



RESEARCH ARTICLE

Anti-inflammatory potential of *Calotropis gigantea* leaf using *in silico* techniques

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Abstract

Secondary metabolites obtained from plants have been extensively studied for their medicinal values. *Calotropis gigantea*, a species native to Cambodia, Pakistan and Malaysia, is a large shrub that grows up to 4 m (13 ft) tall and produces clusters of waxy flowers of white or lavender colour. Traditionally, it has been widely used in folk medicine. *Calotropis gigantea* is rich in bioactive compounds, including flavonoids, cardenolides, oxypregnane, oligoglycosides, triterpenoids, terpenes, sterols and proteinase. Extracts and metabolites derived from its leaves, bark and stem exhibit various biological activities, including anti-bacterial, anti-viral, antifungal, anti-diabetic, anti-inflammatory and anti-cancer. Pharmacological studies have demonstrated its effectiveness in treating diseases like asthma, cold, epilepsy, fever, indigestion, leprosy, piles and various skin diseases. The aim of this study is to evaluate the anti-inflammatory effect of the leaf of the *Calotropis gigantea*. Docking of a ligand with the desired protein is a method of approach for tackling the needs for drug discovery through CADD (Computer-aided drug discovery). In this study, we have identified the potential of flavonoids, Azulene-6, 9,1-octanol-3,7-dimethyl -5, 7 and Cis-vaccenic acid-5,9 from leaves against inflammation using COX pathway through docking. The results have revealed that all of these compounds have anti-inflammatory effects, but based on the binding affinity, azulene has been identified as the most potent target.

Keywords: azulene; CADD; *Calotropis gigantea*; Cis-vaccenic acid -5,9; COX; 1-octanol-3,7-dimethyl -5, 7

Introduction

The leaves, stem, seeds and latex of *Calotropis gigantea* are well known for their toxic and medicinal properties (1). Tropical application of *C. gigantea* latex and leaves has been reported to subdue the inflammatory response (2). Studies have shown that *C. gigantea* leaves exhibit anti-inflammatory activity comparable to the standard drug phenylbutazone (PBZ) in both acute and chronic models of inflammation (3, 4).

C. gigantea is a drought resistant, hard, erect, woolly, multi-branched shrub that grows in diverse climatic zones up to an altitude of about 900 m. The matured stem is woody, round while the tender stem is covered with a whitish powdery or waxy pubescence. The leaves are obovate-oblong, with a smooth upper surface and a cottony underside (Fig. 1). The flowers are purple, white or lilac coloured, present in complex to simple cymose-corymbs, seeds are brown, broad, ovate of about 2.5-3.2 cm with a white tuft of silky hair at the pointed end (5).

Medicinal importance of *C. gigantea*

GC-MS study shows that *Calotropis gigantea* (*C. gigantea*) contains bioactive chemicals with potential therapeutic actions such as anti-inflammatory, antioxidant, antibacterial and wound-healing activities (6, 7). *C. gigantea* has been used traditionally for treating a variety of diseases (8, 9). The whole plant is used to treat skin diseases such as boils, sores. It also serves as a tonic

and purgative. In particular, the stem bark is used as diaphoretic, expectorant and in other ailments such as dysentery, spleen enlargement, convulsions, scabies, ringworm, pneumonia and to induce labour in pregnant women. Fruit pulp is used as an abortifacient. Saponins, glycosides and terpenoids from the powdered root are used for treating elephantiasis, leprosy and dysentery. The latex derived from the plant is used for treating stings, toothache, caries, leprosy, ringworm, syphilis, tumours, rheumatism. It also has antiseptic, vermifuge and purgative properties. According to studies, *Calotropis gigantea* latex has strong anti-inflammatory properties that are on par with those of common anti-inflammatory medications like ibuprofen and phenylbutazone (10).

The flowers are also used for treating jaundice, inflammation, ulcer and asthma (11, 12). The crushed, warmed leaves can be applied on the burns in the form of tincture. These can also be used for curing headaches, rheumatic pains and fever. The flower infusion is used for treating rheumatism, intestinal worms and epileptic attacks (13). Decoction of flowers is used for curing cough and asthma. The plant powder mixed with cow's milk can be used for relieving rheumatism, diarrhoea, dysentery, syphilis, ulcer and leprosy (14). The pictographical representation of the literature review is shown in (Fig. 2).



Fig. 1. *C. gigantea*

Inflammatory pathway

Inflammation is an immune response during the progression of chronic disorders. During chronic disorders, harmful stimuli trigger inflammation and give rise to edema and pain. Under these conditions, the blood vessels increase in size causing inflammation. The mechanism of inflammation involves a series of events. During the early stage of immune response, neutrophils, macrophages and lymphocytes accumulate at the injury site. Arachidonic acid, a polyunsaturated fatty acid found in esterified form, is released when the cell membrane is ruptured.

