RESEARCH PAPER

Phytochemical Profiling, *In-Vitro* COX-2 Inhibition and *In-Silico* Evaluation of the Ethanolic Flower Extract of Rosa indica L.

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ABSTRACT

Background: Pain and inflammation are leading reasons for medical care, and the adverse effects of long-term NSAID and opioid use sustain interest in safer plant-derived alternatives. *Rosa indica* L. (Rosaceae) is traditionally used for wounds, headaches, and inflammatory complaints, but its anti-inflammatory mechanism remains poorly defined.

Objective: To chemically characterise the ethanolic flower extract of *Rosa indica* and evaluate its COX-2 inhibitory potential using integrated *in vitro* and *in silico* approaches.

Methods: Fresh authenticated flowers were cold-macerated in 95 % ethanol and concentrated below 40°C. The extract underwent qualitative phytochemical screening, GC–MS profiling and a concentration-dependent COX-2 inhibition assay with indomethacin as a standard. Major constituents were docked against COX-2 (PDB: 5IKR), assessed for drug-likeness, and the resulting complexes were subjected to RMSF analysis using CABS-flex 3.0.

Results: Screening confirmed phenolics, flavonoids, alkaloids, tannins, saponins, steroids and glycosides. GC–MS identified 27 constituents, including pyrogallol, 2,4-di-tert-butylphenol, 5-hydroxymethylfurfural, DDMP, phenylethyl alcohol, vanillin lactoside, linoleic acid, and chondroitin sulfate. Ethanolic extract of *Rosa indica* inhibited COX-2 with an IC_{50} of 624.5 ± 23.33 µg/mL, compared with 93.35 ± 0.88 % inhibition by indomethacin. Docking energies ranged from −7.0 to −8.7 kcal/mol, led by vanillin lactoside (−8.7) and chondrillasterol (−8.3). Phenylethyl alcohol and 2,4-di-tert-butylphenol showed was most drug-like lead strongest binders were least drug-like. RMSF values remained largely below 1.5 Å.

Conclusion: The ethanolic flower extract of Rosa indica is a weak to moderate, multi-component COX-2 inhibitor whose activity most likely reflects additive contributions of phenolic, glycosidic, fatty acid, as well as steroidal constituents. These findings provide a mechanistic rationale for the traditional anti-inflammatory use of the plant and justify bioassay-guided fractionation and *in-vivo* validation, but do not by themselves establish therapeutic efficacy.

Keywords: *Rosa indica*, COX-2 inhibition, GC–MS; molecular docking, ADME, anti-inflammatory

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INTRODUCTION

Pain is one of the most common causes of seeking medical care globally and constitutes a major public health issue. Around 20 to 30% of age globally circumstance chronic pain, while close to 10% of the world's public expand chronic pain yearly**(1)**. Chronic pain is related to physical disability, psychological distress, decreased productivity, and substantial healthcare costs, considerably affecting the quality of life of individuals**(2)**. The high prevalence and socioeconomic stress of pain emphasise the need for effective, safe, and economical analgesic medicines**(3)**.

The International Association for the Study of Pain (IASP) describe pain as an unpleasant sensory and emotional state related to or accompanied by current or prospective tissue damage. Competent and safe pain management remains a major clinical concern, despite advances in the field **(4)**.

Acute and chronic pain is often treated with readily accessible analgesic drugs, especially opioid analgesics and non-steroidal anti-inflammatory medications (NSAIDs)**(5)**. As opioids provide analgesia by stimulating several opioid receptors in the central and peripheral nervous systems, NSAIDs mainly work by suppressing cyclooxygenase (COX) enzymes and reducing prostaglandin(PG) synthesis**(6)**. Despite the efficacy of these medicines, prolonged use is associated with many negative outcomes, such as intestinal irritation, hepatic as renal toxicity, cardiovascular problems, tolerance, dependency, and the tendency for addiction. Because of such limitations, there is an increasing demand for developing less hazardous and more potent herbal analgesics**(7,8)**.

All conventional and modern medicine still depends extensively on herbal medicinal plants, which have long been the primary sources of therapeutic drugs.**(9)** Flavonoids, phenolic compounds, tannins, alkaloids, and terpenoids are a variety of plant-derived bioactive substances that have exhibited pharmacological actions, such as analgesic, anti-inflammatory, and antioxidant effects**(10,11)**. Traditional medicinal herbal plants are being investigated more and more as a possible solution for synthetic analgesics given to their broad availability and decreased rate of side effects**(12,13)**.

Rosa indica L. (Family: Rosaceae) is a widely propagated plant for decoration that is recognised in conventional medical systems because of its visual aesthetic and aromatic properties**(14)**. In ancient times, the plant's flowers, petals, and leaves have been utilised to treat wounds, headaches, digestive issues, inflammatory conditions, and illness caused by stress**(15)**. Certain naturally active substances, including such as flavonoids, phenolic compounds, tannins, glycosides, and essential oils, which are known to have substantial anti-inflammatory as well as antioxidant effects, have been discovered during in phytochemical analyses**(16)**.

As high-level creation of reactive oxygen species (ROS) contributes to tissue harm and inflammatory response, oxidative stress can be connected to the pathophysiology of pain and inflammation**(17)**. In scavenging free radicals and reducing oxidative stress, natural antioxidants may reduce inflammatory processes associated with pain**(18)**. Therefore, in preliminary research, the antioxidant properties of plant extracts may be an important predictor of their conceivable analgesic impact. In this context, research was carried out to determine the analgesic potential of *Rosa indica's* alcoholic extract by using an *in-vitro* technique. While continuing on to *in- vivo* pharmacological research, the use of *in-vitro* assays provides a simple, inexpensive, consistent, and ethically acceptable technique for the initial evaluation of analgesic activity. The findings of this study could help develop alternative plant-based analgesics and offer scientific evidence in support of *Rosa indica's* conventional use.

Plant description



Figure 1: *Rosa indica*

Rosa indica (Indian rose), a perennial shrub belonging to the Rosaceae family and native to

Asia, is globally cultivated across tropical, subtropical, and temperate zones for its ornamental, commercial, and therapeutic value. Growing upright to a height of 1 to 3 meters, the plant features a robust taproot system, prickle-armed woody stems, and pinnately compound leaves with serrated, glossy leaflets. It flowers throughout the year, especially in cooler months, bearing showy, fragrant, bisexual blooms with numerous stamens and carpels, which later mature into seed-bearing red or orange hips. Beyond its prominence as a cut flower and landscape ornamental, R. indica is economically prized for essential oils, perfumes, rose water, cosmetics, pharmaceuticals, and culinary products like syrups and jams. **(19,20)**

Methods

Chemicals and reagents used in study was maintaining the analytical standards.

