

Abortitristside A and desrhamnosylverbanscoside: The potential COX-2 inhibitor from the leaves of *Nyctanthes arbor-tristis* as Anti-inflammatory agents based on the in-vitro assay, molecular docking and ADMET prediction

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Abstract

The plant *Nyctanthes arbor-tristis* belongs to the genus *Nyctanthes* (Oleaceae family) which is used in the treatment of many diseases like diabetes, cancer, inflammation, intestinal problem and tuberculosis in many countries of Asia. In this analysis, we have investigated the anti-inflammatory activity of various extracts of leaves of *Nyctanthes arbor-tristis* by the in-vitro method as the cyclooxygenase inhibitory potential. The reported phytochemical constituent of the plant was in-silico screened to find the cyclooxygenase inhibitory potential by molecular docking method. The molecular docking study of pre-isolated phytochemicals from the leaves of plants shows that compounds possess a great inhibition potential against COX-1 and COX-2 therefore, an in-vitro comparative study is required to conclude the selective and potential COX-2 inhibitor for the development of new anti-inflammatory drug without causing any gastrointestinal ulcer. Here we deliver a new strategy to design selective COX-2 inhibitor from the plant origin natural compound by using molecular docking technique and in-vitro screening of plant extract. In the other part of the paper abortitristoside A (1) and abortitristoside B (2) were isolated from the leaves part of the plant. The isolated compound possesses good activity against COX-2 and COX-1 (IC₅₀ value 4.51–4.55 ug/ml against COX-2) which is also established by structure-activity relationship analysis.

1. Introduction

Inflammation is a responsive phase of physical, chemical injury, or damage by the attack of a pathogen like bacteria or viruses [1, 2]. The component involved in the inflammation are degradative enzyme and superoxide [3], chemotactic factors [4, 5], vasoactive substances, and neuropeptide substances [6]. To treat the inflammation NSAIDs block the enzyme cyclooxygenase (COX) also known as prostaglandin synthase hence inhibiting the synthesis of prostaglandin at the site of inflammation [7].

The discovery of two different COX isoenzymes that is COX-1 (present throughout the gastrointestinal tract) and COX-2 (present at the site of inflammation) leads to the improvement of NSAIDs. Although only COX-2 involve in inflammation, therefore a COX-2 selective molecule is required to treat the inflammation without causing any ulcer throughout the gastrointestinal tract [8].

Different type of stimuli that triggers the activation of cytosolic phospholipase A₂ (cPLA₂) set amino acid (AA) free from membrane phospholipids [9]. COX-1 or, and COX-2 induced and catalyzed the biosynthesis of endoperoxidase PGH₂ which is highly unstable and converted into prostaglandins (PGD, PGE and PGF), prostacyclin's (PGI) and thromboxane's (TX) by specific catalyst (Fig. 1) [10–13]. Biosynthesis of PGE₂ increases cell proliferation which causes various prolonged inflammatory diseases like the progression of cancer, Alzheimer's disease, arthritis, cardiovascular disorder, neurological disorders and autoimmune [14–18].

Nyctanthes arbor-tritis (family: Oleaceae) is well known medicinal plant generally known as Night jasmine or Harsinger native to India, widely distributed in the sub-Himalayan region and is also used as an

ornamental plant in many regions of Asia [19]. The root part of the plant is used to treat anorexia and fever, the leaf part is used to treat the vermifuge, fever, diaphoretic and diabetes, bark part is used as an expectorant while the whole plant is used to control cancer [20, 21]. Ethnobotanical importance shows that different extracts of the different parts of the plant possess anti-arthritis, antimalarial activity [22], anti-oxidant and anti-inflammatory activity [23, 24]. The sap of greeneries exhibits digestive, diuretic and antivenom properties and is also used as a tonic [25–27]. The plant showed antiviral activity against Semliki Forest virus and Encephalomyocarditis virus while leaves were used to reduce the enlargement of the liver spleen [28]. The plant is very famous for its pharmacological properties from ancient science. Extensive research has been done on the plant *Nyctanthes arbor-tristis* because of its pharmacological properties. In the present study, we have tried to find a potential COX-2 inhibitor by in-vitro analysis of the different extracts of various parts and by virtual screening of phytochemical using molecular docking techniques.

2. Result And Discussion

2.1. Isolation of abortitristoside A (1) and abortitristoside B (2)

The compound (1) was identified as abortitristoside A an iridoid glucoside based on its chemical reaction and spectral analysis. On alkaline hydrolysis with glucosidase gives p-methoxy cinnamic acid confirms the iridoid glucoside skeleton. the UV spectral analysis shows the presence of phenyl group, ester and para-substituted phenyl ring. The IR spectral analysis confirms the presence of ester ($1220, 1180\text{ cm}^{-1}$), ether (1050 cm^{-1}), -CH_3 (1369 cm^{-1}), $\text{C}=\text{C}$ (1660 cm^{-1}), $\text{-C}=\text{O}$ (1727 cm^{-1}), -OH (3428 cm^{-1}) and monosubstituted benzene ring (750 cm^{-1}). The $^1\text{H-NMR}$ spectral analysis of compound 1 shows that it possesses -CH_3 (δ 1.02, 3H, d, $J = 7.0\text{ Hz}$), -OH (δ 3.58–3.68), ester (δ 4.07, 4.52) groups, trans-olefinic protons (δ 6.51, 7.71, $J = 15.7$) and aromatic protons (δ 6.34, 7.16), -OCH_3 (3.72, s, 3.77, s). Further, the presence of -CH_3 (δ 19.3), -OCH_3 (δ 56.0, s), ester (δ 168.5, s) groups, olefinic (δ 145.6, s) and aromatic (δ 114.3–130.3) carbon were confirmed by $^{13}\text{C-NMR}$ spectra. The NMR spectral analysis of compound 2 shows the absence of -OCH_3 (3.77, s) peak in $^1\text{H-NMR}$ spectra and -OCH_3 (δ 56.0, s) peak in $^{13}\text{C-NMR}$ spectra. The mass spectra of compound 1 shows the major abundance of fragments $[\text{a}^+]$ (402), $[\text{b}^+]$ (226), p-methoxy cinnamic acid 178), p-methoxy cinnamoyl moiety (161), $[\text{c}^+]$ (156) as shown in Fig. 2. Evaluating and matching the $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, UV, IR and Mass spectral data from literature confirms that compounds 1 and 2 are abortitristoside A (1) and abortitristoside B (2). These compounds were also isolated from the seed part of the plant *Nyctanthes arbor-tristis* [29, 30].

2.1. COX-1 and COX-2 inhibition activity of extracts, abortitristoside A (1) and abortitristoside B (2)

All the four extracts, abortitristoside A (1) and abortitristoside B (2) isolated from leaves were tested to analyze their inhibitory activity towards COX-1 and COX-2. The tests were performed using the markedly

available COX-(human) inhibitor screening kit (Catalog No. 701070, 701080, Cayman Chemical, PLM Pvt. Ltd., New Delhi, India).

In this method, we analyze the quantity of PGF2 α by using the enzyme immunoassay. Results were expressed in the terms of percentage inhibition of COX by 30 μ M solution of each extract in triplicate observation with a two-digit standard deviation. Further IC₅₀ values were evaluated for the extracts having high inhibitory potential, abortitristoside A (1) and abortitristoside B (2) based on percentage inhibition (Table 1, Fig. 3).

