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Research Article

In Vitro and in Silico Assessment of The Anti-Inflammatory Potential of Ethanolic Leaf Extract of Pithecellobium Dulce

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Abstract

Pithecellobium dulce is a medicinal plant conventionally used for the treatment of various inflammatory and painful conditions. The current study was undertaken to evaluate the anti-inflammatory potential of the ethanolic leaf extract of *Pithecellobium dulce* (ELEPD) using in vitro and *in silico* approaches. In vitro anti-inflammatory activity was assessed using inhibition of egg albumin denaturation and human red blood cell (HRBC) membrane stabilisation assays, with Diclofenac sodium as the reference standard. Molecular docking studies were executed against Cyclooxygenase-2 to identify potential interactions between the major phytoconstituents and inflammatory targets. ELEPD exhibited significant concentration-dependent inhibition of egg albumin denaturation at concentrations ranging from 37.5 to 600 µg/mL, with a maximum inhibition of 68.26±6.66% and an IC_{50} value of 198.5±2 µg/mL. In the HRBC membrane stabilisation assay, the extract demonstrated marked protection against heat-induced hemolysis, producing 84.28% inhibition at 600 µg/mL. Although the standard drug showed comparatively higher activity, the extract displayed substantial anti-inflammatory effects in both models. Docking investigation revealed strong binding affinities of phytoconstituents toward Cyclooxygenase-2, with Kaempferol showing the highest docking score (−9.2 kcal/mol), followed by Kaempferol-3-O-rhamnoside (−8.4 kcal/mol), Alpha-spinasterol (−7.7 kcal/mol), and Dulcitol (−6.3 kcal/mol). These compounds exhibited favourable molecular interactions with active-site residues, suggesting possible COX-2 inhibitory action. The findings indicate that ELEPD possesses significant anti-inflammatory activity, which may be mediated through inhibition of protein denaturation, stabilisation of biological membranes, and suppression of COX-2 activity. This study supports the traditional use of *Pithecellobium dulce* and highlights its potential as a source of novel anti-inflammatory agents.

Keywords: Anti-Inflammatory activity, Egg albumin denaturation, Membrane stabilisation, Autodocking

1 INTRODUCTION

Inflammation is a multifaceted biological defence response initiated when tissues are exposed to harmful stimuli such as pathogens, physical injury, toxic chemicals, or cellular damage. It is characterized by the classical signs of redness, heat, pain, swelling and, in some cases, loss of function. This response is mediated through the release of various chemical mediators, including prostaglandins, histamine, kinins, serotonin, and cytokines, which promote vasodilation, increased vascular permeability, and recruitment of leukocytes to the site of injury[1]. These coordinated events help eliminate injurious agents, remove damaged cells, and initiate tissue repair. Depending on duration and severity, inflammation may be classified as acute or chronic. Acute inflammation is the immediate protective response, whereas chronic inflammation is a prolonged process characterised by persistent tissue injury, cellular infiltration, and fibrosis [2].

Although inflammation is a vital protective mechanism, uncontrolled or excessive inflammation contributes to the pathogenesis of several disorders, such as arthritis, cardiovascular disease, diabetes, asthma, neurodegenerative diseases, and gastrointestinal disorders. Conventional anti-inflammatory drugs such as glucocorticoids and NSAIDs are widely used for treatment; however, their long-term use is associated with adverse effects, including gastric ulceration, bleeding, immunosuppression, renal impairment, and

increased cardiovascular risk [3]. These limitations have prompted the search for harmless and more effective alternatives from natural sources. Medicinal plants are rich in bioactive compounds, including alkaloids, flavonoids, polyphenols, terpenoids, and saponins, many of which exhibit significant anti-inflammatory activity by modulating multiple inflammatory pathways [4,5]. Therefore, the scientific evaluation of plant-based therapies using reliable, economical in vitro methods, such as protein denaturation and membrane stabilisation assays, has gained considerable importance in recent years.

Pithecellobium dulce is a medicinal plant extensively recognised in traditional systems of medicine for its diverse therapeutic applications. Earlier studies have described that the plant possesses antidiabetic, anti-inflammatory, antioxidant, and anti-ulcer activities, indicating its considerable pharmacological potential [6]. Different parts of the herb have been traditionally used to manage toothache, leprosy, earache, and other ailments. The phytochemical investigations have revealed the existence of numerous bioactive secondary metabolites, including saponins, flavonoids, phenolic compounds, glycosides and phytosterols, which are known to contribute to antioxidant and anti-inflammatory effects. These findings suggest that *Pithecellobium dulce* may serve as a valuable natural source for the development of safer anti-inflammatory agents [7].

While earlier reports have established the anti-inflammatory activity of *Pithecellobium dulce* in various experimental models, its precise mechanisms of action remain insufficiently explored. In particular, limited information is available regarding its potential effect on cyclooxygenase-2 (COX-2), a key enzyme involved in the synthesis of inflammatory mediators [8]. Therefore, the current study commenced to evaluate the in vitro anti-inflammatory activity of the ethanolic leaf extract of Pithecellobium dulce using protein denaturation inhibition and RBC membrane stabilisation assays, and to explore the COX-2 inhibitory potential of selected phytoconstituents by molecular docking investigation with PyRx.