Plant material acquisition and verification of *Rosa indica*

The fresh flowers of *Rosa indica* were collected from the campus in Coimbatore in Tamil Nadu, India. The plant material was taxonomically identified and authenticated by Dr.S.S.Hameed, Scientist F and Head of Office and a voucher specimen (BSI/SRC/5/23/2025-26/Tech/563) was deposited for reference.

Flower extract preparation protocol for *Rosa indica*

The shade-dried powdered flowers of *Rosa indica* were treated with cold maceration using ethanol. Approximately 30 gm of powdered flowers were transferred to a clean 500 mL conical flask, and 300 mL of 95% ethanol was added to maintain a 1:10 w/v ratio. The conical flask was covered and retained at room temperature for 7 days with intermittent shaking. After maceration, the mixture was filtered using Whatman No.1 filter paper. The residue was then pressed and rinsed with a small volume of ethanol. The filtrates were concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding 40 °C. The ethanolic extract was then weighed. Finally, the percentage yield was determined, and keep in airtight containers at 4 °C until further examination **(21,22,23,24).**

Preliminary Phytochemical Screening:

The qualitative phytochemical screening of the ethanolic flower extract of *Rosa indica* was carried out to determine the presence or absence of major bioactive compounds **(25,26).**

GC–MS Analysis of *Rosa indica* flower extract

The GC-MS was used to identify the presence of bioactive constituents in the ethanolic extract. The extract was dissolved in HPLC-grade acetone and filtered through a C18 solid-phase extraction cartridge. An aliquot of 1 µL was injected into a GC-MS system (PerkinElmer Clarus 500) equipped with a TG-5MS column. Helium was used as the carrier gas at a flow rate of 1 ml/min. The oven temperature was ramped from 110°C to 280°C. Mass spectra were recorded in electron ionisation mode at 70eV. The compounds were identified by comparing the obtained spectra with those in the NIST mass spectral library **(27**,**28,29)**.

In Vitro COX-2 Inhibition Assay

The ability of ethanolic extract of *Rosa indica* to inhibit the COX-2 enzyme was evaluated using a standard COX-2 inhibition assay kit, following the manufacturer's instructions. Briefly, the assay mixture contained the enzyme, cofactors, and the test extract at different concentrations. A known indomethacin COX-2 inhibitor drug was used as the positive control. The reaction was allowed to proceed, and the absorbance was measured at the recommended wavelength. The percentage inhibition of COX-2 activity was calculated at each concentration, and the IC_{50} value, the concentration required to inhibit 50% of enzyme activity, was determined from the dose-response curve. **(30,31)**

Computational docking analysis

The bioactive compounds identified through GC-MS analysis were subjected to molecular docking to evaluate their possible analgesic activity by targeting the COX receptors, which play a key role in prostaglandin synthesis and pain regulation **(32,33)**. The substance structures were generated using the PubChem database. The 3D structures of the phytochemicals were exported as an SDF file format and optimised to ensure the accurate conformation for docking analysis. Structure-based molecular docking was performed to assess the binding interactions between the compounds and the COX-2 receptors **(34,35)**. The receptors were retrieved from the Protein Data Bank (PDB) maintained by the Research Collaboratory for Structural Bioinformatics (RCSB).

Molecular Docking

The active site of the target protein was prepared, and molecular docking was performed using the Discovery Studio and Pyrex software. The 3D crystal structure of the target protein was retrieved from the Protein Data Bank **36,37)**. During Protein preparation, all water molecules as well as unnecessary heteroatoms were removed using Discovery Studio to obtain a clean protein structure. Polar hydrogen atoms were added and the protein structure atoms were added and the protein structure was optimised for docking**(38,39)**.

The prepared protein and selected phytochemical ligands were then imported into Pyrex, whereas molecular docking was carried out using the AutoDock Vina engine to evaluate the binding affinity and Interactions between the ligands and the target protein**(40,41)**.

Drug-likeness Properties

The drug-likeness characteristics of the selected compounds were predicted by using the SwissADME and MolSoft software. The SMILES for each compound were obtained from the PubChem database to submitted individually to the online platform. The software was used to calculate molecular weight, lipophilicity, hydrogen bond donor, acceptors, Molecular Refractivity as well as, Log P as well as drug likeness score.**(43,44)**

CabsFlex Analysis

The CabsFlex software 3.0 website server was used to evaluate the protein structure's flexibility. The simulations were run with default settings once the protein structure in PDB format was uploaded to the server.To find the location with high structural mobility as well as assess the flexibility of individual amino acid residues. The root mean square fluctuation (RMSF) values were computed**(45,46)**.

Result

Preliminary qualitative screening of the ethanolic extract of Rosa indica revealed a chemically rich extract. All seven classes of secondary metabolites tested for were detected: phenolics, flavonoids, alkaloids, tannins, saponins, steroids and glycosides were each present **(Table 1)**. No class tested for was found to be absent, indicating that ethanol, a solvent of intermediate polarity, efficiently co-extracted both the polar phenolic/glycosidic fraction and the less polar steroidal fraction of the petals.

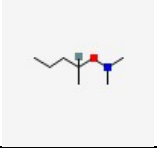
Table 1. Preliminary phytochemical screening of the ethanolic extract of *Rosa indica*. (+) = present

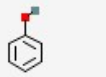
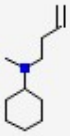
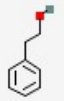
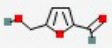
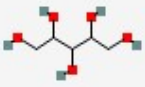
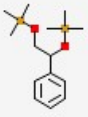
PHYTOCHEMICAL SCREENING	RESULT	INTERFERENCE
Phenolics	Positive (+)	Free and bound phenolic acids present
Flavonoids	Positive (+)	Flavonols/anthocyanin-related-pigments present
Alkaloids	Positive (+)	Nitrogenous bases present
Tannins	Positive (+)	Hydrolysable/condensed tannins present
Saponins	Positive (+)	Persistent froth on shaking
Steroids	Positive (+)	Phytosterol fraction present
Glycosides	Positive (+)	Sugar-conjugated aglycones present

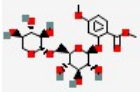
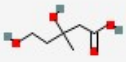
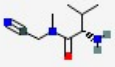
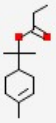
GC–MS profile of the ethanolic extract