Arachidonic acid is oxygenated by several enzymes and converted to inflammatory mediators, eicosanoids (15). These are pro-inflammatory mediators and act as a precursor for two important pathways, called cyclooxygenase (COX-1, COX-2) and lipoxygenase (5-LOX, 12-LOX, 15-LOX). Cyclooxygenase produces prostaglandins (PGs), thromboxane (TX) and lipoxygenase produce leukotrienes (LTs) and hydroperoxyl

fatty acids. The derivatives of these two pathways are involved in numerous inflammatory disease progressions (16). Nonsteroidal anti-inflammatory drugs (NSAIDs) act by blocking COX-1 and COX-2 enzyme pathways or by suppressing the action of PGs and TX. NSAIDs only relieve the symptoms of the disease (7) (Fig. 3). These drugs can cause gastrointestinal mucosal injuries, renal failure, respiratory tract complications, hepatocellular injuries and cardiovascular injuries. Moreover, it causes adverse side effects and its administration relies on careful monitoring (17). Thus it is necessary to develop an anti-inflammatory drug with minimum adverse effects and to halt the disease's progression.

Secondary metabolites in plants possess antioxidant properties, which may contribute to cellular protection against oxidative stress and aging.

In phytomedicine, herbal medicines are obtained from the entire plant or any of its portions, such as the flowers, leaves, roots, bark, fruits and seeds (18, 19). Plant-derived therapeutics from are more effective and safer than synthetic drugs. In the present study, secondary metabolites like Azulene-6, 9,1-octanol-3,7-dimethyl-5, 7 and Cis - vaccenic acid -5, 9 from *C. gigantea* as an anti-inflammatory therapeutic target using docking studies.

Materials and Methods

Protein preparation (Target preparation)

The PDB is the major repository of three dimensional structures of proteins, nucleic acids and other complex structures. This databank stores three dimensional atomic coordinates of proteins and nucleic acids obtained through X-Ray crystallography and NMR experiments (RCSB PDB). The structure of Human COX-1 (6Y3C) obtained from RCSB PDB. Complexes such as non-essential water molecules and hetero atoms are removed using Discovery studio Visualizer and Energy minimization is carried out using PyRx docking tool. The (Fig. 4) depicts the domain structure of Human COX-1.

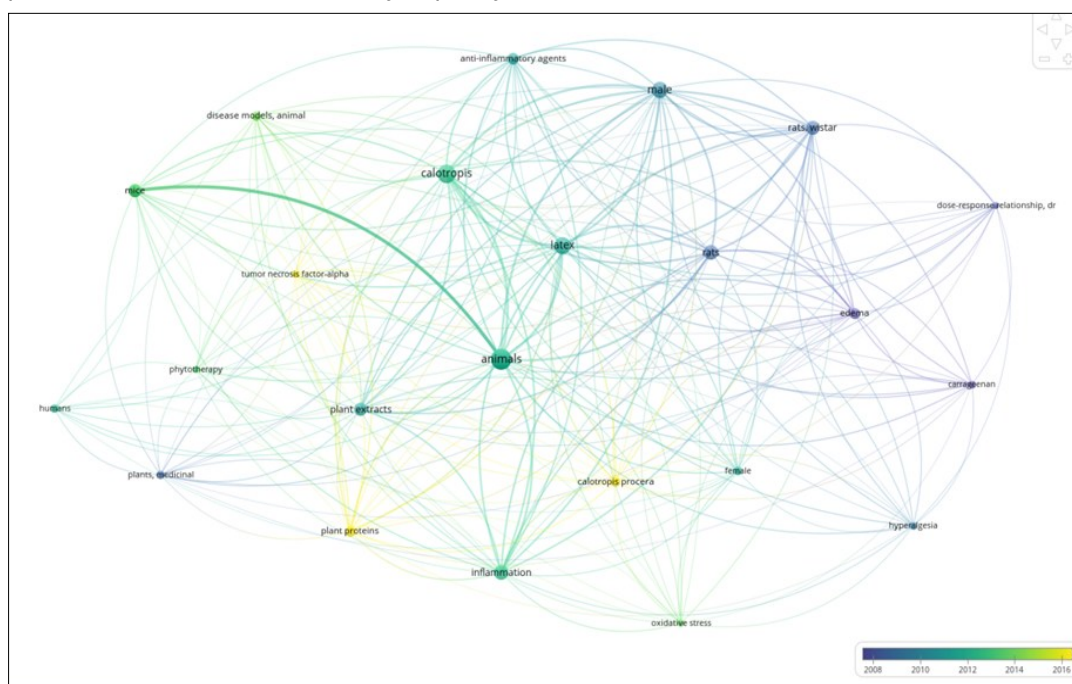


Fig. 2. Pictographical representation of literature review.

