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Review

Integrated *In-Vitro* Anti-Inflammatory Activity and *In-Silico* Investigation Encompassing Pharmacokinetic Properties and Molecular Docking of Phytoconstituents from *Simarouba Glauca* Linn

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Abstract

Simarouba glauca DC. (*Simaroubaceae*), commonly known as Paradise tree, is traditionally used in the management of fever, inflammation, dysentery, malaria and gastrointestinal disorders. The pharmacological reports had demonstrated its anti-inflammatory, antioxidant, antimicrobial, anticancer, hepatoprotective and antidiabetic properties. Phytochemical investigations indicated the presence of quassinoids, flavonoids, triterpenoids, alkaloids, tannins, glycosides and phenolic compounds that contribute to its therapeutic activity. The present study is to evaluate *in-vitro* anti-inflammatory activity and *in-silico* broadcast of *Simarouba glauca* leaves. Aqueous extract of *Simarouba glauca* leaves ($AESG_L$) was prepared by decoction followed by concentration. Anti-inflammatory activity was screened by denaturation methods with egg albumin and gelatin used as protein source.

Anti inflammatory activity is based on the percentage of inhibition of protein denaturation compared with Disprin used as reference. *In-silico examination on* ADMET and molecular docking monitoring were carried out on phytoconstituents that were reported in GC-MS analysis with cyclooxygenase-2 enzyme. $AESG_L$ exhibited significant denaturation of protein in concentration-dependent manner, its IC_{50} values 36 µg/mL and 54 µg/mL with egg albumin and gelatin respectively, demonstrates promising anti-inflammatory effect with Disprin used as reference. ADMET analysis revealed its compatibility and complies with gastrointestinal absorption and with its toxicity profiles for the phytoconstituents. Molecular docking results showed that 45% of compounds exhibited stronger binding affinity toward

COX-2, indicates its potential inhibitory action. Overall, the combined *in-vitro* and *in-silico* findings support the traditional use of *Simarouba glauca* as an anti-inflammatory agent and potential promising source of lead compounds for the development of safer plant-based anti-inflammatory drug.

Keywords: ADMET, Anti-inflammatory activity, *In-silico* studies, Molecular docking, Protein denaturation, *Simarouba glauca*.

Introduction

Inflammation is the protective biological response of body tissues to harmful stimuli such as: Infection, Injury, Chemical or physical damage. Purpose of inflammation - to remove the cause of injury, to eliminate damaged tissue, to initiate tissue repair [1] as a result of the release of chemical mediators from migrating cells and injured tissue. Some of these mediators include histamine, serotonin, Slow reacting substance of anaphylaxis, prostaglandins and several plasma enzyme systems, such as complement system, clotting system, fibrinolytic system and kinin system [2]. Nonsteroidal Anti-inflammatory Drugs such as Ibuprofen and Naproxen etc. are prescribed for orthopaedic conditions such as osteoarthritis, soft-tissue injuries and fractures etc., [3]

Simarouba glauca, belongs to the family *Simaroubaceae*, is a medicinally significant plant recognized for its bioactive components with therapeutic potential. This tree commonly referred to as Shorgum Maram, Tree of Heaven, Dysentery Bark, Paradise Tree, or Lakshmi Taru, is valued for its edible oil and medicinal properties. *Simarouba glauca* is characterized by compound leaves arranged alternately along the stem, with small, fragrant flowers that appear in clusters. It is native to regions including Mexico, South Florida and Central America and is also cultivated in Kenya and various parts of India, particularly in the wastelands of Orissa, Karnataka and Gujarat [4].

Phytochemical investigations indicated the presence of quassinoids, flavonoids, triterpenoids, alkaloids, tannins, glycosides and phenolic compounds that contribute to its therapeutic activity. *In-silico examination on* ADMET and molecular docking monitoring were carried out on phytoconstituents that were reported in GC-MS analysis with cyclooxygenase-2 enzyme.

Simarouba glauca was selected for the evaluation of anti-inflammatory activity based on its traditional medicinal uses. The plant has been widely used in folk medicine for the treatment of conditions such as dysentery, arthritis, fever and other disorders that are closely associated with inflammation.

Therefore, there is a need to explore safer and more effective herbal alternatives. Considering its traditional background and the presence of bioactive phytoconstituents, evaluating the anti-inflammatory potential of *Simarouba glauca* is scientifically justified and therapeutically significant.

Material and methods

Authentication and collection of leaves of *Simarouba glauca* Linn.

Leaves were collected from the village of Nazareth, Thoothukudi, Tamil Nadu in the month of February 2025. The leaves were identified and authenticated by Dr. S. Maheswaran, Scientist, Xavier Research Foundation, St. Xavier's College.Palayamkottai-627002. The herbarium of this specimen was kept in the department for further reference.

Preparation of Aqueous Extract of *Simarouba glauca* Linn. leaves ($AESG_L$)

Fresh mature leaves were collected, washed thoroughly with water and shade dried. The dried leaves were pulverized into a fine powder and 10g of powder were boiled with 200 ml of water for 20 minutes. The extract was then filtered and evaporated to dryness. Batches were prepared as required [5].

In-vitro anti-inflammatory activity of $AESG_L$

Denaturation of egg albumin assay

Procedure

The anti-inflammatory activity of unknown crude extracts can be determined *in-vitro* for inhibition of the denaturation of egg albumin (protein).

- 2 mL of various concentration (25, 50, 75, 100, 150 & 200 µg/mL) of plant extract was pipetted out, treated with 0.2mL of 1-2% egg albumin solution and 2.8 mL of phosphate buffer saline (pH 6.4) were mixed to form a reaction mixture of a total

volume of 5 mL and make up this solution to 10ml by using distilled water.

