Research Article

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MOLECULAR DOCKING STUDIES OF CYCLOOXYGENASE ENZYME (COX-2) INHIBITORY ACTIVITY OF MAIN ACTIVE CONSTITUENTS OF *CALENDULA OFFICINALIS*

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Received on: 15/12/18 Accepted on: 15/01/19

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DOI: 10.7897/2277-4343.100118

ABSTRACT

Recent scientific findings demonstrated that increased use of NSAIDs for relieving the pain, inflammation and fever have greatly affected human beings by producing gastrointestinal, cardiac and many other problems. Therefore the purpose of the present study is to identify some suitable candidates in terms of enhanced efficacy and reduced toxicity on the basis of molecular docking analysis of the main active constituents of *Calendula officinalis* plant. Structures of phytochemicals were drawn using ChemSketch (ACD 2015) freeware and molecular docking was done with AutoDock 4.2 using the Lamarckian genetic algorithm. Among reported anti-inflammatory constituents of *Calendula officinalis* (Astereceae) Faradiol-3 Myristate and Ψ-Taraxasterol exhibited good binding scores (-8.22 and -8.19 kcal/mol respectively) and comparable to the native ligand (Diclofenac, -8.00 kcal/mol) bound to COX-2 (1PXX). Faradiol-3- Palmitate showed hydrophobic interactions and less binding energy (-6.39 kcal/mol). Amino acids interactions also indicated that Faradiol-3 Myristate and Ψ-Taraxasterol fitted to the actual active site of the enzyme and probably less toxic than marketed NSAIDS. Interactions of Faradiol-3 Myristate and Ψ-Taraxasterol with TYR-385 are responsible for enzyme inhibition. These binding scores and amino acids involved in interactions may probably be responsible for inhibition of the COX-2 enzyme activity.

KEYWORDS: COX inhibitors, C. officinalis, Diclofenac, Docking, NSAIDs, Toxicity

INTRODUCTION

The medicinal plants have gained a lot of attention worldwide due to promising pharmacological activities and reduced toxicological profiles. They have been a source of new active constituents for various ailments.[1] *Calendula officinalis* L. (Astereceae) is commonly known as pot marigold plant and has been described many times for its pharmacological properties.[2] *Calendula. officinalis* L. is used medicinally in Europe, China, US and India and have wide range of pharmacological activities viz. antipyretic, antiseptic and many others.[3] In England, the decoction of the flowers was used as a posset drink for the treatment of measles and smallpox, and the fresh juice as a remedy for jaundice, costiveness (constipation) and suppression of menstrual flow.[4] In India, florets are used in ointments for treating wounds, herpes, ulcers, frostbite, skin damage, scars and blood purification. The leaves, in infusion, are utilized for treating varicose veins externally.[4] A number of phytochemical studies have demonstrated the presence of several classes of chemical compounds, like terpenoids, flavonoids, coumarins, quinones, volatile oil, carotenoids and amino acids.[5] The anti-inflammatory activity of *Calendula officinalis* flowers cultured in Europe and Asia has been evaluated and evidenced through the model of edema induction of the ear through croton oil and the model of edema induction of the paw through carrageenan.[6] The flowers of *Calendula officinalis* are found in chapters which may distinguish the varieties through its color and size. They have medicinal use especially as an anti-inflammatory agent, for the treatment of wound, first-degree burns, contusions, and skin rashes. German sanitary authorities recommended its topic use in leg ulcers and its internal use only against inflammatory lesions in the oral and pharynx mucosa.[7-9] The main chemical components found in the flowers are saponins, triterpenes, alcohol triterpenes, fatty acid esters, carotenoids, flavonoids, coumarins, essential oils, hydrocarbons, and fatty acids. [10-12] Ethyl acetate soluble fraction of the methanol extract of *Calendula officinalis* flowers exhibited the most potent inhibition (84%) of 12-otetradecanoyl phorbol-13-acetate (TPA)-induced inflammation (1 µg/ear) in mice with an ID50 value of 0.05 -0.20 mg/ear compared with Indomethacin as reference drug. Furthermore, activity-guided isolation showed that its activity was mainly due to oleanane type triterpenes glycoside.[13] A dose of 1200 µg/ear of an aqueous-ethanol extract showed 20% inhibition in croton oil-induced mouse edema .The activity was attributed to the presence of triterpenoids, the three most active compounds of which were the esters of faradiol-3-myristic acid and faradiol-3-palmitic acid and another compound ψ-Taraxasterol.[14-15] The hydro-alcoholic extract of *Calendula officinalis* flowers, based on the assessment in rats and mice, did not show acute toxicity following administration of an oral dose of up to 5.0g/kg. It didn't show hematological alterations at doses of 0.025, 0.25, 0.5 and 1.0 g/kg.[16] The molecular docking on phytochemicals of *Calendula officinalis* was done to predict the binding modes to the enzyme.