2 PLANT PROFILE:

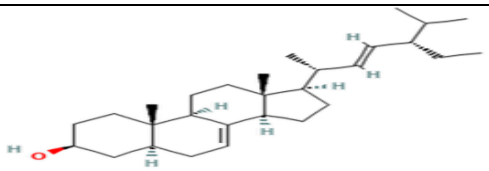
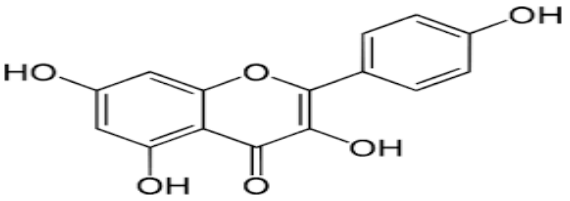
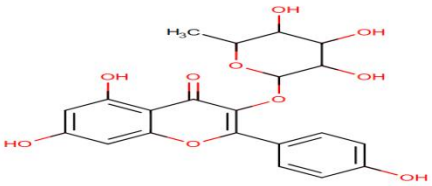
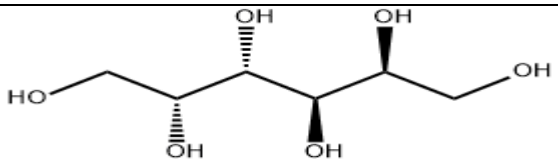
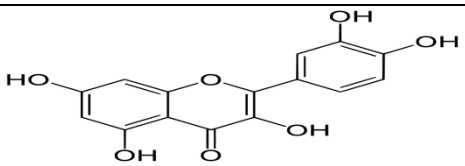
Pithecellobium dulce, commonly known as Jungle Jalebi, Jungle Gilabe, Manila tamarind, or Guamúchil, is a tropical drought-resistant tree belonging to the family Fabaceae. It is native to tropical America, including Mexico, Brazil, Argentina, Colombia, and Bolivia, and has been extensively introduced into India, Southeast Asia, and Africa owing to its adaptability to hot, dry climates. In India, the plant is commonly distributed in states such as Tamil Nadu, Maharashtra, Andhra Pradesh, and other semi-arid regions. The tree thrives in coastal and dry habitats and is valued for both ecological and medicinal purposes. It produces edible pods with sweet pulp and aids as a source of fodder, shade, and soil improvement. The leaves are rich in nutrients and protein, making them useful as animal feed.

Traditionally, several parts of *Pithecellobium dulce* have been used as traditional medicine to treat numerous ailments. The plant bark is commonly employed as an astringent and is employed in the management of diarrhoea, dysentery, fever, toothache, gum disorders, and haemorrhages. The leaves are applied as a plaster for pain relief and are also used in peptic ulcers, leprosy, earache, skin infections, and venereal diseases. The fruit pulp is consumed to reduce fever and control bleeding disorders, while the seeds are traditionally used to cleanse ulcers and relieve chest congestion. In some traditional systems, the plant has similarly been reported to possess abortifacient properties[9].

Modern pharmacological investigations have supported many of these traditional claims and demonstrated that *Pithecellobium dulce* possesses significant anti-inflammatory, antioxidant, antimicrobial, antidiabetic, anti-ulcer, and anti-venom activities. These beneficial effects are mainly attributed to bioactive phytoconstituents, including saponins, flavonoids, phenolic compounds, tannins, sterols and glycosides [10]. Its antioxidant potential may help reduce oxidative stress–mediated cellular damage, while its anti-inflammatory activity may inhibit inflammatory mediators, such as prostaglandins and cytokines [11]. Owing to its nutritional value and broad pharmacological potential, *Pithecellobium dulce* is considered an important medicinal plant with promising therapeutic applications.

Figure 1: *Pithecellobium dulce* Leaves and aerial parts

Table 1: Chemical constituents of *Pithecellobium dulce* leaves [12]

CHEMICAL CONSTITUENT	IUPAC NAME	CHEMICAL STRUCTURE
α-Spinasterol	(22*E*)-5α-Stigmasta-7,22-dien-3β-ol	
Kaempferol	3,4′,5,7-Tetrahydroxyflavone	
Kaempferol 3-rhamnoside	5,7-dihydroxy-2-(4-hydroxyphenyl)-3-[(3,4,5-trihydroxy-6-methyloxan-2-yl)oxy]-4H-chromen-4-one	
Dulcitol	hexane-1,2,3,4,5,6-hexol	
Quercetin	3,3′,4′,5,7-Pentahydroxyflavone	

3 MATERIALS AND METHODS

3.1 Plant material and preparation of extracts

The fresh leaves of *Pithecellobium dulce* were collected from the Andhra Pradesh region of India in October 2025. The plant material was authenticated by Dr M. Madhavi, Y.S.R. Horticultural University, Venkata Ramannagudem, Andhra Pradesh. The leaves were shade-dried, powdered, and sieved to obtain a uniform fine powder. The powdered material (500 g) was Soxhlet-extracted with 99.9% ethanol, a solvent known for its efficiency as a universal extraction solvent for phytochemicals. The extract was concentrated using a rotary evaporator, yielding a dark green, sticky mass with an 11.8% yield. The extract was designated as the ethanolic leaf extract of *Pithecellobium dulce* (ELECG)[12].

3.2 Drugs and Chemicals

For the *in vitro* experiments, the chemicals and reagents used included egg albumin solution (HiMedia Laboratories Pvt. Ltd., India), phosphate-buffered saline (PBS, pH 7.4; HiMedia Laboratories Pvt. Ltd., India), distilled water, Dimethyl sulfoxide (DMSO) (Merck Life Science Pvt. Ltd., India), normal saline (0.9% NaCl), and Diclofenac sodium as the reference standard (Sigma-Aldrich or equivalent analytical grade). All chemicals and reagents used were of analytical grade.

The equipment employed for the *in vitro* studies included adjustable micropipettes with sterile tips (Eppendorf / Thermo Fisher Scientific), test tubes, centrifuge tubes, a UV–Visible spectrophotometer (Shimadzu / Systronics), digital water bath (Remi Equipments Pvt. Ltd., India), laboratory incubator (Labline / Thermolab), centrifuge machine (Remi Instruments Ltd., India), and analytical balance (Sartorius / Shimadzu).

For *in silico* experiments, PyRx 8.0 was employed for molecular docking, and BIOVIA Discovery Studio for ligand–receptor interaction analysis. The crystal structure of Cyclooxygenase-2 (COX-2) was retrieved from the RCSB Protein Data Bank, and ligand structures were obtained from the PubChem online website (https://pubchem.ncbi.nlm.nih.gov/) in .sdf format. All computational studies were executed on a Windows 10 system with 4 GB RAM and a quad-core processor[13].

3.3 Phytochemical analysis:

The ELEPG was screened for major phytochemical constituents, including alkaloids, flavonoids, glycosides, saponins, carbohydrates and tannins [14].

