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Original Article

IN SILICO STUDIES ON PLANT DERIVED COMPONENTS OF *CISSUS QUADRANGULARIS* AGAINST COX-2 ENZYME

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ABSTRACT

Objective: *Cissus quadrangularis*, a perennial plant of grape family is traditionally used as an herbal medicine for treating inflammation caused by hemorrhoids, gastric ulcer and bone disorders. COX-2 is an oxidoreductase enzyme having a role in inflammatory responses. The objective of this study was to show the drug-likeness and the binding of *Cissus quadrangularis* derived biologically active compounds against the inflammation associated target COX-2 enzyme.

Methods: The 3D structure of COX-2 enzyme protein structure was taken from PDB database (PDB ID: 6cox). The structures of plant derived compounds were retrieved from the PubChem database. The Lipinski's properties of about 16 compounds from *Cissus quadrangularis* were checked and those which satisfied the Lipinski's rule of five were subjected to docking experiments. Docking studies had been carried out through AutoDock 4.2.

Results: About 6 compounds showed drug-likeness by satisfying Lipinski's properties. The comparative molecular docking studies were done for 6 compounds which showed drug-likeness through AutoDock 4.2. The comparison reflected that flavonoid and stilbene derivatives bind in the active site region of COX-2 with good binding energy.

Conclusion: The in silico studies on compounds reported from *Cissus quadrangularis* showed that they possess potential medicinal values with anti-inflammatory properties which form insights to develop new leads for COX-2 inhibition.

Keywords: *Cissus quadrangularis,* Inflammation, COX-2, Lipinski's rule of five, Molecular docking.

INTRODUCTION

Cissus quadrangularis Linn., is commonly known as Asthisamhari, a succulent plant belonging to family Vitaceae [1]. *Cissus quadrangularis* has medicinal properties and it is used in the management of weight loss, metabolic syndrome and in Ayurveda for complaints of back and spine [2,3,4].The extract from *Cissus quadrangularis* can be used as analgesic, anti-inflammatory and antipyretic compound [5]. The extract contains triterpenes, flavonoids and stilbenes [6,7,8]. Several biologically active compounds were isolated and they have proved to be responsible for various pharmacological activities [9]. In this work, we have illustrated the binding potential of these compounds as anti-inflammatory molecules by studying their interaction with the COX-2 enzyme.

COX-1 and COX-2 are two isoforms of cyclooxygenase enzyme which is crucial for the production of prostaglandins [10]. COX-1 is involved in protecting the stomach lining and COX-2 is involved in the inflammatory pain found in Central nervous system and in inflammatory cells [11]. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit COX-2 enzymes but result in various side effects such as gastro-intestinal and renal functional suppression. Thus, there is a need to identify plant-derived compounds without any side effects. Plants form primary source of medicine for 65-75% of the world's population for the treatment of various diseases. A series of new compounds can be produced by traditional synthesis but it is time consuming and at high cost. Hence screening of small molecule databases of novel compounds is an alternative process [12].

The drug-likeness of plant derived compounds can be predicted by Lipinski's rule of five which refers to the similarity of compounds to oral drugs. Molecular docking plays an important role in the rational drug design. It predicts the binding orientation of small drug targets to their protein targets. The anti-inflammatory activity of Urosolic acid, a plant derived pentacyclic triterpenoid compound is revealed by binding mechanism of the compound with COX-2 enzyme [13].Molecular docking studies on biologically active compounds from plant *Litsea* Genus against COX-2 enzyme were also done which proves their anti-inflammatory property [14]. In the present study, we have studied the drug-likeness of compounds from *Cissus quadrangularis* using Lipinski's rule of five and the binding mechanism of the compounds with COX-2 enzymes using molecular docking.

MATERIALS AND METHODS

Cissus quadrangularis derived compounds:

Compounds selected for this study are (a) Asarone, (b) Luteolin, (c) Quercetin, (d) Resveratol, (e) Piceatannol, (f) Kampferol, (g) Stigmasterol, (h) Lupeol, (i) Freidalin, (j) Quadrangularin, (k) Hexadecanoic acid, (l) Tetradecanoic acid, (m) Phytol, (n) Oleic acid, (o) Linoleic acid ethyl ester, (p) Octadecanoic acid ethyl ester. The structures and the physiochemical properties of these compounds were retrieved from the PubChem database (www.ncbi.nlm. nih.gov/pubchem) which is shown in Table 1.

