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Deciphering the bioactive potential of *Monosis travancorica*: Phytochemical profiling, Drug-likeness insights, and Cyclooxygenase-2 targeting docking studies

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Bioactive compounds have long been recognized for their therapeutic applications, including curative potential, disease management and drug discovery. This study investigates standard techniques, such as Gas Chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR), and *in silico* analysis, to characterize the phytochemical richness and potential active constituents of the underexplored medicinal plant *Monosis travancorica*. The results indicated that the qualitative phytochemical profiling exhibited various phytoconstituents present in the extracts. The quantitative estimation demonstrated that the ethanolic extract contains an abundant amount of steroids, followed by flavonoids and phenolic compounds. The FTIR profiling revealed thirteen functional groups, while the GC-MS analysis identified twenty-five bioactive compounds, including lauric acid methyl ester, eugenol, 8-pentadecanone, cis-11-eicosenoic acid, vitamin A palmitate, and sabinene, as major compounds. The *in vitro* anti-inflammatory activity, as assessed through albumin denaturation and heat-induced hemolysis assays, shows significant potential in a dose-dependent manner, with the drug-likeness and pharmacokinetic traits of the compounds aligning with ADMET properties. Furthermore, the docking analysis targeting cyclooxygenase-2 (*COX-2*) demonstrated remarkable binding energy towards the proteins. Overall, these analyses suggest that *M. travancorica* holds the most promise as a potential bioactivity and warrants further investigation.

Keywords: Active compounds, Bioavailability, Computational analysis, *Monosis travancorica*

Medicinal plants synthesize a variety of chemical phytoconstituents that play a significant role in their therapeutic benefits. This effectiveness depends on the present traditional knowledge of taxonomic characteristics, plant parts, and their biological efficacy, which is based on the occurrence of primary and secondary metabolites[1]. Secondary metabolites are a basic source for the implementation of various pharmaceutical industries due to their immense medicinal benefits. Rather than contributing directly to beneficial activities, these serve as defensive mechanisms that stimulate the physiological pathways[2]. Fluctuations in identifying the active components among plants, including unprecedented classes of bioactive compounds and imperative trace elements, underscore their wide spectrum of pharmacological properties. Additionally, the components obtained from plants retain extensive medicinal effectiveness in the management of diseases, including diabetes, cancer, inflammation, cardiovascular, and neurological disorders[3]. Advanced instrumentation techniques have made numerous contributions to drug characterization and the discovery of novel therapeutic components in pharmacotherapy.

Inflammation is an intricate physiological condition regulated by the immune system, functioning as a protective mechanism against diverse noxious stimuli, including damaged cells, pathogens, toxic compounds, and various inflammatory processes, that may lead to severe conditions[4]. Cyclooxygenase (*COX*) enzymes are crucial in the production of prostaglandins, which regulate fever, inflammation, pain, and other homeostatic conditions. These enzymes are classified into two types: *COX-1* and *COX-2*. *COX-1* is constitutively expressed and is responsible for the production of prostanoids involved in physiological homeostasis, including gastric mucosal protection and platelet aggregation. In contrast, *COX-2* is highly engaged in inflammation-induced pain and prostaglandin production in inflammatory cells and the central

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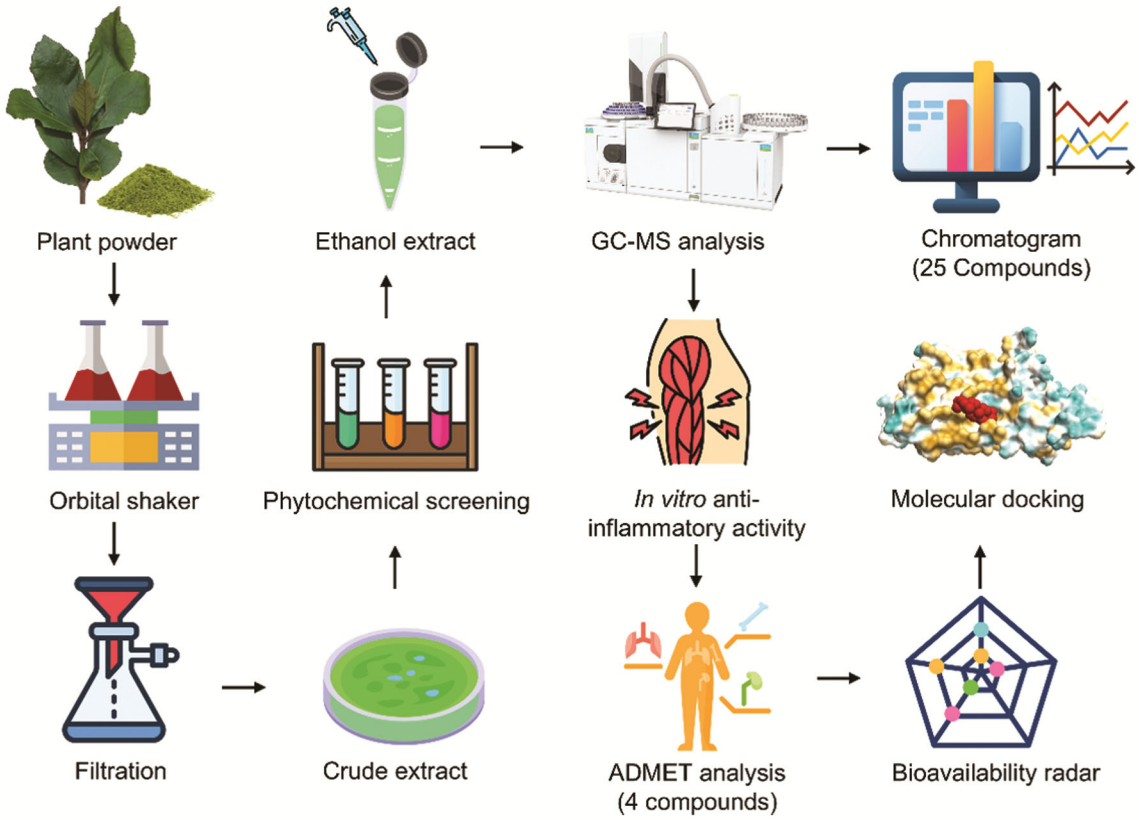

Graphical abstract

nervous system. While inhibiting *COX-1* reduces inflammation, it also undermines the gastric protection, increasing the risk of ulcers and gastrointestinal bleeding. *COX-2* inhibitors such as celecoxib and rofecoxib target inflammation with fewer gastrointestinal side effects and minimal risk of peptic ulceration[5]. Due to this specificity, *COX-2* inhibitors are an effective medication for regulating inflammation and its related symptoms.

Monosis travancorica (Hook.f.) H. Rob. & Skvarla (Synonym: *Vernonia travancorica* Hook.f.) is a small endemic tree from the family Asteraceae, native to the Western Ghats of India. It has elliptic-ovate leaves that are smooth and pubescent along the nerves and can grow up to 8 meters. The tree has whitish-purple flowers in terminal corymbose panicles (Fig. 1). It thrives in high-altitude montane forests, specifically in sheltered, moist areas at elevations ranging from 1000 to 2000 masl[6]. The beneficial potential of different plant parts is ascribed to the presence of numerous types of bioactive phytochemicals such as flavonoids, phenolics, alkaloids, terpenoids, steroids and volatile constituents. These chemical substances combine to enhance the medicinal productivity and

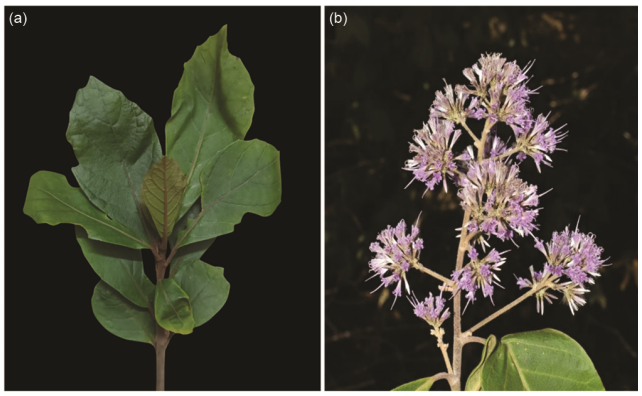

Fig. 1 — Habitat of *M. travancorica*. (a) leaf; and (b) inflorescence

pharmacological advantages of plants[7]. The most volatile entities comprise elongated unsaturated fatty acids, which play an important role in biological systems as fundamental building blocks for a diverse range of beneficial substances and energy storage mechanisms. These fatty acids are essential for the structural and functional integrity of cellular membranes, while also acting as precursors of a number of metabolic pathways[8]. Over the past decade, analytical techniques, including ultraviolet (UV) spectroscopy, FTIR, GC–MS analysis and nuclear

magnetic resonance (NMR) spectroscopy, have made significant progress in detecting and comprehending phytochemical structures[9].