3.4 Egg albumin denaturation inhibition assay[15]

The *in vitro* egg albumin denaturation assay is a widely used preliminary method for evaluating the anti-inflammatory activity of test compounds. Protein denaturation involves the disruption of a protein's native three-dimensional structure, leading to the loss of biological function. During inflammatory conditions, denatured proteins may contribute to tissue damage and the production of autoantigens. Therefore, inhibition of protein denaturation is considered a useful indicator of anti-inflammatory potential [16].

In this assay, egg albumin serves as a model protein substrate. Denaturation is induced under controlled experimental conditions, usually by heat or pH alteration, in the presence or absence of the test sample. Compounds with protein-stabilising properties prevent structural alterations in albumin and thereby inhibit denaturation. The extent of inhibition is quantified spectrophotometrically by measuring absorbance changes. Different concentrations of the test sample are incubated with egg albumin solution, and the percentage inhibition of denaturation is calculated relative to the control. The concentration required to inhibit 50% of denaturation (IC_{50}) may be determined using suitable software such as GraphPad Prism. Diclofenac sodium is commonly used as the reference standard. This assay is based on the rationale that agents capable of preventing protein denaturation may exhibit anti-inflammatory activity, similar to certain nonsteroidal anti-inflammatory drugs (NSAIDs) [17].

3.5 Egg Albumin denaturation inhibition Assay

A stock solution was prepared by dissolving 1 g of dried ELECG in 1 mL of distilled water, followed by serial two-fold dilutions to obtain different test concentrations. A 1% egg albumin solution was prepared by dissolving commercially available egg albumin powder in cold distilled water.[18]

The reaction mixture contained 0.2 mL egg albumin solution, 2 mL of plant extract or Diclofenac sodium (standard), and 2.8 mL phosphate-buffered saline (pH 7.4), making a final volume of 5 mL. The control contained distilled water instead of the test sample. The mixtures were incubated at 37 ± 2°C for 30 min, then heated at 70 ± 2°C for 15 min. After cooling, optical density was measured at 280 nm using a UV–Visible spectrophotometer[19]. The Percentage inhibition of protein denaturation was calculated using:

$$\text{Percentage inhibition} = [(Ac - At) / Ac] \times 100$$

Ac = Absorbance of control
At = Absorbance of test sample

The IC_{50} value was determined from the plot of percentage inhibition versus concentration.

3.6 Membrane Stabilization Assay

The membrane stabilisation assay is based on the ability of test compounds to protect erythrocyte membranes from heat- or hypotonic-induced lysis. The erythrocyte membrane is structurally analogous to the lysosomal membrane; therefore, stabilisation of red blood cell (RBC) membranes suggests that lysosomal membranes may also be stabilised during inflammation. Prevention of lysosomal membrane rupture limits the release of inflammatory mediators and proteolytic enzymes, thereby reducing tissue injury. Hence, compounds that inhibit heat-induced haemolysis are considered to possess anti-inflammatory activity.[20]

Fresh human blood was collected from a healthy volunteer who had not consumed any Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) for at least two weeks prior to the experiment. The blood sample was transferred into centrifuge tubes and centrifuged at 3000 rpm for 10 min. The packed cells were separated and washed three times with an equal volume of normal saline. The washed erythrocytes were then reconstituted with normal saline to obtain a 10% v/v RBC suspension.

The reaction mixture (2 mL) consisted of 1 mL of the test sample at various concentrations (37.5, 70, 150, 300, and 600 µg/mL) and 1 mL of 10% RBC suspension. The control tube has 1 mL of normal saline instead of the test sample. Diclofenac sodium was used as the reference standard.

All tubes were incubated in a water bath at 56°C for 30 min and then cooled below running tap water. The mixtures were centrifuged at 2500 rpm for 5 min, and the absorbance of the supernatant was estimated at 560 nm using a UV–Visible spectrophotometer. Each trial was carried out in triplicate[21]. The percentage inhibition of haemolysis was calculated using the following equation:

$$\% \text{ Inhibition of Haemolysis} = [(Ac - As) / Ac] \times 100$$

Ac = Absorbance of control
As = Absorbance of sample

3.7 Molecular Docking Studies

Molecular docking is a computational approach used to predict the preferred binding orientation of a ligand within the active site of a target protein and to estimate the strength of ligand–receptor interactions[22]. The technique evaluates molecular complementarity based on steric, hydrophobic, electrostatic, hydrogen-bonding, and van der Waals interactions. Binding affinity is expressed as a docking score or binding free energy, with more negative values generally indicating stronger, more stable interactions. Docking studies are widely employed in drug discovery to identify potential bioactive compounds and to understand the molecular basis of pharmacological activity [23].

The crystal structure of the target protein was obtained from the Protein Data Bank and prepared by removing co-crystallised ligands, water molecules, and other non-essential heteroatoms. Polar hydrogens were added, and the processed protein structure was changed into the required docking format. The selected ligands were retrieved from public chemical databases in three-dimensional format and subjected to energy minimisation to obtain stable conformations. The optimised ligands were then modified into docking-compatible files.

Docking simulations were executed by means of PyRx 8.0 and the AutoDock engine. The prepared protein was used as the receptor, and ligands were docked within a defined grid box covering the active site or whole protein region for blind docking. Multiple binding conformations were generated for each ligand. The docked poses were ranked by binding affinity, and the best conformation was chosen based on the lowest binding energy and acceptable RMSD. Protein–ligand interactions, including hydrogen bonds, hydrophobic contacts, and other non-covalent interactions, were further visualised and analysed by means of BIOVIA Discovery Studio [13,24].

3.7.1 Ligand–Receptor Interaction Analysis

The best docked protein–ligand complex obtained from the docking study was subjected to interaction investigation using BIOVIA Discovery Studio 2024. The prepared receptor structure and the selected docked ligand pose were imported into the workspace and superimposed to generate the final complex model. The binding interactions between the ligand and the target protein were then examined using the software's visualisation tools. Both three-dimensional and two-dimensional interaction maps were generated to clearly identify the ligand's orientation within the binding pocket. The 2D interaction diagrams were exported as images for documentation and presentation.

A Detailed analysis of the docked complex was executed to identify key amino acid residues involved in binding. Various non-covalent interactions, including hydrogen bonds, hydrophobic interactions, van der Waals forces, π–alkyl interactions, π–π stacking, ionic interactions, and carbon–hydrogen bonds, were recorded. Bond distances and interacting residues were tabulated to interpret ligand binding affinity and stability within the active site of the receptor.

