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In-silico and *in-vitro* studies of Human 15-lipoxygenase B protein with bioactive phytocompounds having strong anti-inflammatory potential

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***Abstract*: 15-lipoxygenase B (15-LOX B) has been implicated in the pathogenesis of many human diseases, including psoriasis, atherosclerosis, rheumatoid arthritis, alzheimer's dementia, and cancer. Natural biological products present in medicinal plants exhibit a wide range of pharmacological properties that also possess lipoxygenase inhibitory activities. In the current study, we attempted to evaluate the inhibitory activity of six naturally occurring phytocompounds against the crystal structure of *Homo sapiens* (Human) Arachidonate 15-LOX B (PDB ID: 4NRE). Anthraquinone, celastrine, curcumin, ficusin, gilloin, and tinosporide were subjected to molecular docking studies for analyzing their inhibitory potential against the protein, which was compared to NDGA, a known LOX inhibitor, used as the standard. *In-silico* docking studies were performed using Patchdock server. All the compounds were found to have interaction with the protein with all the interacted amino-acid residues being present in the active site cavities of the protein. However, celastrine showed the best interaction on the basis of docking scores and atomic contact energy (ACE) values, followed by gilloin and curcumin, and all of them showed higher affinity towards protein's active pockets, as compared to NDGA. The results of the *in-vitro* LOX inhibition assay, conducted on the plant sources of these phytocompounds, were found to be similar with the *in-silico* docking studies, thereby suggesting that these compounds may prove to be efficacious in inhibiting LOX and therefore can be used as potent inhibitors in drug formulation against inflammatory disorders.**

***Index Terms:* Docking, *Homo sapiens*, Arachidonate 15-lipoxygenase B, Phytocompounds, Anti-inflammatory**

I. INTRODUCTION

Arachidonate 15-lipoxygenase (15-LOX) catalyzes the bioxygenation of polyunsaturated fatty acids (PUFA) such as arachidonic acid (AA) and linoleic acid (LA) to form conjugated hydroperoxydienoic acids. Arachidonate 15-LOXs are classified into two isoforms, 15-LOX A or reticulocyte-type (type-1) and 15-LOX B or epidermis-type (type-2) enzyme. 15-LOX B was cloned from human hair roots and is expressed in skin, prostate, lung, and cornea (Brash et al., 1999). 15-LOX B (15-LOX-2) oxygenates AA to 15-(S)-HPETE (Hydroperoxyeicosatetraenoic acid) that is reduced to 15-(S)-HETE (Hydroxyeicosatetraenoic acid). 15-(S)-HETE is known to be involved in the production of a group of mediators called leukotrienes that act as proinflammatory products. These oxidized metabolites have been implicated as mediators of inflammatory and allergic reactions (Vanderhoek and Bailey, 1984). They are involved in pathogenesis of many human diseases, including psoriasis, atherosclerosis, rheumatoid arthritis, and Alzheimer's dementia (Setsu et al., 2006; Gheorghe et al., 2009; Giannopoulos et al., 2013). Thus, inhibition of 15-LOX-2 protein from *Homo sapiens* (Human) can check the progression of these inflammatory disorders. Medicinal plants have been known to be rich sources of anti-inflammatory compounds which have been used since ancient times in therapeutic medicine to protect against various diseases (Gu et al., 2014). Natural biological products of the medicinal plants exhibit a wide range of pharmacological activities. Since these bioactive compounds possess remarkable lipoxygenase inhibitory activities, they are known to have

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anticancer, anti-inflammatory and anti-aging properties (Schneider and Bucar, 2005). In the present study, an *in-silico* molecular docking was performed on *Homo sapiens* Arachidonate 15-LOX B protein with six selected phytocompounds. Anthraquinone, celastrine, curcumin, ficusin, gilloin, and tinosporide were used to evaluate and compare their lipoxygenase inhibitory activities against a known LOX inhibitor, NDGA (Nordihydroguaiaretic acid) (Mikuni et al., 1998). Simultaneously, an *in-vitro* lipoxygenase inhibition assay was performed using the respective plant sources of the phytocompounds. This attempt was made to discover the therapeutic potentials of the selected phytocompounds that could be used as potential drug candidates for inhibiting 15-LOX-2 protein and its related metabolic pathway, thereby playing an inevitable role in drugs being formulated for treating many inflammatory disorders caused by lipoxygenases.

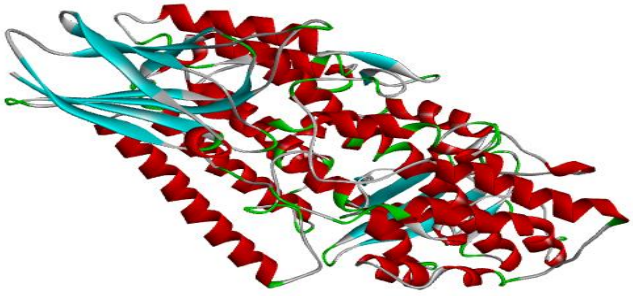


Fig. 1. 3D structure of Arachidonate 15-LOX B protein from *Homo sapiens*.

