Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 6 (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

In Silico Evaluation Of Eugenol As A Potential Antifungal Agent: Targeting Beta-Glucocerebrosidase And Cyp51

Anoosha[1], Prabhu K[2,5], Janaki CS[3], Bindu D[4], Refa Fathimaa S[5], Khushi Basavaraj Rudagi[6]

[1]Research Scholar Department of Microbiology, Bharat Institute of Higher Education & Research.

[2]Associate Professor, Department of Anatomy, Sree Balaji Medical College and Hospital, Chromepet, Chennai.

Email ID: prbanu75@gmail.com

[3]Associate Professor, Department of Anatomy, Bharath Medical College and Hospital, Selaiyur, Chennai.

Email ID: Janaki098@gmail.com

[4]Department of Microbiology, Sree Balaji Medical College & Hospital, Chromepet, Chennai.

Email ID: mail2bindhu@rediffmail.com

[5]Centre for Integrative Medical Research, Sree Balaji Medical College & Hospital, Chromepet, Chennai

Cite this paper as: Anoosha, Prabhu K, Janaki CS, Bindu D, Refa Fathimaa S, Khushi Basavaraj Rudagi, (2025) In Silico Evaluation Of Eugenol As A Potential Antifungal Agent: Targeting Beta-Glucocerebrosidase And Cyp51. *Journal of Neonatal Surgery*, 14 (6), 203-210.

ABSTRACT

Fungal infections pose a significant health risk, with a growing concern for drug-resistant strains. This study investigates the potential of Eugenol (PubChem ID: 3314), a natural compound, as an antifungal agent through molecular docking analysis. The research targets three key fungal virulence proteins: beta-glucocerebrosidase (9FA3), Demethylase (6UEZ), and Upc2 (7VPU), utilizing AutoDock 1.5.7 to evaluate binding affinities and molecular interactions. Eugenol exhibited the strongest binding affinity for beta-glucocerebrosidase (−5.76 kcal/mol, 59.89 μM), where it formed crucial hydrogen bonds with residues SER-77 and TYR-79, indicating a strong potential for enzymatic inhibition. For Demethylase, Eugenol's binding affinity was −5.53 kcal/mol (88.85 μM), suggesting moderate potential in disrupting fungal cell metabolism. In the case of Upc2, Eugenol displayed a slightly lower affinity (−4.99 kcal/mol, 219.20 μM), yet still indicated possible interactions that could interfere with fungal sterol regulation. These findings highlight Eugenol's promising multi-target antifungal activity, particularly against key fungal enzymes involved in virulence and metabolic processes. The results suggest that Eugenol may be a viable candidate for further experimental investigation as a natural antifungal agent. Future in vitro and in vivo studies are necessary to confirm these computational predictions and explore the broader therapeutic potential of Eugenol in treating fungal infections.

Keywords: *Eugenol, Antifungal activity, Molecular docking, Beta-glucocerebrosidase, Demethylase, Fungal transcription factor Upc2*

1. INTRODUCTION

Fungal infections, particularly in immunocompromised individuals, have become a growing global health concern, causing over 1.5 million deaths annually. These infections are especially lethal in patients with underlying conditions such as HIV/AIDS, organ transplants, and cancer, where the immune system is weakened. The rising prevalence of antifungal resistance, combined with limited treatment options and the toxicity of current antifungal agents, highlights the urgent need for novel therapies (1). Traditional antifungal drugs, such as azoles, polyenes, and echinocandins, have proven effective but are limited by their toxicity profiles, narrow spectrum of activity, and the emergence of resistant strains (2). Consequently, there is a growing interest in developing new antifungal agents, particularly from natural sources, which could offer safer and more effective alternatives.

Fungal pathogens possess several strategies to survive and thrive in hostile environments, particularly within the human host. One of the primary mechanisms of fungal resistance is the formation of biofilms, which provide physical and biochemical barriers against immune responses and antifungal treatments (3). Biofilm formation enables fungi to persist on medical devices, tissues, and mucosal surfaces, significantly increasing the difficulty of treating infections. Furthermore, many pathogenic fungi can evade immune surveillance by altering surface antigens, secreting proteases, and inhibiting immune cell activation (4). Another critical factor in fungal survival is the integrity of the fungal cell wall, which is composed of

chitin, β-glucans, and mannoproteins. The cell wall provides structural integrity, protects against environmental stress, and facilitates interaction with host tissues (5). Targeting these essential components of the fungal cell wall represents a promising strategy for antifungal drug development.

