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ORIGINAL ARTICLE

Potential *Cyclooxygenase* (COX-2) Enzyme Inhibitors from *Myrica nagi*-from *in-silico* to *in-vitro* Investigation

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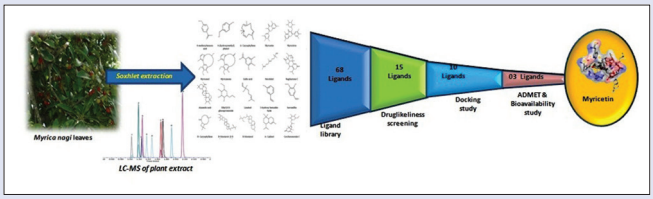
ABSTRACT

Introduction: *Myrica nagi* Thunb. (family *Myricaceae*) are actinorhizal plants showing symbiotic interaction with *Frankia.* Inhibition of cyclooxygenase-2 (COX-2) enzyme is known to be significant in preventing inflammation and in therapeutics. **Objectives:** Our principal focus was to identify COX-2 enzyme inhibitors, safer and natural anti-inflammatory compounds from *M. nagi*. Protein–ligand interaction has a significant role in structure-based drug design. **Materials and Methods:** Sixty-eight phytochemicals were therefore screened and evaluated for their binding energies with COX-2. These phytoconstituents were screened and analyzed for drug Likeliness along with Lipinski's rule of five. The X-ray crystallographic structure of the target COX-2 (protein data bank [PDB] ID: 4PH9), obtained from PDB, was docked with PubChem structures of phytochemicals using AutoDock 4.2 that uses Lamarckian genetic algorithm. Further, myricetin was subjected to *in vitro* anti-inflammatory assay using RAW-264.7 cell lines and inhibitory concentration (IC_{50}) value was also determined. **Results:** The myricetin, myricitrin, and corchoionoside-C inhibited COX-2 with – 6.52, –4.94, and – 4.94 Kcal/mol binding energies, respectively, comparable to ibuprofen. Eventually, bioactivity score and absorption distribution metabolism excretion-toxicity properties showed considerable biological activities as G protein-coupled receptor, nuclear receptor, protease inhibitor, and enzyme inhibitors for myricetin, myricitrin, and corchoionoside-C phytochemicals. Molecular docking revealed hydrophobic interactions followed by four, nine, and four numbers of hydrogen bonds between myricetin, myricitrin, and corchoionoside-C, respectively, within the binding site of COX-2. Flavonol myricetin showed 112 μg/mL as IC_{50} value when it was subjected to *in vitro* cytotoxicity assay. These results clearly demonstrated that myricetin, myricitrin, and corchoionoside-C could act as highly potential COX-2 inhibitors. Therefore, *in silico* and *in vitro* studies revealed that of three best phytochemicals, myricetin could be promising candidate.

Key words: Anti-inflammatory, cyclooxygenase 2, docking, flavonoids, inhibitory concentration, intermolecular energy, myricetin

SUMMARY

- The liquid chromatography–mass spectrometry analysis of the leaf extract of *Myrica nagi* revealed many phytochemicals and the ligand library of 68 compounds were generated from PubChem database
- The ligand library was subjected to drug likeliness with Molsoft tool and top 15 ligands were selected
- Shortlisted ligands were docked with cyclooxygenase-2 (COX-2) enzyme using AutoDock 4.2 tool and then subjected to absorption distribution metabolism excretion-toxicity and bioactivity studies to select the top three best ligands
- *In silico* studies of myricetin and myricitrin indicated anti-inflammatory effects on COX-2 enzyme.



Abbreviations used: COX-2: Cyclooxygenase-2; PDB: Protein data bank; LGA: Lamarckian genetic algorithm; ADMET: Absorption distribution metabolism excretion-toxicity; GPCR: G protein-coupled receptors; PI3K: Phosphoinositide 3-kinase; Akt/PKB: Akt/protein kinase B; JAK1: Janus kinase1; MAP: Mitogen-activated protein; MKK4: Mitogen-activated protein kinase-kinase 4; SMILES: Simplified molecular input line entry specification; GA: Genetic algorithm; LS: Local search; CHARMM: Chemistry at HARvard Macromolecular Mechanics; VDw: Van Der Waals; PDBQT: Protein data bank, partial charge, and atom type format; NMR: Nuclear magnetic resonance; GUI: Graphical user interface; IBP: Ibuprofen; BBB: Blood–brain barrier; RMSD: Root-mean-square deviation; RT: Reverse transcriptase; HIV: Human immunodeficiency virus; RLV: Rauscher murine leukemia virus.