Hence, GC-MS analysis was employed to characterize bioactive constituents of the *M. travancorica* ethanolic leaf extract, representing the first such report for this species. Additionally, *in vitro* anti-inflammatory, *in silico* methods, including ADMET profiling, are employed to characterize the physicochemical features, pharmaceutical potential and pharmacokinetic parameters of the predominant compounds. The molecular docking assessment further evaluates the binding interactions and stability of these key compounds with the *COX-2* protein, assessing their potential anti-inflammatory property.

Materials and Methods

Sample collection and preparation

The well-grown leaves of *M. travancorica* were painstakingly gathered from the Megamalai hills, the Western Ghats, situated in the Theni district, Tamil Nadu, India (coordinates: 9.59°N latitude and 76.28°E longitude). The collected sample was authenticated by the Botanical Survey of India, Southern Regional Centre, Coimbatore (vide no: BSI/SRC/5/23/2023-24/Tech-78). Then, the leaves were meticulously cleaned, shade-dried and ground into coarse powder using a Willy Mill to 50-60 mesh size. The resultant powder was safely kept in an air-tight container for further analysis.

Preparation of crude extract

Thirty grams of fine plant powder were extracted with successive solvents *viz*., petroleum ether, ethyl acetate and ethanol, using a Soxhlet apparatus and the residues were subjected to cold maceration with aqueous. The extracts were then concentrated to dryness under low pressure using a rotary vacuum evaporator after being filtered through Whatman No. 1 filter paper. The solvent was totally evaporated to dryness, and the resultant extract was carefully stored at 4°C in a sealed container for further usage[10].

Qualitative Phytochemical Analysis

To identify the various classes of bioactive chemical components present in the leaves of *M. travancorica*, four freshly obtained crude extracts were subjected to qualitative phytochemical screening using standard techniques[11].

Quantitative Estimation of Phytochemicals

By employing the spectrophotometric technique, the total flavonoid content was ascertained and represented as rutin equivalents (milligrams RE)/g extract[12]. The Folin-Ciocalteu technique was used to determine the total phenolic and tannin contents; phenolics were expressed as gallic acid equivalents (milligrams GAE)/g extract. The condensed tannins were determined by removing the free phenols in a total estimation. The estimation of total saponin content was performed using the standard protocol, such as the vanillin-sulphuric acid method[13], and expressed as diosgenin equivalents (milligrams DE)/g extract. The estimation of total steroids was obtained using the technique[14] and stated as cholesterol equivalents (milligrams CL)/g extract.

Spectroscopic Analysis by FTIR

FTIR spectra were attained using a Nicolet iS50 R spectrometer coupled with OMNIC software, and the attenuated total reflection (ATR) accessory permitted the sample to be in a liquid state and a single drop. Using the deuterated l-alanine-doped triglycine sulphate (DLaTGS) detector, the wavelength of the FTIR band was measured in a spectral range of 4000-400 cm^{-1}. The sample was scanned with a gold-coated Vectra-Piezo interferometer[16].

GC–MS analysis

The ethanolic extract of *M. travancorica* was analyzed using a Perkin Elmer Clarus SQ8C, and the sample introduction was preset in splitless mode. The ratio was set to 1:50. A DB-35 mass spectrometry nonpolar column, featuring dimensions of 0.25 mm outer diameter × 0.25 µm inner diameter × 30 meters in length, was sourced from Agilent Technologies, USA. Ultra-high-purity helium served as the carrier gas, flowing at a steady 1.0 mL/min rate. Mass spectra were analyzed within a range of 50 to 650 Da. The ionization source was kept at 200°C and a vacuum pressure of 40 mTorr. The ionization was performed at an energy level of 70 eV. The raw samples were diluted with a suitable solvent at a 1/100 (*v/v*) ratio and–filtered. A 1 µL aliquot of the filtered diluted extract was drawn into a syringe and introduced into the injector with a split ratio of 30:1. The relative abundance of the components in the filtered extract was determined through peak area normalization, expressed as a percentage. The mass spectrum also has an inbuilt prefilter that reduces the number of neutral particles[17].

Identification of bioactive compounds

The mass spectral data from the GC-MS analysis were decoded using a data management system equipped with dual integrated libraries: National Institute of Standards and Technology version 4 and WILEY version 9, each housing over five million references for spectrum querying and comparison. Compounds with spectral matching scores of 700 or higher were considered confidently identified. The identification of the test material's constituents, including their names, molecular weights, and structures, was achieved without the use of pure standards[18]. The biological activities were sourced from Dr. Duke's phytochemical and ethnobotanical database (https://phytochem.nal.usda.gov). The significant phytocompounds were discriminated and mapped to their corresponding metabolic pathways using MetaboAnalyst 6.0.

***In vitro* Anti-inflammatory activity**

Albumin denaturation assay

The albumin denaturation assay for ethanolic leaf extract of *M. travancorica* was carried out according to the standard protocol described by[4], with a slight modification. Different concentrations of sample aliquots were mixed with 3 mL of 5% aqueous bovine serum albumin. The reaction mixture was incubated at 30°C for 20 min and then heated to 50°C for 20 min. After being allowed to reach room temperature, the optical density values at 660 nm were measured. The experiment was performed in triplicate, and the percent inhibition of albumin denaturation was calculated as follows:

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of sample})} \times 100$$

Heat-induced hemolysis analysis

The red blood cell (RBC) suspension (10% *v/v*) was prepared in normal saline following the procedure[4], with slight modifications. A 3 mL RBC solution was mixed with various concentrations of the sample, then heated at 50ºC for 30 min and allowed to cool to room temperature. The samples were centrifuged at 3000 rpm for 10 min, and the supernatant was collected. The optical density values of the collected supernatant were measured at 560 nm. In both assays, aspirin was used as a standard.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of sample})} \times 100$$

***In silico* ADME Profiling**

The physicochemical attributes, lipophilic tendencies, water solubility, drug-like potential, and pharmacokinetic characteristics of major phytochemicals identified through GC-MS analysis for the ethanolic extract were evaluated using the SwissADME online platform (http://www.swissadme.ch/). This platform, a leading tool for virtual bioactivity screening, was employed to predict ADMET parameters (absorption, distribution, metabolism, excretion, and toxicity), along with other pharmacologically relevant properties[19].

Molecular docking for the *in silico* confirmation

For molecular docking analysis, the cyclooxygenase-2 (COX-2) structure bound to Ibuprofen (PDB ID: 4PH9) was retrieved from the Protein Data Bank (PDB)[20]. Using the Molegro Molecular Viewer (MMV) software, non-essential water molecules and heteroatoms were meticulously removed, and the refined structure was saved in PDB format. The active site, including the binding pocket area and volume, was identified using the CASTp server[21]. To ensure structural reliability, the protein's stereochemical integrity was validated *via* the PROCHECK server[22]. Ligand structures were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Molecular docking analysis was conducted using PyRx 0.8 software and the results were comprehensively visualized through Discovery Studio 2024, which provided precise two-dimensional and three-dimensional interaction structures[23].

