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MOLECULAR DOCKING STUDIES OF CYANOBACTERIA METABOLITIES FOR SKIN CANCER

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ABSTRACT

This work was to find out the flavonoid form derived from the Cyanobacteria *Oscillatoria terebriformis*[6,7]and to test them against SKIN cancer protein. The metabolites were extracted in 100% methanol from the Cyanobacterial biomass of *Oscillatoria terebriformis* isolated from effluent. The crude methanol extract was purified and analysed for the flavonoid form by HPLC. Two predominant compounds (Quercetin) identified in the extracts were screened against the Skin cancer protein (2VCJ) by in-silico docking method. Of the compounds, Quercetin was the most potent having the docking score of −8.4 Kcal/mol. This value was better than the standard drug "Dyclonine". This work recommends the Quercetin for further in vitro and in vivo studies towards its development as anticancer drug.

INTRODUCTION

Cyanobacteria have been identified as one of the most promising group of organism from which novel and biochemically active natural products are isolated.Cyanobacteria such as *Spirulina, Anabaena, Nostoc* and *Oscillatoria* produce a great variety of secondary metabolites [8]. The only comparable group is actinomycetes, which has yielded a tremendous number of metabolites. The rate of discovery from traditional microbial drug producers like actinomycetes and hyphomycetes, which are in the focus of pharmaceutical research for decades, is decreasing and it is time to turn to cyanobacteria and exploit their potential. This is of paramount importance to fight increasingly resistant pathogens and newly emergent diseases [4] because cyanobacteria are largely unexplored, they represent a rich opportunity for discovery. Structure-based drug design is one of the first techniques to be used in drug design. Drug targets are typically key molecules involved in a specific metabolic or cell signaling pathway that is known, or believed, to be related to a particular disease state. Drug targets are most often proteins and enzymes in these pathways.

Drug compounds are designed to inhibit, restore or otherwise modify the structure and behavior of disease related proteins and enzymes. Flavonoids are a large group of non-nutrient compounds naturally obtained from plants as part of their protective mechanisms against stresses of various origins. Hence a study was planned to evaluate the interaction of the selected ligand with a target protein of skin cancer. Therefore, in the present study, an attempt was made to isolate flavonoids from *Oscillatoria terebriformis*, a thermophilic cyanobacterium and to analyze them against the skin cancer by using molecular docking.

MATERIALS AND METHODS

Cyanobacterial Culture

Oscillatoria terebriformis, cyanobacteria were obtained from the culture collection of Vivekananda Institute of Algal technology (VIAT), Chennai (isolated from effluent). Biomass was obtained by growing algal cultures in 20L of water and 0.25g /L of NPK fertilizer was added with a facility to pump the culture with aeration pump. Algae was grown for 20 days and harvested and then biomass was harvested for the extraction of intracellular metabolites.

Extraction of Intracellular secondary Metabolites:

The Cyanobacterial biomass was homogenized with 100% methanol, filtered through Whatman No.1 filter paper and the filtrate was then dried under vacuum at 40^{o}C. This extract was subjected for HPLC analysis.

Content of Flavonoids by HPLC Method:

HPLC analysis was used for the analysis of the intracellular secondary metabolities compounds present in the cyanobacterial extract LDC Milon Roy CM 4000 gradient pump coupled to a Hewlett Packard 1100 diode-array detector Cyanobacterial extracts were eluted at 1 ml/min20 μl injection volume) using as mobile phase a binary solvent system consisting in methanol, water, and phosphoric acid (100:100:1) . Equal volumes (about 20 µL) of each of the Standard solutions was injected and the Test solution into the chromatograph equal volumes (about 20 µL) of each of the Standard solutions was injected and the Test solution into the chromatograph. Record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each flavonoid in samples

Retrieval of Protein Structure:

The target 4, 5 diarylisoxazoleHSP90 chaperone protein (PDB ID: 2VCJ), having the resolution of 2.0 A°, was retrieved from the protein data bank (PDB) (http://www.rcsb.org/pdb/). A standard compound Dyclonine known to have good inhibitory potential against the same skin cancer protein was also docked to compare the effectiveness of the secondary metabolites. Structural and active site studies of the protein were done by using CASTP (Computed Atlas of Surface Topography of Proteins) and Pymol molecular visualization software.

