

In-Silico and In-Vitro Analysis of Schleicheria Oleosa Bark for PLA2 And 5-LOX Enzyme Inhibition

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ABSTRACT

Inflammation is a complex physiological response that plays a central role in many chronic diseases. Targeting key inflammatory enzymes such as phospholipase A2 (PLA2) and 5-lipoxygenase (5-LOX) offers an effective strategy to mitigate the overproduction of pro-inflammatory mediators. In this study, the anti-inflammatory potential of Schleicheria Oleosa bark was investigated through in-vitro enzyme inhibition assays and in-silico molecular docking. Ethanol and aqueous extracts of S. Oleosa bark were prepared using maceration, yielding 18.92% and 15.36%, respectively. The PLA2 inhibition assay revealed that the ethanol extract (SOEE) exhibited higher activity with $69.51 \pm 1.63\%$ inhibition at 100 $\mu\text{g/mL}$ and an IC_{50} of 56.709 $\mu\text{g/mL}$, compared to the aqueous extract (SOAE) with $38.18 \pm 1.39\%$ inhibition and an IC_{50} of 151.157 $\mu\text{g/mL}$. Similarly, SOEE showed superior inhibition of 5-LOX with $57.69 \pm 1.65\%$ inhibition at 100 $\mu\text{g/mL}$ and an IC_{50} of 78.512 $\mu\text{g/mL}$, compared to SOAE ($40.54 \pm 1.39\%$; $\text{IC}_{50} = 152.349 \mu\text{g/mL}$). Molecular docking identified Isorhamnetin 3-O-glucoside, Epicatechin Gallate, and related compounds as strong inhibitors, exhibiting stable interactions with key catalytic residues of PLA2 (CYS43, HIS47, ASP48, LYS62) and 5-LOX (SER171, ASP170, ASN613, ARG401). These findings suggest that S. Oleosa bark contains potent bioactive phytochemicals capable of modulating inflammatory pathways through dual enzyme inhibition. Further in-vivo and clinical investigations are warranted to develop S. Oleosa-derived compounds as potential natural anti-inflammatory therapeutics.

Keywords: Schleicheria Oleosa; PLA2 inhibition; 5-LOX inhibition; molecular docking; phytochemicals; anti-inflammatory activity.

INTRODUCTION

Inflammation is a fundamental protective response of the body to infection, injury, or harmful stimuli, and plays a crucial role in maintaining tissue homeostasis [1]. Although acute inflammation is essential for host defense and tissue repair, its chronic persistence is associated with the onset and progression of several debilitating diseases, including rheumatoid arthritis, asthma, inflammatory bowel disease, cancer, cardiovascular disorders, and neurodegenerative conditions. The underlying molecular mechanisms of inflammation are complex and involve a cascade of signaling events and biochemical mediators that amplify and sustain the inflammatory response [2]. Among these mediators, arachidonic acid metabolites play a pivotal role in regulating vascular permeability,

leukocyte recruitment, and pain perception. The enzymes phospholipase A2 (PLA2) and 5-lipoxygenase (5-LOX) are particularly significant in this pathway, as they regulate the liberation and metabolism of arachidonic acid to produce pro-inflammatory lipid mediators such as prostaglandins and leukotrienes [3]. PLA2 catalyzes the hydrolysis of membrane phospholipids to release arachidonic acid, which serves as the substrate for downstream enzymes including 5-LOX [4]. 5-LOX further metabolizes arachidonic acid into leukotrienes, potent mediators that promote vasodilation, bronchoconstriction, and immune cell activation [5]. The upregulation and overactivation of these enzymes have been strongly implicated in the pathophysiology of chronic inflammatory diseases. Consequently,

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

PLA2 and 5-LOX represent promising pharmacological targets for the development of anti-inflammatory therapies. Several synthetic inhibitors of these enzymes have been developed and evaluated; however, their clinical application is often constrained by adverse effects, poor specificity, high cost, and limited tolerability upon long-term use [6]. These limitations highlight the urgent need for safer and more effective alternatives, ideally derived from natural sources. Over the past few decades, natural products have emerged as a rich reservoir of bioactive compounds with diverse pharmacological activities. Medicinal plants, in particular, offer multi-targeted therapeutic potential and often present fewer side effects compared to synthetic drugs [7]. *Schleichera Oleosa* (Lour.) Oken, commonly known as kusum, is a traditional medicinal plant widely distributed in tropical regions of Asia [8]. Its various parts—bark, leaves, and seeds—have been traditionally used for their anti-inflammatory, antimicrobial, analgesic, and wound-healing properties [9]. Phytochemical investigations have revealed that *S. Oleosa* contains a diverse array of bioactive compounds, including triterpenoids, tannins, fatty acids, and saponins, which may contribute to its therapeutic effects. While its anti-inflammatory activity has been reported in traditional medicine, there is a paucity of scientific studies that elucidate its molecular mechanism of action, particularly in relation to key inflammatory enzymes such as PLA2 and 5-LOX. The present study aims to investigate the anti-inflammatory potential of *Schleichera Oleosa* bark extract by evaluating its inhibitory effects on PLA2 and 5-LOX enzymes using a combination of *in-silico* and *in-vitro* approaches. The study involves the preparation of ethanol and aqueous extracts of the bark, identification of key phytoconstituents, molecular docking to assess their binding affinity with PLA2 and 5-LOX, and biochemical enzyme inhibition assays to determine their inhibitory efficacy in comparison with standard inhibitors. By integrating computational and experimental methodologies, this research seeks to provide a mechanistic understanding of the enzyme-specific activity of *S. Oleosa* bark extract and identify promising natural inhibitors that may serve as leads for future anti-inflammatory drug development.