The inhibitory effectiveness of the extracts was estimated at a concentration of 30 μ M and percentage inhibition values were the means of three observations. Among all the extracts the methanolic extract shows the highest percentage inhibition against COX-2 (88.97%) and COX-1 (80.87%) but reduced selectivity towards COX-2 as compared to celecoxib. Instead of methanolic extract, chloroform extract also showed a remarkable percentage inhibition against COX-2 (85.23%) and COX-1 (69.19%) but lessor than celecoxib. The IC₅₀ Values were evaluated for chloroform, methanolic extracts, abortitristoside A (1) and abortitristoside B (2) against COX-2 which shows that abortitristoside A (1) possess the lowest IC₅₀ value (4.51 μ g/ml) amongst all tested sample but higher than celecoxib (4.17 μ g/ml).

Table 1 The IC₅₀ value and the percentage inhibition by various extracts and celecoxib at the concentration of 30 μ g/ml.

Sample	% Inhibition		IC ₅₀ Value (μ g/ml) (COX-2)
	COX-2	COX-1	
Methanolic Extract	88.97 $\pm$ 1.24	80.87 $\pm$ 0.96	5.10
Ethyl Acetate Extract	67.82 $\pm$ 0.66	80.29 $\pm$ 0.62	–
Chloroform Extract	85.23 $\pm$ 0.89	69.19 $\pm$ 0.66	6.76
Hexane Extract	78.27 $\pm$ 0.54	72.31 $\pm$ 0.99	–
Abortitristoside A (1)	90.67 $\pm$ 0.45	55.58 $\pm$ 0.65	4.51
Abortitristoside B (2)	90.56 $\pm$ 0.43	55.61 $\pm$ 0.64	4.55
Celecoxib	91.69 $\pm$ 0.34	22.35 $\pm$ 0.45	4.17

2.2. Molecular docking analysis of phytochemical against COX-1 and COX-2 isoenzyme

Molecular docking result (Table 2, Fig. 4) of all 21 selected ligands and 7 standard compounds against 3KK6 and 3LN1 isoenzyme were represented in term of Total Binding Energy ($E_{\text{BIND}} = E_{\text{VDW}} + E_{\text{H-BOND}} +$

$E_{\text{ELECTROSTATIC}}$) where the molecule having the lowest binding energy have highest docking score towards the host protein structure. The result shows phytochemical ligands **6** (-9.5 kcal/mol), **21** (-9.2 kcal/mol), **2** (-8.8 kcal/mol) and **1** (-8.3 kcal/mol) have highest binding affinity/docking score for 3KK6 (COX-1 enzyme) while ligands **14** (-10.3 kcal/mol), **1** (-10.2 kcal/mol), **2** (-10.2 kcal/mol) and **6** (-9.8 kcal/mol) have highest binding affinity for 3LN1 (COX-2 enzyme). Active sites of COX-2 contain LEU359, LEU352, ARG120, VAL349, ALA527, LEU384, TRP387, TYR355, TYR385, SER353, MET522, SER530, VAL523, LEU531, GLY526 amino acid residues while COX-1 are SER530, LEU531, TYR355, LEU359, VAL349, LEU352, GLY526, ALA527, LEU384, TYR385, MET522, ILE523, HIS90, MET113, TRP387, ILE517, SER353, PHE381, VAL116, ARG120, PHE518, TYR348 [31]. There are three main domains at COX-2 active site: (1) a hydrophilic domain (possess GLU524, ARG120 and TYR355); (2) a hydrophobic domain (possess ALA201, TYR385, LEU352, PHE518, TYR248 and TRP387) at the beginning of active site; (3) a side pocket having VAL523, HIS90 and ARG513 [32].

Table 2
Molecular Docking Score in terms of Binding Energy (in kcal/mole)
of Hits Phytochemical Ligand and Standard Anti-Inflammatory
Compounds Against 3KK6 (COX-1) and 3LN1 (COX-2).

Phytochemical Ligands	Binding Energy (COX-2 or 3LN1)	Binding Energy (COX-2 or 3LN1)
14	-10.3	-8.1
1	-10.2	-8.3
2	-10.2	-8.8
6	-9.8	-9.5
20	-9.5	-7.5
3	-9.4	-8.1
5	-9.1	-8.8
16	-9.1	-8.3
15	-9	-7.9
18	-9	-7.8
19	-9	-8
17	-8.9	-7.9
10	-8.8	-8.1
4	-8.7	-8
7	-8.5	-7.2
8	-8.4	-6.5
13	-8.4	-7.9
11	-8.3	-7.3
12	-8	-7.7
21	-7.8	-9.2
9	-6.2	-6.6
Celecoxib	-10.9	-9.5
NS398	-8.9	-8.4
Piroxicam	-9	-9.7
Tamoxifen	-9	-8.7

Phytochemical Ligands	Binding Energy (COX-2 or 3LN1)	Binding Energy (COX-2 or 3LN1)
Aspirin	-9.8	-10
Diclofenac	-7.8	-7.5
Indomethacin	-8.6	-8.9

Further ligand-protein residue interaction study (table 4 and Fig: 5, 6) reflex that standard compound Aspirin having highest docking score against COX-1 shows hydrogen bonding and hydrophobic bonding with LYS (A:532), GLN (A:372) and SER (A:126) amino acid residues. In other hand the standard compound Celecoxib hiving highest docking score against COX-2 shows hydrogen bonding and hydrophobic bonds with CYS (D:32), TRP (C:309), MLT (D:33), TYR (D: 122), PRO (D:140), HIS (D:119), VAL (D:119), GLY (D:121), ASP (D:143), ALA (D:142), PRO (D:139), PRO (D:25) and CYS (D:21) amino acid residues.

Following these data, the top two phytochemical ligands possess hydrogen bonding and hydrophobic bonding with ALA(A:199), ALA(A:202) MET(A:391), GLN(A:203), HIS(A:388) HIS(A:383) VAL(A:451), GLU(A:454), HIS(A:386), HIS(A:207), ALA(A:202), LEU(A:390), HIS(A:207, 388), HIS(A: 446), GLU(A:454), THR(A:212), HIS(A:385), ASN(A:382) and PHE(A:210) amino acid residues of the active site of COX-1 and ASP(A:143), ALA(A:142), ASN(A:24, 19), GLN(B:313), CYS(A:32), GLU(B:312), TRP(B:309), SER(A:34), TYR(120), SER(B:34), TRP(A:309), TYR(B:122), ASN(B:24), CYS(B:21), GLN(B:447), GLU(B:451), LEU(B:138), ARG(B:455), CYS(B:32) and TYR(B:116) amino acid residues of the active site of COX-2 to display the good docking score as exhibited by standard one.

Next to these, ADMET properties were predicted for all selected phytochemical ligands and two of the potential anti-inflammatories (Table 3) to know the drug likeliness which shows some of the phytochemical ligands are drug likely molecule and have zero Lipinski violation and some of them shows 1–2 Lipinski violation. The results of ADMET prediction shows that celecoxib and aspirin also possess hepatotoxicity.

Table 3 ADMET value of Selected Phytochemical Ligands and Two Standard Compounds.

