International Journal of Modern Agriculture, Volume 9, No.4, 2020
ISSN: 2305 - 7246

EVALUATION OF ANTIFUNGAL ACTIVITY OF PHYTOCHEMICALS SCREENED FROM *OREGANUM VULGARE* AGAINST VULVO-VAGINAL CANDIDIASIS USING MOLECULAR DOCKING METHODOLOGY

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Abstract

A vertiginous demand of herbal medication, compared to that of synthetic drugs has gained popularity in the developed as well as developing nations. The increasing significance of plants as supplement for chemically formulated drugs, is due to their natural origin, which helps combatting the side effects that are generally associated with medications. The Ethnic world and the tribes in Indian continent are bestowed with a deeper knowledge when it comes to recognizing medicinal plants and their utility in curing various diseases. Phytochemicals are plant based; non-nutritive compounds that are gaining recognition due to enormous health benefits associated with them. In the present study, *Origanum vulgare* plant was screened for its phytochemicals, in order to understand its benefits in the cure of candidiasis. Candidiasis is an infection caused by a group of fungi from the genus Candida. One of the pivotal enzymes associated with the occurrence of Candidiasis is dihydrofolate reductase. Here, molecular docking technique was used to screen the phytochemicals that could efficiently act with the enzyme. The study was carried out using BIOVIA Discovery Studio. The strength of the interaction between the phytochemicals from *Origanum vulgare*, was evaluated against the enzyme based on the values of -CDocker energy and -CDocker interaction energy. Higher values skewed towards the positive side for both the parameters implies that certain phytochemicals such as, apigenin, linalool, luteolin, caryophyllene, germacrene, sabinene, ocimene, sputhalenol have potentials to deactivate the enzyme dihydrofolate reductase and interrupts the life cycle of *Candida*.

Key words: Phytochemical, Oregano vulgare, BIOVIA Discovery studio, Candida

Introduction

World health Organization quotes the traditional medical practice is used in treatment of approximately 85% of patients in India. This significant percentage shows the indigenous relation between local tribe of India with the medicinal plants (WHO, 1996). Many scriptures date back the association of India with therapeutic use of plant towards late 5000-4000 B.C. Plants, being among the richest resource for traditional systems of medicine, has become a has become the cornerstone is modern medicines, folk medicines, pharmaceutical intermediates and in nutraceuticals (Hammer *et al*., 1999; Chhotaray *et al*., Das *et al*., 2020; Dash *et al*., 2020; 2020; Sahoo *et al*., 2020; Tripathy *et al*., 2020). The association of plants and their products with therapeutics could be traced as far back till the beginning of human civilization. The primeval mention of medicinal activity of plants in Hindu culture is quoted precisely in the "Rigveda", which is considered to be written during 4500 - 1600 B.C. and is appraised to be the oldest repository of human proficiency. It is Ayurveda, that laid the founding stone for medicinal science in Hindu culture, hallmarking its role in science of life and in the art of healing. Medicinal plants due to their curative potentials and lesser side effects associated with them, are also considered for their economic value all over the world (Kumar *et al*., 2012). Hence, nature in true sense has bestowed on us a very rich wealth of curative resource which is being explored to the fullest considering their importance.

Oregano belonging to the family Lamiaceae, has therapeutic benefits. It can be used in the cure of microbial infections such as, Candidiasis (Barrata *et al*., 1998). It contains several phytochemicals like apigenin, linalool, luteolin, caryophyllene, germacrene, sabinene, ocimene, sputhalenol (Cleff *et al*., 2010). These phytochemicals could play significant role in combating infections like candidiasis. However, there aren't much report identifying the specific phytochemical that could probably be responsible for its potentials (Adam *et al*., 1998; Harborne *et al*., 1999).

A group of fungi belonging to genus Candida generally cause Candidiasis. Candida infection (Candidiasis) has turned out to be a common fungal infection, affecting the skin, intestinal tract as well as the vagina (Manohar et al., 2001). Candida fungus can invade the human gastro-intestinal tract and skin and vagina. This infection is seen mostly with people having decreased count of lactobacillus in vagina, which could possibly be due to prolonged intake of antibiotics, poor eating habits, stress, lack of sleep, hormonal imbalance near menstrual period or due to a weak immune system. This study focuses on the identification of the phytochemical that are present in *Oreganum vulgare* and could be responsible for cure of Candidiasis caused by *Candida sp.*

Materials and Methods

Software used

Dassault Systemes BIOVIA, Discovery studio tool was used for the present study. The software employs the use of CHARMm molecular mechanics simulation program for the docking. It targets **biomolecules like,** carbohydrates, nucleic acids, peptides, proteins, lipids and ligands as they are present in solution, crystal and membrane environment. It makes use of machine learning techniques for predicting the docking studies.