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INTRODUCTION

Myricaceae is a family of plants that are actinorhizal and having symbiotic interaction with actinomycete. Nodulation process has been observed in all species of *Myrica* as reported previously.[1,2] *Myrica esculenta* and *Myrica nagi* (called Katphala, Kafal, and box berry) are the two major species widely found in China, India, Nepal, and Pakistan. Ancient Ayurvedic medicine has extensively employed *Myrica* species for treating various disorders, such as bleeding piles, burns, gastrointestinal diseases, headaches, skin diseases, and typhoid. Furthermore, it is used as anti-inflammatory agent for asthma, bronchitis, fever, lung infection, dysentery, toothache, wounds, jaundice, catarrhal fever, cough, sore throat, ulcers, cardiac disorders, gonorrhea, diuresis, epilepsy, and paralysis and for regulating the

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menstrual cycle.[3,4] The powdered leaves of *Myrica* species have traditional uses in various ailments.[5]

Phytochemicals are most commonly found as polyphenolic compounds in food and beverages of plant origin. These are not only essential nutrients in plants, but also have numerous activities to avert several diseases.[6-10] They have been structurally classified into different classes, namely phenolic acids, saponins, alkaloids, carotenoids, flavonoids, lignans, terpenes, phytosterols, triterpenoids, and xanthophylls.

Of these, flavonoids are the most extensively investigated molecules and are recorded to have diversified pharmacological activities such as antioxidant, antidiabetic, antiallergic, anti-inflammatory, and anticancer activity.[11,12]

The flavonoids are further classified into seven subclasses, namely flavanols, flavones, anthocyanins, isoflavones, flavanones, flavonols, and flavanonols, based on their structures.[13] Flavonoids, at molecular level, are found to regulate various molecules including interleukins, proteins, and different enzymes. Flavonoids can bind directly to some protein kinases, namely phosphoinositide 3-kinase, Akt/protein kinase B, Janus kinase 1, mitogen-activated protein kinase kinase4, and Raf1. They then modify their phosphorylation site to regulate multiple cell signaling pathways.[14] The flavonols are one such group that has different compounds such as quercetin, kaempferol, myricetin, fisetin, and morin, exhibiting beneficial effects such as anti-inflammatory, antioxidants, antiallergic, antiviral, as well as anticancer activity.[4,12]

Inflammation is one of the pathological features of many diseases and is complicated during pathogenesis of various disorders, such as jaundice, cough, ulcers, and aging.[15] Cyclooxygenase enzyme (COX) exists in two different isoforms, COX-1 and COX-2. It is an endogenous enzyme that catalyze the transformation of arachidonic acid to prostaglandins and thromboxanes.[16] COX-2 enzyme is inducible and gets expressed only on inflammatory stimulus.[17,18]

Molecular docking simulation works in a two-step process that starts with compiling various ligand conformations in the identified binding site of the receptor. Then, ranking of these according to their binding pose energies for each individual molecule is done.[19] Diverse types of commercially available tools such as GOLD, FLEX, AutoDock, HEX, and Argus Lab are extensively employed for evaluating docking binding energies of ligand-receptor conformations.[20] The recognition of the most apt-binding conformations is performed by probing the large conformational space, reporting various binding sites and then meticulously predicting the interaction energy correlated with the respective binding poses.[21] This process is carried out iteratively until obtaining a solution with minimum energy. Molecular docking is the most frequently used approach in drug design because of its competence to predict highly significant, accurate, and different conformations of ligands within the target binding site.[22] Hence, it can be employed to study interaction between phytochemicals and COX-2 enzyme.

Raw-264.7 cells are predominantly used to determine the cytotoxicity and anti-inflammatory activity of secondary metabolites.[23] Previously, our laboratory has reported that compounds of *M. nagi* could be good COX-2 enzyme inhibitors hypothetically.[16] The study was continued using preclinical laboratory to understand the anti-inflammatory activity of the same using crude extract.[24] However, there is a serious need to understand the mechanism of inhibition involving *M. nagi* compounds. This study was therefore initiated or aimed to understand the specific compound involved for its anti-inflammatory activity by *in silico* and *in vitro* mean.

MATERIALS AND METHODS

Selection of target protein

The protein structure of COX-2 (Protein Data Bank [PDB] ID: 4PH9) was obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) PDB (http://www.rcsb.org/pdb/) with X-ray diffraction resolution of 1.81 Å, belonging to *Mus musculus*.[25]

Phytochemical dataset generation

The data obtained from our previous study[16] and other laboratory[26] on *M. nagi* were used to build the dataset of 68 phytochemicals [Figure 1]. The chemical structures and canonical Simplified Molecular Input Line Entry Specification notation of the molecules used in the present study were obtained from NCBI PubChem database for further analysis.

Drug likeliness prediction by Molsoft software

Drug likeliness is defined as an intricate balance of different molecular properties and structural features that determines if the test molecule is similar to the known drugs. Lipinski's rule of five is a thumb rule to evaluate drug likeliness. Lipinski's rules state that an ideal drug molecule need to possesses not >500 Da molecular weight, not >5 hydrogen bond donors, not >10 hydrogen bond acceptors, and LogP not >5. Drug likeliness of the ligands was predicted using "Molsoft" (http://www.molsoft.com) that screens the molecule based on Lipinski's rule. This tool distinguishes drug-like and nondrug-like properties and shows probability scores for a molecule along with the graph depicting drug likeliness.[27]

Preparation of receptor

The native crystal structure of ibuprofen (IBP) bound COX-2 enzyme was retrieved from RCSB PDB[28] (PDB ID: 4PH9). The retrieved PDB file was cleaned to restrict small molecules and hetero atoms manually to obtain the protein for further docking studies.[29,30] PDBSum was availed to understand the binding site region with which the ligand can bind to the COX-2 enzyme. After this process, the ligands and COX-2 enzyme were docked.