Ligands	MW	nRB	nHBA	nHBD	TPSA	MLogP	LD ₅₀	HPT	nLV
1	566.55	10	13	5	190.67	-1.2	2.321	No	2
2	568.52	9	14	7	221.9	-1.88	2.39	No	3
3	552.52	9	13	6	201.67	-1.4	2.43	No	3
4	568.52	9	14	8	232.9	-2.69	2.214	Yes	3
5	566.55	10	13	5	190.67	-1.2	2.318	No	2
6	610.56	11	15	9	245.29	-2.57	2.264	Yes	3
7	406.38	5	11	6	175.37	-2.52	2.26	Yes	2
8	422.38	6	12	7	195.6	-3.28	2.279	Yes	2
9	154.16	0	3	1	46.53	-0.23	2.903	No	0
10	448.38	4	11	7	190.28	-2.1	2.428	Yes	2
11	534.55	9	11	5	172.21	-0.78	2.339	Yes	2
12	536.53	9	12	5	181.44	-0.92	2.21	No	2
13	630.59	12	14	5	207.74	-0.32	2.566	No	2
14	478.45	9	11	7	186.37	-1.01	2.324	Yes	2
15	614.59	11	13	4	187.51	0.43	2.582	No	2
16	782.74	19	17	0	211.79	1.89	2.319	No	2
17	494.49	8	11	4	161.21	-0.23	2.32	Yes	1
18	662.64	16	15	0	185.49	1.3	2.511	No	2
19	510.49	8	12	5	181.44	-0.99	2.324	Yes	2
20	720.67	18	17	0	211.79	0.95	2.423	No	2
21	778.71	21	19	0	238.09	0.61	2.434	Yes	2
Aspirin	180.16	3	4	1	63.60	1.51	2.377	No	0
Celecoxib	381.37	4	7	1	86.36	2.65	2.027	No	0*

* MW= Molecular Weight, nRB= Number of Rotatory Bond, nHBA= Number of Hydrogen Bond Acceptor, nHBD= Number of Hydrogen Bond Donar, TPSA= Total Polar Surface Area, MLogP= Predicted Octanol/Water Partition Coefficient, LD₅₀= Oral Rat Acute Toxicity, HPT= Hepatotoxicity, nLV= Number of Lipinski Violations.

Table 4 List of interacting residues of 3KK6 and 3LN1 with top two hit phytochemical ligands and top two reference compounds.

Ligands	COX-1/2 Interacting residues
1	COX-2 Interacting residues: ASP(A:143, 144) GLY(A:121, B:310) CYS(A:21, 145, 22, 32) ALA(A:142) VAL(A:141) PRO(A:140, 139) ASN(A:24, 19) GLN(A:447, B:313) MET(A:33) GLU(B:308, 31, 312) TYR(A:122) TRP(B:309) SER(A:34)
14	COX-2 Interacting residues: SER(B:34) TRP(A:309) GLN(A:313) GLU(A:308) TYR(B:122) ASN(B:19) ALA(B:142) ASN(B:24) VAL(B:141) CYS(B:22, 145, 21) GLN(B:447) GLU(B:441) LYS(B:454) ASN(B:28) ARG(B:29) LEU(B:138) ARG(B:455) GLY(B:30) CYS(B:32) PRO(B:139) TYR(B:116) GLY(B:14) MET(B:33)
6	COX-1 Interacting residues: LEU(A:390) ALA(A:199) TRP(A:387) ALA(A:202) MET(A:391) THR(A:206) VAL(A:447) GLN(A:203) VAL(A:291) HIS(A:388) HIS(A:383) VAL(A:451) SER(A:455) LEU(A:460) MET(A:458) MET(A:379) GLU(A:454) LYS(A:211) THR(A:212) ASN(A:382) HIS(A:386) PHE(A:210) TYR(A:385) HIS(A:207)
21	COX-1 Interacting residues: ALA(A:202) LEU(A:390) HIS(A:207, 388) VAL(A:291) LEU(A:298, 294, 295, 408) PHE(A:409) MET(A:391) ILE(A:444) HIS(A:443, 406) VAL(A:447) GLU(A:454) LYS(A:211) THR(A:212) HIS(A:385) ASN(A:382) PHE(A:210) THR(A:206) TYR(A:385) ALA(A:199) TRP(A:387)
Aspirin	COX-1 Interacting residues: LYS(A:532) GLN(A:372) SER(A:126) PHE(A:371) GLN(A:370) PRO(B:542) TYR(B:373) PRO(A:127) ILE(A:124) ASN(A:122) SER(A:121)
Celecoxib	COX-2 Interacting residues: CYS(D:32) TRP(C:309) MLT(D:33) TYR(D:122) PRO(D:14) HIS(D:119) VAL(D:119) CYS(D:21) GLY(D:121) ASP(D:143) PRO(D:139) PRO(D:25) ALA(D:142)

2.3 Structure-Activity Relationship

The phytochemicals 1 and 14 contain a propenoate active group for interaction with COX-1/2 enzyme residues (fig. 7). The compound 1-furan-2-yl-3-pyridine-2-yl-propenone (FPP-3) synthesized by Jahng and his colleagues possess the highest COX-2 inhibition potency ($IC_{50}=1.89 \mu M$) which is a preclinical drug for the treatment of chronic inflammatory diseases like rheumatoid arthritis without causing any gastrointestinal ulcer [33, 34]. Further, the FPP-3 confirmed that 5,6-membered aromatic/heterocyclic rings at 1, 3 positions were the basis for its increased potency [35] and 3-substituted larger phenyl group shows better selectivity towards COX-2 isoform to reduce side effects [36]. The presence of (-SO₂Me) group at five or six-membered rings attached to 1 or 3 positions enhanced the selectivity as well as activity of compound towards COX-2 isoform due to hydrogen bonding at the secondary active site of COX-2 [37, 38].

3. Material And Methods

3.1 General

Double beam UV-Visible Spectrometer (Elico SL 218), Bruker Advance-400 (400 MHz for ¹H-NMR and 100 MHz for ¹³C-NMR), Agilent Cary 630 FTIR Spectrometer (Range: 4000-450 cm⁻¹) and Agilent 6520 Q-TOF

Mass Spectrometer with AGILENT 1200 HPLC System (ESI/APCI-HRMS & LC-HRMS) were used to record the spectra.

3.2 Plant material

The plant was collected from the Chauraas Campus, HNB Garhwal University, District Tehri (UK, India) and the identification of the plant was done by the expertise of the Department of Botany, FRI, Dehradun, Uttarakhand and herbarium is deposited (210/Dis./2021/Syst.Bot./Rev.Gen./4-5).

3.3 Isolation and characterization

The leaves of *Nyctanthes arbor-tristis* was dried and powdered (Dry weight =2 kg) and then extracted with n-Hexane, Chloroform, Ethyl acetate and Methanol respectively with 1:5 (wt/vol.) ratio of plant material and the solvent by using Soxhlet extractor. The extracts were concentrated at reduced pressure (70kPa) and temperature by using rotatory evaporator (Roteva®, Medica instrument MFG.). The extracts; n-Hexane (72.30 gm), Chloroform (87.54 gm), Ethyl acetate (78.67 gm) and Methanol (76.43 gm) were stored for further experiment. The methanolic extract was adsorbed on 20 gm of silica gel and chromatographed on column packed with silica gel (100-120 mesh 1.4 Kg) and eluted with mixture of chloroform and methanol with increasing mixture polarity (chloroform-methanol, 98:2, 96:4 to 60:40). The eluent of chloroform-methanol mixture at 92:8 and 82:18 gives crude 1 (light yellow color powdered crystal) and 2 (white color hygroscopic crystal). The crude 1 and 2 were concentrated, washed with 2N HCL and recrystallized with dissolving in hexane. The compound 1 (140 mg) with molecular formula $C_{27}H_{34}O_{13}$ shows following spectral data: MP: 226-229; $[\alpha]_D$: -93° (MeOH); UV (MeOH): 206, 227, 300 and 308 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 1.02 (3H, d, $J = 7.0$ Hz), 2.08 (1H, q, $J = 7.0, 5.5$ Hz), 2.33 (1H, td, $J = 5.4, 2.8$ Hz), 2.94 (1H, dd, $J = 6.9, 5.4$ Hz), 3.12-3.25 (2H, 3.18 (dd, $J = 3.5, 2.7$ Hz), 3.20 (dd, $J = 3.5, 2.7$ Hz)), 3.44 (1H, td, $J = 4.5, 2.7$ Hz), 3.58-3.87 (9H, 3.63 (t, $J = 3.5$ Hz), 3.72 (s), 3.77 (s), 3.81 (d, $J = 4.5$ Hz), 3.81 (d, $J = 4.5$ Hz)), 4.07 (1H, dd, $J = 8.1, 7.0$ Hz), 4.52 (1H, d, $J = 2.7$ Hz), 4.86 (1H, dd, $J = 8.1, 6.9$ Hz), 6.34-6.58 (2H, 6.39 (d, $J = 2.8$ Hz), 6.51 (d, $J = 15.7$ Hz)), 7.16 (2H, ddd, $J = 8.8, 1.2, 0.5$ Hz), 7.49 (2H, ddd, $J = 8.8, 1.7, 0.5$ Hz), 7.71 (1H, d, $J = 15.7$ Hz), 7.95 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.3 (1C, s), 40.0 (1C, s), 40.7-40.8 (2C, 40.7 (s), 40.7 (s)), 52.2 (1C, s), 56.0 (1C, s), 62.3 (1C, s), 71.1 (1C, s), 74.0 (1C, s), 76.7 (1C, s), 78.1 (1C, s), 80.2 (1C, s), 86.2 (1C, s), 96.8 (1C, s), 99.2 (1C, s), 109.7 (1C, s), 114.3 (2C, s), 115.1 (1C, s), 128.7 (2C, s), 130.3 (1C, s), 145.6 (1C, s), 154.1 (1C, s), 159.8 (1C, s), 166.8 (1C, s), 168.5 (1C, s); IR bands (KBr, MeOH): 3424, 2950, 1719, 1662, 1633, 1514, 1450, 1377, 1220, 1180, 1050, 877, 835, 750 and 591 cm^{-1} ; Ms (m/z): 402, 226, 200, 178, 161, 156.

