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Unveiling the *in silico* anti-inflammatory activity of an acetone extract of *Caesalpinia sappan* L.

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Abstract
Caesalpinia sappan L., belonging to the Caesalpiniaceae family, is traditionally used in Asian medicine for the treatment of infectious and inflammatory disorders. Its heartwood, enriched with brazilin, contains diverse phytoconstituents such as proteins, carbohydrates, amino acids, alkaloids, flavonoids, phenols, tannins, glycosides, terpenoids and saponins and the same GC-MS analysis of acetone extract of C. *sappan* revealed 25 compounds. Among them, seven bioactive constituents were subjected to molecular docking against two inflammatory target proteins, Cyclooxygenase-2 (COX-2) and Interleukin-1 beta (IL-1β). Notably, Cholest-4-en-3-one exhibited the strongest binding affinity with both COX-2 (-7.6 kcal/mol) and IL-1β (-6.2 kcal/mol), forming multiple hydrogen bonds and suggesting significant anti-inflammatory potential. Other compounds, such as 1-buten-3-one derivatives and benzoic acid esters, also demonstrated promising interaction. These findings not only provide scientific validation for the ethnomedical use of *C. sappan* but also highlight the importance of *in silico* approaches in identifying natural anti-inflammatory agents. The study emphasizes the relevance of phytochemical evaluation and molecular docking in drug discovery, offering a foundation for future pharmacological investigations. Overall, the result suggests that the acetone extract of *C. sappan* may serve as a potential natural source for anti-inflammatory drug development, warranting further isolation, purification and *in vivo* validation of its active compounds.

Keywords: Acetone extract, anti-inflammatory, *Caesalpinia sappan*, COX-2, GC-MS analysis, IL-1β

Introduction
Medicinal plants represent a valuable reservoir of bioactive compounds and have been used for centuries in traditional healthcare systems. India, with nearly 20,000 documented medicinal species, possesses a rich heritage of traditional practices such as Ayurveda, Siddha and Unani, many of which have influenced modern therapeutic approaches (Karuna Moorthi, 2012; Prejeena *et al*., 2016) [10, 21]. To date, approximately 12,000 plant-derived secondary metabolites have been identified, though this accounts for less than 10% of their estimated total diversity (Mansour Ghorbanpour *et al*., 2017) [15]. Increasing global interest in alternative medicine has further emphasized the importance of plant-derived phytochemicals as a basis for developing new drugs (Bassam Abdul Hassan, 2012) [2].

Caesalpinia sappan L. (family: Caesalpiniaceae), a perennial redwood species native to Southeast Asia, has long been utilized in ethnomedicine. Decoctions of its heartwood are traditionally prescribed for tuberculosis, diarrhoea, diabetes, dysentery and various skin infections (Zanin *et al*., 2012) [26]. Pharmacological investigations have highlighted its wide spectrum of biological activities, including antioxidant, antibacterial, anti-acne, anti-inflammatory, larvicidal, antiviral, anticancer and antimicrobial activities (Mas Rizky Syamsunaarno *et al*. 2021; Heru Sansongko *et al*. 2024; Areeb Husain Thangal *et al*. 2022) [16, 8, 1].

Despite its ethnopharmacological significance, limited mechanistic studies exist to support its anti-inflammatory potential. Since inflammation is largely mediated by proteins such as Cyclooxygenase-2 (COX-2) and Interleukin-1 beta (IL-1β), identifying natural inhibitors of these targets remains a promising therapeutic approach. The present study aimed to perform pharmacognostical, physicochemical and phytochemical evaluation of *C. sappan* L. heartwood, profile its acetone extract using GC-MS analysis, screening of bioactive compounds from the acetone extract and to investigate the anti-inflammatory potential of its bioactive compounds through *in silico* molecular docking against COX-2 and IL-1β.

Materials and Methods

Assortment of plant material

Fresh twigs of *Caesalpinia sappan* were collected from the Vattappoyil region, Kannur district, Kerala, India and identified using standard floras (Gamble, 1957; Matthew, 1983) [4, 18]. A voucher specimen (VCW/BH/Acc. No. 38) was deposited in the Department of Botany, Vellalar College for Women, Thindal, Erode. Photographs of the plants were also taken to supplement the herbarium collection. Dried heartwood was additionally obtained from a local, authenticated herbal store (Erode, Tamil Nadu, South India).

Preparation of plant material and extraction

The heartwood was shade-dried at 31°C for 15 days, ground to 60-mesh size and stored in airtight containers. 20 g of dried wood powder was extracted successively with 200 ml of petroleum ether (42-62 °C) and acetone (56 °C) in a Soxhlet apparatus for 6 hours. Extracts were concentrated at 50 °C, and yields (%) were calculated as

$$\text{Extractive value} = \frac{\text{Weight of extract (g)}}{\text{Weight of plant powder(g)}} \times 100$$

Pharmacognostical and physicochemical evaluation

Macroscopic features of the study plant were followed by the methods described by Trease and Evans (1983) [23] and Wallis (1985). Organoleptic characters (colour, odour, taste, texture) and powder reaction with standard chemical reagents were examined following Kokoshi *et al*. (1958) [13].

Phytochemical screening

Qualitative phytochemical tests were performed using standard protocols described by Harborne (1984) [6] and Kokate *et al.* (1995) [12] to detect proteins, carbohydrates, amino acids, alkaloids, flavonoids, phenols, tannins, glycosides, terpenoids, saponins, coumarins, quinones, anthraquinones and fixed oils and fats.