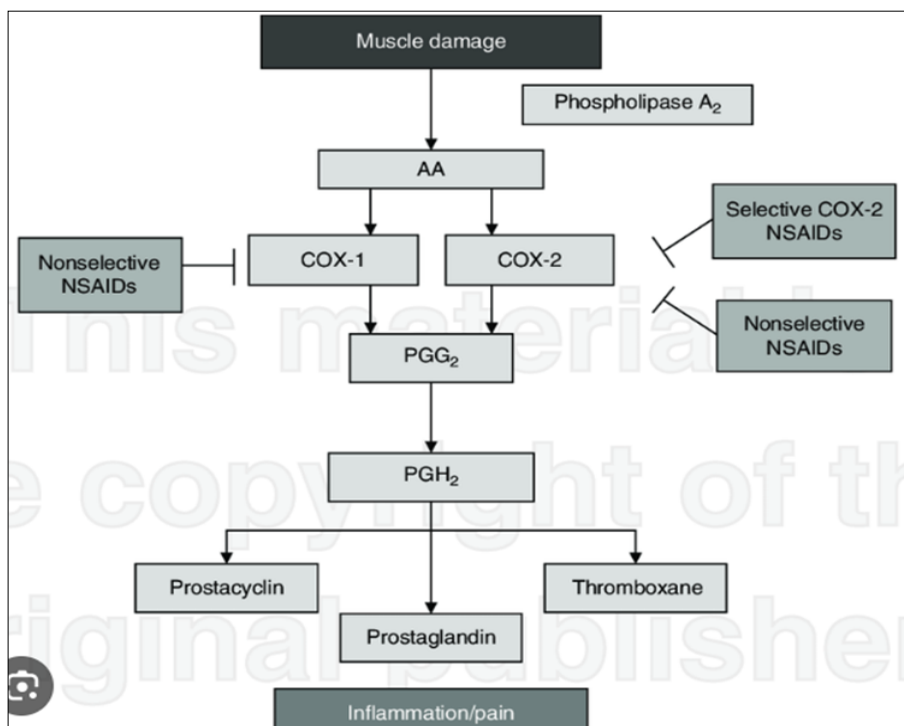


Fig. 3. Inflammatory pathway.



Fig. 4. Protein crystal structure of Human COX-1.

Ligand preparation

Ligands such as Azulene-6. 9 (Fig. 5), 1-octanol-3,7-dimethyl -5. 7 (Fig. 6) and Cis - vaccenic acid -5. 9 (Fig. 7) retrieved from pubchem.ncbi.nlm.nih.gov in SDF format and converted them into PDB format using OpenBabelGUI converter Lead validation is done by using ADMETLab 2.0 and predicted ADME parameters like pharmacokinetic properties and drug like nature of selected ligands. These ligands are opened in PyRx for docking studies and minimization has been done (20, 21).

Ligand- receptor docking

To examine the docking of binding orientation of substrates with Human COX-1 protein, Autodock Vina in PyRx virtual screening tool PyRx 0.8 (<http://pyrx.sourceforge.net>) was chosen. PyRx is a virtual screening software for computational drug discovery that can be used to screen libraries of compounds against potential drug targets. PyRx Tool can dock several ligands with a single protein of interest and has built-in Autodock Vina Wizard and Open Babel, therefore, it was selected for docking,

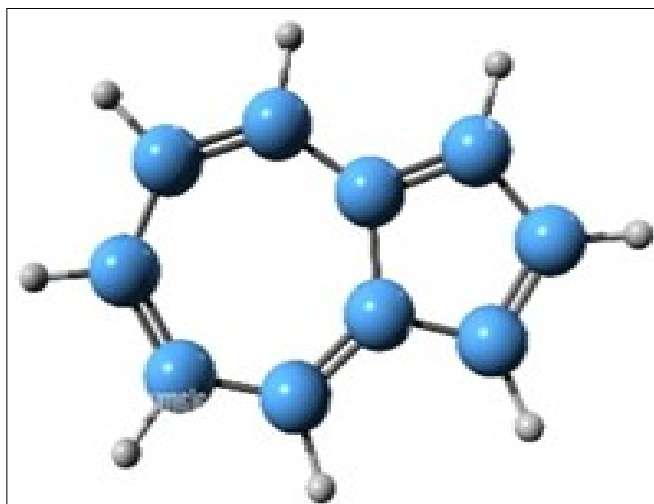


Fig. 5. Azulene-6. 9.