4 RESULTS

4.1 Percentage of yield and characteristics

Ethanolic Leaf Extract of *Pithecellobium dulce* (ELEPD) showed the following characteristics, with a yield of 15.8%.

Table 2: Percent of yield and characters of ELEAI

Form of Extract	Color	Consistency	Yield
Ethanolic	Dark green	Sticky	15.8%

4.2 Preliminary Phytochemical Screening

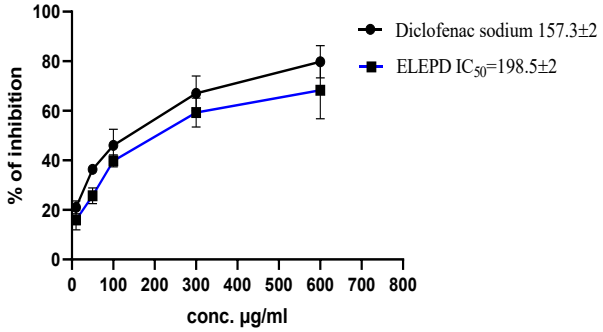
Standard preliminary Photochemical screening was performed the plant extract showed the existence of the following phytoconstituents.

Table 3: Results of Preliminary Phytochemical Screening of ELEPD

S.no	Class of compounds	Presence in Leaf Extract	Test performed
01	Alkaloids	+	Wagner's test
02	Carbohydrates	+	Fehling's test
03	Proteins & Amino acids	+	Ninhydrin test
04	Flavonoids	+	Alkaline reagent test
05	Test for Lipids	+	Salkowski test
06	Terpenoids	+	Horizon test
07	Phenolic compounds	+	Ferric chloride test

ELEPD showed positive for the presence of alkaloids, flavonoids, lipids, terpenoids, phenolic compounds, carbohydrates, proteins and amino acids.

4.3 Inhibition of protein denaturation



Graph 1: Percentage inhibition of egg albumin denaturation by ELEPD and Diclofenac with their IC_{50} values at different concentrations

Using a calorimeter, the percentage inhibition of protein (egg albumin) denaturation is measured. The OD values obtained for the test were incorporated into the formula for percentage inhibition. Mean OD values of the standard and test are compared. It was identified from the study results ethanolic leaf extract of *Pithecellobium dulce* (ELEPD) at various concentrations of 37.5 µg/ml (15.99±2.23), 75µg/ml (25.72±1.84), 150 µg/ml (439.78±1.42), 300 µg/ml (59.29±3.36) and 600µg/ml (68.26±6.66) showed a significant percentage inhibition of egg albumin protein denaturation. A concentration-dependent effect was observed with the plant extract. The reference drug diclofenac sodium at various concentrations 37.5µg/ml (27.04 ±2.61), 75µg/ml (36.34±1.19), 150µg/ml (47.11± 1.67), 300µg/ml

(59.29± 3.37) and 600µg/ml (68.26± 6.66) also exhibited a significant inhibition of protein denaturation, showing the concentration-based effect respectively. The IC_{50} of ELELI was 198.5 ±2 µg, and the IC50 of diclofenac was 157.3 ±2 µg; these values are shown in graph Graph 1.

4.4 Results of Membrane stabilization Assay

Table 4: Effect of ELEPD and Diclofenac on heat-induced hemolysis of erythrocytes.

TREATMENT	CONCENTRATION	ABSORBANCE 560nm	%INHIBITION OF HEAMOLYSIS
ELEPD	37.5µg/ml	0.120±1.64	65.50%
ELEPD	75µg/ml	0.125±1.53	72.05%
ELEPD	150µg/ml	0.101±1.24	80.00%
ELEPD	300µg/ml	0.095±2.67	81.50%
ELEPD	600µg/ml	0.076±1.32	84.28%
Diclofenac Sodium	100µg/ml (STD)	0.009±1.02	98.61%

The stabilisation of RBC membranes was investigated to assess the anti-inflammatory activity of *Pithecellobium dulce*. The extract demonstrated a significant, concentration-dependent membrane-stabilising effect on RBCs exposed to stressful conditions.

When RBC suspensions were heated to 56°C, exceeding normal body temperature, membrane proteins denatured, leading to hemolysis. In the heat-induced RBC hemolysis model, the ethanolic extract showed a pronounced effect, inhibiting hemolysis by 84.28% at 600 µg/mL. In comparison, standard diclofenac sodium showed greater inhibition than the plant extract in all tested concentrations. As this extract was found to inhibit heat-induced denaturation and stabilize the RBC, it is thought to have an anti-inflammatory effect. Anti-inflammatory drugs such as salicylic acid and phenylbutazone have shown a dose-dependent ability to inhibit thermally induced protein denaturation. Similar results were also observed from many plant extracts.

4.5 *In Silico* Docking Results

4.5.1 Docking results of Kaempferol

Table 5: Results of Auto Docking Kaempferol with COX-2 enzyme

S.No	An amino acid of the COX-2 enzyme	Type of bonding interaction
1.	LEU B:294	Pi-Alkyl
2.	VAL B: 444	Pi-Alkyl
3.	LEU B:391	Pi-Alkyl
4.	HIS B:388	Carbon Hydrogen bonds
5.	TYR B: 404	Van der Waals
6.	VAL B:295	Van der Waals
7.	PHE B:395	Van der Waals

