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Original Article

DOCKING STUDIES ON BIOACTIVE COMPOUNDS OF *NYCTANTHES ARBOR-TRISTIS*

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ABSTRACT

Objective: The aim of the present study is to predict the interaction between selected ligand and different types of inflammatory and cancer target proteins.

Methods: *In silico* study of protein-ligand interaction involves molecular docking, where the binding energy and geometry of ligands, substrates or possible drug candidates to target proteins is predicted by using computational chemistry methods.

Results: A highest binding energy of-4.35 kcal/mol at 10th run was observed with $C_{15}H_{24}O_{11}$ compound from dried stem methanolic extract on *Nyctanthes arbor-tristis* against 1JNX receptor protein. 4.37 kcal/mol at 10th run was observed with $C_{21}H_{14}O_4$ compound from dried fruit methanolic extract against 1CX2. 2ITO lung cancer protein with ligand from dried fruit methanolic showed a highest binding energy of-6.77 kcal/mol at 10th run with different bond interactions. Among all the drugs 2ITO showed its effectiveness in binding with selected cancer target proteins.

Conclusion: The results reveal *in silico* study support the interaction of protein-ligand that is binding and interaction of ligands from *N. arbor-tristis* with inflammatory and cancer targets in molecular docking studies.

Keywords: Molecular Docking Studies, *Nyctanthes arbor-tristis*, Glycosides and Phenols, Methonolic extracts

INTRODUCTION

Computational methods are useful to identify the structural disease mechanisms and helps in designing the drugs that disrupt pathogenesis. The tool that is used to predict the structure of protein-protein interfaces is known as protein docking. Among the biological macromolecules, proteins are one among them responsible for various biochemical reactions with their structure, signaling and catalyzing reactions. Adopting the free-energy confirmation at lowest level through the amino acid polymer after translation is called protein folding. Various molecular forces such as Vander Waals, electrostatics, and hydrogen-bonding energies are responsible for the confirmation.

Nowadays modern drug designing helps in designing the potent inhibitors [1], hence, the present study was carried to evaluate the selected ligand interaction with various inflammatory and cancer target proteins.

The most common type of diagnosed and hereditary cancer is breast cancer, a second major cause among women that even leads to death [2]. *Homo sapiens* are the biological sources of 1JNX and 2ITO receptor proteins. 1JNX is a Type 1 susceptibility Protein whereas 2ITO is an Epidermal Growth Factor Receptor (EGFR) [3, 4]. The pharmacological targets of NSAIDs are cyclooxygenase enzymes (COX). The addition of two oxygen molecules to arachidonic acid by COX with a set of reactions develops inflammation. The enzyme survives in two isoforms, among which COX-2 (1CX2) [5] helps in prostaglandin production in inflammatory diseases. Only 60% of the homology will be observed between two isoforms: COX1 and COX2. From a variety of species such as rat, sheep, and humans COX2 will be cloned. The molecular weight of COX2 is 72 kDa. Several growth factors, cytokines, and endotoxins (bacteria) will regulate the expression of COX-2. The iridoid glucosides from *Nyctanthes arbor-tristis* and identified the increased reactive oxygen species and cellular redox homeostasis imbalance in *Leishmania* parasite [6]. The molecular modeling and network-based approach in explaining the medicinal properties of *Nyctanthes arbor-tristis, Lippia nodifera* for Rheumotoid arthritis [7] were well established. However, in view of diversified phytochemicals in different parts of the plants, their extensive studies are required to understand their characteristics and bio-prospects. Many more such investigations are needed to specify the nature and properties of ligands existing in various parts of the plants and add them to the list of chemicals useful for the development of drugs to treat inflammatory and cancer-related ailments.

MATERIALS AND METHODS

Preparation of protein structure

Using X-ray diffraction method the structures are determined and the crystal structure of receptors such as anti-inflammatory COX-2 protein (PDB ID: 1CX2), breast cancer protein (PDB ID: 1JNX) and lung cancer protein (PDB ID: 2ITO) are retrieved from the RCSB protein data bank. In the final stage, all the water molecules were removed, and hydrogen atoms were added to the target protein molecule.