Results and Discussion

Qualitative and Quantitative Phytochemical Screening

The qualitative phytochemical screening of *M. travancorica* leaf extracts revealed the presence of alkaloids, carbohydrates, glycosides, proteins, saponins, coumarins, chalcones, quinones, steroids, terpenoids, flavonoids, phenols, tannins, and acidic compounds. At the same time, fixed oils and fats were absent in all solvent extracts (Table 1). The total flavonoid content, expressed in terms of GAE, was highest in ethanolic extracts, followed by aqueous and ethyl acetate extracts, with the lowest content in petroleum ether extracts, as calculated using the linear regression equation $y=0.0043x$ ($r^2=0.9628$). Similarly, the total phenolic content, expressed as RE, was maximal in ethanolic extracts, followed by aqueous and ethyl acetate extracts, and minimal in petroleum

Table 1 — Results of qualitative phytochemical screening of various extracts of *M. travancorica* leaves

Groups	Test	Petroleum ether	Ethyl acetate	Ethanol	Aqueous
Alkaloids	Wagner's test	◐	●	◐	●
	Dragendroff's test	○	●	■	◐
	Tannic acid test	●	◐	●	○
Carbohydrates	Barfoed's test	○	●	●	●
	Molisch test	◐	◐	●	○
	Fehling's test	○	■	■	■
	Silver mirror test	○	◐	■	◐
Glycosides	Borntrager's test	●	○	○	○
	Baljet reagent test	○	○	■	◐
	Keller-kiliani test	◐	■	■	●
Protein	Biuret test	○	○	○	○
	Millon's test	○	○	●	○
	Ninhydrin test	○	○	○	○
	Xanthoproteic test	○	◐	◐	◐
Saponin	Frothing test	●	○	○	◐
Coumarins	NaOH test	○	◐	■	◐
Chalcones	NH_3OH test	○	◐	■	●
Phytosterols & Terpenoids	Libermann-Burchard test	◐	◐	■	○
	Salkowski test	■	◐	◐	○
	Copper acetate test (diterpenes)	○	○	◐	●
Phenols & Tannins	Gelatin test	○	○	○	○
	Braymer's test	○	◐	■	◐
	Lead acetate test	●	◐	■	●
Flavonoids	Shinoda's test	○	■	◐	○
	Alkaline reagent test	●	●	■	◐
Flavonols & Flavones	$AlCl_3$ test	◐	◐	■	●
Fixed oils & Fats	Saponification test	○	○	○	○
Quinones	HCl test	○	○	◐	○
	H_2SO_4 test	●	◐	■	●
Acidic compounds	Carboxylic acid test	○	○	●	○

*Note: (■) High; (◐) Moderate; (●) Low; (○) Absent

ether extracts, calculated using y=0.0181x (r^2=0.9751). The tannin content, expressed as GAE equivalents, was also highest in ethanolic extracts, followed by aqueous and ethyl acetate extracts, with the lowest in petroleum ether extracts. The saponin content, expressed as DE equivalents, was greater in petroleum ether extracts, followed by aqueous and ethanol extracts, with the lowest in ethyl acetate extracts, calculated using y=0.0073x (r^2=0.9888). Lastly, the total saponin content, expressed as CE equivalents, was most abundant in ethanolic extracts, followed by ethyl acetate and aqueous extracts, with petroleum ether extracts showing the least content, as calculated using y=0.0227x (r^2=0.9445) (Table 2). Aliyu *et al*[24] reported the presence of various phytochemicals in the aerial parts of *Vernonia ambigua, V. blumeoides, V. oocephala*. Plant metabolites, such as pigments, serve as secondary metabolites that attract pollinators and defend against herbivores. Although not essential for plant growth and development, flavonoids often play an important role in defense, UV filtration, signaling, and detoxification[25]. Known for their antioxidant capabilities, phenols and tannins have significant potential to prevent infection by acting as metal chelators under extreme stress from heavy metals[26]. Saponins predominantly inhibit the development and reproduction of cancer cells by interfering with their cholesterol-rich membranes[27]. The steroids obtained from the medicinal plants exhibit exceptional applications in drug discovery and management[28]. In the present study, the quantitative estimation of steroidal content manifested a higher level in the ethanolic extract. Recent studies indicate that the steroidal compounds and their derivatives exhibit significant antimicrobial and antidiabetic activity in the *Vernonia amygdalina* extract[29].

Functional Groups Identification

Based on the qualitative and quantitative phytochemical analysis, the ethanol extract was the most effective choice for further investigation due to its substantial amount of phytochemical contents than the other three solvents. The FTIR spectrum results identify the presence of thirteen distinct bands corresponding to their various functional groups, as described in (Table 3 and Fig. 2). The band absorption at 2970 and 2885 is due to the O-H and C-H stretching of the alkane and carboxylic acids present in the extract. Between 2500 and 1500 frequencies, no peaks are formed. Due to the presence of an amine group and a nitro compound, the peaks at 1387 and 1327 cm^{-1} correspond to the N-O and Ar-N stretching vibrations. The peaks at 1273, 1087, and 1049 indicate the presence of the C-O stretch responsible for the ethers, esters and alcohols. The bands at

Table 2 — Quantitative analysis for various extracts of *M. travancorica* leaves

Crude extract	Petroleum ether	Ethyl acetate	Ethanol	Aqueous
Total flavonoid (mgRE/g)	10.09±0.1	13.22±0.5	44.15±1.3	14.91±0.8
Total phenol (mgGAE/g)	5.00±0.3	21.54±0.2	48.80±3.3	22.54±1.2
Tannin (mgGAE/g)	1.02±0.1	15.81±0.7	39.78±4.1	16.47±1.6
Total saponin (mgDE/g)	63.37±3.2	14.24±4.9	18.63±5.6	25.84±0.8
Total steroid (mgCE/g)	7.1±0.2	51.39±2.1	109.1±2.7	47.04±1.1

*Note: Values are mean ± SD of three independent experiments

Table 3 — FTIR peak values and functional groups of ethanolic extract of *M. travancorica*

S. No.	Frequency (cm^{-1})	Class	Structure	Intensity	Chemical bond details
1.	2970.38	Alkanes	RCH_2CH_3	strong	CH stretch
2.	2885.51	Carboxylic acids	RCO-OH	s (broad)	dimer OH
3.	1381.03	Nitriles	N - O nitro compound	s (broad)	Aliphatic nitro
4.	1327.03	Amines	Ar_2NH	strong	Ar-N stretch
5.	1273.02	Esters	RCOOR	strong	C-O stretch
6.	1087.85	Ethers	R-O-R	strong	C-O stretch
7.	1049.28	Alcohols	RCH_2OH	strong	C-O stretch
8.	879.54	Amines	RNH_2, R_2NH	s (broad)	N-H wag amines
9.	802.39	Aromatics	1,2,3,4 – tetrasubstituted	medium	C-H out of a plane
10.	601.79	Alkynes	RC≡CH	strong	C-H bend
11.	563.21	Alkyl halides	R-Br	strong	C-Br stretch
12.	509.21	Miscellaneous	S-S disulfide	weak	S-S disulfide asymmetric
13.	455.21	Alkyl halides	R-Br	strong	C-Br stretch

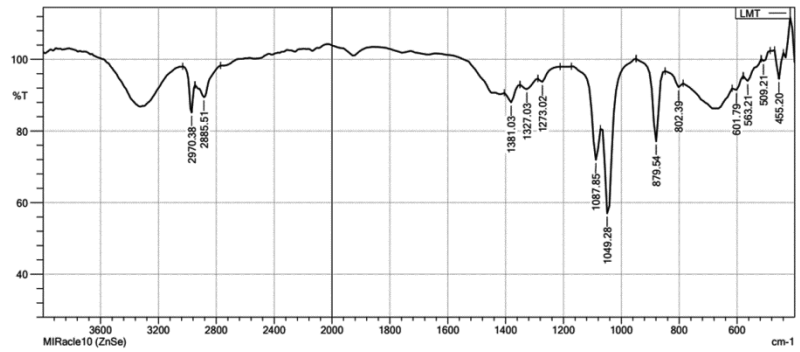

Fig. 2 — FTIR analysis of ethanolic extract of *M. travancorica*

879 and 802 represented N-H wagging from amines and C-H out-of-plane bending from aromatics. Alkynes and miscellaneous compounds exhibit C-H bending and S-S disulfide asymmetric stretching, as seen by the band formation at 601 and 509. The peaks at 563 and 455 are the detection of the C-Br stretch due to alkyl halides.