The compound 2 (163 mg) with molecular formula $C_{26}H_{32}O_{14}$ shows following spectral data, MP: 226-229; $[\alpha]_D$: -69° (MeOH); ^1H NMR (400 MHz, $CDCl_3$): δ 1.02 (3H, d, $J = 7.0$ Hz), 2.08 (1H, quintd, $J = 7.0, 5.5$ Hz), 2.33 (1H, td, $J = 5.4, 2.8$ Hz), 2.94 (1H, dd, $J = 6.9, 5.4$ Hz), 3.12-3.25 (2H, 3.18 (dd, $J = 3.5, 2.7$ Hz), 3.20 (dd, $J = 3.5, 2.7$ Hz)), 3.44 (1H, td, $J = 4.5, 2.7$ Hz), 3.58-3.87 (6H, 3.63 (t, $J = 3.5$ Hz), 3.72 (s), 3.81 (d,

$J = 4.5$ Hz), 3.81 (d, $J = 4.5$ Hz)), 4.07 (1H, dd, $J = 8.1, 7.0$ Hz), 4.52 (1H, d, $J = 2.7$ Hz), 4.88 (1H, dd, $J = 8.1, 6.9$ Hz), 6.39 (1H, d, $J = 2.8$ Hz), 6.57 (1H, d, $J = 15.7$ Hz), 6.77 (1H, dd, $J = 8.4, 0.5$ Hz), 7.28 (1H, dd, $J = 1.9, 0.5$ Hz), 7.63-7.80 (2H, 7.69 (dd, $J = 8.4, 1.9$ Hz), 7.74 (d, $J = 15.7$ Hz)), 7.95 (1H, s); ^{13}C NMR (100 MHz, CDCl_3): δ 19.3 (1C, s), 40.0 (1C, s), 40.7-40.8 (2C, 40.7 s), 52.2 (1C, s), 62.3 (1C, s), 71.1 (1C, s), 74.0 (1C, s), 76.7 (1C, s), 78.1 (1C, s), 80.2 (1C, s), 86.2 (1C, s), 96.8 (1C, s), 99.2 (1C, s), 109.7 (1C, s), 114.8 (1C, s), 115.1 (1C, s), 115.8 (1C, s), 128.2 (1C, s), 128.7 (1C, s), 145.6 (1C, s), 145.8 (1C, s), 146.3 (1C, s), 154.1 (1C, s), 166.8 (1C, s), 168.5 (1C, s); IR bands (KBr, MeOH): 3350, 2920, 1690, 1630, 1520, 1440, 1270, 1080, 950, 860, 810 and 770 cm^{-1} ; Ms (m/z): 404, 226, 210, 178, 163, 156.

3.4 Cyclooxygenase inhibition assay

The repressive action of various extracts against COX-2 was calculated by the fair marketable quality COX-(human) inhibitor screening kit (Catalog No. 701070, 701080, Cayman Chemical, PLM Pvt. Ltd., New Delhi, India) based on the producer's directions. The prepared reaction buffer contains 160 μL , 0.1 M Tris-HCl (pH 8.0), 5mM EDTA and 2 mM phenol. COX-1/2 (10 μL) was added in 10 μL , 30 μM solution of each extract in the presence of 10 μL Heme solution. All these test solutions were relaxed for 10 minutes at 37°C . Further, 10 μL arachidonic acid and 30 μL stannous chloride were added to test solution (sample) and blank solution (control) to start the reaction to produce the PGF 2α by the reduction of PGH 2 and relaxed exactly for 5 minutes at 37°C . The amount of produced PGF 2α was calculated for sample and control by immunoassay method using the UV microplate reader at the wavelength 410 nm to calculate the percentage inhibition. The following equation was used to calculate the percentage inhibition:

$$\% \text{ Inhibition} = \{([PGF2\alpha]_{\text{control}} - [PGF2\alpha]_{\text{sample}}) / [PGF2\alpha]_{\text{control}}\} \times 100$$

Where $[PGF2\alpha]_{\text{control}}$ is PGF 2α produced in blank solution (without extracts) and $[PGF2\alpha]_{\text{sample}}$ is PGF 2α produced in the test sample solution (with extracts). The test compounds, dissolved in 5% DMSO, were evaluated at a concentration of 30 μM .

3.5 ADMET Calculation

In order to know drug likeliness of molecule/ligand PKCSM [39] and Swiss ADME [40] was used to predict the molecular weight, water partition coefficient (MLogP), H-bond donors, H-bond acceptor, total polar surface area (TPSA), Lipinski (drug-likeness), hepatotoxicity and Oral Rat Acute Toxicity (LD_{50}) [41].

3.6 Molecular docking study

3.6.1 Geometry optimization of reported molecules

The designing of all compounds was done by using ACD Chemskech and their optimization was done by choosing molecular mechanics (MM2) as a force field. These optimized compounds were used for docking. The following 21 reported phytochemicals of *Nyctanthes arbor-tristis* were used for the docking: abortitristoside A (1), abortitristoside B (2), abortitristoside C (3), abortitristosideD (4), abortitristoside E (5), nyctoside A (6), 6-beta-hydroxyloganin (7), nyctanthoside (8), rnygolne (9), astragalin (10), 7-o-trans-cinnamoyl-6b-hydroxyloganin (11), 6-o-trans-cinnamoyl-6b-hydroxyloganin (12), 6,7-di-o-benzoylnyctanthoside (13), desrhamnosylverbanscoside (14), arborside A (15), arborside A tetraacetate (16), arborside B (17), arborside B tetraacetate (18), arborside C (19), arborside C pentaacetate (20), arborside D hexaacetate (21) [10].

