

High-Throughput Screening by *In silico* Molecular Docking of *Eryngium Foetidum* (Linn.) Bioactives for Cyclooxygenase-2 Inhibition

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ABSTRACT

Several studies are in progress worldwide to find natural healing agents with better safety profiles. Our current study aimed to screen and evaluate *Eryngium foetidum* Linn. bioactives reported for therapeutic drug discovery by *In silico* docking. Ligands /bioactives were prepared by following the appropriate procedures and finally *In silico* molecular docking to cyclooxygenase (COX)-2 was performed and analyzed by FleX X. Of the docked bioactives, caryophyllene oxide in particular, showed high binding affinity of -7.00 kcal/mol against 1PXX corroborating *in vitro* COX-2 inhibition and providing a theoretical contribution in understanding the ligand-protein interactions. The docked pose resembled the orientation similar to that observed with diclofenac ligand (inhibitor of COX-2). The ligand was docked deeply within the binding pocket region forming interactions with ALA₅₂₇-GLY₅₂₆-LEU₃₅₂-SER₅₃₀-TYR₃₄₈-TRP₃₈₇-VAL₃₄₉-VAL₅₂₃ and Ser353. Our docking result was found to have three hydrogen bonding sites with SER₅₃₀-TYR₃₄₈ and TYR₃₈₅, indicating COX-2 inhibition with the highest fitness score of 50.64. Furthermore, all the bioactives were subjected to

iLOG predictor of the Swiss ADMET website software generating *In silico* ADME properties and testing their capacity to exhibit drug likeliness. The data supports caryophyllene oxide to be a potent anti-inflammatory compound worthy of further clinical trials.

Key words: *Eryngium foetidum*, Swiss Dock, cyclooxygenase-2 inhibitor, iLOG predictor of the Swiss ADMET, Caryophyllene oxide.

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INTRODUCTION

Inflammation is the defense response of the body, characterized by redness, swelling, heat, pain and loss of function to eliminate or limit the spread of an injurious agent. Cyclooxygenases (COX) or prostaglandin endoperoxide synthase (PGHS) exists in two isoforms COX-1 and COX-2, which are key enzymes in the synthesis of prostaglandins and the main mediators of inflammation, pain and increased body temperature (hyperpyrexia).¹ Given the need for more effective and/or less toxic pain therapies, a great deal of emphasis has been placed on identifying novel molecular targets that could provide new analgesics. Natural products, including medicinal plants, have been the primary source for obtaining new drugs with therapeutic potential throughout history. It is estimated that approximately half of the drugs in use are derived from natural products. According to the World Health Organization (WHO), poverty and lack of access to modern medicine leads the 65% to 80% of the world population in developing countries to critically depend on plants for primary health care.²⁻⁴

Eryngium foetidum Linn., a tropical perennial and annual herb of the family Apiaceae with a pungent unique aroma is widely used in herbal medicines and is beneficial in the treatment of a number of ailments.^{5,6} Studies have reported the use of *E. foetidum* in treatment of several anti-inflammatory disorders. Garcia *et al*⁷ evaluated a hexane extract rich in bioactives for anti-inflammatory capacity. The topical anti-inflammatory activity of n-hexane extract was evaluated for anti-inflammatory capacity in auricular oedema in mose as both single and multiple applications. It reduced oedema both in acute and chronic models and decreased meloperoxidase activity. Aqueous leaf extract suppressed pro-inflammatory capacity in Caco-2 cells and exhibited a modest antioxidant effect. The topical anti-inflammatory activity of n-hexane extract when evaluated

by auricular oedema in mouse as both single and multiple applications of phlogistic agent and a reduced oedema both in acute and chronic models with decreased meloperoxidase activity indicated the capacity of this herb against topical inflammatory processes. An aqueous leaf fraction suppressed pro-inflammatory capacity with modest antioxidant effect in Caco-2 cells. Monocyte chemo attractant protein-1 (MCP-1), interleukin (IL)-8 and reactive oxygen species were suppressed to various degrees by *E. foetidum* phytochemicals partitioned in mixed micelles.⁸