MATERIALS AND METHODS

Collection, authentication, and preparation of extract

The dried *Schleichera Oleosa* bark was collected, cleaned to remove debris, and shade-dried at room temperature to preserve bioactive compounds. The dried bark will then be ground into a fine powder using a mechanical grinder and sieved for uniformity. For maceration, a weighed amount (100 g) of the powdered bark will be placed in a clean glass container and soaked in ethanol at a 1:10 (w/v) ratio and water separately, ensuring complete immersion. The mixture will be left to stand at room temperature for 48–72 hours with occasional stirring. After maceration, the extract will be filtered using muslin cloth or Whatman filter paper to remove plant residues, and the process will be repeated twice with fresh solvent to maximize compound extraction. The combined filtrates will be concentrated using a rotary evaporator under reduced pressure at 40–50°C. The concentrated extract will be further dried using a water bath or lyophilization to obtain a solid or semi-solid extract, which will be stored in an airtight container at 4°C until further analysis [10].

In-Vitro PLA2 inhibition assay

The PLA2 inhibitory activity was assessed following the method described by De Arango and Radvany [11]. The substrate solution consisted of lecithin (3.5 mM), sodium taurodeoxycholate (NaTDC, 3 mM), sodium chloride (NaCl, 100 mM), calcium chloride (CaCl₂, 10 mM), and phenol red (0.055 mM) as a colorimetric indicator, prepared in 100 mL of distilled water. The reaction mixture was adjusted to pH 7.6, and 0.2 µg of pG-IB was dissolved in 10% acetonitrile at a concentration of 0.002 µg/µL. A 2 µL aliquot of PLA2 enzyme solution was incubated with 2 µL of the SOEE and SOAE at room temperature for 20 minutes. Following incubation, 200 µL of the prepared PLA2 substrate was added, and enzymatic hydrolysis was monitored kinetically for 5 minutes. The absorbance was recorded at 558 nm, and the percentage inhibition of PLA2 activity was calculated.

Enzyme activity (EA) = Optical Density (0 min) – Optical Density (15 min)/ 15 min



% inhibition = $\frac{\text{EA -ve control} - \text{EA Sample}}{\text{EA -ve control}} \times 100$

***In-Vitro* 5-LOX inhibition assay**

The 5-lipoxygenase (5-LOX) inhibitory activity of SOEE and SOAE was evaluated following the method described by Wisastra *et al.*, with modifications. The assay measured the residual enzymatic activity after incubation with the extract for 10 to 15 minutes at 25°C. The reaction was based on the conversion of linoleic acid into hydroperoxy-octadecadienoate (HPOD), and the absorbance change was monitored using a UV-visible spectrophotometer at 234 nm. The assay buffer consisted of 50 mM Tris buffer (pH 7.5) containing 2 mM EDTA and 2 mM CaCl₂. The 5-LOX enzyme, prepared at 20,000 U/mL, was diluted in the buffer at a 1:4000 ratio. The *S. Oleosa* bark extract was tested at concentrations of 10, 25, 50, 75, and 100 µg/mL, prepared by dissolving in dimethyl sulfoxide (DMSO) and further diluting in assay buffer. The linoleic acid substrate (20 mg/mL) was diluted in ethanol before use. The reaction mixture consisted of 1 mL of the diluted enzyme solution, 100 µL of 2 mM ATP, 100 µL of the extract at the specified concentration, and 790 µL of Tris buffer, followed by a 10-minute incubation. After 10 seconds of mixing the enzyme with the extract, 10 µL of a 20 mM substrate solution was added to initiate the reaction. The change in absorbance was recorded, and the enzyme activity in the absence of inhibitors served as the positive control. Montelukast was used as the reference standard for comparison [12].

***In-silico* molecular docking analysis**

The *in-silico* molecular docking analysis of *Schleichera Oleosa* phytoconstituents against phospholipase A2 (PLA2) and 5-lipoxygenase (5-LOX) was performed using the SWISSdock web server. The 3D structures of target enzymes (PLA2 and 5-LOX) were retrieved from the Protein Data Bank (PDB) in .pdb format. The structures were prepared by removing water molecules, adding hydrogen atoms, and optimizing the energy using molecular modeling software. The bioactive compounds present in *S. Oleosa* were identified from the literature, and their 2D structures were retrieved from PubChem or other chemical databases, followed by conversion to 3D .mol2 or .pdb format using Open

Babel. The prepared ligand and receptor files were uploaded to SWISSdock, and docking was performed under default parameters, using the CHARMM force field to predict binding interactions. The docking results were analyzed using the Molegro visualization tool, where docking scores, binding energies, and molecular interactions (hydrogen bonds, hydrophobic interactions) were examined. The binding affinity of *S. Oleosa* phytoconstituents was compared with standard inhibitors of PLA2 and 5-LOX to determine their potential as anti-inflammatory agents [13].