PyRx enables medicinal chemists to run virtual screening from any platform and helps users in every step of this process which starts from data preparation to job submission and analysis of the results. PyRx includes docking wizard with easy-to-use user interface which makes it a valuable tool for Computer-Aided Drug Design (CADD). PyRx also includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for rational drug design.

The crystal structure of protein Human COX-1 (6Y3C) was opened in PyRx virtual screening tool as a starting protein structure in pdbqt format. The ligands, Azulene-6. 9, 1-octanol-3,7-dimethyl-5. 7 and Cis - vaccenic acid -5. 9 were also opened and converted to pdbqt format. The grid box was opened and the centre of the target site was assigned along with the dimensions. The centres of the box were assigned for 6Y3C (X = -30.4260, Y = -48.2516, Z = 2.0788) together with the exhaustiveness equalling to 8. The dimensions of the box were set to 95 × 89 × 25 Å. The docking was performed with autodock vina in PyRx virtual screening

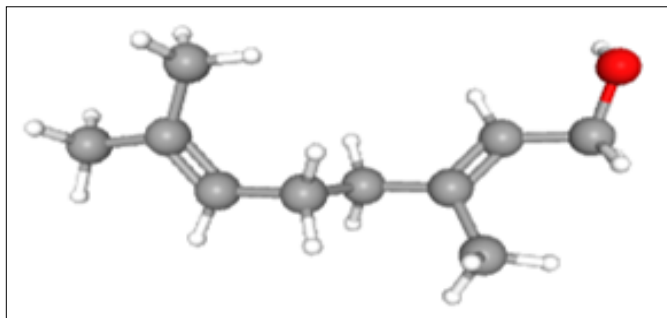


Fig. 6. 1-octanol-3,7-dimethyl -5.

tool. In this study, three ligands were compared with COX1 receptor (22). The Autodock program's dock function (S, kcal/mol) score function was utilized to assess the ligands' binding affinities with the COX1 receptor: Cis-vaccenic acid -5.9, Azulene-6.9 and 1-octanol-3,7-dimethyl-5.7.

The best free energy of binding values would be obtained in PyRx virtual screening tool GUI and log files. The DS Visualizer 4.0 was used to perform for all figures. Azulene-6.9, 1-octanol-3,7-dimethyl -5.7 and Cis - vaccenic acid -5.9 derived from *C. gigantea* are docked with Cyclooxygenase-1 as target for identifying anti-inflammatory activity.

Results

Docking results

The accuracy of a docking result is normally evaluated by the binding affinity and RMSD between the experimentally observed docking and the x-ray ligand, which usually uses RMSD cut-off value in a range of 2 - 3 Å. RMSD values indicate how reproducible or consistent each predicted pose is across different docking runs or confirmations. A large RMSD value indicates that the best-scoring pose may be less stable or is more variable. A low RMSD value signifies a more consistent pose. Ligand 1-Octanol-3,7-dimethyl having two poses with equal binding affinity -5.5, but pose 2 is a different orientation in the binding site with higher RMSD, 4.952 RMSDL/1.221 RMSDU.

In the case of azulene both poses score equally well binding affinity with -6.2, pose 2 is having slightly different from pose 1 (RMSD-2.18/0.13). Fig. 8 depicts the visualization of 3D interaction of azulene with COX1. The 2D interaction shows (Fig. 9) hydrophobic interactions like pi-alkyl

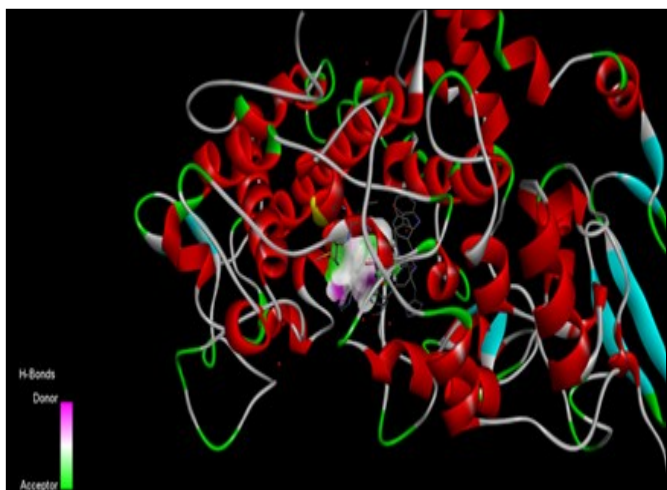


Fig. 8. 3D docking result of 6y3c with azulene.