GC-MS analysis

The acetone extract (1µL diluted in 1 mL of acetone) was analysed on a Thermal GC Trace Ultra version 5.0 system coupled with a Thermo MS DSQ II detector, equipped with a DB-35MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas at 1 mL/min. The oven was programmed from 150 ºC (2 min), to 220 ºC at 5ºC/min, then to 260 ºC at 10 ºC/min, with a final hold at 350 ºC for 20min. Mass Spectra were recorded at 70 eV across m/z 50-650. The relative percentage of each component was calculated by comparing its average peak area to the total peak areas (Jennings and Shibamoto, 1980) [9]. Compounds were identified by comparing mass spectra with NIST Library 2008, WILEY8, and FAME databases (Massada, 1976) [17].

Molecular docking

Molecular docking was performed to predict the optimal binding orientation between selected bioactive compounds from *Caesalpinia sappan* and the target proteins, interleukin-1 beta (IL-1β) and cyclooxygenase-2 (COX-2), using Auto Dock Vina (version 4.2) and the results were analysed in BIOVIA Discovery Studio (version 2021).

Ligand preparation

Phytochemical compounds identified in the wood extract of *Caesalpinia sappan* L. through GC-MS analysis. PubChem database (https://pubchem.ncbi.nlm.nih.gov) was used to reclaim 3D structures of ligands and saved in SDF format. Energy minimization was performed using the integrated Open Babel GUI plugin within the PyRx 0.8 software suite. Following minimization, files were saved in Protein Data Bank (PDB) format for further molecular docking studies.

Protein preparation

Protein structures are critical components in docking analysis. Crystal structures of Interleukin 1 beta (PDB ID: 9ILB) and Cyclooxygenase-2 (COX-2) were retrieved from the Protein Data Bank (PDB; http://www.rcsb.org). Heteroatoms and water molecules were removed, followed by the addition of hydrogen atoms and Kollman charges and structures were validated in Discovery Studio before saving in PDB format.

Docking

The prepared ligands and target proteins were subjected to molecular docking using Auto Dock Vina. Multiple conformations of each ligand were generated during the docking process, and the final energy refinement of the ligand poses was performed. The docking scores corresponding to the best binding poses of each bioactive compound with the target proteins were calculated and recorded.

Results

Macroscopical studies

The macroscopic characteristics of *Caesalpinia sappan* are summarized in Table 1. The plant is a shrubby tree (up to 8m tall) with a hard, greyish-brown stem, dark green bipinnate leaves, and yellow flowers in terminal panicles. Fruits are glabrous pods with ellipsoid brown seeds. Representative plant parts are shown in Plate 1.

Plate 1: Flowering twig of the study plant *Caesalpinia sappan* L.

Table 1: Macroscopic characteristics of *Caesalpinia sappan* L

Feature	Description
Habit	Shrubby tree
Height	Grows up to 8 m
Stem	**Colour:** Greyish brown **Odour:** Characteristic **Shape:** Rounded, Solid **Texture:** Hard **Trunk:** Up to 14 cm in diameter, distinct ridges, many prickles
Leaf	Compound, alternate, stipulate, bipinnate, simple, oblong, emarginate **Size:** 20–45 cm long, 10–20 cm broad, 8–16 pairs, up to 20 cm long pinnae **Surface:** Glabrous **Stipules:** Spiniform, 3–5 mm long **Rachis:** 30–40 cm long, pinnae 10–14 pairs of oblongs, oblique at base, rounded to emarginate at apex **Spine:** Present at junction between pinnae pairs on the upper side **Texture:** Glabrous **Taste:** Bitter **Odour:** Characteristic **Colour:** Dark green
Inflorescence	Terminal panicle
Flowers	Yellow, racemes pubescent, 5-merous, bisexual flowers, calyx-5, corolla-5, calyx tube 3 mm long, stamens-10
Fruit	Pod, Glabrous, polished brown when ripe
Seeds	Ellipsoid, flattened, brown

Physico-chemical studies

Organoleptic evaluation of heartwood powder revealed an orange-red coarse powder with bitter taste and characteristic odour (Table 2). The petroleum ether extract was whitish yellow and semi-solid, whereas the acetone extract appeared brown and semi-liquid (Table 3).

Reaction with chemical reagents produced distinct color changes (Table 4), including a dark pink with HCL, reddish-black with H_2SO_4 and crimson-brown with HNO_3. Extractive value determination indicated that acetone yielded the highest extract (8.1%), followed by petroleum ether (7.3%) summarized in Table 5.

Table 2: Organoleptic characters of heartwood powder of *C. sappan* L.

Characters	Observations
Colour	Orange-red
Texture	Coarse powder
Taste	Bitter
Odour	Characteristic

Table 3: Organoleptic characters of successive solvent extracts of *C. sappan* heartwood powder

Extraction medium	Colour	Consistency	Odour
Petroleum ether	Whitish yellow	Semi-solid	Characteristic
Acetone	Brown	Semi-liquid	Characteristic

Table 4: Behaviour of *C. sappan* heartwood powder with different chemical reagents

Powder + Chemical reagents used	Nature of the colour of Powder
Powder as such	Orange-red
Concentrated HCl	Darkish pink
Concentrated H_2SO_4	Reddish black
Concentrated HNO_3	Crimson brown
Acetic acid	Brownish orange
10% Sodium hydroxide	Dark violet
Aqueous solution	Pink

Table 5: Extractive values of the heartwood powder of *C. sappan* using. Different solvents

Method of extraction	Solvents used	Yield (%)
Continuous hot percolation using the Soxhlet apparatus	Petroleum ether	7.3
	Acetone	8.1

Preliminary phytochemical investigation

Qualitative tests (Table 6) confirmed the presence of proteins, carbohydrates, alkaloids, phenols, terpenoids, saponins, anthraquinones and fixed oils. The acetone extract revealed a broader phytochemical profile compared to petroleum ether.