Preparation of ligand structure

The advanced Java based chemical editor used in this study for drawing the chemical structures is Marvin Sketch. It supports various file types such as MOL, MOL2, SDF, SMILES, MRV, InChi, CML and PDB. Using this, the ligand generation and three-dimensional optimizations were done and saved in. mol file. Ligand geometry optimizations were performed by Autodock 4.0 software using Force-Field (FF) based scoring functions.

The protein-fixed, ligand-flexible docking calculations were performed using Autodock 4.0. It describes the target protein by docking the ligand to a set of grids. The end docking results were obtained by pre-calculating these grids by an auto grid.

Protein-ligand interaction using auto dock 4.0

Proteins such as 1CX2, 1JNX, 2ITO were docked against the obtained three ligands using Autodock 4.0 to find and explored the protein ligand interactions with binding geometries. The predicted active site *i. e* involved in the protein-ligand complex was mainly targeted by the docking process.

RESULTS AND DISCUSSION

The aim of the present study was to predict the interaction between selected ligand and different types of inflammatory and cancer target proteins. The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work, receptors such as Crystal structures of COX-2 receptor having protein ID: 1CX2, Breast cancer protein ID: 1JNX, Lung cancer

protein ID: 2ITO were retrieved from the RCSB protein data bank. Based on the overall quality of the X-ray crystal structure and stereochemistry these proteins were selected. Docking simulation of *N. arbor-tristis* with ligands (1S,4aS,5R,6S,7S,7aS)-ethyl1,5,6-trihydroxy-7-(propionyl oxy) 1,4a,5,6,7,7a-hexahydrocyclopenta [c] pyran-4-carboxylate (317 ($C_{14}H_{20}O_8$)) from *N. arbor-tristis* leaves, 8-(5,7-dihydroxy-3-vinylnaphthalen-1-yl)-2H-chromen-2-one (331 ($C_{21}H_{14}O_4$)) from *N. arbor-tristis* fruit and 7-ethyl-3a,7a-dihydroxy-3-(2,3,4,5,6-pentahydroxycyclohexyloxy) tetrahydro-2H-furo[3,2-c] pyran-6(3H)-one (381 ($C_{15}H_{24}O_{11}$)) from *N. arbor-tristis* stem with three proteins 1CX2, 1JNX and 2ITO produced different binding complexes using flexible ligand searching methods with RMSD-tolerance of 2.0 Å out of 10 docking runs.

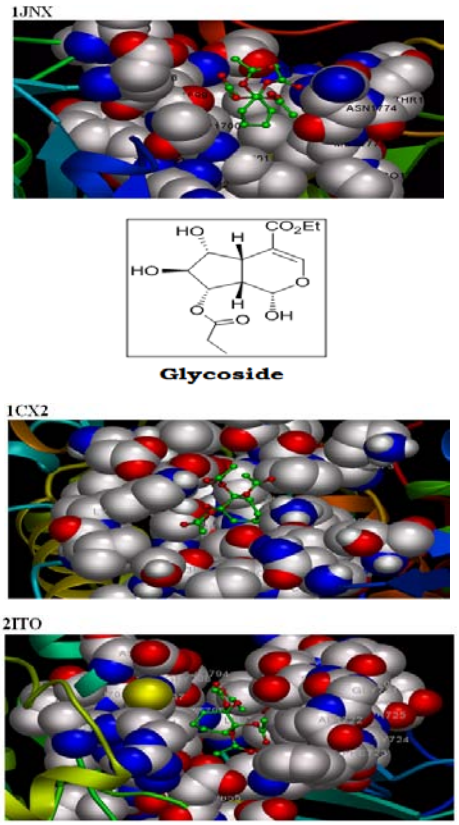

Fig. 1: Molecular docking of ligand from dried leaf methanolic extract of *N. arbor-tristis* ($C_{14}H_{20}O_8$) with receptor proteins

The main chemical compound (1S,4aS,5R,6S,7S,7aS)-ethyl1,5,6-trihydroxy-7-(propionyloxy) 1,4a,5,6,7,7a-hexahydrocyclopenta [c] pyran-4-carboxylate (M. Wt. 317 ($C_{14}H_{20}O_8$)) obtained from *N. arbor-tristis* leaves.