RESULTS AND DISCUSSION

Extraction of *S. Oleosa* extract

The extraction of *Schleichera Oleosa* bark produced ethanol and aqueous extracts with yields of 18.92% and 15.36%, respectively. The process involved macerating 100 g of powdered bark in ethanol or water (1:10 w/v), followed by filtration and rotary evaporation. The higher ethanol yield indicates its superior efficiency in extracting a broader range of phytochemicals. Ethanol, being semi-polar, extracts both water- and fat-soluble compounds such as flavonoids, alkaloids, phenolics, and terpenoids. Water, a polar solvent, primarily extracts carbohydrates and glycosides, leading to a lower yield. The extraction method proved effective for isolating bioactive materials from the bark. The ethanol extract likely contains a richer phytochemical profile with strong therapeutic potential. The aqueous extract, although lower in yield, may offer unique bioactive components. Both extracts can be valuable for pharmacological screening. This efficient extraction highlights the medicinal promise of *S. Oleosa* bark for developing natural anti-inflammatory and antimicrobial agents.

***In-Vitro* PLA2 inhibition assay**

The PLA2 inhibition assay showed a clear concentration-dependent inhibition by both *S. Oleosa* ethanol (SOEE) and aqueous (SOAE) extracts. At 10 µg/mL, SOEE inhibited 27.64%, while SOAE showed 9.41%. Inhibition increased with concentration, with SOEE consistently showing higher activity. At 50 µg/mL, SOEE inhibited 47.39% and SOAE 23.19%, indicating stronger potency of SOEE. At 100 µg/mL, SOEE achieved 69.51%



inhibition versus 38.18% for SOAE. The IC₅₀ of SOEE (56.709 µg/mL) was markedly lower than SOAE (151.157 µg/mL), confirming superior efficacy. This suggests ethanol extraction yields more potent PLA2-inhibiting compounds. Both extracts exhibited dose-dependent inhibition, reflecting the

presence of active phytochemicals. SOEE's greater activity likely results from its richer bioactive composition. These findings highlight the therapeutic potential of *S. Oleosa* bark as a natural PLA2 inhibitor.

Table 1: PLA2 inhibition assay of *S. Oleosa* extracts

Concentration (µg/mL)	Ethanol Extract (%) ± SD	Aqueous Extract (%) ± SD
10	27.64 ± 1.05	9.41 ± 0.72
25	33.83 ± 1.19	16.07 ± 0.85
50	47.39 ± 1.35	23.19 ± 1.02
75	58.04 ± 1.48	30.39 ± 1.21
100	69.51 ± 1.63	38.18 ± 1.39

Mechanism of Action

PLA2 catalyzes the hydrolysis of membrane phospholipids, releasing arachidonic acid—a key precursor of prostaglandins, thromboxanes, and leukotrienes via COX and LOX pathways. Inhibiting PLA2 can suppress inflammation early in the cascade [14, 15]. *S. Oleosa* extracts may inhibit PLA2 through multiple mechanisms.

Competitive inhibition: Phytochemicals like flavonoids and terpenoids mimic natural substrates, blocking the catalytic site [16].

Allosteric modulation: Some compounds may bind to allosteric sites, reducing enzyme affinity [17].

Metal ion chelation: Polyphenols and tannins can chelate Ca²⁺, impairing PLA2 activity [18].

Membrane stabilization: Secondary metabolites may reduce phospholipid accessibility to PLA2 [19].

Oxidative inhibition: Antioxidants in *S. Oleosa* lower ROS levels, indirectly suppressing PLA2 activation [20].

These combined mechanisms indicate the extract's broad-spectrum anti-inflammatory potential.

In-Vitro 5-LOX inhibition assay

The 5-LOX inhibition assay showed concentration-dependent activity for Montelukast, SOEE, and SOAE. At 10 µg/mL, Montelukast inhibited 37.63%, SOEE 14.61%, and SOAE 10.39%. At 100 µg/mL, inhibition increased to 87.16% for Montelukast, 57.69% for SOEE, and 40.54% for SOAE. IC₅₀ values were 28.897 µg/mL (Montelukast), 78.512 µg/mL (SOEE), and 152.349 µg/mL (SOAE), confirming higher potency of SOEE over SOAE. Ethanol extracted more active compounds responsible for 5-LOX inhibition. Aqueous extract showed weaker activity, reflecting solvent influence on phytochemical yield. SOAE required higher concentrations to achieve similar inhibition levels. Both extracts still demonstrated meaningful inhibition, indicating promising anti-inflammatory potential. These findings support further investigation of *S. Oleosa* bioactive constituents and mechanisms.