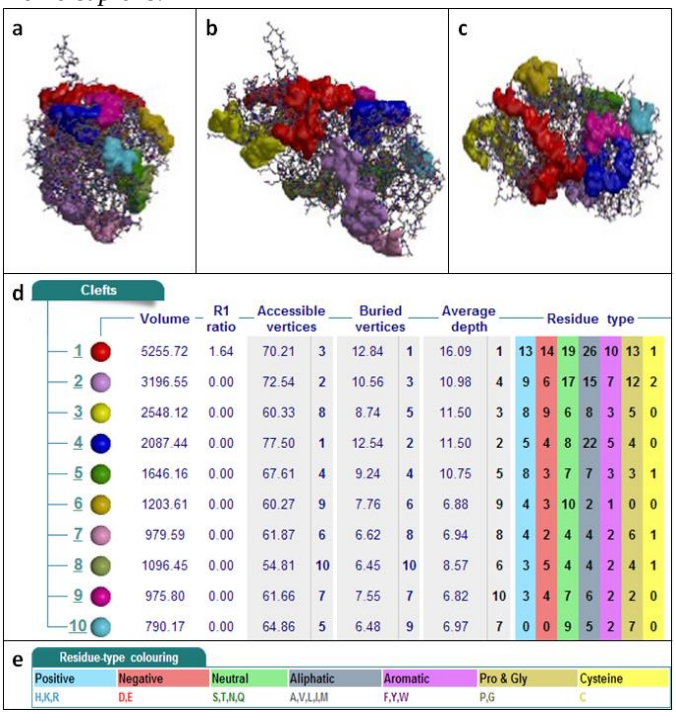


Fig. 2. The active sites/clefts of *Homo sapiens* 15-LOX-2 protein, predicted through PDBSum, are represented in three different orientations (a), (b) & (c); (d) provides the details about 10 clefts represented in different colors; (e) shows the residue types depicted by different colors.

II. Materials and Methods

In the current study, an investigation was done to evaluate the interaction of the selected six bioactive phytocompounds against *Homo sapiens* 15-LOX-2 crystal protein using *in-silico* docking studies.

A. Retrieval of the 15-LOX B protein structure

The reported crystal structure of protein Arachidonate 15-lipoxygenase-B (15-LOX-2) from *Homo sapiens* was obtained from Protein Data Bank (http:// www. pdb. org/ pdb/ home/home.do) with PDB ID- 4NRE and amino acid length of 696 aa. It was further used for molecular docking (Arora et al.., 2012, Kobe et al., 2014).

B. Molecular docking studies

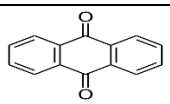
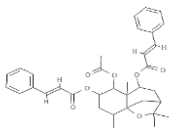
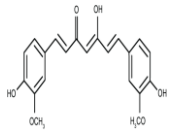
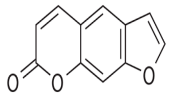
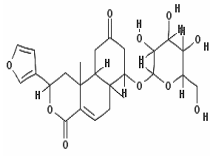
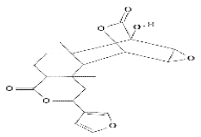
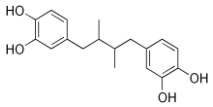
1) Active site analysis

Active site analysis to identify the active site cavities of the *Homo sapiens* 15-LOX-2 protein was done using MetaPocket 2.0 (http://projects.biotec.tu-dresden.de/metapocket/) and PDBSum server (http://www.ebi.ac.uk/pdbsum/) (Zhang et al., 2011; Kumari et al., 2013). Simulations were performed using Discovery Studio 3.0 (Arora et al., 2012). Top three and top ten active sites of the protein were predicted using MetaPocket 2.0 and PDBSum servers, respectively. MetaPocket 2.0 is a meta server that identifies ligand binding sites on a protein surface. It follows a consensus method, in which the predicted binding sites from eight methods: LIGSITEcs, PASS, Q-SiteFinder, SURFNET, Fpocket, GHECOM, ConCavity and POCASA are combined together to improve the prediction success rate. All these methods are evaluated on two datasets of 48 unbound/bound structures and 210 bound structures. However, PDBSum server provides information about the orientation of the ligand (phytocompounds) that binds with the protein at its different active sites during docking interactions.

2) Docking calculation and protein-protein interaction

Docking analysis of the *Homo sapiens* 15-LOX-2 protein with selected six compounds, namely, anthraquinone, celastrine, curcumin, ficusin, gilloin and tinosporide, was done to examine the interaction of the phytocompounds with the protein. The molecular details of the phytocompounds used in the study are given in Table I. Docking calculations were evaluated using Patchdock server (Duhovny et al., 2002). Docking parameters like docking score, interaction surface area and atomic contact energy (ACE) were calculated for all the compounds. The functional protein-protein interaction analysis was performed through STRING (Search Tool for the Retrieval of Interacting Genes/proteins) server to find out the other proteins that regulates LOX signaling pathway. The active proteins were selected on the basis of their confidence scores by analyzing several parameters such as occurrence, co-expression, gene fusion, text mining and experiments (Szklarczyk et al., 2015).

