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Original Article

Molecular Docking of Seed Bioactives as Dual COX-2 and LOX-3 Inhibitors in Context to Osteoarthritis

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ABSTRACT

Introduction: Potent anti-inflammatory seed bioactives through dual inhibition of cyclooxygenase (COX)-2 and lipoxygenase (LOX)-3 were evaluated by a computational approach, routinely used to reduce cost and time in drug discovery. **Methods:** The strategy employed in this study could be split into the two categories of screening and docking. **Results:** The analysis resulted in seven Lipinski compliant hits (epicatechin, 3, 4-Dihydroxy phenyl acetic acid, gallic acid, 3, 4-Dihydroxy benzoate, procyanidin, ascorbic acid, oxacyclohexadecan-2-one, 16 ethyl). They were docked to the crystal structure of COX-2; 1PXX and LOX-3; 1JNQ and scored to identify structurally novel ligands that make similar interaction to the known ligands (diclofenac) and many have different interactions with other parts of the binding sites on crystal structure. These ligands were prepared by following the appropriate procedures and *in silico* molecular docking analysis were performed to by a FleX X tool. 3,4-Dihydroxyphenyl acetic acid and epicatechin had the highest binding energy with hydrogen bonding to crucial SER530 and TYR385 amino acid residues of COX-2. Gallic acid and epicatechin are promising lead compounds for LOX-3 inhibition with a binding pose depicting hydrogen bonding to ASP766, GLN716 and GLN514 were encouraging. *In vivo* enzyme inhibition studies recorded microgram/mL for 3,4-dihydroxyphenyl acetic acid and epicatechin for inhibiting COX-2 were 28 and 35.25 microgram/mL respectively in comparison to diclofenac with 1.3 microgram/mL ($p \leq 0.05$). Gallic acid and epicatechin inhibited LOX-3 with IC_{50} of 25 and 42 microgram/mL respectively in comparison to diclofenac with 1.5 microgram/mL ($p \leq 0.05$). **Conclusion:** The current data supports the presence of potent bioactives naturally and conclusive proof could be provided by further clinical studies.

Key words: Cyclooxygenase-2, Lipoxygenase-3, 3, 4-dihydroxyphenyl acetic acid, Epicatechin Gallic acid, Swiss Dock.

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INTRODUCTION

Chronic inflammatory diseases such as osteoarthritis (OA) result in progressive degradation of human cartilage leading to joint pain.[1] Arachidonic acid (AA) is generated from these damaged cell membrane phospholipids by the action of phospholipase A2.[2] It is subjected to metabolism by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes generating mediators, prostaglandin (PG) E2, thromboxanes (TXs) (TXA2), prostacyclins (PGI2) and inflammatory leukotrienes, leukotriene (LT)B4, LTC4 and LTD4.[3–5] Pharmacological management for inflammation includes nonsteroidal anti-inflammatory drugs (NSAIDS) and also selective COX-2 inhibitors that inhibit PGs formation without affecting LOX activity.[6] However, if COX-1 enzyme inhibition occurs, shunting of AA metabolism via LOX pathway results, aggravating toxicity due to lack of PGs and production of excess of LTs[1]. This causes changes in normal human physiology,[7] including homeostasis,[8] gastroprotection, renal homeostasis[9] and bone formation with pathophysiological processes contribute to pain and inflammation in osteoarthritis.[10] The reported side effects due to use of NSAIDS inducing gastric ulcers due to high concentrations of LTB4 in their walls results leading to chemotactic movement of leukocytes to stomach resulting inducing ulceration.[11] It has been envisaged that a molecule capable of inhibiting LOX and COX-2 could be analgesic and possess anti-inflammatory properties with a favorable toxicity profile in comparison to current available treatments.[1,7-10] Thus, current reports focus on identification of dual inhibitors with COX-2 and LOX-3 inhibition.

Currently, an increased global interest in identifying pharmacological potent compounds as preventive medicine without side effects has warranted attention. There are recommendations to increase consumption of foods rich in bioactive components for overall maintenance of health. Mankind, through trial and error, has found medicinal properties in seeds, barks, roots and leaves of certain plants and traditional knowledge has given clues to the discovery of these valuable drugs.[12-13] Seeds, as a new source of anti-inflammatory and analgesic bioactives, may also have beneficial bioactives. Several bioactives have been proposed to have a beneficial effect on joint health and osteoarthritis (OA).[14] Research into the preventing and slowing of OA through dietary intervention has gained attention due to the high quality data in this field.[15]