GC–MS analysis

The GC–MS chromatogram identified twenty-five compounds present in the *M. travancorica* leaf ethanolic extract (Fig. 3), and the details of peak area, retention duration, and the compounds' chemical classification are summarized in (Tables 4 & 5). The phytocompounds associated with biological characteristics are presented in (Table 6). The major peak area of bioactive compounds includes lauric acid methyl ester (2.98%), followed by eugenol (2.56%), 8-pentadecanone (2.10%), cis-11-eicosenoic acid (1.76%), vitamin A palmitate (1.21%), and sabinene (1.10%). Pinacidil (0.11%), α- pinene (0.15%), and L-ascorbic acid, 6-octadecenoate (0.15%) were present at lower percentages. These identified constituents exert pivotal roles in a spectrum of pharmacological activities. The majority of the compounds come under the fatty acyl class, followed by phenol lipids, steroids, and steroid derivatives (Fig. 4), and these compounds were found to be associated with pathways (Fig. 5) like Ascorbate and aldarate metabolism, and cutin, suberin, wax

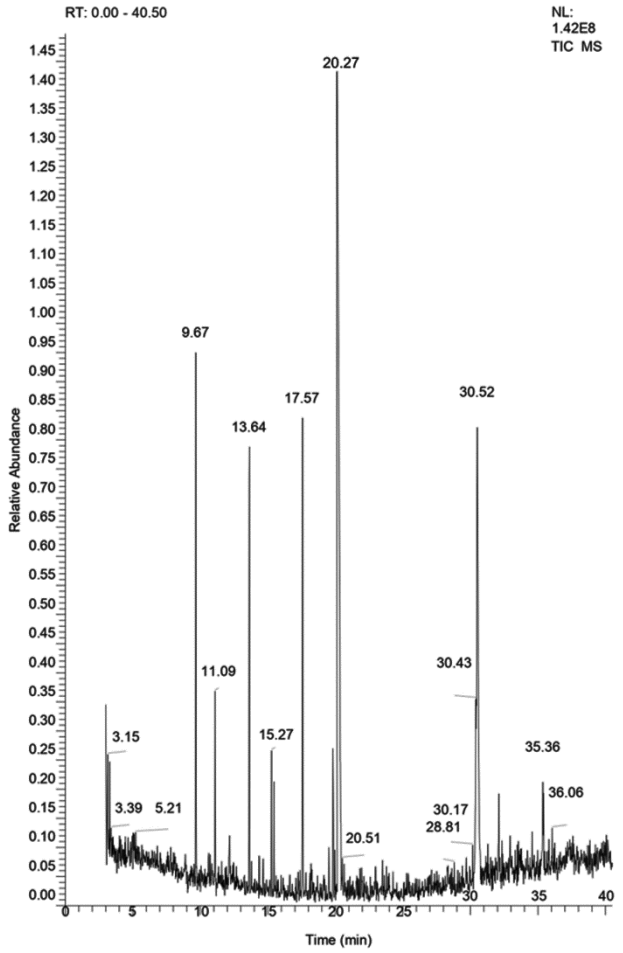

Fig. 3 — GC–MS chromatogram of the ethanolic leaf extract of *M. travancorica*

biosynthesis, majorly with the pathway impact of 0.13 and 0.12, respectively. Other identified compounds were associated with the phenylpropanoid biosynthesis, flavone and flavonoid biosynthesis, the biosynthesis of unsaturated fatty acids, and glutathione metabolism.

Among the identified compounds, lauric acid methyl ester, which is a saturated fatty acid, has anti-inflammatory, antioxidant, and antimicrobial properties[30] and is used in various applications, such as in the manufacture of lubricants, metalworking fluids, solvents, and oiling agents. Eugenol is a phenolic aromatic compound known for its multifaceted properties, including anti-inflammatory, anticancer, antimicrobial, and antioxidant effects[31]. Consequently, it has been extensively utilized across various domains, including cosmetology and pharmacology[32]. Pentadecanone compounds isolated from *Elipta alba* L., which belongs to the Asteraceae family, have shown efficacy against *Escherichia coli*, a bacterium associated with diarrhoeal illnesses, indicating their potential for antimicrobial usage[33]. Cis-11-eicosenoic acid or gondoic acid, which is primarily found in the oil-yielding plants and nuts. It is chiefly used as a moisturizing agent in the cosmetic industry, and the compound possesses potential anti-inflammatory activity in metabolic pathways, as well as antimicrobial and anti-psoriasis properties[34,35]. Vitamin A palmitate (retinyl palmitate) is predominantly used in eye-related disorders and is used to treat various dermatological diseases, including acne and wrinkles, and to enhance collagen production[36]. Sabinene (bicyclic monoterpene) exhibits antioxidant, antifungal, and angiogenic properties, suggesting its potential in novel drug development[37].

In vitro Anti-inflammatory Activity

The inhibitory effects of the ethanolic leaf extract of *M. travancorica* against albumin denaturation and heat-induced hemolysis were evaluated and are presented in (Table 7 and Fig. 6). The anti-albumin denaturation potential of the plant extract at different concentrations (50 to 1000 µg/mL) showed the highest inhibition at 1000 µg/mL, with 60.53% protection, whereas the lowest inhibition was observed at 25 µg/mL (7.57%). In comparison, the non-steroidal anti-inflammatory drug (aspirin), used as the standard, exhibited relatively higher inhibition of 92.36% and 94.58% at 750 and 1000 µg/mL, respectively. Similarly, in the heat-induced hemolysis assay, the plant extract demonstrated maximum inhibition at 1000 µg/mL (40.09%) and the lowest inhibition at 25 µg/mL (0.21%). In comparison, the standard drug aspirin showed higher inhibition at 750 and 1000 µg/mL, with values of 95.54% and 96.25%, respectively.

Denaturation of tissue proteins is a major consequence of inflammation-related illness, during which the protein loses its biological function. Such proteins act as an important marker for identifying the onset of inflammation[38,39]. In the present study, the inhibition of albumin denaturation and heat-induced protein denaturation was used as a measure of the anti-inflammatory activity for the ethanolic leaf extract of *M. travancorica*. The plant extract demonstrated a significant inhibitory property at doses ranging from 25 to 1000 µg/mL when compared to the standard drug, aspirin. The inhibitory impact is dose-dependent and the noted inhibition can be associated with the phytochemical elements present in the extract[40].