High-throughput “absorption, distribution, metabolism and excretion” (ADME) screening as an effective paradigm for filtering compounds for drug discovery process,^{9,10} employs the prediction of binding modes and binding affinities of each compound in the dataset by means of docking to an X-ray crystallographic structure.¹¹ Various studies reported in the literature state the importance of dataset size such as 10,000 compounds using FlexX,¹² 44,000 compounds using Surfflex,¹³ among others. Therefore, an alternative approach is to eliminate unpromising compounds before docking by restricting the dataset to drug-like compounds by filtering the dataset based on appropriate property and sub-structural features and by performing diversity analysis. These approaches can be highly effective in reducing the dataset to be docked to the order of 10³ to 10⁴ compounds.¹⁴

In the present study, screening of a hundred and six *E. foetidum* compounds for anti-inflammatory activity was attempted using Swiss ADME software. Docking of the identified bioactives to the target site in the protein and further scoring was achieved using the Flex X tool to evaluate the binding in terms of score on crystal structure of COX-2 1PXX. One of the steps in designing COX-2 inhibitors is to utilize the three dimensional structural information of the target molecule. Protein

Data Bank (PDB) hosts a few of the COX-2 crystal structures like ICVU (PDB ID) with bound substrate arachidonic acid, 1PXX bound to inhibitor diclofenac (DIF) and 6COX bound to SC-558.¹⁵ Along with providing a framework for the design of novel COX-2 inhibitors, these offer an insight substrate specificity and the mechanism of COX-2 enzymatic reaction.

MATERIALS AND METHODS

Inhibition of COX2

Colorimetric COX (ovine) inhibitor screening assay kit (Cayman Chemical, catalog No. 760111) was used to measure enzyme activity and inhibition according to the manufacturer's protocols. Celecoxib, standard inhibitors of COX2 was tested as positive controls. Caryophyllene oxide, menthol, methyl acetate and α -terpinyl acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used for COX inhibition studies.

Docking

Ligand preparation for docking

A set of one hundred and six bioactives identified in *Eryngium foetidum* Linn. was screened. However, as few as five: caryophyllene oxide, cedrane, menthol, methyl acetate, daucol and α -terpinyl acetate were finalized for docking. The 3D-coordinates for these compounds in the PDB format were obtained through drawing window of chemsketch and further explored for gross biological activity comprising of Lipinski rules of 5, drug likeness and drug score.

Molecular docking

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

Target protein minimization

The protein 1PXX (Figure 1a) was loaded into the prepare molecule module of the Biosolve IT software. Lead IT chain A of the protein was selected for preparation and docking. The binding site comprised of the binding pocket where the reference ligand, diclofenac (DIF) is bound in this particular chain (Figure 1b). The energy of this binding site was minimized, and the atomic coordinates of the amino acids of this binding pocket were converged, and the protein was thus prepared for docking.

Active Site prediction

From the binding site analysis of 1PXX it was observed that binding pockets were identified and the largest binding pockets were selected for docking studies. Possible binding residues of receptor was searched and they were PHE₅₁₈, ALA₅₂₇, TRP₃₈₇, MET₅₂₂, TYR₃₄₈, TYR₃₈₅, TRP₃₈₇, TRP₃₈₅, VAL₃₄₉, LEU₃₅₂, VAL₅₂₃, GLY₅₂₆, SER₅₃₀, SER₅₂₃, SER₅₅₃, SER₅₃₀ and GLY₅₂₆.

Drug likeliness evaluation by Swiss ADME

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

RESULTS

Inhibition of COX2 activity

The bioactives caryophyllene oxide, menthol, methyl acetate, α - terpinyl acetate all significantly inhibited COX-2 enzymatic activity (Figure 2). However, caryophyllene oxide was the most potent inhibitor with $IC_{50}=1.75\text{ }\mu\text{g.mL}^{-1}$, in comparison to celecoxib with $IC_{50}=0.5\text{ }\mu\text{g.mL}^{-1}$ at ($p<0.0001$) (Figure 2).