Table I. Details of the selected phytocompounds with their PubChem IDs used for *in-silico* study

Compound name	Mol. formula	Mol. weight (g/mol)	Chemical structure	PubChem ID
Anthraquinone	$C_{14}H_8O_2$	208.21		6780
Celastrine	$C_{35}H_{40}O_7$	572.68		10555115
Curcumin	$C_{21}H_{20}O_6$	368.379		969516
Ficusin	$C_{11}H_6O_3$	186.16		6199
Gilloin	$C_{25}H_{32}O_{10}$	492.51		317629
Tinosporide	$C_{20}H_{22}O_7$	374.38		167631
NDGA	$C_{18}H_{22}O_4$	302.36		4534

C. In-vitro lipoxygenase (LOX) inhibition study

1) Plant material

For the *in-vitro* lipoxygenase (LOX) inhibition study, five different plants were selected that are known to be highly rich in the phytocompounds used in the *in-silico* docking study. Different plant parts of the five plants (*Aloe vera*, *Celastrus paniculatus*, *Curcuma longa*, *Tinospora cordifolia*, *and Psoralea corylifolia*) were used for *in-vitro* LOX inhibition study. Table II shows the plants used, their families, their parts used and the selected phytocompounds they are known to constitute.

2) Preparation of plant extract

The different plant materials from the five plants were weighed to 10 g, followed by their fine crushing and grinding using pestle and mortar. The fine powder of the plant materials was extracted in 100 ml of methanol for the preparation of the methanolic extract of different plants. The prepared extracts were filtered through double layered muslin cloth and were subjected to evaporation for 3-4 days at 40°C (Alanis et al., 2005). The extracts were preserved in brown bottles at 4 °C until use.

3) In-vitro lipoxygenase (LOX) inhibition assay

Lipoxygenase inhibitory activity of the methanolic extracts of five selected plants was analyzed using LOX inhibition assay. In the assay, linoleic acid served as the substrate and soybean lipoxygenase as enzyme. The enzyme solution was pre-incubated for 5 min at 25 °C, with methanolic extracts of all the plants. In these test samples, the reaction was initiated by the addition of 0.2 µM linoleic acid solution and 0.2 M borate buffer (pH 9.0). The reaction mixture was incubated at 25 °C for 5 min. An ethanolic solution of linoleic acid was used to stop the reaction and the absorbance was measured at 234 nm (Kemal et al., 1987). Five different concentrations (10, 25, 50, 100, 200 µg/ml) of the methanolic extracts of all the five plants were used. NDGA served as a positive control in the assay. IC_{50} values of the extracts were calculated that indicate the concentration required to inhibit 50% LOX activity. The percentage inhibition of lipoxygenase activity was calculated using the formula:

$$\text{Lox inhibition } (\%) = \frac{OD_{Control} - OD_{Sample}}{OD_{Control}} X\ 100$$

D. Statistical analysis

The data were expressed as mean ± SE (standard error). The experiments were performed in triplicate and were repeated twice.

Table II. Plant sources from which the selected phytocompounds are derived and their parts used for *in-vitro* lipoxygenase inhibition assay

Plant source	Common Name	Plant part used	Related phyto-compound	Reference
Aloe vera	Aloe	Leaf	Anthraquinone	Arora and Rai, 2012
Celastrus paniculatus	Malkangani	Seed	Celastrine	Jurenka, 2009
Curcuma longa	Turmeric	Root	Curcumin	Khushboo et al., 2010
Tinospora cordifolia	Giloe	Stem	Gilloin, Tinosporide	Ruan et al., 2007
Psoralea corylifolia	Bakuchi	Seed	Ficusin	Schneider and Bucar, 2005

III. Results

A. Retrieval and refinement of 15-LOX-2 protein structure

15-lipoxygenase B (15-LOX-2) protein crystal structure from *Homo sapiens* (PDB ID: 4NRE) was retrieved from the structure database of NCBI. It was refined using Discovery studio for active site analysis and docking studies (Fig.1) The results of the simulations of 15-LOX-2 protein used in the present study has

been already discussed in detail in our previous publication (Arora et al., 2012).

B. Molecular docking studies

1) Active site analysis

MetaPocket 2.0 and PDBSum servers and Discovery studio 3.0 were used for active site prediction. MetaPocket 2 predicted the top three active sites of 15-LOX-2 protein with their respective active site residues and residue numbers (Table III). PDBSum server demonstrated top ten active site cavities/clefts in three different orientations. The server was also helpful in predicting the respective volumes of the 10 active sites. Site 1 cavity of the protein was found to be the largest with maximum volume whereas site 10 was the smallest. PDBSum analysis also demonstrated the type and number of amino acid residues present in each active site (Fig. 2). It has been observed that the results of the top 3 predicted active sites through PDBSum were showing similarity with active sites identified through MetaPocket 2.0 server and Discovery studio 3.0 (Fig. 3).

