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Anti-Inflammatory Potential of *Anisomeles malabarica* (L.): Integrated Phytochemical, ADMET and Molecular Docking Analysis Targeting IL-6

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In this study, phytochemical analysis of *Anisomeles malabarica* (L.) led to the identification of 25 bioactive compounds. The presence of key secondary metabolites including flavonoids, alkaloids, terpenoids, tannins, steroids, anthraquinones and phenols was confirmed, highlighting the plant's rich pharmacological profile. Among various extracts tested, the ethanol extract exhibited the most significant anti-inflammatory activity, suggesting its potential for further pharmacological and therapeutic exploration. The bioactive compounds that satisfied Lipinski's rule of five were isolated and subjected to further evaluation. Their physico-chemical properties, toxicity profiles, and drug-likeness were predicted using *in silico* ADMET analysis tools. Molecular docking studies demonstrated strong binding affinities of several compounds toward Interleukin-6 (IL-6), a key pro-inflammatory cytokine implicated in the pathogenesis of rheumatoid arthritis, suggesting their potential as therapeutic agents for inflammatory disorders. When compared to control tofacitinib (-6.17 Kcal/mol), bioactive compounds such as dinaphthofuran (-9.48 Kcal/mol) and isoxazole[4,3-*a*]phenazine,1-phenyl (-9.07 Kcal/mol) have shown a substantial binding affinity to Interleukin-6, making them potentially useful for anti-inflammatory therapeutics. RBC membrane stabilization assays showed concentration-dependent protection against hypotonic-induced haemolysis, supporting the extract's ability to prevent cellular damage and inflammation. Overall, *A. malabarica* leaf extracts demonstrated significant anti-inflammatory potential, with dinaphthofuran and isoxazolo[4,3-*a*]phenazine,1-phenyl identified as lead candidates for further drug development targeting rheumatoid arthritis.

Keywords: *Anisomeles malabarica*, Rheumatoid arthritis, Anti-inflammatory, RBC stabilization assay, ADMET, Molecular docking.

INTRODUCTION

Herbal plants have historically served as the primary source of medicine and continue to play a significant role in traditional healthcare systems worldwide [1,2]. The plant compounds that have therapeutic value or that serve as building blocks for the production of helpful medications are considered medicinal plants [3]. Anti-inflammatory drugs play a crucial role in managing rheumatoid arthritis (RA) by alleviating joint inflammation, pain and tissue damage [4], a chronic autoimmune disorder characterized by the immune system's erroneous attack on synovial joints that leads to persistent inflammation, swelling and progressive joint degeneration [5]. Now-days, the development of new anti-inflammatory drugs is essential to address the adverse effects, resistance and limited selectivity associated with current treatments. In this context, plants-based drugs can provide targeted modulation of specific molecular pathways involved in the chronic inflammation [6,7].

Anisomeles malabarica (L.) is considered one of the most promising medicinal plants, traditionally passed down through generations for its therapeutic uses [1,8]. Recent studies have supported the efficacy of bioactive compounds isolated from *A. malabarica*, confirming its potential in modern medicinal applications [8-10]. The aim of this study was to investigate the phytochemical composition and anti-inflammatory potential of *A. malabarica* (L.) leaf extracts, identify bioactive compounds with drug-like properties and evaluate their therapeutic potential against inflammatory disorders, particularly rheumatoid arthritis through *in vitro* assays and *in silico* approaches including ADMET analysis and molecular docking against Interleukin-6.

EXPERIMENTAL

Plant collection and extraction sample: The plant *Anisomeles malabarica* was sourced from reclaimed lands and

forest areas located in Mazhavanthangal, India. A plant specimen was authenticated by Dr. Prabhakaran, a taxonomist, Department of Botany, Ramakrishna Mission Vivekananda College (Autonomous), Chennai, India. The dried leaf powder of *A. malabarica* was extracted using ethanol as solvent through the maceration method, where the sample was kept at 32 ºC for 24 h in an orbital shaker. Then the samples were filtered by Whattman No. 1 paper and dried the sample [11].

Preliminary analysis: *A. malabarica* leaf extract was subjected to the phytochemical screening to identify the biologically active components including flavonoids, phenols, steroids, terpenoids, anthraquinones and alkaloids using GC-MS [12].

Molecular properties and drug-likeness prediction: The study investigated the molecular characteristics of bioactive compounds identified in the ethanolic extract of *A. malabarica* (L.). To evaluate their drug-likeness, physico-chemical properties and ADMET profiles, *in silico* tools including the SwissDock platform and Lipinski's rule of five were employed. The Lipinski criteria serve as a predictive model for assessing oral bioavailability and include the following parameters: (i) no more than 5 hydrogen bond donors (–OH and –NH groups), (ii) no more than 10 hydrogen bond acceptors (N and O atoms), (iii) molecular weight less than 500 Da, (iv) calculated LogP not exceeding 5 and (v) a molar refractivity range between 40 and 130 [13,14].

Molecular docking: AutoDock4 was employed as the computational tool for protein-ligand docking simulations. A total of 25 predominant bioactive compounds, identified through GC-MS analysis of the ethanolic extract, were selected for molecular docking studies. These simulations aimed to evaluate the potential interactions between the selected phytochemicals and key therapeutic target proteins involved in inflammatory pathways. The docking procedures were carried out using AutoDock4 and the resulting protein-ligand complexes were analyzed and visualized using BIOVIA Discovery Studio Visualizer v21.1.0.20298 (BIOVIA, San Diego, CA, USA) [15].