Was docked against three receptors (1CX2, 1JNX and 2ITO) and analyzed for binding energy values. The glycoside obtained from DLM showed highest binding energy of-2.43 kcal/mol at 10th run against 1JNX receptor protein followed by 2ITO with-2.24 kcal/mol and 1CX2 with-1.67 kcal/mol. As shown in table 1 these ligands interact at different sites in modeled proteins and visualized the docked complex in fig. 1 and table 1.

The main chemical compound 8-(5,7-dihydroxy-3-vinylnaphthalen-1-yl)-2H-chromen-2-one (M. Wt. 331 ($C_{21}H_{14}O_4$)) obtained from *N. arbor-tristis* fruit was docked against three receptors (1CX2, 1JNX and 2ITO) and analyzed for binding energy values. The phenolic compound obtained from DFM showed highest binding energy of-6.77 kcal/mol at 10th run against 2ITO receptor protein followed by 1CX2 with-4.37 kcal/mol and with 1JNX-3.69 kcal/mol. As shown in table 2, these ligands interact at different sites in modeled proteins and visualized the docked complex in fig. 2 and table 2.

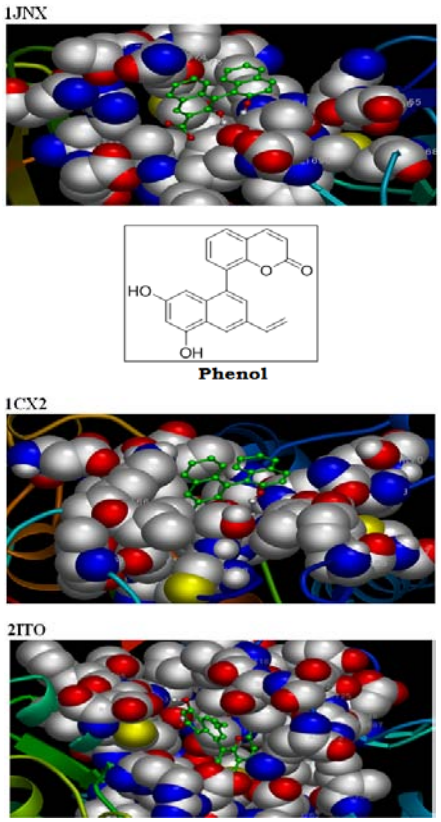

Fig. 2: Molecular docking of ligand from dried fruit methanolic extract of *N. arbor-tristis* with receptor proteins ($C_{21}H_{14}O_4$)

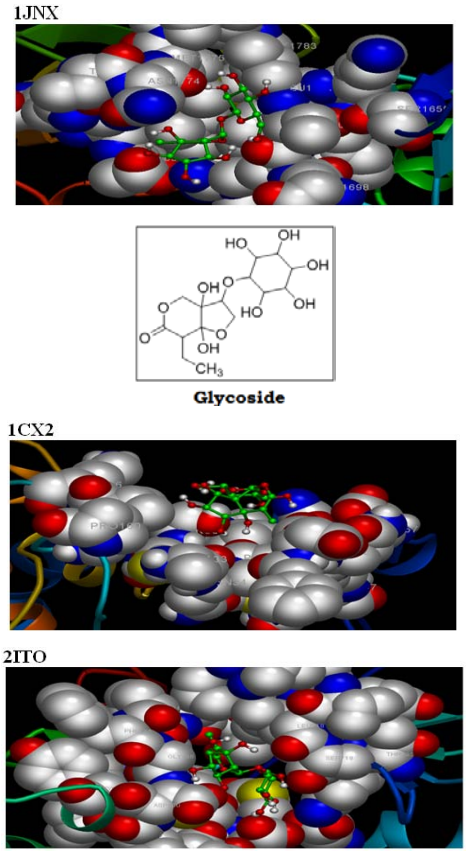

Fig. 3: Molecular docking of ligand from dried stem methanolic extract of *N. arbor-tristis* with receptor proteins ($C_{15}H_{24}O_{11}$)

Table 1: Docking results of receptors with ligand of dried leaves methanolic extracts