Table III. Active site residues of 15-LOX-2 crystal protein from *Homo sapiens* predicted through MetaPocket 2.0 server

Site No.	Active site residues
Site 1	SER^{9}, THR^{10}, GLY^{11}, GLU^{12}, ALA^{13}, PHE^{14}, GLY^{15}, VAL^{27}, GLU^{32}, ALA^{47}, GLY^{48}, ALA^{49}, GLU^{50}, LEU^{66}, ARG^{68}, ALA^{86}, PHE^{88}, ARG^{90}, TRP^{91}, PRO^{105}, CYS^{106}, TYR^{107}, GLN^{108}, TRP^{109}, LEU^{110}, GLU^{111}, GLY^{112}, ALA^{113}, GLY^{114}, THR^{115}, LEU^{116}, VAL^{117}, LEU^{118}, GLN^{119}, PRO^{132}, VAL^{133}, GLN^{136}, GLN^{137}, GLN^{139}, GLU^{140}, GLU^{141}, ALA^{144}, ARG^{145}, MET^{148}, TYR^{149}, VAL^{167}, GLU^{168}, GLU^{171}, LEU^{172}, ASN^{173}, ILE^{174}, LYS^{175}, TYR^{176}, SER^{177}, THR^{178}, ALA^{179}, LYS^{180}, ASN^{181}, ALA^{182}, ASN^{183}, PHE^{184}, TYR^{185}, THR^{385}, THR^{388}, LEU^{389}, HIS^{394}, CYS^{395}, PHE^{399}, LYS^{400}, LEU^{401}, LEU^{402}, ILE^{403}, PRO^{404}, HIS^{405}, THR^{406}, ARG^{407}, TYR^{408}, THR^{409}, LEU^{410}, HIS^{411}, LEU^{415}, LEU^{419}, PHE^{487}, GLY^{615}, ASP^{616}, GLN^{617}, ARG^{618}, TYR^{623}, PRO^{624}, ASP^{625}, GLU^{626}, HIS^{627}, PHE^{628}, THR^{629}, GLU^{630}, GLU^{631}, ALA^{632}, ARG^{634}, ARG^{635}, ILE^{637}, ALA^{638}, GLN^{641}, GLU^{671}
Site 2	TYR^{154}, TRP^{158}, PRO^{159}, HIS^{160}, CYS^{161}, PHE^{184}, TYR^{185}, ALA^{188}, GLY^{189}, PHE^{192}, LYS^{196}, LEU^{201}, ASP^{202}, LYS^{204}, VAL^{302}, ILE^{303}, ASN^{304}, GLY^{305}, PHE^{365}, GLU^{369}, HIS^{373}, LEU^{374}, HIS^{378}, LEU^{379}, GLU^{382}, ILE^{412}, ASN^{413}, LEU^{415}, ALA^{416}, ARG^{417}, LEU^{419}, LEU^{420}, ILE^{421}, GLY^{424}, GLN^{425}, VAL^{426}, VAL^{427}, ASP^{428}, ARG^{429}, SER^{430}, THR^{431}, PHE^{438}, HIS^{553}, GLN^{560}, PRO^{596}, ASN^{598}, ALA^{599}, ASP^{602}, VAL^{603}, LEU^{605}, ALA^{606}, LEU^{607}, LEU^{609}, LEU^{610}, ILE^{676}
Site 3	ARG^{220}, ARG^{221}, THR^{222}, PRO^{223}, ALA^{224}, HIS^{227}, ALA^{228}, HIS^{231}, GLU^{234}, ALA^{236}, PHE^{237}, SER^{240}, GLN^{241}, LEU^{243}, ASN^{244}, GLY^{245}, LEU^{246}, GLN^{319}, GLY^{322}, CYS^{323}, GLY^{324}, PRO^{325}, LEU^{326}, LEU^{327}, ARG^{361}, PRO^{469}, GLY^{470}, TYR^{472}, CYS^{549}, SER^{550}, HIS^{553}, ALA^{554}, SER^{557}, ALA^{558}, PHE^{561}, ASP^{562}, SER^{563}, ASN^{569}, TRP^{608}, LEU^{609}, SER^{611}, LYS^{612}, GLU^{613}, PRO^{614}, ASP^{616}, GLN^{617}, ARG^{618}, PRO^{619}, LEU^{658}, VAL^{659}, LEU^{660}, PRO^{661}, TYR^{662}, THR^{663}, TYR^{664}, LEU^{665}, ASP^{666}, PRO^{667}, PRO^{668}, LEU^{669}, ILE^{670}, GLU^{671}, SER^{673}, VAL^{674}, SER^{675}, ILE^{676}

2) Molecular docking and protein-protein interaction

Molecular docking studies were performed through Patchdock server. On the basis of docking scores, the best docked model for each compound was considered for analysis and visualization. Further, interaction surface area and atomic contact energy of all the compounds were compared. Docking studies between 15-LOX-2 protein and the selected phytocompounds revealed strong interactions with the protein's predicted active sites. Among the 3 active sites predicted through MetaPocket 2.0, it was observed that site 1 of the protein was predominantly active in the molecular interactions with the compounds. From the seven docked compounds, including NDGA, six showed interactions with site 1, except ficusin which was found to interact with site 2 of the protein. Moreover, in case of celastrine, it was observed that one residue of site 3 of the protein was also involved in docking. Although it was observed that all the interacted residues in the docked complexes of the protein and phytocompounds were present in the active site cavities of the protein, however in case of celastrine it was noticed that the interacted residues PHE87 and ARG89 were not present in any of the 3 active site cavities of the protein. The active site residues of the protein and the interacted residues of all the compounds are given in Table IV.