Ligand preparation: The selected major bioactive compounds identified in the ethanolic extract, along with the reference drug tofacitinib (PubChem ID: 9926791), were retrieved from the PubChem database for further analysis. The corresponding 2D chemical structures of the compounds (PubChem IDs: 192762, 550070, 622697, 626689, 248040, 550401 and 550198) were utilized for molecular docking studies [16].

Protein preparation: The crystal structure of the target inflammatory protein, Interleukin-6 (IL-6), was also obtained from the Protein Data Bank (PDB ID: 00001ALU) *via* the RCSB PDB database [17]. The protein structure was prepared using the Protein Preparation Wizard integrated within AutoDock4, involving standard steps of preprocessing, optimization, and energy minimization based on established protocols. Docking simulations were conducted using Glide (version 4), a module of AutoDock, where receptor grids were generated with default parameters for each prepared protein structure. Flexible ligand docking was performed using the Glide-Standard Precision scoring method. This approach allowed assessment of potential interactions between the target protein and selected ligands without imposing rigid constraints, making it well-suited for virtual screening applications. Tofacitinib, an FDA-approved anti-inflammatory agent, was used as a reference ligand. Compounds were ranked based on their docking scores, with the most favourable interactions identified by the highest negative binding energy values [18].

RBC membrane stabilization assay: The membrane-stabilizing potential of the samples was evaluated using the human red blood cell (RBC) membrane stabilization assay. Fresh blood collected in Alsever's solution was centrifuged at 3000 rpm for 10 min and the packed RBCs were washed thrice with isotonic phosphate buffer (10 mM, pH 7.4). A 10% RBC suspension was prepared in the same buffer. The assay mixture consisted of 1 mL of RBC suspension, 1 mL of extract at various concentrations and 1 mL of hypotonic solution (distilled water). After incubation at 37 ºC for 1 h, the mixture was centrifuged at 3000 rpm for 10 min. The extent of hemolysis was determined by measuring the absorbance of the supernatant at 540 nm. The percentage of membrane stabilization was calculated by comparing the absorbance values of treated and control samples (eqn. 1) [19].

$$\text{Stabilization (\%)} = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100 \quad (1)$$

RESULTS AND DISCUSSION

Preliminary results: Phytochemical analysis of the ethanolic leaf extract of *A. malabarica* confirmed the presence of alkaloids, steroids, glycosides, saponins and flavonoids, all of which possess significant pharmacological activities (Table-1). Moreover, integration of modern extraction and analytical technologies can facilitate the efficient isolation and characterization of these bioactive constituents, supporting the development of novel therapeutic agents and drug lead compounds.

TABLE-1
IDENTIFICATION OF THE PHYTOCHEMICAL CONSTITUENTS FROM THE EXTRACTED MATERIALS

Name of the compound	Hexane	Acetone	Ethanol
Saponin	–	–	–
Terpenoids	–	–	++
Tannins	+	+	+
Steroids	–	–	++
Glycosides	–	–	–
Alkaloids	+	+	++
Flavonoids	–	+	+++
Anthraquinones	+	–	++
Phenol	+	+	++

(Presence indicates + and Absences indicate –)

GC-MS analysis: On the basis of comparison of the mass spectra with the NIST library, a total of 25 compounds were identified from the ethanolic extract of *A. malabarica*. Each compound was quantified according to its peak area percentage in the total ion chromatogram (Fig. 1). The major bioactive constituents identified, along with their relative abundances, are summarized in Table-2.

ADMET and drug likeness prediction: To evaluate the oral bioavailability and drug-likeness of the identified bioactive compounds from the ethanolic extract of *A. malabarica*,

TABLE-2
ISOLATED MAJOR COMPOUNDS IN THE ETHANOL EXTRACT OF LEAF OF *Anisomeles malabarica*

