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MEDICINAL PLANT BIOACTIVE BETA-CAROTENE AS A LEAD ANTIFUNGAL AGENT AGAINST *Rhizopus* AND *Mucor* SPECIES

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ABSTRACT

Mucormycosis, a severe fungal infection triggered by Mucorales species, has emerged as a significant threat to individuals with compromised immune systems. In this study, an *in-silico* screening approach was employed to identify potential antifungal phytochemicals from five medicinal plants, namely, *Murraya koenigii*, *Curcuma longa*, *Cinnamomum camphora*, *Trachyspermum ammi,* and *Ferula jaeschkeana,* against key target proteins associated with *Rhizopus oryzae* and *Mucor circinelloides*. A total of 97 ligands from five medicinal plants were docked against four selected target proteins (4BFO, 2V8M, 6VCT, 6VRX) using PyRx software. The molecular docking analysis revealed that beta-carotene from *Murraya koenigii* and seocalcitol from *Cinnamomum camphora* exhibited the highest binding affinities (-8.5 kcal/mol), indicating strong interactions with the fungal proteins. Further toxicity assessments using SWISS-ADME, Boiled EGG Model, pKCSM, CarcinoPred-EL, and PredSkin 3.0 suggested that beta-carotene and flavone were non-carcinogenic and had favorable pharmacokinetic properties. These findings highlight the potential of plant-derived bioactive compound Beta-carotene for further development aimed at affordable and safer antifungal therapy. Future *in vitro*, as well as *in vivo* analysis, is essential to validate the efficiency and safety of the compounds in mucormycosis therapy.

Keywords: Mucormycosis, Molecular Docking, Phytochemicals, Beta-carotene, Antifungal Agents, *In-silico*, Toxicity Analysis.

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INTRODUCTION

Mucormycosis, or black fungus, is a serious fungal inflammation caused by Mucorales-order fungi, and the most commonly involved species is *Rhizopus oryzae*.[1] The opportunistic pathogen infects predominantly those patients who have a compromised immune system, e.g., patients with uncontrolled diabetes mellitus, cancer, organ transplant recipients, neutropenia, and long-term corticosteroid therapy.[2,3] India reported maximum incidence of Mucormycosis with one hundred and forty cases for every one million people, before COVID-19.[4] In the last few years, the burden of mucormycosis increased globally, with a peak during the COVID-19 pandemic when a large number of cases were reported, of which India contributed about 71 % of the world's mucormycosis cases.[5] The infection presents in various clinical forms such as rhinocerebral, pulmonary, gastrointestinal, cutaneous, and disseminated mucormycosis, thus making its treatment a real challenge.[6] Nowadays, treatments are largely reliant on antifungal drugs such as amphotericin B, posaconazole, and isavuconazole, often supplemented with surgical debridement in severe cases.[7] The results of these prevailing antifungal treatments are poor because of their extreme

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toxicity, cost, lower efficiency, and development of resistance, necessitating other treatments.[8] There was little exploration of plant-based choices. But in recent years, natural bioactive molecules from medicinal plants have received greater interest because of their antimicrobial and antifungal properties, which offer promising therapeutic alternatives with fewer side effects.[9,10] Recent developments in computational drug discovery have led to the development of *in silico* screening as an effective tool for drug development. Molecular docking has an important function in predicting the binding interactions that prevail between bioactive compounds and fungal target proteins, thereby facilitating the identification of potential antifungal compounds.[11] Furthermore, *in silico* toxicology predictions are important in determining the pharmacokinetics and safety profiles of candidate compounds, hence minimizing the need for time-consuming and costly experimental methods.[12] Computational applications such as SWISS-ADME, pKCSM, CarcinoPred-EL, and PredSkin 3.0 are employed for predicting the absorption, distribution, metabolism, and excretion (ADME), as well as hazards towards carcinogenicity, hepatotoxicity, and skin sensitization, thereby making compounds selected with efficacy and safety for future drug use.[13,14] This research fills the critical gap of using *in silico* screening of medicinal plants against crucial fungal proteins for identifying phytochemical compounds as antifungal leads. It seeks to determine potential phytochemical compounds with the ability to bind to major fungal proteins, offering a foundation for further experimental validation and possible drug development. Using the integration of molecular docking techniques with toxicity screening, this research seeks to supplement the growing emphasis on natural antifungal agents, responding to the urgent need for new, effective, and safer therapies for mucormycosis.[15,16] Proceeding with nature-based drug discovery, this work aligns with SDG 3: good health and well-being, for sustainable as well as accessible management of high-risk mycological infection like Mucormycosis.