Table 6: Qualitative phytochemical screening of two different solvent extracts of *C. sappan* heartwood powder

Phyto constituents	Test /Reagent	Petroleum ether	Acetone
Protein	Biuret test	-	+
Carbohydrate	Barfoed's test	_-	+
Amino acids	Ninhydrin test	-	-
Alkaloids	Wagner's reagent	+	+
Flavonoids	Ammonium hydroxide test	-	-
Phenols	Lead acetate test	+	+
Tannins	Ferric chloride test	-	-
Glycosides	Borntrager's test	_-	_-
Terpenoids	Liebermann-Burchard's test	+	-
Saponins	Foam test	+	-
Coumarin	NaOH test	_	-
Quinone	Sulphuric acid test	-	-
Anthraquinone	Borntrager's test	+	+
Fixed oils and fats	Stain test	_	+

Note: (+) Presence of chemical compound; (-) Absence of chemical compound

GC–MS screening

The acetone extract yielded 25 compounds (Table7, Fig.1). The major constituents included benzoic acid, 4-ethoxy-ethy ester (99.2%), hexadecenoic acid methyl ester (98.5%) and diethyl phthalate (98.2%) Cholest-4-en-3-one (86.5). These compounds are associated with antioxidant, antimicrobial and anti-inflammatory properties.