S. No.	Compound name	m.f.	RT (min)	Biological activity
1	S-Propylthio-l-cysteine	$C_6H_{13}NO_2S_2$	16.589	Blood pressure, antioxidant and anti-inflammatory
2	Methyl4-hydroxybutanoate	$C_5H_{10}O_3$	16.589	Antiviral, antiallergic
3	Hexanoic acid, 2-methyl	$C_7H_{14}O_2$	16.674	Perfumes, oil lubricants and friction agents
4	2,2,4-Trimethyl-3-hydroxy-n-valeronitrile	$C_5H_{10}O_3$	16.674	No activity
6	N-Hexadecanoic acid	$C_8H_{15}NO$	17.080	No activity
7	9,15-Octadecadienoic acid, methyl ester, (z,z)	$C_{19}H_{34}O_2$	18.240	Antioxidant, antimicrobial and anti-inflammatory
8	9,12-Octadecadienoic acid, methyl ester, (e,e)	$C_{19}H_{34}O_2$	18.335	Antioxidant, anticancer and anti-inflammatory
9	Methyl 11-oxo-9 undecenoate	$C_{12}H_{20}O_3$	18.400	Antifungal and antibacterial
10	Naphthalene, decahydro-1-pentadecyl	$C_{14}H_{28}O_2$	18.645	Antibacterial
11	Tridecanoic acid, methyl ester	$C_{16}H_{12}O_4$	22.662	Antioxidant, anticancer and anti-inflammatory
12	4*H*-1-Benzopyran-4-one,5-hydroxy-7-methoxy-2-phenyl	$C_{20}H_{120}O$	22.662	Anticancer, anti-inflammatory, antibacterial, anti-Alzheimer's disease, antioxidant,
13	Dinaphtho[2,1-*b*:1',2'-*d*]furan	$C_{19}H_{11}N_3O$	22.992	Anticancer, anti-inflammatory, antibacterial, anti-Alzheimer's disease, antioxidant, insecticidal antifungal and antidiabetic
14	Isoxazolo[4,3-*a*]phenazine,1-phenyl	$C_{20}H_{14}N_2O$	22.992	Anticancer, anti-inflammatory, antibacterial, antioxidant
15	Quinazolin-4(3*H*)-one, 2,3-diphenyl	$C_{11}H_{19}O_2Cl_3$	23.457	No activity
16	Acetic acid, trichloro-, nonyl ester	$C_{20}H_{13}NO_2$	23.522	Antiproliferative and anticancer
17	Benzamide, n-(9*H*-fluoren-9-on-1-yl)	$C_{17}H_{36}O$	24.963	Antimicrobial and anti-inflammatory
18	N-heptadecanol-1	$C_{27}H_{46}O_2$	25.268	Antimicrobial and anti-inflammatory
19	2*H*-1-Benzopyran-6-ol,3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltride	$C_{23}H_{46}$	26.353	Antimicrobial, antioxidant and anti-inflammatory
20	11-Tricosene	$C_{26}H_{54}O$	26.353	Anti-inflammatory, antioxidant, insecticidal
21	Hexacosanol	$C_6H_7N_5O$	26.503	Antiviral
22	2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-*c*)triazine	$C_{30}H_{48}O_2$	28.074	Anticancer
23	Ergosta-7,22-dien-3-ol, acetate, (3β,5α)	$C_{30}H_{48}O$	28.499	Antioxidant, antimicrobial antiproliferative
24	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a	$C_{17}H_{30}O_3$	28.99	Anticancer, anti-inflammatory and antibacterial
25	2r-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclo	$C_{15}H_{26}O_3$	28.99	Antibacterial, insecticidal and antioxidant

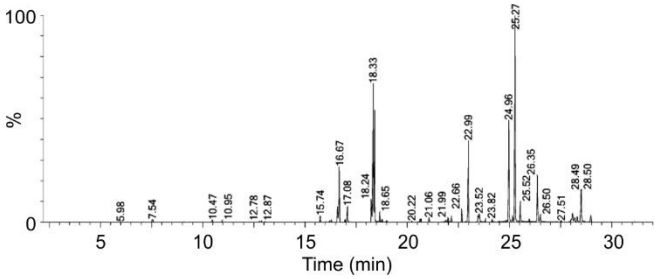

Fig. 1. GC-MS chromatogram ethanolic extract of *Anisomeles malabarica*

Lipinski's rule of five was applied. Compounds fulfilling these criteria are considered more likely to be effectively absorbed upon oral administration [12]. Compounds fulfilling the Lipinski's rule of five are more likely to be orally absorbed, as shown in Table-3.

The ADMET properties of 12 selected compounds were assessed *in silico* and the physico-chemical properties are shown in Table-4. The remaining three compounds, *e.g.* methyl 4-hydroxybutanoate, methyl 11-oxo-9-undecenoate and tridecanoic acid methyl ester, were excluded, as their key physico-chemical parameters, including C*sp*² atom count, number of rotatable bonds, molar refractivity and the number of heavy and aromatic atoms, fell outside the acceptable ranges. These compounds exhibited topological polar surface area (TPSA) values ranging from 0.00 to 55.62 Å^2 and XLogP3 values between 0.90 and 4.67. The solubility (Log S) values ranged from -2.06 to -5.51, indicating moderate to low aqueous solubility. The fraction of sp^3-hybridized carbon atoms (fraction Csp^3) varied from 5% to 30%, and the number of rotatable bonds ranged between 1 and 16.

Remarkably, a substantial number of these compounds showed favourable gastrointestinal absorption and efficient blood-brain barrier (BBB) permeability [20]. A bioavailability score of 0.55 was observed for most of the tested phytochemicals. Among them, 2-dinaphthofuran and isoxazolo[4,3-*a*]-phenazine,1-phenyl- demonstrated higher scores of 0.904 and 9.07, respectively, indicating strong oral bioavailability potential [21]. Regarding metabolic interactions, several compounds showed no significant inhibition of cytochrome P450 enzymes, particularly CYP2D6. However, compounds like quinazolin-4(3*H*)-one, 5-hydroxy-7-methoxy-2-phenyl and 4-hydroxy-1-benzopyran-4-one were found to inhibit CYP2D6 and CYP-3A4 enzymes, respectively.