List of phytochemicals

Phytochemicals are plant based secondary metabolites that bestow several protective effects to the plants. Plants consisting of several beneficial phytochemicals, when consumed by humans and animals, helps ward off several threats to health and has healing effects on several damages caused to the cells (Leung *et al*., 1996). Certain studies conducted on *Oreganum vulgare* have identified phytochemicals like, apigenin, linalool, luteolin, caryophyllene, germacrene, sabinene, ocimene, sputhalenol in it. It has been established that *Oreganum vulgare* plant belonging to Lamiaceae family has potentials in curing Candidiasis. This work centers on identifying the particular phytochemicals present in *Oreganum vulgare* plant that are responsible for inhibiting or controlling of Candidiasis.

Enzyme found in Candida

Candidiasis, which is caused due to infection by *Candida* is possible due to the several major metabolic cycles of the fungi taking place inside the host cells. These metabolic cycles require certain key enzymes for their regulation. The RCSB enzyme database was used for identifying and listing out different enzymes required for the survival of *Candida sp*. It has been reported that the enzyme dihydrofolate reductase (protein database code 3QLW) is involved in DHFR catalyses and in the transfer of hydride from NADPH to dihydrofolate. This reaction occurs along with a protonation in order to produce tetrahydrofolate that is very crucial for survival of this particular fungi.

Molecular docking

In the present study, Computational docking technique was exploited to screen the phytochemicals from *Oreganum vulgare* plant, that can act as ligand to form strong covalent interaction with the fungal protein in order to inhibit the growth of the fungi. Dassault Systemes BIOVIA, Discovery studio tool was used for the purpose of identifying the interaction in molecular level, the result was obtained by performing molecular docking. During the study, the sdf files were at first acquired from PubChem. The protein database code for the enzyme dihydrofolate reductase was extracted from RCSB site. For identifying the active site present in the enzyme, the "receptor cavity" option, that is present under "receptor-ligand interaction" menu of Discovery Studio was used. Molecular docking was performed based on the CDocker protocol of the software that is categorized under "receptor-ligand interaction". During the docking process, the enzyme molecule was presumed as the receptor and the phytochemical was presumed to be the ligand. The scores obtained for "-CDOCKER_ENERGY" and "-CDOCKER_INTERACTION_ENERGY" were used to estimate the quality of molecular docking. The scores showing greater positive value were due to a good and stable interaction between the small molecule and the receptor. Thus, such type of interaction implies for the presence of crucial phytochemical responsible for curing of the disease.

Results and discussion

Figure 1 depicts the active sites that are present in dihydrofolate reductase enzyme, that are visible as light green color. CDOCK is a molecular dynamics (MD) technique, that is a simulated-annealing-based algorithm. The value of -CDOCKER energy is calculated with respect to the internal ligand strain energy along with the receptor-ligand interaction energy. -CDOCKER interaction represents the energy value of the non-bonded interaction existing between the small molecule and the protein molecule. Based on the scores of interactions that were obtained, the most efficient association was selected by observing the scores with high positive value of -CDOCKER energy and with scores showing minimum difference between -CDOCKER energy and -CDOCKER interaction energy (Bhaskar et al, 2019).

