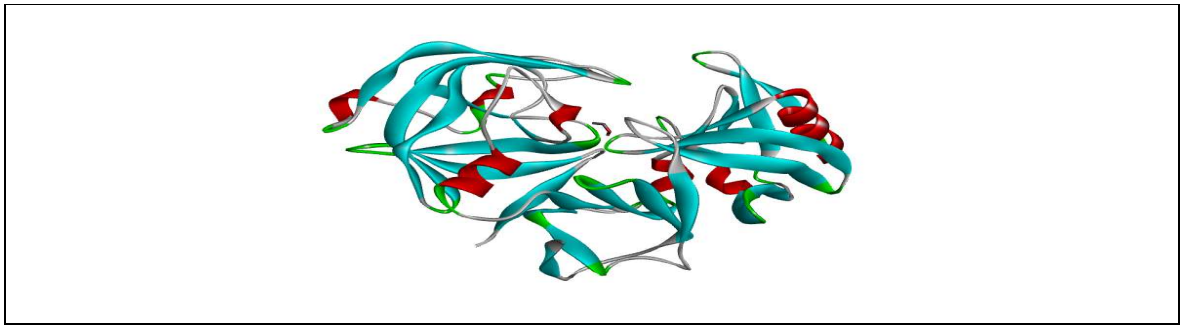


Figure 4: Molecular docking of Secreted aspartyl proteinase-5 (SAP5)1. PDB ID: 2QZX with Tween 80 shows 3D model of the interactions and the 2D interaction patterns and H-bond interaction



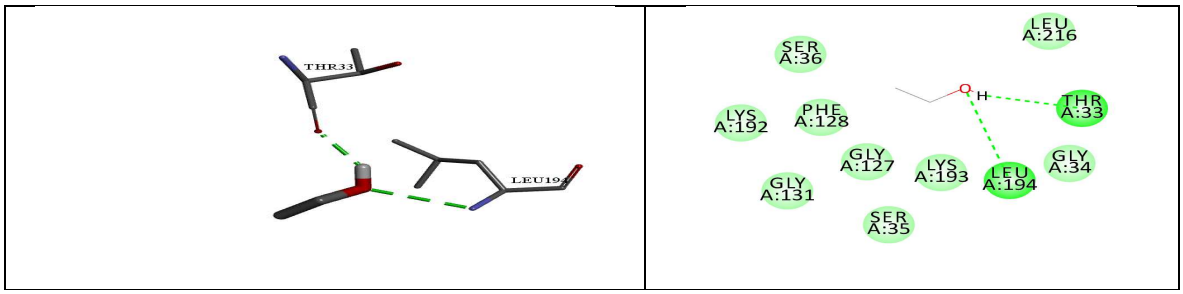


Figure 5: Molecular docking of Secreted aspartyl proteinase-5 (SAP5)1. PDB ID: 2QZX with Ethanol shows 3D model of the interactions and the 2D interaction patterns and H-bond interaction