Drug-likeness was further supported by favourable physicochemical parameters. Most of the compounds had molecular weights below 500 g/mol, which supports the efficient absorption, diffusion and transport across biological membranes [22]. The lipophilic nature of these molecules contributes to optimal solubility, selectivity and membrane permeability, all

TABLE-3
LIPINSKI RULE OF FIVE OF BIOACTIVE COMPOUNDS ISOLATED FROM ETHANOLIC EXTRACT OF *A. malabarica*

S. No.	Compound name	m.w.	H-Donor	H-Acceptor	LogP	Molar mass
1	S-Propylthio-l-cysteine	195	3	3	1.1897	50.818
2	Methyl 4-hydroxybutanoate	118	1	3	-0.0681	28.336
3	2,2,4-Trimethyl-3-hydroxy-n-valeronitrile	144	1	2	5.2874	40.941
4	Methyl 11-oxo-9-undecenoate	212	0	3	2.6452	59.538
5	Tridecanoic acid, methyl ester	284	0	2	4.8867	86.875
6	4*H*-1-Benzopyran-4-one,5-hydroxy-7-methoxy-2-phenyl	268	1	4	3.0169	74.036
7	Dinaphtho[2,1-*b*:1',2'-*d*]furan	268	0	1	4.4685	84.019
8	Isoxazolo[4,3-*a*]phenazine, 1-phenyl	297	0	4	4.5912	90.047
9	Quinazolin-4(3*H*)-one, 2,3-diphenyl	298	0	3	4.4253	92.332
10	Benzamide, n-(9*H*-fluoren-9-on-1-yl)	300	0	2	4.8894	93.501
11	2r-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexene	282	1	3	3.7092	81.299
12	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	222	1	1	4.0877	70.338

TABLE-4
PHYSIO-CHEMICAL PROPERTIES OF BIOACTIVE COMPOUNDS ISOLATED FROM ETHANOLIC EXTRACT OF *A. malabarica*

S. No.	Compound name	No of heavy atom	No of arom. heavy atom	Csp^3	Rotatable bonds	Molar refract-tivity	Bioavail-ability score
1	S-Propylthio-l-cysteine	11	0	0.83	6	50.62	0.55
2	2,2,4-Trimethyl-3-hydroxy-n-valeronitrile	10	0	0.88	2	41.22	0.85
3	4*H*-1-Benzopyran-4-one,5-hydroxy-7-methoxy-2-phenyl	20	16	0.06	2	76.44	0.55
4	Dinaphtho[2,1-*b*:1',2'-*d*]furan	21	21	0.00	0	88.73	0.55
5	Isoxazolo[4,3-*a*]phenazine,1-phenyl	23	23	0.00	1	90.05	0.55
6	Quinazolin-4(3*H*)-one, 2,3-diphenyl	23	22	0.00	2	92.78	0.55
7	Benzamide, n-(9*H*-fluoren-9-on-1-yl)	23	18	0.00	3	89.52	0.55
8	2r-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexene	20	0	0.82	5	83.72	0.55
9	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	16	0	0.73	3	72.06	0.55

of which are essential for effective drug delivery. Toxicity predictions indicated that the majority of the compounds could be safely administered orally, with minimal risk of adverse gastrointestinal effects [22]. Furthermore, approximately 85% of the compounds demonstrated the capability to cross the BBB, suggesting potential action in the central nervous system [23]. These results are presented in Table-5. Following the screening of nine compounds, two compounds *viz*. 4H-1-benzopyran-4-one,5-hydroxy-7-methoxy-2-phenyl and benzamide, N-(9H-fluoren-9-on-1-yl) were excluded due to failing the AMES toxicity test (Table-6) [24]. The remaining seven compounds were selected for docking studies. The skin, functioning as a selective barrier, also plays a critical role in transdermal delivery. Evaluation of skin permeability showed that the compounds were generally not well-suited for transdermal application. The predicted bioavailability values ranged between 0.55 and 0.85, aligning with ADMET predictions generated using the SwissADME platform. These findings reflect the overall pharmacokinetic behaviour the compounds including absorption, distribution, metabolism, excretion and toxicity,

TABLE-5
DRUG-LIKENESS SCORE OF BIOACTIVE COMPOUNDS ISOLATED FROM ETHANOLIC EXTRACT OF *A. malabarica*

S. No.	Compound name	PSA	TPSA	Mol LogS in Log (mol/L)	Volume (A^3)	Drug like ness score
1	S-Propylthio-l-cysteine	43.86	37.3	-2.84	262.08	4.25
2	2,2,4-Trimethyl-3-hydroxy-n-valeronitrile	33.25	44.02	-1.65	183.28	3.95
3	4*H*-1-Benzopyran-4-one,5-hydroxy-7-methoxy-2-phenyl	45.88	26.3	-4.13	270.89	4.27
4	Dinaphtho[2,1-*b*:1',2'-*d*]furan	37.61	25.63	-3.39	252.19	3.58
5	Isoxazolo[4,3-*a*]phenazine,1-phenyl	39.13	13.14	-5.28	273.37	3.90
6	Quinazolin-4(3*H*)-one, 2,3-diphenyl	24.74	34.89	-3.93	290.91	5.20
7	Benzamide, n-(9*H*-fluoren-9-on-1-yl)	35.79	46.63	-4.53	294.36	4.47
8	2r-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexene	36.99	46.52	-4.05	360.18	4.09
9	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	17.55	20.23	-4.73	305.54	4.37

TABLE-6
ADSORPTION DISTRIBUTION METABOLISM EXCRETION TOXICITY-PREDICTION FOR BIOACTIVE COMPOUNDS ISOLATED FROM ETHANOLIC EXTRACT OF *A. malabarica*

