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**Research Article**

# Screening of potential antifungal compounds for uropathogens isolated from traditional Indian Medicinal plant of *Carica papaya: In silico* study

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**Abstract**

Urinary tract infections are one the most tedious infection and painful in India. The insufficient knowledge and misdiagnosis lead to the results of indiscriminate use in antibiotics which develop resistance. Fungal diseases are the most dangerous since the patterns of the infections varies person to person. Fungal spores in dermis can leads to severe infection and develop scars in moist and damp area. Herbal drugs and naturopathy makes sense now a day and found reliable for such infections, resistance in synthetic drugs are higher as compared to herbal ones. This research work used various active compounds selected from plant leaves of *Carica papaya* exhibiting antifungal activity. Molecular docking studies were performed. Selected compounds were validated and confirmed for drug design using ADME analysis virtually. A good reliable binding affinity was found among the compounds and fungal targets from *Aspergillus* and *Candida* selected on the base of virulence factors.

**Keywords**: Urinary Tract Infections, Candidiasis, Molecular docking studies

## INTRODUCTION

Fungal infections are the most deadly diseases and lifelong. Women are more prone to such infections due to the unhygeine and moist environment. *Aspergillus* and *Candida* are the common pathogens. *Candida* yeast plays a vital role in women's UTI, there are various types of UTI , Complicated and Uncomplicated UTI, virulence factors of the *Candida* includes enzymes and several protein secreated by the yeast. *Candida albicans* have special sets of glycosylphosphatidylinositol (GPI)-linked cell surface glycoproteins that allow it to adhere to the surfaces. Als3 proteins act as invasins that help with the invasion to the host epithelial and endothelial cells. Once the colonization takes place the cells are the most powerful. As per the epidemiology several antifungal agents receives resistance. The increased use of triazoles in prophylactic and empiric antifungal treatments in high-risk patients has led to emergence of antifungal resistance. Glucosamine 6 phosphate synthase confirms the role of hexosamine metabolism. This allows the fungal cell to multiply Hence isomerase domain of glucosamine 6 phosphate synthase selected for the study [(1)].

A like candidiasis one of the major diseases in women is aspergillosis. The cytochrome P450 isoenzymes are a conserved group of proteins that plays role in various metabolisms. Generally in fungus, ergosterols synthesized which maintains the membrane fluidity and cell membrane formation such sterol formation can be inhibited using drug. Hence to develop plant based drug, receptor target selected as crystal structure of lanosterol 14 $\alpha$-demethylase transmembrane[2].

The current study designed to initiate the formulation of the herbal composition of which the complete Phytochemical analysis shows that there are various anti fungal and anti bacterial compounds present in the leaves of *Carica papaya*[3]. *Carica papaya* is majorly used plant since ancient times. The research work underwent the screening *invitro* for the said leaves and tried to put forward herbal antimicrobial drug against MDR uropathogens[4]. Microbiological studies were performed previously and it is found that, there are certain bioactive compound which having MDR antifungal activity. Computational studies were tried to support literature for future studies

**Materials and Methods**

**Preparation of protein structure:** Protein structures were important in disease biology. The research work selected 2 protein targets from *Candida albicans and A. fumigates* were retrieved from database Protein Data Bank (PDB) (PDB: http://www.rcsb.org/ pdb/home/home. do) was (PDB id 1MOQ, 4K0F of the target proteins. All water molecules were removed and on final stage hydrogen atoms were added to receptor molecule. Table1.

**Preparation of ligand structure**: Three active natural compounds of the plant extract in *C. papaya* were selected on the basis of their biological activity reported and molinspiron drawing tool used to draw the 3D structures.

**Protein ligand interaction:** The two proteins and two ligands were subjected to docking studies individually using AUTODOCK 4.0 docking server, was based on the quantum mechanics, it predicts the molecular structures, active energies, geometry of structure, coordinates of atoms, bond length and bond angle present in their pocket[5].