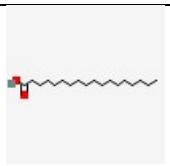
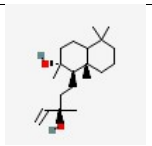
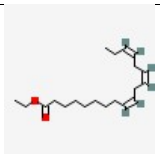
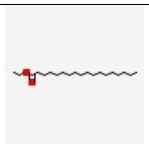
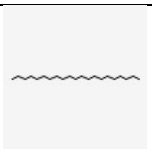
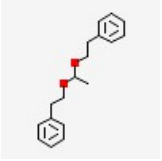
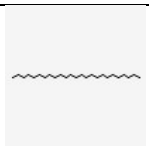
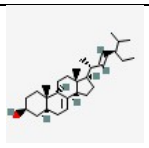
GC–MS analysis of Ethanolic Extract of *Rosa indica* resolved twenty-seven constituents between 5.059 and 30.770 min **(Table 2)**. The chromatogram was dominated by three chemically distinct groups: (i) low-molecular-weight oxygenated compounds eluting early (phenol, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, 2-hydroxy-gamma-butyrolactone, 4H-pyran-4-one derivative, 5-hydroxymethylfurfural, pentane-1,2,3,4,5-pentaol); (ii) aromatic and phenolic constituents of the mid-region (phenylethyl alcohol, 1-phenyl-1,2-ethanediol, 1,2,3-benzenetriol, 3-methoxy-2-methylphenol, 2,4-di-tert-butylphenol, vanillin lactoside); and (iii) a late-eluting lipophilic fraction of fatty acid ethyl esters (hexadecanoic, linoleic, linolenic and octadecanoic acid ethyl esters), long-chain alkanes (heneicosane, pentacosane) and the phytosterol chondrillasterol. Molecular weights ranged from 94.11 g/mol (phenol) to 476 g/mol (vanillin lactoside).

Table 2. Phytochemical composition of Rosa indica ethanolic extract as determined by GC–MS analysis.

S.NO	Phytochemical constituents	CHEMICAL FORMULA	Molecular weight	Retention time	Chemical structures
1	N,N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine 2-[(Dimethylamino)oxy]pentane	$C_7H_{17}NO$	131g/mol	5.059	

2	Phenol	C_6H_6O	94.11 g/mol	5.099	
3	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C6H8O4	144	5.177	
4	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102.09 g/mol	5.334	
5	Cyclohexanamine, N-3-butenyl-N-methyl	C11H21N	167	7.198	
6	Phenylethyl Alcohol	$C_8H_{10}O$	122.16 g/mol	8.011	
7	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C6H8O4	144	8.634	
8	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11 g/mol	10.265	
9	Pentane-1,2,3,4,5-pentaol	$C_5H_{12}O_5$	152.15 g/mol	10.646	
10	1,2-Ethanediol, 1-phenyl	C8H10O2	138	11.822	

11	1,2,3-Benzenetriol	C6H6O3	126	13.035	
12	3-methoxy-2-methylphenol	C8H10O2	138	13.919	
13	2,4-Di-tert-butylphenol	C14H22O	206	15.238	
14	Vanillin lactoside	C20H28O13	476	16.439	
15	3,5-dihydroxy-3-methylpentanoic acid	C6H12O4	148	17.097	
16	2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)	C13H20O	192	17.392	
17	2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	C13H22O	194	17.563	
18	Hydrazinecarboxamide, 2-(2-methylcyclohexylidene)	C8H15N3O	169	17.776	
19	2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl	C13H22O2	210	18.418	

20	Hexadecanoic acid, ethyl ester	C18H36O2	284	22.004	
21	Linoleic acid ethyl ester	C20H36O2	308	23.978	
22	9,12,15-Octadecatrienoic acid, ethyl ester	C20H34O2	306	24.049	
23	Octadecanoic acid, ethyl ester	C20H40O2	312	24.357	
24	Heneicosane	C21H44	296.6 g/mol	25.549	
25	Phenylethylacetal	C18H22O2	270	25.930	
26	Pentacosane	C25H52	352.7 g/mol	27.615	
27	Chondrillasterol	C29H48O	412.7 g/mol	30.770	

In vitro COX-2 inhibitory activity

The COX-2 inhibitory potential of ETHANOLIC EXTRACT OF ROSA INDICA was assessed over the concentration range 15.62–1000 µg/mL using a colourimetric COX-2 inhibitor screening assay. The reference standard indomethacin produced a mean inhibition of 93.35 ± 0.88%, confirming assay validity. It inhibited COX-2 in a clear concentration-dependent manner: inhibition was minimal at the lowest concentration tested (15.62 µg/mL, 0.62 ± 0.14%) and rose progressively across 31.25, 62.5, 125, 250, 500 and 1000 µg/mL. The half-maximal inhibitory concentration (IC_{50}) was calculated as 624.5 ± 23.33 µg/mL (**Figure 2**), indicating moderate COX-2 inhibitory activity for the crude extract relative to the pure standard.

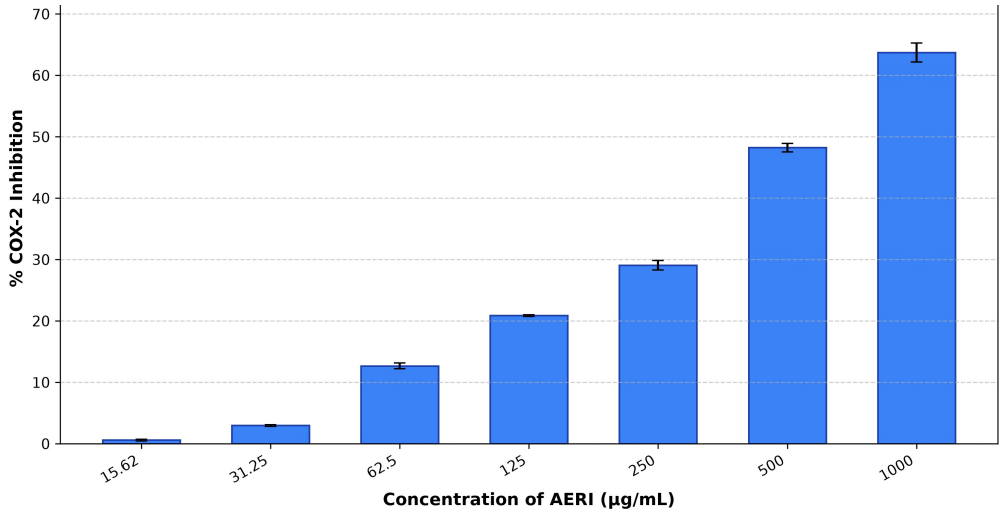


Figure 2. Concentration-dependent COX-2 inhibition by the alcoholic extract of Rosa indica (15.62–1000 µg/mL) with indomethacin as positive control.

Molecular docking against COX-2 (PDB: 5IKR)

Six GC–MS-identified constituents (N, 2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl), Linoleic acid ethyl ester, 9,12,15-Octadecatrienoic acid, ethyl ester, Y and Chondrillasterol) were docked into the active site of human COX-2 (PDB ID: 5IKR) (**Table 3**). All six ligands returned favourable binding energies between −7.0 and −8.7 kcal/mol (Table 3). Vanillin lactoside showed the strongest affinity (−8.7 kcal/mol), followed by chondrillasterol (−8.3 kcal/mol) and phenylethylacetal (−7.3 kcal/mol). 2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl) (P) scored −7.5 kcal/mol, while the two unsaturated fatty acid ethyl esters, linoleic acid ethyl ester and 9,12,15-octadecatrienoic acid ethyl ester, each scored −7.0 kcal/mol. The consistently negative scores across structurally diverse ligands suggest that COX-2 inhibition by the extract is a multi-component effect rather than the action of a single constituent.