Compound name	Absorption				Distribution	
	Caco2 perm	Intestinal absorption	Skin perm	p-gP	VDss (human) (LogL/kg)	BBB perm
S-Propylthio-l-cysteine	0.8661	High	0.380842	No	56.37844	Yes
2,2,4-Trimethyl-3-hydroxy-n-valeronitrile	0.6471	High	0.591886	No	50.05816	Yes
Dinaphtho[2,1-*b*:1',2'-*d*]furan	0.6839	Low	0.8954	Yes	98.06126	No
Isoxazolo[4,3-*a*]phenazine,1-phenyl	0.6024	High	0.823526	Yes	44.24195	Yes
Quinazolin-4(3*H*)-one, 2,3-diphenyl	0.8029	High	0.418309	No	38.1931	Yes
2r-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexene	0.6468	High	0.778283	No	11.12834	Yes
1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	0.6594	High	0.848891	No	90.15122	Yes

Compound name	Metabolism		Excretion	Toxicity		
	CYP2D6 substrate	CYP3A4 Substrate	Renal oct2 sub	Ames Tox	Herg	Rat hepato tox
S-Propylthio-l-cysteine	0.132	0.288	0.9259	No	0.028	2.0526
2,2,4-Trimethyl-3-hydroxy-n-valeronitrile	0.152	0.428	0.9349	No	0.058	2.0269
Dinaphtho[2,1-*b*:1',2'-*d*]furan	0.124	0.414	0.7550	No	0.676	2.3900
Isoxazolo[4,3-*a*]phenazine,1-phenyl	0.134	0.463	0.7761	No	0.68	1.9764
Quinazolin-4(3*H*)-one, 2,3-diphenyl	0.224	0.588	0.8253	No	0.593	2.8063
2r-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexene	0.37	0.786	0.8213	No	0.37	2.2424
1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	0.47	0.595	0.7993	No	0.525	1.8322

which may influence their therapeutic efficacy and safety profiles [25].

Molecular docking: The anti-inflammatory effects of bioactive compounds were analyzed computationally by docking. For Interleukin-6 (PDB ID: 1ALU), molecular docking analysis revealed that several bioactive compounds exhibited significant binding affinities and the results are shown in Table-7. Tofacitinib, a well-known anti-inflammatory drug, was used as a control. In rational drug design, the binding free energy values lower than -6.0 kcal/mol are generally considered indicative of strong binding affinity and are commonly used as a threshold for identifying promising lead compounds [26]. In this study, several compounds demonstrated binding energies below -6.0 kcal/mol, suggesting a strong inhibitory potential against IL-6. Among the screened compounds, dinaphtho[2,1-*b*:1′,2′-*d*]-furan and isoxazolo[4,3-*a*]phenazine,1-phenyl exhibited the most favorable binding affinities, outperforming the control drug tofacitinib (5 mg), a known IL-6 inhibitor.

Fig. 2 shows the 2D and 3D structure of protein-ligand interactions of bioactive compounds. The overall binding to the receptor was further aided by hydrogen bonding interactions with ARG40, ASN61, ARG168 and HIS164. The docking result revealed two pi-sigma interactions with ASN61 and a pi-alkyl contact with ASN63, LYS66, LUS167 and LYS171 also exhibited alkyl interactions [27]. These results highlight the potential role of these two phytoconstituents in contributing to the anti-inflammatory effects of the ethanolic leaf extract. While a few compounds exhibited relatively lower affinities, the majority of the docked ligands demonstrated enhanced binding efficiency compared to tofacitinib. These findings are consistent with previous studies reporting the IL-6 inhibitory activity of structurally similar phytochemicals [28]. To substantiate the observed experimental anti-inflammatory activity, molecular docking was employed to elucidate the underlying interaction mechanisms between the bioactive compounds and IL-6. The computational outcomes reinforce the potential of these natural compounds as lead candidates for the development of novel anti-inflammatory therapeutics [29,30].

RBC membrane stabilization assay: Since the components of lysosomal and red blood cell (RBC) membranes are similar, the anti-inflammatory efficacy of *A. malabarica* (L.) extract was evaluated by avoiding heat-induced hypotonicity and RBC membrane lysis. The ethanol extract of *A. malabarica* may help stabilize RBC membranes by preventing the release of lytic enzymes and inflammatory mediators. Phytochemical analysis revealed the presence of steroids, flavonoids, alkaloids and terpenoids, with flavonoids widely recognized for their potent anti-inflammatory and antioxidant properties. The haemolysis assay, a dose-dependent method for assessing anti-inflammatory activity, showed that increasing concentrations of diclofenac sodium generally reduced or maintained low levels of haemolysis, indicating a membrane-stabilizing effect. However, at higher doses, some compounds may induce haemolysis due to cytotoxicity [31].

In this study, diclofenac sodium was used as a reference (Fig. 3) to evaluate the anti-inflammatory and safety profiles of the plant extracts. Ethanol extract of *A. malabarica* demonstrated a significant reduction in RBC haemolysis (upto 70-90%), followed by ethyl acetate and aqueous extracts. At concentrations of 10-100 µg/mL, haemolysis increased slightly likely due to enhanced interaction with the RBC phospholipid bilayer. These findings suggest that *A. malabarica* leaf extracts may have therapeutic potential in managing inflammatory conditions [32].