Table 4 — List of compounds identified through GC-MS analysis

S. No	Retention time	Name of the compound	Molecular formula	Molecular weight	Probability %	Peak area %
1	5.67	Alpha-Pinene	$C_{10}H_{16}$	136.00	2.74	0.15
2	9.67	Eugenol	$C_{10}H_{12}O_2$	164.00	30.89	2.56
3	11.09	Hexanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256.00	37.97	0.28
4	12.21	L-Ascorbic acid, 6- octadecanoate	$C_{24}H_{42}O_7$	442.00	2.44	0.15
5	13.04	Vitamin A palmitate	$C_{36}H_{60}O_2$	524.00	1.45	1.21
6	15.00	Oleic Acid	$C_{18}H_{34}O_2$	282.00	75.57	0.87
7	17.67	Cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310.00	10.14	1.76
8	19.82	Ferulic acid	$C_{10}H_{10}O_4$	194.00	22.56	0.51
9	19.97	Luteolin	$C_{15}H_{10}O_6$	286.00	26.66	0.54
10	20.27	Lauric acid methyl ester	$C_{13}H_{26}O_2$	214.00	29.75	2.98
11	20.51	Pentadecanoic acid methyl ester	$C_{16}H_{32}O_2$	256.00	19.17	0.71
12	21.89	Pinacidil	$C_{13}H_{19}N_5$	245.00	11.00	0.11
13	23.15	Gemcitabine	$C_9H_{11}F_2N_3O_4$	263.00	11.20	0.31
14	23.85	Cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.00	2.26	0.71
15	24.26	Stigmasterol	$C_{29}H_{48}O$	412.69	0.65	0.51
16	28.81	Ethyl iso-allocholate	$C_{27}H_{48}O_5$	452.70	0.35	0.62
17	30.17	Tetradecane	$C_{14}H_{30}$	198.39	0.53	0.98
18	30.43	Alpha-Methyl mannofuranoside	$C_7H_{14}O_6$	194.18	6.60	0.24
19	30.67	8-Pentadecanone	$C_{15}H_{30}O$	226.39	0.46	2.10
20	32.07	Pregn-5-en-20-one, 16,17-epoxy-3-hydroxy-,	$C_{21}H_{32}O$	344.49	0.46	0.78
21	33.17	Norgestrel	$C_{21}H_{28}O_2$	312.45	0.30	0.98
22	34.51	Phenol, 3-methyl-5-(1-methyl ethyl)-, methylcarbamate	$C_{12}H_{17}NO$	207.00	13.26	0.19
23	35.52	Sabinene	$C_{10}H_{16}$	136.00	16.86	1.10
24	36.00	Alpha-eudesmol	$C_{15}H_{26}O$	222.00	2.41	0.52
25	38.61	Cis-nerolidol	$C_{15}H_{26}O$	222.00	1.05	0.57

Table 5 — Chemical Classification of the compounds identified in GC-MS analysis

S. No	Name of the compound	CID	Chemical classification
1.	8-Pentadecanone	13162	Organooxygen compounds
2.	Alpha-Methyl mannofuranoside	143402	Organooxygen compounds
3.	Alpha-eudesmol	92762	Prenol lipids
4.	Cis-11-Eicosenoic acid	5282768	Fatty Acyls
5.	Cis-nerolidol	5320128	Prenol lipids
6.	Cis-Vaccenic acid	5282761	Fatty Acyls
7.	Ethyl iso-allocholate	6452096	Steroids and steroid derivatives
8.	Eugenol	3314	Phenols
9.	Ferulic acid	445858	Cinnamic acids and derivatives
10.	Gemcitabine	60750	Pyrimidine nucleosides
11.	Hexanoic acid, ethyl ester	31265	Fatty Acyls
12.	L-Ascorbic acid, 6-octadecanoate	54725318	Fatty Acyls
13.	Lauric acid methyl ester	8139	Fatty Acyls
14.	Luteolin	5280445	Flavonoids
15.	Norgestrel	13109	Steroids and steroid derivatives
16.	Oleic Acid	445639	Fatty Acyls
17.	Pentadecanoic acid metyl ester	23518	Fatty Acyls
18.	Phenol, 3-methyl-5- (1-methyl ethyl), methylcarbamate	4944	Phenol ethers
19.	Pinacidil	4826	Pyridines and derivatives
20.	Pregn-5-en-20-one, 16,17-epoxy-3- hydroxy	94199	Steroids and steroid derivatives
21.	Sabinene	18818	Prenol lipids
22.	Stigmasterol	5280794	Steroids and steroid derivatives
23.	Tetradecane	12389	Saturated hydrocarbons
24.	Vitamin A palmitate	5280531	Fatty Acyls
25.	Alpha-Pinene	6654	Prenol lipids

Table 6 — Biological activity of the identified compounds

S. No	List of the compounds	Therapeutics value
1	Alpha-Pinene	Treatment of bladder, kidney, and urinary stones, Antimicrobial, Anticoagulative, anti-inflammatory, Antitumour, Antioxidant, Gastroprotective and Neuroprotective activity
2	Eugenol	Antioxidant, antimicrobial, anesthetic, anti-inflammatory, neuroprotective, anti-diabetic, and anti-cancerous
3	Hexanoic acid, ethyl ester	Antimicrobial and Antioxidant; acts as a neurotransmitter in the central nervous system
4	L-Ascorbic acid, 6-octadecanoate	Antioxidant activity
5	Vitamin A palmitate	A fat-soluble form of vitamin C, antioxidant food additive
6	Oleic Acid	Antimicrobial, Anticarcinogenic, Antimalarials, Antineoplastics, Antipruritic, Anti-hypercholesterolemic, Anti-inflammatory, Antiseborrheic, Menopausal disorders treatment
7	Cis-11-Eicosenoic acid	Anti-inflammatory
8	Ferulic acid	Antioxidation and Anti-inflammation
9	Luteolin	Anti-inflammatory, anti-cardiovascular, anticancerous, and anti-neurodegenerative
10	Lauric acid methyl ester	Used mainly for the production of soaps and cosmetics
11	Pentadecanoic acid methyl ester	Antibacterial and antifungal
12	Pinacidil	Antihypertensive, cyanoguanidine drug, reduced blood pressure
13	Gemcitabine	Anticancer
14	Cis-Vaccenic acid	Anti-inflammatory
15	Stigmasterol	Antifungals, antiparasitic
16	Ethyl iso-allocholate	Anti-inflammatory and antitumor activities. Used in perfumery and as a food additive.
17	Tetradecane	Used in cosmetics - skin and hair care products
18	Alpha-Methyl mannofuranoside	Antimicrobial
19	8-Pentadecanone	Antioxidant, anti-inflammatory
20	Pregn-5-en-20-one, 16,17-epoxy-3-hydroxy	These steroid shots can protect the body from numerous ailments such as inflammation, pain, and swelling. Treatment of various kinds of cancers, such as leukemia and lymphoma. Dermatologists use cortisone eczema treatment and atopic dermatitis treatment
21	Norgestrel	Used as birth control pills and menopause hormone therapy
22	Phenol, 3-methyl-5- (1-methyl ethyl), methylcarbamate	Antimicrobial, anti-inflammatory, antioxidant
23	Sabinene	Antioxidant, antiangiogenic, antimicrobial, anti-inflammatory, antifungal
24	Alpha-eudesmol	Anti-inflammatory
25	Cis-nerolidol	Antimicrobial activity; used as a flavouring agent and in perfumery, as well as in non-cosmetic products such as detergents and cleansers

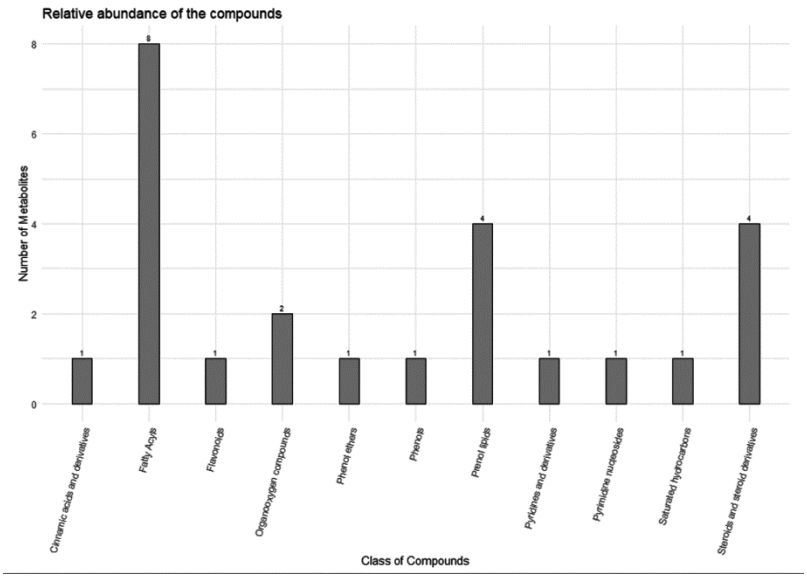

Fig. 4 — Relative abundance of identified compounds

ADMET analysis

The ADMET parameters provide vital information about the phytochemical's ability to convert into viable medicines. The present study investigated the pharmacokinetic and physicochemical characteristics, water solubility and toxicity assessments of four prevalent compounds such as lauric acid methyl ester, eugenol, 8-Pentadecanone, and sabinene. These components were evaluated through Swiss ADME and pkCSM servers; the physicochemical results are depicted in (Table 8).