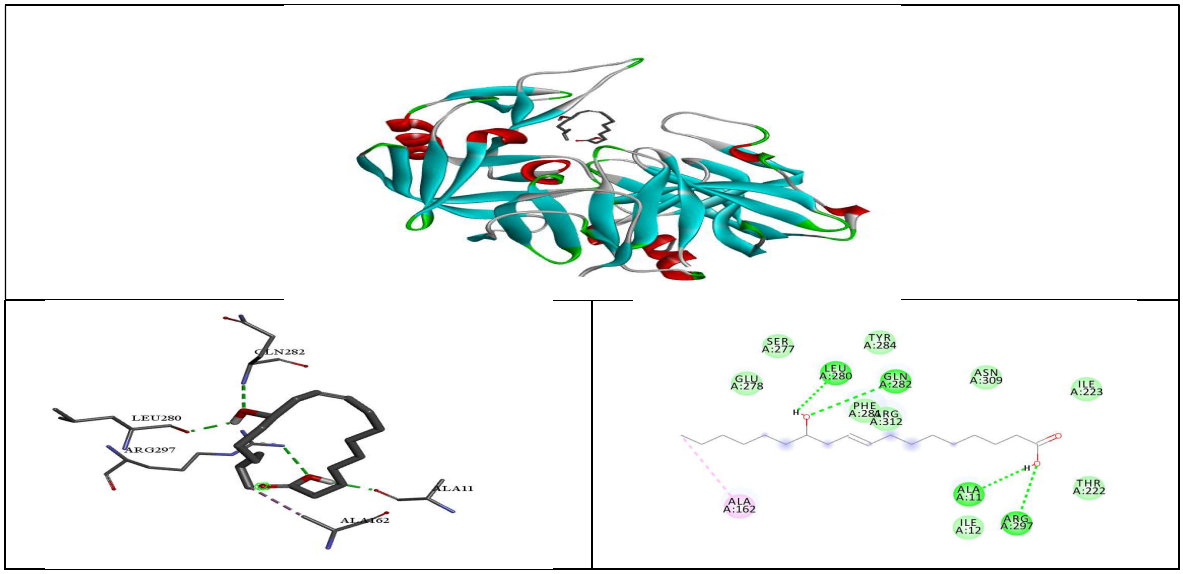


Figure 6: Molecular docking of Secreted aspartyl proteinase-5 (SAP5)1. PDB ID: 2QZX with Ricinoleic acid shows 3D model of the interactions and the 2D interaction patterns and H-bond interaction

3.2. Interpretation

Common mycobiota like *Candida spp.* invade human skin, the vagina, the stomach, and the oral cavity, among other mucosal surfaces. Since fungal infections can cause invasive fungal illnesses or candidemia, candidiasis has become a more common worldwide issue. In specific patient populations, the incidence of candidemia has increased, resulting in high mortality rates of over 70%. Numerous cases of infections connected to Candida have been linked to notable species as *Candida albicans, Candida krusei, Candida glabrata, Candida parapsilosis,* and *Candida tropicalis*. A relatively recent development in the field of medical microbiology is medical mycology. The second part of the 20th century saw the recognition of fungal illnesses as clinically significant, mostly as a result of advancements in medical technology. However, the AIDS pandemic has opened up the discipline of clinical mycology throughout the previous 20 years. A whole new area of host vulnerability and illness was revealed by the finding that lowering the CD4+ lymphocyte population of the

cell-mediated immune system might predispose patients to a variety of opportunistic fungal infections. Consequently, there has been a noticeable upsurge in fundamental research on pathogenic fungus, namely Aspergillus fumigatus, Cryptococcus neoformans, and Candida species. As a result of this research, numerous basic biological processes occurring in the primary fungal infections, especially *Candida albicans*, have been uncovered. The secreted aspartyl proteinases (Sap), phospholipase B enzymes, and lipases are the three most important extracellular hydrolytic enzymes that *Candida albicans* produces. This study focuses on the Sap proteins, which are the main virulence factors of *Candida albicans* and are encoded by a family of 10 SAP genes. Of these, the Sap proteins have been the topic of the most thorough research.

Candida albicans is typically a commensal resident of mucosal surfaces, but when specific host variables are out of balance, it may often lead to surface infections. These superficial infections have the potential to spread and develop into dangerous systemic infections in some situations. The secreted aspartyl proteinases (Saps), which are expressed by 10 SAP genes, are important virulence factors of *Candida albicans* that seem to play essential roles in the pathogenesis of this opportunistic fungus. When oral and cutaneous candidiasis is introduced into three-dimensional models, these genes are differently regulated in vitro and in vivo in both human samples and infected mouse tissue.

4. DISCUSSION

From the docking search, the best conformation with the lowest docked energy was selected.It was shown that the binding energy displayed by the protein and ligand is good at Luliconazole (-5.52 kcal/mol) after docking Protein Secreted aspartyl proteinase-5 (SAP5) (PDB ID: 2QZX) with Luliconazole (-5.52), Tween 80 (-4.79), Ethanol (-2.27), and Ricinoleic acid (-2.85). The number of torsions is selected between 0 and 6, and if a ligand exhibits more than 6, it is reduced to 6. Additionally computed and discussed are hydrogen bond interactions; the presence of H-bonds indicates a stable association between a protein and ligand. For an effective docking visualization, 2-D and 3-D pictures, as well as representations of protein-ligand interactions, are represented using the Discovery Studio 2020 Client and Chimera software.

5. CONCLUSIONS

The present study gives a brief idea regarding the docking interaction of the proposed compound topical broad-spectrum antifungal agent Luliconazole. Studies assist in lead optimization by guiding the advanced modification of chemical structures to improve potency, selectivity,

and pharmacokinetic properties while maintaining favorable binding interactions with the target protein. Recent advancements in high-throughput screening have led to the development of lipophilic Active Pharmaceutical Ingredients (APIs). Based on the docking study the phases system analyzed for ternary phase diagrams helped identify the different phases present in a system and the regions of phase stability under specific conditions. Utilizing two advanced techniques that offer insights into the thermodynamic principles governing phase equilibrium and enabling the rational design and optimization of materials and processes.

6. ACKNOWLEDGMENT

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