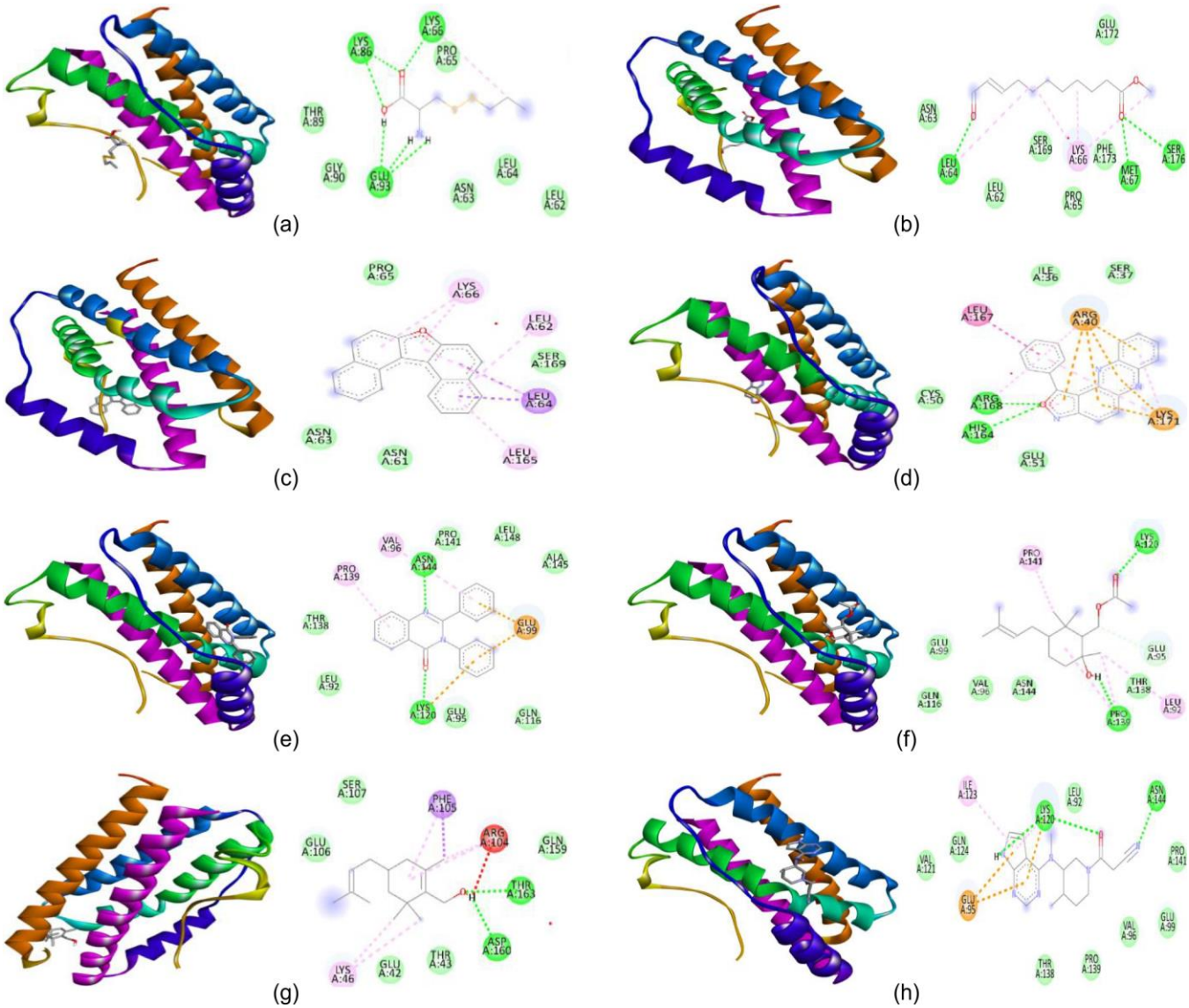

Fig. 2. 2D and 3D structure of the bioactive compounds (a) S-propylthio-l-cysteine, (b) 2,2,4-trimethyl-3-hydroxy-n-valeronitrile, (c) dinaphtho-[2,1-*b*:1',2'-*d*]furan, (d) isoxazolo[4,3-*a*]phenazine,1-phenyl, (e) quinazolin-4(3*H*)-one, 2,3-diphenyl, (f) 2r-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexene, (g) 1,3,3-trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene, (h) tofacitinib (control)