MATERIALS AND METHODS

Retrieval of Molecular Target

The crystal structure of the cyclooxygenase enzyme (COX-2 PDB ID: 1PXX) involved in the inflammatory process was retrieved from the protein data bank (www.rcsb.org).[17]

Analysis of target active binding sites and target optimization

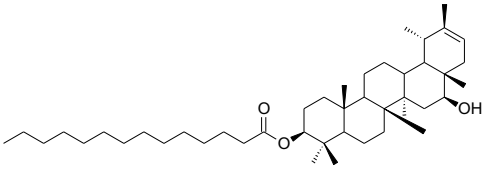
Active sites of the enzyme were identified employing Discovery Studio 4.5 and only A chain was used for docking purposes which also has a native ligand (Diclofenac). Molecular target had to be prepared before the initiation of the docking process, which involved the removal of water molecules, removal of unwanted heteroatoms attached with the target because these may hinder the entire docking process, and adding the hydrogens in the target.

Ligand preparation and optimization

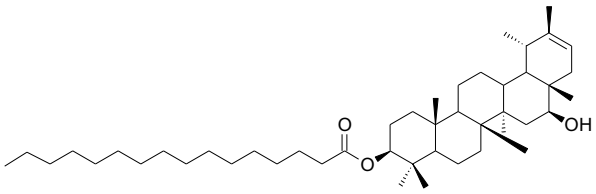
The main active constituents of Calendula officinalis (faradiol-3-Myristate, faradiol-3- Palmitate, and Ψ-Taraxasterol) responsible for anti-inflammatory activity were prepared using ChemSketch Freeware (ACD2015) and optimized for energy minimization using the MM2 force field and save in the .mol format which further converted into .pdb format through OpenBable GUI Figure1.

Molecular docking analysis

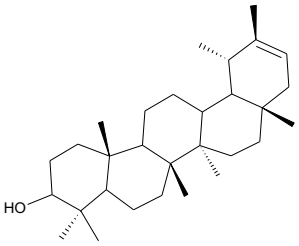
AutoDock v 4.2.6 software was used to carry out the molecular docking study by which binding modes and best conformers in terms of lowest binding energy (-kcal/mol) and binding to the active site can be identified. To find out possible enzyme inhibitory mechanism of investigational ligands, ligands were docked on entire molecular target (blind docking) and further identified whether they made interactions in the active site or anywhere else in the enzyme. This version of AutoDock facilitates the automatic distribution of charges on the molecular target. Further, the active constituents (ligands) were loaded and their torsions along with rotatable bonds were assigned and the files were saved as ligand.pdbqt. After refining, macromolecule was saved as .pdb execution file. The macromolecule was loaded and stored as macromolecule.pdbqt after assigning hydrogen bonds and Gasteiger charges. The docking parameters were defined as coordinates of the center of binding site with x = 126, y = 126, z = 126 and binding radius = 0.375 Å. All AutoDock output file (.dlg) were then analyzed through Analysis option provided in MGLTools-1.5.6 rc3. Top-scoring molecules in the largest cluster were analyzed. Complexes (in .pdbqt format) of the different docked conformers of the ligands with the protein were manually prepared and converted to .pdb format through pdbqt_to_pdb.py script. Rigid dockings were performed using Genetic Algorithms and keeping other docking parameters in default. Followed by, setting up of docking parameter files with search parameter as genetic algorithm and docking parameter utilizing Lamarckian genetic algorithm. The Lamarckian genetic algorithm (LGA) was applied to deal with all the proteins–ligands interactions. Docked structures of the inhibitors were generated after a short number of evaluations. The 2D images of macromolecules and ligands interactions, to identify the active site and to verify further whether anti-inflammatory molecules were found to be present in the same active site or anywhere else, were obtained through Discovery Studio 4.5 Visualizer prior to loading to AutoDock execution window.



