

## PREDICTION OF ANTIFUNGAL ACTIVITY OF *OCIMUM SANCTUM* TARGETING VULVO-VAGINAL CANDIDIASIS USING COMPUTATIONAL DOCKING TECHNIQUES

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### Abstract

Phytochemicals are non-nutritive plant-based compounds which are known to have therapeutic properties. *Ocimum sanctum* is popular due to the presence of many medicinal qualities in it. This plant extract contains various phytochemicals that help maintain its medicinal value. Candidiasis, caused by *Candida sp.* has emerged as a common fungal infection these days. One of the key enzymes that is involved in its metabolic pathway is dihydrofolate reductase. The molecular docking of the phytochemicals that were screened from *Ocimum sanctum* was carried out against the enzyme from *Candida sp.* using BIOVIA Discovery Studio. The efficiency of the interaction between the ligand and the protein was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for the selected parameters indicated that phytochemicals such as, apigenin, cirsilineol, cirsimaritin, eugenol, luteolin, rosmarinic acid can effectively deactivate the enzyme thereby interrupting the life cycle of *Candida*.

**Key words:** Phytochemical, BIOVIA, Discovery studio, *Ocimum sanctum*, *Candida*

### Introduction

The present lifestyle conditions have become fast paced, comfortable, monotonous and stressful. The prevailing work conditions along with unhealthy life style that lacks physical activity is causing deterioration of health, rather than bringing any health benefits. Life style diseases such as blood pressure, diabetes, obesity etc are increasing day by day which is further leading to other associated diseases. One of the common diseases prevailing these days is fungal infection caused by *Candida sp.* More than 1.5 million people have lost their lives and billions have got affected by fungal diseases. Though the cases of fungal diseases are increasing, the medical facility is not available to sufficient number of people who could have easily got cured if treatment was available at the correct time. Serious fungal infections are even reported as a consequence of some major health issues like asthma, corticosteroid therapies, AIDS, cancer and organ transplantation. Early diagnosis of the infection can allow for a prompt antifungal medication; however, this often gets delayed due to insufficient resources (Armstrong *et al.*, 2014; Marr *et al.*, 2002).

Folk medicines, as they are dependent on naturally available medications (including plants) have become a reliable resource in developing nations (Panda *et al.*, 2016). Most of these countries, necessarily depend on herbal products for health care (Arulselvan *et al.*, 2016). The increasing dependence on herbal therapies, despite the development of commercial drugs, is due to the side effects associated with synthetic drugs. Hence, for the prevention and treatment of various ailments, traditional medicines are used (Bongomin *et al.*, 2017; Chhotaray *et al.*, 2020; Das *et al.*, 2020; Dash *et al.*, 2020; Sahoo *et al.*, 2020; Tripathy *et al.*, 2020). India is a rich source of knowledge, when it comes to herbal understanding (Sahoo *et al.*, 2010). A number of literatures since primeval times, such as Ayurveda, Unani and Sidha have quoted the use of herbs for treatment of several ailments. Ayurvedic texts like the, 'Charak Samhita', 'Sushrut Samhita', 'Satmya Darpan Samhita', 'Vaisajya

Ratnabali,' 'Sarangadhara Samhita', 'Bhavaprakasha Samhita', 'Rasatarangini' precisely explain numerous remedies. In fact, many plant-based medications are still in demand for the treatment of diseases like arthritis, inflammatory conditions, bronchitis, tuberculosis, congestive cardiac failure and cancer. Fungal infections are one such common disease, that is affecting people around the world, particularly in rural localities of developing nations. The prevailing of fungal infections in such areas is due to poor sanitation, inadequate amount of nutritious food and inattentiveness to dietary food supplements. Fungal infection can be seen infecting people of all age groups.

The medicinal value of plants mainly lies in the chemical substances known as phytochemicals, that produce a definite physiological action on the human body. Plant based therapeutic constituents can be derived from any part of plant like its leaves, flowers, fruits, bark, roots, and seeds (Hussain et al., 2001). Various medicinal plants and their phytoextracts are reported to have numerous medicinal properties such as anti-microbial, anti-inflammatory, anti-oxidant, anti-diabetes and anti-cancer activity. Medicinal plants, due to the presence of several beneficial phytochemicals in them, have become the foundation of many modern drugs. More than 25% of modern drugs are reported to have botanical origins. Hence, the demand for research in the field of medicinal plants are huge. Also, plants are balanced with potential health benefits because of which they have found growing needs in the supplementary diet as well as in nutraceuticals. Based on this potential demand, research into the biological activity, quality, clinical efficacy and safety plants is hugely required. Newly emerging scientific techniques and approaches have made it convenient to study the biological activity of plants in much details. These techniques have found applications in the investigation of constituents and determination of biological activity of therapeutic plants. Evidence based on these techniques have revealed several beneficial effects of plants. Plants having curative effect on inflammation, microbial infection, cancer have received greater attention (Tewari et al., 2012).