Table IV. Details of the identified active site residues with their active sites and compound wise interacted residues

Compound name	Site no.	Interacted residues present in active sites	Interacted residues absent in active sites
Anthraquinone	Site 1	PHE^{365}, GLU^{369}, HIS^{373}, LEU^{374}, HIS^{378}, ILE^{412}, LEU^{420}, VAL^{426}, VAL^{427}, THR^{431}, PHE^{438}, ALA^{606}, LEU^{610}, ILE^{676}	–
Celastrine	Site 1	THR^{10}, GLY^{11}, GLU^{12}, ALA^{47}, GLN^{108}, TYR^{176}, THR^{178}, ALA^{179}, GLY^{615}, ASP^{616}, GLN^{617}, TYR^{623}, PRO^{624}	PHE^{87}, ARG^{89}
	Site 3	PRO^{614}	
Curcumin	Site 1	TYR^{107}, GLN^{108}, TRP^{109}, LEU^{110}, GLU^{111}, GLY^{114}, THR^{115}, LEU^{116}, ARG^{145}, TYR^{149}, ASN^{173}, ILE^{174}, LEU^{389}, HIS^{394}, PHE^{399}, ILE^{403}, THR^{406}, ARG^{407}, TYR^{408}, ASP^{625}, HIS^{627}	–
Ficusin	Site 2	PHE^{365}, GLU^{369}, HIS^{373}, LEU^{374}, HIS^{378}, LEU^{420}, THR^{431}, PHE^{438}, VAL^{603},	–

		LEU[607],	LEU[610],	ILE[676]	
Gilloin	Site 1	PHE[88], GLN[108], MET[148], ASN[173], SER[177], PHE[399], TYR[408], HIS[627]	CYS[106], TRP[109], TYR[149], ILE[174], LEU[389], ILE[403], ARG[618],	TYR[107], ARG[145], LEU[172], LYS[175], HIS[394], ARG[407], ASP[625],	–
Tinosporide	Site 1	PHE[88], ARG[145], ASN[173], HIS[394], ARG[407], HIS[627]	TYR[107], MET[148], ILE[174], PHE[399], TYR[408],	GLN[108], TYR[149], LEU[389], ILE[403], ASP[625],	–
NDGA	Site 1	CYS[106], LEU[116], GLU[141], MET[148], LEU[389], ILE[403],	TYR[107], VAL[117], ALA[144], TYR[149], HIS[394], HIS[627]	GLN[108], GLN[137], ARG[145], ASN[173], PHE[399],	–

The docking simulations for all the phytocompounds revealed that celastrine showed the best interaction amongst the other six compounds, with highest docking score and maximum interaction surface area, which was followed by gilloin, curcumin, tinosporide, NDGA, anthraquinone, and ficusin. Considering, docking scores and interaction surface area values, the ranking of all the compounds followed the same order. However, in case of the atomic contact energy (ACE), it was observed that anthraquinone interacted with the protein with minimum contact energy, and was followed by curcumin, gilloin, ficusin, tinosporide, NDGA, and celastrine. Surprisingly, celastrine that was found to have the best docking score and maximum area coverage has the maximum contact energy. The docking calculations of the various compounds are given in Table V. The docking interactions of all the selected compounds with 15-LOX-2 protein are illustrated in Fig. 4. NDGA, which is a known LOX inhibitor, was used as a standard for the assessing the docking results. On analysis of the hydrogen bonding interactions between the compounds and the protein, it was observed that amongst the seven compounds, only two compounds were involved in the bonding. While only one atom of curcumin bonded with ARG145 residue, three atoms of gilloin were involved in H-bonding with two protein residues ARG145 and TYR408 (Table VI).

The functional protein interactive network as analyzed by STRING server revealed extreme similarity of LOX15 B with LOX 15 protein of Human (Fig. 5). Additionally, LOX protein was also found to have interaction with LOX12, LOX5, CYP4F2, CYP4F3, CYP4F8, PTGS1 and PTGS2 protein which is involved in the metabolism of polyunsaturated fatty acids. Furthermore, LOX protein also showed interaction with IL-1B, IL4, and STAT3 which are required for B-cell activation processes that regulates synthesis of macrophages, a cell type which is known to be critically involved in inflammation. The production of allergic responses depends upon the coordinated production of cytokines such as IL4 and IL1B to promote effector T cell development and the recruitment of inflammatory cells .