Molecular docking simulation

Docking is a virtual screening process of ligand dataset and predicting the agonists and antagonists based on some scoring functions.[22] AutoDock[31] was used for docking of ligands with COX-2 enzyme. AutoDock works on Lamarkian genetic algorithm (LGA), which is a blend of genetic algorithm and adaptive local search that identifies the conformational space of the ligand and enzyme for proper interaction.[31]

Before docking process, the ligand molecules were prepared using the "prepare ligand" module. Enzyme–ligand interactions were refined by molecular dynamics using Chemistry at HARvard Macromolecular Mechanics (CHARMM) in Biovia discovery suite. Force fields are energies applied on each particle and their positional relationships, indicating energies between the atoms.[32] The form of the potential energy function CHARMM uses the same equation indicated as per our previous studies.[33]

$$V = \sum_{\text{bonds}} k_b (b - b_0)^2 + \sum_{angles} k_\theta (\theta - \theta_0)^2 + \sum_{dihedrals} k_\phi [1 + \cos(n\phi - \delta)] + \sum_{impropers} k_\omega (\omega - \omega_0)^2 + \sum_{Uery-Bradley} k_u (u - u_0)^2 + \sum_{nonbonded} \in [(\frac{R_{\min_{ij}}}{\tau_{ij}})^{12} - (\frac{R_{\min_{ij}}}{\tau_{ij}})^6] + \frac{q_i q_j}{\in \tau_{ij}}$$

In the equation, k_b is the bond force constant and $b - b_0$ is the distance of atom displaced from the equilibrium. The next term in the equation is bond angles where k_0 is the angle force constant and $\theta - \theta_0$ denotes the angle between three bonded atoms from equilibrium.