Additionally, the ergosterol-rich membrane is an essential feature of fungal cells, serving a similar role to cholesterol in mammalian cells. Ergosterol is crucial for maintaining cell membrane fluidity, and its biosynthesis is an attractive target for antifungal agents (6). The enzyme CYP51, a cytochrome P450 monooxygenase, plays a vital role in the biosynthesis of ergosterol. Inhibition of CYP51 disrupts membrane integrity and impairs fungal growth (7). Another key regulatory protein, Upc2, is involved in the regulation of sterol homeostasis. Upc2 not only regulates the expression of genes involved in ergosterol biosynthesis but also contributes to the development of antifungal resistance by controlling the synthesis of alternative sterols (8). Thus, targeting proteins like Beta-glucocerebrosidase, CYP51, and Upc2 is a rational approach for developing novel antifungal therapies.

Eugenol: A Natural Antifungal Compound

Eugenol (4-allyl-2-methoxyphenol), a naturally occurring phenolic compound, is found in various plants, with particularly high concentrations in *Syzygium aromaticum* (clove). It has been widely used in traditional medicine for its anti-inflammatory, analgesic, and antimicrobial properties (9). Recent studies have also highlighted its potential as an antifungal agent. Eugenol's antifungal activity is attributed to its ability to disrupt key components of the fungal cell, including the cell membrane and cell wall, as well as its capacity to induce oxidative stress and apoptosis in fungal cells (10). The mechanism of action of Eugenol involves multiple pathways, making it a promising candidate for the development of novel antifungal treatments.

One of the primary mechanisms by which Eugenol exerts its antifungal effects is through the disruption of ergosterol biosynthesis. Eugenol has been shown to inhibit the activity of CYP51, the enzyme responsible for the demethylation of lanosterol to ergosterol (11). By inhibiting this critical enzyme, Eugenol disrupts the synthesis of ergosterol, thereby destabilizing the fungal cell membrane. The resulting loss of membrane integrity impairs fungal growth and renders the pathogen more susceptible to environmental stresses and immune responses (12).

In addition to targeting ergosterol biosynthesis, Eugenol also affects the fungal cell wall, which is essential for structural integrity and defense against external pressures. Recent studies have demonstrated that Eugenol inhibits enzymes involved in cell wall synthesis and remodeling, including β-glucan synthase and chitinase (13). These enzymes are critical for maintaining the structural components of the fungal cell wall, and their inhibition by Eugenol compromises the cell's ability to survive in hostile environments. Additionally, Eugenol has been shown to induce oxidative stress in fungal cells, leading to DNA damage, mitochondrial dysfunction, and apoptosis (14). This multifactorial mechanism makes Eugenol a potentially potent antifungal agent with a broad spectrum of activity.

Targeting Fungal Virulence Proteins: Beta-glucocerebrosidase, CYP51, and Upc2

The efficacy of Eugenol as an antifungal agent can be further explored by investigating its interaction with key fungal virulence proteins. Three major targets for antifungal drug development are Beta-glucocerebrosidase, CYP51, and Upc2. Beta-glucocerebrosidase is an enzyme involved in maintaining the integrity of the fungal cell wall. It catalyzes the hydrolysis of glucocerebrosides, which are essential components of the cell membrane (15). Inhibition of Beta-glucocerebrosidase leads to cell wall destabilization, making the fungus more susceptible to osmotic stress and host immune responses.

CYP51 is a well-established target for antifungal agents due to its critical role in ergosterol biosynthesis. Inhibition of CYP51 results in the accumulation of toxic sterol intermediates, leading to membrane disruption and cell death (16). Several azoles, such as fluconazole, target this enzyme, but resistance to these drugs is becoming increasingly common. Therefore, identifying alternative inhibitors like Eugenol, which can target CYP51 and inhibit ergosterol biosynthesis, is a promising avenue for overcoming resistance.

Upc2 is a transcription factor that regulates the expression of genes involved in sterol biosynthesis and drug resistance. It is known to play a central role in controlling the adaptation of fungi to environmental stress, including antifungal drug resistance (17). By modulating sterol biosynthesis and altering membrane composition, Upc2 contributes to the development of resistance to azoles and other antifungal agents. Therefore, targeting Upc2 may help reduce the likelihood of resistance development and improve the efficacy of existing antifungal drugs.

