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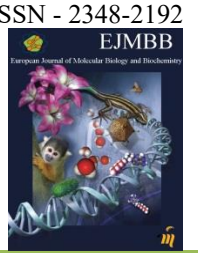

IN SILICO EVALUATION OF TERMINALIA CHEBULA PHYTOCONSTITUENTS AS POTENTIAL ANTIFUNGAL AGENTS AGAINST CANDIDA ALBICANS

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ABSTRACT

Candida albicans is the cause of candidiasis, which is one of the most significant fungal infections, with the potential to cause severe complications, particularly in immunocompromised people. The growing resistance of antifungals highlights the use of alternative therapies. The paper explores the antifungal effects of chebulic acid, gallic acid and ellagic acid, which are antimicrobial agents of Terminalia chebula, against Als3 adhesin of C. albicans (PDB ID: 4LEB) through the simulation of molecular docking. The findings demonstrated that the chebulic acid had the strongest binding affinity and hydrogen bonding reaction, next was fluconazole, a common antifungal. The results indicate that chebulic acid is more stable in interaction and thus it is a promising agent in the treatment of systemic candidiasis. This research gives credit to the prospects of natural compounds in augmenting antifungal treatment and eliminating drug-resistant strains.

INTRODUCTION

Candidiasis is a fungal infection that is mainly induced by Candida albicans which is a commensal fungus that inhabits different places of the human body, such as the skin, mouth, gastrointestinal tract and the vaginal mucosal region. In the healthy state of affairs, C. albicans can be present in the body harmlessly as a part of microbiota. But in case of an immune system failure or the microbial flora balance is disturbed, which may occur by the long-lasting use of broad-spectrum antibiotics, corticosteroids, chemotherapy, diabetes and immunodeficiency diseases such as HIV, the fungus may proliferate and lead to an infection [1, 2].

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Candidiasis may present in a number of different forms depending on the site that is affected. Oral thrush, vaginal candidiasis and cutaneous candidiasis are examples of mild infections which occur in healthy people under transient immunologic or hormonal changes. Even more serious ones like invasive candidiasis or candidemia are the ones where C. albicans gets into the bloodstream and internal organs and is a serious threat especially with hospitalized or immunocompromised patients. These infections have the potential to result in high mortality and morbidity particularly in intensive care units [3,4].

The dimorphic nature of Candida albicans is one of the most important components of its pathogenicity, since it enables the microorganism to change between the yeast and hyphae forms contributing to its infectiousness and immune response evasion. Moreover, it has the capacity to attach

itself in biofilms to medical devices, such as catheters and implants, which makes it resistant to antifungal therapy. The increase in the rate of antifungal resistance has only aggravated the process of treatment, leaving the creation of new antifungal agents and other alternative medicines as an emergency requirement [5,6].

Therefore, candidiasis is a serious community health complication. As a response to the increasing incidence of drug resistance strains, scientists are going into studies examining the potential of natural products such as Termsina chebula and phytoconstituents in their potential utility to directly improve or substitute existing antifungal treatments as an antifungal agent.

Terminalia chebula

Haritaki or *Terminalia chebula* is a famous Ayurvedic, Unani and Siddha medicine. This Indian native and tree belonging to the family of Combretaceae is known by the title of the King of Medicine in Ayurveda because of its vast and extensive therapeutic uses. The fruits of *T. chebula* are especially highly appreciated because they possess high antioxidant, antimicrobial, anti-inflammatory, immunomodulatory and antifungal activity.

Recent scientific researchers have investigated the efficacy of *T. chebula* in treating Candida albicans which is a pathogenic yeast causing candidiasis, especially in immunocompromised people. In a study conducted by Fitoterapia (Regar et al), the antifungal effect of *T. chebula* was assessed in vitro, in vivo and in silico [1]. The results indicated that T. chebura was a strong inhibitor of the C. albicans growth even without drugs. Besides, it exhibited synergistic action when used together with traditional antifungal agents such as fluconazole and it lowered fungus load in infected animal models indicating its possible use as a systemic antifungal agent [7-12].

The antifungal effect of the plant has various modes of action: destruction of fungal walls and membranes, blocking ergosterol synthesis, inhibition of hypertrophic development of the hyphae and induction of oxidative stress, which are ultimately accompanied by the apoptosis of fungal cells. All these multifaceted measures render T. chebura less susceptible to the development of resistance.