- The control solution was prepared by combining 0.2mL of 1-2% egg albumin solution, 2.8 mL of phosphate buffer saline and make up this solution to 10ml by using distilled water.
- The reaction mixtures were then incubated at 37±2°C for 30 min and will be heated in a water bath at 70±2°C for 15 min.
- After cooling, the absorbance was measured at 280 nm by a suitable UV/Vis spectrophotometer using distilled water as the blank.
- The following equation was used to determine the % inhibition of protein denaturation.

$$\text{Percentage of protein inhibition} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Then plant extract/positive control concentration for 50% inhibition (IC_{50}) was determined by plotting concentration against percentage of protein inhibition [6] and its results are presented in table 1 & 2.

Denaturation of gelatin assay

Procedure :

- One percent aqueous gelatin solution was prepared.
- The reaction mixture pH (6.3) was adjusted with 1N hydrochloric acid.
- 2 mL of various concentration (25, 50, 75, 100, 150 & 200 µg/mL) of plant extract was pipetted out, treated with 0.45ml of gelatin solution were incubated 20 minutes at 37ºC and heated for 20 minutes at 51ºC.
- 2.5ml of phosphate buffer was added, after the samples were cooled. The absorbance was measured at 660nm.
- Instead of test extract, distilled water was added for the control. Disprin was taken as a standard.
- The protein denaturation inhibition percentage was determined by the following formula.

$$\text{Percentage of protein inhibition} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

- Then plant extract/positive control concentration for 50% inhibition (IC_{50}) was determined by plotting concentration against percentage of protein inhibition and its results are presented in table 3 & 4.

***In-silico* screening method**

ADMET Analysis

ADMET analysis was performed using the swissADME online server and the toxicity data of each phytochemical was obtained from the Protox II online server. Lipinski's rule of five, ADME/T and drug-like properties were focused on determining compounds' drug ability.

Molecular docking

Molecular docking prediction is a computer-based method used to predict how a small molecule (ligand) binds to a biological target (protein or enzyme) and how strong that binding is. The binding affinity is the predicted strength of interaction between the ligand and the target, usually expressed as binding energy (kcal/mol).Binding affinity indicates how stable the ligand–protein complex is.

It is expressed as negative values

More negative value = stronger and more stable binding [7, 8].

Results and Discussion

***In-Vitro* Anti-Inflammatory activity of AESG$_L$**

Denaturation of egg albumin assay of disprin

Table 1. Determination of *in-vitro* denaturation of egg albumin using Disprin.

Concentration (µg/mL)	Absorbance (280nm)			Percentage of protein inhibition			Percentage of protein inhibition (Mean ± SEM *)
25	0.098	0.104	0.115	69.37	67.5	64.06	66.98 ± 1.55
50	0.068	0.069	0.067	78.75	78.44	79.06	78.75 ± 0.18
75	0.036	0.036	0.039	88.75	88.75	87.81	88.43 ± 0.31
100	0.061	0.061	0.058	80.93	80.93	81.87	81.25 ± 0.31
150	0.046	0.046	0.048	85.62	85.62	85	85.42 ± 0.21
200	0.044	0.044	0.046	86.25	86.25	85.62	86.04 ± 0.21
IC_{50}							19 g/mL

***Indicates results of triplicate.**

Chart 1: Determination of *in-vitro* denaturation of egg albumin by Disprin.