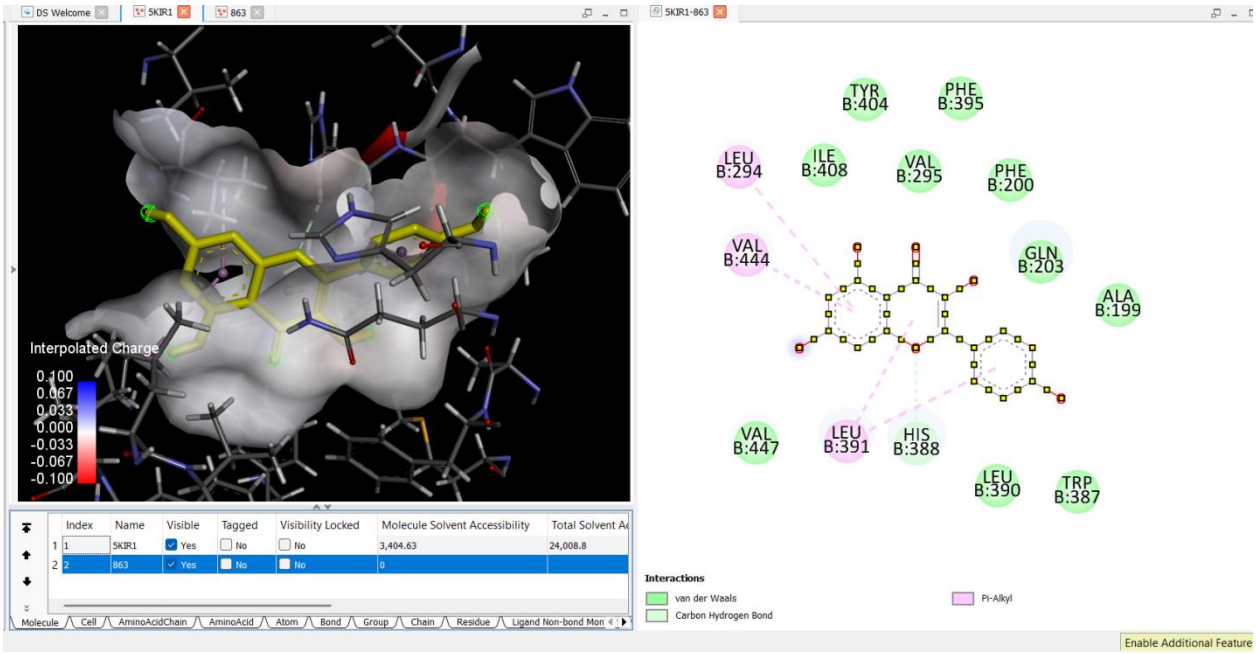


Figure 2: 3D and 2D integration of Kaempferol with COX-2 enzyme

The autodocking results show a strong interaction between Kaempferol and the COX-2 enzyme (PDB ID: 5IKR) with the B-Chain, exhibiting the highest binding energies. (−9.2 kcal/mol). Kaempferol exhibited favourable molecular interactions with the active-site residues of Cyclooxygenase-2 via multiple Pi-alkyl, carbon-hydrogen, and van der Waals interactions, suggesting a stable binding affinity and potential inhibitory activity against COX-2-mediated inflammation.

4.5.2 Docking Results of Dulcitol

Table 6: Results of Auto Docking Dulcitol with COX-2 enzyme

S.No	An amino acid of the COX-2 enzyme	Type of bonding interaction
1.	ASN B:43	Conventional hydrogen bond
2.	GLU B: 465	Conventional hydrogen bond
3.	GLY B: 45	Conventional hydrogen bond
4.	AGR B: 44	Attractive Charges
5.	ASP B: 125	Attractive Charges
6.	LYS B:137	Attractive Charges
7.	GLN B: 42	Van der Waals
8.	HIS B:468	Van der Waals
9.	LYS B:41	Carbon Hydrogen bonds
10.	LEU B:152	Carbon Hydrogen bonds
11.	CYS B: 47	Carbon Hydrogen bonds

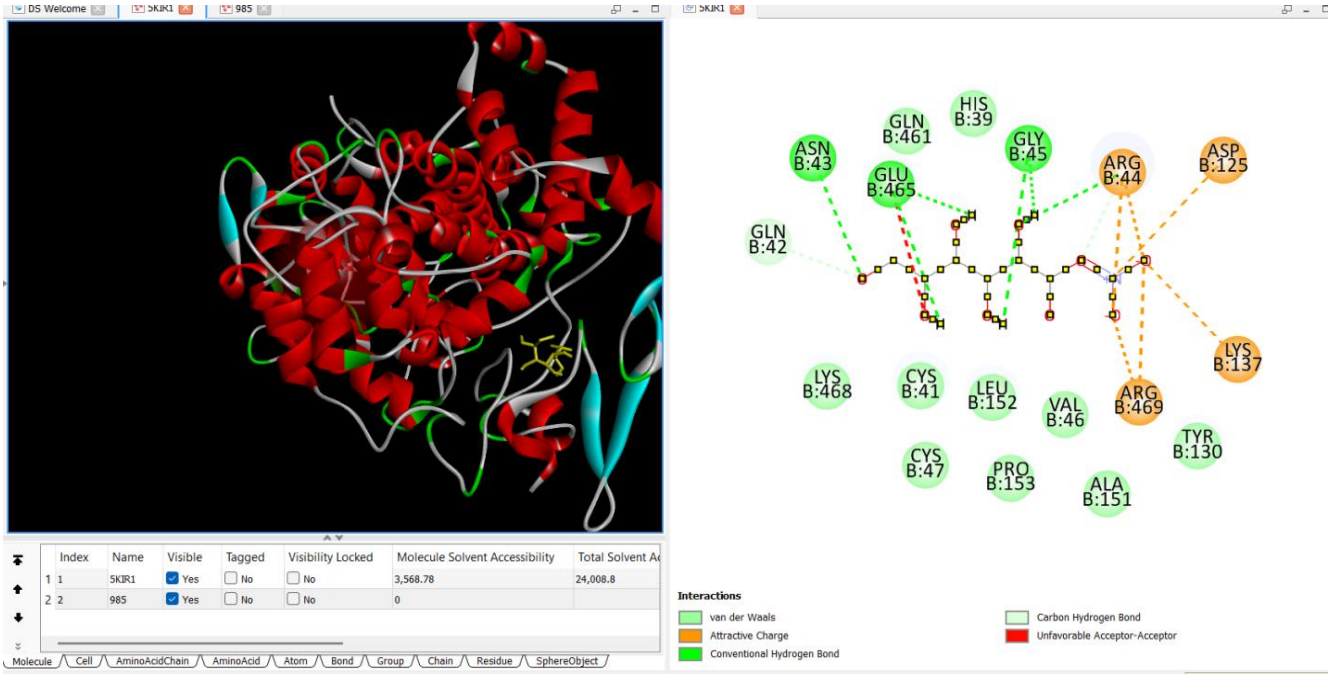


Figure 3: 3D and 2D integration of Dulcitol with COX-2 enzyme

The autodocking results show a strong interaction between Dulcitol and the COX-2 enzyme (PDB ID: 5IKR) with the B-Chain, exhibiting the highest binding energies (−6.3 kcal/mol).