More than 200 bioactive compounds in *T. chebula* have been determined by phytochemical analysis. Some of the well-known antifungal ingredients are chebulic acid, chebulagic acid, gallic acid, ellagic acid, corilagin and flavonoids such as quercetin. These substances are important in the inhibition of fungal growth, membrane disruption and ROS production, which increases antifungal action [13-15]. *Terminalia chebula* holds a lot of potential as a natural antifungal agent, particularly against Candida strains, which are resistant to antifungals and integration into complementary antifungal treatment is encouraged.

MATERIALS AND METHODS

Protein Preparation

The molecular docking experiment was initiated by the preparation of the target protein structure. The Protein Data Bank (PDB) was searched to retrieve the crystal structure of the Als3 adhesin of Candida albicans (PDB ID: 4LEB), that includes hepta-threonine-complexed residues of the protein, 1293 in total. Als3 is a severe virulence factor, which participates in host cell adhesion and biofilm and it is an excellent target of antifungal drug discovery. The Molegro Virtual Docker (MVD) software was used to import the protein in the software through the File Import Molecule Protein functions. A number of refining and optimization procedures were performed to make docking simulations accurate. Co-crystallized ligands and non-essential water molecules were eliminated, as well as irrelevant heteroatoms and protein chains that may cause any interference. The Repair → Add Missing Hydrogens tool was used to add polar hydrogen atoms to the structure to make sure that the charges were distributed and geometry was correct. Structural consistency was automatically ensured by assigning atom types and bond orders. The largest cavity was chosen with the highest level of volume and the lowest energy profile to identify possible ligand binding zones, so the "Detect Cavities" functionality was used and the largest cavity was docked. This binding site was regarded as the most biologically important site to interact with antifungal phytocompounds.

Ligand Preparation

The chosen ligands, which include chebulic acid, gallic acid and ellagic acid, have been selected on the basis of literature available, which indicates that they have a significant antifungal activity, especially on the medicinal plant *Terminalia chebula*. The PubChem database was searched to obtain the chemical structures of these bioactive compounds in 2D or 3D format and then with standard chemical file format like. mol or .sdf so that they can be compatible with docking software. To optimize the ligands to molecular docking, energy minimization of the ligand was done by using Chem3D software, with MM2 or MMFF94 force field option generating geometry minimally favored and energetic favorable conformers of the ligand. This involved optimization of bond angles and lengths, reduction of steric clashes and transformation of 2D structures to correct 3D representations where required. After energy minimization was done, the ligands were loaded into Molegro Virtual Docker (MVD) by clicking on the File menu and then on Import Molecule followed by Ligand. In this process, hydrogen atoms that were missed were added and valency problems resolved to make the molecules have the right geometry and to be compatible with docking simulation. Fluconazole, which is a developed antifungal agent, was used as a standard reference compound to compare and confirm the docking performance and binding affinity of the target natural ligands.

Molecular Import and Binding Site

The final protein structure (4LEB) and the energy-minimized ligands, namely, chebulic acid, ellagic acid and gallic acid were effectively imported into the Molegro Virtual Docker (MVD) workspace to achieve the docking simulations. Preparatory steps were also made before docking was done and this was done to be accurate and physiologically relevant. Initially the protonation of both the protein and the ligands was adjusted to a physiological pH of 7.4 which is important in ensuring that the protein and the ligands maintain their respective electrostatic interactions and hydrogen bonding patterns during docking. Hydrogenation and charge corrections throughout were done to provide structural integrity and to provide proper molecular behavior in the docking environment. The binding site of Als3 adhesin protein was then defined with the help of the docking wizard → select protein → define binding site tool in MVD. This was an very important step in order to concentrate the docking algorithm in regions of biological interest. The active site was manually positioned by a set of coordinate points (X, Y, Z) and a reasonable radius of 8-12 A was set to cover the main cavity of Als3 protein. This region was selected according to the cavity identification and structural similarity and the docking simulation was focused on the right interface of the interaction.

Docking Setup

Molegro Virtual Docker (MVD) was started with a new docking project by going into pathway Docking > Start Docking Wizard > Create New Docking Job. In the case of the simulation, the MolDock SE (Simplex Evolution) algorithm was chosen as it has been demonstrated to have a high degree of exploration of the ligand conformational space and a high degree of optimal binding pose identification with a high level of accuracy. There are a few important parameters, which were set to have a precise and efficient docking process. The MolDock Score and Re-Rank Score were used as the main scoring functions to measure binding affinities on the basis of non-bonded interactions, steric complementarity and energy components. There were to be 20 independent runs on the docking simulation with population size of 50 and with a maximum of 1500 iterations per run to ensure that sufficient number of ligand conformations is searched. A cutoff of 100 was set to select the better poses and the 10 best poses of each ligand were stored to be analyzed further. Further, the docking of constraints was also allowed to give preference to the interactions with particular active site residues, which have been shown to be involved in ligand binding. This set of parameters was tested to provide a optimal balance between computation speed and the extent of conformational discovery to provide reliable biologically significant docking solutions.