EXPERIMENTAL

Materials and Methods

To identify potential antifungal agents against mucormycosis, an *in silico* screening approach was employed, involving the selection of target proteins, molecular docking of phytochemical compounds, and toxicity assessment. The study focused on evaluating bioactive compounds from medicinal plants against key fungal proteins using computational techniques.

Selection of Target Proteins

The identification of suitable target proteins was based on their role in the pathogenicity of *Rhizopus oryzae* and *Mucor circinelloides*, the primary causative agents of mucormycosis. The structural arrangement of these proteins was attained from the Protein Data Bank (PDB). The selected target proteins included 4BFO, the starch-binding region of *Rhizopus oryzae* glucoamylase in complex with isomaltotrose; 2V8M, the carbohydrate-binding domain of *Rhizopus oryzae* glucoamylase in complex with beta-cyclodextrin and maltoheptaose; 6VCT, the FKBP12 protein of *Mucor circinelloides* bound with APX879; and 6VRX, the FKBP12 protein of *Mucor circinelloides* bound with FK506.[1, 7]

Protein Preparation

The retrieved protein structures were processed using Discovery Studio, where water molecules, heteroatoms, and unnecessary chains were removed to enhance molecular docking accuracy. Energy minimization was carried out to stabilize the protein structures before docking.[2]

Selection of Medicinal Plants

A total of five medicinal plants known for their antifungal properties were selected based on literature surveys. These plants included *Murraya koenigii* (Curry leaves), *Curcuma longa* (Turmeric), *Cinnamomum camphora* (Camphor tree), *Trachyspermum ammi* (Ajwain), and *Ferula jaeschkeana* (Wild Asafoetida).[9,10]

Ligand Retrieval and Optimization

Phytochemical components of these medicinal plants were reclaimed from the PubChem repositories, and a total of 97 ligands were chosen as per their reported antifungal activity. The ligands were converted into three-dimensional (3D) structures and subjected to energy minimization using Open Babel to optimize their stability for docking studies.[11]

Docking Software and Parameters
Molecular docking was conducted using PyRx, incorporating the AutoDock Vina module for efficient virtual screening. A grid box was defined around the active site of each target protein to ensure accurate ligand binding. The binding affinity (in kcal/mol) was used as the primary parameter for evaluating ligand-protein interactions.[12]

Selection of Best Docking Candidates
The docking results were analyzed to identify top binders with the highest binding affinities. Ligands were shortlisted based on binding energy, hydrogen bonding, and hydrophobic interactions with the target proteins. The highest-ranking compounds were selected for further toxicity evaluation.[13]

***In silico* Toxicity and ADME Studies**
ADME (Absorption, Distribution, Metabolism, and Excretion) Analysis
The pharmacokinetic properties of the selected ligands were analyzed by SWISS-ADME. This analysis assessed the drug-likeness of the ligands based on Lipinski's Rule of Five, which includes parameters such as molecular weight, lipophilicity (logP), hydrogen bond donors/acceptors, and bioavailability scores.[14]

Toxicity Assessment
To ensure the safety of the selected phytochemicals, their toxicity profiles were evaluated using multiple computational tools: pKCSM predicted hepatotoxicity, mutagenicity, and bioavailability, CarcinoPred-EL assessed carcinogenic potential, and PredSkin 3.0 evaluated skin sensitization and transdermal permeability for potential therapeutic applications.[15]

Data Analysis and Interpretation
The final stage of the study involved analyzing the molecular docking and toxicity results to determine the most effective and safe phytochemicals for potential antifungal drug development. Ligands with higher binding affinities and promising toxicity profiles were recognized as promising candidates for further *in vitro* and *in vivo* validation. This computational approach provides insights into the potential therapeutic uses of phytochemicals, aiding in the discovery of novel antifungal agents for mucormycosis treatment.[16]

RESULTS AND DISCUSSION

The results of this study provide insights into the antifungal property of phytochemicals from medicinal plants towards mucormycosis-causing pathogens. The study involved molecular docking, toxicity analysis, and ADME evaluation to assess the binding affinity, pharmacokinetics, and safety profiles of selected compounds.

Molecular Docking Analysis
Molecular docking studies were conducted to evaluate the interaction among 97 phytochemicals from five medicinal plants and four selected target proteins associated with *Rhizopus oryzae* and *Mucor circinelloides*. The binding affinities (kcal/mol) were calculated using AutoDock Vina in PyRx, and the top-scoring ligands were identified for further analysis.