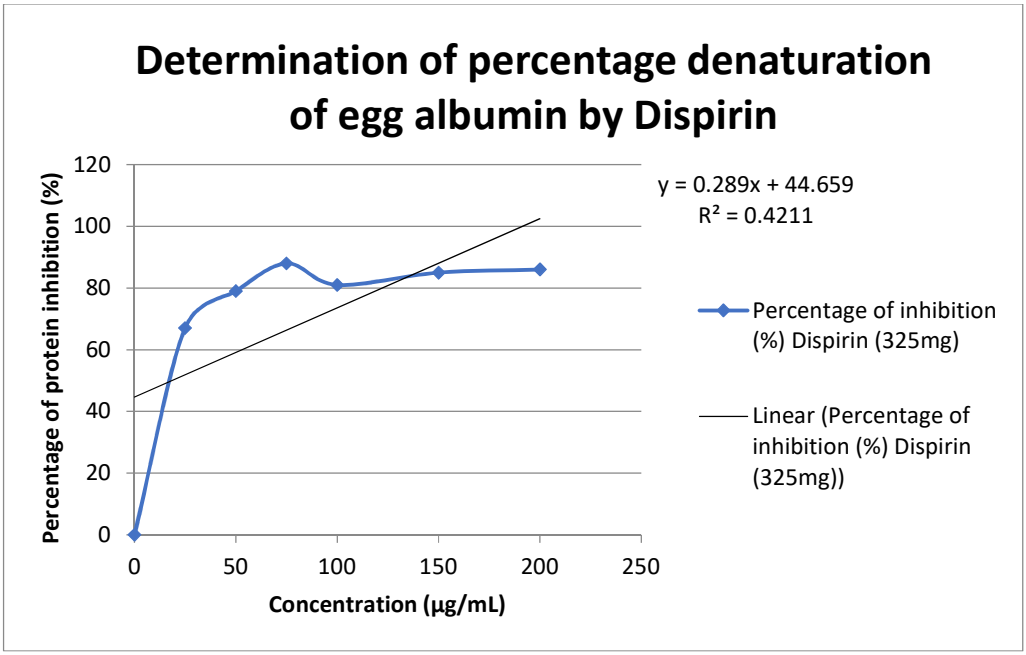


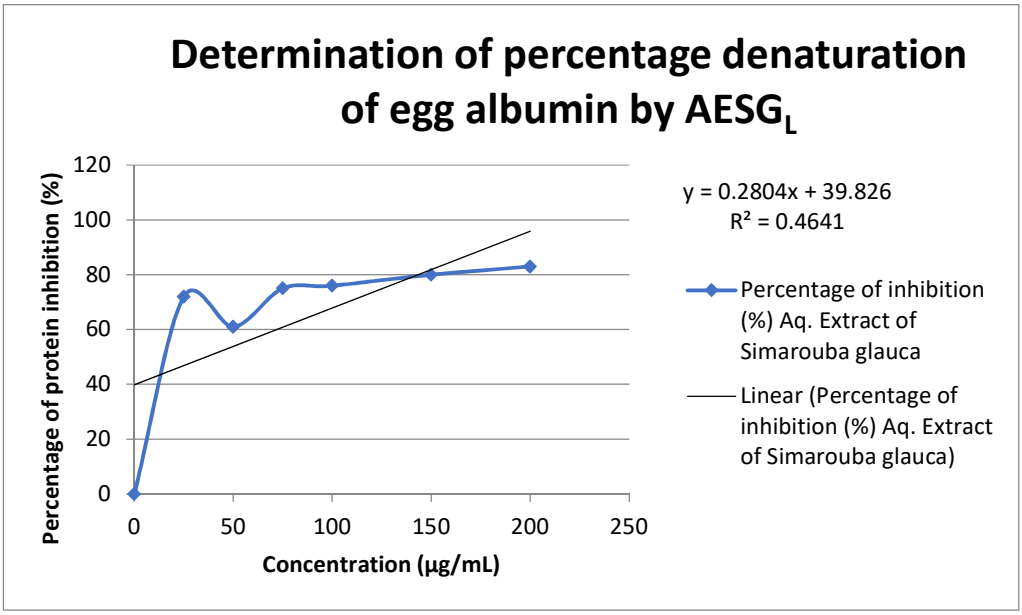
Table 2. Determination of *in-vitro* denaturation of egg albumin by AESG$_L$.

Concentration (µg/mL)	Absorbance (280nm)			Percentage of protein inhibition			Percentage of protein inhibition (Mean ± SEM *)
25	0.088	0.093	0.086	72.5	70.94	73.13	72.19 ± 0.65
50	0.122	0.124	0.126	61.88	61.25	60.63	61.25 ± 0.36

75	0.075	0.075	0.090	76.56	76.56	71.88	75 ± 1.56
100	0.077	0.077	0.075	75.94	75.94	76.56	76.15 ± 0.21
150	0.063	0.064	0.069	80.31	80	78.44	79.58 ± 0.58
200	0.052	0.052	0.058	83.75	83.75	81.88	83.13 ± 0.62
IC_{50}							36.357µg/mL

***Indicates results of triplicate.**

Chart 2: Determination of *in-vitro* denaturation of egg albumin by $AESG_L$.



It is observed that *S.glauca* inhibited denaturation of egg albumin with an IC_{50} of 36.357µg/mL, compared to disprin used as standard (IC_{50} - 19 µg/mL), indicating its promising anti-inflammatory effect.

Denaturation of gelatin assay

Table 3. Determination of denaturantion of gelatin by Dispirin.

Concentration (µg/mL)	**Absorbance (280nm)**			**Percentage of protein inhibition**			**Percentage of protein inhibition** (Mean ± SEM *)
25	0.139	0.142	0.145	63.42	62.63	61.84	62.63 ± 0.45612
50	0.129	0.127	0.131	66.05	66.57	65.52	66.05 ± 0.303122
75	0.118	0.112	0.115	68.94	70.53	69.73	69.73 ± 0.45901
100	0.101	0.103	0.105	73.42	72.89	72.36	72.89 ± 0.306005
150	0.097	0.100	0.094	74.47	73.68	75.26	74.47 ± 0.45612
200	0.096	0.094	0.092	74.73	75.52	75.78	75.52 ± 0.315727
IC_{50}							45.17µg/Ml