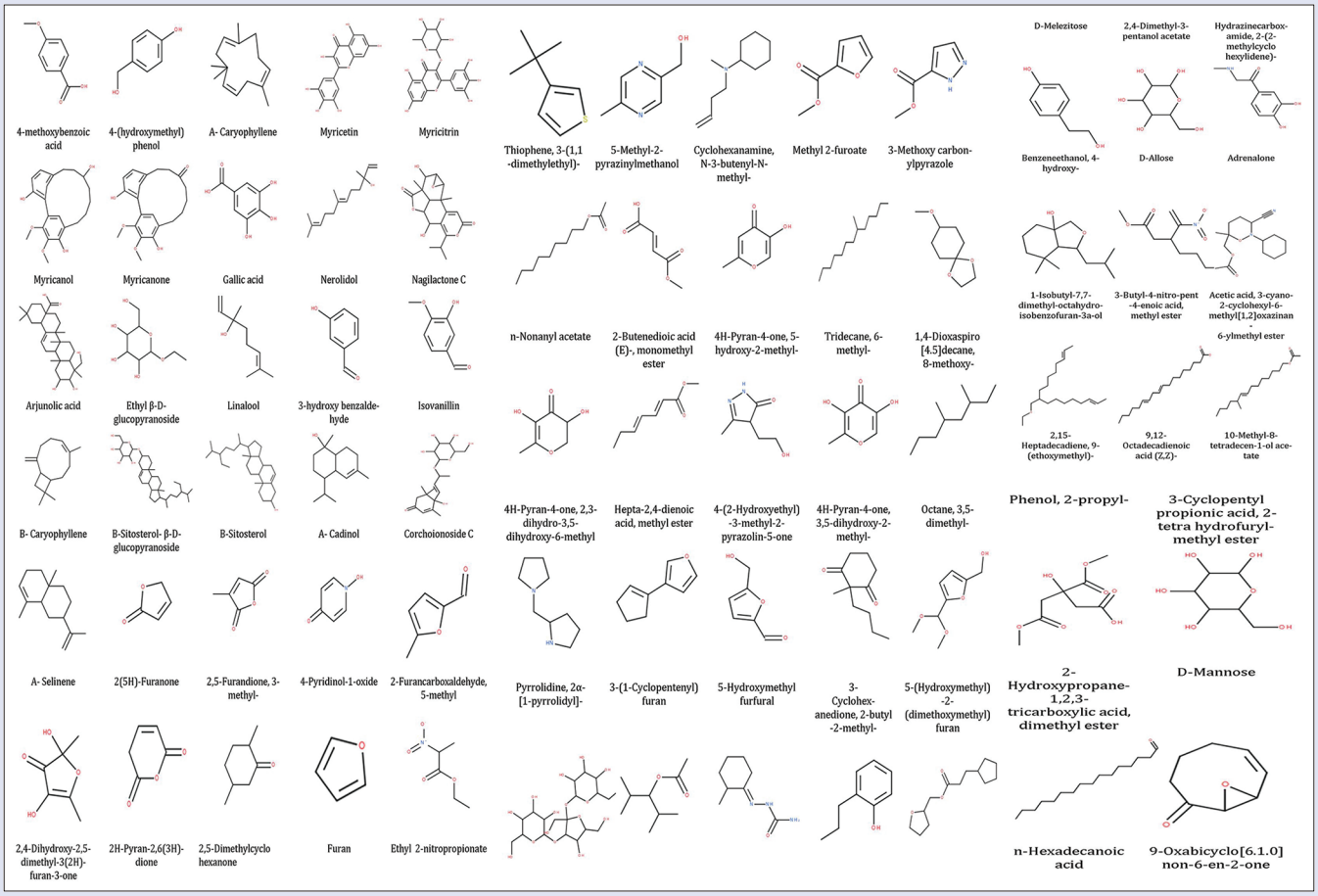

Figure 1: Structures of 68 phytochemical dataset from *Myrica nagi*

The third term in the equation represents dihedrals (a. k. a. torsion angles) where k_ϕ implies dihedral force constant, n is function multiplicity, ϕ indicate dihedral angle and δ is phase shift.

The fourth expression represents impropers, which shows out of plane bending, where k_ω denotes force constant and $\omega - \omega_0$ shows out of plane angle.

The Urey-Bradley element contains the next term, where K_U displays respective force constant and U represents the harmonic potential of distance between 1 and 3 atoms. Nonbonded synergies between pairs of atoms (i, j) are shown by the last two expressions.

By denotation, the non-bonded forces are only applied to atom pairs apportioned by at least three bonds. The Van Der Waals energy is determined with the help of standard 12–6 Lennard-Jones (L J) potential and with Coulombic potential's electrostatic energy. In the equation mentioned above, Lennard-Jones potential is given by $R_{\min_{ij}}$ and is not the minimum of the potential, while L J potential crosses the x-axis (i.e., where the potential is zero). To run the molecular dynamics simulations, the parameter file has all the specified terms in the energy function (i.e., the equation denoted above).

AutoDock was performed in four steps:

1. Coordinate files preparation, where PDB, partial charge, and atom type format (PDBQT) files from traditional PDB files
2. Precalculation of atomic grid affinities of the ligands and embedding of protein in a three-dimensional (3D) grid
3. Docking of ligands that is performed using one of the most efficient method, namely LGA
4. Result analysis is performed by clustering the solutions of COX-2 enzyme and ligands interaction.[30] Figure 2 depicts the graphical representation of different methods or approaches used in our study.

Prediction of bioactivity for the selected ligands

Molinspiration was used to determine bioactivity of the selected ligands. It analyzes physicochemical molecular descriptors for a given ligand; such as, ion channel modulator, G-protein coupled receptors ligand, kinase inhibitor, nuclear receptor ligand, and enzyme inhibitor (http://www.molinspiration.com).

Pharmacokinetics and toxicity analysis

Ligands that inhibit the receptors cannot be extrapolated as good drug candidate, unless the ligand has good bioavailability and desirable duration of action. For the identification of possible effects of ligand molecules in virtual, the absorption distribution metabolism excretion-toxicity (ADMET) properties of the compounds were inferred using an online freeware, AdmetSAR (http://http://lmmd.ecust.edu.cn/admetsar1).[34]

Determination of cell cytotoxicity

Cell Seeding

For adherent cells, 100–200 µl of desired cell suspension (RAW-264.7) in a 96-well plate at a cell density (25,000–50,000 cells per well), without the test agent were seeded and allowed the cells to adhere to the plate for 24 h.

Incubation

The plate was incubated for the required period at 37°C in a 5% CO_2 atmosphere. After 24 h, the growth medium was removed. 100 µl of freshly prepared five different concentrations (300 µg, 100 µg, 10 µg, 1 µg, and 0.1 µg) of purified myricetin were seeded in 96-well plate in triplicate.

Cytotoxicity assay

After the incubation, plates were removed from the incubator and 10% of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent was added to the total volume. This volume was maintained as same as the volume used while determining optimum cell density. The plate was wrapped with aluminum foil to avoid exposure to light and re-incubated for 2–4 h. For adherent cells, aspiration of the culture medium without disturbing the monolayer was carried out. Then, solubilization solution was added in an amount equal to the culture volume and was stirred gently in a gyratory shaker to enhance dissolution. The absorbance was read using spectrostar Nano ELISA Plate reader at 570 nm and inhibitory concentration (IC_{50}) value was carried out.[23]