Table 3. Molecular docking scores of selected *Rosa indica* constituents against COX-2 (PDB ID: 5IKR). Hydrogen-bond and hydrophobic interaction counts/residues to be inserted from the interaction analysis.

S.no	Ligand	Pdb id	Binding score	No.of hydrogen bond	No.of residues	No.of hydrobobic bond	No.of residues
1	Vanillin lactoside	5IKR	-8.7	7	ASN B: 375 GLY B: 225 HIS B: 226 GLY B:536 VAL B: 538 TYR B: 373 LEU A :224	0	-
2	2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)		-7.5	-	-	4	LEU A:145 LEU B:145 MET B:458 ALA B:219
3	Linoleic acid ethyl ester		-7	1	ILE B: 124	1	PRO A:542
4	9,12,15-Octadecatrienoic		-7	-	-	2	PRO B:542 ALA B:543

	acid, ethyl ester					
5	Phenylethylacetal	-7.3	2	PRO A:156 GLY A:136	6	ARG A:469 LYS A:468 LEU A:152 PRO A:153 CYS A: 36 CYS A:47
6	Chondrillasterol	-8.3	-	-	2	PRO A:542 ARG B:44

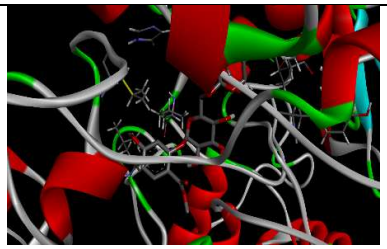
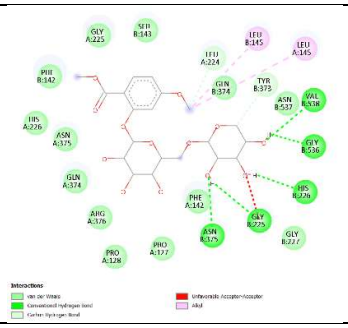
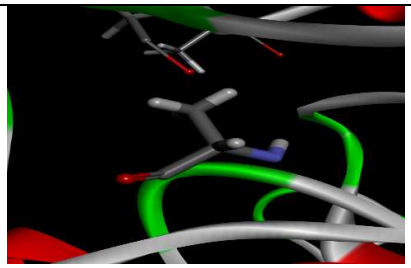
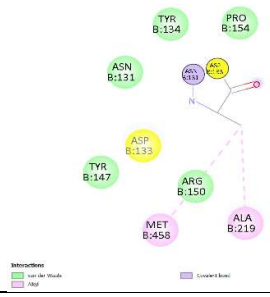
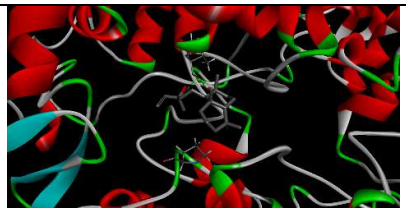
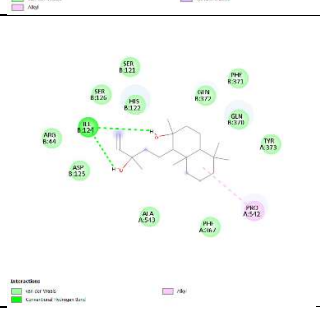
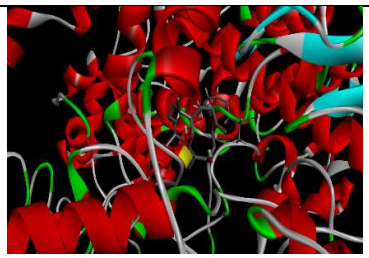
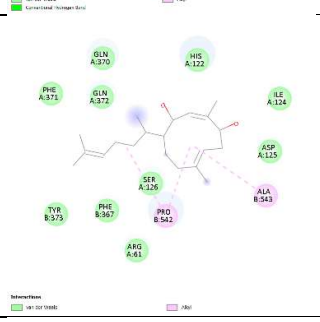
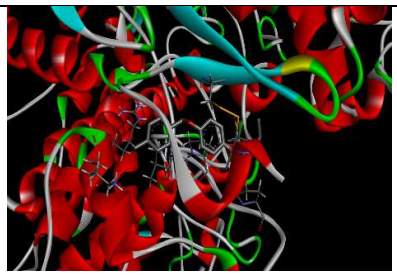
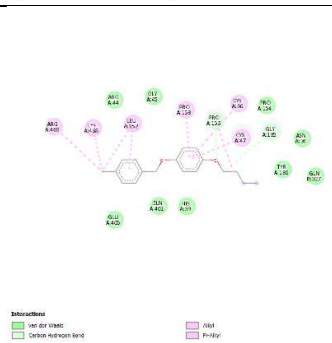
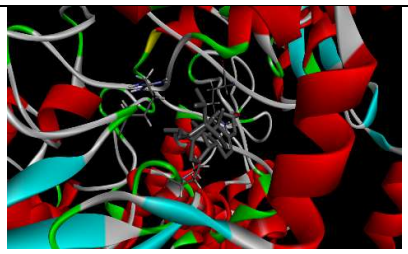
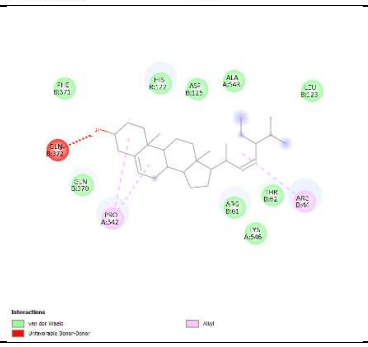
s.no	Compound name	3d	2d
1	Vanillin lactoside RESEARCH PAPER		
2	2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)		
3	Linoleic acid ethyl ester		
4	9,12,15-Octadecatrienoic acid, ethyl ester		
5	Phenylethylacetal		
6	Chondrillasterol		

Figure 3. Three-dimensional and two-dimensional interaction poses of ligands within the COX-2 active site.