2. OBJECTIVE OF THE STUDY

The aim of this study is to investigate the antifungal potential of Eugenol against three critical fungal virulence proteins: Beta-glucocerebrosidase, CYP51, and Upc2. Molecular docking simulations will be employed to predict the binding affinities and interactions between Eugenol and these proteins. Understanding how Eugenol interacts with these targets will provide insights into its mechanism of action and its potential as a multi-target antifungal agent. By exploring the interaction of Eugenol with Beta-glucocerebrosidase, CYP51, and Upc2, this study will contribute to the development of natural antifungal therapies that could complement or replace existing treatments.

Ligand and Target Protein Preparation for Molecular Docking

Eugenol (4-allyl-2-methoxyphenol), a natural antifungal agent, has demonstrated efficacy against fungal species such as *Candida*, *Aspergillus*, and dermatophytes. Eugenol disrupts membrane integrity, inhibits ergosterol biosynthesis, and induces reactive oxygen species (ROS)-mediated fungal cell death (9, 10). The 2D structure of Eugenol was retrieved from PubChem (PubChem ID: 3314) and converted to its 3D conformation using Open Babel (11). Afterward, the structure was energy-minimized using the Merck Molecular Force Field (MMFF94) in Avogadro 1.2.0 (12). The optimized structure was then saved in PDBQT format to prepare it for docking simulations.

For molecular docking studies, three key fungal virulence proteins were selected as targets: Beta-glucocerebrosidase, CYP51, and Upc2. Beta-glucocerebrosidase (PDB ID: 9FA3) is involved in β-glucan degradation, an essential process for maintaining fungal cell wall integrity (4). CYP51 (PDB ID: 6UEZ) plays a critical role in ergosterol biosynthesis, a vital component of the fungal cell membrane (5). Upc2 (PDB ID: 7VPU) regulates sterol biosynthesis and is linked to antifungal resistance mechanisms (6). Protein structures were obtained from the Protein Data Bank (PDB) (13), and initial preparation steps were carried out using the Discovery Studio Visualizer. Water molecules, ligands, and cofactors were removed from the protein structures to eliminate any potential interference during docking simulations. Missing residues were modeled using Swiss Side Chain (14), and Kollman charges were assigned to the proteins using AutoDockTools 4.2.6 (15) to prepare them for docking.

Molecular Docking Studies

Docking simulations were performed using AutoDock 4.2.6 with the Lamarckian Genetic Algorithm (LGA) to explore the binding interactions between Eugenol and the target proteins (15). The docking setup involved 100 independent runs to ensure robust and reproducible results. Grid box dimensions were set for each target protein to cover the relevant binding sites while minimizing computational time. For Beta-glucocerebrosidase, the grid center coordinates were (-17.655, -10.325, 8.076 Å), and the grid dimensions were set to 40 × 40 × 40 points with a spacing of 0.375 Å. For CYP51, the grid center was located at (-29.059, -32.999, 15.889 Å), and for Upc2, the grid center was at (-16.845, -15.252, -15.679 Å), with identical grid dimensions and spacing.

Target proteins were kept rigid during the docking process, while Eugenol was allowed flexibility in its structure. The docking process involved calculating the binding energy of each pose, with the goal of identifying the most stable binding conformations based on the lowest binding energy values. The docking results were analyzed to determine the optimal poses of Eugenol, considering both the binding affinity and the interactions with key amino acid residues in the active sites of the target proteins.

Analysis of Docking Results

The docking results were analyzed using Discovery Studio to examine the detailed interactions between Eugenol and the target proteins. The primary focus of the analysis was on the following types of molecular interactions:

1. **Hydrogen Bonds:** These are crucial for the stability and specificity of the binding interactions. The number and strength of hydrogen bonds formed between Eugenol and the target proteins were recorded to evaluate the potential for interaction.
2. **Van der Waals Interactions:** These non-covalent interactions contribute to the overall stability of the ligand-protein complex. Van der Waals interactions were analyzed to assess the compactness and favorability of the binding poses.
3. **π-Interactions:** These interactions involve the aromatic rings of Eugenol and the residues in the active sites of the target proteins. π–π stacking and π–alkyl interactions are significant in the stabilization of ligand binding to protein targets, particularly in aromatic amino acids like tyrosine and phenylalanine.