**Drug likeness Analysis**

Molinspiron was a tools used which is a freeware to perform the properties for drug likeness a rule of five. Analysis was done online using a server dedicated account[6].

**Absorption, Distribution, Metabolism, and Excretion (ADME)** was calculated using admet SAR tool various parameters were screened to analyse the drug like properties of the phytocompounds[7].

**Computational Methods**

Docking of the selected compounds and target selected were carried out using Autodock Server[8] Gasteiger partial charges were added to the ligand. Cleaning of the targets and ligand were done using Accelrys Discovery studio visualizer 4.0. Essential hydrogen atoms, Kollman united atom type charges, and salvation parameters were added with the aid of Auto Dock tools maps of 20 Å grid points and 0.375 Å spacing were generated using the Auto grid program automatically. Auto Dock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively[9].

Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied. Also, due to high data output only the best score were selected and bonds were measured.

**Results**

Structures of the two different proteins of uropathogens downloaded from PDB shown in table1.Selected 2 targets were characterized well and necessary precaution were taken by adding hydrogen while handling target proteins. Discovery studio visualization tool were used. Two pathogens were from the same family was taken, hence a common virulence factor selected and chosen for the study.

*Invitro* analysis of the *Carica papaya* plant leaves shows various active components available for fungus especially *Aspergillus and Candida* spp. Molecular docking was carried out using Lamarckian genetic algorithm (LGA), with Autodock tools, free energy of bindings were recorded for all the 8 compounds with 2 targets. Only remarkable docking scores were shown and discussed. 1H-Indole,3-[4-(1H-indol-3-yl)-2-thiazolyl found as the most suitable compound for all the targets with high dock score and various hydrogen bond formation i.e. -7.25kcal/mol. N amino morphiline also shows good score by forming strong hydrogen bonding with respective interacting amino acid chains.

Drug likeness activity were performed and all the compounds obeys Lipinski rule 5 and in the range of drug parameters, Out of three compounds 2-Methoxy-4-vinylphenol shows better score and definitely act as drug. This was good against *Aspergillus* target. Table 3 depicts ADMET analysis range of results includes aqueous solubility among the all compounds, Blood brain barrier analysis generally used for penetration studies, level of 0-1 shows high penetration, in this study all the compounds shows high penetration levels hence considered to cross the barrier and act on Central nervous system. CYP450 are the important catalysers for drug metabolism, levels are from 0 and 1. In this study N-Aminomorpholine proved as inhibitors level and can act as good antifungal.

**Table 1 Protein Retrieved from PDB**

| Sr No | Name of Protein | PDB Id |
|---|---|---|
| **1** | Glucosamine 6 phosphate synthase | 1MOQ |
| **2** | Lanosterol 14 $\alpha$-demethylase transmembrane domain | 4F0K |