High-throughput ADME screening provides an effective paradigm for filtering compounds for drug discovery process.[16-17] The technique employed is based on the prediction of binding modes and binding affinities of each compound in the dataset by means of docking to an X-ray crystallographic structure.[18] Various studies reported in literature stated the importance of dataset size such as 10,000 compounds using Flex and X,[19] 44,000 compounds using Surflex[20] and several others. Therefore, an alternative approach to eliminate unpromising compounds before docking by restricting the dataset to drug-like compounds is by filtering the dataset based on appropriate property and sub-structural features and by performing diversity analysis. These approaches can be highly effective in reducing the dataset to be docked.[21]

In the present study, bioactives of tamarind,[22] watermelon,[23] musk melon,[23] papaya[24] and amla[25] seeds were assimilated. They were screened for drug likeliness by Swiss ADME analysis. Nearly 96 seed bioactives were considered to be screened for anti-inflammatory activity using Swiss ADME software. Docking of the identified bioactives to the target site in the protein and further scoring was done by using Flex X tool to evaluate the binding in terms of score on crystal structure of COX 2, 1PXX and LOX-3, 1JNQ. One of the steps in designing of COX-2 and LOX inhibitors was to utilize the 3D structural information of the target molecule. Protein Data Bank (PDB) hosts a few of the COX-2 crystal structures like ICVU (PDB ID) with bound substrate arachidonic acid, 1PXX bound to inhibitor diclofenac (DIF) and lipoxygenase with PDB ID 1JNQ.[26] Along with providing a framework for the design of novel inhibitors, they

offer an insight on understanding of substrate specificity and mechanism of enzymatic reaction. The 1PXX bound to inhibitor diclofenac (DIF) and lipoxygenase with PDB ID 1JNQ were applied for our studies. Seed bioactives with particularly best docking properties were screened for their COX-2 and LOX inhibition in cell free assay as reported earlier.[26-28]

METHODOLOGY

Docking

Flex and X tool of the LeadIT software and Swiss ADME analysis were applied.

Ligand preparation for docking

A set of ninety six bioactives identified in seeds was screened, however as few as seven: epicatechin, 3-4 dihydroxy phenyl acetic aicd, gallic acid, 3-4 dihydroxy benzoate, procyanidin, vitamin c, oxacyclohexadecan-2-one, 16 ethyl and standard- Diclofenac were finalized for docking. The 3D-coordinates for these compounds in the PDB format were obtained through drawing window of chemsketch and further explored for biological activity, which is comprised of Lipinski rules of 5, drug likeness and drug score.

Molecular docking

In silico docking experiments were performed using Flex and X tool of the LeadIT software. The ligands were downloaded from Pubchem and H atoms were added to them as required. The molecules were then model built and minimized by running a 1000 cycles of energy minimization by steepest descent approximation, and were converged to a gradient of 0.02 using the tool, Chimera UCSF 1.6.2., and the AMBERff99SB Force field for this procedure. Gastiger charges were added to the ligands and they were saved in the Mol2 format. These were then uploaded into the Flex and X docking tool of the LeadIT software.

Target protein minimization

The protein 1PXX and 1JNQ were loaded into the prepare molecule module of the BiosolveIT software - LeadIT chain A of the protein was selected for preparation and docking. The binding site comprised of the binding pocket where the reference ligand, diclofenac was bound in this particular chain. The energy of this binding site was minimized, the atomic coordinates of the amino acids of this binding pocket were converged and the protein was thus prepared for docking.

Active site prediction

From the binding site analysis of 1 PXX, the binding pockets were identified and the largest binding pockets were selected for docking studies. Possible binding residues of receptor were searched. They are 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, SER 530, GLY 526. The possible binding pockets in LOX-3 protein with PDB ID 1JNQ were ILE 857, LEU 773, GLN 716, HIS 523, HIS 518, GLN 514, ILE 572, PHE 576 of chain A

Drug likeliness evaluation by Swiss ADME

Drug likeliness properties of the ligand based on Swiss ADME analysis include their chemical properties like, its molecular weight being < 500 Daltons, with < 5 hydrogen bond donors, < 10 hydrogen bond acceptors and QPlogPo/w < 5. The *n*-octanol/water partition coefficient (log *P*o/w) is a key physicochemical parameter for drug discovery depicts lipophilicity indices of the ligand as within the range. The parameters measured for the ligand's solubility in water identifies the ligand to be an ideal drug.

In vitro assays

Chemicals and reagents

Linoleic acid, diclofenac, 3, 4-Dihydroxyphenyl acetic acid, epicatechin and Gallic acid were purchased from Sigma (St. Louis, MO, USA). 15-lipoxygenase (Soybean) purchases from Himedia (Mumbai, India). Human cyclooxygenase (COX)-2 inhibition kit was obtained from Cayman Chemicals, Ann Arbor, MI, USA. All other chemicals used were analytical grade.