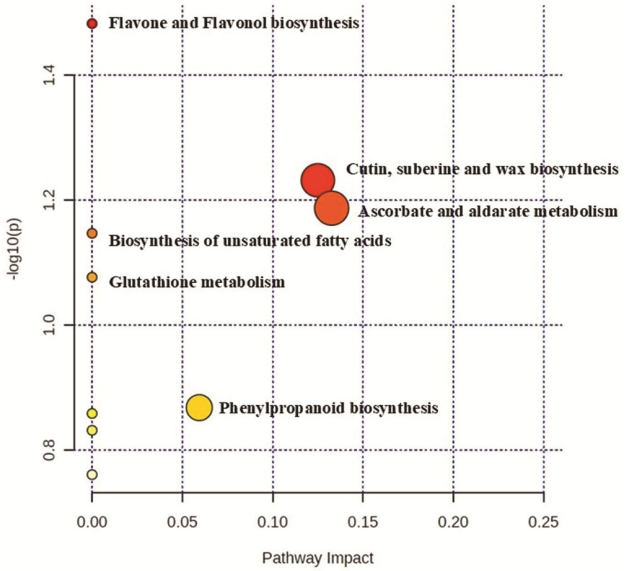

Fig. 5 — Pathway analysis

Table 7 — Effect of ethanolic leaf extract of *M. travancorica* on albumin denaturation and heat-induced hemolysis assay

Concentration (µg/mL)	Albumin denaturation assay (% of inhibition)		Heat-induced hemolysis assay (% of inhibition)	
	Aspirin	Ethanolic leaf extract	Aspirin	Ethanolic leaf extract
25	6.03 ± 0.35	7.57 ± 0.06	7.01 ± 0.81	0.21 ± 0.02
50	14.24 ± 1.4	11.76 ± 0.01	30.37 ± 0.53	2.53 ± 0.04
75	39.38 ± 2.11	14.62 ± 0.23	47.21 ± 0.32	6.77 ± 0.04
100	62.81 ± 1.06	23.89 ± 0.11	55.64 ± 0.55	14.23 ± 0.02
250	87.24 ± 0.22	41.31 ± 0.08	69.59 ± 0.18	21.59 ± 0.04
500	91.46 ± 0.43	45.84 ± 0.08	85.73 ± 0.31	23.71 ± 0.04
750	92.36 ± 0.04	50.43 ± 0.23	95.54 ± 0.56	28.28 ± 0.05
1000	94.58 ± 0.21	60.53 ± 0.09	96.25 ± 0.27	40.09 ± 0.02

*Values are expressed as mean ± SD (n=3)

Based on Lipinski's criteria, the drug component must satisfy at least two parameters designated as significant drug candidates[41]. In our study, upon analysis, the physicochemical properties of each compound adhered to Lipinski's rule, establishing them as potential drug-like molecules. Only sabinene shows a single violation in the partition coefficient aspect (MLOGP > 4.15) of the selected components; the remaining components show no violations of Lipinski's rule. A similar study was conducted on the *Vernonia amygdalina* compounds, which obey Lipinski's features, demonstrating good oral bioavailability[42]. The absorption criteria of the compounds were evaluated based on their lipophilicity, water solubility, and pharmacokinetic properties. The lipophilicity was examined by consensus logPo/w (partition coefficient between n-octanol and water) to determine how the compounds engage with water and fats. All the compounds possessed the logP values above two (logP > 2), suggesting high hydrophilic character, while lauric acid methyl ester and 8-pentadecanone exhibited the highest values (>5), displaying excessive hydrophilic nature. The water solubility of the compounds was analyzed using the three-parameter, including ESOL, Ali, and SILICOS-IT[43]. All four compounds exhibit high aqueous solubility (Log S between -2 to -4), and findings were presented in (Table 9).

In pharmacokinetic profiling, all compounds exhibit exceptional gastrointestinal absorption (GIA) and blood-brain barrier (BBB) permeability, except sabinene, which shows low intestinal absorption, but still has the ability to cross the BBB. The P-gp is an ATP-driven glycoprotein efflux carrier found in body tissues such as the kidney, liver, and BBB[38]. Among

Table 8 — Physicochemical and pharmacokinetic properties of bioactive compounds in *M. travancorica*

S. No	Compound name	Molecular weight (g/mol)	Lipinski	BBB	GIA	PGP	Log *P*o/w (XLOGP3)	TPSA (Å^2)	Fraction Csp3	Rotatable bonds
1	Lauric acid methyl ester	214.34	Yes	Yes	High	No	5.41	26.3	0.92	11
2	Eugenol	164.2	Yes	Yes	High	No	2.27	29.46	0.2	3
3	8-Pentadecanone	226.4	Yes	Yes	High	No	6.18	17.07	0.93	12
4	Sabinene	136.23	Yes	Yes	Low	No	3.09	0	0.8	1

*Note: Lipinski Compliance: 'Yes' indicates zero violations; BBB: 'Yes' suggests favorable blood-brain barrier permeability; GIA: 'High' denotes good absorption; PGP-: 'No' means no retention in CNS due to P-glycoprotein efflux; Lipophilicity: XLOGP3 between -0.7 and +5.0 is optimal; Polarity: TPSA between 20–130 Å² is ideal; Saturation: Csp3 fraction of at least 0.25 is favorable; Flexibility: No more than 9 rotatable bonds are preferred

Table 9 — Water Solubility of major phytochemical components of *M. travancorica*

S. No	Compounds	ESOL				Ali				SILICOS-IT			
		Log S (ESOL)	Solubility		Class	Log S (Ali)	Solubility		Class	Log S (SILICOS-IT)	Solubility		Class
			mg/mL	mol/L			mg/mL	mol/L			mg/mL	mol/L	
1.	Lauric acid methyl ester	-3.85	3.02E-02	1.41E-04	Soluble	-5.72	4.11E-04	1.92E-06	Moderately soluble	-4.4	8.57E-03	4.00E-05	Moderately soluble
2.	Eugenol	-2.46	5.69E-01	3.47E-03	Soluble	-2.53	4.90E-01	2.98E-03	Soluble	-2.79	2.65E-01	1.61E-03	Soluble
3.	8-Pentadecanone	-4.35	1.02E-02	4.52E-05	Moderately soluble	-6.32	1.08E-04	4.76E-07	Poorly soluble	-5.47	7.71E-04	3.40E-06	Moderately soluble
4.	Sabinene	-2.57	3.71E-01	2.72E-03	Soluble	-2.76	2.38E-01	1.75E-03	Soluble	-2.48	4.55E-01	3.34E-03	Soluble

*Note: Water Solubility: Log S up to -6 is acceptable

Table 10 — Cytochrome P450 attributes of bioactive constituents in *M. travancorica*

S. No	Compound name	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log *Kp* (skin permeation)	Bioavailability score
1	Lauric acid methyl ester	Yes	No	No	No	No	-3.77	0.55
2	Eugenol	Yes	No	No	No	No	-5.69	0.55
3	8-Pentadecanone	Yes	No	No	No	No	-3.29	0.55
4	Sabinene	No	No	No	No	No	-4.94	0.55

*Note: 'No' signifies the compound does not interact with cytochrome enzymes. 'Yes' indicates that the compound impedes cytochrome enzymes. Lower (more negative) log *Kp* values represent minimal skin permeability. The bioavailability score of 0.55 suggests good oral administration potential

the analyzed compounds, all exhibited favorable absorption potential and proficiency to pass through the BBB. The compounds' metabolism was appraised using the important Cytochromes (CYPs) P450 enzymes, including CYP1A2, CYP2C9, CYP2C9, CYP2D6, and CYP3A4. Hampering these enzymes may decrease the toxicity level of the compounds. Advantageously, all the compounds demonstrated no inhibition to those enzymes, except for the inhibition in CYP1A2. Additionally, the bioavailability radar suggests favorable oral bioavailability[45] and details were summarized in (Table 10) and (Fig. 7). Furthermore, the bioavailability score of 0.55 and the BOILED-Egg plot highlight the compounds' better intestinal absorption and BBB (Fig. 8). Toxicity assessment is very important in drug development. All the compounds showed non-mutagenicity, except for eugenol, but among the hepatotoxicity, all displayed non-toxicity (Table 11). They additionally possessed lower acute and chronic toxicity levels, with the LD_{50} higher than 2 mol/kg and above 1.5 mg/kg/day, suggesting the compounds' potential safety profile[46]. These findings indicate that the compounds' bioavailability, pharmacokinetics, and safety evaluation.