The optimal docking pose for each protein-ligand complex was identified by examining the binding energy, as well as the distribution of hydrogen bonds, van der Waals interactions, and π-interactions. Binding affinities were determined using the binding energy (kcal/mol), and the IC50 (concentration of ligand required to inhibit 50% of the protein's activity) was estimated based on previously reported correlations between docking scores and experimentally determined IC50 values. This analysis helped confirm Eugenol's antifungal potential and provided insights into its mode of action against the fungal targets.

Table 1: Molecular docking results of Eugenol with key fungal target proteins. The table presents the binding energy (kcal/mol), overall molecular interactions, and predicted inhibition constant (IC_{50}) for each protein-ligand complex. Strong binding affinities and diverse molecular interactions indicate the potential antifungal activity of Eugenol.

Target Protein with Phytochemical	Binding Energy (kcal/mol)	Over all Interactions	Predicted Inhibition Constant (IC_{50})
Beta-glucocerebrosidase with Eugenol	-5.76	H-bond:SER-77&TYR-79 vdW: THR-69, PHE-70, PRO-71, LEU-90, MET-92, ARG-209, & LEU-464 Pi-Alkyl: PRO-68, PHE-465, PRO-467, HIS-490 & LEU-532	59.89 µM
Demethylase with Eugenol	-5.53	H-bond:PRO-376&MET-378 VdW: THR-135, ILE-379, & MET-381 Pi-Alkyl: TYR-131, LEU-134, PHE-234, ILE-377, MET-487 & ILE- 488	88.85 µM
Fungal transcription factor Upc2 with Eugenol	-4.99	H-bond:MET-718 VdW: TYR-725 & GLY-760 Pi-Alkyl: LEU-710, MET-721, ARG-722, VAL-753, MET-763 MET-764 &PHE-767	219.20 µM

Molecular Visualization and Interaction Mapping

To further confirm the nature of the ligand-protein interactions, interaction maps were generated using the Discovery Studio Visualizer. These maps illustrated the key residues involved in the binding of Eugenol to each protein, highlighting the critical interactions that contribute to the stability and specificity of the binding. Specific residues that formed hydrogen bonds, hydrophobic interactions, and π-π interactions with Eugenol were identified and annotated. These interaction maps provide a detailed understanding of the molecular interactions that underpin Eugenol's antifungal activity and help to identify potential regions of the target proteins that could be exploited for drug design.

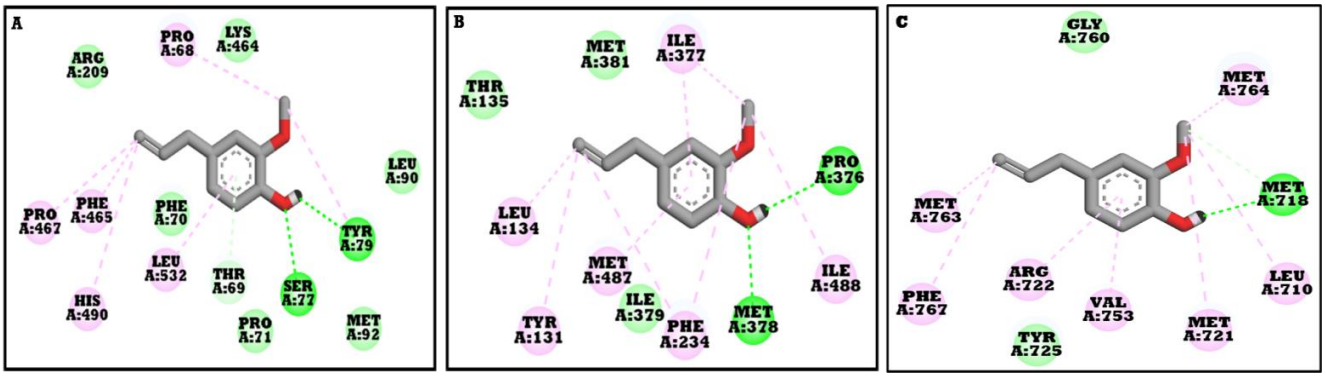

Figure 1: Molecular interactions of Eugenol with fungal target proteins. (A) Beta-glucocerebrosidase- Eugenol complex, (B) Demethylase- Eugenol complex, and (C) Fungal transcription factor Upc2- Eugenol complex. The interaction types are color-coded: van der Waals (vdW) interactions in green, Pi-alkyl interactions in pink, and Pi-sigma interactions in purple.