Table 2: 5-LOX inhibition assay of *S. Oleosa* extracts

Concentration (µg/mL)	Ethanol Extract (%) ± SD	Aqueous Extract (%) ± SD	Montelukast (%) ± SD
10	14.61 ± 0.92	10.39 ± 0.75	37.63 ± 1.25
25	26.83 ± 1.15	16.03 ± 0.89	49.27 ± 1.47
50	38.16 ± 1.32	26.61 ± 1.05	60.85 ± 1.61
75	47.93 ± 1.48	33.28 ± 1.23	74.91 ± 1.78
100	57.69 ± 1.65	40.54 ± 1.39	87.16 ± 1.92



Mechanism of Action

5-LOX catalyzes the oxidation of arachidonic acid to leukotrienes, key mediators in inflammatory diseases like asthma, arthritis, and IBD. Its inhibition can reduce leukotriene synthesis and inflammation [21-23]. *S. Oleosa* extracts may inhibit 5-LOX through multiple mechanisms.

Competitive inhibition: Flavonoids, polyphenols, and terpenoids compete with arachidonic acid for the active site, interacting with the catalytic iron center [21].

Iron chelation: Polyphenols and tannins chelate iron and neutralize ROS, suppressing enzyme activity [22].

Allosteric modulation: Certain metabolites bind allosteric sites, inducing conformational changes that reduce substrate affinity [22].

Membrane stabilization: Bioactive compounds may prevent 5-LOX translocation to membranes, hindering substrate interaction [23].

These combined mechanisms highlight the anti-inflammatory potential of *S. Oleosa* extracts.

In-silico molecular docking study

PLA2 inhibition

Among the tested phytoconstituents, Isorhamnetin 3-O-glucoside exhibited the highest binding affinity (-17.590 kcal/mol), followed by Epicatechin Gallate (-15.850 kcal/mol), (+)-Epicatechin-3-O-gallate (-15.450 kcal/mol), and 4a,5-Dihydroriboflavin (-15.090 kcal/mol). These top five ligands demonstrated strong interactions with PLA2, indicating their potential as effective inhibitors.

Table 3: Molecular docking data of PLA2 inhibition protein

Ligand	Binding affinity (Kcal/mol)
4-Hydroxybenzoic acid	-6.590
3,4-Dihydroxybenzoic acid	-7.910
Vanillic Acid	-8.100
p-Coumaric acid	-8.510
Syringic acid	-9.250
Caffeic Acid	-9.480
9-Octadecanone	-10.490
Betulinic Acid	-10.560
Lupeol	-10.810
Betulin	-10.970
Taraxerone	-11.080
Scillarenin	-11.520
Methyl palmitate	-11.820
Lupeol Acetate	-11.840
Noscapine	-12.260
10-Deacetylbaecatin-III	-12.300
Glutathione	-13.020
Fumaric acid, decyl 2-methylallyl ester	-13.340
Aloesin	-14.560
Deoxyuridine-5'-Diphosphate	-14.630
Daidzin	-14.680
4a,5-Dihydroriboflavin	-15.090
(+)-epicatechin-3-O-gallate	-15.450
Epicatechin Gallate	-15.850
isorhamnetin 3-O-glucoside	-17.590