Drug-likeness and ADME prediction

The six docked constituents were evaluated against Lipinski-type criteria (MW < 500 g/mol, HBA < 10, HBD < 5, log P < 5, molar refractivity < 150 cm³/mol) together with bioavailability score (> 0.12) and drug-likeness model score (> 0.18) **(Table 4).** Compounds 2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl), Linoleic acid ethyl ester and Phenylethylacetal satisfied the molecular-weight, hydrogen-bonding and refractivity criteria and returned the maximum bioavailability score of 0.55. Vanillin lactoside, despite the best docking score, violated the hydrogen-bond acceptor (13) and donor (6) limits and returned a lower bioavailability score of 0.17, consistent with its highly glycosylated, polar structure (log P −1.84). Chondrillasterol exceeded the lipophilicity threshold (log P 7.74) but recorded the highest drug-likeness model score (0.62) and a bioavailability score of 0.55. Phenylethylacetal was borderline on lipophilicity (log P 5.36) and returned a negative drug-likeness model score (−1.09), as did linoleic acid ethyl ester (−0.97).

Table 4. Predicted drug-likeness and physicochemical parameters of the selected Rosa indica constituents.

Compound name	Molecular weight g/mol	Hydrogen bond acceptor	Hydrogen bond donor	Log p	Molar refractivity (cm3/mol)	Bio availability	Drug-likeness model score
Rule	**<500**	**<10**	**<5**	**<5**	**<150**	**> 0.12**	**> 0.18**
Vanillin lactoside	476.43	13	6	-1.84	104.77	0.17	0.19
2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)	192.3	1	1	3.27	61.48	0.55	0.33
Linoleic acid ethyl ester	308.5	2	2	4.22	95.43	0.55	-0.97
9,12,15-Octadecatrienoic acid, ethyl ester	306.48	2	2	-	97.04	0.55	-
Phenylethylacetal	270.37	2	0	5.36	83.3	0.55	-1.09
Chondrillasterol	412.69	1	1	7.74	132.75	0.55	0.62

Residue flexibility analysis of the protein–ligand complexes (CABS-flex 3.0)

The residue-level flexibility of the protein–ligand complexes was assessed using CABS-flex 3.0 through root-mean-square fluctuation (RMSF) analysis. RMSF quantifies the average deviation of each amino acid residue from its mean position during the simulation; low RMSF values denote conformational rigidity and structural stability, whereas elevated values are characteristic of surface-exposed loops and terminal segments.

The RMSF profile of Vanillin lactoside indicated that the majority of residues fluctuated below 1.5 Å, reflecting a well-maintained protein fold. A single prominent peak of approximately 4.0 Å was observed in the region spanning residues 350–360. These deviations were confined to loop regions, while α-helical and β-sheet elements remained comparatively rigid, indicating that ligand binding preserved the structural integrity of the protein.

Phenylethyl alcohol and 2,4-di-tert-butylphenol showed displayed a fluctuation pattern closely comparable to that of Vanillin lactoside. Most residues remained below 1.5 Å, with a pronounced peak of approximately 4.0 Å near residue 350. The secondary structural elements were largely unperturbed, consistent with the formation of a stable protein–ligand complex.

Linoleic acid ethyl ester exhibited moderate

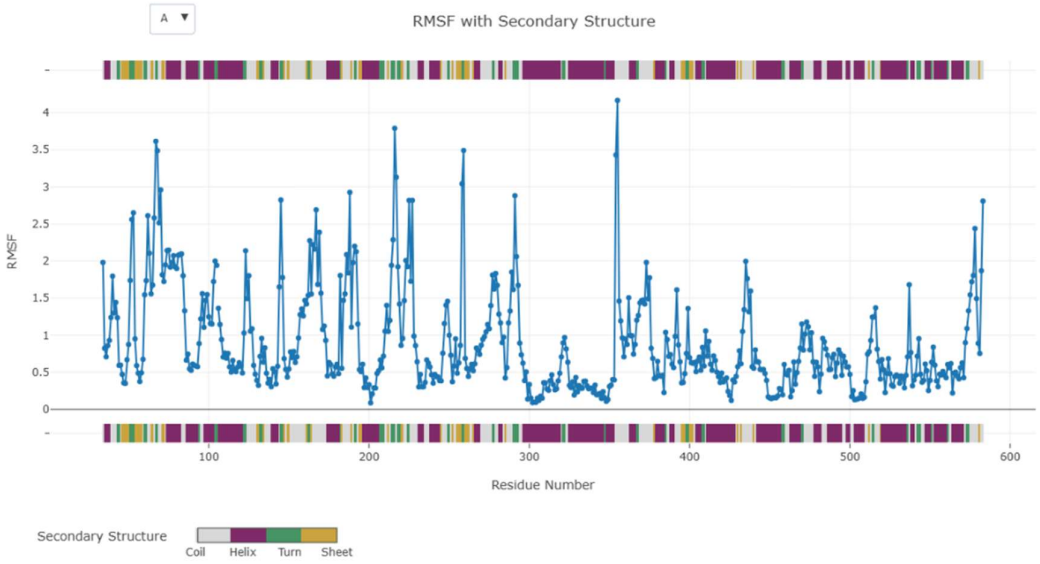
flexibility, with residue fluctuations predominantly in the range of 0.2–1.5 Å. A single major peak of approximately 4.5 Å was recorded at residues 170–180, indicating enhanced mobility within this loop region. Apart from this localized deviation, the remainder of the structure remained stable.

9,12,15-Octadecatrienoic acid, ethyl ester showed generally low residue fluctuations, with most RMSF values below 1.5 Å. The maximum fluctuation of approximately 4.0 Å occurred at residues 60–70, while the remaining residues displayed only moderate mobility. Conserved secondary structural regions exhibited minimal displacement, suggesting stable ligand accommodation within the binding site.

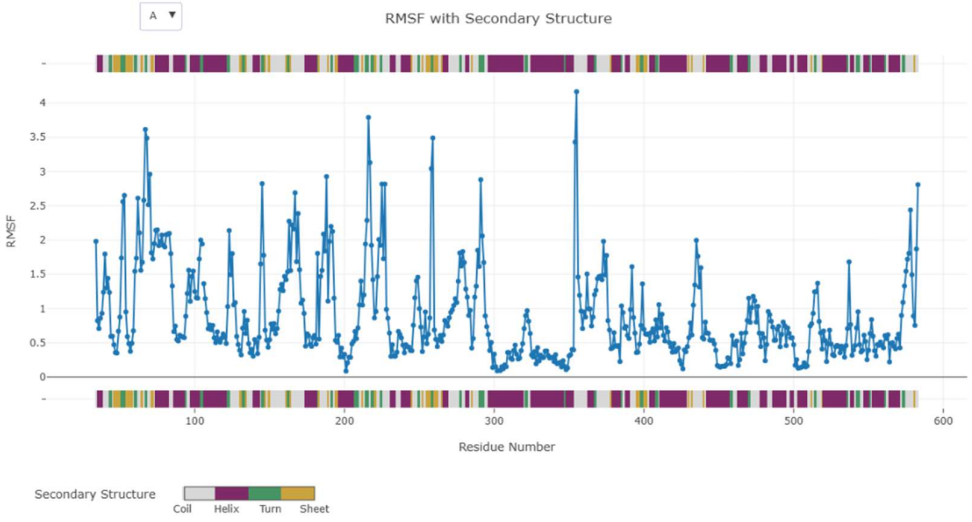
Phenylethylacetal maintained consistently low RMSF values across most of the protein. The highest fluctuation, approximately 3.5 Å, was localized to the N-terminal region, whereas the central core remained comparatively rigid — indicative of good overall structural stability with restricted local flexibility.