Molecular docking

The interaction between proteins and ligands is essential when developing novel drugs to treat various disorders[47]. In this study, the interaction between the

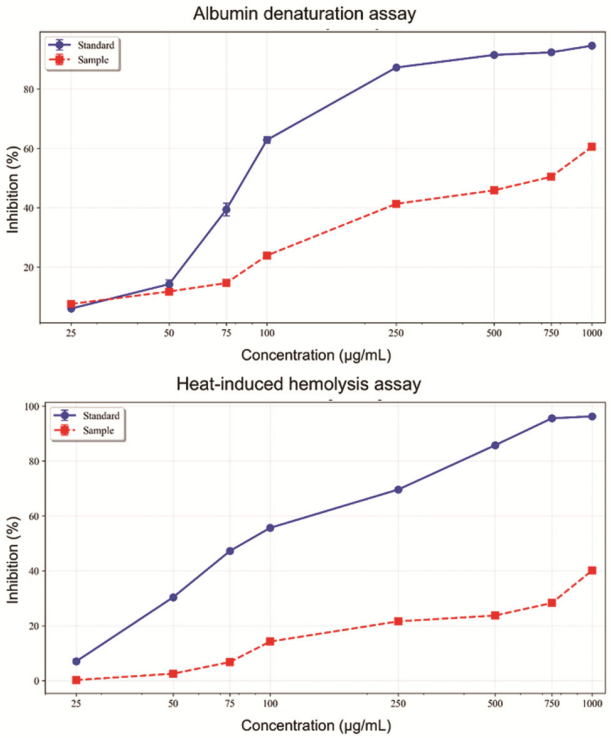

Fig. 6 — *In vitro* anti-inflammatory effect of *M. travancorica* leaf ethanolic extract

COX-2 protein and phytocompounds complements the anti-inflammatory property, offering molecular-level elucidation. The binding energy between a compound and its active binding region of the receptor is ascertained by examining hydrogen bonding

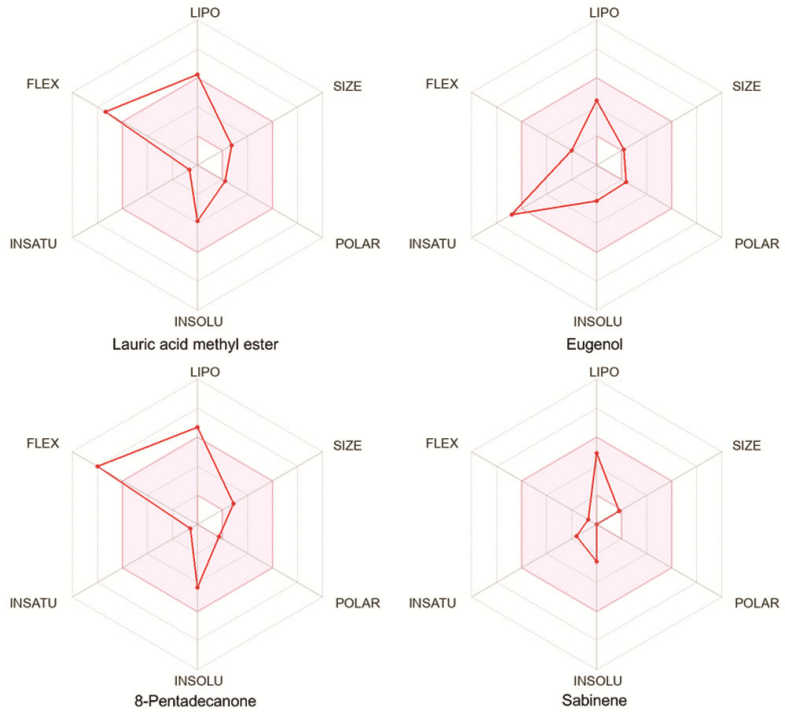

Fig. 7 — The bioavailability radar of selected phytochemical components

Table 11 — Toxicity prediction of *M. travancorica* bioactive compounds

S. No	Compound name	AMES toxicity	Maximum tolerated dose (Human) (Log mg/kg/day)	Oral Rat Acute Toxicity (LD_{50}) (mol/kg)	Oral Rat Chronic Toxicity (LOAEL) (Log mg/kg_bw/day)	Hepatotoxicity
1	Lauric acid methyl ester	No	0.351	1.661	2.707	No
2	Eugenol	Yes	1.024	2.118	2.049	No
3	8-Pentadecanone	No	0.421	1.495	1.128	No
4	Sabinene	No	0.369	1.549	2.309	No

*Note: AMES toxicity "Yes" indicates non-mutagenic properties; Higher maximum tolerated dose (human), acute toxicity (LD_{50}), and chronic toxicity (LOAEL) values suggest lower toxicity and better tolerability; Hepatotoxicity "No" implies the compound is unlikely to cause liver damage

attachment and its amino acid residues. In this context, the CASTp tool was used to analyze the active binding sites of the three-dimensional structure of the target protein, which were illustrated in (Fig. 9a). In 153 possible binding pockets were identified, with pocket 1 demonstrating the ideal ligand binding site, a volume of 3575.84 Å³ and an area of 4346.11 Å². These confirm that the target protein displayed significant structural integrity and possible active regions. Furthermore, the three-dimensional structure of the Ramachandran plot (Fig. 9b) was created to illustrate the conformational preferences of the proteins' backbone dihedral angles phi (φ) and psi (ψ) angles, across various amino acid residue types and a high-quality protein was determined by above 90% of its residues within the favored areas[48]. In the present study, the protein manifested promising structural stability such as 89.6% of residues lying within the maximum permissible areas and the non-permissible regions only 0.4%, further suggesting that the protein structure is precisely consistent and geometrically stable.

The docking results unveiled that the interaction between all the compounds and the target protein (PDB ID: 3TTZ) presented significant binding affinities, ranging from -5.08 to -7.57 kcal/mol. The findings were illustrated in (Fig. 10) and summarized in (Table 12). The lowest binding energy ligands indicate the most effective and strongest structural stability towards the target protein[49]. Remarkably, eugenol exhibited a robust binding affinity of -7.57 kcal/mol, binding to the amino acid residues THR 207, followed by the lauric acid

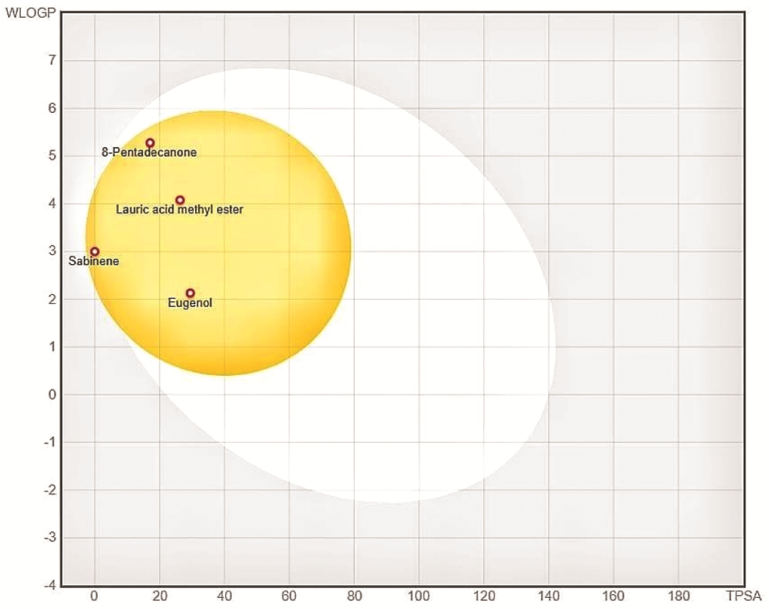

Fig. 8 — Boiled-egg graph of Gastrointestinal absorption and BBB permeability of the selected phytoconstituents