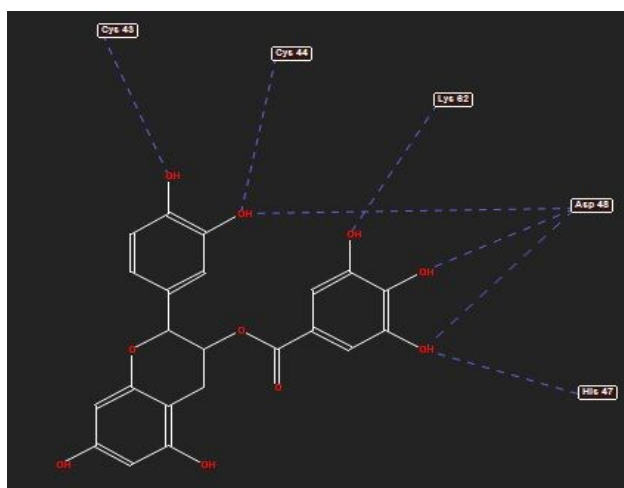


Figure 1: 2D interaction of (+)-Epicatechin-3-O-gallate in active site of PLA2

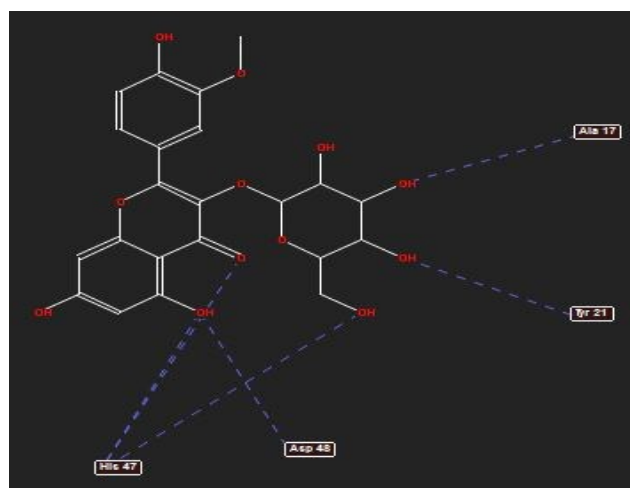


Figure 4: 2D interaction of isorhamnetin 3-O-glucoside in active site of PLA2

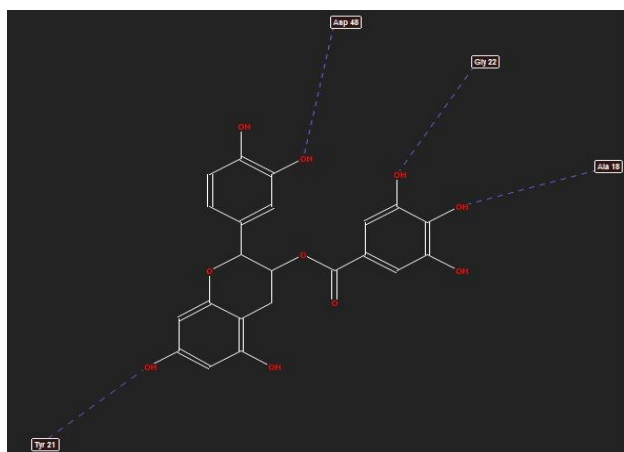


Figure 2: 2D interaction of Epicatechin gallate in active site of PLA2

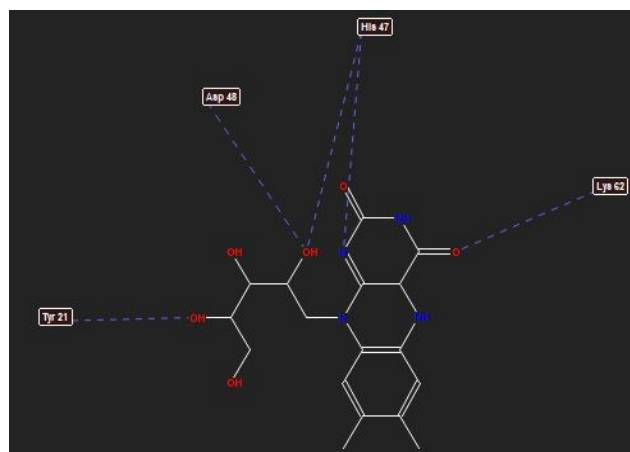


Figure 5: 2D interaction of 4a,5-Dihydroriboflavin in active site of PLA2

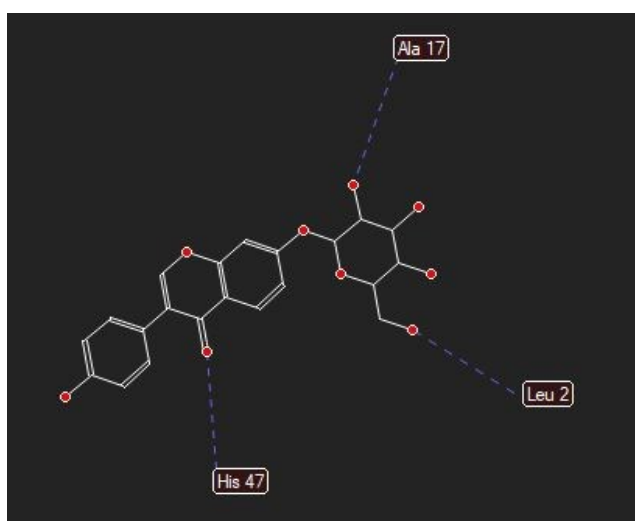


Figure 3: 2D interaction of Diadzin in active site of PLA2

(+)-Epicatechin-3-O-gallate (-15.450 kcal/mol)

Interacting residues: CYS43, CYS44, LYS62, ASP48, HIS47

(+)-Epicatechin-3-O-gallate exhibited a strong interaction with CYS43 and CYS44, which are crucial for maintaining the structural integrity of PLA2. The interaction with ASP48 and HIS47 suggests possible involvement in catalytic inhibition, as these residues are part of the active site of the enzyme. Additionally, its binding to LYS62 may enhance stability through electrostatic interactions. The presence of multiple hydroxyl groups in epicatechin gallate likely contributes to strong hydrogen bonding, reinforcing its inhibitory potential. Given its natural antioxidant and anti-inflammatory properties, (+)-Epicatechin-3-O-gallate may serve as an effective PLA2 inhibitor.