Among all complexes examined, Chondrillasterol produced the highest RMSF peak, approximately 6.0 Å at residues 60–80, reflecting pronounced mobility of the N-terminal loop. Nevertheless, residues beyond position 100 consistently remained below 1.5 Å, demonstrating that the structural core of the protein was preserved throughout the simulation.

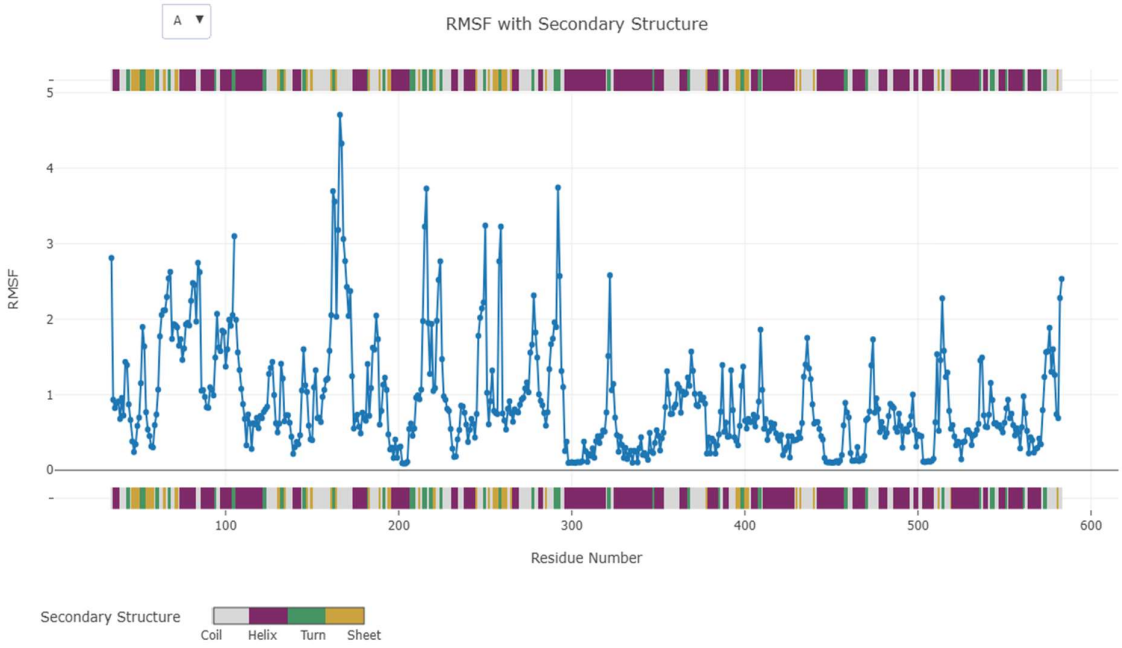
Vanillin lactoside



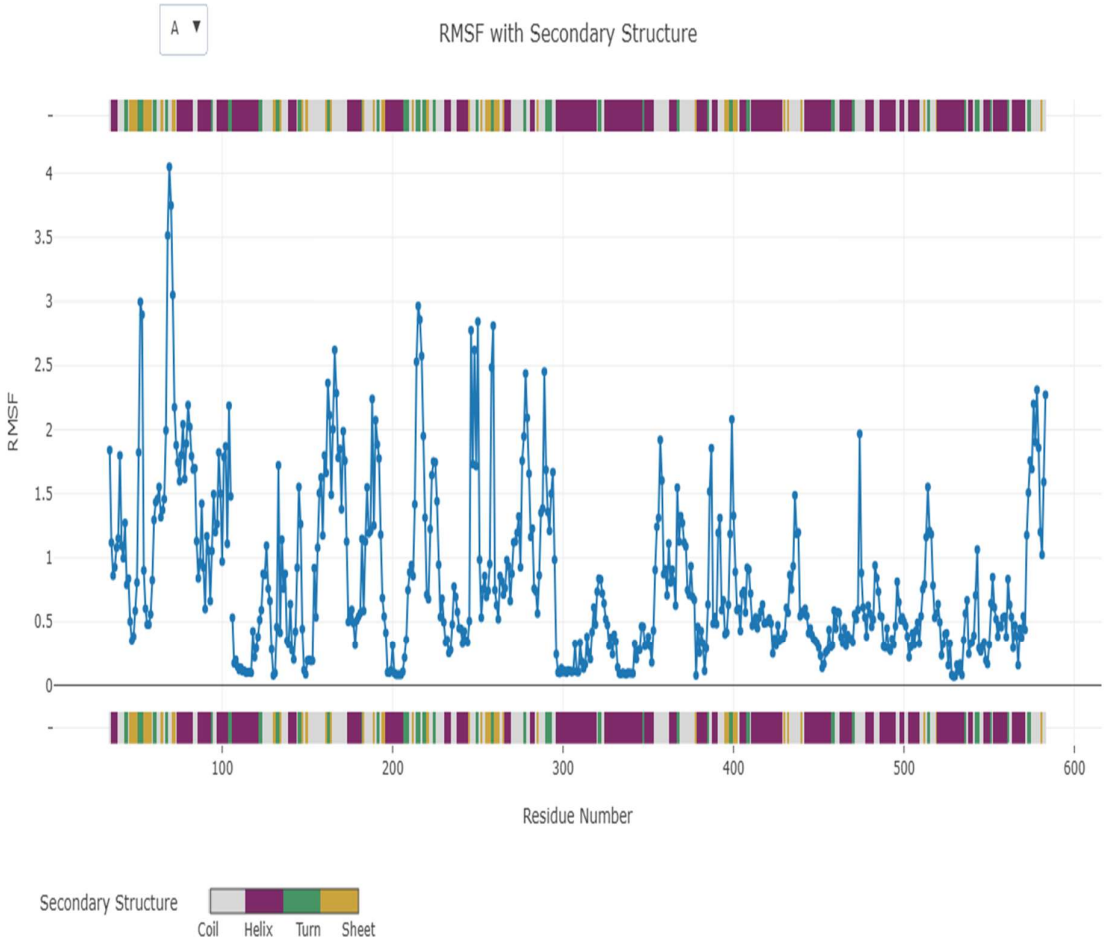
2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1- yl)