Anti-inflammatory activity

Lipoxygenase (LOX) inhibition

A spectrophotometric assay for determination of soybean LOX (5 µg) activity with 0.2 µM linoleic acid (substrate) in buffer [(0.2 M borate buffer (pH 9.0)] was carried out. Diclofenac (0.1 to 10µg), 3,4-dihydroxyphenyl acetic acid, epicatechin and gallic acid (0.1 to 100µg) were used for inhibition studies, and the values of hydroperoxide content and LOX activity were calculated.[29]

Human cyclooxygenase (COX)-2 inhibition

COX-2 inhibition was measured using a colorimetric human COX-2 inhibitor screening assay kit (Cayman, Ann Arbor, MI, USA). Diclofenac (0.1 to 10µg), 3, 4-Dihydroxyphenyl acetic acid, epicatechin and gallic acid (0.1 to 100 µg) were used for inhibition studies as per manufacturer's protocol. The absorbance at 415 nm was read using a microtitre plate reader Varioskan Flash with SkanIt Software 2.4.3 RE.[29]

Statistical analysis

Data were reported as mean (± *SD*) of three independent experiments. Comparisons for IC_{50} values, LOX, and human COX-2 inhibition were by one-way analysis of variance with Duncan's Multiple Range Test ($p \leq 0.05$) using software SPSS 15.0.

RESULT

The results of all ninety six compounds were established by FleX X scoring parameter (Table 1). The compound obtained the highest score was further subjected to docking analysis. For a control study, inhibitor diclofenac was docked to the proteins, cyclooxygenase (COX)-2; 1PXX and lipoxygenase (LOX)-3; 1JNQ, an exercise that resulted in reproducing the crystal structure poses for the inhibitor. To the crystal structure of 1PXX and 1JNQ, the inhibitor diclofenac bound to all four chains of the structure, but none of the binding sites come in the interface of two domains of two different chains. An essential feature of the original binding site is the conservation of hydrogen bonding residues and the aromatic stacking interactions, which were also observed in the binding modes of the compounds in the study.

High-throughput screening

Structure-functional relationship of identified ninety six seed bioactives along with diclofenac (standard inhibitor) were evaluated to know their biological activity against the cyclooxygenase (COX)-2 and lipoxygenase (LOX)-3 enzyme using the 3D crystal structure of the receptor retrieved from protein data bank (PDB) site, crystal structures, COX-2, PDB code, 1PXX (Figure 1a) and LOX-3, PDB code, 1JNQ (Figure 2a). The analysis resulted in seven Lipinski compliant hits (epicatechin, 3, 4-dihydroxy phenyl acetic acid, gallic acid, 3, 4-dihydroxy benzoate, procyanidin, ascorbic acid, oxacyclohexadecan-2-one, 16 ethyl). In the control, docking of standard inhibitor, diclofenac to both 1PXX (Figure 1e) and 1JNQ (Figure 2e) was carried out. This exercise resulted in poses as references to be used for docking of identified seed bioactives. The docking and scoring pattern between COX-2 and LOX-3 protein crystal structures and the seed bioactives identified similar interaction in comparison to diclofenac.

Table 1: Swiss ADME data of the following compounds.

Sl no	Name of the compound	Log S ESOL	No of H bond acceptors	No of H bond donors	GI tract absorption	Lipophilicity Consensus Log $P_{o/w}$	Lipinski drug likeliness
1	Epicatechin	-2.22	6	5	High	0.85	0 violations
2	3,4 dihydroxy benzoate	-2.27	4	2	High	1.40	1 violation
3	3,4 Dihydroxy phenyl acetic acid	-1.66	4	3	High	0.65	0 violations
4	Procyanidin	-4.90	13	10	Low	1.14	1 violations
5	Ascorbic acid	0.23	6	4	High	-1.28	1 violations
6	Oxacyclohexadecan-2-one, 16 ethyl	-2.75	1	0	High	2.53	o violations
7	Gallic acid	-1.64	5	4	High	0.21	1 violation

The Lipinski, Ghose, veber, Egan, Muegge rules for drug-like molecules have also approved the ligand. GI tract absorbtion is high except in case of procyanidin it is low and bioavailability of the ligand resulted in the partition coefficient (QPlogPo/w) ranges from – 0.21- to 2.53 and water solubility (QPlogS), critical for estimation of absorption and distribution of drugs within the body, ranged between -6.5 and 0.5. Topological polar Surface Area of the ligand is also appreciable. All these pharmacokinetic parameters are within the acceptable range signifying the ligand to be a typical Drug molecule.