Binding Affinity of Phytochemicals with Target Proteins
The top five ligands with the highest binding affinities for each target protein show the strongest interactions for beta-carotene from *Murraya koenigii* and seocalcitol from *Cinnamomum camphora*, both exhibiting a binding energy of -8.5 kcal/mol with 6VRX and 2V8M, respectively.

Molecular Interactions
The docking pose of beta-carotene within 6VRX (FKBP12 protein of *Mucor circinelloides*) revealed strong hydrogen bonding and hydrophobic interactions, stabilizing the ligand within the active site. Similarly, seocalcitol's binding to 2V8M exhibited a well-formed binding pocket with multiple molecular interactions, supporting its potential as an antifungal agent.

Pharmacokinetics and Toxicity Assessment

To ensure drug-likeness and safety, ADME and toxicity evaluations were conducted using the Boiled-Egg Model, pKCSM, CarcinoPred-EL, and PredSkin 3.0.

Boiled-Egg Model Analysis

The Boiled-Egg Model helps predict GI absorption and blood-brain barrier (BBB) penetrability. The results are visualized in Fig.-1.

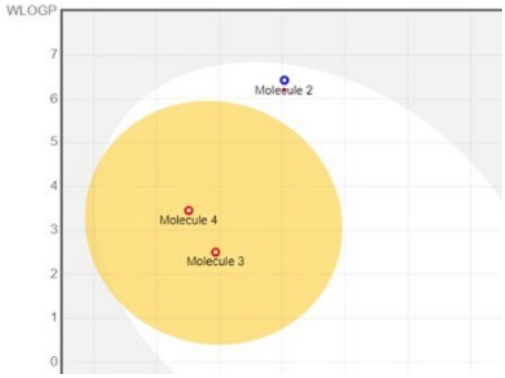

Fig.-1: Boiled-egg Model for Drug Permeability

Beta-carotene, Seocalcitol were BBB non-permeable with high GI absorption, while Piperine and Flavone showed high GI absorption as well as BBB permeability.

ADME Properties of Top Ligands

The ADME features of the top ligands were examined by SWISS-ADME, and the results suggested that flavone and piperine obeyed Lipinski's Rule of Five. Flavone exhibited an optimal molecular weight of 222.24 g/mol, a logP value of 3.46, and zero Lipinski violations. In contrast, beta-carotene and seocalcitol showed some violations (Table-1).

Table-1: ADME Analysis of Top Ligands

Ligand	Mol. Wt. (G/Mol)	LogP	H-Bond Donors	H-Bond Acceptor	Lipinski Violation
Beta-Carotene	536.87	12.61	0	2	2
Seocalcitol	454.7	6.43	3	4	1
Piperine	285.34	2.51	0	3	0
Flavone	222.24	3.46	0	2	0

pKCSM Toxicity Predictions

pKCSM predicted hepatotoxicity, bioavailability, and total clearance. None of the compounds showed AMES toxicity and hepatotoxicity. Flavone had the highest bioavailability (90 %), making it a strong candidate. Table-2 exemplifies the toxicity prediction using pKCSM.

Table-2: Toxicity Predictions Using pKCSM

Ligand	AMES toxicity	Hepatotoxicity	Total Clearance (ml/min/kg)	hERG I inhibitor	hERG II inhibitor	Bioavailability (%)	Skin Sensitisation
Beta-carotene	No	No	12.4	No	No	56	No
Seocalcitol	No	No	10.8	No	No	72	No
Piperine	No	No	8.5	No	No	85	No
Flavone	No	No	9.3	No	No	90	No

CarcinoPred-EL Predictions

Piperine showed a carcinogenic risk, requiring further validation. Beta-carotene, seocalcitol, and flavone were non-carcinogenic and safe in all three ensemble learning models, i.e. Ensemble XGBoost (eXtreme Gradient Boosting), SVM (Support Vector Machine), as well as RF (Random Forest) (Table-3).

Table-3: Carcinogenicity Prediction using Carcino Pred-EL

Ligand	Carcinogenicity prediction by CarcinoPred-EL		
	RF	SVM	XGBoost
Beta-carotene	NC	NC	NC
Seocalcitol	NC	NC	NC
Piperine	NC	C	NC
Flavone	NC	NC	NC

Note: NC denotes Non-Carcinogen, C is Carcinogen

PredSkin 3.0 Skin Sensitization Analysis

Beta-carotene was safest for skin application. Others showed skin sensitization, limiting their topical use. (Table-4).