In silico docking of bioactives from *Eryngium foetidum* Linn. and their binding modes

Structure and function relationships of *Eryngium foetidum* Linn. compounds with diclofenac were evaluated for their biological activity against the COX-2 using the 3D structure of the receptor retrieved from protein data bank site of COX-2 enzyme (PDB code: 1PXX; Figure 1a). The docked binding mode was established to link the docking score function with these selected compounds and protein. For a control study, inhibitor diclofenac (DIF) were docked to the protein, an exercise that resulted in reproducing the crystal structure (Figure 3a) poses for this compound. Furthermore, analysis of the binding pattern between COX-2 protein and ligands suggested that the binding pattern also varied with the ligand nature; caryophyllene oxide docked onto COX-2 protein (Figure 1b); cedrane docked to COX-2 protein (Figure 1c; 3b); menthol docked to COX-2 protein (Figure 1e); methyl acetate docked to COX-2 protein (Figure 1c; 3c); daucol docked to COX-2 protein (Figure 1f; 3d) and α -terpinyl acetate docked to COX-2 (Figure 3e). Out of all the docked compounds, caryophyllene oxide showed the highest binding affinity of -7.00 kcal/mol to 1PXX. The docked pose resembles the orientation observed with the diclofenac ligand. The ligand was docked deeply within the binding pocket region, forming interactions with ALA₅₂₇, GLY₅₂₆, LEU₃₅₂, SER₅₃₀, TYR₃₄₈, TRP₃₈₇, VAL₃₄₉, VAL₅₂₃ and Ser353 (Figure 3b).

The results of all compounds were established by Flex X scoring parameter (Table 1). The compound that obtained the highest score was further subjected to analysis. The binding mode was compared with standard COX-2 inhibitor, diclofenac. To the crystal structure of 1PXX, the inhibitor diclofenac is bound to all four chains of the structure (Figure 3a), but none of the binding sites come in the interface of two domains of two different chains. An essential feature of the original binding site is the conservation of hydrogen bonding residues and the aromatic stacking interactions. These binding modes were also observed for the compounds in the study.

The binding energies of the caryophyllene oxide, cedrane, menthol, methyl acetate, daucol and α -terpinyl acetate with 1PXX were -7.00, -6.74, -6.76, -6.52, -6.62 and -6.26 respectively in comparison to COX-2 inhibitor diclofenac with binding energy of -7.75 (Table 1). Furthermore, the molecules were subjected to the iLOG predictor of the Swiss ADMET website software generating *In silico* ADME properties reporting the various parameters for drug like characteristics such as Lipinski's rule of 5, pharmacophoric groups attached on the ligand, size of the dataset and compound libraries among others (Table 2). It also substantiated the above bioactives to be safe. Therefore, it is suggested that suitable research could be carried out with the reported bioactives and further studies would declare the possibility of such studies in identifying the anti-inflammatory drug candidate.

DISCUSSION

High throughput screening methods are routinely and extensively used to reduce the cost and time of drug discovery. An increase in the number of molecules reported in a typical drug discovery program necessitates the need for *In silico* determinations of ADME to be regularly generated with the studies. The present study clearly demonstrates that the