PubChem is a public database which makes database search for a broad range of properties including compound structure, name, fragments, molecular weight, chemical formula, X Log P, hydrogen bond donor and acceptor count. It has own online editor with smiles format and our compounds are converted to. pdb format using this converter.

Lipinski's properties such as molecular weight, log P and number of hydrogen bond donors and acceptors were taken from the PubChem database for *Cissus quadrangularis* derived plant compounds.

COX-2 enzyme protein structure:

The 3 dimensional structure of the COX-2 enzyme was taken from the Protein Data Bank (PDB) database (www.rcsb.pdb) which is given in Figure 1. The RCSB PDB is a repository for the 3D structural data of large biological macromolecules such as proteins and nucleic acids. It provides simple and advanced searches based on annotations related to sequence, structure and function The PDB ID is 6cox which is a complex of COX-2 enzyme with selective inhibitor SC-558 [15].

Fig. 1: Cyclooxygenase-2 (Prostaglandin Synthase-2) in complex with a COX-2 selective inhibitor

The active site region of COX-2 enzyme is given in Figure 2. The docking process was done by submitting the pdb coordinates of protein and ligand to AutoDock 4.2. The binding energy was obtained for each ligand and the contact analysis of the docked complexes done using Discovery Studio 3.1 visualizer.

Docking Studies – AutoDock 4.2

Molecular docking studies were performed for the active plant components with COX-2 enzyme by AutoDock 4.2. It is an automated docking tool which works by Lamarckian Genetic Algorithm. It predicts how small molecules such as substrates or drug candidates bind to a receptor of known 3D structures.

The precise interaction of bioactive agents or candidate molecules with their targets is important in the drug development process. AutoDock combines two methods to achieve these goals: rapid grid-based energy evaluation and efficient search of torsional freedom.

AutoDock 4.2 using the Lamarckian Genetic Algorithm and empirical free energy scoring function will provide docking results for ligands with approximately 10 flexible bonds.

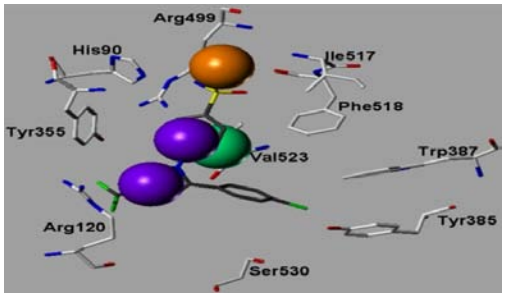

Fig. 2: COX -2 enzyme in complex with selective inhibitor SC-558 showing the active site region of COX-2 enzyme

Discovery Studio Visualizer 3.1

Discovery Studio Visualizer is a free, molecular modeling environment, for both small molecule and macromolecule applications. Discovery Studio is a well-known suite of software for simulating molecule and macromolecule systems. It is developed by Accelrys which specializes in scientific software products. It is used regularly in a range of academic and commercial entities, but is most relevant to pharmaceutical and biotechnology industries. It generates 2D receptor-ligand interaction plots and analyzes the ligand binding patterns between a protein and its bound ligands.

RESULTS

Molecular weight, Log P, Number of H bond donor and H bond acceptor are tabulated in Table 1. Compounds which obey Lipinski's rule of five were alone subjected to docking experiment.