***Indicates results of triplicate.**

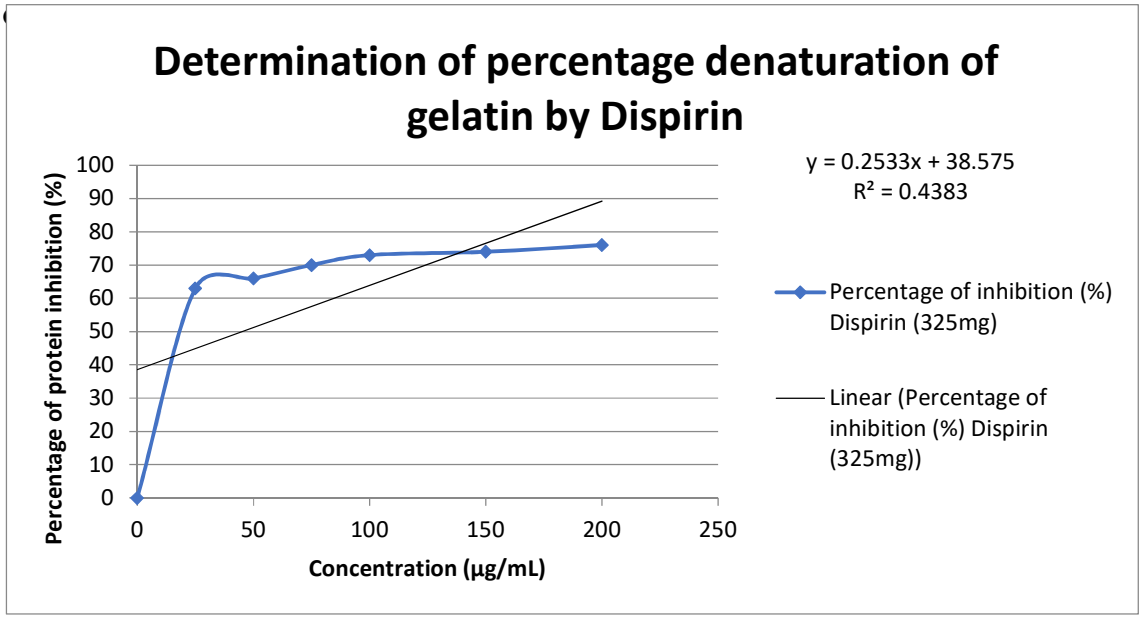
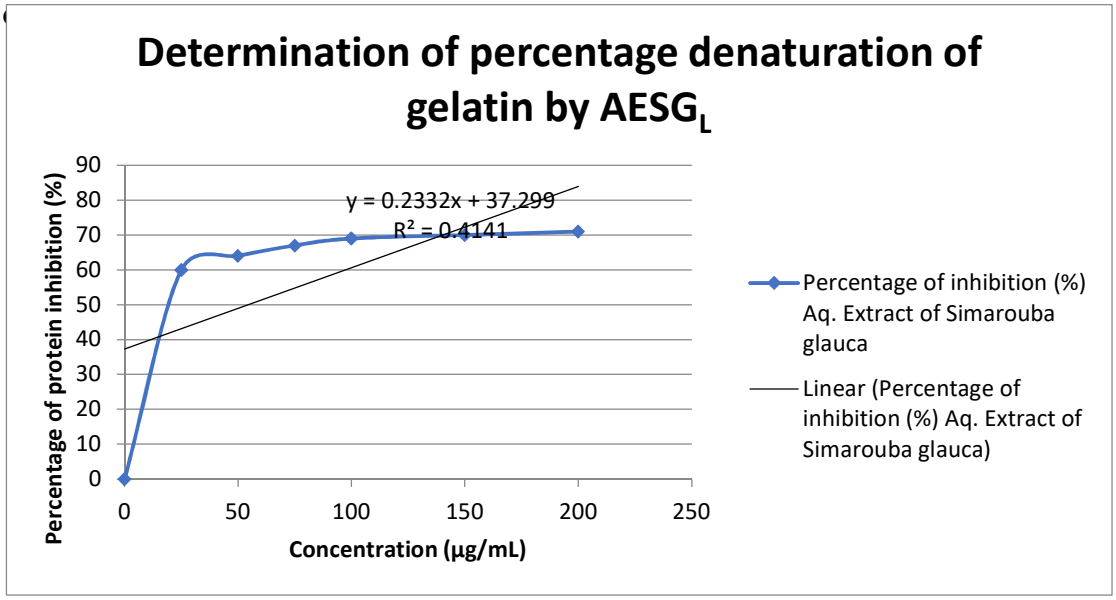


Table 4. Determination of denaturation of gelatin by AESG$_L$.

Concentration (µg/mL)	Absorbance (280nm)			Percentage of protein inhibition			Percentage of protein inhibition (Mean ± SEM *)
25	0.150	0.153	0.156	60.52	59.73	58.94	59.73 ± 0.45612
50	0.138	0.138	0.140	63.68	64.21	63.16	63.68 ± 0.303122
75	0.129	0.123	0.126	66.05	67.63	66.84	66.84 ± 0.45612
100	0.115	0.117	0.119	69.73	69.21	68.68	69.20 ± 0.303122
150	0.112	0.115	0.109	70.52	69.73	71.31	70.52 ± 0.45612
200	0.110	0.112	0.108	71.05	70.52	71.57	71.05 ± 0.303122
IC_{50}							54.54 µg/mL

***Indicates results of triplicate.**



It is observed that *S.glauca* inhibited denaturation of gelatin with an IC_{50} of 54.54 µg/mL, compared to disprin used as standard (IC_{50} - 45.17 µg/mL), indicating its promising anti-inflammatory effect.

The phytoconstituents that were reported from GC-MS analysis of *Simarouba glauca* Linn. [9] are screened by *in-silico* studies and its ADMET Analysis is shown in table 5.

***In-silico* screening method**

Table 5. ADMET Analysis data of *Simarouba glauca* Linn.

S.no.	Name of the Phytoconstituents	Lipinski's rules				Lipinski's Rule violations	GI absorption	BBB Permeability	CYP3A34 inhibitor	Bioavailability score
		MW g/mol	HBA	HBD	LogP					
		< 500	≤ 10	≤ 5	≤ 5					
1.	3,3-Dimethyl-1-(5-phenyl-tetrahydrofuran-2-yl)-butan2-one	246.34	2	0	3.27	0	High	Yes	No	0.55
2.	2,2-Dimethoxybutane	118.17	2	0	1.32	0	High	Yes	No	0.55
3.	Methyl 2-chloro-2-methyl-3-[(4-methylbenzene) sulfonyl] propanoate	290.76	4	0	2.41	0	High	Yes	No	0.55
4.	4-Methylindeno [3',2':5,6] pyrazino[2,3-e] [2,1,3] benzothiadiazol-11-one	304.33	5	0	2.76	0	High	No	Yes	0.55
5.	Thiocyanic acid, [1-(4-amino-1,2,5-oxadiazol-3-yl)-1H1,2,3-triazol-5-yl] methyl ester	223.22	6	1	0.09	0	Low	No	No	0.55