Faradiol-3-myristate



Faradiol-3-palmitate



Ψ-Taraxasterol

Figure 1: Structures of active constituents of *Calendula officinalis* (Astereceae)

RESULT AND DISCUSSION

The active site of COX-2 enzyme was identified and which is comprised **PHE518, ALA 527, TRP387, MET 522, TYR348, TYR 385, TRP 387, TRP 385,VAL 349, LEU 352, VAL 523, GLY 526, SER 530, SER 523, SER 353, SER530, GLY 526.** Macromolecule was successfully docked with constituents having anti-inflammatory activity i.e.Faradiol-3Myristate, Faradiol-3- Palmitate and Ψ-Taraxasterol. Faradiol-3-Myristate was found to be having better binding energy (-8.22 kcal/mol) with the COX-2 subunit and formed H-bond with His207 of COX-2 subunit. Similarly, Faradiol-3 Palmitate was docked with COX-2 subunits and the binding score was found to be -6.39 kcal/mol, without any hydrogen bond formation whereas Ψ-Taraxasterol was docked with the COX-2 enzyme and found to be having a binding energy of -8.19 kcal/mol and formed a hydrogen bond with Glu290 (Table1).In this study analysis of enzyme ligands interactions revealed that Faradiol-3Myristate and Ψ-Taraxasterol (8th conformer) were actually bound at the active site whereas Faradiol-3- Palmitate was found to be bound anywhere, therefore, it may be proposed for not having a good binding affinity towards enzyme. (Figures:2.1– 2.8). Apart from this it is also suggested that Faradiol-3-Myristate is the active constituents among others, which can be responsible for the reported anti-inflammatory activity as it was found to be fitted in the active site and have interactions with Tyr385 including other amino acid residues, similar to the native ligand i.e. Diclofenac, but have more binding energy compared to that of native ligand, Diclofenac (-8.00 kcal/mol) and interacted with the amino acid residues which are supposed to be involved in inflammation process.

Table 1: Binding affinities between phytochemicals and proteins

S.N.	Ligand	M.W	LogP	HBA	HBD	Amino acids engaged in Interaction	H-bond formed between	Binding Affinity (-kcal/mol)	Hydrogen bond Distance (Å)
1.	Faradiol-3-Myristate	652.58	16.496	3	1	Thr206, His207, Phe210, Tyr409, His388, Tyr385, Val444, Leu294, Val447, Leu406, Gln203	1pxx:A:HIS207: NE2,F3M-2: :LIG1:O,1pxx:A: HIS207:HE2	8.22	2.038
2.	Faradiol-3-Palmitate	680.61	17.634	3	1	Phe404, Val447, Val444, Leu408, Gln203, His207, Val291, Gln289, Lys211, thr212, His214, Ala443	----	7.2	----
3.	Ψ-Taraxasterol	426.39	11.751	1	1	His207, Phe210, Tyr385, Ile274, His388, His386, Glu290, Val291, Arg222, Thr212	Tarax-2: :LIG1:O,1pxx:A: GLU290:OE2,Ta rax-2: :LIG1:H	8.19	2.165
4.	Diclofenac	296.15	4.51	3	2	Thr206, His207,Phe210,Gln 203, Ala199, His388,Leu391, Tyr385	1pxx:A:HIS388: NE2,Diclofenac-2: :LIG1:O,1pxx:A: HIS388:HE2	8.00	1.854