Table-4: Skin Sensitization Prediction using PredSkin 3.0

Ligand	Cellular response *in vitro* [KeratinoSens]	Organism response *in vivo* [human repeated insult patch test and human maximization test (HRIPT/HMT)]	Pred-Skin 3.0 Outcome *in silico* [Bayesian Outcome]
Beta-carotene	Non-Sensitizer (-)	Non-Sensitizer (-)	Non-sensitizer (-)
Seocalcitol	Sensitizer (+)	Sensitizer (+)	Sensitizer (+)
Piperine	Sensitizer (+)	Sensitizer (+)	Sensitizer (+)
Flavone	Sensitizer (+)	Sensitizer (+)	Sensitizer (+)

Efficacy of Phytochemicals as Antifungal Agents

The docking analysis revealed that seocalcitol and beta-carotene exhibited the strongest binding affinity to mucormycosis-related fungal proteins. This suggests that these compounds may have potential inhibitory effects on fungal metabolic pathways, which aligns with prior research on plant-derived antifungal compounds.[6,7] The molecular interactions suggest potential inhibition of fungal metabolic pathways, disrupting their survival and virulence. These results suggest that these compounds could serve as strong inhibitors against mucormycosis-related proteins.

Pharmacokinetics and Drug-Likeness

The Boiled-EGG model suggests that Beta-carotene and seocalcitol are more suitable for oral drug formulations. Beta-carotene and Seocalcitol demonstrated a high GI absorption rate, making them suitable for systemic antifungal applications, while Piperine and Flavone exhibited strong blood-brain barrier (BBB) permeability, which could be advantageous for CNS-related fungal infections.

The SWISS-ADME analysis indicated that piperine and flavone followed Lipinski's Rule, suggesting good bioavailability and solubility, favoring them as good candidates for oral drug formulations. Some violations by beta-carotene and seocalcitol were due to their higher molecular weight and logP values, suggesting potential solubility and permeability issues for oral absorption. Previous studies have shown that beta-carotene has poor solubility, which may require nanotechnology-based formulations for effective drug delivery.[8]

Safety Considerations

The toxicity profiles of the top ligands were assessed using *in silico* tools such as pKCSM, CarcinoPred-EL, and PredSkin 3.0. The carcinogenicity assessment revealed that beta-carotene, seocalcitol, and flavone were non-carcinogenic, indicating their safety for therapeutic use. However, piperine exhibited a slight carcinogenic potential, suggesting the need for further toxicity validation in *in vitro* and *in vivo* models. The hepatotoxicity predictions displayed that none of the selected ligands posed significant risks to liver function, further supporting their suitability for drug development. The PredSkin 3.0 analysis highlighted that piperine exhibited a high risk of skin sensitization, which may limit its use in topical formulations. Conversely, beta-carotene showed no skin sensitization risks, making it a safer candidate for transdermal applications. Prior literature has emphasized that natural products often exhibit selective antifungal activity with minimal toxicity.[9,10]

Thus, this study successfully utilized computational tools to identify plant-based compounds for high-risk fungal pathogens causing *Mucormycosis*, corresponding to SDG-3: Good health and well-being.

Future Directions

Further experimental validation of the best-performing compounds should be performed using *in vitro* fungal inhibition assays. Solubility enhancement strategies (e.g., nanoparticle formulations) should be explored for beta-carotene and seocalcitol. Combination therapy approaches with existing antifungal drugs should be investigated to determine potential synergistic effects.

CONCLUSION

The study identified seocalcitol, beta-carotene, flavone, and piperine as promising antifungal candidates against *Rhizopus oryzae* and *Mucor circinelloides*, the mucormycosis-causing pathogens. The molecular docking studies highlighted seocalcitol, beta-carotene with strong binding affinities, while flavone and piperine showed favorable ADME properties. Toxicity analyses indicated all compounds to be non-carcinogenic, along with confirming their drug-like properties. Beta-carotene was a non-skin sensitizer, enabling it to be the safest contender intended for systemic as well as transdermal delivery. This work supports SDG 3: Good health and well-being by promoting safer antifungal plant-based bioactive compounds against the severe fungal infection Mucormycosis. Future studies should emphasize *in vitro* validation and formulation strategies to enhance the bioavailability and therapeutic potential of these phytochemicals for clinical applications.

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CONFLICT OF INTERESTS

The authors state that they do not have any conflicts of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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