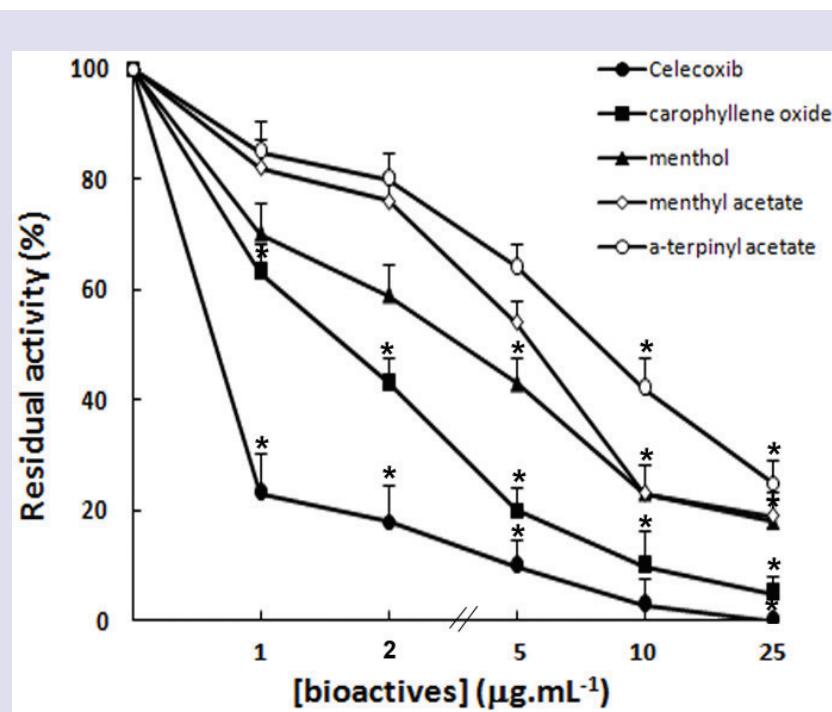
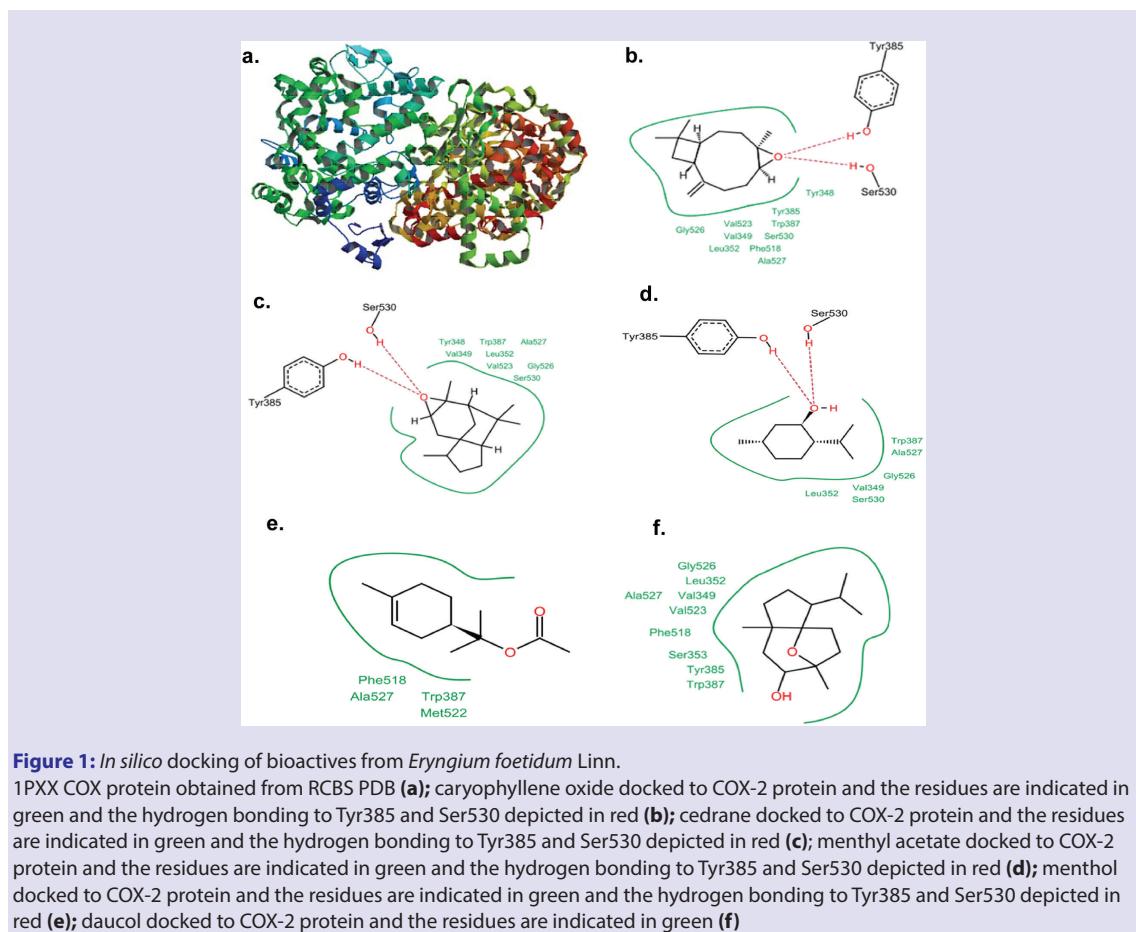