Tulsi belonging to the family Lamiaceae, is used to cure disease like candidiasis and blood purifying agent. Tulsi is reported to contain phytochemicals such as rosmarinic acid, apigenin, eugenol, carnosic acid, cirsilineol, cirsimaritin, leuteolin. These phytochemicals play major role in curing several diseases including candidiasis (Amit *et al.*, 2013). However, there are limited reports that have identified the specific phytochemical in it, that are responsible to cure candidiasis.

Candidiasis, is a common fungal disease that affects the skin, intestinal tract and the vagina. Candida fungus typically invades the human gastro-intestinal tract along with the skin and vagina, where it sheds through the vaginal secretions. Humans beings get infected by this infection, due to decrease in the *Lactobacillus* community in the vagina, which could be associated with poor eating habits, hormonal imbalance, stress, lack of sleep or even due to a weak immune system. This study focuses on the identification of the phytochemical from *Ocimum sanctum* that are involved in the cure of Candidiasis caused by *Candida*.

## Materials and Methods

### Software used

Dassault Systemes BIOVIA, Discovery studio was used for analysis. The software utilizes the CHARMM molecular mechanics simulation program. It targets **biological molecules such as**, peptides, proteins, ligands, carbohydrates, nucleic acids and lipids, as they occur in the solution, crystal, and membrane environment. It makes the use of machine learning techniques in order to predict the molecular interaction.

### List of phytochemicals

Phytochemicals are naturally occurring plant based chemical components that when consumed by humans, can provide adequate nutrition as well as protection from pathogens. Reports suggest that *Ocimum sanctum* contains phytochemicals such as rosmarinic acid, apigenin, eugenol, carnosic acid, cirsilineol, cirsimaritin, leuteolin. It has already been established that *Ocimum sanctum* has potentials in controlling

Candidiasis. This work is focused on screening of specific phytochemicals responsible for inhibiting and controlling Candidiasis infection.

#### *Enzyme found in Candida*

Candidiasis, caused as a result of *Candida* infection is due to the various metabolic cycles in the fungal life cycle for its survival. These metabolic cycles are regulated by several enzymes. In the present study, with the help of RCSB enzyme database list of different enzymes found in *Candida sp* were screened. It has been reported that enzyme dihydrofolate reductase (protein database code 1M78) catalyses the transfer of hydride from NADPH to dihydrofolate with an accompanying protonation that produces tetrahydrofolate, which is very crucial for the survival of the particular microbe.

#### *Molecular docking*

For the purpose of evaluation of level of interaction between the phytochemical and the enzyme, Molecular docking method has been used. With the help of Dassault Systemes BIOVIA, Discovery studio the interaction strength was analysed. For the analysis, the sdf files for the phytochemicals that are present in *Ocimum sanctum* were initially downloaded from PubChem. The protein database code for the enzyme was extracted from RCSB site. The active site present in the enzyme was identified using “receptor cavity” tool, that is present under “receptor-ligand interaction” menu. Molecular docking was carried out by using CDocker protocol of BIOVIA Discovery Studio, that is present under “receptor-ligand interaction”. The enzyme molecule served as the receptor, whereas the phytochemical served as the ligand. The “-CDOCKER\_ENERGY” and “-CDOCKER\_INTERACTION\_ENERGY” were analysed and used as the criterion for estimating the quality of molecular docking. Greater positive values of these criterions represent good interaction between the ligand and the receptor. Lesser difference between “-CDOCKER\_ENERGY” and “-CDOCKER\_INTERACTION\_ENERGY” indicated a stable interaction between the ligand and the enzyme. Thus, the interactions with higher values indicated a greater efficiency of the phytochemical in curing the disease.