6.	4,7-Dimethoxy-6-nitro-2H-1,3-benzodioxole-5carbonitrile	252.18	7	0	0.54	0	High	No	No	0.55
7.	5-(4-Fluorophenyl)-1-methyl-3H,5H,6H,8H-[1,3] diazino[4,5-d] pyrimidine-2,4,7-trione	290.25	4	3	0.70	0	High	No	No	0.55
8.	3(9bH)-Dibenzofuranone	302.28	6	3	1.60	0	High	No	Yes	0.56
9.	4-Methyl-Z-4-hexadecen-1-ol	254.45	1	1	5.51	1	High	Yes	No	0.55
10.	Heneicosanoic acid, methyl ester	340.58	2	0	7.35	1	Low	No	No	0.55
11.	Phthalic acid, isobutyl octadecyl ester	474.72	4	0	8.26	1	Low	No	No	0.55
12.	Phytol	296.53	1	1	6.25	1	Low	No	No	0.55
13.	Carbonic acid, but-2-yn-1-yl tetradecyl ester	310.47	3	0	5.84	1	High	Yes	No	0.55
14.	Cinnamic acid	652.64	15	7	0.04	3	Low	No	No	0.17
15.	N, N'-Di-O-nitrophenyloxamide	330.25	6	2	0.85	0	Low	No	No	0.55
16.	L-Menthyl 3-ethyl-4-oxotricyclo (4.3.0.0(1,)) nonane-5carboxylate	346.50	3	0	4.71	0	High	Yes	No	0.55
17.	2,2,4-Trimethyl-6-(1-oxo-3-phenylprop-2-enyl)cyclohexane-1,3,5-trione	298.33	4	2	2.79	0	High	Yes	Yes	0.85
18.	4-(4-Chlorophenyl)-4-hydroxypiperidine, Otrimethylsilyl-	283.75	3	1	2.45	0	High	Yes	No	0.55
19.	Ethyl homovanillate, TMS derivative	282.41	4	0	2.74	0	High	Yes	No	0.55
20.	13,14-Epoxyursan-3-ol, acetate	470.73	3	0	6.66	1	Low	No	No	0.55

Discussion of ADMET analysis of *Simarouba glauca* Linn.

The ADMET profiling of phytoconstituents identified from *Simarouba glauca* Linn. was performed using SwissADME software. This analysis provides the suitability of these phytoconstituents as potential anti-inflammatory drug candidates.

- **Lipinski's Rule of Five:** It is widely used to predict oral drug-likeness. In the present study, the majority of compounds (1–8 and 15–19) exhibited zero violations, indicating

compliance with optimal physicochemical parameters such as molecular weight (<500 g/mol), hydrogen bond donors (≤5), hydrogen bond acceptors (≤10) and LogP (≤5). These results suggest favorable membrane permeability, adequate solubility and strong potential for oral administration. Compounds 9 - 13, and 20 showed one violation, primarily due to elevated lipophilicity (LogP >5). Although increased lipophilicity may reduce aqueous solubility and dissolution rate, a single violation is generally acceptable and does not affect oral bioavailability. These compounds may require formulation optimization to enhance solubility. Compound 14 exhibited three violations, including high molecular weight and excessive hydrogen bonding capacity, indicating poor permeability and limited oral absorption, suggesting that it may not be suitable for oral drug development without structural modification or advanced delivery systems.

- **Gastrointestinal (GI) Absorption:** High GI absorption was predicted for compounds 1- 4, 6 - 9, 13, 16 - 19 indicating efficient intestinal permeability and potential for rapid systemic availability following oral administration. In contrast, compounds 5, 10, 11, 12, 14, 15 and 20 demonstrated low GI absorption, suggesting limited intestinal uptake and the possible need for absorption-enhancing strategies.

- **Blood–Brain Barrier (BBB) Permeability:** Most compounds (1–8, 10–12, 14, 15 and 20) were predicted to be non-permeable to the blood–brain barrier. This characteristic is advantageous for drugs intended for peripheral inflammatory conditions, as it reduces the probability of central nervous system (CNS) adverse effects. Conversely, compounds 9, 13, 16, 17, 18 and 19 were predicted to cross the BBB, suggesting potential applicability in neuroinflammatory or CNS-related disorders, although CNS-related side effects must be carefully considered.

- **CYP3A4 Inhibition:** Cytochrome P450 3A4 (CYP3A4) plays a critical role in hepatic drug metabolism. Most compounds did not inhibit CYP3A4, indicating a lower probability of drug–drug interactions and improved metabolic safety. However, compounds 4, 8 and 17 were predicted to inhibit CYP3A4, which may increase the risk of metabolic interactions when co-administered with other drugs metabolized by this enzyme.

- **Bioavailability Score:** Nearly all compounds exhibited a bioavailability score of 0.55, indicating a moderate probability of achieving adequate oral bioavailability. Notably, compound 17 demonstrated a higher bioavailability score (0.85), highlighting its strong potential as an orally active candidate. In contrast, compound 14 showed a low bioavailability score (0.17), consistent with its multiple Lipinski violations and predicted poor absorption.

Collectively, the ADMET analysis suggests that the majority of phytoconstituents from *Simarouba glauca* Linn. possess favorable drug-likeness properties. Compounds with zero Lipinski violations, high GI absorption, absence of CYP3A4 inhibition and moderate bioavailability scores represent promising lead molecules for further pharmacological and molecular docking studies. Among them, particularly compound 17 appears promising due to its high predicted bioavailability and favorable absorption characteristics.These findings support the potential of *Simarouba glauca* Linn. phytoconstituents as viable candidates for the development of novel anti-inflammatory agents, warranting further *in-vitro* and *in-vivo* validation studies.

Table 6. Target prediction data of *Simarouba glauca* Linn.