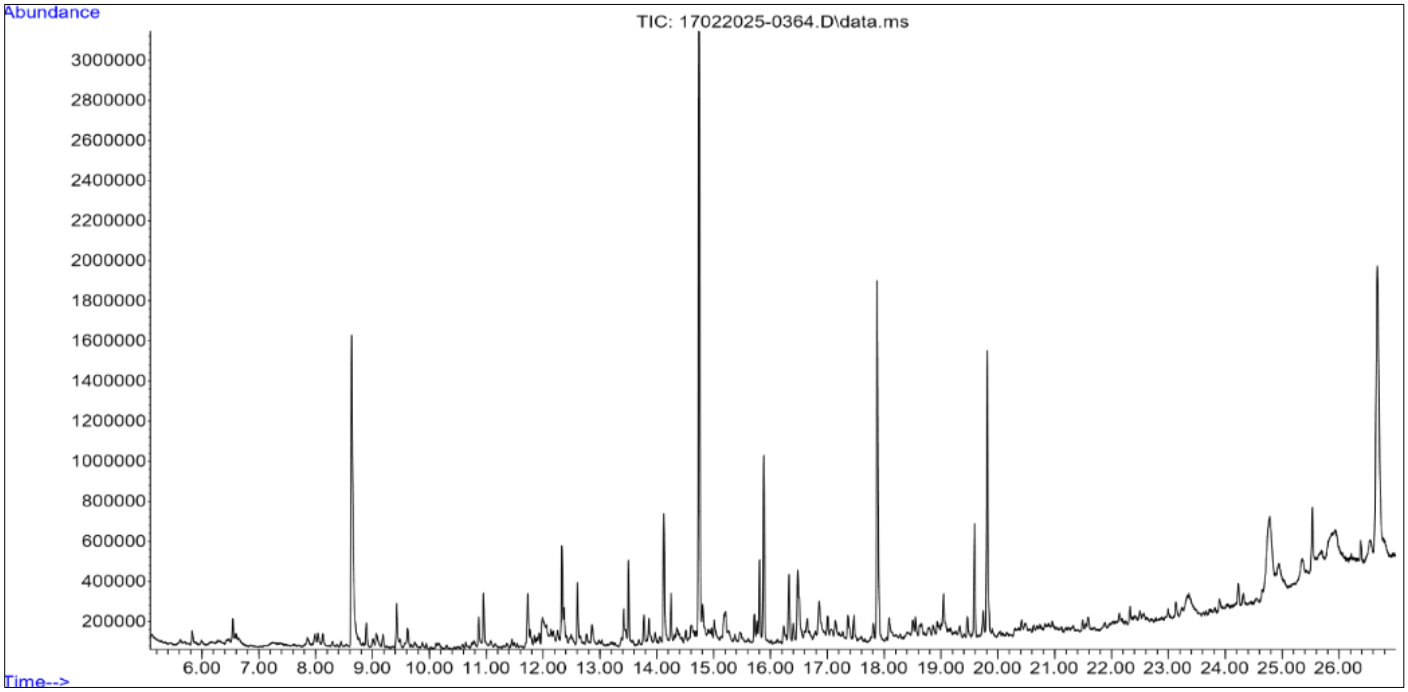
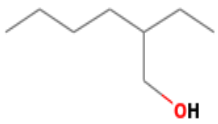
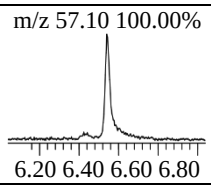
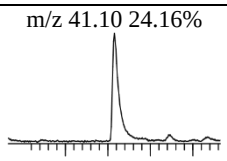
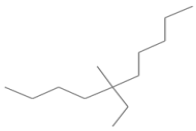
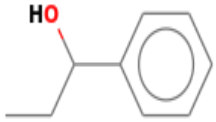
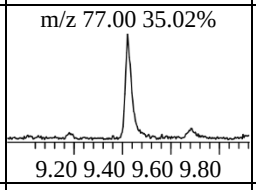
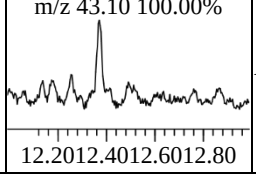
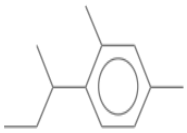
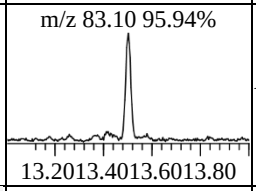
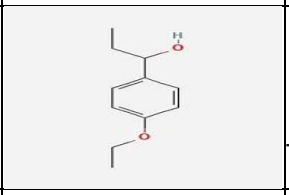
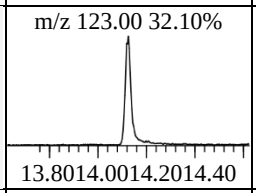
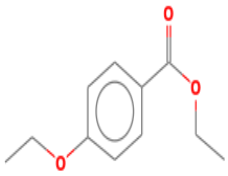
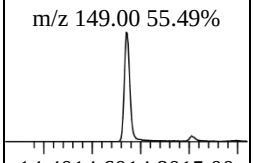
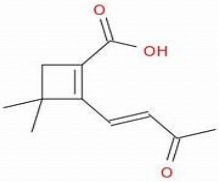
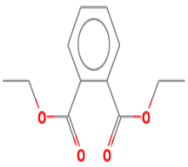
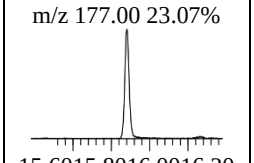
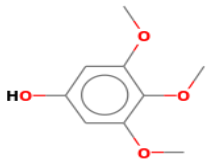
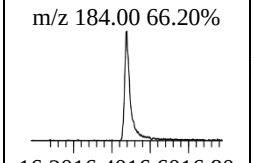
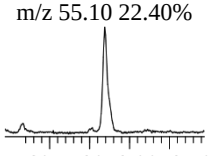
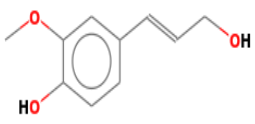
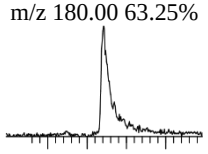
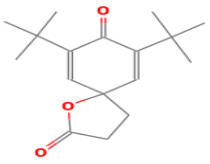
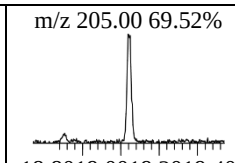
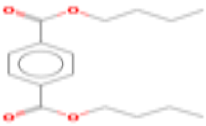
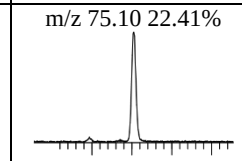
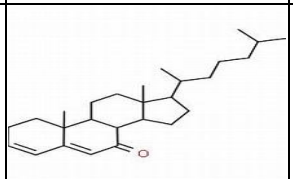
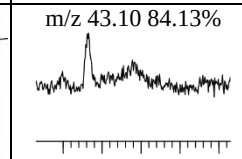
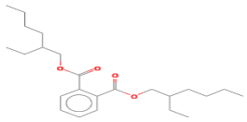
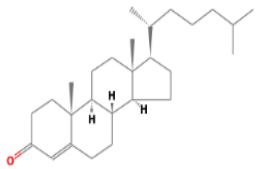
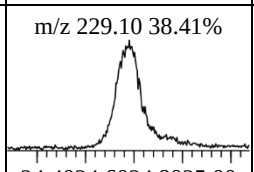
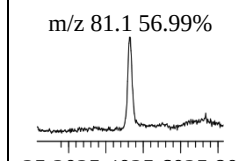
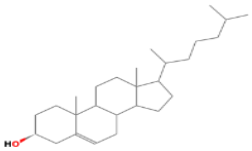
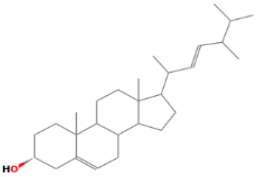
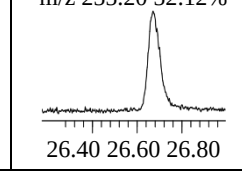


Fig 1: GC-MS Chromatogram of heartwood acetone extract of *C. sappan* L.

Table 7: GC-MS analysis of the acetone extract of *C. sappan* L. heartwood powder

S. No.	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure	Hit Spectrum	Nature of Compound	Activity
1.	6.540	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	130.2279	86.0			Alcohol	Plasticizers
2.	8.630	2-Pyrrolidinone, 1-methyl-	C_5H_9NO	99.1311	95.6			Heterocyclic compounds	Antimicrobial activity and Anti-inflammatory
3.	8.892	Decane, 5-ethyl-5-methyl-	$C_{13}H_{28}$	184.3614	86.2			Non-polar compounds	Ayurvedic medicines
4.	9.424	Benzene methanol, alpha. -ethyl-	$C_9H_{12}O$	136.1910	92.1			Aromatic	Anesthetic and Antimicrobial
5.	10.953	1-Tetradecanol	$C_{14}H_{30}O$	214.3874	93.5		-	Alcohol	Antibacterial and Anti-inflammatory
6.	11.729	Octadecane	$C_{18}H_{38}$	254.4943	89.2			Alkanes and Allene's	Antimicrobial, Microbial metabolism and Anti-inflammatory