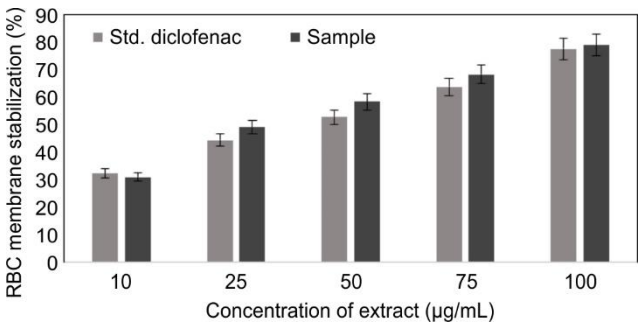

Fig. 3. Percentage of RBC membrane stabilization assay

Conclusion

In this study, the phytochemical constituents and anti-inflammatory properties of ethanol extracts from *Anisomeles malabarica* leaves were investigated. GC-MS analysis identified isoxazolo[4,3-*a*]phenazine,1-phenyl and dinaphthofuran as the major bioactive compounds. These molecules demonstrated strong binding affinity toward Interleukin-6 (IL-6), a key pro-inflammatory cytokine, comparable to the standard drug Tofacitinib. Their interaction suggests potential to reduce synovial inflammation in rheumatoid arthritis and support immune modulation. Drug-likeness analysis and ADMET profiling indicated that both compounds possess favourable pharmacokinetic and safety properties, qualifying them as promising drug candidates. Notably, isoxazolo[4,3-*a*]phen-azine,1-phenyl and dinaphthofuran achieved the best docking scores, reinforcing their potential as novel anti-inflammatory leads. The hemolysis assay further supported these findings by demonstrating the compounds' ability to stabilize red blood cell membranes under hypotonic and heat-induced stress, an indirect indicator of lysosomal membrane stabilization and suppression of inflammatory mediator release. The observed reduction in hemolysis

TABLE-7
MOLECULAR DOCKING AND INTERACTION OF BIOACTIVE COMPOUNDS ISOLATED FROM *A. malabarica* (L)

Target gene	Compound	van der Waals Interaction	Binding energy (KCal/mol)	No. of H bonds	Hydrogen interaction	Total no. of residues
1ALU	S-Propylthio-l-cysteine	THR 89, GLY 90, ASN 63, LEU 64, LEU 62, PRO 65	-4.51	3	GLU 93, LYS 86, LYS 66	THR 89, GLY 90, ASN 63, LEU 64, LEU 62, PRO 65, GLU 93, LYS 86, LYS 66
	2,2,4-Trimethyl-3-hydroxy-n-valeronitrile	THR 163, THR 43, ARG 104, GLU 106	-4.52	3	GLU 42, SER 107, SER 108	THR 163, THR 43, ARG 104, GLU 106, GLU 42, SER 107, SER 108, PHE 105, LYS 46
	Dinaphtho[2,1-*b*:1',2'-*d*]furan	ASN 61, ASN 63, PRO 65, SER 169	-9.48	2	ARG 40, ASN 61	LEU 165, LEU 64, LEU 62, LYS 66, ASN 61, ASN 63, PRO 65, SER 169
	Isoxazolo[4,3-*a*]phenazine,1-phenyl	CYS 50, GLU 51, ILE 36, SER 37	-9.07	2	ARG 168, HIS 164	ARG 40, LYS 171, LEU 167, ARG 168, HIS 164, CYS 50, GLU 51, ILE 36, SER 37
	Quinazolin-4(3*H*)-one, 2,3-diphenyl	THR 138, LEU 92, GLU 95, GLN 116, ALA 145, LEU 148, PRO 141	-6.68	2	LYS 120, ASN 144	VAL 96, PRO 139, GLU 99, LYS 120, ASN 144, THR 138, LEU 92, GLU 95, GLN 116, ALA 145, LEU 148, PRO 141
	2r-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexene	GLU 99, GLN 116, VAL 96, ASN 144, THR 138	-5.80	3	LYS 120, PRO 139, GLU 95	LEU 92, PRO 141, LYS 120, PRO 139, GLU 95, GLU 99, GLN 116, VAL 96, ASN 144, THR 138
	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	GLN 159, THR 43, GLU 42, GLU 106, SER 107	-6.24	2	ASP 160, THR 163	GLN 159, THR 43, GLU 42, GLU 106, SER 107, ASP 160, THR 163, PHE 105, ARG 104, LYS 46
	CN Tofacitinib 5 mg	VAL 121, GLN 124, THR 138, PRO 139, VAL 96, GLU 99, PRO 141, LEU 92	-6.17	2	LYS 120, ASN 144	LYS 120, ASN 144, VAL 121, GLN 124, THR 138, PRO 139, VAL 96, GLU 99, PRO 141, LEU 92, ILE 123, GLU 95,