S.no.	Name of the phytoconstituents	Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*
1.	3,3-Dimethyl-1-(5-phenyl-tetrahydrofuran-2-yl)-butan2-one	Melatonin receptor 1A	MTNR1A	P48039	CHEMBL1945	Family A G protein-coupled receptor	0.0535560755162
2.	2,2-Dimethoxybutane	Epoxide hydrolase 1	EPHX1	P07099	CHEMBL1968	Protease	0.0
3.	Methyl 2-chloro-2-methyl-3-[(4-methylbenzene) sulfonyl] propanoate	Metabotropic glutamate receptor 5 (by homology)	GRM5	P41594	CHEMBL3227	Family C G protein-coupled receptor	0.0535560755162
4.	4-Methylindeno [3',2':5,6] pyrazino[2,3-e] [2,1,3] benzothiadiazol-11-one	Orexin receptor 2	HCRTR2	O43614	CHEMBL4792	Family A G protein-coupled receptor	0.11150186548
5.	Thiocyanic acid, [1-(4-amino-1,2,5-oxadiazol-3-yl)-1H1,2,3-triazol-5-yl] methyl ester	Fructose-1,6-bisphosphatase	FBP1	P09467	CHEMBL3975	Enzyme	0.0428942118511
6.	4,7-Dimethoxy-6-nitro-2H-1,3-benzodioxole-5carbonitrile	Thrombin	F2	P00734	CHEMBL204	Protease	0.0535560755162
7.	5-(4-Fluorophenyl)-1-methyl-3H,5H,6H,8H-[1,3] diazino[4,5-d] pyrimidine-2,4,7-trione	Melatonin receptor 1A	MTNR1A	P48039	CHEMBL1945	Family A G protein-coupled receptor	0.0978745343258
8.	3(9bH)-Dibenzofuranone	Protein kinase C gamma	PRKCG	P05129	CHEMBL2938	Kinase	0.127302569502
9.	4-Methyl-Z-4-hexadecen-1-ol	Protein-tyrosine phosphatase 1B	PTPN1	P18031	CHEMBL335	Phosphatase	0.117056357516
10.	Heneicosanoic acid, methyl ester	Cannabinoid receptor 1	CNR1	P21554	CHEMBL218	Family A G protein-coupled receptor	0.104671941128
11.	Phthalic acid, isobutyl octadecyl ester	Protein-tyrosine phosphatase 1B	PTPN1	P18031	CHEMBL335	Phosphatase	0.120225750913
12.	Phytol	Cannabinoid receptor 2	CNR2	P34972	CHEMBL253	Family A G protein-coupled receptor	0.0978745343258

13.	Carbonic acid, but-2-yn-1-yl tetradecyl ester	Adrenergic receptor beta	ADRB2	P07550	CHEMBL210	Family A G protein-coupled receptor	0.11150186548
14.	Cinnamic acid	Protein kinase C alpha	PRKCA	P17252	CHEMBL299	Kinase	0.534649608444
15.	N, N'-Di-O-nitrophenyloxamide	Epidermal growth factor receptor erbB1	EGFR	P00533	CHEMBL203	Kinase	0.104671941128
16.	L-Menthyl 3-ethyl-4-oxotricyclo (4.3.0.0(1,)) nonane-5carboxylate	Protein-tyrosine phosphatase 1B	PTPN1	P18031	CHEMBL335	Phosphatase	0.164184051624
17.	2,2,4-Trimethyl-6-(1-oxo-3-phenylprop-2-enyl)cyclohexane-1,3,5-trione	Integrin alpha-4/beta-1	ITGB1 ITGA4	P05556 P13612	CHEMBL1907599	Membrane receptor	0.11150186548
18.	4-(4-Chlorophenyl)-4-hydroxypiperidine, Otrimethylsilyl-	Dopamine D2 receptor	DRD2	P14416	CHEMBL217	Family A G protein-coupled receptor	0.622743757482
19.	Ethyl homovanillate, TMS derivative	Phosphodiesterase 10A	PDE10A	Q9Y233	CHEMBL4409	Phosphodiesterase	0.112041901328
20.	13,14-Epoxyursan-3-ol, acetate	Acetylcholinesterase	ACHE	P22303	CHEMBL220	Hydrolase	0.128531577649

Discussion of Target Prediction *Simarouba glauca* Linn.

- The probable molecular targets of phytoconstituents identified from *Simarouba glauca* Linn. were predicted using the in silico platform SwissTargetPrediction to elucidate their possible mechanisms underlying anti-inflammatory activity.
- The predicted targets predominantly belong to G-protein coupled receptors (GPCRs), kinases, phosphatases, hydrolases, and membrane receptors, many of which are directly involved in inflammatory signaling and immune modulation.
- Protein Kinase C (PKC) isoforms are key mediators in intracellular inflammatory signaling cascades, including activation of NF-κB and subsequent production of pro-inflammatory cytokines such as TNF-α and IL-6. Modulation or inhibition of PKC activity can attenuate inflammatory responses by suppressing cytokine release and downstream signaling events. Compound 8 was predicted to interact with PKC-γ (PRKCG), while compound 14 showed a high probability of interaction with PKC-α (PRKCA) (probability score 0.5346). The relatively high probability score for compound 14 suggests strong biologically relevant interaction.
- Compounds 9, 11 and 16 were predicted to target protein tyrosine phosphatase 1B (PTPN1), with compound 16 showing the highest probability among them. PTPN1 is involved in the regulation of immune cell signaling and inflammatory mediator production. Inhibition of PTPN1 has been associated with modulation of chronic inflammatory pathways, suggesting that

these phytoconstituents may exert immunomodulatory effects.