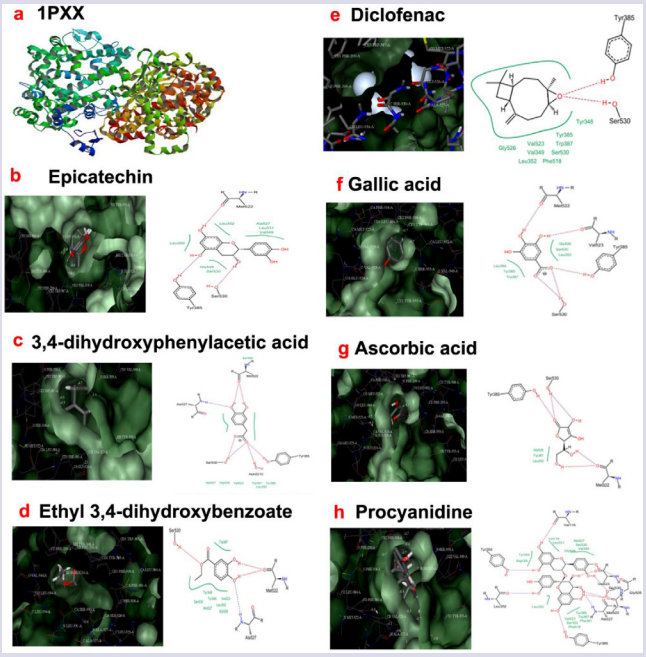

Figure 1: *In silico* docking of seed bioactives to 1PXX COX-2 protein. AA in green. COX-2, 1PXX (a); Diclofenac docked, H- bonding to Tyr385 and Ser530 (e); Epicatechin, H- bonding to Tyr385, Ser530 and Met522 (b); 3,4-diphenylacetic acid, H- bonding to Tyr385, Ser530, Met522 and Ala527 (c); Ethyl 3,4-dihydroxygenzoate, H- bonding to Ser530, Met522 and Ala527 (d); Gallic acid, H- bonding to Ser530, Met522, Tyr385 and Val523 (f); Ascorbic acid, H- bonding to Ser530, Met522 and Tyr385 (g); Procyanidine, H- bonding to Tyr385, Leu352, Tyr385, Ala527, Met522, Ser530, Gly526, Val523 and Val115 (h).

Computational docking of seed bioactives to COX-2, 1PXX

Epicatechin docked onto 1PXX protein (Figure 1b); 3,4-Dihydroxyphenyl acetic acid docked to 1PXX protein (Figure 1c); ethyl 3,4-dihydroxybenzoate docked to 1PXX protein (Figure 1d); gallic acid docked to 1PXX protein (Figure 1f); ascorbic acid docked to 1PXX protein (Figure 1g) and procynidine docked to 1PXX (Figure 1h) are presented. Out of all the docked compounds, 3, 4-dihydroxyphenyl acetic acidand gallic acid showed the highest binding affinity of -24.3976 and -21.4301 kcal/mol respectively to 1PXX. The docked pose resembles the orientation observed with the diclofenac ligand. The ligands, 3, 4-dihydroxyphenyl acetic acidand gallic acid were docked deeply within the binding pocket region, forming interactions with $ALA_{527}SER_{530}$ TYR_{385} $_{Met522}$ (Figure 1c) and SER_{530} TYR_{385} $_{Met522}Val523$ (Figure 1f).

Computational docking of seed bioactives to LOX-3, 1JNQ

Epicatechin docked onto 1JNQ protein (Figure 1b); 3,4-dihydroxyphenyl acetic acid docked to 1JNQ protein (Figure 1c); ethyl 3,4-dihydroxybenzoate docked to 1JNQ protein (Figure 1d); gallic acid docked to 1JNQ protein (Figure 1f); ascorbic acid docked to 1JNQ protein (Figure 1g) and procyanidin docked to 1JNQ (Figure 1h) are presented. Out of all