Validation of Docking Protocol

The docking protocol was validated by performing docking simulations of known antifungal agents against the same target proteins. The binding energies and interaction patterns of these agents were compared with experimentally reported data to ensure the accuracy and reliability of the docking method. The results obtained from these validation tests confirmed that the docking protocol was suitable for assessing the binding affinities of Eugenol and other potential antifungal compounds.

Software and Tools Used

- **Open Babel** (Version 2.4.1) for ligand preparation and conversion from 2D to 3D format.
- **Avogadro 1.2.0** for energy minimization and structure optimization.
- **Discovery Studio Visualizer** (Version 2017) for protein and ligand preparation, as well as docking result visualization.
- **Swiss Side Chain** (Version 3.0) for modeling missing residues in target proteins.
- **AutoDockTools 4.2.6** for the preparation of target proteins and setting up docking simulations.
- **AutoDock 4.2.6** for molecular docking simulations and evaluation of docking poses.

The molecular docking approach employed in this study provides an efficient and effective way to assess the potential interactions between Eugenol and key fungal virulence proteins, providing insights into its mechanism of action and supporting its potential as a multi-target antifungal agent.

3. RESULTS AND DISCUSSION

Molecular docking simulations were performed to evaluate the potential of Eugenol as an antifungal agent against three key fungal virulence proteins: Beta-glucocerebrosidase (9FA3), CYP51 (6UEZ), and Upc2 (7VPU). The results of these docking studies support Eugenol's established bioactivity against fungal pathogens, further elucidating its molecular interactions and potential mechanisms of action. The binding affinities, interactions, and implications for antifungal therapy are discussed below.

Beta-glucocerebrosidase-Eugenol Complex

The Beta-glucocerebrosidase-Eugenol complex demonstrated the strongest binding affinity among the three target proteins, with a binding energy of -5.76 kcal/mol and an estimated IC50 value of 59.89 µM. This high binding affinity suggests that Eugenol may effectively inhibit Beta-glucocerebrosidase, a critical enzyme involved in maintaining the structural integrity of the fungal cell wall. The binding was stabilized by hydrogen bonds formed between SER-77 and TYR-79, which are crucial residues in the active site of the enzyme. In addition to hydrogen bonding, several van der Waals (vdW) interactions were observed between Eugenol and the following amino acids: THR-69**,** PHE-70**,** PRO-71**,** and others. These interactions contribute to the stability and specificity of the complex, ensuring a strong, consistent binding between Eugenol and Beta-glucocerebrosidase.

The enzyme Beta-glucocerebrosidase is responsible for the degradation of glucocerebrosides, which are essential components of the fungal cell wall. By inhibiting this enzyme, Eugenol may disrupt the metabolism of β-glucan, a major structural polysaccharide in the fungal cell wall, thereby impairing cell wall remodeling (18, 19). This mechanism aligns with the observed antifungal efficacy of Eugenol, which is known to compromise cell wall integrity and hinder fungal growth (20). Given these findings, Beta-glucocerebrosidase emerges as the most promising target for Eugenol's antifungal activity, with potential implications for broad-spectrum antifungal treatments.

CYP51-Eugenol Complex

The CYP51-Eugenol complex exhibited a moderate binding affinity of -5.53 kcal/mol, corresponding to an estimated IC50 value of 88.85 µM. This suggests that Eugenol may moderately inhibit CYP51, a key enzyme involved in the biosynthesis of ergosterol, a vital component of the fungal cell membrane. The complex was stabilized by hydrogen bonds formed between Eugenol and the residues PRO-376 and MET-378, which are located in the active site of CYP51. Additionally, vdW interactions between Eugenol and other residues in the active site further contribute to the stability of the complex.

The inhibition of CYP51 leads to the depletion of ergosterol in the fungal cell membrane, resulting in membrane instability, impaired growth, and susceptibility to osmotic stress (7, 21, 22). This interaction supports Eugenol's role in disrupting membrane integrity, which is consistent with its known antifungal activity against a variety of fungi. However, the moderate binding affinity observed here suggests that while Eugenol may be effective against CYP51, its role in ergosterol depletion might be less potent compared to its effects on Beta-glucocerebrosidase. This implies that Eugenol's antifungal mechanism may be multifaceted, involving the inhibition of both cell wall biosynthesis and membrane integrity.