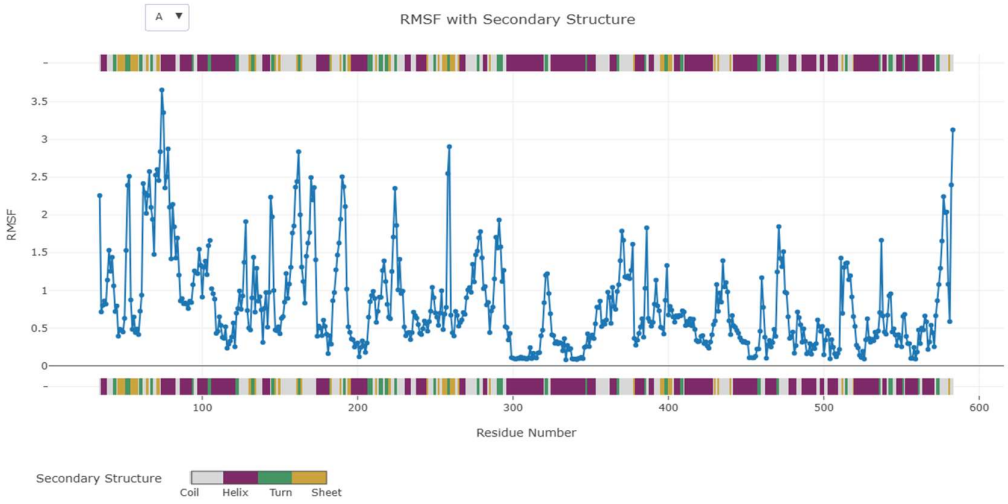
Linoleic acid ethyl ester



9,12,15-Octadecatrienoic acid, ethyl ester



Phenylethylacetal



Chondrillasterol

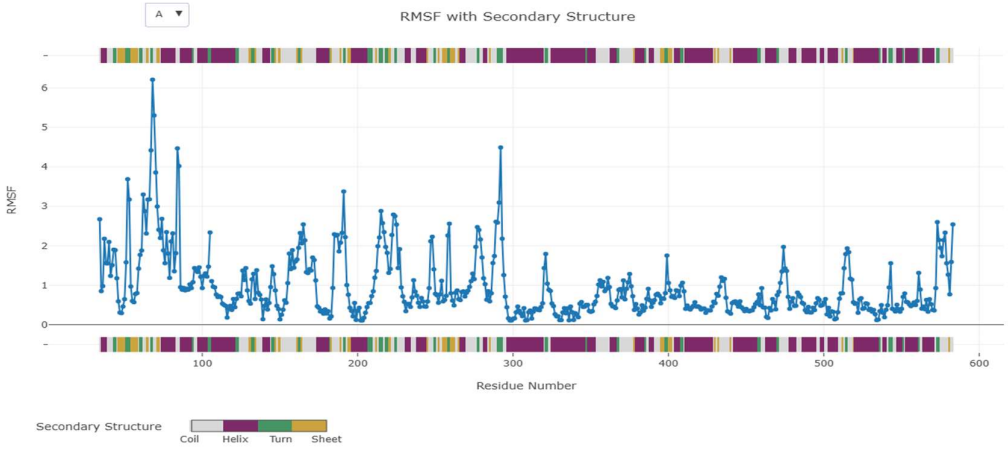


Figure 4. CABS-flex 3.0 RMSF profiles and corresponding structural models for the Vanillin lactoside, 2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1- yl), Linoleic acid ethyl ester, 9,12,15-Octadecatrienoic acid, ethyl ester, Phenylethylacetal, Chondrillasterol compounds

DISCUSSION

The present study set out to characterise the ethanolic extract of Rosa indica chemically and to establish, both experimentally and computationally, a rationale for its anti-inflammatory use. The results provide a coherent chain of evidence linking extract composition to COX-2 inhibition.

Qualitative screening indicated the simultaneous presence of phenolics, flavonoids, alkaloids, tannins, saponins, steroids and glycosides. Such a broad metabolite spectrum is characteristic of Rosa species, whose petals are recognised as concentrated sources of polyphenols and glycosylated aromatics**(47)**. Phenolics and flavonoids are of particular interest here because they are the most consistently reported plant-derived inhibitors of prostaglandin biosynthesis**(48)**, acting either by direct competition at the cyclooxygenase channel or by suppressing COX-2 induction at the transcriptional level. The concurrent detection of steroids and saponins accounts for the lipophilic fraction observed later by GC–MS and adds a second, membrane-directed mode of anti-inflammatory action.

The GC–MS profile substantiates the qualitative findings at molecular resolution. The phenolic screen is explained by phenol, 1,2,3-benzenetriol (pyrogallol), 3-methoxy-2-methylphenol and 2,4-di-tert-butylphenol; the glycoside screen by vanillin lactoside; and the steroid screen by chondrillasterol. Several of these are known bioactives in their own right. 2,4-Di-tert-butylphenol is a well-documented antioxidant and anti-inflammatory metabolite**(49)**Pyrogallol is a potent radical scavenger**(50)** 5-hydroxymethylfurfural and the 4H-pyran-4-one derivative (DDMP) are Maillard-type products with reported antioxidant and anti-inflammatory activity**(51)**. Phenylethyl alcohol is the principal aroma constituent of rose and possesses antimicrobial activity. The late-eluting fatty acid ethyl esters – particularly linoleic and α-linolenic acid ethyl esters – are recognised modulators of eicosanoid metabolism**(52)**, since polyunsaturated fatty acids compete with arachidonic acid for the cyclooxygenase active site. Chondrillasterol, a Δ7-phytosterol, has documented anti-inflammatory and anti-nociceptive properties**(53)**. The extract therefore contains at least four mechanistically plausible classes of COX-2 modulators.

COX-2 inhibitory activity

Ethanolic extract of *Rosa indica* produced a clean, concentration-dependent inhibition of COX-2 across the tested range, with an IC_{50} of 624.5 ± 23.33 µg/mL. While this value is high compared with indomethacin, which achieved 93.35 ± 0.88% inhibition**(54),** the comparison is not like-for-like: indomethacin is a purified single molecule, whereas ethanolic extract of *Rosa indica* is a crude multi-component matrix in which the active constituents represent only a small mass fraction of the material weighed out. Expressed based on active constituents rather than total extract, the intrinsic potency is considerably greater than the crude IC_{50} suggests. The very low inhibition at 15.62 µg/mL (0.62 ± 0.14%) followed by a steady rise is typical of extracts in which several weak-to-moderate inhibitors act additively; a single high-affinity inhibitor would instead produce a steeper curve with an earlier inflexion. The finding classifies the ethanolic extract of *Rosa indica* as a moderate COX-2 inhibitor and supports the traditional topical and oral use of rose preparations for inflammatory complaints, while indicating that fractionation would be required to obtain pharmacologically competitive potency.