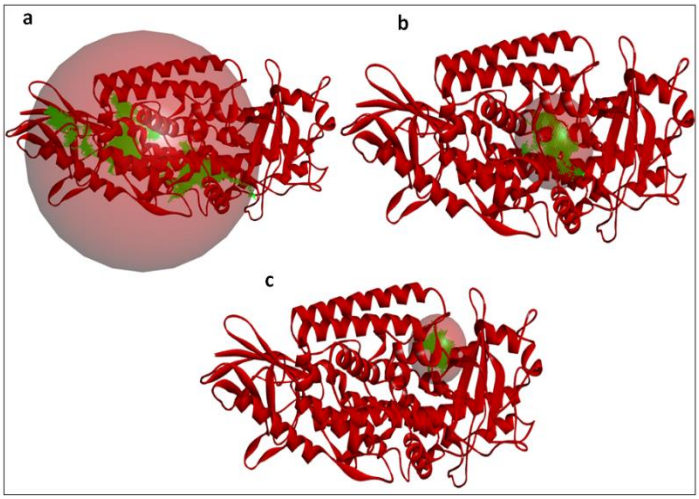


Fig. 3. Top three active sites visualization, a- site 1, b- site 2, and c- site 3, using Discovery studio 3.0. Green color indicates prominent ligand-binding region, whereas circular surface in pink color shows the surface area of the identified active sites.

Table V. Docking calculations of *Homo sapiens* 15-LOX-2 protein with phytocompounds using Patchdock server

Compound	Score	Area (cubic angstrom)	ACE (kcal/mol)
Celastrine	7010	860.10	-57.97
Gilloin	6198	755.30	-139.27
Curcumin	5896	677.70	-158.47
Tinosporide	5070	608.90	-127.48
NDGA	4990	531.30	-82.20
Anthraquinone	3968	458.20	-160.77
Ficusin	3724	411.70	-128.03

Table VI. Ligand/phytocompound atoms, residues and residue atoms of the protein involved in hydrogen bonding

Ligand name	Ligand atom	Residue	Residue atom
Curcumin	O5	ARG[145]	HE
Gilloin	O9	ARG[145]	HE
	O9	ARG[145]	HH 11
	O2	TYR[408]	HH

C. In-vitro lipoxygenase inhibition assay

Lipoxygenase inhibition assay was performed to evaluate the anti-inflammatory activities of the plant sources of the selected phytocompounds. On comparing the percentage inhibition of lipoxygenase enzyme by the methanolic extracts of the five selected plants at the concentration of 50 µg/ml, it was observed

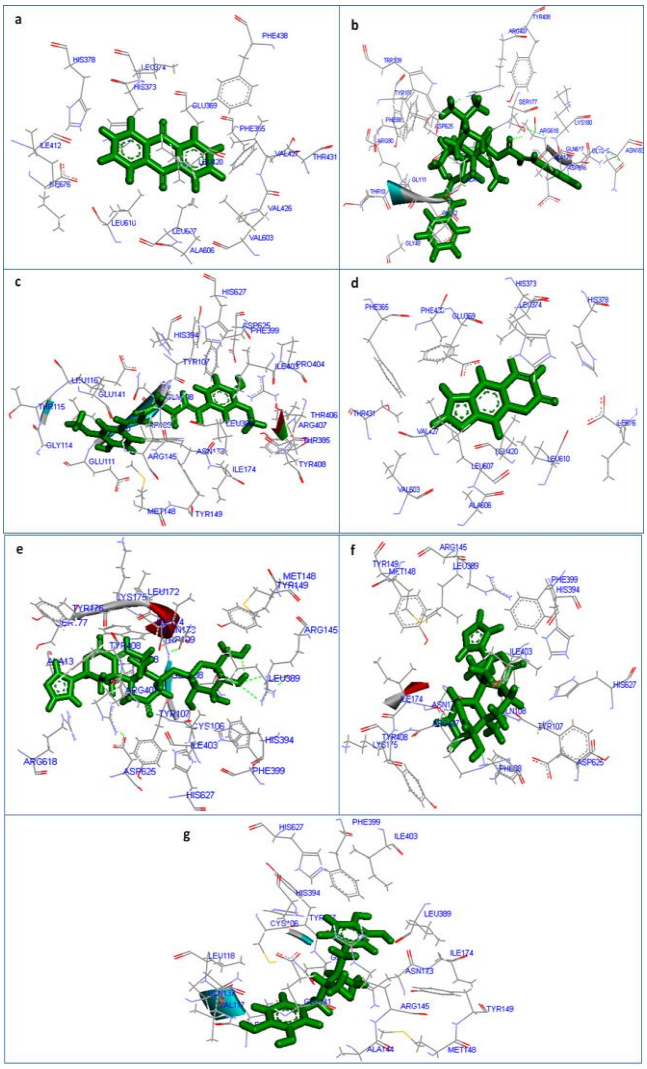


Fig. 4. Interacted amino acid residues of *Homo sapiens* 15-LOX-2 crystal protein with selected phytocompounds, (a) anthraquinone, (b) celastrine, (c) curcumin, (d) ficusin, (e) gilloin, (f) tinosporide, and (g) NDGA. Phytocompounds and active site residues are shown in green and blue colors, respectively.