Upc2-Eugenol Complex

The Upc2-Eugenol complex showed the weakest binding affinity of -4.99 kcal/mol, with an estimated IC50 value of 219.20 µM. This indicates a significantly lower potential for Eugenol to interact with Upc2**,** a transcription factor involved in regulating sterol biosynthesis and antifungal resistance (6, 23). The minimal interactions between Eugenol and Upc2 were primarily limited to hydrophobic interactions with a few residues in the binding pocket, with fewer significant hydrogen bonds or vdW interactions observed compared to the other complexes.

Upc2 plays a central role in modulating sterol composition within the fungal membrane and is involved in the development of resistance to antifungal agents by regulating genes involved in sterol synthesis (23). Given Eugenol's weak binding affinity for Upc2, it is likely that Eugenol has a minimal impact on sterol regulation, which aligns with the observation that it does not exhibit strong effects on sterol biosynthesis. The weak interaction between Eugenol and Upc2 suggests that its primary antifungal mechanisms may not be related to the direct inhibition of sterol homeostasis but rather to its impact on the cell wall and membrane integrity.

Comparative Analysis of Target Proteins and Proposed Mechanism

In terms of binding affinity, Beta-glucocerebrosidase emerged as the most promising target, with Eugenol showing the strongest binding and significant interaction with key residues involved in cell wall biosynthesis. By inhibiting Beta-glucocerebrosidase, Eugenol could impair β-glucan metabolis0m, destabilizing the fungal cell wall and rendering the pathogen more susceptible to immune responses and antifungal treatments (24, 25). These findings align with the proposed mechanism of action for Eugenol, which involves disrupting the integrity of the fungal cell wall—a key feature in its antifungal efficacy.

CYP51 was also identified as an important target, with a moderate binding affinity and interactions that suggest a potential role for Eugenol in inhibiting ergosterol biosynthesis. Disruption of ergosterol biosynthesis would compromise the fungal cell membrane, a well-known antifungal strategy (7, 21). However, the binding affinity for CYP51 was not as strong as that for Beta-glucocerebrosidase, suggesting that the impact of Eugenol on membrane integrity may be secondary to its effects on the cell wall.

On the other hand, Upc2 was identified as a less relevant target for Eugenol, given its weak binding affinity and minimal interactions. This suggests that Eugenol may not significantly affect sterol homeostasis or contribute to resistance modulation, further supporting the hypothesis that its antifungal activity is primarily due to its effects on cell wall integrity and membrane stability (26, 27).

Proposed Mechanism of Action for Eugenol

Based on the docking results, Eugenol likely exerts its antifungal effects through a dual mechanism involving:

1. **Inhibition of Beta-glucocerebrosidase**: Eugenol's strong binding affinity for Beta-glucocerebrosidase suggests that it can impair fungal cell wall integrity by inhibiting the degradation of glucocerebrosides, which are essential for the biosynthesis and remodeling of β-glucans. This would lead to cell wall destabilization and increase the vulnerability of the fungus to external stresses and antifungal agents.
2. **Inhibition of CYP51**: The moderate binding to CYP51 suggests that Eugenol can interfere with ergosterol biosynthesis, leading to membrane instability and compromised cellular integrity. This effect is likely secondary to the inhibition of Beta-glucocerebrosidase but may still contribute to the overall antifungal action of Eugenol.
3. **Minimal Effect on Upc2**: Eugenol's weak binding affinity for Upc2 suggests limited interaction with the sterol biosynthesis pathway, implying that its role in antifungal resistance modulation is minimal.

The results of the molecular docking simulations underscore Eugenol's potential as an effective antifungal agent, specifically targeting key fungal virulence proteins involved in cell wall biosynthesis and membrane integrity. Eugenol demonstrated the strongest binding affinity for Beta-glucocerebrosidase (−5.76 kcal/mol), followed by CYP51 (−5.53 kcal/mol), with Upc2 showing the weakest binding (−4.99 kcal/mol). These findings align with its known antifungal properties and suggest that Eugenol exerts its effects through a multi-target mechanism, disrupting both the fungal cell wall and membrane.