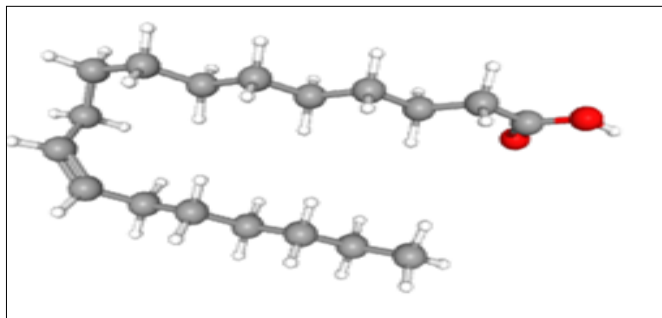
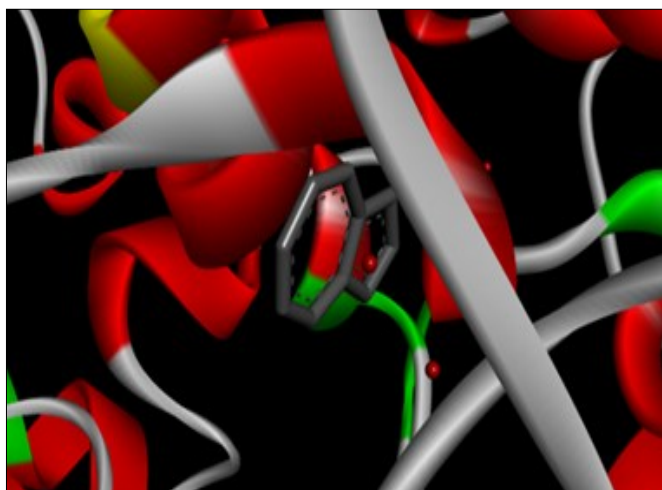


Fig. 7. Cis - vaccenic acid -5.

interaction at Alanine102, stabilizing the ligand in the nonpolar part of the pocket. The Pi-sulphur bond at Methionine 388, forms the key interacting residue, highly stabilize the interaction of azulene with COX1, thus increase its binding affinity with receptor. The major residues seen in the active site are Alanine 199, Glutamine208, leucine 390, Tryptophan 387. Cis- Vaccenic acid, pose 1 is the stronger binder (-6.2) and serves as the reference pose due to its RMSd 0.0. Pose 2 not only has weaker binding (-5.4) but also differs significantly RMSD-7.65/4.32. Fig. 10 depicts the 3D docking interaction of COX1 with Cis- Vaccenic acid. At positions 423 Glutamine and 483 histidine forms hydrogen bond interaction between hydroxyl group of the ligand, Cis- Vaccenic acid with receptor COX1 (Fig. 11). The hydrogen bond interaction stabilizes the ligand's position with the pocket. At positions 471 Isoleucine, 469 Asparagine, 426 Glutamine, 421 Cysteine forms alkyl bond between the ligand and the hydrophobic residues of the receptor. This indicates strong stabilizing hydrogen bonding interaction which are essential for binding affinity and specificity. Polar residues like histidine, glutamine, asparagine forming electrostatic interaction, indicates key spatial arrangement and possible binding site. 2D visualization of the interaction suggest ligand Cis- Vaccenic acid also having moderate to strong binding, though the unfavourable features could weaken the overall efficiency. Fig. 12 depicts the 3D docking interaction of the ligand - Octanol -3,7dimethyl with the receptor COX1. The 2D interaction of 1- Octanol -3,7dimethyl (Fig. 13) shows water-mediated binding where water molecules (HOH A :815 and 810) play a central role in interaction binding. Pi-stigma and pi-alkyl interaction of the ligand with the receptor at the positions 207 and 386 Histidine, stabilize the binding.