Figure 2: Inhibition of COX-2 activity. Bioactives from *Eryngium foetidum* Linn., caryophyllene oxide, menthol, menthyl acetate and a - terpinyl acetate was measured using a COX-2 enzyme inhibitor assay. Residual COX-2 activity (%) = (RLU_{sample}/RLU_{control}) – 100. Data from three experiments are expressed as mean ± SEM. Significant inhibition is indicated by * with a *p* value <0.0001.

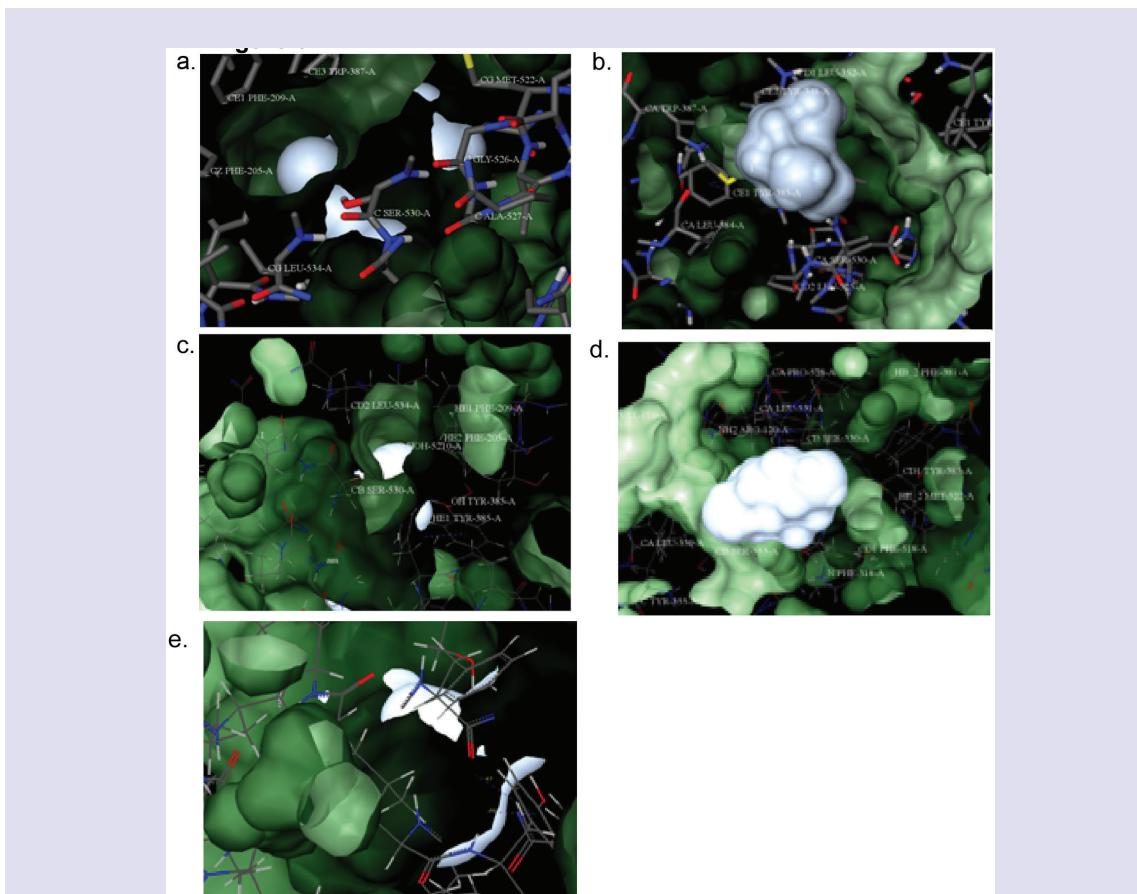


Figure 3: Binding modes of four bioactives from *Eryngium foetidum* Linn., diclofenac docked onto 1PXX protein (a); cedrane docked to COX-2 protein (b); menthol acetate docked to COX-2 protein (c); daucol docked to COX-2 protein (d); a-terpinyl acetate docked to COX-2 protein (e). Ligands are shown in white module and the site residues in stick representation in all panels.

Table 1: Swiss ADME data of the following compounds

Name of the compound	Log S ESOL	No. of H bond acceptors	No. of H bond donors	GI tract absorption	Lipophilicity Qlog Po/w	Lipinski drug likeliness
Carophyllene oxide	-3.45	1	0	High	3.15	0 violations
Cedrane	-4.69	0	0	High	3.23	I violation
Menthol	-2.88	1	1	High	2.53	0 violations
Menthyl acetate	-2.73	2	0	High	2.73	0 violations
a-terpinyl acetate	-3.35	2	0	High	2.92	0 violations
Daucol	-2.75	1	0	High	2.53	0 violations

The Lipinski, Ghose, Veber, Egan and Muegge rules for drug-like molecules have also approved the ligand. Gastro-intestinal (GI) tract absorption is high and bioavailability of the ligand resulted in the partition coefficient (Q_{Plog} Po/w) ranges from -2.0 to 6.5 and water solubility (Q_{Plog} S) i.e., critical for estimation of absorption and distribution of drugs within the body, ranged between -6.5 and 0.5. Topological Polar Surface Area of the ligand is also appreciable. All these pharmacokinetic parameters are within the acceptable range signifying the ligand to be a typical drug molecule.