Compounds Screened:

Four compounds namely Rutin, Quercetin, Kaemferol and Luteolin isolated from *Oscillatoria terebriformis* were screened against the skin cancer protein. The pubchem database was used for retrieving the structure of the ligand molecules. The selected chemical structures were generated from the software (Open Babel). The molecular docking was performed using PyRx a widely distributed public domain of molecular docking software. The inhibitor and target protein were geometrically optimized and docked using PyRx.

Docking Methods:

Virtual-screening is an emerging approach and is extensively used to reduce cost, and time in drug discovery. PyRx is virtual screening software for Computational Drug Discovery (CDD), which can be used to screen libraries of compounds against potential drug targets. It uses a large body of already established open source software such as Auto Dock 4 and AutoDock Vina. These two are used as docking software.

Active Site Prediction:

Active site of the target protein was predicted by using "Active site prediction tool" from SCFBio Server (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp) which requires a. pdb file as an input and this tool explains the total number of active sites along with information on their amino acid sequence, cavity points, and the average volume of the cavity.

Ligand Binding Sites Prediction:

After docking the docked structure was saved as ".pdb" file and further explored to predict the binding sites using "Ligand Explorer" software. The predicted binding sites, based on the binding energy, and amino acids make up the binding cavity. Here ligand binding site represents the site where the ligands most efficiently bind with the protein, among all the active sites.

Drug Like-liness Prediction:

Ligand property was predicted by using "Lipinski Drug Filters" (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp). Lipinski rule of five helps in distinguishing drug-like and non-drug-like properties and predicts high probability of success or failure due to drug likeliness for molecules. The Lipsinki filter helps in early preclinical assessment and thereby avoiding costly late-stage preclinical and clinical failures.

RESULTS AND DISCUSSION

Oscillatoria terebriformis was analyzed for flavonoids contents by RP-HPLC. Reversed-phase HPLC has been used in a number of occasions for the analysis of flavonoids in cyanobacteria. It has been applied especially for the identification of flavonoids derivatives. In the present investigation, flavonoids were quantified at 254nm using peak area by comparison to a calibration curve derived from the *O*scillatoria terebriformis the main difference was in peak eluted at 3.336 min.All the 4 isolated compounds of Flavonoids were screened in docking analysis against Skin cancer protein .Of which, displayed good docking scores (Table 2). The flavonoids present in *Oscillatoria terebriformis* were analyzed in HPLC and the compound (Quercetin) which showed the best result against skin cancer protein is shown in Figure 2. HPLC result revealed the presence of flavonoids in *Oscillatoria terebriformis*. Cyanobacteria produce a wide variety of bioactive compounds, which include 40% lipopeptides, 5.6% amino acids, 4.2% fattyacids, 4.2% macrolides and 9% amides.

Cyanobacterial lipopeptides include different compounds like cytotoxic (41%), antitumor (13%), antiviral (4%), antibiotics (12%) and the remaining18% activities include antimalarial,

antimycotics, multi-drug resistance reversers, antifeedant, herbicides and immunosuppressive agents [2].Cyanobacteria are also known to produce antitumor, antiviral and antifungal compounds. Many of the pharmaceutically interesting compounds in cyanobacteria are peptides, including cyanobacterial toxins and important candidates for anti-cancer drugs. Hsp90 is a very promising and relevant target in cancer therapy because it is required for the stability and/or activity of many client proteins that are important for cancer cells.

By inhibiting Hsp90, several pathways can therefore be targeted with a single inhibitor. This is of importance because a frequent cause of failure of targeted therapy is that the therapies inhibit only one signaling pathway, whereas several may be deregulated at the same time in a cancer cell. In addition, oncoproteins are often expressed as mutant forms, which are more dependent on Hsp90 for stability than their normal counterparts. Cancer cells are also subjected to more stresses (for example due to acidosis, hypoxia and nutrient deprivation) than normal cells and are consequently more dependent on the activity of the Hsp90 chaperone machinery [9]. Finally, it has been suggested that Hsp90 inhibitors preferentially bind to Hsp90 in cancer cells rather than in normal cells, possibly because Hsp90 would be in an activated complex in cancer cells, having a greater affinity for nucleotides and inhibitors[5] .Inhibitors of the Hsp90 molecular chaperone are showing considerable promise as potential chemotherapeutic agents for cancer [1] .