4.5.3 Docking results of Kaempferol-3-O-rhamnoside

Table 7: Results of Auto Docking Kaempferol-3-O-rhamnoside with COX-2 enzyme

S.No	An amino acid of the COX-2 enzyme	Type of bonding interaction
1.	GLU B:346	Van der Waals
2.	GLN B: 565	Van der Waals
3.	SER B:581	Van der Waals
4.	TYR B: 355	Van der Waals
5.	GLY B: 354	Carbon Hydrogen Bond
6.	ASP B: 347	Pi-Anion bond
7.	LYS B: 358	Alkyl bond
8.	ASN B: 350	Conventional Hydrogen bond
9.	GLN B: 192	Conventional Hydrogen bond
10.	HS B: 351	Pi-Sigma

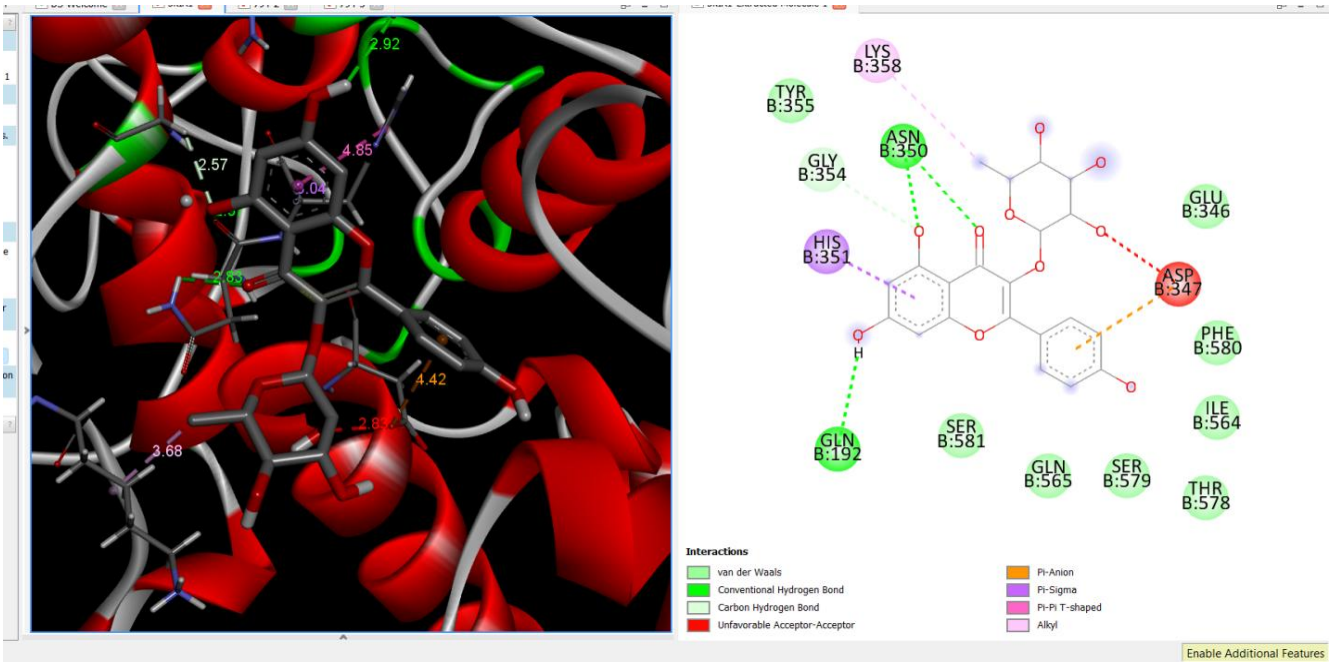


Figure 4: 3D and 2D integration of Kaempferol-3-O-rhamnoside with COX-2 enzyme

The autodocking results show a strong interaction between Kaempferol-3-O-rhamnoside and the COX-2 enzyme (PDB ID: 5IKR) with the B-Chain, exhibiting the highest binding energy. (−8.4 kcal/mol,)

4.5.4 Docking Results of Alpha Spinasterol

Table 8: Results of Auto Docking Alpha spinasterol with COX 2 enzyme

S.No	An amino acid of the COX-2 enzyme	Type of bonding interaction
1.	PRO B:528	Van der Waals
2.	PHE B: 470	Van der Waals
3.	GLU B: 524	Carbon Hydrogen Bond
4.	SER B: 119	Carbon Hydrogen Bond
5.	AGR B: 120	Pi-Cation
6.	VAL B: 89	Cpi-Alkyl
7.	ASP B: 347	Pi-Anion bond
8.	LYS B: 358	Alkyl bond
9.	ASN B: 350	Conventional Hydrogen bond
10.	GLN B: 192	Conventional Hydrogen bond
11.	HIS B: 351	Pi-Sigma