Epicatechin Gallate (-15.850 kcal/mol)

Interacting residues: TYR21, ALA18, GLY22, ASP48

Epicatechin Gallate demonstrated strong binding to TYR21 and ALA18, which are known to contribute to ligand stabilization through hydrophobic interactions. The interaction with ASP48, a key catalytic residue, suggests a direct inhibitory effect on PLA2 enzymatic activity. Additionally, its binding with GLY22 may further stabilize the ligand within the binding pocket. The structural similarity between Epicatechin Gallate and (+)-Epicatechin-3-O-gallate indicates that both compounds could share a common mechanism of enzyme inhibition via hydrogen bonding and hydrophobic interactions.

Daidzin (-14.680 kcal/mol)

Interacting residues: ALA17, LEU2, HIS47

Daidzin exhibited binding interactions with ALA17 and LEU2, suggesting strong hydrophobic interactions that stabilize its binding within the active site. The interaction with HIS47, a key catalytic residue, indicates that Daidzin may disrupt PLA2 enzymatic function, leading to reduced inflammatory activity. Daidzin is an isoflavonoid glycoside, known for its anti-inflammatory and antioxidant effects, which may contribute to its PLA2 inhibitory potential. The presence of sugar moieties in its structure could enhance hydrogen bonding, further strengthening its affinity.

Isorhamnetin 3-O-glucoside (-17.590 kcal/mol)

Interacting residues: ALA17, TYR21, ASP48, HIS47

Isorhamnetin 3-O-glucoside, with the strongest binding affinity (-17.590 kcal/mol), interacted with

key residues ASP48 and HIS47, suggesting a direct inhibition of PLA2 enzymatic function. Its binding with ALA17 and TYR21 indicates hydrophobic stabilization, further enhancing its interaction within the active site. The presence of multiple hydroxyl groups likely facilitates hydrogen bonding, strengthening the ligand-protein complex. Given its flavonoid nature, isorhamnetin 3-O-glucoside is likely to exhibit anti-inflammatory properties, making it a strong candidate for PLA2 inhibition.

4a,5-Dihydoriboflavin (-15.090 kcal/mol)

Interacting residues: TYR21, LYS62, ASP48, HIS47

4a,5-Dihydoriboflavin showed interaction with ASP48 and HIS47, indicating a potential role in inhibiting PLA2 catalytic activity. The interaction with TYR21 suggests involvement in hydrophobic interactions, while the binding with LYS62 may contribute to electrostatic stability. As a derivative of riboflavin (Vitamin B2), this compound is known for its antioxidant and anti-inflammatory properties, which may further enhance its role in mitigating PLA2-mediated inflammation.

5-LOX inhibition

The molecular docking study assessed the interaction between various phytoconstituents and 3O8Y, the protein associated with 5-lipoxygenase (5-LOX) inhibition. A more negative binding affinity indicates a stronger and more stable interaction between the ligand and the target protein. The top five phytoconstituents and standard with the highest binding affinities are: (+)-Epicatechin-3-O-gallate (-14.50 kcal/mol), 10-Deacetylbaccatin-III (-14.42 kcal/mol), Deoxyuridine-5'-Diphosphate (-14.09 kcal/mol), Isorhamnetin 3-O-glucoside (-13.93 kcal/mol), Epicatechin Gallate (-12.97 kcal/mol).

Table 4: Molecular docking data of 5-LOX inhibition protein

Ligand	Binding affinity (Kcal/mol)
4-Hydroxybenzoic acid	-5.59
3,4-Dihydroxybenzoic acid	-6.46
Vanillic Acid	-7.37
Syringic acid	-7.64
p-Coumaric acid	-7.91
Caffeic Acid	-8.81
Scillarenin	-8.92



Betulinic Acid	-9.24
Lupeol	-9.3
Betulin	-9.48
Methyl palmitate	-9.7
9-Octadecanone	-9.71
Taraxerone	-9.87
Lupeol Acetate	-10.16
Daidzin	-10.36
Fumaric acid, decyl 2-methylallyl ester	-10.99
Noscapine	-11.38
Aloesin	-11.65
Glutathione	-12.03
4a,5-Dihydoriboflavin	-12.40
Epicatechin Gallate	-12.97
isorhamnetin 3-O-glucoside	-13.93
Deoxyuridine-5'-Diphosphate	-14.09
10-Deacetylbaccatin-III	-14.42
(+)-epicatechin-3-O-gallate	-14.50

(+)-Epicatechin-3-O-gallate (-14.50 kcal/mol)

Interacting residues: SER171, ASP170

(+)-Epicatechin-3-O-gallate demonstrated a strong binding affinity and interacted with SER171 and ASP170, both of which are crucial for the enzymatic activity of 5-LOX. ASP170 is a key catalytic residue involved in substrate stabilization, and its interaction with the ligand suggests potential inhibition of enzyme function. SER171 plays a role in hydrogen bonding, which may enhance ligand stability within the active site. As a flavonoid with potent antioxidant and anti-inflammatory properties, (+)-Epicatechin-3-O-gallate likely inhibits leukotriene biosynthesis, reducing inflammation associated with conditions such as asthma and arthritis.

suppressing leukotriene-driven inflammation in cancer and other inflammatory diseases.