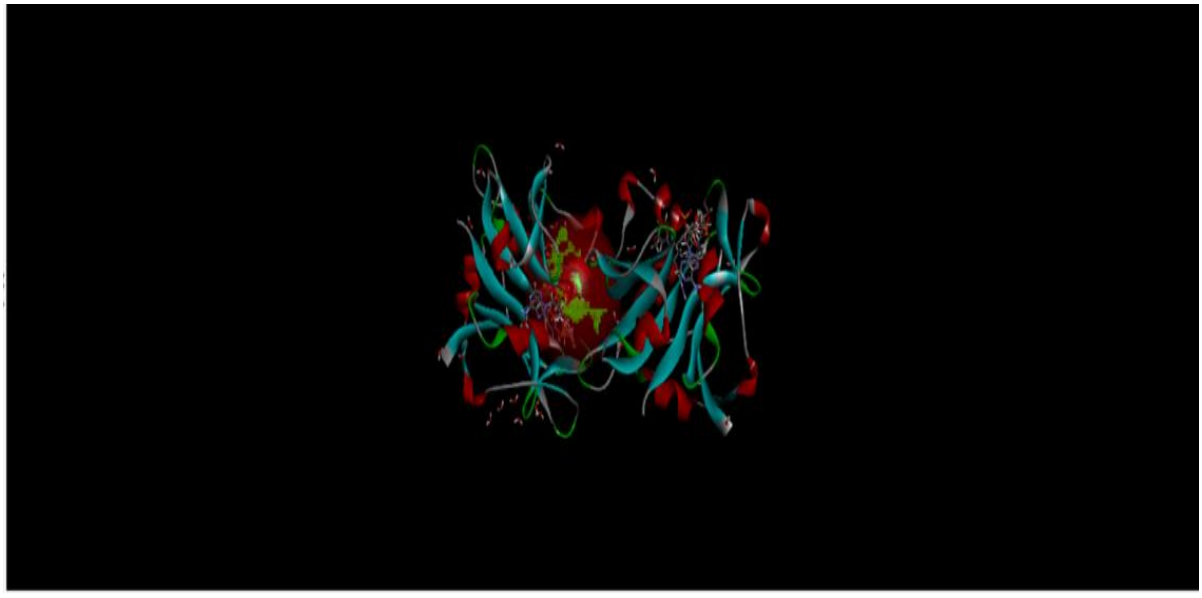

Figure 1. Active site of dihydrofolate reductase enzyme

Table 1 shows that the interaction between the enzyme dihydrofolate reductase and the ligand leuteolin had higher positive value of -CDOCKER energy (21.8177) and also a minimum difference (2.735) between the

values of -CDOCKER interaction energy and -CDOCKER energy. The efficiency of the phytochemical leuteolin was followed by apigenin. Hence, based on the present study, we can estimate that leuteolin and apigenin have potentials to effectively deactivate the enzyme dihydrofolate reductase and interrupt the biological metabolism of *Candida*. However, the other phytochemicals that are present in *Oreganum vulgare*, i.e. Caryophyllene, germacrene, linalool, ocimene, sabinene and spathulenol do not show much effectiveness in deactivating the enzyme. Thus, the essential phytochemicals that show potentials in acting against candidiasis infection caused by *Candida sp.* are leuteolin and apigenin.

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

SL NO	LIGAND	-CDOCKER ENERGY	-CDOCKER INTERACTION ENERGY	Difference between -CDOCKER interaction energy and -CDOCKER energy
1	**Apigenin**	**21.2476**	**26.7547**	**5.5071**
2	**Caryophyllene**	**-16.7031**	**19.9409**	**36.644**
3	**Germacrene**	**-20.8776**	**21.3671**	**42.2447**
4	**Linalool**	**-11.6864**	**18.2474**	**29.9338**
5	**Luteolin**	**21.8177**	**24.5527**	**2.735**
6	**Ocimene**	**-22.0574**	**16.2372**	**38.2946**
7	**Sabinene**	**-14.356**	**14.0222**	**28.3782**
8	**Spathulenol**	**-36.6297**	**23.972**	**60.6017**

Conclusions

Oreganum vulgare plant has medicinal action against candidiasis caused by the fungi *Candida*. In this study the phytochemicals present in *Oreganum vulgare* were screened for their activity against the fungi. Dassault Systemes BIOVIA, Discovery studio was used to carry out the molecular docking process. The results of this study infer that leuteolin and apigenin can effectively interact with the enzyme to inhibit its metabolic activity. Whereas, the other phytochemicals such as, caryophyllene, germacrene, linalool, ocimene, sabinene and spathulenol cannot deactivate the enzyme to a large extent and are less effective. Hence, the study concludes that the presence of phytochemicals leuteolin and apigenin provide medicinal values to *Oreganum vulgare* against Candidiasis caused by *Candida.*

Acknowledgement

The authors are thankful to Senior Management Team of Centurion University of Technology and Management for their kind support.

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