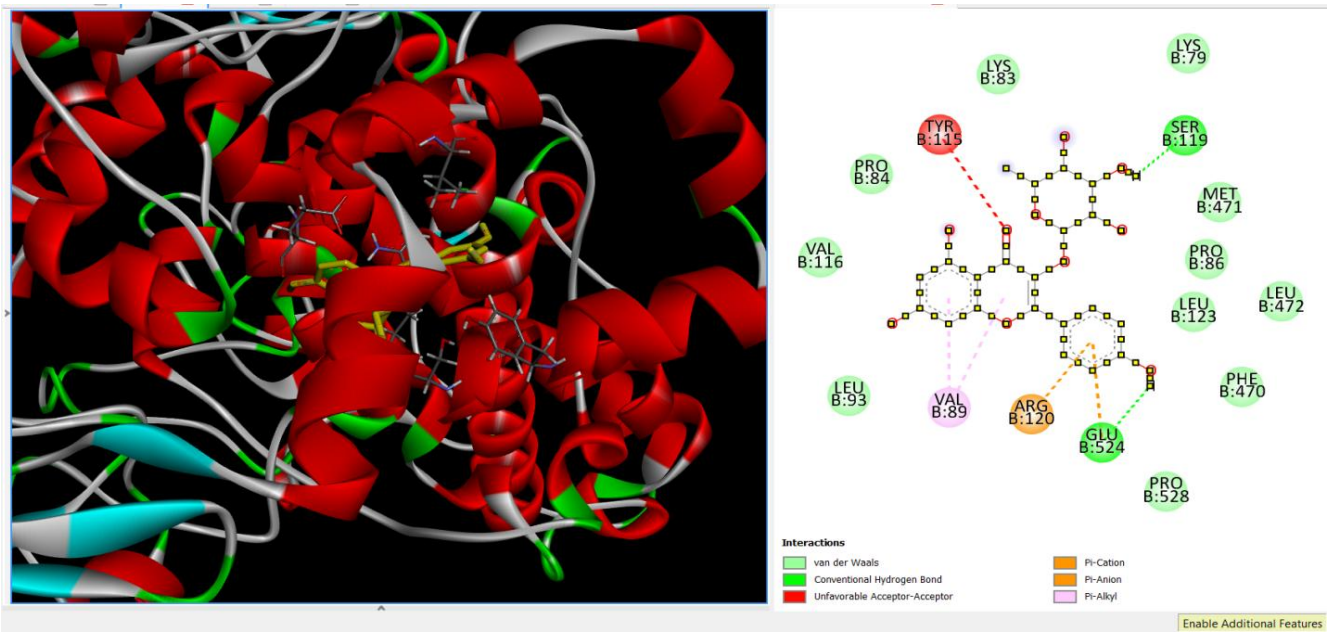


Figure 5: 3D and 2D integration of Alpha Spinasterol with COX-2 enzyme

The autodocking results show a strong interaction between Alpha Spinasterol and the COX-2 enzyme (PDB ID: 5IKR) with the B-Chain, exhibiting the highest binding energy (−7.7 kcal/mol).

4.5.5 Docking Results of Quercetin

Table 9: Results of Auto Docking Quercetin with COX-2 enzyme

S.No	An amino acid of the COX-2 enzyme	Type of bonding interaction
1.	ALA B:202	Pi-Pi-T-Shaped
2.	LEU B: 391	Van der Waals
3.	ALA B: 199	Van der Waals
4.	LEU B: 390	Van der Waals
5.	GLN B: 203	Van der Waals
6.	TYR B: 385	Van der Waals
7.	THR B: 206	Van der Waals
8.	PHE B: 210	Van der Waals
9.	HIS B: 386	Van der Waals
10.	ASN B: 382	Van der Waals
11.	HIS B: 207	Pi-Donor Hydrogen Bond

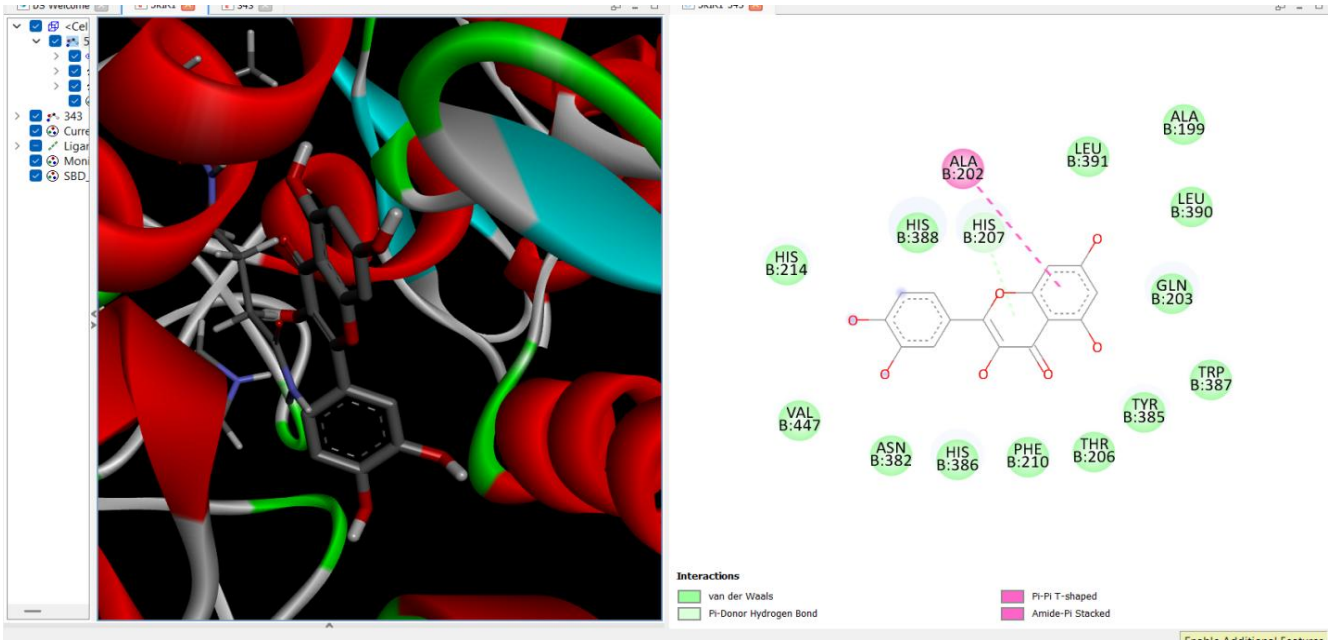


Figure 6: 3D and 2D integration of quercetin with COX-2 enzyme

The autodocking results show a strong interaction between quercetin and the COX-2 enzyme (PDB ID: 5IKR) with the B-Chain, exhibiting the highest binding energy (−9.0 kcal/mol).