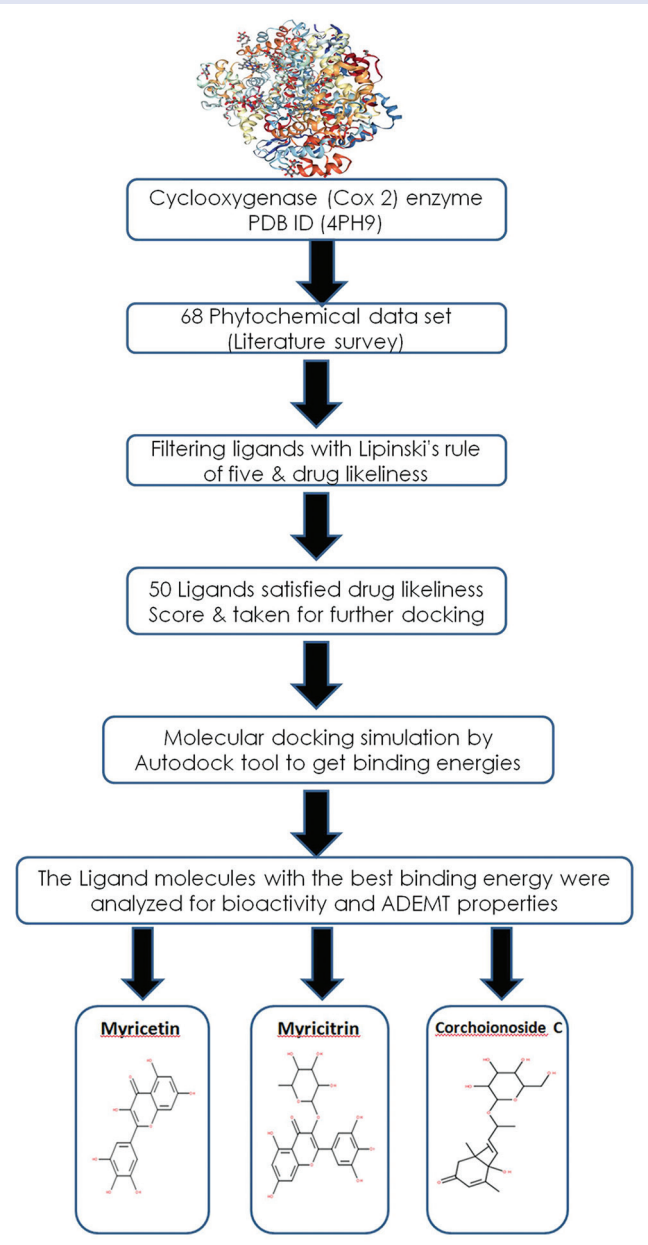

Figure 2: Flowchart of the methodology used in the study

RESULTS AND DISCUSSION

Structure of the target protein

RCSB is a global repository for 3D structure of macromolecules (proteins and DNA) and their complexes, resolved by X-ray crystallography, NMR spectroscopy, and cryoelectron microscopy.[9,35] In the current study, the X-ray crystallographic structure of COX-2 enzyme[36] bound with IBP was retrieved from the PDB with PDB ID: 4PH9[25] and has been exploited as the therapeutic target.

Drug likeliness of phytochemicals

During the initial part of the study, we hypothesized drug likeliness for all the 68 phytochemicals using Molsoft tool. Majority of ligand molecules showed positive for drug likeliness and did not deviate much with Lipinski's rule. Threshold values >−1.05 [Table 1] were considered significant and 15 ligands were selected for further analysis. Biomolecular properties of these phytochemicals were also investigated which is essential for rational drug design.

Virtual screening and docking

Docking explicitly with AutoDock is useful in various situations in rational drug discovery, namely screening thousands of phytochemicals or ligand molecules against specific target protein, screening a small set of ligand molecules against multiple protein molecules, active site sampling, and ligand–protein interaction prediction.[31]

In the current study, docking simulation was carried out using AutoDock 4.2. The docking process is performed using one of the most efficient method namely LGA. AutoDock 4.2[37] is frame worked to use an extended PDB format, namely PDBQT, for coordinate files. In AutoDock 4.2, force field is applied differently on aromatic and aliphatic carbon atoms and different for polar and nonpolar atoms. PDBQT files also have information on torsional degrees of freedom for all the atoms of protein. The graphical user interface for AutoDock is used for creating PDBQT files from traditional PDB files.

Selected receptor (COX-2) was prepared for docking process with the help of different modules present in AutoDock. Commercially available IBP was used as standard in the current study. During the AutoGrid

Table 1: Top 15 phytochemicals selected based on drug-likeness score in Molsoft tool

Compound names	Drug likeliness model score
β-Sitosterol	0.88
Myricitrin	0.78
Arjunolic acid	0.59
Myricanol	0.57
β-Sitosterol-β-D-glucopyranoside	0.51
Corchoionoside C	0.33
Myricanone	0.28
Ethyl β-D-glucopyranoside	0.09
Gallic acid	0.07
Myricetin	−0.04
Nagilactone C	−0.27
α-Cadinol	−0.35
3-Cyclohexanedione, 2-butyl-2-methyl-	−0.59
α-Selinene	−0.77
Nerolidol	−1.03

process, the protein is embedded in a 3D grid; affinity grids are estimated for each type of atom in the ligand; such as, carbon, hydrogen, oxygen, and nitrogen and also for electrostatic and de-solvation potentials. The energetics of a specific ligand pose is calculated using the values obtained from the grids.[31]

AutoDock was run multiple times to obtain different docked conformations or poses. The conformations obtained after docking are sorted based on the order of increasing energy (kcal/mol) and the lowest energy (kcal/mol) conformation is seen in the first frame [Table 2].[38]

The threshold value was decided with respect to binding energies (kcal/mol) of different receptor-ligand complexes. The best docked ligand poses of 15 out of 68 phytochemicals [Figure 1] were then ranked according to their binding energies with COX-2 enzyme. The current study revealed that the IBP showed the binding energy of −7.99 kcal/mol. Table 2 indicates top 10 ligands exhibiting docking energy values above −4.90 kcal/mol. Hence, these ligand molecules were selected for further investigation as they exhibited good drug likeliness and ranked based on high to low docking energy scores: α-Selinene > β-sitosterol > α-cadinol > β-sitosterol-β-D-glucopyranoside > 3-cyc lohexanedione,butyl-2-methyl- > myricetin > nerolidol >myricanol > myricitrin > corchoionoside-C >myricanone. α-Selinene has emerged as most prospective drug candidate that can be further evaluated and enforced against COX-2 enzyme.