7.	12.330	Benzene, 2,4-dimethyl-1-(1-methylpropyl)-	$C_{12}H_{18}$	162.2713	86.5		-	Aromatic	Antimicrobial, antiviral, Cytotoxic, antioxidant and Anti-inflammatory
8.	13.502	Cetene	$C_{16}H_{32}$	224.4253	95.0			Alkanes and Allene's	Surfactant
9.	14.125	1-(4-Ethoxyphenyl) propan-1-ol	$C_9H_{12}O_2$	152.186	86.5			Aromatic Alcohol	Antimicrobial and Anti-inflammatory
10.	14.740	Benzoic acid, 4-ethoxy-, ethyl ester	$C_{11}H_{14}O_3$	194.2271	99.2			Carboxylic Acid and Esters	Antioxidant, Antibacterial and Food Preservation
11.	15.206	1-Buten-3-one, 1-(2-carboxy-4,4-dimethylcyclobutenyl)-	$C_{10}H_{12}O_2$	164.196	61.8		-	Ketone	Antimicrobial, Antioxidant and Synthetic utility
12.	15.880	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.2372	98.2			Plasticizers and Phthalates	Plasticizer and Toxicity
13.	16.477	Phenol, 3,4,5-trimethoxy-	$C_9H_{12}O_4$	184.1892	83.0			Phenols and Phenolic Derivatives	Anticancer, Antimicrobial, Antiviral and Anti-inflammatory
14.	17.464	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256.4671	76.9			Alcohol	Antibacterial, Antioxidant and Anti-inflammatory
15.	17.876	Hexadecenoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	98.5			Carboxylic Acid and Esters	Antifungal, Antioxidant and Anti-inflammatory
16.	18.087	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxy phenol	$C_{10}H_{12}O_3$	180.2005	87.0			Phenol	Antioxidant, Anti-inflammatory, Antimicrobial and Cancer Prevention

17.	19.041	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8 Dione	$C_{17}H_{24}O_3$	276.3707	88.5			Lipophilic and Spirocyclic	Antimicrobial and Aroma Component
18.	19.587	1,4-Dibutyl benzene-1,4-dicarboxylate	$C_{16}H_{22}O_4$	278.3435	93.7		-	Aromatic diester	Plasticizer
19.	19.809	Methyl stearate	$C_{19}H_{38}O_2$	298.5038	97.6			Carboxylic Acid and their Esters	Antimicrobial and Emollient
20.	23.360	Cholesta-3,5-dien-7-one	$C_{27}H_{42}O$	382.61	71.0			Steroid derivative	Antimicrobial, Anti-inflammatory and Cholesterol Metabolism
21.	24.223	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.5561	63.9		-	Phthalate ether	Endocrine Disruption, Carcinogenic Potential and Toxicity
22.	24.776	Cholest-4-en-3-one	$C_{27}H_{44}O$	384.6377	86.5			Terpenes and Steroids	Antimicrobial Activity, Metabolic Benefits, Liver Health and Skin Health
23.	25.530	Squalene	$C_{30}H_{50}$	410.7180	74.6			Terpenes and Steroids	Antioxidant, Anti-inflammatory, Cholesterol Regulation and Immune System Support
24.	25.938	Cholesterol	$C_{27}H_{46}O$	386.6535	69.7			Terpenes and Steroids	Hormone Production, Vitamin D Synthesis and Bile Acid Formation
25.	26.672	Ergosta-5,22-dien-3-ol, (3. beta.,22E)-	$C_{28}H_{46}O$	398.6642	93.4			Terpenes and Steroids	Antioxidant, Anti-inflammatory, Cholesterol-Lowering and Anticancer potential

***In silico* molecular docking**

Seven bioactive compounds identified through GC–MS analysis were selected for docking with IL-1β and COX-2 proteins. Cholest-4-en-3-one showed the highest affinity for COX-2(-7.6 kcal/mol) with five hydrogen bonds and IL-1β (-6.2kcal/mol) with twelve hydrogen bonds. (Tables 8 and 9) represents docking scores and H-bond interactions. (Fig 2 and 3) shows 2D and 3D Structure of best docking interaction.