- The interaction of compound 12 (phytol) with cannabinoid receptor 2 (CNR2) and compound 10 with cannabinoid receptor 1 (CNR1) is particularly relevant, as cannabinoid receptors play a crucial role in immune regulation. Activation of CB2 receptors in immune cells suppresses cytokine release and reduces inflammatory cell migration, thereby contributing to anti-inflammatory effects.
- Compound 13 was predicted to interact with the β2-adrenergic receptor (ADRB2), which is known to regulate cytokine production and inflammatory mediator release. β2 receptor activation has been associated with suppression of pro-inflammatory cytokines, supporting a possible anti-inflammatory mechanism.
- Compound 6 was predicted to target thrombin, which participates in inflammation-associated coagulation pathways. Compound 20 was associated with acetylcholinesterase (ACHE), suggesting possible involvement in the cholinergic anti-inflammatory pathway, where increased acetylcholine levels suppress cytokine production. Compound 19 showed affinity toward phosphodiesterase 10A (PDE10A), and modulation of phosphodiesterases can elevate intracellular cAMP levels, thereby reducing inflammatory mediator synthesis.
- Compound 17 was predicted to target integrin α4/β1, a membrane receptor involved in leukocyte adhesion and migration. Inhibition of integrin-mediated cell adhesion may reduce inflammatory cell infiltration into tissues, contributing to attenuation of inflammatory responses.
- Interestingly, compound 18 demonstrated the highest probability score (0.6227) for interaction with the dopamine D2 receptor (DRD2). Although primarily associated with neurological functions, dopaminergic signaling also modulates immune responses and inflammatory pathways, indicating a potential neuro-immune regulatory mechanism.

Overall, the Swiss target prediction analysis suggests that the phytoconstituents of *Simarouba glauca* Linn. may exert anti-inflammatory effects through multi-target mechanisms, including modulation of intracellular signaling kinases, regulation of immune receptors, inhibition of pro-inflammatory enzymes and interference with leukocyte migration pathways. The identification of targets such as PKC isoforms, cannabinoid receptors, PTPN1, integrins and adrenergic receptors provides mechanistic support for the experimentally observed anti-inflammatory activity. These findings highlight the potential of *Simarouba glauca* Linn. phytoconstituents as multi-target anti-inflammatory agents and warrant further experimental validation through *in-vitro* and *in-vivo* studies.

Table 7. Toxicity prediction data on the selected compounds as per Protox server results

S.NO	PHYTOCONSTITUENTS	CLASSIFICATION	TARGET SITE	PROBABILITY
1	3,3-Dimethyl-1-(5-phenyl-tetrahydrofuran-2-yl)-butan 2-one	Toxicity end points	BBB-barrier	0.96
		Toxicity end points	Ecotoxicity	0.71
2	2,2-Dimethoxybutane	Toxicity end points	BBB-barrier	0.95
3	Methyl 2-chloro-2-methyl-3-[(4-methylbenzene) sulfonyl] propanoate	Toxicity end points	BBB-barrier	0.86

4	4-Methylindeno [3',2':5,6] pyrazino[2,3-e] [2,1,3] benzothiadiazol-11-one	----------	---------	---------
5	Thiocyanic acid, [1-(4-amino-1,2,5-oxadiazol-3-yl)-1H 1,2,3-triazol-5-yl] methyl ester	Toxicity end points	BBB-barrier	0.85
		Toxicity end points	Carcinogenicity	0.72
6	4,7-Dimethoxy-6-nitro-2H-1,3-benzodioxole-5 carbonitrile	Toxicity end points	Mutagenicity	0.88
		Toxicity end points	Ecotoxicity	0.74
7	5-(4-Fluorophenyl)-1-methyl-3H,5H,6H,8H- [1,3] diazino[4,5-d] pyrimidine-2,4,7-trione	Organ toxicity	Neurotoxicity	0.93
		Organ toxicity	Respiratory toxicity	0.78
		Toxicity end points	BBB- barrier	0.8
		Toxicity end points	Clinical toxicity	0.73
8	3(9bH)-Dibenzofuranone	Toxicity end points	BBB- barrier	0.7
		Toxicity end points	Nutritional toxicity	0.73
		Molecular Initiating Events	Pregnane X receptor (PXR)	0.75
		Metabolism	Cytochrome CYP1A2	0.71
		Metabolism	Cytochrome CYP2C9	0.86
9	4-Methyl-Z-4-hexadecen-1-ol	Toxicity end points	BBB-barrier	0.9

10	Heneicosanoic acid, methyl ester	Toxicity end points	BBB-barrier	0.97
		Toxicity end points	Ecotoxicity	0.75
11	Phthalic acid, isobutyl octadecyl ester	Toxicity end points	BBB-barrier	0.86
		Toxicity end points	Carcinogenicity	0.83
12	Phytol	Toxicity end points	BBB-barrier	0.91
13	Carbonic acid, but-2-yn-1-yl tetradecyl ester	Toxicity end points	BBB-barrier	0.94
14	Cinnamic acid	Organ toxicity	Nephrotoxicity	0.73
		Organ toxicity	Cardiotoxicity	0.91
		Toxicity end points	Immunotoxicity	0.99
		Molecular Initiating Events	Transtyretrin(TTR)	0.74
15	N, N'-Di-O-nitrophenyloxamide	Toxicity end points	Mutagenicity	0.83
16	L-Menthyl 3-ethyl-4-oxotricyclo (4.3.0.0(1,)) nonane-5 carboxylate	Toxicity end points	BBB-barrier	0.93
		Molecular Initiating Events	GABA receptor	0.71
17	2,2,4-Trimethyl-6-(1-oxo-3-phenylprop-2-enyl) cyclohexane-1,3,5-trione	Toxicity end points	BBB-barrier	0.9
		Toxicity end points	Ecotoxicity	0.7

18	4-(4-Chlorophenyl)-4-hydroxypiperidine, O trimethylsilyl-	----------	----------	----------
19	Ethyl homovanillate, TMS derivative	Toxicity end points	BBB-barrier	0.8
		Molecular Initiating Events	Thyroid hormone receptor Alpha	0.73
20	13,14-Epoxyursan-3-ol, acetate	Toxicity end points	BBB-barrier	0.92
		Toxicity end points	immunotoxicity	0.99

Discussion of Toxicity Prediction of *Simarouba glauca* Linn.