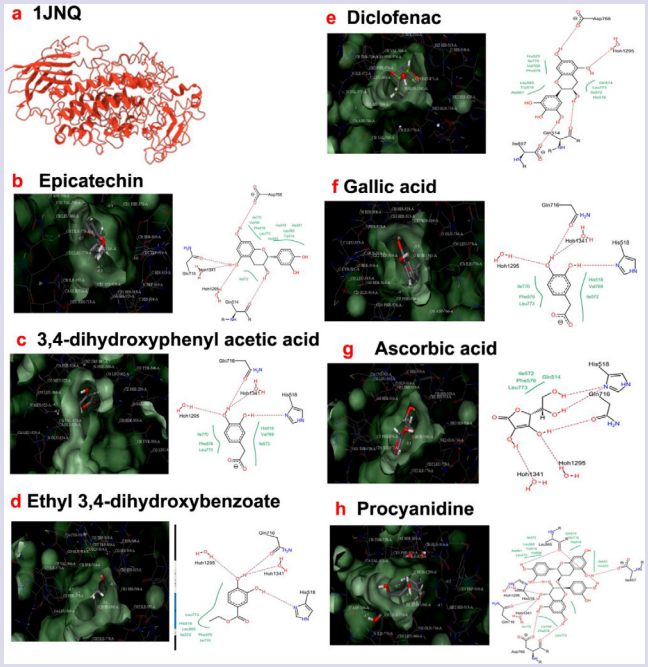

Figure 2: *In silico* docking of seed bioactives to 1JNB LOX-3 protein. AA in green. LOX-3 IJNB (a); Diclofenac docked, H- bonding to Asp766, Ile857, Gln514 (e); Epicatechin docked, H- bonding to Asp766, Gln514 (b); 3,4-diphenylacetic acid docked, H- bonding to Gln716, His518 (c); Ethyl 3,4-dihydroxygenzoate docked, H- bonding to Gln716, His518 (d); Gallic acid docked, H- bonding to Gln716, His518 (f); Ascorbic acid docked, H- bonding to His518, Gln716 (g); Procyanidine docked, H- bonding to Leu565, His518, Gln716, Gly526, Asp766, Ile857 (h).

the docked compounds, epicatechin and gallic acid showed the highest binding affinity of-25.4918and -21.4301 kcal/mol respectively to 1JNQ. The docked pose resembles the orientation observed with the diclofenac ligand. The ligands epicatechin and gallic acid was docked deeply within the binding pocket region, forming interactions with ASP766, GLN716, GLN514 (Figure 2b) and GLN716, HIS518 (Figure 2f).

The seed bioactives were subjected to the iLOG predictor of the Swiss ADMET website software generating *in silico* ADME properties reporting the various parameters for drug like characteristics such as Lipinski's rule of 5, pharmacophoric groups attached on the ligand, size of the dataset and compound libraries among others (Table 2 and Table 3). It also substantiated the safety aspect of the said seed bio actives.

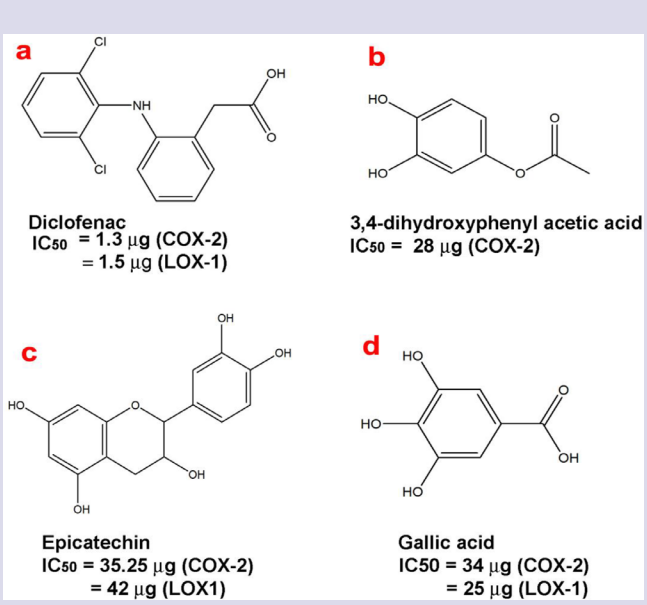

Figure 3: *In vitro* Inhibition Studies- Inhibition of Human COX-2 and soybean LOX-3 enzyme activity by seed bioactives. The IC_{50} values for standard drug diclofenac (a) and seed bioactives 3,4-dihydroxyphenyl acetic acid (b); epicatechin (c), gallic acid (d) reported based on their capacities to inhibit Cyclooxygenase (COX)-2 and Lipoxygenase (LOX)-3 enzymes under *in vitro* conditions. Data from three experiments are expressed as mean ± SEM. Significant inhibition is indicated by * with a p value ≤ 0.05.

In vitro inhibition studies

Inhibition of Human COX-2 enzyme activity by seed bioactives

Inhibitory effects of tested molecules by *in vitro* enzymatic activities measured against human COX-2 indicated diclofenac (standard inhibitor) with IC_{50} of 1.3 mof 1.(Figure 3a), 3,4-dihydroxyphenyl acetic acid with IC_{50} of 28mof 28(Figure 3b), epicatechin with IC_{50} of 35.25 mg/mL (Figure 3c) and gallic acid with IC_{50} of 34mof 34(Figure 3d).

Inhibition of soybeanLOX-3 enzyme activity by seed bioactives

Inhibitory effects of tested molecules by *in vitro* enzymatic activities measured against soybean LOX-3 indicated diclofenac (standard inhibitor) with IC_{50} of 1.5mof 1.(Figure 3a), epicatechin with IC_{50} of 42mof 42(Figure 3c) and gallic acid with IC_{50} of 25mof 25(Figure 3d).