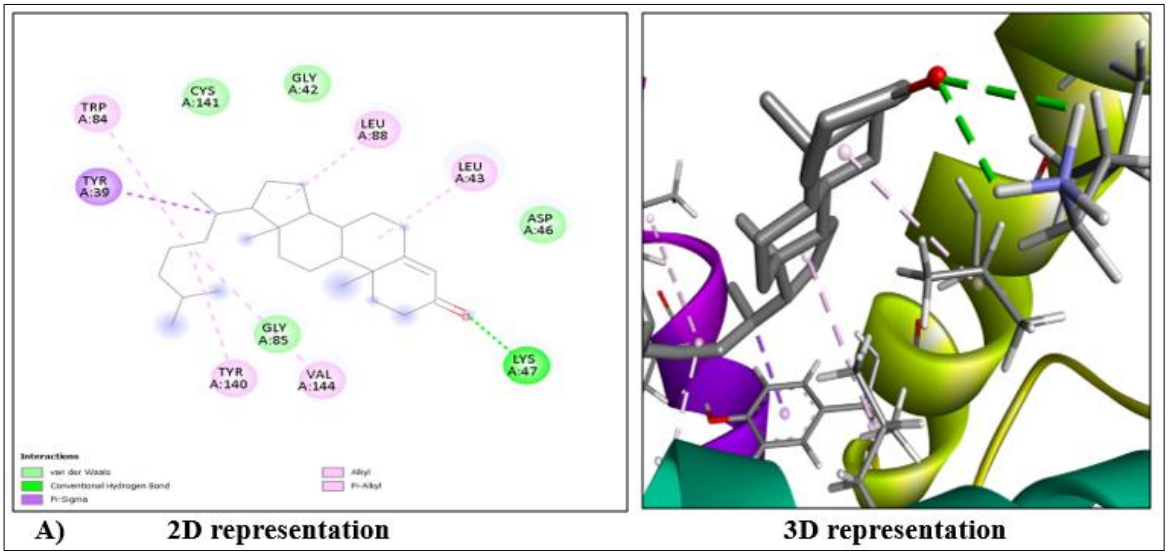


Fig 2: A) 2D and 3D representation of the best molecular docking compound Cholest-4-en-3-one.

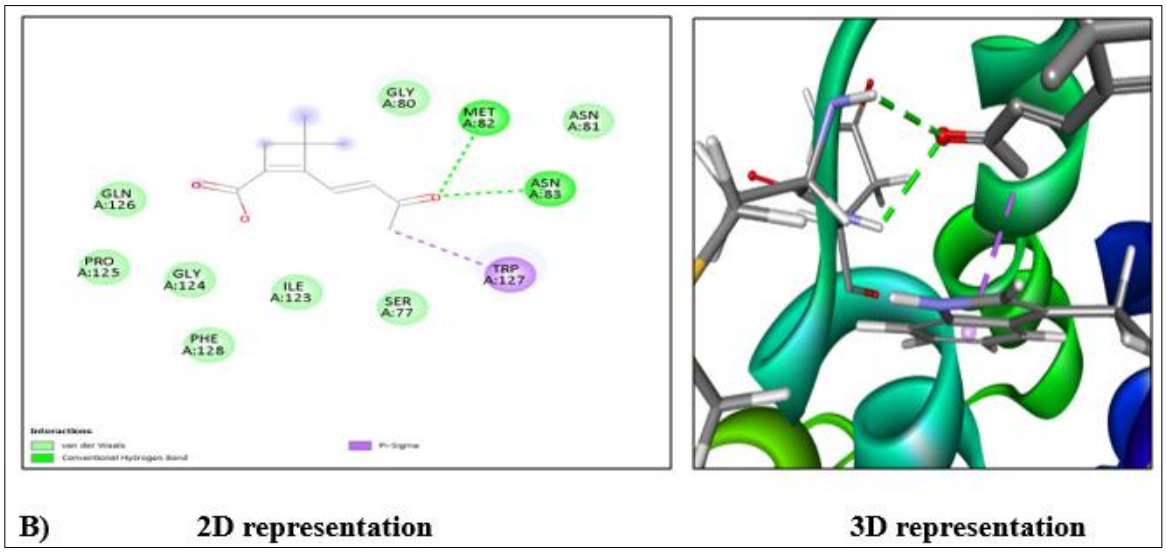


Fig 2: B) 2 D and 3D representation of the best hydrogen bonding compound 1-Buten-3-one, 1-(2-carboxy-4,4-dimethylcyclobutenyl).

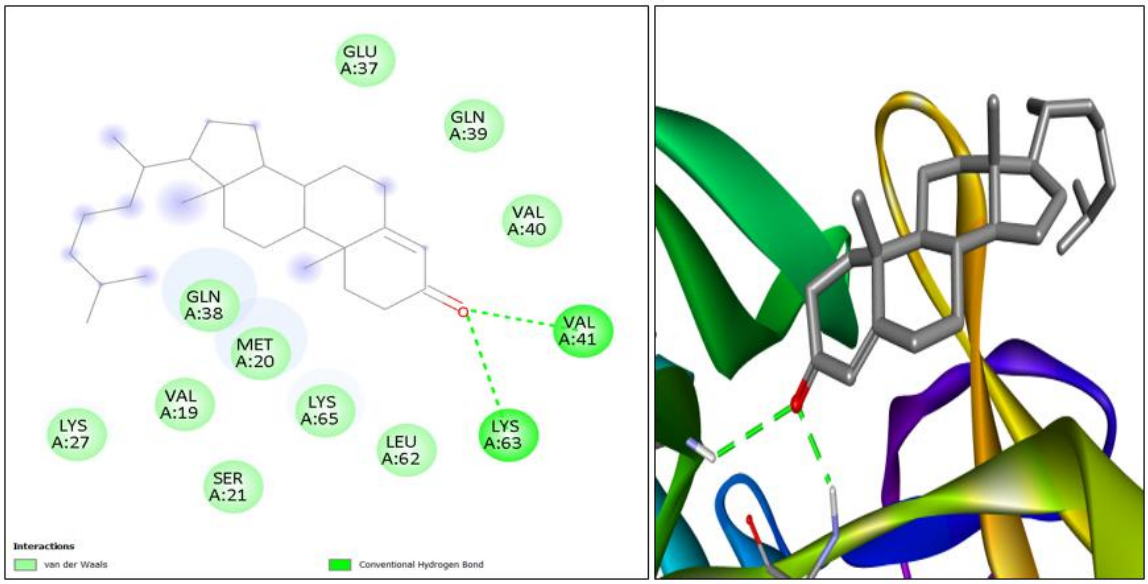


Fig 3: 2D and 3D representation of the best molecular docking and the best hydrogen bonding compound Cholest-4-en-3-one.