In-silico toxicity prediction was performed to assess the safety profile of phytoconstituents identified from *Simarouba glauca* Linn. Several compounds showed high blood–brain barrier (BBB) permeability, indicating good absorption and distribution, but also suggesting a possible risk of neurotoxicity. Some phytochemicals exhibited predicted mutagenic, carcinogenic, immunotoxic, and organ-specific toxicities, including nephrotoxicity and cardiotoxicity. These effects may be linked to interactions with molecular targets such as thyroid hormone receptors, GABA receptors, and transthyretin. Additionally, predicted interactions with cytochrome P450 enzymes (CYP1A2 and CYP2C9) indicate a potential for altered drug metabolism and drug–drug interactions. Ecotoxicity observed in certain compounds highlights possible environmental concerns. *in-silico* toxicity prediction of *Simarouba glauca* Linn. phytoconstituents revealed that most compounds possess favorable safety profiles, acceptable organ toxicity risk, and good pharmacokinetic potential.

Although a few compounds showed predicted toxicity endpoints, these were moderate and manageable, indicating the need for dose optimization and further experimental validation.

Overall, the results strongly suggest that selected phytoconstituents of *Simarouba glauca* can be considered promising and relatively safe lead compounds for future drug discovery, particularly after structural optimization and *in-vitro* and *in-vivo* confirmation.

Table 8. Binding interaction of the selected compounds with human cyclooxygenase-2 (PDP ID 5IKR) enzyme

S.no	Name of the phytoconstituents	Binding affinity (kcal/mol)
1.	3,3-Dimethyl-1-(5-phenyl-tetrahydrofuran-2-yl)-butan- 2-one	-7.5
2.	2,2-Dimethoxybutane	-3.9

3.	Methyl2-chloro-2-methyl-3-[(4-methylbenzene) sulfonyl]propanoate	-6.4
4.	4-Methylindeno [3',2':5,6] pyrazino[2,3-e] [2,1,3] benzothiadiazol-11-one	------
5.	Thiocyanic acid, [1-(4-amino-1,2,5-oxadiazol-3-yl)-1H- 1,2,3-triazol-5-yl] methyl ester	-6.8
6.	4,7-Dimethoxy-6-nitro-2H-1,3-benzodioxole-5- carbonitrile	-6.6
7.	5-(4-Fluorophenyl)-1-methyl-3H,5H,6H,8H- [1,3] diazino[4,5-d] pyrimidine-2,4,7-trione	-8.4
8.	3(9bH)-Dibenzofuranone, 2-acetyl-1,7,9-trihydroxy- 8,9b-dimethyl-	-8.8
9.	4-Methyl-Z-4-hexadecen-1-ol	-4.9
10.	Heneicosanoicacid,methylester	--------
11.	Phthalic acid, isobutyl octadecyl ester	-------
12.	Phytol	-5.2
13.	Carbonic acid, but-2-yn-1-yl tetradecyl ester	-------
14.	Cinnamic acid	-------
15.	N,N'-Di-O-nitrophenyloxamide	-9.3
16.	L-Menthyl 3-ethyl-4-oxotricyclo (4.3.0.0(1,)) nonane-5- carboxylate	-7.4
17.	2,2,4-Trimethyl-6-(1-oxo-3-phenylprop-2-enyl)- cyclohexane-1,3,5-trione	-7.4
18.	4-(4-Chlorophenyl)-4-hydroxypiperidine, O- trimethylsilyl-	--------
19.	Ethylhomovanillate, TMSderivative	-------
20.	13,14-Epoxyursan-3-ol, acetate	-------
TEST	DISPIRIN	-5.7

Comparing docking results with Dispirin (COX-2)

- Molecular docking analysis was carried out using PyRx to investigate the binding affinity of selected plant-derived constituents against cyclooxygenase-2 (COX-2), with dispirin used as the reference standard.
- Dispirin exhibited a binding energy of −5.7 kcal/mol, which served as the benchmark for comparison.
- It is showed that 45% of phytoconstituents demonstrated binding energies more negative than −5.7 kcal/mol, indicating stronger binding affinity towards the COX-2 active site when compared to dispirin. This enhanced binding suggests the formation of more stable ligand–protein complexes and potentially improved inhibitory activity.
- The table 8 further highlights that the median binding energy of the plant

constituents lies below that of dispirin, confirming the overall superior binding performance of the selected compounds. The presence of lower outlier values represents compounds with particularly strong interactions, suggesting their potential as lead molecules for anti-inflammatory drug development.

- These findings support the hypothesis that certain plant-derived constituents may exhibit comparable or superior COX-2 inhibitory potential relative to dispirin.
- The docking results revealed that several phytoconstituents exhibited stronger binding affinity towards COX-2 than dispirin (−5.7 kcal/mol).

Conclusion

The present study demonstrated that the aqueous extract of *Simarouba glauca* Linn. leaves exhibited significant, concentration-dependent in vitro anti-inflammatory activity, as evidenced by the egg albumin denaturation and gelatin denaturation assays. Furthermore, molecular docking analysis indicated that the phytochemicals present in the extract possess potential cyclooxygenase-2 (COX-2) inhibitory activity. The selected compounds also exhibited favourable toxicity profiles and pharmacokinetic parameters, thereby supporting their drug ability. Overall, the results indicate a strong possibility for the discovery of lead anti-inflammatory agents from the leaves of *Simarouba glauca* Linn.

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