that *T. cordifolia* exhibited maximum lipoxygenase inhibition activity, followed by *C. longa* and *C. paniculatus* with percent inhibition of 85.7 ± 1.31%, 79.17 ± 1.21%, and 75.8 ± 1.16%, respectively. All of these three plants inhibited LOX either better or to the same extent as that of the positive control, NDGA, with 7.5 µg/ml concentration (80.3 ± 1.1%). However, *P. corylifolia* and *A. vera*, though inhibited the lipoxygenase enzyme, but the percent inhibition was much lesser than NDGA (Fig. 6). IC_{50} of *T. cordifolia* (24.31 ± 0.79 µg/ml) was minimum amongst all the plant extracts, thereby exhibiting maximum LOX inhibition. However, *A. vera* showed maximum IC_{50} of 70.05 ± 0.46 µg/ml (Table VII).

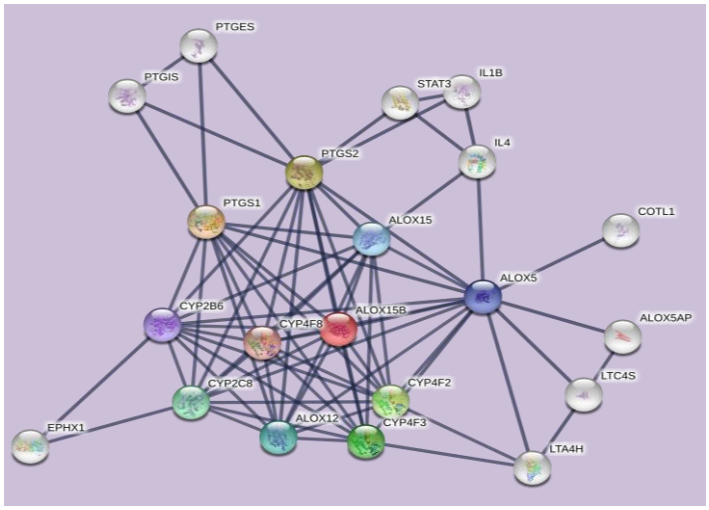


Fig. 5. Functional interactive network of LOX with other protein family members as found on STRING server.

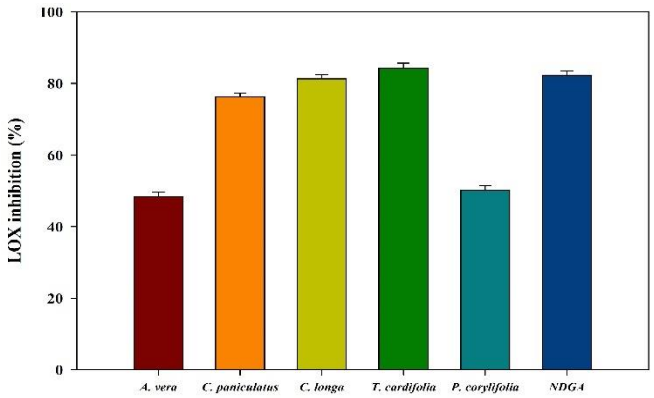


Fig. 6. Lipoxygenase inhibition (%) by the methanolic extracts of *Aloe vera*, *Celastrus paniculatus*, *Curcuma longa*, *Tinospora cordifolia*, and *Psoralea corylifolia*, at the concentration of 50 µg/ml for each extract. NDGA (at 7.5 µg/ml concentration) served as a positive control.

IV. Discussion

Lipoxygenase, which is a key enzyme in initiating the immune responses in the body, has been targeted *in-vitro*, *in-vivo*, and *in-silico*, by many researchers till date, to study its inhibition mechanisms and to discover its potent inhibitors. Reports show that the medicinal plants derived compounds have proved to be good inhibitors of lipoxygenases (Schneider and Bucar, 2005; Devasagayam and Sainis, 2002). The study on the docking interactions between the selected phytocompounds and the protein, suggests that the active site 1 of Human 15-LOX-2 protein was the most prominent site that was involved in interaction with all the compounds, except ficusin. Furthermore, the active site 1 of the protein was also observed to be the largest site with highest site volume (PDBSum analysis). This could even explain the cause of site 1 to be majorly involved in the interactions.