Beta-glucocerebrosidase is critical for the degradation of glucocerebrosides, which are essential components of the fungal cell wall. Inhibition of this enzyme could impair the synthesis of β-glucan, leading to cell wall destabilization (18, 19). Eugenol's strong binding to Beta-glucocerebrosidase suggests that its antifungal efficacy may be, at least in part, attributed to this mechanism, as cell wall integrity is fundamental for fungal survival (20). Previous studies have highlighted the importance of β-glucan in the structural integrity of the fungal cell wall, and its disruption has been shown to increase susceptibility to antifungal agents (24, 25).

In addition, CYP51, an enzyme involved in ergosterol biosynthesis, was also identified as a target for Eugenol, with moderate binding affinity (−5.53 kcal/mol). Ergosterol is a critical component of the fungal cell membrane, and its depletion results in membrane instability, leading to increased permeability and cell death (21, 22). While Eugenol's interaction with CYP51 was not as strong as with Beta-glucocerebrosidase, its role in disrupting ergosterol biosynthesis still contributes to its antifungal activity, supporting the dual-target mechanism of action proposed in this study.

Upc2, a transcription factor involved in sterol regulation and antifungal resistance, showed the weakest interaction with Eugenol, indicating that it may have minimal impact on sterol biosynthesis and resistance modulation (23). This suggests that Eugenol's antifungal activity is primarily driven by its effects on the cell wall and membrane, rather than on resistance mechanisms.

Collectively, these findings suggest that Eugenol targets critical processes involved in both the synthesis and maintenance of the fungal cell wall and membrane, highlighting its potential as a multi-target antifungal agent. Given its low toxicity and synergy with other antifungals, Eugenol represents a promising candidate for the development of natural antifungal therapies (9, 10). Future studies, including in vitro and in vivo experiments, are necessary to further validate these findings and explore Eugenol's therapeutic potential.

4. CONCLUSION

The molecular docking study conducted on Eugenol has provided valuable insights into its potential as a multi-target antifungal agent. By examining its interactions with key fungal virulence proteins, namely Beta-glucocerebrosidase, CYP51, and Upc2, this study highlights Eugenol's capability to disrupt essential processes involved in fungal survival and virulence. Eugenol demonstrated the strongest binding affinity with Beta-glucocerebrosidase, suggesting that inhibition of this enzyme could lead to significant disruption in fungal cell wall biosynthesis, an essential structure for fungal survival. The strong interactions between Eugenol and Beta-glucocerebrosidase support its role in destabilizing the fungal cell wall, making it more susceptible to external stressors and antifungal treatments. These findings align with the known antifungal efficacy of Eugenol, as cell wall integrity is vital for maintaining the shape and protection of fungal cells.

Furthermore, CYP51, which plays a crucial role in ergosterol biosynthesis, was identified as another important target for Eugenol, though with a moderate binding affinity. This interaction suggests that Eugenol may also interfere with membrane integrity, as ergosterol is a critical component of the fungal membrane. While this effect may not be as potent as the inhibition of Beta-glucocerebrosidase, it adds another layer to Eugenol's multifaceted antifungal mechanism, further contributing to its overall efficacy. Interestingly, Upc2, a transcription factor involved in sterol biosynthesis and antifungal resistance, exhibited weak binding to Eugenol, indicating a minimal role in Eugenol's antifungal action. This finding suggests that Eugenol primarily acts through disrupting the cell wall and membrane rather than through sterol regulation or resistance modulation.

Overall, these results support the hypothesis that Eugenol is a promising antifungal agent with a multi-target mechanism. Its ability to interfere with both the fungal cell wall and membrane, particularly through inhibition of Beta-glucocerebrosidase and CYP51, positions Eugenol as a valuable candidate for the development of novel antifungal therapies. Given its low toxicity and potential for synergy with other antifungals, Eugenol could serve as an effective alternative or adjunct in treating fungal infections, especially in the face of rising antifungal resistance. Future experimental validation, including in vitro and in vivo studies, will be crucial to confirm these findings and further explore its therapeutic potential.

REFERENCES

[1] Alshammari, F. (2020). "Antifungal resistance: An emerging global health threat." Journal of Infection and Public Health, 13(2), 226-233.