DISCUSSION

Cyclooxygenase (COX), COX-1, COX-2 and lipoxygenase (LOX) 5-LOX have been studied as therapeutic targets for development of anti-inflammatory drugs as aspirin, indomethacin, ibuprofen, celecoxib among others.[30] Given the complex biological role of both COX-2 and LOX-5, it would be helpful to have specific inhibitors to better delineate their role in human OA. Theorell *et al.*[31] succeeded in crystallizing and characterizing lipoxygenase (LOX) from soybeans and since then among plant LOXs, soybean lipoxygenase isozyme 3 (LOX-3) can be regarded as the mechanistic paradigm nonheme iron. Due to lack of sufficiently purified human enzymes most of the structural research has been done on soybean LOX-3.[31] Hence in the present study, in continuation with our previous report[28] 1PXX (crystal structure of COX-2) and 1JNQ (crystal structure of LOX-3) were used.

An increase in the number of molecules reported[12] in a typical drug discovery program necessitates the need for *in silico* determinations of ADME to be regularly generated with the studies. The present study clearly demonstrates that application of *in silico* approach utilized was successful in finding novel COX-2 and LOX-3 inhibitors from seeds, a byproduct of food industries. Initial screening with a set of 96 compounds resulted in seven potential bioactives that was further streamlined to three potent anti-inflammatory molecules.

CONCLUSION

The Flex and X docking score determined in this study could be correlated with the biological activities. The detailed analysis of docking program could add to the known knowledge in understanding the nature of COX-2 and LOX-3 activities. The most potent seed bioactives, 3,4-dihydroxy-

Table 2: Docking scores of most potent derivatives with COX-2 enzyme.

Sl no	Name of the compound	Docking Score	Match score	Lipo score	Ambig score	Clash score	Rot score
1	Diclofenac	-21.4028	-24.1755	-11.4798	-8.9300	7.9824	9.8000
2	Epicatechin	-25.4918	-28.7876	-10.7807	-7.0822	5.3590	8.4000
3	3,4 Ethyl dihydroxy benzoate	-16.8652	-20.5224	-6.1965	-4.1584	3.0121	5.6000
4	3,4 Dihydroxy phenyl acetic acid	-14.1047	-18.3109	-5.0892	-3.8390	2.1435	5.6000
5	Procyanidin	-3.5349	-30.3794	-22.5327	-16.9346	41.3118	19.6000
6	Ascorbic acid	-13.5002	-23.4302	-3.3395	-4.1962	3.6386	8.4000
7	Oxacyclohexadecan-2-one, 16 ethyl	6.4796	-5.4000	-14.6613	-5.3948	25.1367	1.4000
8	Gallic acid	-21.4301	-23.4736	-5.5162	-4.7043	2.6608	4.2000

Description of the score: Total Score (E_TOTAL) Total score of the docking solution. **Match Score** (E_MATCH) Contribution of the matched interacting groups. **Lipo Score** (E_LIPO) Contribution of the lipophilic contact area. **Ambig Score** (E_AMBIG) Contribution of the lipophilic–hydrophilic (ambiguous) contact area. **Clash Score** (E_CLASH) Contribution of the clash penalty. **Rot Score** (E_ROT) Ligand conformational entropy score.

Table 3: Docking scores of most potent derivatives with Lipoxygenase enzyme.

Sl no	Name of the compound	Binding affinity	Match score	Lipo score	Ambig score	Clash score	Rot score
1	Diclofenac	-24.9233	-20.5518	-12.6435	-3.5239	3.5960	2.800
2	Epicatechin	-20.3993	-21.0676	-11.2730	-7.6642	5.8055	8.4000
3	3,4 Ethyl dihydroxy benzoate	-15.7629	-19.4411	-6.8197	-5.3081	4.8061	5.6000
4	3,4 Dihydroxy phenyl acetic acid	-24.3976	-28.0559	-5.7541	-4.4566	2.8691	5.6000
5	Procyanidin	-22.2142	-45.4862	-23.5170	-14.2379	36.0647	19.6000
6	Ascorbic acid	-11.0415	-16.8072	-5.0305	-5.3685	2.3276	8.4000
7	Oxacyclohexadecan-2-one, 16 ethyl	2.5193	-5.4000	-11.3742	-4.4516	17.0513	1.4000
8	Gallic acid	-21.4301	-23.4736	-5.5162	-4.7043	2.6608	4.2000

Description of the score: Total Score (E_TOTAL) Total score of the docking solution. **Match Score** (E_MATCH) Contribution of the matched interacting groups. **Lipo Score** (E_LIPO) Contribution of the lipophilic contact area. **Ambig Score** (E_AMBIG) Contribution of the lipophilic–hydrophilic (ambiguous) contact area. **Clash Score** (E_CLASH) Contribution of the clash penalty**. Rot Score** (E_ROT) Ligand conformational entropy score.

phenyl acetic acid, epicatechin and gallic acid identified in the present study could be upgraded to clinical studies leading to development of potent osteoarthritic drugs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