Table VII. Lipoxygenase inhibition (%) and IC_{50} values of methanolic extracts of the selected plants

Medicinal plant	Concentration of extract (µg/ml)	LOX inhibition (%)	IC_{50} (µg/ml)
NDGA (Positive control)	2.5	23.63 ± 1.02	4.76 ± 0.02
	5	56.4 ± 0.13	
	7.5	80.3 ± 1.1	
	10	100 ± 0.11	
Aloe vera	10	8.89 ± 2.03	70.0 ± 0.46
	25	23.12 ± 1.07	
	50	51.8 ± 0.95	
	100	82.4 ± 1.22	
	200	100 ± 0.23	
Celastrus paniculatus	10	22.9 ± 1.03	32.2 ± 1.92
	25	38.2 ± 0.84	
	50	75.8 ± 1.16	
	100	98.5 ± 1.31	
	200	100 ± 0.15	
Curcuma longa	10	27.01 ± 1.7	31.7 ± 1.07
	25	41.14 ± 0.99	
	50	79.17 ± 0.21	
	100	100 ± 0.17	
Tinospora cordifolia	10	31.69 ± 1.29	24.3 ± 0.79
	25	48.65 ± 1.17	
	50	85.7 ± 1.31	
	100	100 ± 0.21	
Psoralea corylifolia	10	9.27 ± 1.34	66.6 ± 0.84
	25	22.81 ± 1.41	
	50	53.15 ± 1.15	
	100	89.21 ± 0.97	
	200	100 ± 0.13	

In case of celastrine, apart from the site 1 residues, site 3 residues were also involved in the interaction, which implies that celastrine interacts with larger surface area coverage, involving two active sites. Incidentally, docking calculations also show that celastrine interacts with the maximum surface area, in comparison to the other compounds. This could also be the possible reason behind its stronger docking, as is evident with its highest docking score.

However, the docking results also reveal that celastrine has the maximum atomic contact energy. Curcumin and gilloin are the other phytocompounds, after celastrine, which showed very good interactions with higher docking scores, larger surface area coverage and lesser atomic contact energy. They were noted to have positioned at second and third places in the ranking order for all the three docking parameters, with regards to the other compounds. Interestingly, both the compounds were found to have hydrogen bonding interactions between their atoms and the active site residues of the protein. While only one atom of curcumin was involved in H-bonding, three atoms of gilloin had H-bonding interactions with the protein residues.

Moreover, ARG145 was the common residue involved in interactions in case of both the compounds. The additional H-bonding in these two compounds justifies their stronger docking interactions with the protein. Docking studies conducted on various inflammatory enzymes with curcumin, have already revealed its strong inhibitory properties (Elumalai et al., 2012). The other compounds, *i.e.* tinosporide, NDGA, and anthraquinone, were found to have the same ranking order in case of docking score and surface area coverage parameters. Furthermore, while the score of the two docking parameters of these compounds were lesser than the earlier discussed 3 compounds, their score were much higher than ficusin, which apparently has the weakest interaction with the protein. However, its anti-lipoxygenase activity is already reported (Yang et al., 2011). It was further observed that anthraquinone showed least ACE which indicates that it binds with the protein with minimum energy, that could suggest its strong interaction with the protein and hence its efficient inhibition. Some other studies on anthraquinone have also managed to unveil its anti-inflammatory properties (Duan et al., 2009; Wei and Shibamoto, 2010; Das et al., 2011).

The results of the *in-vitro* lipoxygenase inhibition assay, conducted with the plant sources of the phytocompounds used for molecular docking, are in accordance with the *in-silico* studies. It was observed that the three plants (*T. cordifolia*, *C. longa*, *and C. paniculatus*) that performed very well in the *in-vitro* lipoxygenase inhibition assay as indicated by their percent LOX inhibition and IC_{50} values, in comparison to the positive control, NDGA, are the plants that are rich sources of the phytocompounds gilloin, curcumin, and celastrine, respectively. The plant source of gilloin (glucosoid), has already been reported to be a lipoxygenase inhibitor in *in-vitro* studies (Kumar et al., 2011) and also possess strong anti-inflammatory properties (Upadhyay et al., 2010). Likewise, many investigations done on *C. longa* and *C. paniculatus*, the plant sources of curcumin and celastrine, respectively, have also revealed their LOX inhibition properties (Bezakova et al., 2014; Khattak et al., 2005; Min et al., 1999) and anti-inflammatory activities (Araujo and Leon, 2001; Ahmad et al., 1994; Weng et al., 2013).

CONCLUSION

From the *in-silico* study conducted, it could be summarized that although all the selected phytocompounds have interacted well with 15-LOX-2 protein, however celastrine, gilloin, and curcumin were the ones that docked the protein in a highly appreciable manner with very good docking scores. Interestingly, the plant sources of these three phytocompounds (*C. paniculatus*, *T. cordifolia*, and *C. longa*, respectively) also have been noted to have very well performed in the *in-vitro* LOX inhibition assay, thereby supporting and validating the results of the *in-silico* study. Moreover, it was also observed that in both *in-silico* and *in-vitro* studies, the phytocompounds and their respective plants inhibited the lipoxygenase enzyme far much better than the already known LOX inhibitor drug NDGA. It may therefore be concluded that the phytocompounds used in

the study, especially celastrine, gilloin, and curcumin could be considered as good inhibitors of human 15-LOX-2 protein and hence could prove to be promising candidates for drug formulations for possibly all the anti-inflammatory disorders.

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