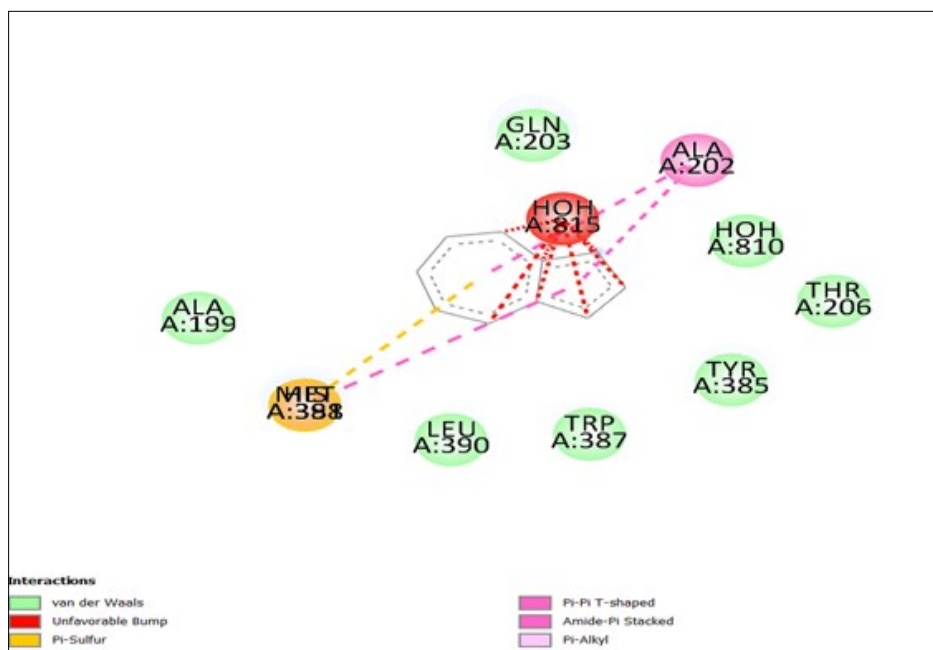


Fig. 9. 2D docking result of 6y3c with azulene.

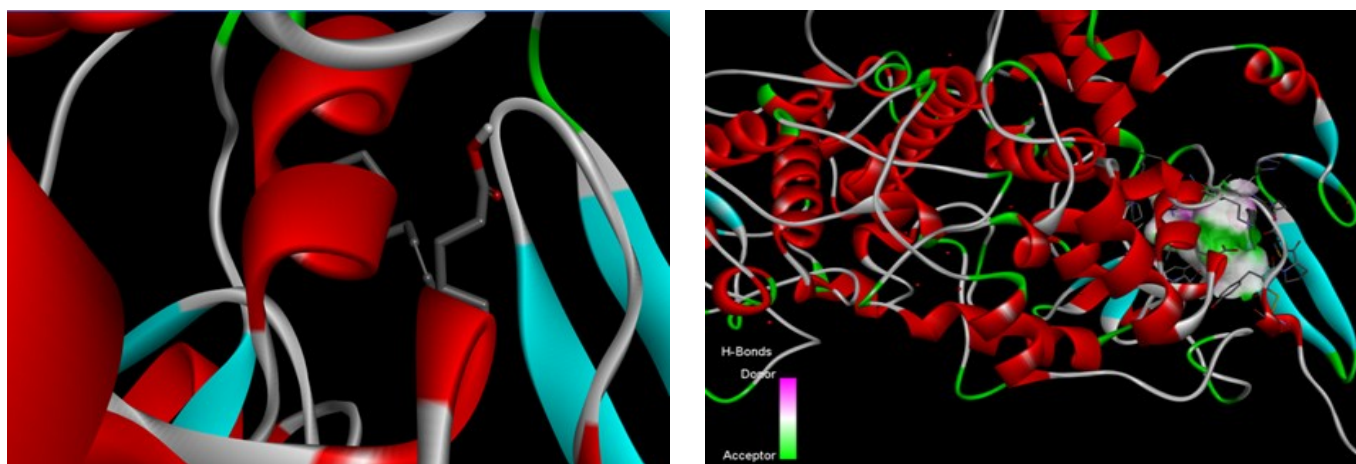


Fig. 10. 3D docking result of 6y3c with cis-Vaccenic acid.