Ligand	Receptor	Amino acids	
Ligand-1	1CX2	42	GLN
		166	LYS
		465	GLU
		468	LYS
		474	PRO
		499	ASP
	1JNX	1699	ARG
		1701	LEU
		1704	PHE
		1774	ASN
		1775	MET
		1835	ARG
		1839	LEU
	2ITO	718	LEU
		719	SER
		726	VAL
		745	LYS
		797	CYS
		844	LEU
		854	THR
		855	ASP

Table 2: Docking results of receptors with ligand of dried fruit methanolic extract

Ligand	Receptor	Amino acids	
Ligand-2	1CX2	38	SER
		39	ASN
		40	PRO
		42	GLN
		68	ASN
		165	VAL
		166	LYS
		465	GLU
		468	LYS
	1JNX	1699	ARG
		1700	THR
		1701	LEU
		1704	PHE
		1774	ASN
		1775	MET
		1839	LEU
	2ITO	718	LEU
		719	SER
		726	VAL
		743	ALA
		745	LYS
		762	GLU
		766	MET
		788	LEU
		790	THR
		793	MET
		844	LEU
		854	THR
		855	ASP

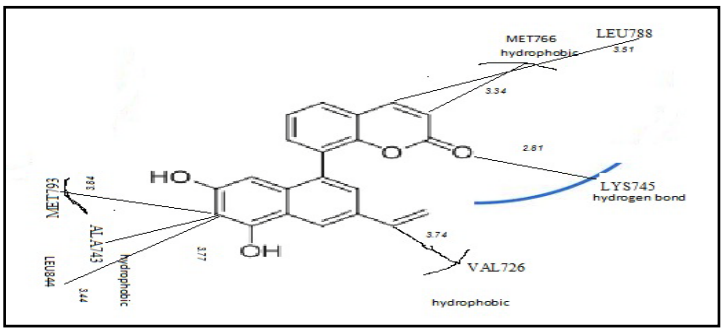

Fig. 4: Interactions between 2ITO receptor and ligand atom of DFM

Table 3: Docking results of receptors with ligand of dried stem methanolic extract

Ligand	Receptor	Amino Acids	
Ligand-3	1CX2	35	PRO
		38	SER
		40	PRO
		55	TYR
		67	GLU
		68	ASN
		165	VAL
	1JNX	1699	ARG
		1700	THR
		1701	LEU
		1704	PHE
		1774	ASN
		1775	MET
		1835	ARG
		1836	GLU
		1839	LEU
		718	LEU
	2ITO	719	SER
		726	VAL
		745	LYS
		797	CYS
		800	ASP
		844	LEU
		855	ASP

The main chemical compound 7-ethyl-3a, 7a-dihydroxy-3-(2,3,4,5,6-pentahydroxycyclohexyloxy) tetrahydro-2H-furo[3,2-c] pyran-6(3H)-one (381 ($C_{15}H_{24}O_{11}$)) obtained from *N. arbor-tristis* stem was docked against three receptors (1CX2, 1JNX and 2ITO) and analyzed for their binding energy values. The glycoside compound obtained from DSM showed highest binding energy of-5.73 kcal/mol at 10^{th} run against 2ITO receptor protein followed by 1JNX with-4.35 kcal/mol and with 1CX2-3.45 kcal/mol. As shown in table 3 these ligands interacted at different sites in model proteins and visualized the docked complex in fig. 3 and table 3.

By using anticancer and anti-inflammatory receptors, the ligands of main chemical compounds from *N. arbor-tristis* methanolic extracts were docked, and the results were tabulated and showed in table 4. A highest binding energy of-4.35 kcal/mol at 10^{th} run was observed with $C_{15}H_{24}O_{11}$ compound from DSM against 1JNX,-4.37 kcal/mol at 10^{th} run was observed with $C_{21}H_{14}O_4$ compound from DFM against 1CX2 and of the three receptors used, 2ITO lung cancer protein with ligand from dried fruit methanolic extracts showed a highest binding energy of-6.77 kcal/mol at 10^{th} run with different bond interactions at residues.