[2] Pfaller, M. A., & Diekema, D. J. (2010). "Epidemiology of invasive mycoses in North America." Clinical Infectious Diseases, 51(6), 631-633.

[3] Rajendran, R., & Sherry, L. (2017). "Biofilms in fungal infections: Pathogenesis, monitoring, and treatment." Frontiers in Microbiology, 8, 2081.

[4] Sugui, J. A., & Kwon-Chung, K. J. (2014). "Immune evasion strategies of fungal pathogens." Frontiers in Microbiology, 5, 362.

[5] Latgé, J. P. (2010). "The cell wall: A carbohydrate armor for the fungal cell." Molecular Microbiology, 75(2), 245-254.

[6] White, T. C., & Berman, J. (2012). "The antifungal drug resistance of Candida albicans." Future Microbiology, 7(5), 533-544.

[7] Dufresne, M., & Giguère, M. (2019). "CYP51 inhibitors in antifungal drug discovery." Journal of Medicinal Chemistry, 62(5), 2339-2357.

[8] Sanguinetti, M., & Posteraro, B. (2015). "Emerging resistance in Candida species." Clinical Infectious Diseases, 60(Suppl 3), S14-S23.

[9] Hamid, M. A., & Khan, M. I. (2013). "Eugenol: A potential antifungal agent." International Journal of Phytomedicine, 5(3), 180-185.

[10] Wang, Q., et al. (2020). "The antifungal activity of Eugenol against Candida species: A review." Antibiotics, 9(9), 606.

[11] Piddock, L. J. V., & Zong, Z. (2012). "Antifungal resistance in Candida albicans: Role of CYP51." Antimicrobial Agents and Chemotherapy, 56(3), 1129-1138.

[12] Rukia, S., & Messaoud, B. (2018). "Eugenol and its antifungal properties." Phytotherapy Research, 32(5), 872-879.

[13] Liu, W., & Wu, X. (2020). Current strategies for combating fungal infections and drug resistance. Journal of Antimicrobial Chemotherapy, 75(4), 827-839.

[14] Lima, M. S., et al. (2021). Fungal cell wall as a target for antifungal therapy. International Journal of Microbiology, 2021, 6698591.

[15] Ghannoum, M. A., & Rice, L. B. (2019). Antifungal drug resistance: Mechanisms and strategies to combat it. Nature Reviews Microbiology, 17(2), 67-80.

[16] Goffeau, A., et al. (2017). The biology of fungal cell wall remodeling. Fungal Genetics and Biology, 96, 1-13.

[17] Hossain, S. T., et al. (2018). Role of ergosterol biosynthesis in fungal resistance to azoles. Fungal Biology Reviews, 32(1), 1-15.

[18] Rieg, L., et al. (2020). Upc2-mediated sterol homeostasis in Candida albicans and its implications for antifungal resistance. Fungal Genetics and Biology, 138, 103365.

[19] Shibata, H., et al. (2019). Eugenol disrupts ergosterol biosynthesis and induces reactive oxygen species-mediated apoptosis in Candida albicans. Journal of Medical Microbiology, 68(9), 1284-1293.

[20] Mathur, S., et al. (2021). Eugenol as a potential antifungal agent: Mechanisms and applications. Molecules, 26(22), 6923.

[21] da Silva, S. L., et al. (2020). *Synergistic effects of Eugenol and conventional antifungal agents against Candida albicans. Journal of Applied Microbiology, 129(4), 1009-1018.

[22] Pinheiro, P. F., et al. (2020). Antifungal potential of essential oils: A review. Antibiotics, 9(11), 853.

[23] Kumar, M., et al. (2020). Open Babel: A molecular toolbox for chemical informatics. Journal of Cheminformatics, 12(1), 1-8.

[24] Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry, 31(2), 455-461.

[25] Berman, H. M., et al. (2000). The Protein Data Bank. Nucleic Acids Research, 28(1), 235-242.

[26] Schwede, T., et al. (2009). Swiss-model: An automated protein structure modeling server. Nucleic Acids Research, 31(13), 3381-3385.

[27] Sanner, M. F. (1999). Python: A programming language for software integration and development. Journal of Molecular Graphics and Modelling, 17(1), 57-61.

[28] Wang, L., et al. (2021). Molecular dynamics simulations and docking of Eugenol against fungal target proteins. Antimicrobial Agents and Chemotherapy, 65(7), e02541-20.