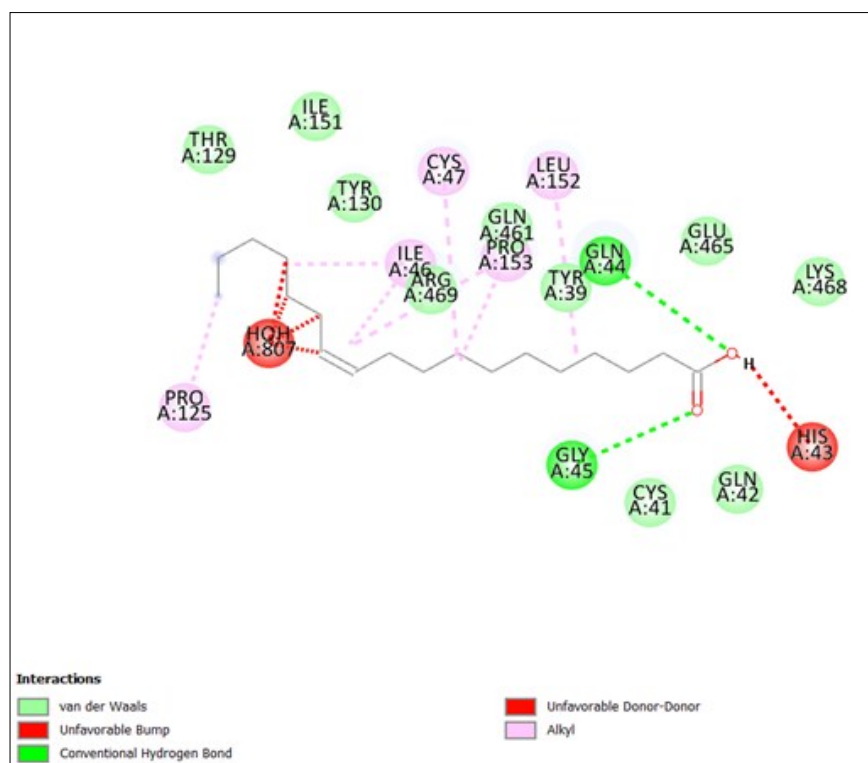


Fig. 11. 2D docking result of 6y3c with cis-Vaccenic acid.

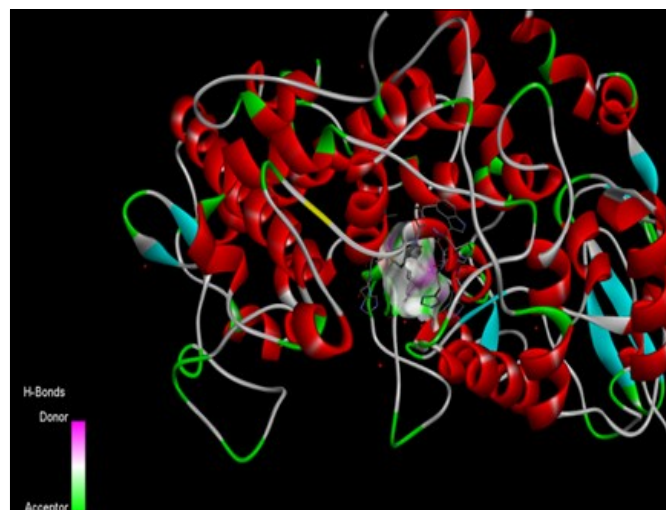
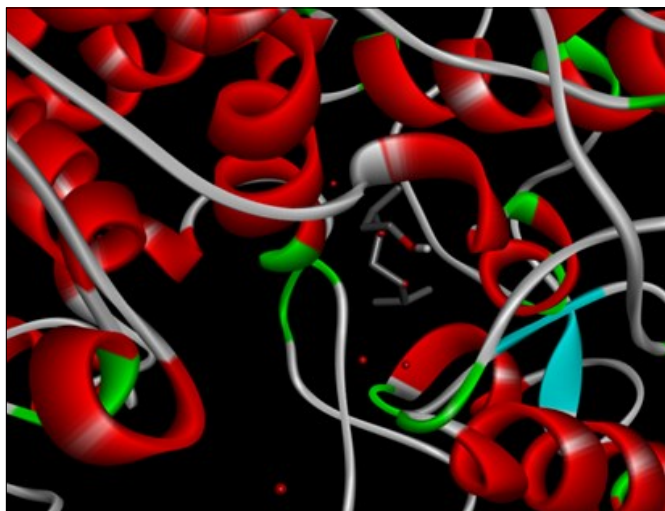


Fig. 12. 3D docking result of 6y3c with 1-octanol-3,7-dimethyl-5.

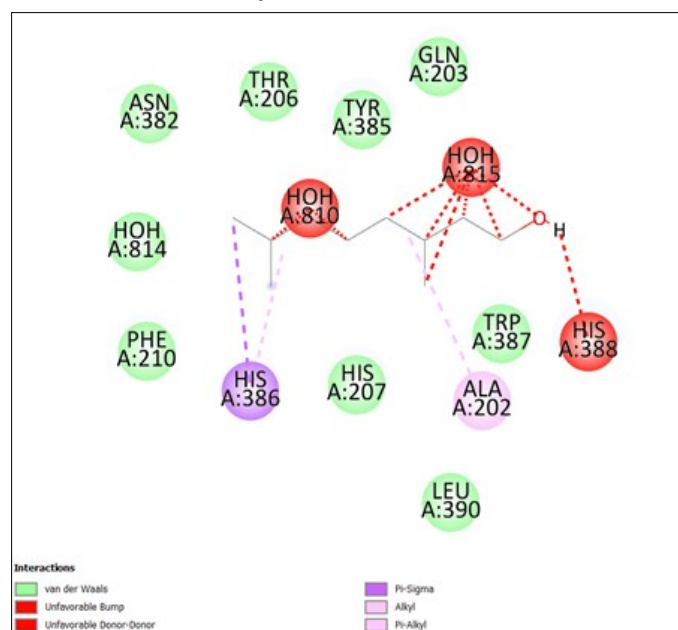


Fig. 13. 3D docking result of 6y3c with 1-octanol-3,7-dimethyl-5.