In silico approach utilized was successful in finding novel COX-2 inhibitors from *Eryngium foetidum*. Initial screening with a set of 106 compounds resulted in six potential bioactives suitable for further docking studies. This demonstrates the value of this technological approach for screening COX-2 inhibitors. Caryophyllene oxide had a particularly high binding affinity of -7.00 kcal/mol to 1PXX and thus can be proposed to have anti-inflammatory capacity. Our docking result was found to having three hydrogen bonding with SER₅₃₀ TYR₃₄₈ and TYR₃₈₅, demonstrating specific COX-2 inhibition.

CONCLUSION

High-throughput screening using Swiss ADME followed by molecular docking using FleX X has proved to be useful in finding some possible lead compounds. The docked poses resemble similar orientations as observed with the inhibitor diclofenac (DIF) binding in 1PXX. Caryophyllene oxide bound with the lowest energy and largest size in the cluster and had hydrogen bonding contacts of S530 and Y385 in the site which are very crucial for COX-2 inhibition. This compound is therefore a promising lead compound. The Flex X docking score determined in this

Table 2: Docking scores for the most potent bioactives from *Eryngium foetidum*

Name of the compound	Full fitness (Kcal/mol)	Binding energy (ΔG Kcal/mol)	Total Score	Match score	Lipo score	Ambig score	Clash score	Rot score
Diclofenac	-2060.34	-7.75	-18.6534	-21.8448	-14.1317	-4.0843	6.0723	2.8000
α -terpinyl acetate	-2368.58	-6.26	-9.9832	-12.9755	-7.7962	-3.1035	4.2921	4.2000
Methyl acetate	-2342.90	-6.52	3.9960	-0.7000	-3.9441	-1.0957	0.1359	4.2000
Menthol	-2350.40	-6.76	-5.9171	-9.3022	-8.0456	-2.5609	5.7914	2.8000
Daucol	-2285.96	-6.62	1.8769	-1.4000	-10.2143	-3.6533	8.9455	2.8000
Cedrane	-2308.20	-6.74	-8.9108	-8.2686	-11.9434	-3.2803	9.1786	0.0000
Caryophyllene oxide	-2290.18	-7.00	-12.6983	-9.4823	-12.0014	-3.0272	6.4126	0.0000

Description of the score:

Total Score (E_TOTAL) Total score of the docking solution.