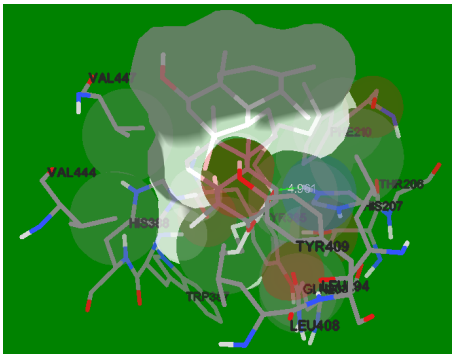


Figure 2.1: Binding mode of Faradiol-3- Myristate with Cox-2 enzyme (1pxx)

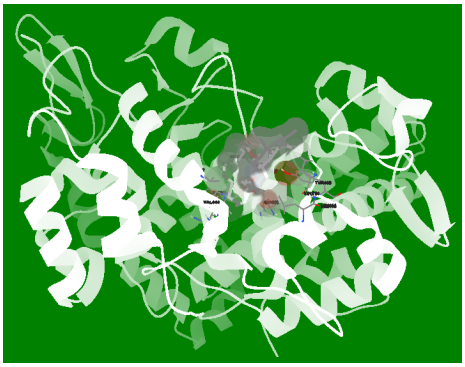


Figure 2.2: Secondary Structure of the binding mode of Faradiol-3-Myristate with Cox-2 enzyme (1pxx)

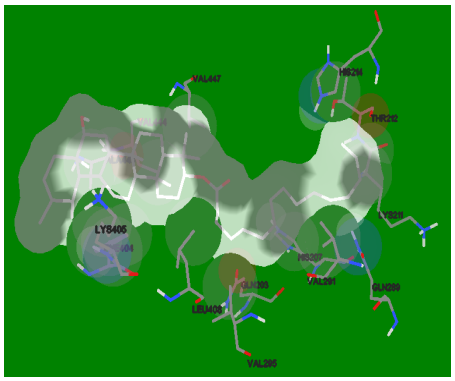


Figure 2.3: Binding mode of Faradiol-3- Palmitate with Cox-2 enzyme (1pxx)

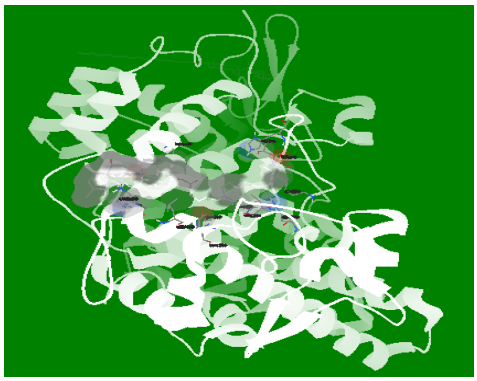


Figure 2.4: Secondary Structure of the binding mode of Faradiol-3-Palmitate with Cox-2 enzyme (1pxx)

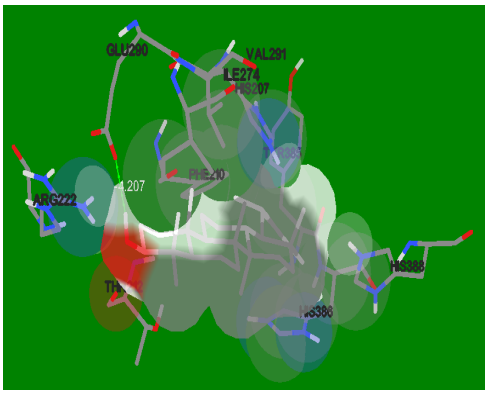


Figure 2.5: Binding modes of Ψ-Taraxasterol with Cox-2 enzyme (1pxx)