5 DISCUSSION

Pithecellobium dulce, commonly known as Jungle Jalebi or Manila tamarind, belongs to the family Fabaceae and has long been recognized in conventional medicine for its comprehensive therapeutic potential. Earlier pharmacological studies have described antidiabetic, anti-inflammatory, antioxidant, antimicrobial, and anti-ulcer activities, indicating that the plant contains multiple bioactive phytoconstituents with medicinal relevance. Different parts of the plant have been conventionally used to manage toothache, earache, skin disorders, and inflammatory conditions. In addition, the leaves possess appreciable nutritional value and are used as protein-rich fodder for animals. These ethnopharmacological claims support the scientific evaluation of the plant as a potential source of anti-inflammatory agents [10,11].

Inflammation is a complex protective response initiated by tissue injury, infection, or chemical insult. It is clinically characterised by pain, redness, swelling, heat, itching, and functional loss. At the cellular and molecular level, inflammation involves membrane destabilisation, protein denaturation, lysosomal enzyme release, leukocyte infiltration, and increased production of inflammatory mediators such as prostaglandins, tumour necrosis factor-alpha (TNF-α), interleukins, histamine, and reactive oxygen species. Among these pathways, induction of cyclooxygenase-2 (COX-2) plays a central role by catalyzing the synthesis of pro-inflammatory prostaglandins. Therefore, inhibition of protein denaturation, stabilization of biological membranes, and suppression of COX-2 activity are established strategies for identifying potential anti-inflammatory compounds [2,8].

Based on these mechanisms, the current study assessed the anti-inflammatory activity of the ethanolic leaf extract of Pithecellobium dulce using *in vitro* and in silico approaches. The *in vitro* assays included inhibition of egg albumin denaturation and a human red blood cell (HRBC) membrane stabilisation model, whereas molecular docking studies were performed against the Cyclooxygenase-2 target.

Protein denaturation is a chief cause of inflammatory and arthritic disorders, as denatured proteins can act as autoantigens and perpetuate inflammatory responses. In the present investigation, the ELEPD exhibited significant concentration-dependent inhibition of egg albumin denaturation. The percentage inhibition produced by the extract at concentrations of 37.5, 75, 150, 300, and 600 µg/mL was 15.99±2.23, 25.72±1.84, 39.78±1.42, 59.29±3.36, and 68.26±6.66, respectively. These findings indicate progressive protection against thermal protein denaturation with increasing concentration. The standard drug, Diclofenac sodium, also showed marked inhibition at the corresponding concentrations, confirming the model's validity. The IC_{50} value of the extract was 198.5±2 µg/mL, whereas diclofenac showed a lower IC_{50} of 157.3±2 µg/mL, indicating comparatively higher potency of the standard drug. Nevertheless, the extract demonstrated appreciable anti-denaturation activity, suggesting the presence of bioactive phytochemicals capable of stabilising protein conformation under stress conditions.

Membrane stabilization is another important mechanism of anti-inflammatory action, since

erythrocyte membranes are structurally analogous to lysosomal membranes. Stabilisation of RBC membranes prevents lysosomal membrane rupture and the subsequent release of inflammatory mediators. In the heat-induced hemolysis model, exposure of RBC suspensions to 56°C caused membrane protein denaturation and hemolysis. The ethanolic extract significantly protected erythrocytes against heat-induced lysis in a concentration-dependent manner, with maximum inhibition of 84.28% at 600 µg/mL. Although Diclofenac sodium provided greater protection at all tested concentrations, the extract's substantial membrane-stabilising effect confirms its anti-inflammatory potential. This activity may be attributed to flavonoids, phenolics, sterols, and triterpenoids, which are known to strengthen membrane integrity and reduce oxidative damage.

To further elucidate the probable molecular mechanism, phytoconstituents of Pithecellobium dulce were subjected to docking analysis against Cyclooxygenase-2. Among the tested compounds, Kaempferol exhibited the highest binding affinity, with a docking score of −9.2 kcal/mol, suggesting a strong interaction with the enzyme's active site. It formed multiple Pi-alkyl, carbon-hydrogen, and van der Waals interactions with key amino acid residues, indicating stable ligand-receptor binding and probable inhibition of COX-2 catalytic activity. Kaempferol-3-O-rhamnoside also showed favourable binding energy (−8.4 kcal/mol), followed by Alpha-spinasterol (−7.7 kcal/mol), Dulcitol (−6.3 kcal/mol) and quercetin (−9.0 kcal/mol). These findings indicate that multiple constituents of the extract may synergistically contribute to anti-inflammatory activity by inhibiting COX-2.

The strong *in vitro* membrane-protective and anti-denaturation effects, together with favourable docking interactions against COX-2, suggest that the ethanolic leaf extract of Pithecellobium dulce possesses promising anti-inflammatory potential. The activity may primarily be mediated through inhibition of protein denaturation, stabilization of cellular membranes, antioxidant protection, and suppression of prostaglandin synthesis via COX-2 blockade. Overall, the present findings scientifically validate the traditional use of the plant in the treatment of inflammatory disorders and support further studies aimed at isolating active constituents, conducting mechanistic investigations, and evaluating its in vivo pharmacological effects.

6 CONCLUSION:

It is anticipated that compounds with membrane-stabilizing properties and the ability to prevent protein denaturation can provide substantial protection to cell membranes against Inflammation. Such compounds are well recognized for their ability to inhibit the release of phospholipases, enzymes that initiate the production of inflammatory mediators. *Pithecellobium Dulce* has demonstrated significant membrane-stabilizing properties and inhibition of protein denaturation, indicating its potential anti-inflammatory activity. *In silico* studies on the binding of the COX-2 enzyme to key constituents—kaempferol, kaempferol-3-O-rhamnoside, and quercetin —have revealed high binding energies (more than −8.0 kcal/mol), further suggesting their role in COX-2 inhibition. This effect is likely attributable to the presence of major phytochemicals in the extract, which inhibit phospholipase release and thereby reduce the formation of inflammatory mediators.

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Author's contributions: SM conceived and designed the study, supervised the experiments, analyzed the data, and drafted the manuscript. CV and CS performed the *in vitro* experiments and assisted with data analysis and manuscript preparation. SM and DK performed the Insilico docking work and contributed to data interpretation and manuscript writing. AV provided scientific guidance and revised the manuscript. All authors read and approved the final manuscript

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