Docking and Interaction Analysis

After docking simulations were performed, Molegro Virtual Docker (MVD) produced several binding poses per ligand with ranking according to MolDock Scores with the lowest values denoting the strongest predicted binding affinities. View Ligand Interactions tool was used to examine each pose in detail and provided detailed visualization of the major molecular interactions such as hydrogen bonding, hydrophobic contacts and electrostatic between the active site of the Als3 protein and the ligand and individual amino acid residues. Other critical parameters used in the analysis were bond length, bond angle and identification of specific interacting residue. More so, the energy contributions through different sources, which include van der Waals forces, hydrogen bonding, torsional strain and electrostatics were calculated to determine the binding stability and specificity of each ligand. The most optimal dock conformations were then contrasted with the natural orientation of the ligand in the binding site to confirm the proper docking and be structurally relevant. All the results such as 2D interaction charts, binding energy curves, as well as structural information of the docked complexes were exported in standard formats such as. mol2, pdb and high-resolution image files, which were appropriate to document, present and publish [16-19].

RESULTS AND DISCUSSION

Table 1: Ranking of Ligands and poses against crystal structure of the Als3 adhesin from Candida albicans based on MolDock score

Protein: 4LEB

Ligand	Ligands Name	MolDock Score	Rerank Score	H Bond
71308174	Chebulic Acid	-94.87	-38.73	-13.18
370	Gallic Acid	-70.56	-58.76	-13.44
5281855	Ellagic Acid	-67.68	-54.07	-12.37
3365	Fluconazole	-82.19	-61.53	-3.89

