



***In silico* Analysis of Phytochemicals from Neem Leaves against Sterol 14- α Demethylase of *Microsporum sp* Causing Skin Disease**

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Authors' contributions

This work was carried out in collaboration among all authors. Author SD conceived and planned the study, performed the computational framework and analyzed the statistical data. Author RKS carried out the implementation. Author SD wrote the first draft of the manuscript. Author PBS managed the analyses of the study. Author SD took lead in the literature searches. Author KVDP read and approved the final manuscript and provided assistance throughout the study. While author DB supervised and guided the whole study. All authors read and approved the final manuscript.

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ABSTRACT

This analysis aims at evaluating the effects of Neem leaves extract on Skin disease. Skin disease is caused by *Microsporum sp*. The phytochemicals of Neem leaves were interacted with sterol 14- α demethylase enzyme involved in sterol biosynthesis metabolic pathway of *Microsporum sp*. Sterol 14- α demethylase was taken as receptor and phytochemicals present in Neem leaves were considered as ligands. All the interactions were done in Biovia discovery Studio 2020 and the process is known as molecular Docking. Molecular Docking provides us an opportunity to identify the potential phytochemical or component which can act as powerful tool against the pathogen. Out of all the phytochemicals, Glutamic acid and Oleic Acid of Neem leaves inhibits or blocks the mechanism or action of sterol 14- α demethylase enzyme of *Microsporum sp*. There is high possibility that these phytochemicals can potentially inhibit others enzymes involved in various metabolic pathways of *Microsporum sp*.

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1. INTRODUCTION

Life is never slow in 21st century. From transportation to communication, everything can happen in couple of minutes. Humans have been relying on chemical drugs for fast recovery from their ailments. These drugs have proved harmful to the biological cycles of the body in the long run. Thus plant based drugs are safer alternative over chemical based drugs. Plant extracts are used to cure the diseases now.

Neem (*Azadirachta indica*) belongs to family Meliaceae has wide range of medicinal properties. Ayurveda, Unani and Homoeopathic medicine has described Neem as one of the most widely used plant in treatment of various ailments. Various parts of the neem tree like leaves, seeds, flowers, fruits, barks and roots are used to prepare medicines [1,2]. Use of neem leaves against various skin diseases have been used in ancient times and the information was passed on from one generation to another. Neem leaves have been incorporated in various regimen of Indian women and used in treatment of various skin related disorders. Studies have revealed that Neem leaves contains phytochemical like I- Ascorbic acid, Beta-Sisterol, Glutamic Acid, Nimbin, Oleic acid, Stigmasterol and so on [3]. Neem leaves are known to treat skin diseases, inflammation, dental disorders, blood sugar level, infections and fever [4,5].

Dermatophytoses are caused by molds of the genera *Microsporum*, *Trichophyton* and *Epidermophyton*. About 40 known species are worldwide in distribution where as others are confined to a particular region. Common human pathogens narrow down to 10. The dermatophytes invade the stratum corneum (outermost layer of the epidermis) of the skin and other keratinized tissues, such as nails and hair. Many systemic and topical agents are available for treatment of these infections. The choice of treatment and its duration depends on the site of infection, etiological agent and the extent of the disease [6]. *Microsporum sp* cause fungal infections which are superficial in nature. The incidence of this pathogen is higher in hot and humid climates. They invade the superficial skin tissue by producing keratinases which in turn degrades the keratin [7].

This study focuses on the identification of the phytochemical from *Neem Leaves* responsible to cure Skin disease caused by *Microsporum sp.*

2. MATERIALS AND METHODS

2.1 Software Used

All the operations were carried out in Discovery studio module of Biovia 2020 software (Dassault Systemes of France). Biovia 2020 discovery studio is one of the user-friendly software. Its user interface is quite easy to carry out the molecular docking. The software utilizes machine learning techniques to predict the level of molecular interaction between the receptor (enzyme) and Ligand (Phytochemicals).

2.2 Methodology

2.2.1 List of phytochemicals

Plants produce a number of chemicals which may or may not be directly involved in their metabolism. Phytochemicals are the secondary metabolites produced by plants as a response to flight or fight mechanism against their predators. Phytochemicals are generally bio-active compounds which can affect animal biochemistry and metabolism. Hence they are widely examined to prove their ability towards our health benefits. It becomes important for us to include them in our foods, as potential nutritionally active ingredients. When we consume them they passed on to our systems from plant products. Published works showed that Neem Leaves contains I- Ascorbic Acid, Beta-Sisterol, Glutamic Acid, Nimbin, Oleic Acid, Stigmasterol and so on [8]. It has already been established that Neem Leaves plant belonging to Meliaceae family has potential to help controlling Skin disease. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Skin disease.

2.2.2 Enzyme found in *Microsporum sp* causing skin disease

From published books and papers we can say that Skin disease is caused due to *Microsporum sp* infestation [9]. The survival of pathogen inside its host is highly dependent on certain metabolic pathways. These metabolic pathways require certain enzymes as its co-factor to function

properly. Brenda enzyme database helped us to identify and list different enzymes found in *Microsporium sp.* It has been found that sterol 14-alpha demethylase (protein database code 5EQB) is involved in Sterol biosynthesis metabolism [10]. This metabolism proves to be very crucial for the pathogen thus blocking or inhibiting that pathway results in death of the particular microbe.