3.6.2 Protein Preparation

The groundwork of protein was done employing UCSF Chimera 1.11.2 in dock prep module, where the deletion of inherent ligand attached to crystal structure, hydrogen addition, water, solvents, replacing partial residues employing Dunbrack rotamer library, renovation of bromo-UMP to UMP, renovation of selenomethionine to methionine, alteration of methylselenyl-dCMP to CPM. AMBER.ff14SB force field were used to dispensed the charges on protein molecule [42]. Then the augmented protein was used for docking study.

3.6.3 Molecular Docking, Docking parameters & Post Docking modeling

The docking of all reference compounds and the selected phytochemicals of *Nyctanthes arbor-tristis* was performed using PyRx against COX-1 (PDB ID – 3KK6) and COX-2 (PDB ID – 3LN1). This software used generic algorithms for the docking. The defined parameters in PyRx for docking with Grid center (X=26.0916, Y=157.3507, Z= 10.1225 for COX-1 and X= 48.1391, Y=33.8036, 25.3268 for COX-2), Dimension (X=82.5649, Y=89.4395, Z=118.3433 Å for COX-1 and X=133.5613, 89.4395, 118.3433 Å for COX-2), population size (n = 200), generations (g = 10) and number of solutions for each compound (s = 1). Based on the above set parameters, the compounds were screened. The post dock modeling of docked poses was executed by Discovery studio visualizer v3.5. The top four compounds were chosen by considering the lowest energy of docking/binding. The binding energy of a ligand to the protein is given by:

$$E_{\text{BINDING}} = E_{\text{VDW}} + E_{\text{H-BOND}} + E_{\text{ELECTROSTATIC}}$$

where E_{VDW} means for Vander Waal energy, $E_{\text{H-BOND}}$ means for hydrogen bonding energy

and $E_{\text{ELECTROSTATIC}}$ means for electro statistic energy [43].

4. Conclusion

As in the case of inflammation COX-2 enzyme is responsible while COX-1 involve to maintain normal physiology. Most of the known anti-inflammatory drugs inhibit both the isoenzyme and cause a peptic ulcer or gastrointestinal ulcer therefore a selective drug molecule is required. The in-vitro analysis of all the four fractions of the leave of *Nyctanthes arbor-tristis* shows that the methanolic fraction has the greatest potential to inhibit the COX-2 as compared to other fractions. Further in-vitro and in-vivo analysis of methanolic fraction in the different models also may provide a good therapeutic option to treat the inflammation. The molecular docking study of pre-isolated phytochemicals from the leave of the plant also shows that compounds **6** (-9.5 kcal/mol), **21** (-9.2 kcal/mol), **2** (-8.8 kcal/mol) and **1** (-8.3 kcal/mol) have the highest binding affinity/docking score for 3KK6 (COX-1 enzyme) while ligands **14** (-10.3 kcal/mol), **1** (-10.2 kcal/mol), **2** (-10.2 kcal/mol) and **6** (-9.8 kcal/mol) have highest binding affinity for 3LN1 (COX-2 enzyme) but compound 1, 2 and 14 also shows docking selectivity for COX-2 express the novelty for finding good NSAIDs. Therefore, the methanolic fraction was subjected to isolation and two compounds (abortristoside A and abortristoside B) were isolated and tested against COX-1 and COX-2. The in-vitro screening against COX-1 and COX-2 reflexes that compound 1 and compound 2 have selectivity for COX-2 over COX-1 with IC₅₀ near to celecoxib which is also established by structure-activity relationship analysis. Further in-vitro analysis of 1 and 2 in the different models also may provide a good therapeutic option to treat the inflammation Therefore, in-vivo analysis of abortristoside A (1) and abortristoside B (2) in the different models may provide a good therapeutic option to treat the inflammation without causing any gastrointestinal ulcer. Desrhamnosylverbanscoside a verbascoside reported phytochemical of the *Nyctanthes arbor-tristis* shows the highest docking score which isolation and screening against COX-1/2 may give the potential anti-inflammatory agent. Further, we provide a new strategy to design potential selective COX-2 inhibitors from the plant-origin natural compound by using molecular docking technique, in-vitro screening of plant extract and isolated compounds in this paper.

Declarations

Ethical Approval

Not applicable

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Rahul Kumar Vishwakarma], [Aayasha Negi] and [Devendra Singh Negi]. The first draft of the manuscript was written by [Rahul Kumar Vishwakarma] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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New Delhi.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability

Please see the material and method section to find the availability of the code used for molecular docking.

Competing interest

There is no conflict of interest from any authors.