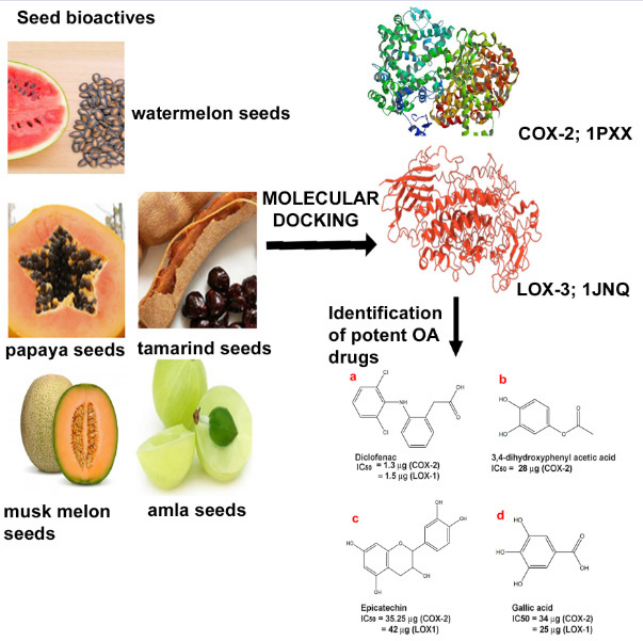
ABBREVIATIONS USED

COX-2: cyclooxygenase-2; **LOX-3;** lipoxygenase-3; **Flex X:** software package to predict protein ligand interactions; **1PXX:** crystal structure of diclofenac bound to the cyclooxygenase active site of COX-2; **ADME(T):** absorption, distribution, metabolism and excretion-toxicity.