2.2.3 Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract, which act as a ligand and forms a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia 2020 software was used for identifying molecular interaction and molecular docking was performed. First step involves making a list of phytochemicals present in Neem Leaves from various research papers. Second steps involves download of the sdf files for the phytochemicals found in the Neem Leaves plant from various website like PubChem, MolInstincts etc. The protein database code of Sterol 14-alpha demethylase enzyme was identified from the RCSB-PDB website. The active site of the enzyme was identified via "receptor cavity" protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the C-Docker protocol of Biovia software under "receptor-ligand

interaction". The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The "-CDOCKER_ENERGY" and "-CDOCKER_INTERACTION_ENERGY" were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

3. RESULTS AND DISCUSSION

Fig. 1 shows the active site of Sterol 14-alpha demethylase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the non-bonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy [11].

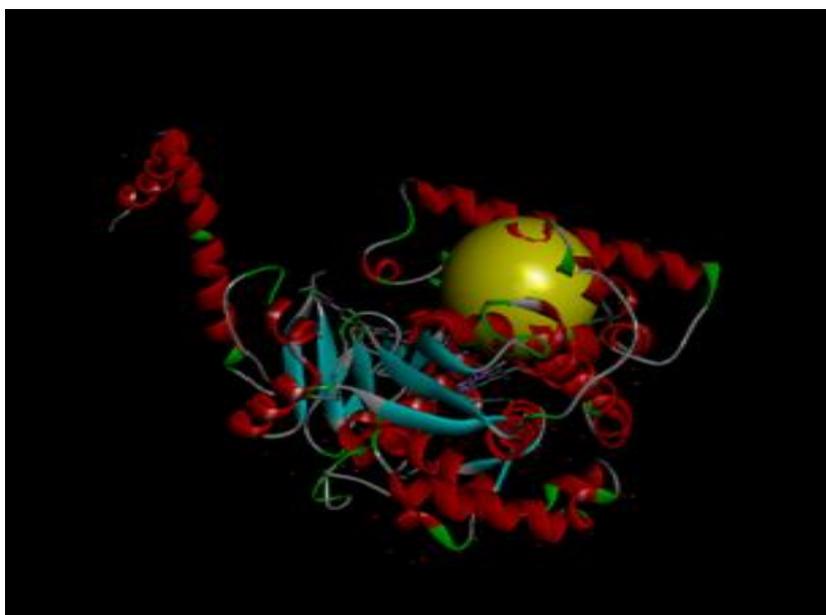


Fig. 1. Active site of Sterol 14-alpha demethylase enzyme

Table 1. Results of CDocking of phytochemicals with Sterol 14-alpha demethylase (receptor)

SI No	Ligand	- C DOCKER energy	- C DOCKER interaction energy	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	1-Ascorbic acid	7.40462	30.9059	23.50128
2	Beta-sisterol	Failed	Failed	
3	Glutamic acid	29.1584	27.6594	1.499
4	Nimbin	Failed	Failed	
5	Oleic acid	15.75	37.5964	21.8464
6	Stigmasterol	Failed	Failed	

Table 1 show that Sterol 14-alpha demethylase-Glutamic Acid interaction has the highest positive value of -CDOCKER energy (29.1584) and minimum value of the difference (1.499) between - C DOCKER interaction energy and - C DOCKER energy followed by. Thus the results indicated that Glutamic Acid and Oleic Acid can effectively deactivate the Sterol 14-alpha demethylase enzyme thereby interrupting the biological cycle of *Microsporum sp.* higher positive values for indicated that it was the most active ingredient against *Microsporum sp.* On the other hand, I- Ascorbic Acid can deactivate the enzyme to a small extent. Nimbin, Beta- sisterol and Stigmasterol cannot interact with Sterol 14-alpha demethylase. Thus, the key phytochemicals preventing Skin disease caused by *Microsporum sp* are Glutamic acid and Oleic Acid.

4. CONCLUSION

Neem has properties like analgesic, anthelmintic, antibacterial, antiyeast, antiulcer, antifungal, antihyperglycemic, anti-inflammatory, antiviral, antimalarial, diuretic, antinematodal, antipyretic, antispasmodic, insecticidal and hypercholesteremic [12]. It was previously known that Neem Leaves has medicinal action against Skin disease [13]. Skin disease is caused by *Microsporum sp.* This study was carried out to provide the theoretical basis of this observation using Discovery studio module of Biovia 2020 software. It was found that Glutamic Acid and Oleic Acid can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. I- Ascorbic Acid was found to be not much effective in deactivating the enzyme of the microbe. Beta- sisterol, Nimbin and Stigmasterol cannot deactivate the enzyme. The presence of Glutamic Acid and Oleic Acid provided the medicinal values to Neem Leaves against Skin disease caused by *Microsporum sp* and can be used in making of drugs.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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