Hence, the present work tested the metabolites against 4, 5-diarylisoxazole Hsp90 chaperone is a skin cancer protein. Totally 47 active sites were predicted in the target protein by the "Active site prediction tool". PyRx is virtual screening software was used to dock flavonoid derivative compounds against the skin cancer protein (4, 5-diarylisoxazoleHsp90 chaperone). The docking interaction of the protein and ligand, and the predicted ligand binding site residues are shown in **Figures (1) and (2)** respectively. The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in Table 1.of the four compounds, Quercetin was the most potent having the least docking score of **-8.4 Kcal/mol**. This value was better than that of the potent drug "Dyclonine" [3] which showed the docking score of **-7.3 Kcal/mol**. Lesser the docking score more is the binding capacity of the ligand.Hence, the present study suggested that Quercetin could be considered for further *in vitro* and *in-vivo* studies towards development of anti skin cancer drug.

CONCLUSION

The present study showed that flavonoid derivative obtained from *Oscillatoria terebriformis* could be a potent inhibitor against skin cancer protein on the basis of docking scores. We anticipate that further exploration of the functions and molecular mechanisms of the compound will facilitate a better understanding for the control of skin cancer and in development of anticancer drugs.

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Table 1 -Distribution of Flavonoids in *Oscillatoria terebriformis*

S.No	Flavonoids(Compounds)	Quantity (mg/ml)
1.	RUTIN	0.072
2.	QUERCETIN	0.78
3	KAEMFEROL	0.012
4	LUTEOLIN	0.0125

Table 2 - Results of Lipinski Drug Filters of Flavonoids from *Oscillatoria terebriformis* Extract

Compound Name	LogP	Molecular Weight(g/mol)	Hydrogen Donor/acceptor	Molar Refractivity
RUTIN	8.044	610.0	10,6	136.886
QUERCETIN	0.974	302.00	5,2	73.910
KAEMFEROL	1.269	286.2363	4,2	72.246
LUTEOLIN	2.125	286.2363	4,2	72.476

Table 3 - Docking results of Flavonoids from *Oscillatoria terebriformis* Compounds Against Skin Cancer Protein

S.No	Compound With Pubchem ID	Chemical Formula	Binding Energy (Kcal/mol)
1	RUTIN (CID 5280805)	$C_{27}H_{30}O_{16}$	-7.9
2	QUERCETIN) (CID 5280343)	$C_{15}H_{10}O_7$	-8.4
3	KAEMFEROL (CID 5280863)	$C_{15}H_{10}O_6$	-7.4
4	LUTEOLIN (CID 5280445)	$C_{15}H_{10}O_6$	-7.9
5	DYCLONINE (CID 3180) (Marketed Drug)	$C_{18}H_{27}NO_2$	-7.3

FIGURE 1- AMINO ACIDS IN THE BINDING POCKET (THR 360, ASN331, SER 324, ASP355, LEU321, VAL 350) RCSB LIGAND EXPLORER

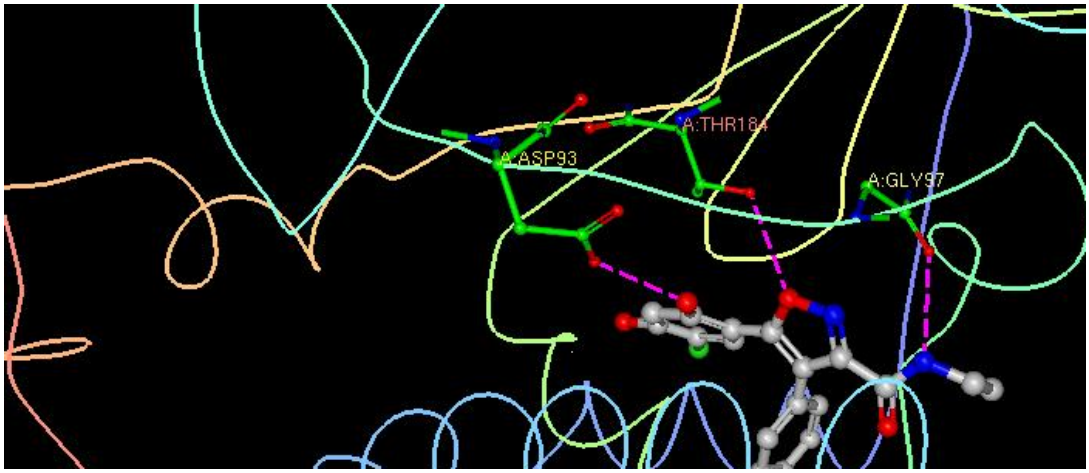

FIGURE 2- PROTEIN -LIGAND INTERACTION OF Quercetin and EGFR