REFERENCES

1. Brune K. Safety of anti-inflammatory treatment - new ways of thinking. Rheumatol. 2004;43(1):i16-i20.
2. Baynes JW, Dominiczak MH. Medical Biochemistry 2nd. Edition. Elsevier Mosby. 2005:555-60.
3. Wlodawer P, Samuelsson B. On the organization and mechanism of prostaglandin synthetase. J Biol Chem. 1973;248(16):5673-8.
4. Smith WL, Song I. The enzymology of prostaglandin endoperoxide H synthases-1 and -2. Prostaglandins Lipid Mediators. 2002;68:115-28.
5. Powell WS, Rokach J. Biosynthesis, biological effects, and receptors of hydroxyeicosatetraenoic acids (HETEs) and oxoeicosatetraenoic acids (oxo-ETEs) derived from arachidonic acid. Biochim Biophys Acta. 2015;1851(4):340-55.
6. Bailey DM, Chakrin LW. Arachidonate lipoxygenase. Ann Rep Med Chem. 1981;16:213-227.
7. Martel-Pelletier J, Lajeunesse D, Reboul P, Pelletier JP. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. Ann Rheumatol Dis. 2003;62(6):501-9.
8. Parente L. Pros and cons of selective inhibition of cyclooxygenase-2 versus dual lipoxygenase/cyclooxygenase inhibition: is two better than one? J Rheumatol. 2001;28(11):2375-82.
9. Bertolini A, Ottani A, Sandrini M. Selective COX-2 inhibitors and dual acting anti-inflammatory drugs: critical remarks. Curr Pharm Design. 2002;9:1-11.
10. Raisz LG. Prostaglandins and bone:physiology and pathophysiology. Osteoarthritis Cartilage. 1999;7(4):419-21.
11. Hernandez-Diaz S, Rodriguez LA. Association between nonsteroidal anti-inflammatory drugs and upper gastrointestinal tract bleeding/perforation: An overview of epidemiologic studies published in the 1990s. Arch Intern Med. 2000;160(14):2093-9.
12. Shailasree S, Ruma K, Kini KR, Niranjana SR, Prakash HS. Potential anti-inflammatory bioactives from medicinal plants of Western Ghats, India. Pharmacogn Commun. 2012;2:71-7.
13. Aggarwal BB, Prasad S, Reuter S, Kannappan R, Yadev VR, Park B, Kim JH, Gupta SC, Phromnoi K, Sundaram C, Prasad S, Chaturvedi MM, Sung B. Identification of novel anti-inflammatory agents from Ayurvedic medicine for prevention of chronic diseases: "reverse pharmacology" and "bedside to bench" approach. Curr Drug Targets. 2012;12(11):1595-653.
14. Duncan K. Medical nutrition therapy for rheumatic disease. In: Mahan K, Escott-stump S, Editors. Krause's Food and Nutrition Therapy. Saunders, Philadelphia. 2008:1042-66.
15. Sadat BE, Haghighian MK, Alipoor B, Mahdavi AM, Jafarabadi A, Moghaddam A. Effects of sesame seed supplementation on clinical signs and symptoms in patients with knee osteoarthritis. Int J Rheumatic dis. 2013;16(5):578-82.
16. Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Tox Methods. 2000;44(1):235-49.
17. Butina D, Segall MD, Frankcombe K. Predicting ADME properties *in silico*: methods and models. Drug Discovery Today. 2002;7(11):83-8.
18. Eddershaw PJ, Beresford AP, Bayliss MK. ADME/PK as part of a rational approach to drug discovery. Drug Discovery Today. 2000;5(9):409-14.
19. Rummey C, Nordhoff S, Thiemann M, Metz G. *In silico* fragment-based discovery of DPP-IV S1 pocket binders. Bioorg Med Chem Lett. 2006;16(5):1405-9.
20. Kellenberger E, Springael JY, Parmentier M. Identification of non-peptide CCR5 receptor agonists by structure-based virtual screening. J Med Chem. 2007;50(6):1294-303.
21. Waszkowycz B. Towards improving compound selection in structure-based virtual screening. Drug Discovery Today. 2008;13(5-6):219-26.
22. Sundjaroen Y, Haubner R, Wurtele G, Hull WE, Erben G, Spiegelhalder B, *et al*. Isolation and structure elucidation of phenolic antioxidants from tamarind (*Tamarindus indica* L.) seeds and pericarp. Food Chem Toxicol. 2005;43(11):1673-82.
23. Albishri HM, Almaghrabi OA, Moussa TA. Characterization and chemical composition of fatty acids content of watermelon and muskmelon cultivars in Saudi Arabia using gas chromatography/mass spectroscopy. Pharmacogn Mag. 2013;9(33):58-66.
24. Singh O, Ali M. Phytochemical and antifungal profiles of the seeds of *Carica papaya* L. Indian J Pharm Sci. 2011;73(4):447-51.
25. Arora A, Kumar I, Sen R, Singh J. *Emblica officinalis* (amla): physiochemical and fatty acid analysis from arid zone of Rajasthan. Int J Basic App Chem Sci. 2011;1(1):89-92.
26. Bhagvat R, Saqib A, Karigar CS. Molecular docking studies of novel palmitoylated ligands for cyclooxygenase-2. Chem Biol Drug Discovery. 2012;79(6):1043-8.
27. Kumar KA, Reddy TC, Reddy GV, Reddy DB. High throughput screening assays for cyclooxygenase-2 and 5-lipoxygenase, the targets for inflammatory disorders. Indian J Biochem Biophys. 2011;48:256-261.
28. Pavan R, Rai RV, Saqhib A, Jyothi L, Swamy MS, Karigar CS, *et al*. High throughput screening by *in silico* molecular docking of *Eryngium foetidum* (Linn.) bioactives for cyclooxygenase-2 inhibition. Pharmcogn Commn. 2016;6(4):232-7.
29. Sekhar S, Sampathkumara KK, Niranjana SR, Prakash HS. Bioactive Potential of Medicinal Plants from Western Ghats Region. India. J Herbs, Spices Med Plants. 2014;20(3):221-34.
30. Brune K, Hinz B. The discovery and development of anti-inflammatory drugs. Arthritis Rheumatism. 2004;50(8):2391-9.
31. Theorell H, Holman RT, Akeson A. Cristalline lipoxygenase. Acta Chemica Scand. 1947;1:571-6.

PICTORIAL ABSTRACT



SUMMARY

- The analysis of reported seed bioactives by insilico drug discovery method resulted in seven Lipinski compliant hits (epicatechin, 3,4-Dihydroxy phenyl acetic acid, gallic acid, 3,4-Dihydroxy benzoate, procyanidin, ascorbic acid, oxacyclohexadecan-2-one, 16 ethyl). 3,4-Dihydroxyphenyl acetic acid for COX-2 inhibition along with gallic acid and epicatechin inhibiting LOX-3 could be evaluated as drugs against chronic inflammatory diseases such as osteoarthritis (OA).

ABOUT AUTHORS

Dr. Shailasree Sekhar is recipient of UGC-CSIR NET JRF-SRF grants for her doctoral studies from CSIR-CFTRI, 2000. Currently, as Scientist at The Institution of Excellence, University of Mysore with the thrust area identified as the biodiversity of Western Ghats medicinal plants (MP), with immunological affections and cancer prevention properties, due to location advantage of this hot spot to the University, she has been actively involved in compilation of their scientific data as reviews. Recently, she has brought out a database on medicinal plants of Western Ghats in an efficient way. Screening of MP with immunological affections used by Western Ghats tribal population has resulted in identification of several of them with inflammation/ cancer inhibiting property. Fingerprinting their metabolites has been her priority. She has published several scientific reports/ papers in peer-reviewed journals, has 2 patents and is an adhoc reviewer of various journals. She has to her credit grants from National scientific agencies under Government of India.