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Figures

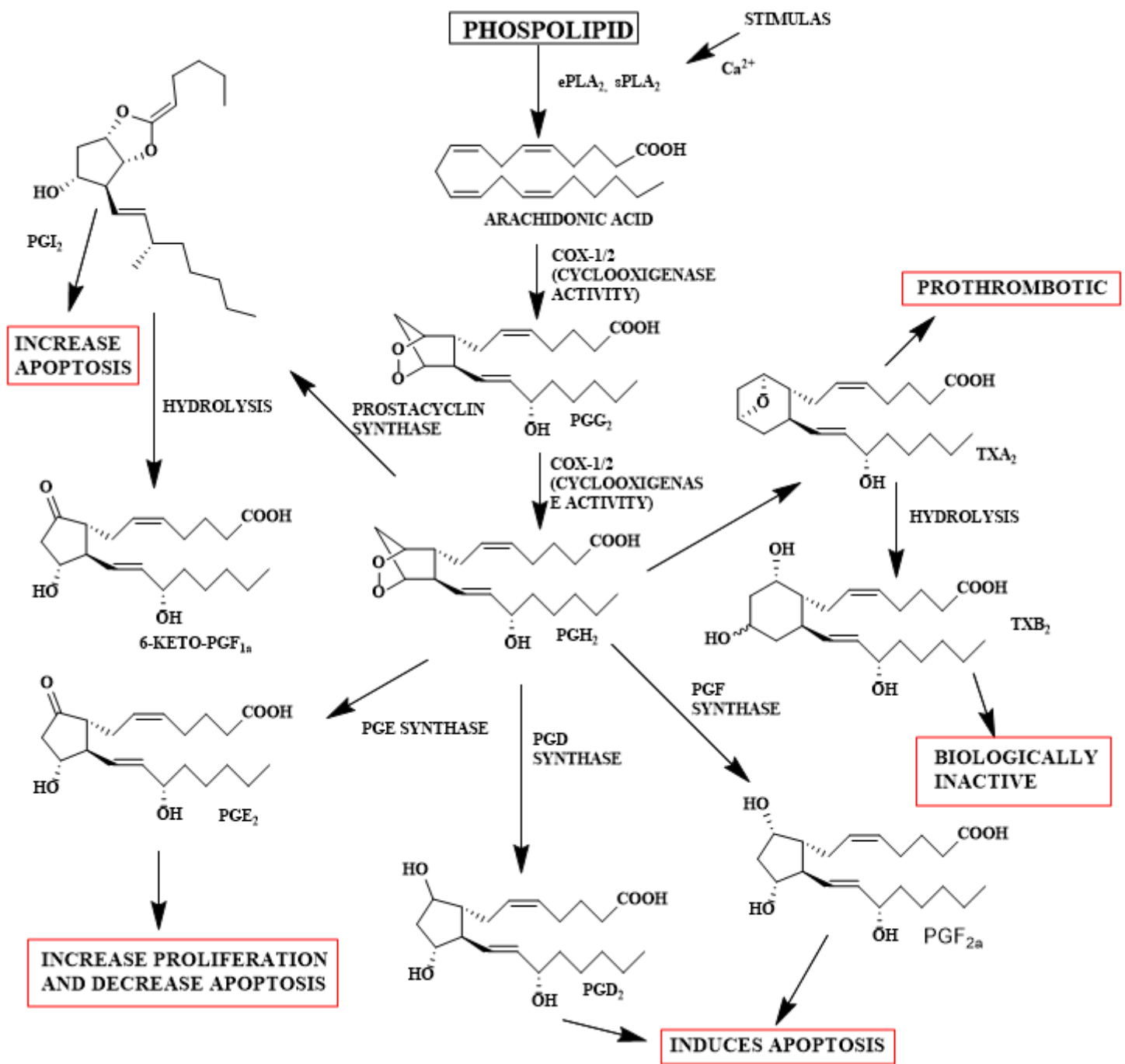


Figure 1

Cyclooxygenase Pathway for the biosynthesis of PGs, PGLs and TXs

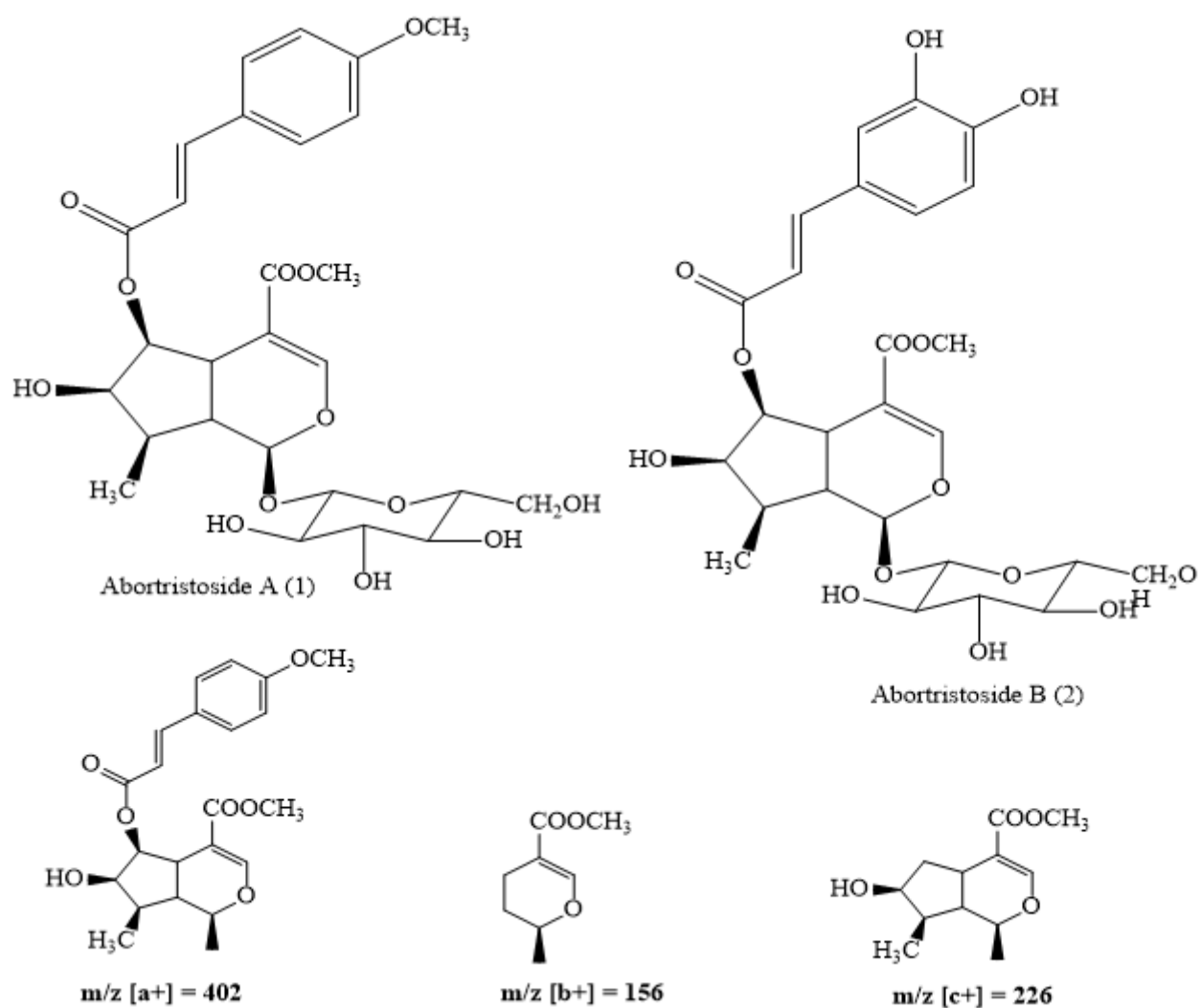


Figure 2

Structure of compounds 1 and 2, m/z peaks of fragments of compound 1

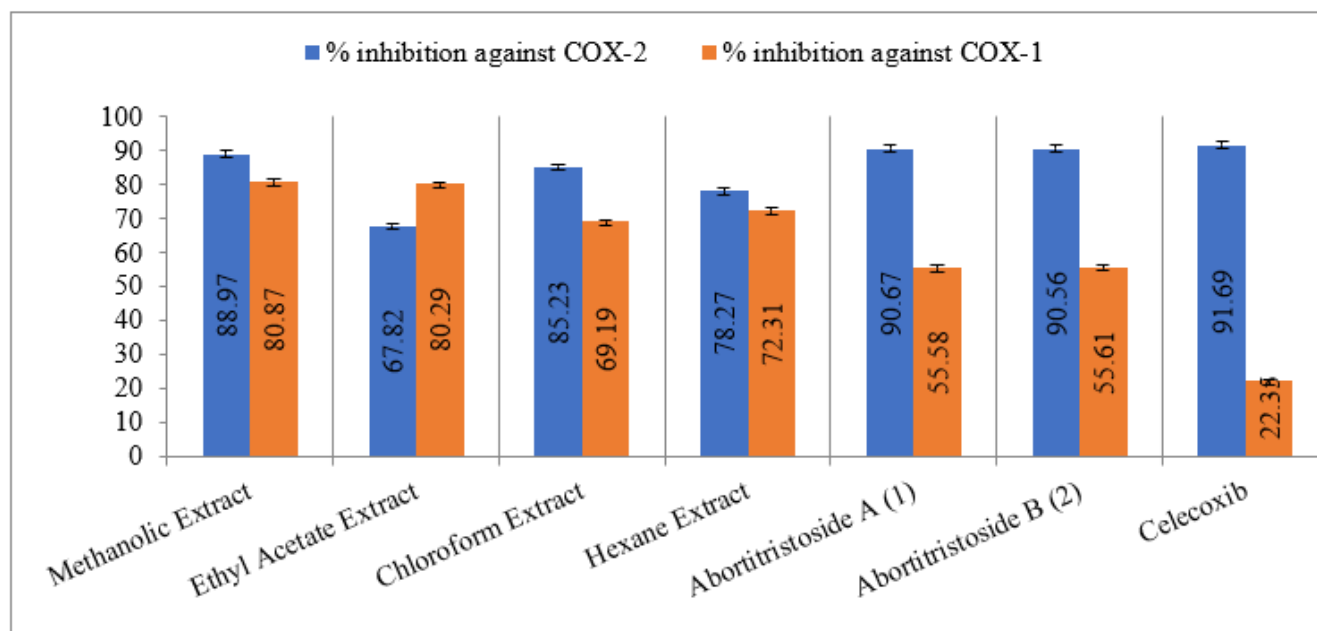


Figure 3

Comparative analysis of the percentage inhibition by various extracts (1-4) and celecoxib (5) at the concentration of 30ug/ml against COX-1 and COX-2.