Table 1: Lipinski properties of the active plant components

S. No.	Compound	Molecular weight (< than 500 Da)	Log P (< than 5)	No. of H bond donor (< than 5)	No. of H bond acceptor (< than 10)
1.	Asarone	208.25	3	0	3
2.	Luteolin	286.24	1.4`	4	6
3.	Quercetin	302.235	1.5	5	7
4.	Resveratol	228.243	3.1	3	3
5.	Piceatannol	244.24	2.9	4	4
6.	Kampferol	286.23	1.9	4	6
7.	Stigmasterol	412.69	**8.6**	1	1
8.	Lupeol	426.72	**9.9**	1	1
9.	Quadrangularin	454.47	4.9	**6**	6
10.	Freidalin	426.72	**9.8**	0	1
11.	Hexadecanoic acid	256.42	**6.4**	1	2
12.	Tetradecanoic acid	228.37	**5.3**	1	2
13.	Phytol	296.53	**8.2**	1	1
14.	Oleic acid	282.46	**6.5**	1	2
15.	Linoleic acid ethyl ester	308.5	**7.3**	0	2
16.	Octadecanoic acid ethyl ester	312.53	**8.9**	0	2

Of all the 16 compounds taken from *Cissus quadrangularis*, six compounds (1-6) satisfy Lipinski's rule of five for drug-likeness which is shown in Table 1. The values of the Lipinski's properties which deviated from the Lipinski's rule of five are highlighted in Table 1. Hence compounds which do not follow the Lipinski's properties were not considered for further study.

The plant components which showed drug-likeness from *Cissus quadrangularis* were a) Asarone, b) Luteolin, c) Quercetin, d)Resveratol, e) Piceatannol, and f) Kampferol. The structures of the selected compounds are shown in Fig. 3

The binding energy for each chosen compound with the COX-2 enzyme using AutoDock 4.2 is given in Table 2. Docking studies show that the ligands bind to the active site region of COX-2 enzyme with good binding energy. Also all the ligands bind in the same hydrophobic pocket which is the active site region of COX-2 enzyme.

The docking models of the plant compounds a)Asarone, b) Luteolin, c) Quercetin, d)Resveratol, e)Piceatannol, f)Kampferol in 3D and 2D view are shown in Figure 4,5,6,7,8 and 9. The hydrogen contacts of the ligands are tabulated in Table 3.

Hydrogen bonds indicate the strength of contact between the ligand and the receptor and the catalytic activity of an enzyme can be predicted by the hydrogen bonds between them. The arrow in the 2D view denotes the hydrogen bond interactions and the other aminoacids form hydrophobic contacts with the compounds.

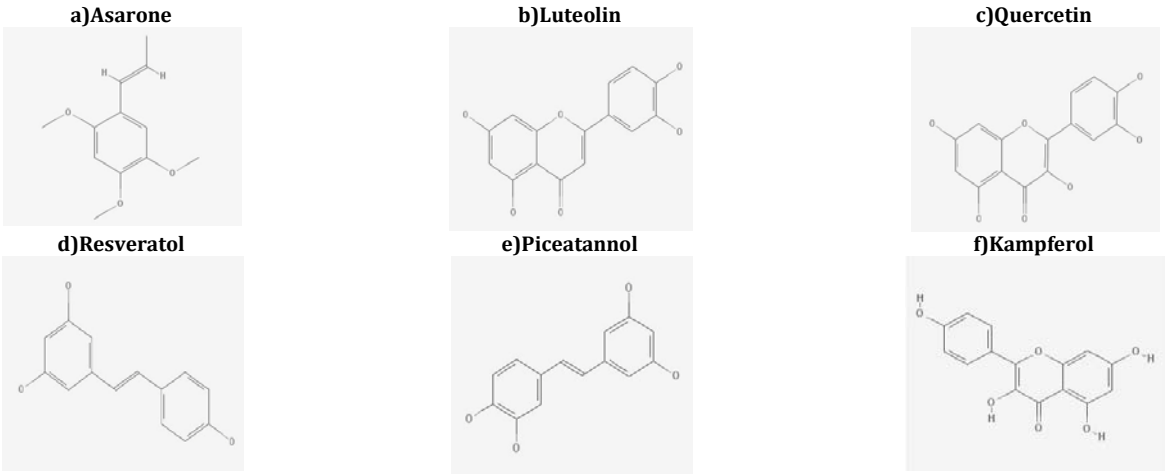

Fig. 3: The structures of compounds a)Asarone, b) Luteolin, c) Quercetin, d)Resveratol, e)Piceatannol, f)Kampferol.