In addition, one need not trust completely the docking predictions. Instead, one should evaluate if the generated docked conformations are realistic or not. That is, there are no negative or positive charged groups nearby to aliphatic or neutral regions. α-Selinene was ranked first and thirteenth based on binding score and drug likeliness score, respectively, and it did not have any residues interacting within the active site of the receptor. However, β-sitosterol, α-cadinol and β-sitosterol-β-d-gluco pyranoside, 3-cyclohexanedione, 2-butyl-2-methyl-, and myricanone had good binding scores as well as drug likeliness scores. However, it did not display much impressive bioactivity [Table 3] and ADMET properties [Table 4]. Hence, it has been eliminated from further analysis.

Table 2: Top 10 molecules with their binding energy using AutoDock

Compound	Docking score (kcal/mol)
α-Selinene	−7.89
β-Sitosterol	−7.52
α-Cadinol	−7.43
β-Sitosterol-β-D-glucopyranoside	−6.91
3-Cyclohexanedione, 2-butyl-2-methyl-	−6.62
Myricetin	−6.52
Nerolidol	−6.13
Myricanol	−5.27
Myricitrin	−4.94
Corchoionoside C	−4.94
Myricanone	−4.90

Absorption distribution metabolism excretion-toxicity analysis

The ADMET parameters were evaluated using knowledge-based online server AdmetSAR. Myricetin and myricitrin are predicted to display high human intestinal absorption with a probability of 0.96 and 0.90, while corchoionoside-C displayed poor intestinal absorption. Blood–brain barrier (BBB) is a regulatory system that separates the brain from the

Table 3: Bioactivity of selected ligand molecules using molinspiration tool

Compound name	BBB	HIA	Caco2 permeability	CYP inhibitory promiscuity	Ames toxicity	Acute oral toxicity	Aqueous solubility (Log S)	Rat acute toxicity LD_{50}, (mol/kg)	Subcellular localization
α-Selinene	+	+	+	Low	0.834	III	−5.3561	1.5125	Lysosome
β-Sitosterol	+	+	+	Low	0.9132	I	−4.7027	2.6561	Lysosome
α-Cadinol	+	+	+	Low	0.9157	III	−3.909	2.2009	Lysosome
β-Sitosterol-β-D-glucopyranoside	+	+	-	Low	0.7989	III	−4.4041	2.9113	Mitochondria
3-Cyclohexanedione, 2-butyl-2-methyl-	+	+	+	Low	0.8808	III	−2.2288	2.0728	Mitochondria
Myricetin	-	+	-	High	0.722	II	−2.9994	3.02	Mitochondria
Nerolidol	+	+	+	Low	0.9185	III	−3.1456	1.6795	Lysosome
Myricanol	+	+	+	Low	0.511	III	−3.6068	2.3347	Mitichondria
Myricitrin	-	+	-	Low	0.9319	III	−3.4974	2.5458	Mitochondria
Corchoionoside C	-	-	-	Low	0.5759	III	−2.3613	2.3884	Mitochondria
Myricanone	+	+	+	Low	0.5555, toxic	III	−3.5029	2.44	Mitochondria

BBB: Blood-brain barrier (+: permeability; -: non permeability); HIA: Human intestinal absorption (+: Absorption; -: non absorption); CYP: Cytochrome P 450; Caco2 permeability (+: permeability; -: non permeability)

Table 4: Hypothetical absorption distribution metabolism excretion-toxicity properties of selected ligands

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
α-Selinene	−0.24	0.12	−0.97	0.34	−0.51	0.28
β-Sitosterol	0.14	0.04	−0.51	0.73	0.07	0.51
α-Cadinol	−0.09	0.05	−0.87	0.39	−0.63	0.4
β-Sitosterol-β-D-glucopyranoside	0.15	−0.21	−0.47	0.33	0.11	0.41
3-Cyclohexanedione, 2-butyl-2-methyl-	−0.63	−0.21	−1.32	−0.32	−0.76	−0.04
Myricetin	−0.06	−0.18	0.28	0.32	−0.2	0.3
Nerolidol	−0.17	0.21	−0.64	0.42	−0.43	0.39
Myricanol	0.27	0.20	0.07	0.37	0.15	0.27
Myricitrin	−0.2	−0.08	0.08	0.14	−0.06	0.38
Corchoionoside C	0.21	0.26	−0.32	0.45	0.14	0.7
Myricanone	0.12	0.05	−0.23	0.2	0.02	0.16

GPCR: G-protein coupled receptors

direct contact of blood in the circulatory system, thus safeguarding the brain from unwanted particles.[36] All the three molecules were predicted to be BBB negative, assuring that its administration is safe for the brain. These ligands also showed to be noncarcinogenic with some amount of oral, AMES, and rat toxicity.