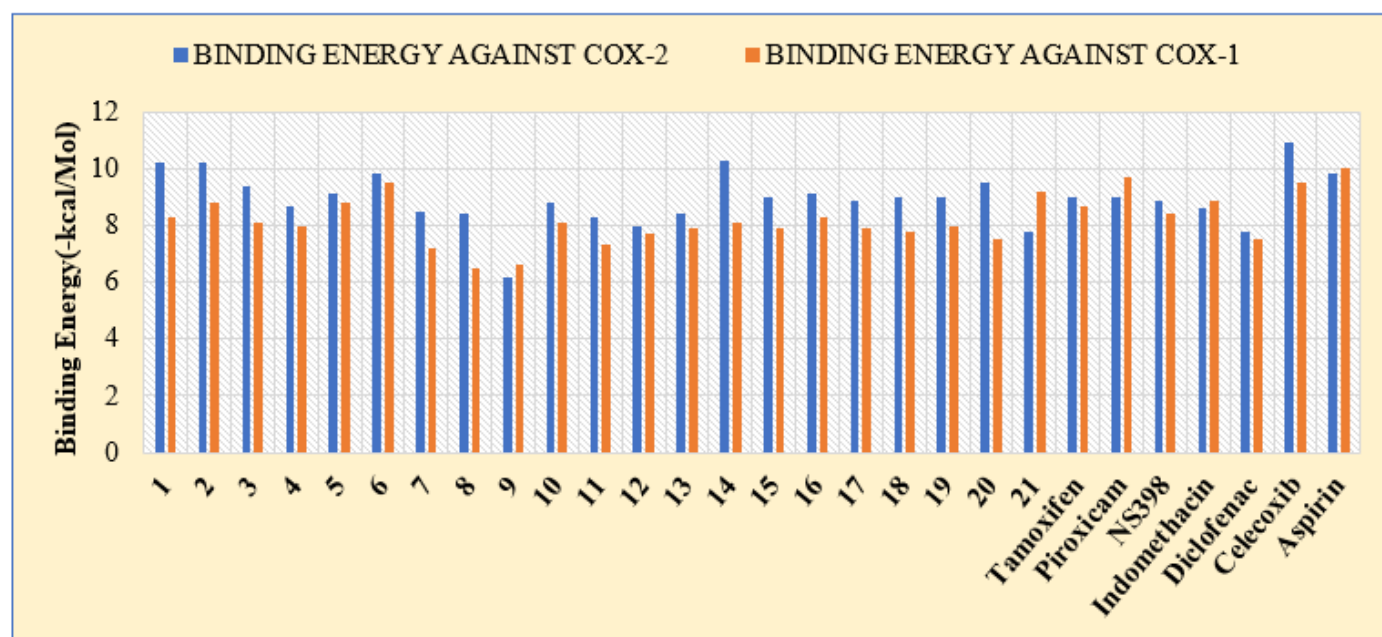


Figure 4

Comparative analysis of Molecular Docking Score in Terms Total Binding Energy (TBE) of 1-21 Hits Phytochemical Ligands and standard compounds against COX-1 and COX-2.

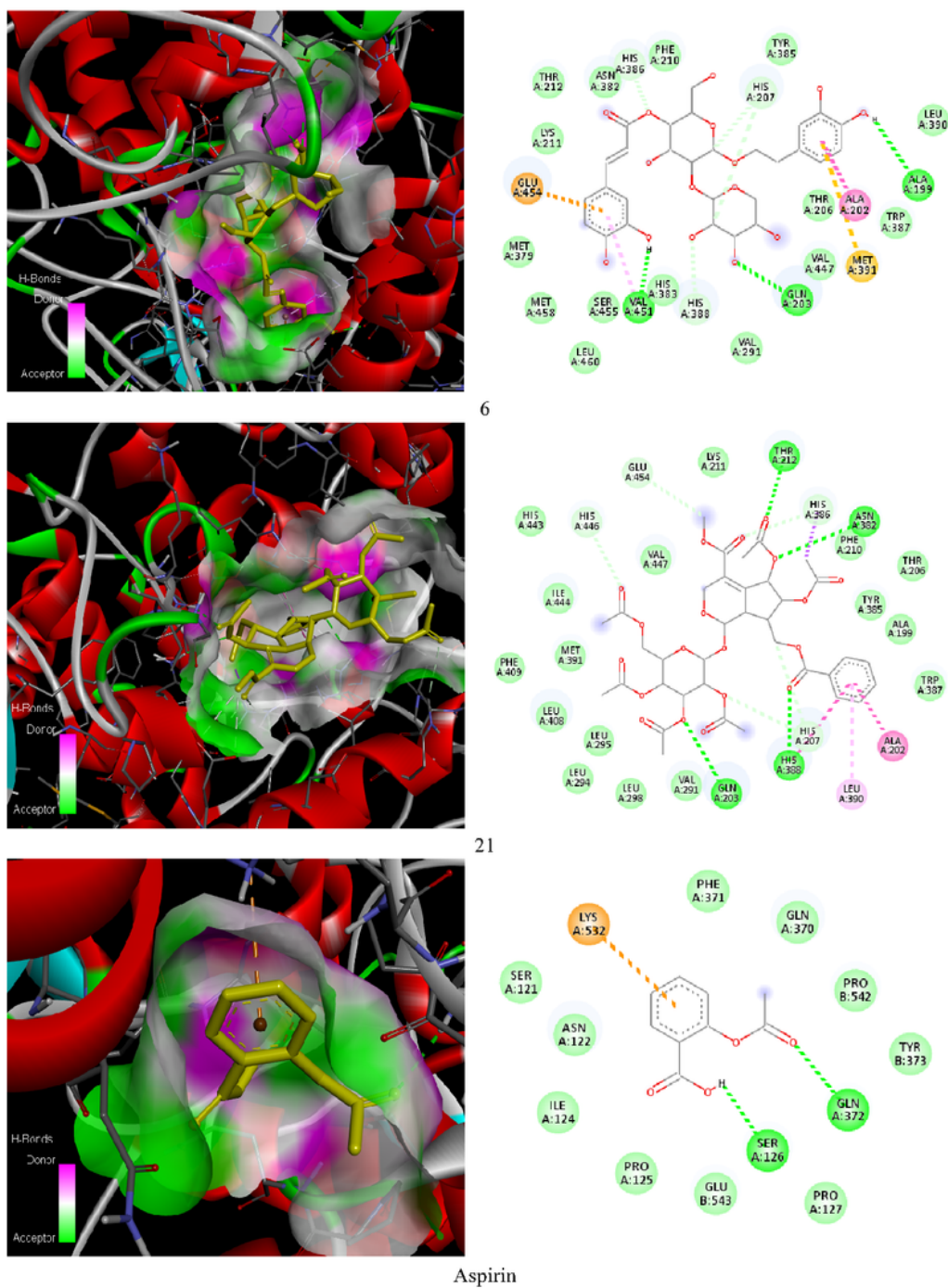
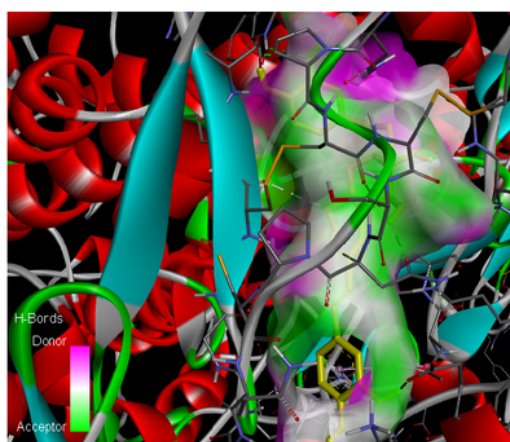
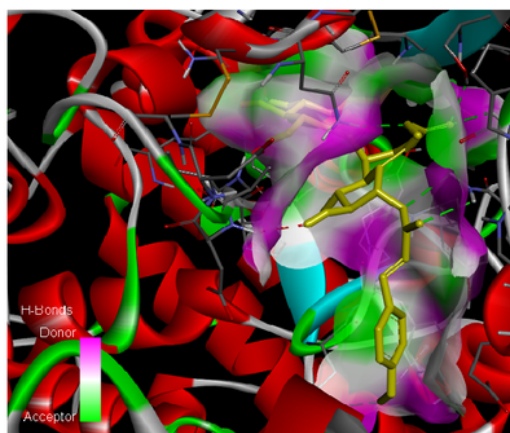
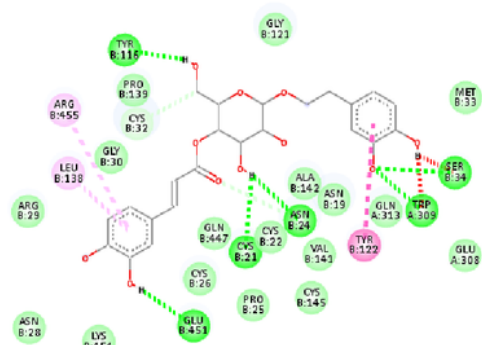