Table 8: Docking score and H bond interaction of phytocompounds with Cyclooxygenase-2 protein (COX-2)

S. No.	Name of the Compound	Docking Score (in kcal/mol)	No. of Hydrogen bond	Interacting Amino acids
1.	Diethyl Phthalate	-5.1	8	TYR 39, LEU 88, TRP 84, GLY 85, ARG 86, ASN 83, MET 82, TYR 140
2.	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8 Dione	-6.9	9	ASN 81, GLN 126, PRO 125, GLY 124, ILE 123, ASN 83, GLY 80, ASP 79, MET 82
3.	Benzoic acid, 4-ethoxy-, ethyl ester	-5.1	8	SER 77, ASN 83, GLY 80, ASN 81, MET 82, GLN 126, GLY 124, ILE 123
4.	Cholest-4-en-3-one	-7.6	5	CYS 141, GLY 42, ASP 46. LYS 47, GLY 85
5.	1-(4-Ethoxyphenyl) propan-1-ol	-6.5	7	THR 142, LEU 38, GLY 42, LEU 43, LEU 88, TYR 39, TRP 84
6.	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxy phenol	-5.7	10	PRO 125, GLY124, ASN 81, MET 82, ASN 83, SER 77, GLY 80, ILE 123, ALA 122, GLY 78
7.	1-Buten-3-one, 1-(2-carboxy-4,4-dimethyl cyclo butenyl)-	-5.8	11	GLY 80, MET 82, ASN 81, ASN 83, TRP 127, SER 77, ILE 123, PHE 128, GLY 124, PRO 125, GLN 126

Table 9: Docking score and H bond interaction of phytocompounds with Interleukin 1 beta (IL-1β) protein (9 ILB)

S. No.	Name of the Compound	Docking Score (in kcal/mol)	No of Hydrogen bond	Interacting Amino acids
1.	Diethyl Phthalate	-4.5	7	PRO 131, VAL 132, THR 79, LYS 77, PRO 78, LEU 80, LEU 134
2.	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8 Dione	-5.2	7	GLN 149, PHE150, SER 152, VAL 41, GLN 39, MET 36, ARG 11
3.	Benzoic acid, 4-ethoxy-, ethyl ester	-4.4	6	LYS 65, LEU 62, LYS 63, VAL 41, GLN 39, GLN 38
4.	Cholest-4-en-3-one	-6.2	12	GLU 37, GLN 39, VAL 40, VAL 41, LYS 63, LEU 62, LYS 65, MET 20, GLN 38, VAL 19, SER 21, LYS27
5.	1-(4-Ethoxyphenyl) propan-1-ol	-6	10	LYS 63, MET 20, VAL 41, ARG 11, GLN 149, THR 9, VAL 151, MET 36, GLN 38, GLU 37
6.	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxy phenol	-5.4	7	GLN 39, GLN 38, VAL 19, MET 20, LYS 65, VAL 41, LYS 63
7.	1-Buten-3-one, 1-(2-carboxy-4,4-dimethyl cyclo butenyl)-	-4.9	10	PRO 78, THR 79, PHE 133, PRO 131, VAL 132, LEU 26, GLN 81, GLU 25, LEU 80, LEU 134

Discussion

The macroscopic and organoleptic features of *C. sappan* observed in this study agree with earlier descriptions agree with earlier descriptions (Mas Rizky Syamsunarno *et al*., 2021; Areeb Husain Thangal *et al*., 2022) [16, 1] and partially align with reports on the related species *Caesalpinia bonducella* (Ganesh Wadkar and Fahim Sayyad, 2017) [5]. Physico-chemical evaluations, including diagnostic colour reactions, confirmed the presence of phenolic constituents, which are essential quality markers for standardization.

Extractive values revealed that acetone provided a higher yield than petroleum ether, consistent with the role of solvent polarity in enhancing bioactive recovery (Hayouni *et al*., 2007) [7]. The acetone extracts also exhibited a broader phytochemical spectrum, supporting previous reports on the richness of secondary metabolites in C. *sappan* (Saravanakumar and Helan Chandra, 2013; Ganesh Wadkar and Fahim Sayyad, 2017; Mithun Singh Rajput *et al.*, 2022) [22, 5, 9]

GC-MS profiling identified compounds such as benzoic acid derivatives, hexadecenoic acid methyl ester and diethyl phthalate, which are associated with antioxidant, antimicrobial and anti-inflammatory activities. These findings are in line with reports on related species, including *Delonix elata* and *Clerodendrum phlomidis* (Kilimozhi *et al*., 2009) [11], as well as *Cassia fistula* (Ferdosi *et al*., 2023) [3].

Molecular docking highlighted Cholest-4-en-3-one as the most promising compound, showing strong affinity toward COX-2 and IL-1β targets, suggesting anti-inflammatory potential. These results correspond with previous docking studies on phytochemicals from *Parkia timoriana* and *Moringa oleifera* (Laldinfeli Ralte *et al*., 2022; Naglaa Hamdy, 2024) [14, 20], reinforcing the therapeutic significance of plant-derived polyphenols.

Conclusion

Caesalpinia sappan plays a vital part in traditional Ayurvedic and Unani systems of holistic health and herbal medicine across Southeast Asia, valued for its diverse biological activities, including antibacterial, antifungal, anti-inflammatory, antileukemic, antineurotic, antiviral and antiseborrhoeic agents. In this study, systematic physicochemical evaluation of C. *sappan* heartwood provided standardized parameters for quality control, authentication and prevention of adulteration. These data serve as a valuable reference for researchers, pharmaceutical industries and healthcare practitioners concerned with the application and integrity of traditional herbal remedies.