### **Results and Discussion**

Figure 1 shows the active site present in dihydrofolate reductase enzyme, that appears light green in color. CDock, a CHARMM based docking engine, is a molecular dynamics simulated-annealing-based algorithm that is optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

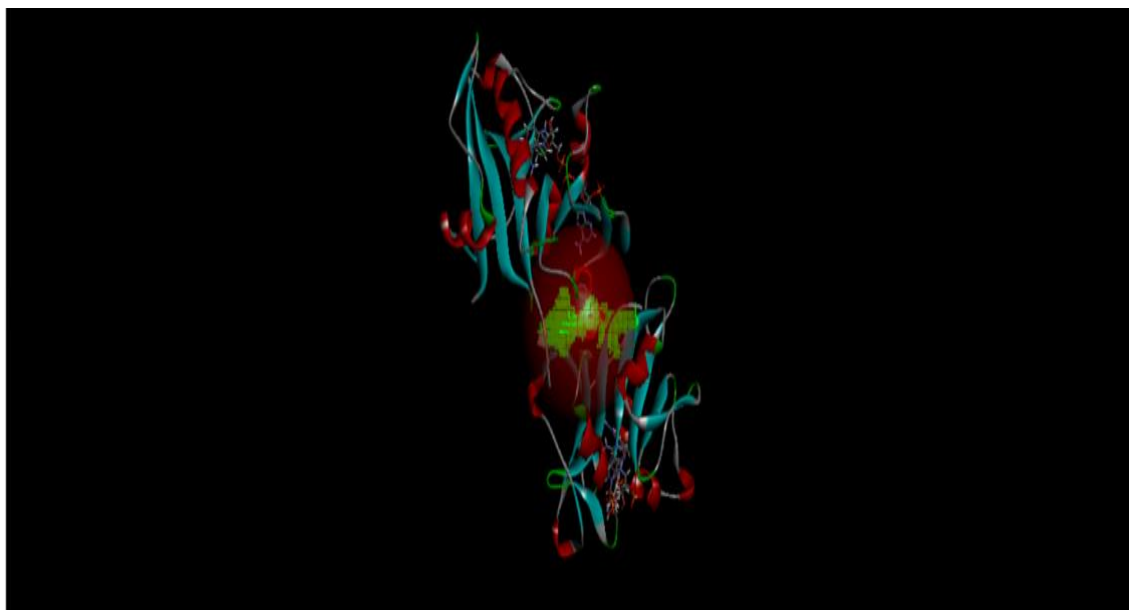


Figure1. Active site of dihydrofolate reductase enzyme

Calculation of -CDOCKER energy was carried out based on the values of internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction reveals the energy of non-bonded interaction existing between the protein and the ligand. The gauge for measuring the best interaction was selected based on a) greater positive value of -CDOCKER energy and b) minimum difference between -CDOCKER energy and -CDOCKER interaction energy (Bhaskar *et al.*, 2019). Table 1 shows that the interaction between dihydrofolate reductase and rosmarinic acid has the highest positive value of -CDOCKER energy (44.8438) and a minimum value of the difference (2.0713) between -CDOCKER interaction energy and -CDOCKER energy, which is followed by leuteolin. Thus, based on the present study, results indicate that rosmarinic acid and Leuteolin can effectively deactivate dihydrofolate reductase enzyme by interrupting the biological cycle of *Candida*. Values skewed towards the positive side for rosmarinic acid indicates that it was the most effective phytochemical against *Candida*. On the other hand, apigenin, cirsileneol, cirsimaritin, eugenol can deactivate the enzyme to a certain extent. Carnosic acid could not show any interaction with dihydrofolate reductase enzyme. Thus, the key phytochemicals that could prevent candidiasis infection caused by *Candida* are rosmarinic acid and leuteolin.

Table 1. Results of CDocking of phytochemicals with dihydrofolate reductase (receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Rosmarinic acid	44.8438	46.9151	2.0713
2	Luteolin	35.64	38.995	3.355
3	Eugenol	14.3914	23.8465	9.4551
4	Cirsimaritin	26.3815	40.0162	13.6347
5	Cirsileneol	24.9754	40.7378	15.7624
6	Apigenin	29.9057	34.7145	4.8088
7	Carnosic acid	Failed	Failed	NA

## Conclusions

*Ocimum sanctum* has medicinal action against many diseases including candidiasis. Candidiasis is caused by the fungi *Candida*. The present could provide a theoretical basis of this observation. Using Discovery studio module of BIOVIA software, molecular docking operation was performed to identify the phytochemical (apigenin, carnosic acid, cirsilineol, cirsimaritin, eugenol, leuteolin, rosmarinic acid, which can significantly interact with the vital enzyme (dihydrofolate reductase) of the microbe. It was found that Rosmarinic acid and Luteolin can form stronger bond with the enzyme and can successfully inhibit the metabolic cycle of the microbe. Apigenin, cirsilineol, cirsimaritin, eugenol deactivate have the potential to deactivate the enzyme to a certain extent and is less effective, whereas, carnosic acid does not have effect on the enzyme. Thus, this study provides evidence that the presence of Rosmarinic acid and Luteolin can provide the medicinal values to *Ocimum sanctum* against Candidiasis caused by *Candida*.

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