MOLECULAR DOCKING STUDIES OF NATURAL ANTIMICROBIAL COMPOUNDS FROM AZADIRACHTA INDICA AGAINST SELECTED TARGET PATHOGEN PSEUDOMONAS FLUORESCENS

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ABSTRACT

In drug development, in-silico approaches play a key role to explore molecular aspects of targeting specific proteins through various tools and software. It also analyze the bioactivities and inhibitory effects across mechanisms underlying for treatment of several bacterial diseases. Medicinal plants have been the single most productive source of leads for the drug development thus played a vital role in treating and preventing a wide range of diseases throughout the world. In the present investigation, A.indica was examined for their antibacterial activity particularly against P.fluorescens. In this study the bioactive compounds were identified from Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The identified compounds are docked against AprX enzyme through molecular docking. The identified compound α-D-Glucopyranoside, α-D-glucopyranosyl is a best bioactive compound against AprX enzyme based on energy values.

This research work was helpful attempt to the drug discovery against pathogenic P. fluorescens.

KEYWORDS: Azadirachta indica, P.fluorescens, α-D-Glucopyranoside, α-D-glucopyranosyl, AprX enzyme
INTRODUCTION

Molecular docking is the technique employed to predict and analyze the interactions between receptors of known three dimensional protein structure and ligands. It provides most detailed possible view of drug receptor interactions and also has created a new rational approach to drug design.[1] Infectious diseases are leading cause of death world-wide. Bacterial diseases occur when pathogenic bacteria invades the body, begin to secrete or excrete toxins and to grow in tissues that are normally sterile.

_Pseudomonas fluorescens_ is an aquaculture pathogen that can infect many fish species, including Indian major carps, black carp and common carp. Infection of fish by _P. fluorescens_ leads to the development of the so-called Red Skin Disease, which can occur at any time in a year and especially in fish injured due to improper handling and transportation. When the normal environmental conditions change, the disease often leads to mortality, thus causing heavy economic losses.[2] _Pseudomonas_ considers one of the most pathogenic bacteria affecting fish farms especially the Indian major carps.

Many infectious diseases were effectively treated with herbal remedies from ancient times. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for the development of new drugs. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.[3]

India has a rich flora that is widely distributed throughout the country. Herbal medicines are basis of treatment and cure variety of diseases. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.[4] Plants are very significant to human, because they have several dynamic constituents which are precursor to synthesize many drug. Misuse or over use of currently available antibiotics made most of the pathogenic bacteria to develop resistance against them. This situation made mankind to explore different sources of cost-effective, efficient and less toxic antimicrobial agents. Medicinal plants play a major role and constitute the backbone of traditional medicine.[5]

_Azadirachta indica_ (A.Juss) belonging to Meliaceae family is very important medicinal plant which is traditionally used to treat different diseases. Neem is a widely distributed in tropical and sub-tropical regions. The chemical constituents extracted from neem contain many biologically active compounds including alkaloids, triterpenoids, flavonoids, phenolic
compounds, carotenoids, steroids and ketones. These phytoconstituents are threatful to infectious microbes.[6] Molecular docking has been a focus of attention for many years. This docking program is very flexible and able to predict protein ligand complex structures with appropriate accuracy and speed. The present investigation was to analyze the binding affinity of the plant synthesized compounds 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, Phytol, Butanoicacid, 3-methyl-,3,7-dimethyl-2,6-octadienyl ester, 2(R),3(S)-1,2,3,4 Butanetetrol against the enzyme AprX.

MATERIALS AND METHODS
Collection, identification and authentication of selected plant
Fresh, healthy and young leaves of *Azadirachta indica* were collected from Saliyamangalam, Thanjavur district, Tamil Nadu, India and authenticated by professionals in the Department of Botany, St. Joseph’s College, Tiruchirappalli, India. The voucher specimen number of the plant is DK001.