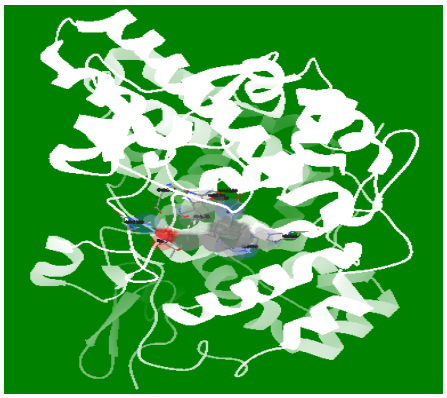


Figure 2.6: Secondary Structure of the binding mode of Ψ-Taraxasterol with Cox-2 enzyme (1pxx)

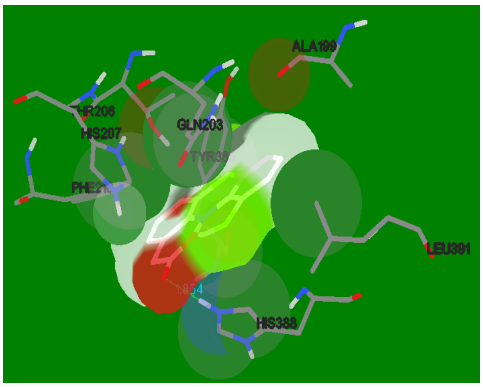


Figure 2.7: Binding mode of Diclofenac with Cox-2 enzyme (1pxx)

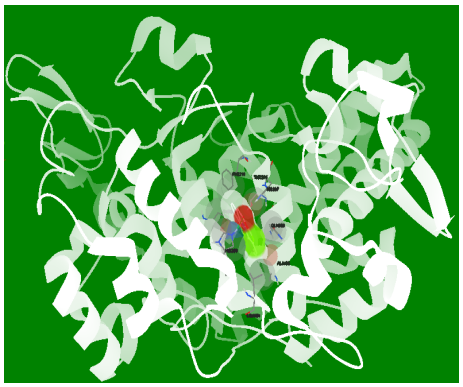


Figure 2.8: Secondary Structure of the binding mode of Diclofenac with Cox-2 enzyme (1pxx)

CONCLUSION

From above molecular docking studies, it is concluded that the phytoconstituents i.e. Faradiol-3 Myristate, Faradiol-3 Palmitate and Ψ-Taraxasterol possess significant binding scores on COX-2 subunits. Among all phytoconstituents, Faradiol-3-Myristate has better binding affinity with COX-2 receptor. Suitable extraction and synthetic modification of phytoconstituents may lead to the development of appropriate clinical candidates since natural compounds are believed to be devoid of the toxicological profile. These constituents can show meaningful in-vivo and in-vitro studies after extraction and optimization synthetically. Since, most of the NSAIDs possess adverse effects like hepatotoxicity, gastrointestinal disorders etc. there is a need of candidates from a natural source which will show better pharmacological and toxicological profile than other NSAIDs.

ACKNOWLEDGEMENT

Authors wish to thank honorable vice chancellor, ITM University Gwalior for providing adequate computational facilities and cordial support during the research work.

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Cite this article as:

Anurag Agrawal *et al*. Molecular docking studies of cyclooxygenase enzyme (Cox-2) inhibitory activity of main active constituents of *Calendula officinalis*. Int. J. Res. Ayurveda Pharm. 2019;10(1):75-79 http://dx.doi.org/10.7897/2277-4343.100118

Source of support: Nil, Conflict of interest: None Declared