At postions 387 and 385, Tryptophan and Tyrosine at 385 forms Pi based hydrophobic contacts, also stabilizing the complex. Glutamine 203 and Asparagine 392, polar residues which are part of the extended polar environment dominates the binding pocket, indicating a potential environment that balances hydrophobic and polar compatibility. Though binding affinity scores of 1- Octanol -3,7dimethyl is less comparing to other ligands, it also shows moderate binding interaction with the receptor. Thus by raw binding affinity scores alone, azulene is predicted to bind most favourably, followed by cis-viccinic acid, then 1-octanol-37-dimethyl. The RMSD values and binding affinity of docking result of 3 ligands with Cyclooxygenase-1 were shown in Table 1.

Table 1. Ligand-enzyme binding energy and RMSD of 6Y3C and ligands

Ligand	Binding Affinity	RMSD/ub	RMSD/lb
6y3c_(1) _cis_vaccenic_acid_uff_E=75.97	-6.2	0	0
6y3c_(1) _cis_vaccenic_acid_uff_E=75.97	-5.4	7.648	4.319
6y3c_(1)_azulene_uff_E=243.74	-6.5	0	0
6y3c_(1)_azulene_uff_E=243.74	-6.5	2.184	0.127
6y3c_(1)_1-Octanol-37-dimethyl_uff_E=93.18	-5.5	0	0
6y3c_(1)_1-Octanol-37-dimethyl_uff_E=93.18	-5.5	4.952	1.221

Discussion

The molecular docking study revealed that all selected ligands exhibited binding affinity towards key inflammatory mediators, COX1. Among the tested compounds, azulene showed the strongest binding affinities with the target (-6.5 kcal/mol). 1-octanol-37-di methyl and cis-viccinic acid, though exhibiting comparatively moderate affinities (ranging from -5.5 to -6.2 kcal/mol), still demonstrated notable interactions with inflammatory receptors, COX1. These findings support the potential anti-inflammatory role of these phenolic compounds, especially the flavonoid glycosides, in modulating key targets associated with inflammation, making them promising candidates for further investigation in the context of neurodegenerative diseases such as Alzheimer's. The findings underscore the therapeutic promise of phenolic compounds, particularly flavonoid glycosides derived from HPVCO, as potential inhibitors of inflammatory targets. These bioactive molecules warrant further *in vitro* and *in vivo* studies to validate their efficacy and explore their mechanisms of action in Alzheimer's disease models. NSAIDs, like diclofenac and celecoxib, have been linked to a higher risk of heart-related side effects. Long-term use of NSAIDs can reduce kidney blood flow, potentially leading to kidney

damages. It is evident from the ADMET analysis, azulene is less toxic than NSAIDS. Furthermore, Ames toxicity indicated that the compound is not mutagenic, suggesting a lower risk of causing genetic mutations. Human dose tolerance indicated that the compound may have a higher potential for toxicity at elevated dose, 0.507 log mg/kg/day. azulene does not inhibit hERG I or II channels suggesting a lower risk of drug-induced cardiac arrhythmias. Hepatotoxicity indicates no observed liver toxicity, suggesting the compound is not hepatotoxic. It shows moderate chronic and acute toxicity in rats, moderate aquatic toxicity in *T. Pyriiformis* but low in minnows, suggest that the azulene has environmental toxicity is moderate for protozoa and low for fish. (Table 2).

Table 2. Pharmacokinetics and Toxicity prediction using pKSCM ADMET Predictor

Toxicity	AMES toxicity	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	0.507	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	1.66	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	2.271	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	No	Categorical (Yes/No)
Toxicity	Skin Sensitisation	Yes	Categorical (Yes/No)
Toxicity	T.Pyriiformis toxicity	0.347	Numeric (log ug/L)
Toxicity	Minnow toxicity	1.425	Numeric (log mM)

Hence, from our study, we have identified the anti-inflammatory effects of three secondary metabolites from *C. gigantea* through docking studies. In this, azulene has shown higher affinity with Cyclooxygenase-1(COX-1). When the toxicity is compared, azulene is superior to NSAIDS, therefore, it has the potential to become the major anti-inflammatory medication. Further *in vivo* and *in vitro* studies have to be done and to make this compound close to clinical trials.

Conclusion

The results of the current study have revealed that the secondary metabolites from *C. gigantea* contain a potential inhibitor for human cyclooxygenase-1. *C. gigantea* extract can be validated as a new alternative to existing anti-inflammatory drugs. This study will contribute to the development of novel pharmaceuticals for managing these diseases, potentially offering safer and more effective alternatives to existing therapies. Additionally, this study may shed light on the molecular mechanisms underlying the diseases and provide insights into their treatment.

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Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

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