Figure 5

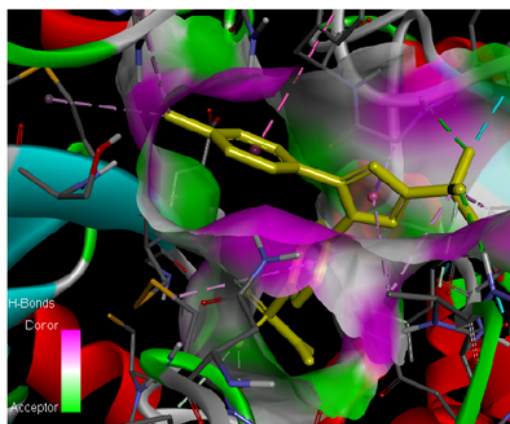
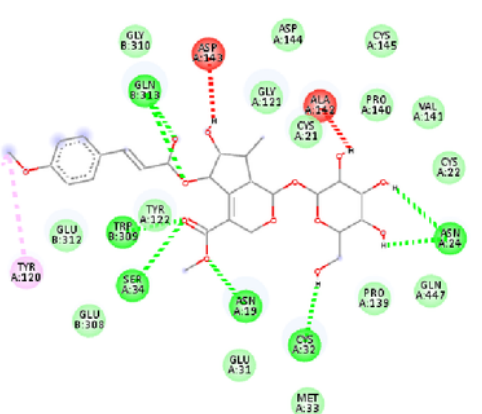
Protein-Ligand Interaction Diagram of Top Two Hit Phytochemical Ligand and Aspirin with COX-1 (3KK6).



14



1



Celecoxib

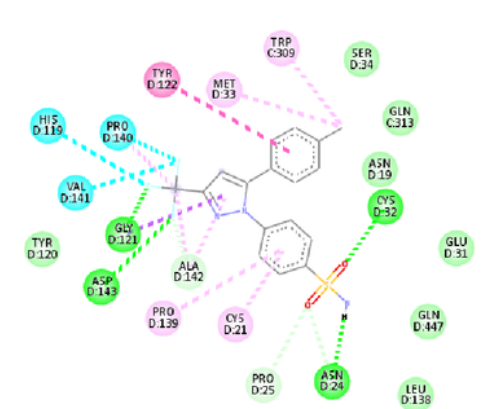


Figure 6

Protein-Ligand Interaction Diagram of Top Two Hit Phytochemical Ligand and Celecoxib with COX-2 (3LN1).

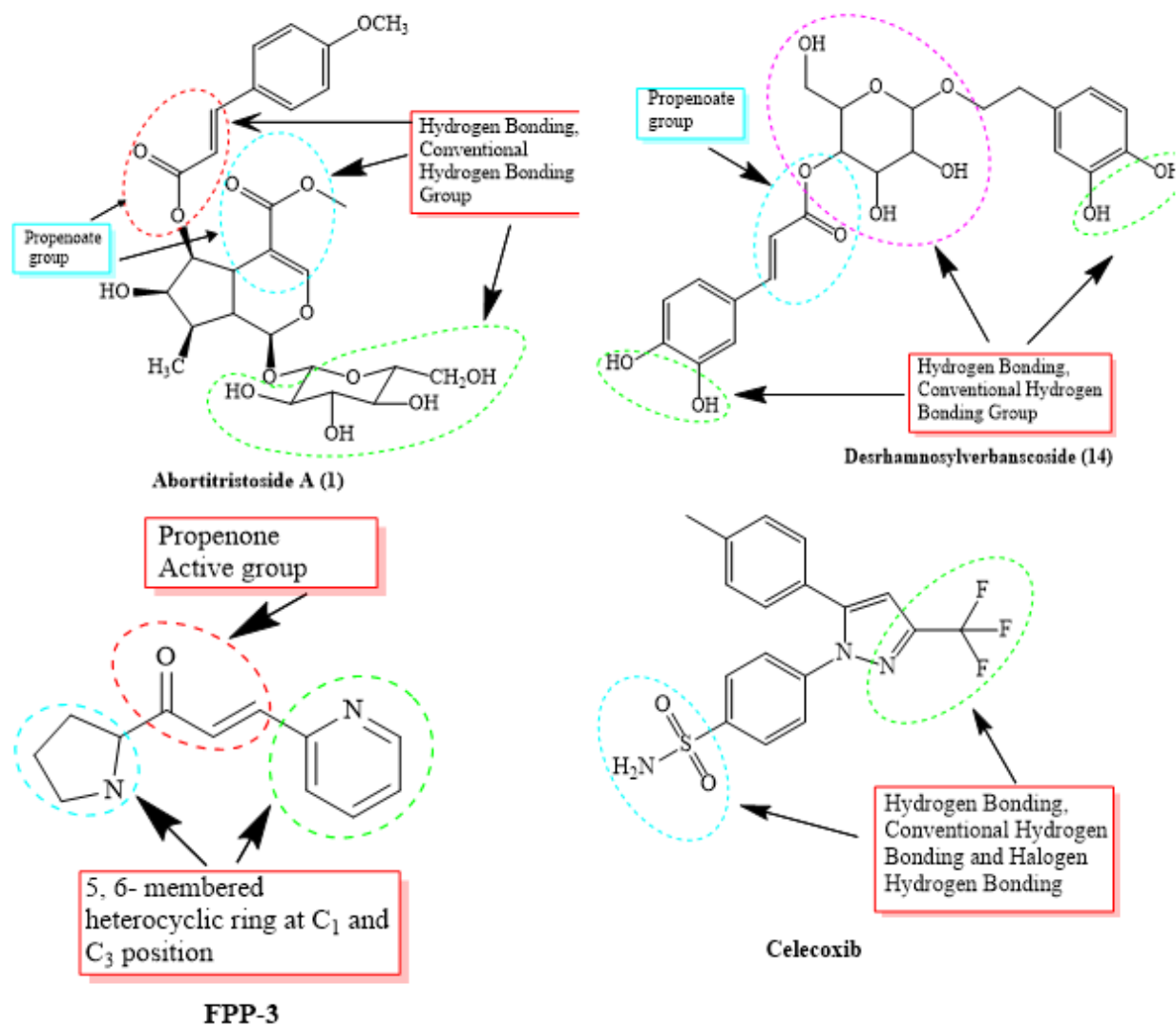


Figure 7

Structure-activity relationship of abortristoside A and desrhamnosylverbanscoside with FPP-3 and celecoxib.

Supplementary Files

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