Molecular docking analyses identified Cholest-4-en-3-one as the most promising bioactive compound, exhibiting strong binding affinity towards (COX-2) and (IL-1β), thereby supporting its potential role as an anti-inflammatory agent. These results validate the traditional therapeutic use of *C. sappan* and highlight its potential for modern drug discovery.

Future research will focus on the isolation, purification and pharmacological evaluation of active compounds, particularly from the acetone extract, to further elucidate their mechanisms of action and therapeutic applicability.

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Data availability

The data that support the findings of this study are available within the article.

Declarations

Conflict of interest

The authors declare no competing interests

References

1. Thangal AH, Prasanth S, Prasanth L, Anu. Pharmacogenetic, phytochemical and pharmacological aspects of *Caesalpinia sappan* plant. Int J Pharmacognosy. 2022;9(12):213-219.
2. Hassan BAR. Medicinal plants (importance and uses). Pharm Anal Acta. 2012;3:10.
3. Ferdosi MFH, Khan IH, Javaid A. Bioactive components of ethyl acetate extract of *Cassia fistula* flowers. J Anim Plant Sci. 2023;33(3):511-517.
4. Gamble JS. Flora of the presidency of Madras. Calcutta: Botanical Survey of India; 1957.
5. Wadkar GH, Sayyad FJ. Pharmacognostic, physicochemical and phytochemical investigation of root bark of *Caesalpinia bonducella*. Int J Pharmacognosy Phytochem Res. 2017;9(1):26-30.
6. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 2nd ed. London, New York: Chapman and Hall; 1984.
7. Hayouni EA, Abedrabba M, Bouix M, Hamdi M. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. Food Chem. 2007;105:1126-1134.
8. Sasongko H, Hedianti DP, Safitri LI. *Caesalpinia sappan* reduces fever with an anti-inflammatory and antioxidant mechanism: a review. J Pharm Sci Clin Res. 2024;1:119-141.
9. Jennings W, Shibamoto T. Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography. Sci Direct. 1980;1-27.
10. Karunamoorthi K. The counterfeit anti-malarial is a crime against humanity: a systematic review of the scientific evidence. Malar J. 2012;1-13.
11. Kilimozhi D, Parthasarathy V, Manavalan R. Active principles determination by GC-MS in *Delonix elata* and *Clerodendrum phlomidis*. Asian J Res Chem. 2009;2(3):344-348.
12. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 3rd ed. Pune: Nirali Prakashan; 1995.
13. Kokoshi CJ, Kokoshi RJ, Sharma FJ. Fluorescence of powdered vegetable drugs under UV radiation. J Am Pharm Assoc. 1958;48(10):715-717.
14. Ralte L, Khiangte L, Thangjam NM, Kumar YA, Singh T. GC-MS and molecular docking analyses of phytochemicals from the underutilized plant *Parkia timoriana* revealed candidate anticancerous and anti-inflammatory agents. Sci Rep. 2022;1-21.
15. Ghorbanpour M, Hadian J, Nikabadi S, Varma A. Medicinal plants and environmental challenges. Springer; 2017. p. 1-24.
16. Syamsunarno MRAA, Safitri R, Kamisah Y. Protective effects of *Caesalpinia sappan* Linn. and its bioactive compounds on cardiovascular organs. Front Pharmacol. 2021;12:1-14.
17. Massada Y. Analysis of essential oil by gas chromatography and spectrometry. Amsterdam: Wiley; 1976.
18. Matthew KM. Flora of Tamil Nadu Carnatic. Tiruchirapalli: Rapinat Herbarium, St. Joseph's College; 1981-1983.
19. Rajput MS, Nirmal NP, Nirmal SJ, Santivarangkna C. Bio-actives from *Caesalpinia sappan* L.: recent advancements in phytochemistry and pharmacology. S Afr J Bot. 2022;151:60-74.
20. Hamdy N. Exploring the anti-inflammatory potential of the polyphenolic compounds in *Moringa oleifera* leaf: in silico molecular docking. Egypt J Basic Appl Sci. 2024;11:367-385.
21. Prejeena V, Suresh SN, Varsha V. Preliminary phytochemical screening, antimicrobial and GC-MS analysis of *Costus pictus* D DON. Int J Recent Adv Multidiscip Res. 2016;3(12):2092-2099.
22. Saravanakumar S, Chandra HJ. Screening of antimicrobial activity and phytochemical analysis of *Caesalpinia sappan* L. J Chem Pharm Res. 2013;5(2):171-175.
23. Trease GE, Evans IC. Pharmacognosy. 12th ed. London: Bailliere Tindall; 1983. p. 21-22.
24. Wallis TE. Textbook of pharmacognosy. New Delhi: CBS Publishers and Distributors; 1985. p. 5.
25. Yuniati Y, Azmi DD, Nurandriea E, Qadariyah L, Mahfud. Parametric study and characterization of sappan wood (*Caesalpinia sappan* Linn.) natural red colorant extract with ultrasonic assisted extraction method. ASEAN J Chem Eng. 2023;23(1):103-112.
26. Zanin B, de Carvalho A, Martineli PS, dos Santos MH, Lago JH, Sartorelli P, Viegas C, Soares M. The genus *Caesalpinia* L. (Caesalpiniaceae): phytochemical and pharmacological characteristics. Molecules. 2012;17:7887-7902.