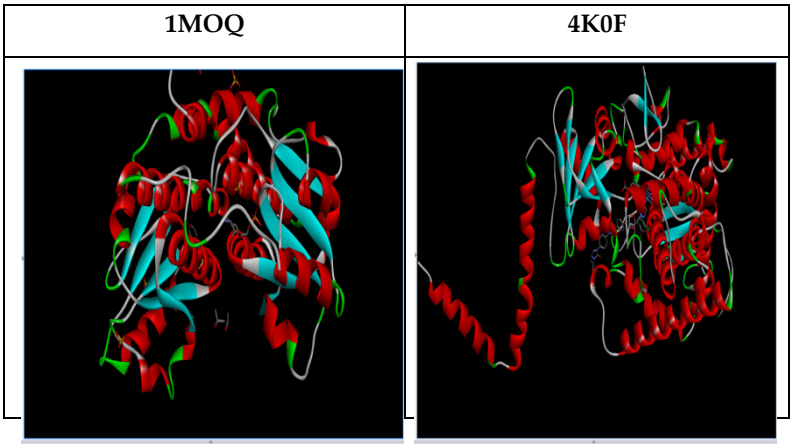


**Table 2 Protein Ligand Interaction Docking Scores**

**A. Docking studies results of *Candida albicans* PDB ID: 1MOQ with selected phytocompounds**

| Ligands /Protein | Est. Free Energy of Binding kcal/mol | vdW + Hbond + desolv Energy kcal/mol | Amino acids |
|---|---|---|---|
| N-Aminomorpholine | -4.30 | -4.23 | Ser349 |
| 2-Methoxy-4-vinylphenol | -3.78 | -4.13 | Ser349,347 Thr352, |
| 1H-Indole,3-[4-(1H-indol-3-yl)-2-thiazolyl | -7.27 | -7.1 | Lys603, Asp 354 |

**B. Docking studies results of *Aspergillus species* PDB ID: 4K0F with selected phytocompounds**

| Ligands /Protein | Est. Free Energy of Binding kcal/mol | vdW + Hbond + desolv Energy kcal/mol | Amino acids |
|---|---|---|---|
| N-Aminomorpholine | -5.14 | -4.61 | His 317,Phe 236, Val 510 |
| 2-Methoxy-4-vinylphenol | -6.72 | -8.42 | Tyr 140,Thr 318,Leu 158 Phe 184 |
| 1H-Indole,3-[4-(1H-indol-3-yl)-2-thiazolyl | -4.82 | -5.30 | Phe 243 Arg 98 Trp 65 |

**Table 3: ADME analysis values of phytocompounds**

| Ligands/ Test | Aqueous Solubility | BBB+ | CYP450 | AMES toxicity | Carcinogens |
|---|---|---|---|---|---|
| N-Aminomorpholine | -0.6090 | 0.9873 | 0.9148 | 0.5348- | 0.8709 - |
| 2-Methoxy-4-vinylphenol | -1.9439 | 0.8480 | 0.7598 | 0.9132- | 0.8519- |
| 1H-Indole,3-[4-(1H-indol-3-yl)-2-thiazolyl | -3.7499 | 0.8941 | 0.7353 | 0.5764 | 0.5548 |

## Discussion

In 2008, the potential antibacterial activities and phytochemical studies on leaf of *Carica papaya* species, components quantitatively analyzed were amino acids, a-amylase, ß-amylase, carbohydrate, glutamine, protein, proline and phenolic compounds. The components qualitatively analysed were alkaloids, anthroquinone, catachol, flavonoids, phenols, saponins, steriods, triterpenoids and tannins. Impression of the study were *Carica papaya, Cynodon dactylon, Euphorbia hirta*, *Melia azedarach* and *Psidium guajava* shows antimicrobial activity to the test organism [(10)]. The leaves of papaya contain many active components that can act as a potent antioxidant in blood and also promising therapy for to reduce lipid peroxidation level[(11)].

The antifungal activities of dry and green leaf of *Carica papaya* leaves have been studied in the extract of papaya. Fungus *Candida albicans and Aspergillus flavus* was included in the study[(12,13)]. Selected anti microbial molecules was docked with the fungal targets, promising results were noted shown in tables all the targets were retrieved and docked. 1H-Indole,3-[4-(1H-indol-3-yl)-2-thiazolyl which is already showing good antifungal activity showed good molecular interaction with hydrogen bond formation with score -7.27 kcal/mol. Scores more than -4.0 in docking studies can be considered as a good score.

Drug likeness activity of the molecules utilized for the docking need to be screened. Here docked compound such as indole and phenolic groups directly binds with the enzymes with good free energy of binding. Selected compounds were freely bound with the targets known for the virulence factors.

## Conclusion

In 21st Century the greatest inclination towards the herbal care is growing, and antibiotic resistance is at alarming stage. *Carica papaya* was found to be a basket of benefits since ancient periods but yet lot to be discovered. The current study has initiated and found a good antimicrobial result, hence on the basis of analysis and results of insilico studies, selected compounds need to be recommended for further pharmacological studies.

## Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper

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