Table 4: Docking result of 1CX2, 1JNX, 2ITO receptors with ligands from *Nyctanthes arbor-tristis* methanolic extracts

Chemical structure	Est. of free energy	Inhibition constant K_i	Vdw+Hbond+ obsl energy	Electrostatic energy	Total internal energy
1JNX					
$C_{14}H_{20}O_8$	-2.43	16.41	-4.27	-0.00	-4.28
$C_{15}H_{24}O_{11}$	-4.35	647.46	-5.11	-0.00	-5.11
$C_{21}H_{14}O_4$	-3.69	1.96	-4.90	-0.10	-5.01
1CX2					
$C_{14}H_{20}O_8$	-1.67	59.92	-3.45	-0.49	-3.74
$C_{15}H_{24}O_{11}$	-3.45	2.97	-4.22	-0.07	-4.29
$C_{21}H_{14}O_4$	-4.37	626.04	-5.20	+0.01	-5.19
2ITO					
$C_{14}H_{20}O_8$	-2.24	22.69	-4.32	-0.29	-4.61
$C_{15}H_{24}O_{11}$	-5.73	63.40	-6.77	-0.58	-7.35
$C_{21}H_{14}O_4$	-6.77	10.97	-7.59	-0.10	-7.69

Among all the drugs 2ITO showed its effectiveness in binding with selected cancer target proteins. The results of the present *in silico* study support the interaction of protein-ligand that is binding and interaction of ligands (*N. arbor-tristis*) with inflammatory and cancer targets will be predicted by using these molecular docking studies. Similar to the present studies Radhakrishnan and Ismail (2015) [8] analyzed the *in silico* experiments and identified the cosmetic potential of Southeast Asian herbs. The bioassay-guided isolation of mosquito larvicidal compound from the acetone leaf extract of *Elaeagnus indica* Bull was evaluated and its detailed *in silico* study was reported [9]. Certain important compounds were isolated from medicinal plants and extensively studied their properties against inflammatory arthritis and regulatory effects of glucose and triglycerides in mice (*Mus musculus*) [10, 11]. The phytochemical and pharmacological potential ethanolic extracts of *Nyctanthes arbor-tristis* were investigated through *in silico* experimentations, and their clear potentials on diabetes and cancer were proved [12-14]. In another study [15] the parameter for the identification of weak binding chemical entities, are illustrated with the discovery of a new lead compound for NF-kappaB. Further biochemical analyzes based on EMSA were performed, and biological effects were tested on the compound exhibiting the best docking score. All experimental analysis was in fairly good agreement with molecular modeling findings. In another study [16] they isolated two compounds Of the two compounds, taraxerol showed the greatest inhibition of GSK-3 protein in molecular docking and dynamics studies. As it showed minimum binding −(12.59 kJ mol−1) and docking (−11.25 kJ mol−1) energy with an acceptable affinity towards the active pocket, taraxerol can be considered a good inhibitor of GSK-3.

CONCLUSION

Docking simulation of *N. arbor-tristis* with ligands (1S,4aS,5R,6S,7S,7aS)-ethyl1,5,6-trihydroxy-7-(propionyloxy) 1,4a,5,6,7,7a-hexahydrocyclopenta [c] pyran-4-carboxylate (317 ($C_{14}H_{20}O_8$)) from *N. arbor-tristis* leaves, 8-(5,7-dihydroxy-3-vinylnaphthalen-1-yl)-2H-chromen-2-one (331 ($C_{21}H_{14}O_4$)) from *N. arbor-tristis* fruit and 7-ethyl-3a,7a-dihydroxy-3-(2,3,4,5,6-pentahydroxycyclohexyloxy) tetrahydro-2H-furo[3,2-c] pyran-6(3H)-one (381 ($C_{15}H_{24}O_{11}$)) from *N. arbor-tristis* stem with three proteins 1CX2, 1JNX and 2ITO produced different binding complexes using flexible ligand searching methods with RMSD-tolerance of 2.0 Å out of 10 docking runs. This pre-study of dry lab procedure helps us to move on for wet lab or *in vitro* procedure to confirm the same as well to find out the exact compound responsible for its bioactivity. However, based on the results of these *in silico* studies, further studies are desirable to prove the anticancer and anti-inflammatory potential against different types either by *in vitro* or *in vivo* studies.

CONFLICT OF INTERESTS

Declared none

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