Table 2: Dock scores of the compounds

S. No.	Compound	Dock score
1	Asarone	-5.88
2	Luteolin	-8.62
3	Quercetin	-8.69
4	Resveratol	-7.57
5	Piceatannol	-7.58
6	Kampferol	-3.59

DISCUSSION

Cyclooxygenase (COX), also known as Prostaglandin (PG) H synthase enzymes aids in the conversion of arachidonic acid to prostaglandins [16]. Various phyto pathological processes such as inflammatory and cardiovascular responses are regulated by prostaglandins. Mammalian cells contain COX-1 which is a constitutive enzyme whereas COX-2, an inducible enzyme is abundant in macrophages and inflammatory sites [17].

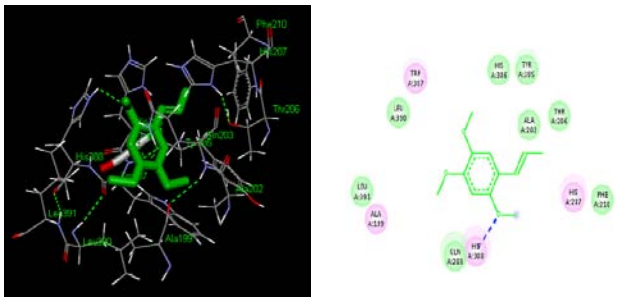

Fig. 4: Interaction of Asarone with COX-2 enzyme

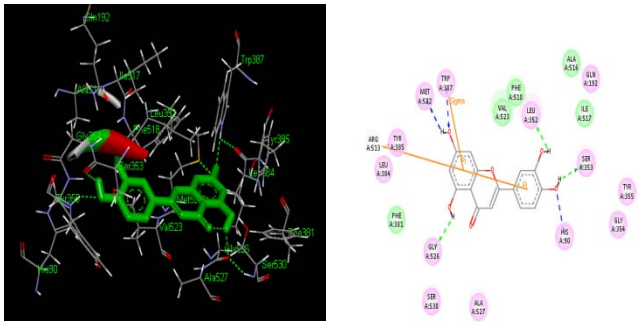

Fig. 5: Interaction of Luteolin with COX-2 enzyme

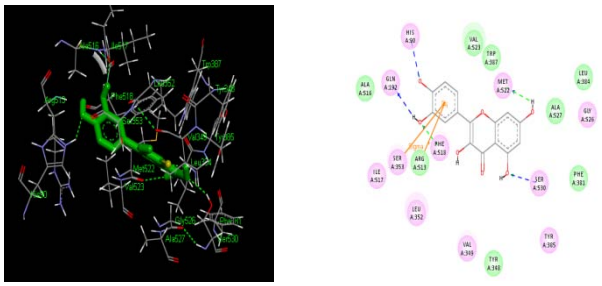

Fig. 6: Interaction of Quercetin with COX-2 enzyme

Table 3: Hydrogen bonds of the ligands

S. No.	Compound	Hydrogen contacts
1	Asarone	His 388
2	Luteoiln	His 90, Leu 352, Ser 353, Trp 387, Met 522, Gly 526.
3	Quercetin	His 90,Gln 192,Phe 518, Met 522,Ser 530
4	Resveratol	His 90, Gln 192, Ser 353, Phe 518, Gly 526.
5	Piceatannol	His 90, Gln 192, Ser 353, Gly 526.
6	Kampferol	-

COX-2 enzyme plays an important role in carcinogenesis and their levels are up-regulated in various carcinomas. Thus, apart from inflammation, suppressing levels of COX-2 will be a better method for inhibiting carcinogenesis [18]. It has been estimated that NSAIDs can cause 3500 hospitalizations and 400 deaths from ulcer bleeding per annum in the UK in those aged 60 years and above. The cardiac risk for NSAIDs has received a lot of attention both in media and within the medical profession. About 30-60% of NSAID users have gastrointestinal effects such as dyspepsia and some abdominal discomfort [19]. The current study dealt with the in silico investigation of *Cissus quadrangularis* plant compounds for inflammation inhibitor to avoid any undesirable side effects. The docking studies using AutoDock 4.2 reveals that flavonoids such as Luteolin and Quercetin bind strongly to COX-2 enzyme with good binding energy of -8.69 and -8.62 respectively. Both form more hydrogen bonds on interaction with best cavity.