Molecular docking rationale

Docking against COX-2 (PDB: 5IKR)**(55)** provided a structural explanation for the in vitro observation. All six selected ligands bound the active site with energies of −7.0 to −8.7 kcal/mol, values generally regarded as indicative of stable ligand–protein complexes. Vanillin lactoside (−8.7 kcal/mol) was the best scorer, which is consistent with its large, highly hydroxylated scaffold capable of extensive hydrogen bonding within the polar mouth of the cyclooxygenase channel. Chondrillasterol (−8.3 kcal/mol) achieved a comparable score by an opposite mechanism – its rigid, bulky steroid nucleus is well matched to the hydrophobic tunnel lined by Val349, Leu352, Tyr385, Trp387 and Leu531 that normally accommodates the arachidonate chain. The intermediate scores of the fatty acid ethyl esters Linoleic acid ethyl ester and 9,12,15-Octadecatrienoic acid, ethyl ester (−7.0 kcal/mol each) are readily interpreted as substrate mimicry, since these molecules closely resemble the natural substrate and can therefore occupy the same channel competitively. The narrow spread of scores across chemically unrelated ligands reinforces the interpretation drawn from the assay data: the extract's activity is the summed contribution of multiple moderate binders rather than the action of one dominant inhibitor. Detailed hydrogen-bond and hydrophobic-contact counts for each complex are to be reported alongside the 2D/3D interaction maps in Figure 2, and will allow the individual residue-level contributions to be assigned.

Drug-likeness considerations

The *in-silico* ADME analysis introduces an important qualification. The two strongest binders are the two least drug-like molecules. Vanillin lactoside violates two Lipinski parameters**(56)** (13 hydrogen-bond acceptors, 6 donors) and carries a low bioavailability score of 0.17, predicting poor passive membrane permeability and limited oral absorption despite its excellent docking score and acceptable drug-likeness model score of 0.19. Chondrillasterol lies at the opposite extreme, with a log P of 7.74 that greatly exceeds the lipophilicity limit and predicts poor aqueous solubility, although its drug-likeness model score of 0.62 was the highest in the set. Compound P is the most balanced candidate overall: it satisfies

every Lipinski criterion (MW 192.3, HBA 1, HBD 1, log P 3.27, MR 61.48) and returns favourable bioavailability (0.55) and drug-likeness (0.33) scores alongside a respectable binding energy of −7.5 kcal/mol. Compounds Linoleic acid ethyl ester and Phenylethylacetal satisfy most physicochemical criteria but return negative drug-likeness model scores (−0.97 and −1.09), reflecting the poor resemblance of simple long-chain esters to established drug scaffolds. Taken together, compound P emerges as the most promising lead for optimisation, chondrillasterol as a candidate requiring formulation support such as nanoencapsulation or lipid-based delivery, and vanillin lactoside as a compound better suited to topical or local application where systemic absorption is not required.

CABS-flex 3.0 RMSF analysis**(57)** demonstrated that all six compounds preserved the overall structural stability of the target protein. The predominance of RMSF values below 1.5 Å across the residue range indicates limited atomic fluctuation and conformationally stable complexes. The elevated values detected at discrete positions correspond principally to solvent-exposed loops and terminal residues, which are intrinsically more mobile than helical and β-strand elements and therefore do not constitute destabilization of the fold.

Compounds produced comparable RMSF profiles characterized by isolated, region-specific fluctuations, indicating that ligand binding effectively restrained protein motion. Compound Linoleic acid ethyl ester introduced a single pronounced flexible segment at residues 170–180 while retaining stability elsewhere. Chondrillasterol displayed the greatest local deviation (~6.0 Å) at the N-terminus; because this mobility was restricted to terminal residues and did not propagate to the structural core, the complex is nonetheless considered stable.

Collectively, these observations indicate that all tested compounds form stable complexes with the target protein, with flexibility confined to peripheral rather than core regions — an outcome that supports the reliability of the corresponding docking poses. The comparatively lower fluctuation profiles of compounds Vanillin lactoside, 2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl), 9,12,15-Octadecatrienoic acid, ethyl ester, and Phenylethylacetal suggest superior conformational stability and potentially stronger binding characteristics, whereas the greater flexibility associated with compound Chondrillasterol implies somewhat weaker structural stabilisation while still yielding an overall stable complex.

Limitation

The study has several limitations. Activity was measured for the crude extract only. The individual constituents were neither isolated nor assayed, so the contribution of each compound remains inferential.COX-1 was not screened. So, no selectivity index could be calculated, and cytotoxicity was not assayed; the specificity of inhibition cannot be excluded. The GC-MS identifications are library-based, therefore tentative in the absence of authentic standards. Docking, ADME prediction and CABS-Flex simulation are computational and require experimental confirmation, and no *in-vivo* analgesic model was performed. These aspects define the scope of ongoing work.

Conclusion

The present study provides an integrated chemical and mechanistic rationale for the traditional anti-inflammatory use of *Rosa indica* flowers. Ethanolic extraction recovered both polar phenolic/glycosidic and less polar steroidal fractions, and GC–MS resolved the qualitative screening into 27 identifiable constituents spanning at least four mechanistically plausible classes of COX-2 modulators. Ethanolic extract of *Rosa indica* inhibited COX-2 in a clean concentration-dependent manner (IC_{50} 624.5 ± 23.33 µg/mL); the shallow inhibition curve, together with the narrow spread of docking energies (−7.0 to −8.7 kcal/mol) across chemically unrelated ligands, indicates that the effect arises from the additive action of several moderate binders rather than a single dominant inhibitor.

In-silico ADME analysis qualifies this promise: the strongest binders are the least drug-like. Vanillin lactoside, despite the best docking score, violates two Lipinski parameters and is better suited to topical or local application, while chondrillasterol's high lipophilicity (log P 7.74) would require formulation support such as nanoencapsulation or lipid-based delivery. Compound P emerges as the most balanced lead, combining full Lipinski compliance with a binding energy of −7.5 kcal/mol. CABS-flex analysis confirmed that all six complexes are conformationally stable, with flexibility restricted to peripheral loops and terminal residues, supporting the reliability of the docking poses.

Overall, *Rosa indica* flower extract can be classified as a moderate COX-2 inhibitor of multi-component origin. Bioassay-guided fractionation to enrich the active constituents, *in-vivo* analgesic and anti-inflammatory validation, and toxicity profiling are the logical next steps toward developing a competitive plant-based anti-inflammatory agent.

FUNDING

None

CONFLICT OF INTEREST

The list of authors has no conflict of interest

ETHICAL APPROVAL

Not Applicable

INFORMED CONSENT

Nil

DATA AVAILABILITY STATEMENT

The data that support the funding are available upon request from the corresponding author.

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