Hydrogen bonding and electrostatic interactions are considerable driving forces and act in placing the ligand molecule in unanticipated binding pose. There are various metrics for determining whether docked conformations for a given ligand are "good" or "bad." These can be explored and explained by RMSD, free energy of binding (kcal/mol) and many more. One can also look for the presence or absence of different potential interactions to understand the binding mechanism.[39]

Molecular docking simulation

Molecular docking simulation revealed the interacting residues in the binding site of COX-2 enzyme [Table 5]. For IBP, residues such as ARG121 and TYR356 provided only three hydrogen bonds; α-selinene, which showed the second best binding energy next to standard drug IBP, did not have any interaction residues. However, myricetin showed interactions with HIS90, GLN193, MET523, and SER531 providing four hydrogen bonds. Another flavonoid myricitrin [Figure 3] interacted with HIS90, ARG121, TYR356, TYR386, ILE518, PHE519, and SER531 with nine hydrogen bonds. However, Corchoionside-C provides four H-bonds by interacting with residues such as ARG 121, TYR 356, and VAL 524. Figure 4 shows the ligplot and residues interacting in the binding site of COX-2 enzyme for the selected ligands. These interactions clearly display that the molecules interact with conserved amino acids in COX-2 binding pocket and hence, apprehend to inhibit the functionality of the target enzyme.

Determination of cell cytotoxicity by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

IC_{50} value of myricetin was carried out using MTT cytotoxicity assay to obtain percentage of cell viability and also percentage of inhibitory concentration (%IC) for each dilution. As the concentration of drug increases the %IC of RAW-264.7 cells also increased from 6.5% to 64.6%, respectively. The IC_{50} value of myricetin was found to be 102 μg/mL.

CONCLUSION

Myricetin occurs throughout the plant kingdom as it is synthesized primarily by members of the families *Anacardiaceae*,[40] *Myricaceae*,[41] *Primulaceae*,[42] *Polygonaceae*,[43] and *Pinaceae*.[44]

Myricetin, a flavanol (subclass of flavonoids) derived from *M. nagi* leaves, has lot of pharmacological activities, such as antimicrobial, antioxidant, and anticancerous.[45] It is extensively studied *in vitro* for their various properties, namely, anti-inflammatory, anti-angiogenesis, enzyme activation, anti-proliferation activity, anti-invasiveness, and induction of apoptosis. *In vitro* studies have also shown that myricetin regulates cancer initiation and development. Myricetin showed antiviral and antibacterial properties against various organisms. D'Souza *et al.*[46] proved myricetin to be very active at a concentration of 30 μg/mL against *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa,* and *Shigella flexineri.* Antitubercular activity was evaluated exposing myricetin to *Mycobacterium tuberculosis.* A structure–activity relationship proved that the responsible factor for this is hydroxyl groups present in the structure of myricetin.[47] Myricetin showed strong inhibition of reverse

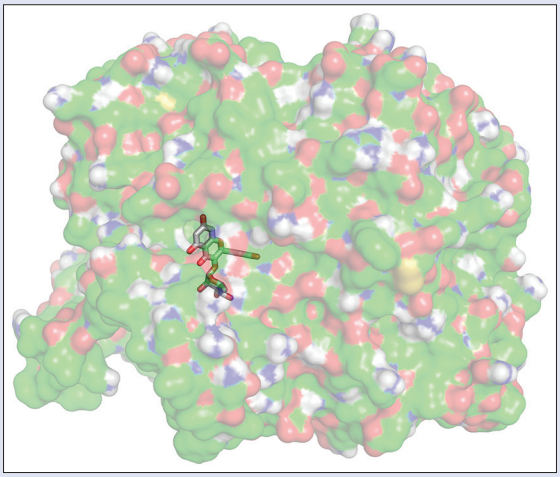

Figure 3: Surface view of cyclooxygenase-2 (Protein Data Bank ID: 4PH9) with myricitrin in the binding site

Table 5: Number of H-bonds and its distances in best selected molecule based on their energies

Compound	Number of hydrogen bonds	Interacting residues	H-bond distance (Å)
Myricitrin	09	O10 ATOM -OG (SER 531)	2.63
		O9 ATOM -OH (SER 531)	3.30
		O9 ATOM -OG (SER 531)	2.50
		O12 ATOM -N (PHE 519)	2.97
		O12 ATOM -N (ILE 518)	2.94
		O7 ATOM -OH (TYR 356)	3.24
		O7 ATOM -NH2 (ARG 121)	2.91
		O7 ATOM -NE (ARG 121)	3.11
		O11 ATOM -NE2 (HIS 90)	2.97
Myricetin	04	O8 ATOM -O (MET 523)	3.06
		O7 ATOM -OG (SER 531)	2.75
		O5 ATOM -OE1 (GLN 193)	2.55
		O4 ATOM -NE2 (HIS 90)	2.73
Corchoionoside-C	04	O8 ATOM -O (VAL 524)	3.24
		O2 ATOM -OH (TYR 356)	3.01
		O3 ATOM -NE (ARG 121)	2.73
		O3 ATOM -NH2 (ARG 121)	3.07
Ibuprofen (standard)	03	O ATOM -O (TYR 356)	2.42
		O ATOM -N (ARG 121)	3.01
		O ATOM -N (ARG 121)	2.97