The catalytic activity of an enzyme molecule can be accessed by their hydrogen bonds in a docking study [20]. Recent studies have shown that some flavonoids attenuate inflammatory responses by acting as modulators of pro inflammatory gene expression[21]. In addition to flavonoids, stilbene derivatives such as Resveratol and Piceatannol also proved to be good inhibitors whose hydrogen bonds are with

residues His 90, Gln 192, Ser 353, Phe 518 and Gly 526. These residues form active site residues for COX-2 enzyme.

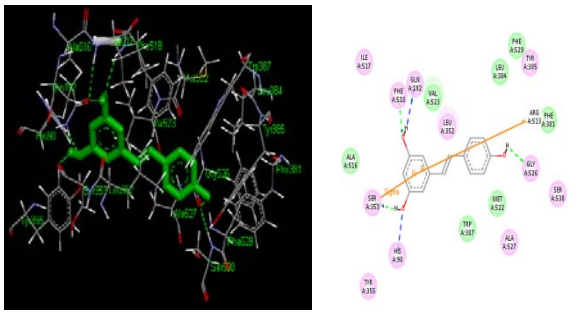

Fig. 7: Interaction of Resveratol with COX-2.

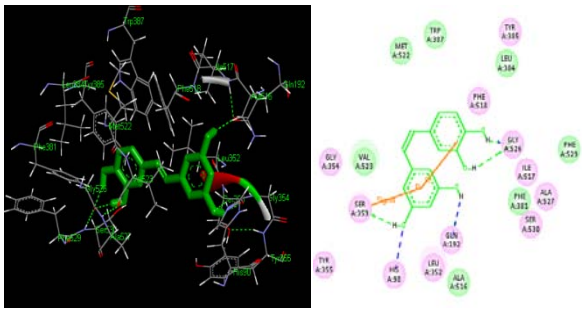

Fig. 8: Interaction of Piceatannol with COX-2.

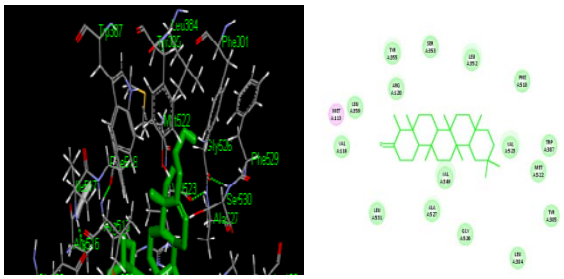

Fig. 9: Interaction of Kampferol with COX-2

The compound asarone, an ether compound also scored good binding energy but forms only one hydrogen bond so that its structure can be modified to act as a better drug with anti-inflammatory activity. The foliage leaves of *Sabina virginiana L.Antoine* containing asarone was found to be anti-inflammatory in mice paw edema test. Thus asarone can be used as an effective inhibitor of COX-2 [22]. Kampferol do not form any hydrogen bond and its docking score is also less when compared to other *Cissus quadrangularis* derived compounds. The active compounds from *Cissus quadrangularis* such as Luteolin, Quercetin, Resveratol, Piceatannol and Asarone have been found to be more potent as COX-2 inhibitors through comparative analysis in this docking experiment and they hold lots of promise to develop as COX-2 inhibitor.

CONCLUSIONS

The pharmacological activities of the medicinal plant, *Cissus quadrangularis* are antioxidant, anti-inflammatory, antioxidant, free radical scavenging, antiulcer, bone healing, analgesic and diuretic. In this study, we focused on the anti-inflammatory properties of the plant. The plant compounds showed better binding features with the COX-2 enzyme. Thus these compounds can be effectively used as drugs for treating inflammation. The insights gained in this work can be further used in experimental studies to develop leads of drugs against COX-2 enzyme.

CONFLICT OF INTERESTS

Declared None

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