Match Score (E_MATCH) Contribution of the matched interacting groups.

Lipo Score (E_LIPO) Contribution of the lipophilic contact area.

Ambig Score (E_AMBIG) Contribution of the lipophilic-hydrophilic (ambiguous) contact area.

Clash Score (E_CLASH) Contribution of the clash penalty.

Rot Score (E_ROT) Ligand conformational entropy score.

study can be correlated with the biological activities. Some false positives and false negatives were observed, but considering the limitations of the available docking program, the results are encouraging. The detailed analysis of the resulted COX-2-ligands may improve our knowledge in understanding the binding interactions in detail. The most potent derivatives in this study could be subjected to further pharmacological evaluations to develop highly potent anti-inflammatory drugs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATION USED

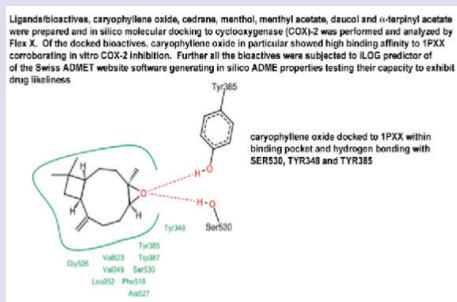
COX-2: Cyclooxygenase-2; Flex X: software package to predict protein ligand interactions; 1PXX, crystal structure of diclofenac bound to the cyclooxygenase active site of COX-2; ADME(T), absorption, distribution, metabolism and excretion-toxicity; sc-558, COX-2 selective inhibitor.

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PICTORIAL ABSTRACT



ABBREVIATIONS

Cyclooxygenase-2 : COX-2; **ADME:** absorption, digestion, metabolism, excretion; **ADMET:** absorption, distribution, metabolism & excretion-toxicity

SUMMARY

- Studies to find natural healing agents with better safety profiles are present day concern.
- Our current study aimed to screen and evaluate *Eryngium foetidum* Linn. bioactives reported for therapeutic drug discovery by *in silico* docking and analyzed by FleX X to cyclooxygenase (COX)-2.
- Caryophyllene oxide showed high binding affinity of -7.00 kcal/mol against 1PXX corroborating *in vitro* COX-2 inhibition and providing a theoretical contribution in understanding the ligand-protein interactions.
- The docked pose resembled the orientation similar to that observed with diclofenac ligand (inhibitor of COX-2).
- The ligand was docked deeply within the binding pocket region forming interactions with ALA₅₂₇ GLY₅₂₆ LEU₃₅₂ SER₅₃₀ TYR₃₄₈ TRP₃₈₇ VAL₃₄₉ VAL₅₂₃ and Ser353. Our docking result was found to have three hydrogen bonding sites with SER₅₃₀ TYR₃₄₈ and TYR₃₈₅, indicating COX-2 inhibition with the highest fitness score of 50.64.
- Furthermore, all the bioactives were subjected to iLOG predictor of the Swiss ADMET website software generating *in silico* ADME properties and testing their capacity to exhibit drug likeliness.

ABOUT AUTHORS



Dr. Pavan Rangahanumaiah is Post Doctoral Fellow at Institution of Excellence, University of Mysore, Mysuru-570006, India. His research is directed towards screening of bioactives from medicinal plants of Western Ghats for anti-inflammatory property. His work has identified several leads. He is presently employing co-crystallization of protein-ligand complexes and XRD analysis of lead molecules to better understand their spatial arrangement and interactions.



Dr. Shailasree Sekhar has PhD in Biochemistry from the CSIR-CFTRI, India. Presently as Scientist at Institution of Excellence, University of Mysore, Mysuru- 570006, India she is working in the thrust area of Western Ghats medicinal plants, due to location advantage of this hot spot to the University. She has compiled their scientific data as reviews and has brought a database hyperlinked to source. Her research involves pharmacognosy of natural products and their molecular interactions with identified therapeutic targets.