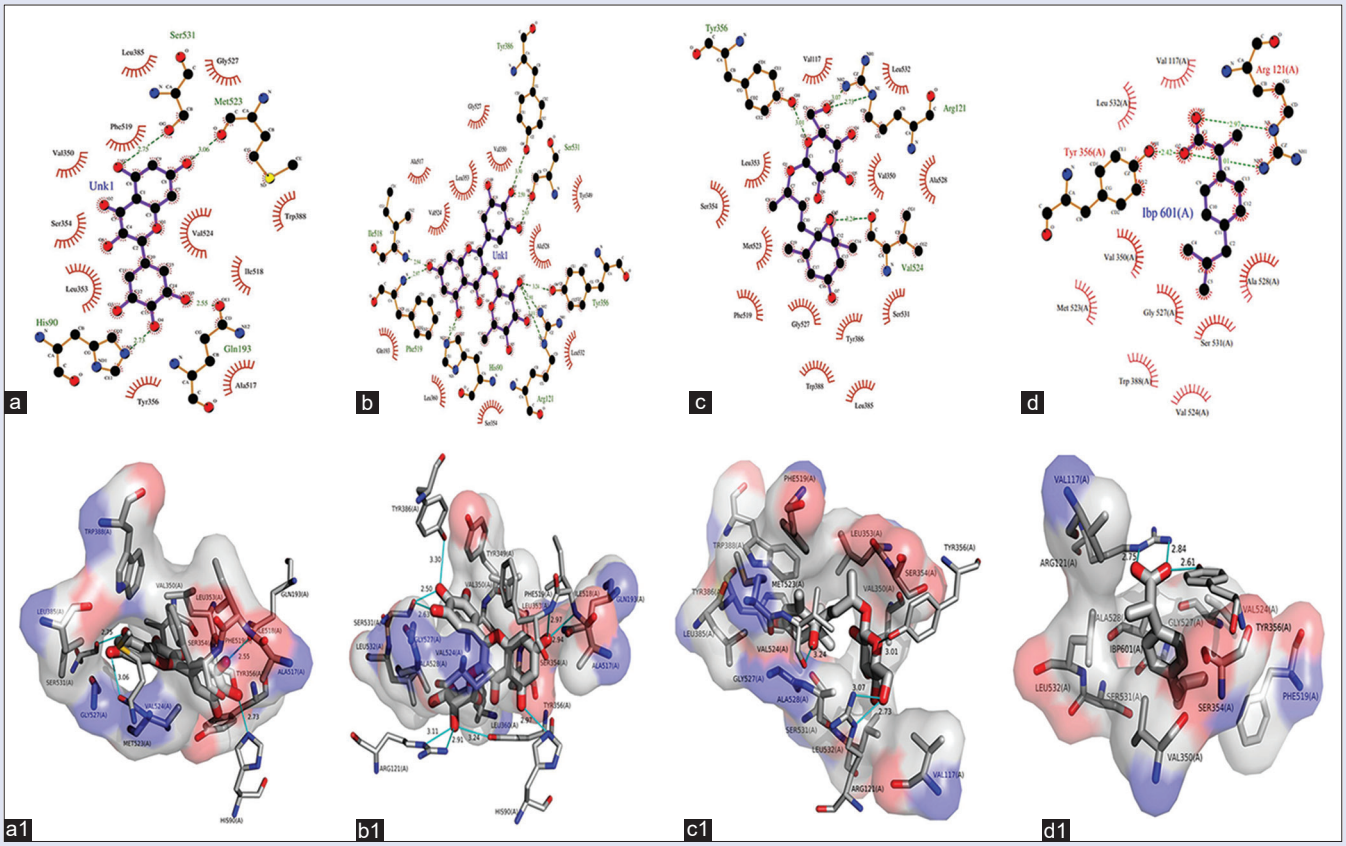

Figure 4: Ligplot of myricetin (a), myricitrin (b) and corchoionoside-C (c) and residues interacting in the binding site for myricetin (a. 1), myricitrin (b. 1) and corchoionoside-C (c. 1) of cyclooxygenase-2 enzyme for the best selected ligands along with the standard ibuprofen (d and d. 1)

transcriptase from human immunodeficiency virus, and Rauscher murine leukemia virus. Furthermore, the flavonoid inhibited DNA polymerase I and DNA polymerase-α.[48]

Myricetin fulfills all the drug likeliness and ADMET properties and emerges as one of the most potent anti-inflammatory agents in our analysis. The myricitrin exhibits good binding ability with poor bioactivity which can be modulated in *in vitro* and *in vivo* studies.

Overall, we conclude that the myricetin possess the desired properties to be the potent anti-inflammatory drug against human COX-2 enzyme for the treatment of acute and chronic inflammation process. Cytotoxicity activity of myricetin from *M. nagi* using MTT assay showed IC_{50} value as 102 µg/mL proving its potential as probable anti-inflammatory drug candidate.

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Conflicts of interest

There are no conflicts of interest.

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