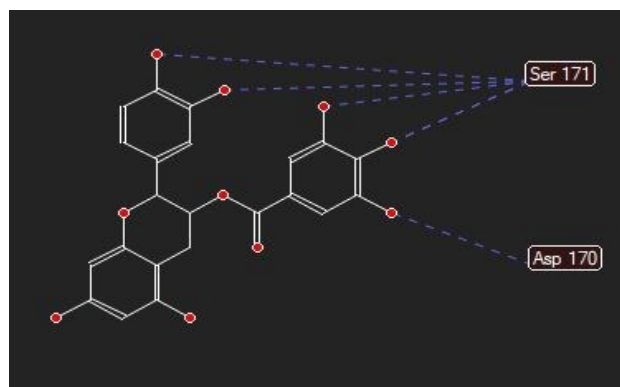


Figure 6: 2D interaction of (+)-Epicatechin-3-O-gallate in active site of 5-LOX

10-Deacetylbaccatin-III (-14.42 kcal/mol)

Interacting residues: SER171, ASN613, ARG401

10-Deacetylbaccatin-III exhibited interactions with SER171, ASN613, and ARG401, indicating strong inhibition potential. ASN613 and ARG401 contribute to the enzyme's structural integrity, and their binding suggests a disruption in the enzyme's function. The interaction with SER171 further stabilizes the ligand within the active site. Given that 10-Deacetylbaccatin-III is a taxane derivative with known anticancer and anti-inflammatory effects, its ability to target 5-LOX suggests a potential role in