2D, 3D and Secondary Interactions between ligands and proteins

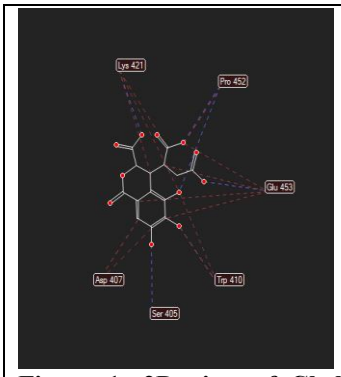

Figure 1: 2D view of Chebulic acid Vs 4LEB

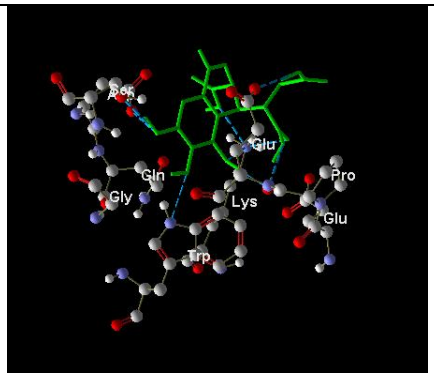

Figure 2: 3D view of Chebulic acid Vs 4LEB

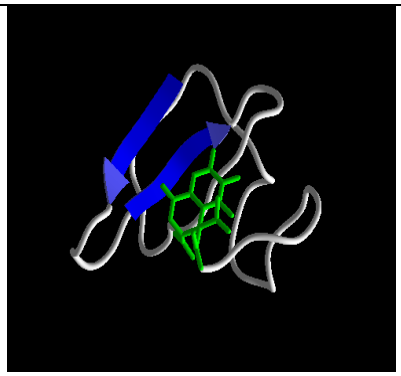

Figure 3: Secondary view of Chebulic acid Vs 4LEB

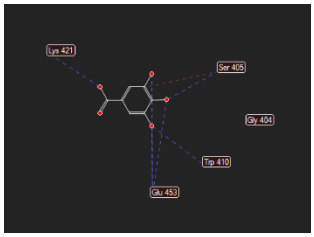

Figure 4: 2D view of Gallic acid Vs 4LEB

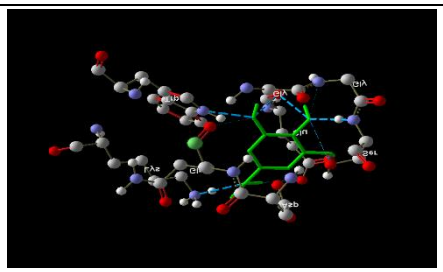

Figure 5: 3D view of Gallic acid Vs 4LEB

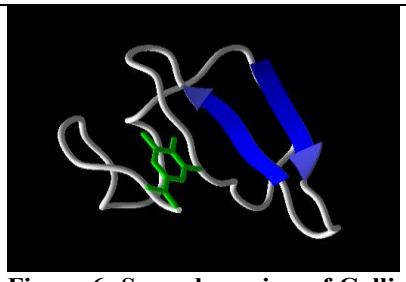

Figure 6: Secondary view of Gallic acid Vs 4LEB

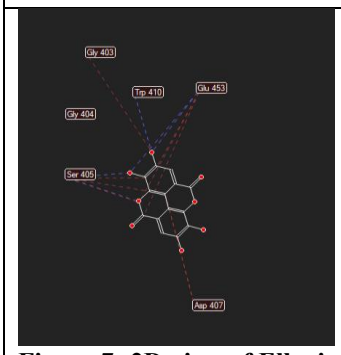

Figure 7: 2D view of Ellagic acid Vs 4LEB

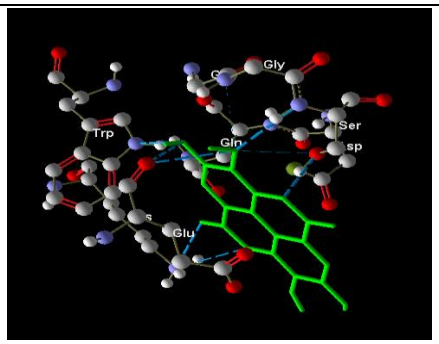

Figure 8: 3D view of Ellagic acid Vs 4LEB

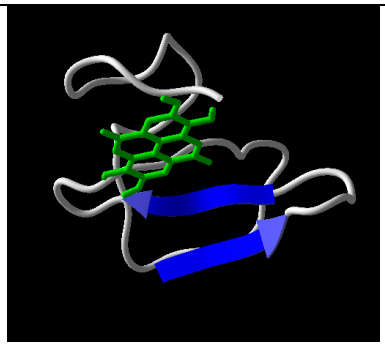

Figure 9: Secondary view of Ellagic acid Vs 4LEB

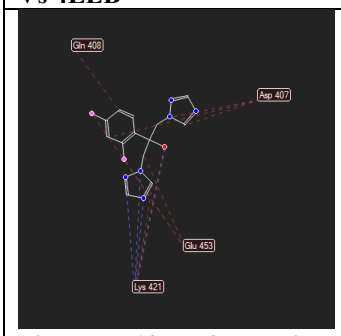

Figure 10: 2D view of Fluconazole Vs 4LEB

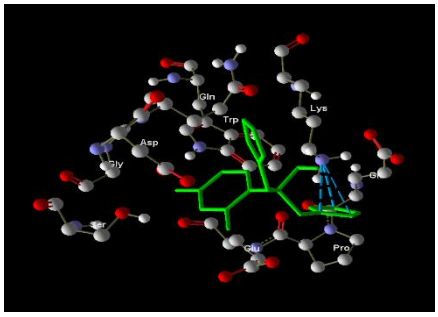

Figure 11: 3D view of Fluconazole Vs 4LEB

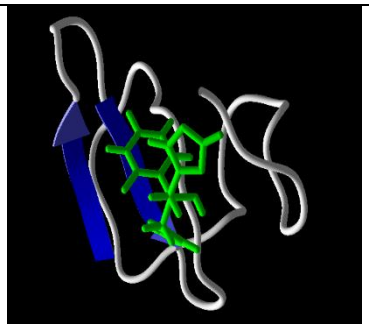

Figure 12: Secondary view of Fluconazole Vs 4LEB

The MolDock Scores, Re-rank Scores and Hydrogen Bond (H Bond) values of the ligands indicate that there are significant differences in the binding affinities and interaction patterns of the ligands based on the data presented in the table 1 and from figure 1-12 respectively. The best binding affinity is in chebulic acid with MolDock Score, -94.87 and Re-Rank Score, -38.73, which means that there would be a high probability of interaction with the target protein. Also, it has the highest H bond value -13.18 as compared to the other ligands, indicating that chebulic acid has a large number of hydrogen bonds and this is what makes it remain stable in the binding site. The standard

reference drug fluconazole is also relatively high binding with a MolDock Score of -82.19 and a Re-Rank Score of -61.53 as well, yet it exhibits a lower number of hydrogen bonds (-3.89) than the natural compounds. Gallic acid and ellagic acid are intermediate binding affinities with MolDock Scores of -70.56 and -67.68, respectively and Re-Rank Scores of -58.76 and -54.07. The H Bond values of both compounds are also similar H -13.44 and H -12.37, respectively, indicating that, although the interaction between the compounds is also important, it is slightly less stable than that of chebulic acid in hydrogen bonding. Comprehensively, the chebulic acid is the most promising ligand on both binding affinity and hydrogen bonding, then there is fluconazole, which is a good reference ligand and lastly, gallic and ellagic acids have moderate potential.