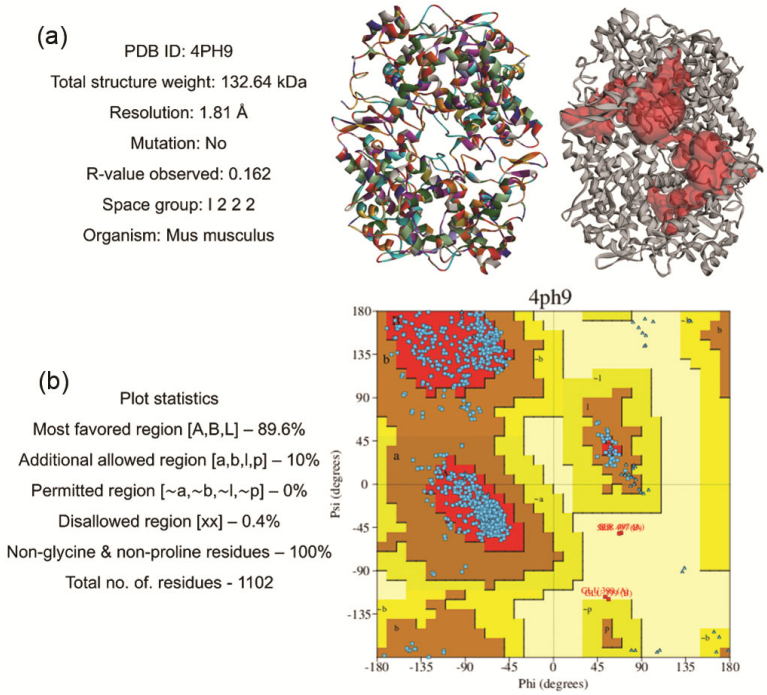

Fig. 9 — The 3D structure and key binding site analysis of the target protein using the Ramachandran plot. (a) Three-dimensional structure of protein; and (b) Active binding sites of the protein

Table 12 — Docking scores and amino acid interactions with the target protein

S. No	Compound name	Binding Affinity (kcal/mol)	No. of. Hydrogen bonds	Hydrogen bond interaction	Steric interaction
1	Lauric acid methyl ester	-6.18	1	ALA 200	ALA 203, TYR 386
2	Eugenol	-7.57	1	THR 207	TYR 386
3	8-pentadecanone	-5.08	1	-	GLY 45
4	Sabinene	-5.88	0	ASN 43, GLU 466, GLN 42	TRP 388, ALA 200

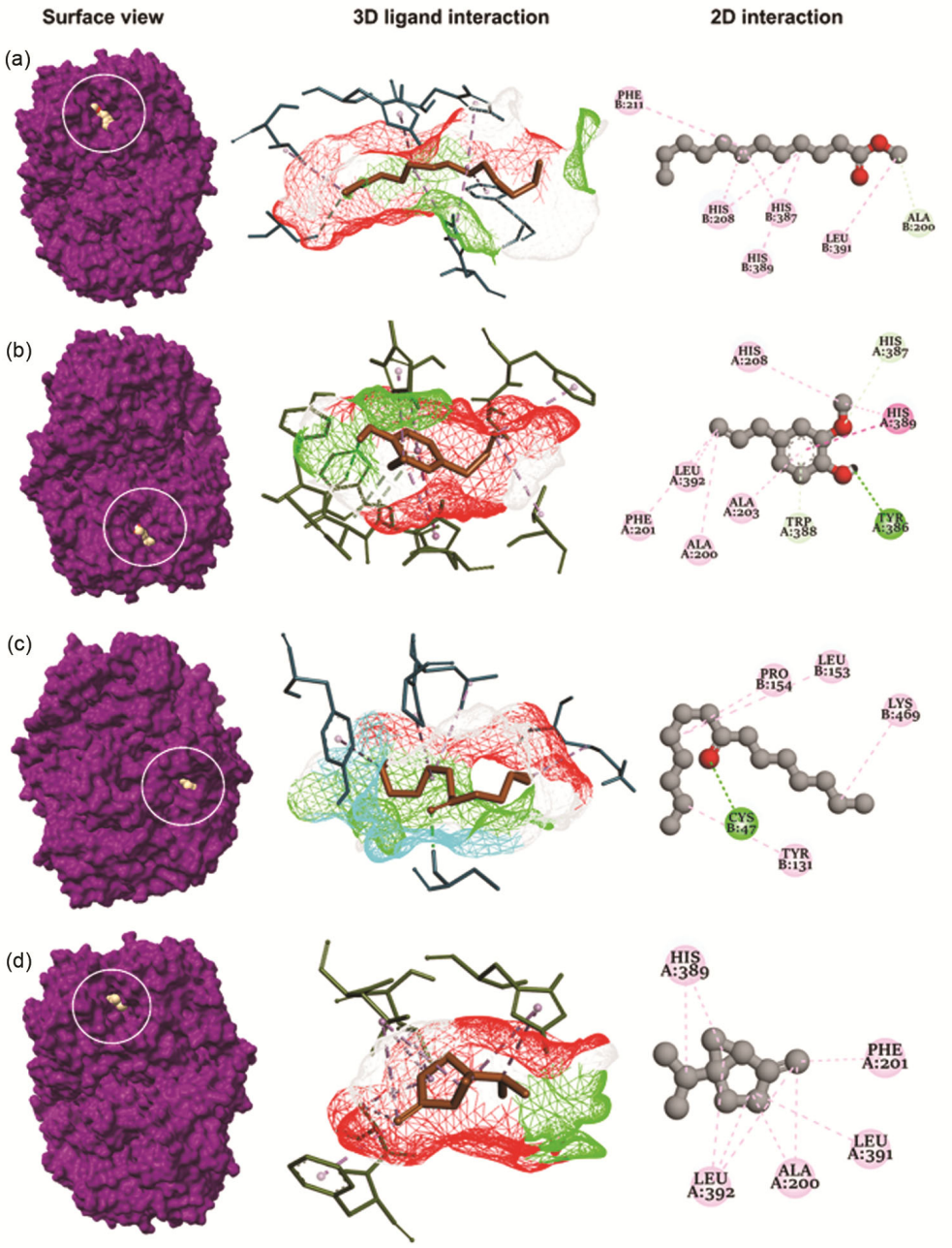

Fig. 10 — Interactions between the target protein (4PH9) and phytocompounds. (a) lauric acid methyl ester; (b) eugenol; and (c) 8-pentadecanone; and (d) sabinene

methyl ester, which manifested a moderate binding energy of -6.23 kcal/mol, associating with ALA 200. Furthermore, the sabinene has an energy of -6.18 kcal/mol and does not engage with any amino acids. Finally, the 8-pentadecanone exhibits a binding energy of -5.08 kcal/mol, with the interacting residues being ASN 43, ASN 466, and GLN 42. The hydrogen bonds are responsible for the major interactions between the ligand and protein, which possess the geometric and structural validity[50]. In our study, the value is below 3.6 Å, suggesting significant compatibility of the interactions.

Conclusion

The ethanolic *M. travancorica* leaf extract revealed a rich phytochemical composition, with GC-MS analysis classifying twenty-five active constituents. Noteworthy dose-dependent anti-inflammatory activity was observed *in vitro* through albumin denaturation and heat-induced

hemolysis. *In silico* ADME appraisal implied favorable drug-likeness and oral bioavailability of major constituents in accordance with Lipinski's Rule of Five. Molecular docking analysis underlined eugenol, lauric acid methyl ester, 8-pentadecanone and sabinene as promising anti-inflammatory leads for further validation.

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Conflict of interest

All authors declare no conflict of interest.

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