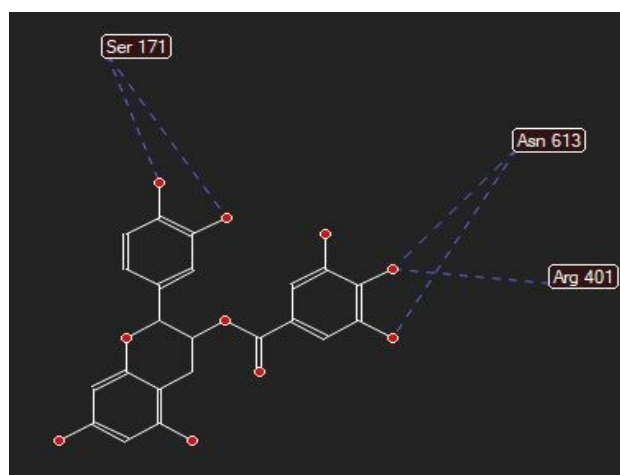


Figure 7: 2D interaction of Epicatechin gallate in active site of 5-LOX



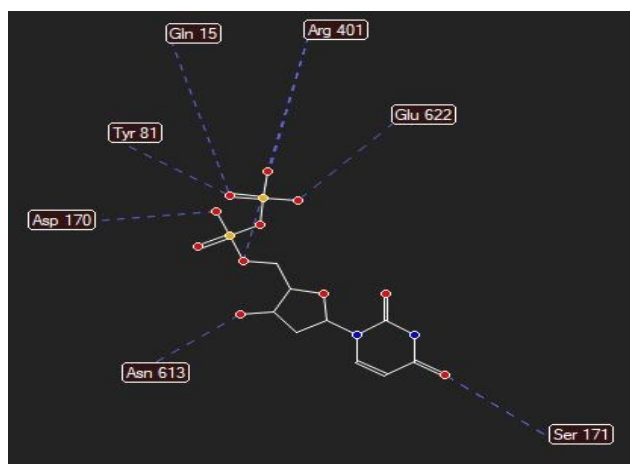


Figure 8: 2D interaction of Deoxyuridine-5'-Diphosphate in active site of 5-LOX

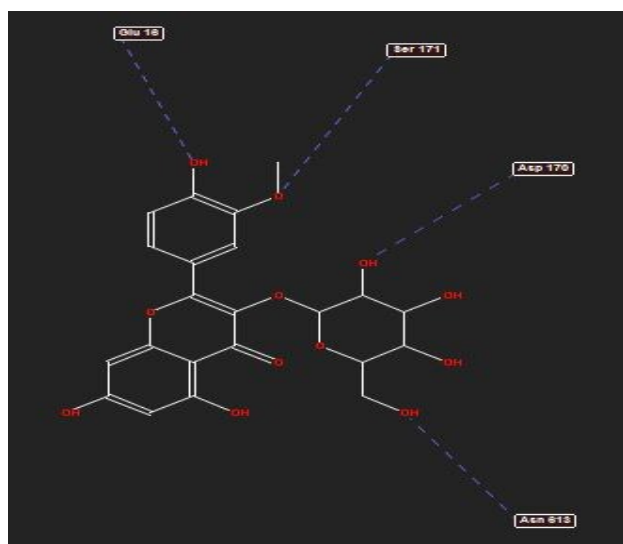


Figure 9: 2D interaction of isorhamnetin 3-O-glucoside in active site of 5-LOX

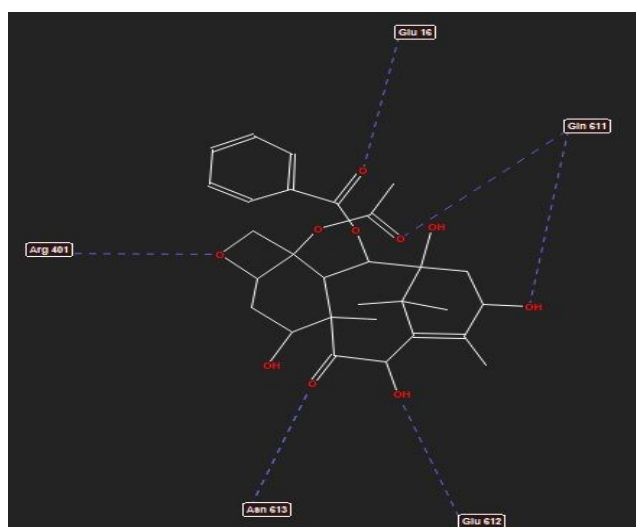


Figure 10: 2D interaction of 10-Deacetylbaecatin-III in active site of 5-LOX

Deoxyuridine-5'-Diphosphate (-14.09 kcal/mol)

Interacting residues: ASN613, ASP170, TYR81, GLN15, ARG401, GLU622

Deoxyuridine-5'-Diphosphate formed multiple interactions with key catalytic and structural residues of 5-LOX, making it a strong inhibitor candidate. ASP170 and TYR81 play critical roles in the enzymatic reaction, and their interaction suggests direct interference with the catalytic mechanism. The presence of GLN15, ARG401, ASN613, and GLU622 suggests additional stabilization through hydrogen bonding and electrostatic interactions. As a nucleotide derivative, Deoxyuridine-5'-Diphosphate may act as a competitive inhibitor, blocking substrate access to the active site and preventing leukotriene synthesis.

Isorhamnetin 3-O-glucoside (-13.93 kcal/mol)

Interacting residues: GLU16, SER171, ASP170, ASN613

Isorhamnetin 3-O-glucoside showed interactions with ASP170, SER171, and ASN613, indicating a strong inhibitory effect on 5-LOX activity. The interaction with GLU16 suggests additional hydrogen bonding, which may enhance its stability within the active site. Given its flavonoid nature, Isorhamnetin 3-O-glucoside likely inhibits inflammation through both direct enzyme inhibition and antioxidant mechanisms, making it a potential candidate for natural anti-inflammatory drug development.

Epicatechin Gallate (-12.97 kcal/mol)

Interacting residues: ARG401, GLU16, GLN611, ASN613, GLU612

Epicatechin Gallate demonstrated strong interaction with ARG401 and ASN613, indicating potential enzyme inhibition through structural destabilization. The additional interactions with GLU16, GLN611, and GLU612 suggest a network of hydrogen bonds and electrostatic interactions that may enhance its inhibitory effect. Like other flavonoids, Epicatechin Gallate is known for its ability to modulate inflammatory pathways, and its strong binding affinity to 5-LOX suggests a role in reducing leukotriene-induced inflammation.

CONCLUSION

The present study establishes *Schleichera Oleosa* bark as a promising natural source of anti-inflammatory agents through its dual inhibition of PLA2 and 5-LOX pathways. The ethanol extract demonstrated superior yield and bioactivity compared to the aqueous extract, emphasizing the role of solvent polarity in extracting potent phytochemicals. SOEE exhibited significantly higher enzyme inhibition and lower IC₅₀ values for both PLA2 and 5-LOX, underscoring its therapeutic potential. Molecular docking further revealed strong interactions between key phytoconstituents—particularly Isorhamnetin 3-O-glucoside and Epicatechin Gallate—and critical active site residues of both enzymes. These interactions suggest that these compounds may effectively disrupt pro-inflammatory mediator synthesis. The observed bioactivity aligns with their strong binding affinities and stable interactions within the catalytic pockets. This dual mechanism highlights *S. Oleosa* as a valuable candidate for developing plant-based anti-inflammatory therapeutics. While the findings provide strong *in-vitro* and *in-silico* evidence, further isolation, structural elucidation, and *in-vivo* validation are warranted. Such studies may lead to the development of novel, safe, and effective anti-inflammatory agents derived from *S. Oleosa* bark.

ACKNOWLEDGEMENT

The authors are thankful for Mohammed Sathak AJ college of Pharmacy for providing facility to complete the research work.

AUTHOR CONTRIBUTIONS

All authors contributed significantly to the conception and design of the study, formulation development, data acquisition, analysis, and interpretation.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this work.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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HOW TO CITE: Sundhararajan R., Gayathri K., Vanjinathan M.*, In-Silico and In-Vitro Analysis of Schleicheria Oleosa Bark for PLA2 And 5-LOX Enzyme Inhibition, J. Pharm. Sci., 2025, 1 (10), 708-718. <https://doi.org/10.5281/zenodo.17464931>