DISCUSSION

Candidiasis is a fungal disease which is mainly produced by Candida albicans as a commensal fungus that usually stays in different areas of the human body like the skin, mouth, gastrointestinal tract and the mucosa in the vagina. In the healthy organism, C. albicans can live without causing any harm as the microbiota of the body. But, in case of an immunosuppressed state of the immune system, or a disruption in the balance of microbial flora, because of long-term intake of broad-spectrum antibiotics, corticosteroids, chemotherapy, diabetes and immunodeficiency diseases, e.g., HIV, the amount of the fungus may increase and lead to an infection [1,2]. The different levels in which candidacy can present itself are based on the area of attack. Mild Candida infections like oral thrush, vaginal Candida and cutaneous Candida usually arise in normal healthy individuals during interim aspects of immune or hormonal variations. The more serious types include invasive candidiasis or candidemia where C. albicans invades the bloodstream and internal body organs, which is very dangerous, especially in hospitalized or immunocompromised patients. High mortality and morbidity in the intensive care units are also possible due to these infections [3,4].

The rationale of the study was to determine the antifungal properties of *Terminalia chebula* (*T. chebula*) against Candida albicans in the presence of an important protein of the host, Als3 adhesin (PDB ID: 4LEB), which is a vital factor in the host adhesion and biofilm formation. Due to the growing difficulty of developing antifungal resistance, especially C. albicans, natural compounds are subject to vast investigations in order to complement or substitute the current antifungal treatments [5-10]. The outcome of the molecular docking study showed that there were high disparities in the binding affinities and the hydrogen bond interactions of the chosen ligands. The compound that showed the most promising results in the binding affinity was chebulic acid with the MolDock Score of -94.87 and the hydrogen bond interaction of -13.18, then came fluconazole, the widely used antifungal medication. Although fluconazole interacts with the active site of the protein with a high interaction frequency (MolDock Score of -82.19), it formed less hydrogen bonds, which demonstrates the high binding capacity of chebulic acid. By contrast, similar binding affinities and high binding hydrogen bonding were also found in gallic acid and ellagic acid although they were a little less stable than chebulic acid to interact [11-15].

The simulations of the Molegro Virtual Docker (MVD) verified the significance of hydrogen bonding, hydrophobic contacts and electrostatic interactions in the determination of the effectiveness of the binding as well as specificity of the ligands to the Als3 adhesin. The optimally docked poses of each ligand were analyzed in terms of their structural significance such that the molecular docking findings were of biological value and precise prediction of the interaction between the ligand and the protein. The better interaction profile of Chebulic acid renders it a lead drug that can be experimented upon again to treat systemic candidiasis infections as well as other Candida-related infections and most importantly Candida drug-resistant strains [16-19].

The results are in agreement with the earlier researches which showed that phytoconstituents of *T. chebula* have high inhibitory effects against C. albicans whose growth is inhibited by breaking down the key cell wall components, inhibiting the production of ergosterol and causing oxidative stress thereby restricting the growth of the fungi and consequently improving fungicidal effect. These findings also elucidate the fact that the interaction of natural products such as chebulic acid and standard antifungals such as fluconazole may or may not have synergies, as previous studies have hypothesized in the in vitro and in vivo models of C. albicans management.

On balance, this paper has underscored the chebulic acid as a potential natural antifungal aid that can be successfully used as a complementary treatment to overcome Candida infections particularly with the emerging antifungal resistance. The experimental confirmation of these in silico results and the clinical research should be conducted in the future with the aim of assessing the possibility of *T. chebula*-based therapy in antifungal therapy.

CONCLUSION

This research has shown that the bioactive compounds of *Terminalia chebula*, including chebulic acid, gallic acid and ellagic acid, possess strong antifungal activity against Candida albicans Als3 adhesin, with molecular docking simulations revealing this interaction. The binding affinity and hydrogen bonding of Chebulic acid were the highest and hence the agency had greater potential to be an effective antifungal agent. The findings show that natural products such as chebulic acid can either be used to supplement or supplement traditional antifungal treatment, particularly in the context of treating drug-resistant strains. More experimental validation and clinical research should

be conducted to determine completely the therapeutic feasibility of *T. chebula* in the treatment of candidiasis.

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