Preparation of plant extracts
The shade dried leaves were taken and grinded well to fine powder. About 500 g of dry powder was extracted with ethanol (80%) using Soxhlet apparatus at 70°C by continuous hot percolation. The extraction was continued for 24 hrs and then the filtered extract was kept in hot air oven at 40°C for 24 hrs to evaporate the excess ethanol from it. The obtained dark brown residue was kept separately in airtight containers and stored in a deep freezer.

GC-MS analysis
Clarus 500 Perkin-Elmer (Auto System XL) was used to carry out GC-MS analysis.

Structure Elucidation
The 2D structures of phytochemical compounds were obtained from Chemspider database (http://www.chemspider.com/). Then 2D structures are converted to 3D structures using swiss pdb viewer (http://www.spdbv.vital-it.ch/). They act as a ligand. Sequence and 3-D structure of particular protein are provided by the UniProt KB/Swiss-Prot database. AprX is retrieved from UniProt KB / Swiss-Prot database (http://www.uniprot.org/). Hex docking program (hex.loria.fr/ dist50/) is used to dock AprX with these bioactive compounds.
Docking studies

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present study, AprX is selected as receptor and the bioactive compounds from leaves of A.indica are selected as ligands. The receptor was docked against ligands and the energy values were obtained using the docking software.

RESULTS AND DISCUSSION

Phytocompounds play a significant role in the field of drug development. In recent years, a major attention has been drawn to phytocompounds due to no side effects in therapeutic. These bioactive compounds are essential in defense mechanism and contribute to produce many novel drugs against dangerous diseases.[7]

In the last few years, greatest issue faced worldwide in the field of aquaculture is fish diseases that occurred due to antibiotic resistant bacteria and this issue continues to increase due to the absence of a more effective and safer use of antibiotics.[8] Neem could be used potentially in preventive treatment of Epizootic Ulcerative Syndrome (EUS) and recommended for use in small ponds.[9] Neem seed oil has been shown to exert antibacterial activity. Biological activity of some Neem compounds include immunostimulant activity, antiviral, antifungal, antiulcer activity.[10] The dried bark and leaves of Neem contain a bitter amorphous resin, an alkoholid margisine and margosic acid that are used as an antisectic and germicide.[11] Methanol and ethanol extracts of neem, Azadirachta indica express good in vitro activity against Pseudomonas sp.[12]

Bioinformatics is seen as an emerging field with the potential to significantly improve drugs, brought to the clinical trials and eventually released to the marketplace. Computer Aided Drug Design is one of the specialized discipline that uses computational methods to study drug receptor interactions and is heavily dependent on bioinformatics tools, applications and databases.[13] Nowadays, molecular docking approaches are consistently used in modern drug design to know drug receptor interaction. The literature reviews express that these computational techniques can robustly support and facilitate the design of novel, more potent inhibitors by revealing the mechanism of drug receptor interaction.[14]

In the current state, the flexible docking program is able to predict protein ligand complex structures with reasonable accuracy and speed. In order to find out the best effective drug, Hex docking was carried out. In this docking, α-D-Glucopyranoside, α-D-glucopyranosyl and
4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl bioactive compounds from neem were taken into consideration for docking as ligand molecules. These ligands have been used to target AprX which bound to the receptor to inhibit its function. The nature of the complex between the drug and the receptor molecule was identified via docking and the inhibition nature of the ligands and their binding affinities were calculated using free energy simulations.

In the present study, α-D-Glucopyranoside, α-D-glucopyranosyl from Neem showed a maximum e-value (−223.37) followed by 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (-107.19).

Figures: Molecular Dockings
CONCLUSION
The present study concluded that the Neem compounds render antimicrobial action against *P. fluorescens*. Hence it can be used as drug to treat aquatic bacterial pathogens. The antibacterial activity results indicated that the tested compounds showed the most promising antibacterial activities. These observations may promote a further development of our research in this field. Further development of this group of compounds can serve as templates for the construction of better drugs which may have better pharmacological profile than standard drugs to combat bacterial infection. After studying the docking poses and binding modes of the docked compounds, the necessity of hydrogen bond formation for enhancing the activity of this class of compounds can be highly advocated.

CONFLICT OF INTEREST STATEMENT
We declare that we have no conflict of interest.

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