confirms their membrane-protective and anti-inflammatory potential.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. N. Chaachouay and L. Zidane, *Drugs Drug Candid.*, **3**, 184 (2024); https://doi.org/10.3390/ddc3010011
2. M, Ekor, *Front. Pharmacol.*, **4**, 177 (2014); https://doi.org/10.3389/fphar.2013.00177
3. B. Santhanalakshmi, G. Sivanandhan, S. Manojkumar, S. Anitha, G. Sharmistha, N. Selvaraj and G. Kapildev, *Plant Biosyst.*, **158**, 925 (2024); https://doi.org/10.1080/11263504.2024.2379365
4. R.B. Mrid, N. Bouchmaa, H. Ainani, R.E. Fatimy, G. Malka and L. Mazini, *Biomed. Pharmacother.*, **151**, 113126 (2022); https://doi.org/10.1016/j.biopha.2022.113126
5. J. Bullock, S.A.A. Rizvi, A.M. Saleh, S.S. Ahmed, D.P. Do, R.A. Ansari and J. Ahmed, *Med. Princ. Pract.*, **27**, 501 (2018); https://doi.org/10.1159/000493390
6. A.J. Olędzka and M.E. Czerwińska, *Int. J. Mol. Sci.*, **24**, 4666 (2023); https://doi.org/10.3390/ijms24054666
7. R. Direito, S.M. Barbalho, M.E. Figueira, G. Minniti, G.M. de Carvalho, B. de O. Zanuso, A.R. de O. dos Santos, N. de G. Corrêa, V.D. Rodrigues, R. de A. Goulart, E.L. Guiguer, A.C. Araújo, H. Bosso and L.F. Laurindo, *Metabolites*, **13**, 728 (2023); https://doi.org/10.3390/metabo13060728
8. M. P. Madhivardhana, K. E. Kiranvikas, Z. H. Khan, and R. S. Mangal, *Pharmacogn. Res.*, **17**, 769 (2025); https://doi.org/10.5530/pres.20252254
9. A. Sudha and P. Srinivasan, *Pharmacogn. Mag.*, **10(Suppl 3)**, S596 (2014); https://doi.org/10.4103/0973-1296.139795
10. R. Bhuvaneshwari and R. Anandhan, *Environ. Ecol.*, **42**, 547 (2024); https://doi.org/10.60151/envec/RNSW4006
11. P. Kavya, R.C. Theijeswini and M. Gayathri, *Front Chem.*, **12**, 1458505 (2024); https://doi.org/10.3389/fchem.2024.1458505
12. C.Y. Jia, J.Y. Li, G.F. Hao and G.F. Yang, *Drug Discov. Today*, **25**, 248 (2020); https://doi.org/10.1016/j.drudis.2019.10.014
13. A. Belal, *Pharmazie*, **73**, 635 (2018); https://doi.org/10.1691/ph.2018.8061
14. M. Abdul-Hammed, I.O. Adedotun, M. Olajide, C.O. Irabor, T.I. Afolabi, I.O. Gbadebo, L. Rhyman and P. Ramasami, *Nat. Prod. Res.*, **36**, 3110 (2022); https://doi.org/10.1080/14786419.2021.1935933
15. S. Jose, M. Gupta, U. Sharma, J. Quintero-Saumeth and M. Dwivedi, *J. Mol. Struct.*, **1254**, 132369 (2022); https://doi.org/10.1016/j.molstruc.2022.132369
16. H. Prabhavathi, K.R. Dasegowda, K.H. Renukananda, K. Lingaraju and H.R. Naika, *J. Biomol. Struct. Dyn.*, **39**, 5471 (2021); https://doi.org/10.1080/07391102.2020.1790424
17. J. Xu, Q. Chen, Y. Qiu, Z. Wang, M. Zeng, F. Qin, J. Chen and Z. He, *Food Chem.*, **475**, 143279 (2025); https://doi.org/10.1016/j.foodchem.2025.143279

18. R.R. Deshpande, A.P. Tiwari, N. Nyayanit and M. Modak, *Eur. J. Pharmacol.*, **886**, 173430 (2020); https://doi.org/10.1016/j.ejphar.2020.173430
19. I. Rjeibi, S. Ncib, A. Ben Saad and S. Souid, *Lipids Health Dis.*, **16**, 206 (2017); https://doi.org/10.1186/s12944-017-0596-1
20. G. Xiong, Z. Wu, J. Yi, L. Fu, Z. Yang, C. Hsieh, M. Yin, X. Zeng, C. Wu, A. Lu, X. Chen, T. Hou and D. Cao, *Nucleic Acids Res.*, **49(W1)**, W5 (2021); https://doi.org/10.1093/nar/gkab255
21. A. Daina, O. Michielin and V. Zoete, *Sci. Rep.*, **7**, 42717 (2017); https://doi.org/10.1038/srep42717
22. V. Srivastava, A. Yadav and P. Sarkar, *Mater. Today Proc.*, **49**, 2999 (2022); https://doi.org/10.1016/j.matpr.2020.10.055
23. L. Jia and H. Gao, *Methods Mol. Biol.*, **2390**, 447 (2022); https://doi.org/10.1007/978-1-0716-1787-8_20
24. C.P.M. da Silva, G.M. das Neves, G.L. Poser, V.L. Eifler-Lima and S.M.K. Rates, *Med. Chem.*, **19**, 1002 (2023); https://doi.org/10.2174/1573406419666230601092358
25. M. Sharif, P. John, A. Bhatti, R.Z. Paracha and A. Majeed, *Front. Pharmacol.*, **15**, 1488790 (2024); https://doi.org/10.3389/fphar.2024.1488790
26. G. Karakaya, A. Türe, A. Ercan, S. Öncül and M.D. Aytemir, *Bioorg. Chem.*, **88**, 102950 (2019); https://doi.org/10.1016/j.bioorg.2019.102950
27. A.H. Li, S. Moro, N. Forsyth, N. Melman, X.D. Ji and K.A. Jacobson, *J. Med. Chem.*, **42**, 706 (1999); https://doi.org/10.1021/jm980550w
28. D.L. Boyle, K. Soma, J. Hodge, A. Kavanaugh, D. Mandel, P. Mease, R. Shurmur, A.K. Singhal, N. Wei, S. Rosengren, S. Krishnaswami, I. Kaplan, Z. Luo, J. Bradley and G.S. Firestein, *Ann. Rheum. Dis.*, **74**, 1311 (2015); https://doi.org/10.1136/annrheumdis-2014-206028
29. R. Fleischmann, J. Kremer, J. Cush, H. Schulze-Koops, C.A. Connell, J.D. Bradley, D. Gruben, G.V. Wallenstein, S.H. Zwillich, K.S. Kanik, ORAL Solo Investigators, *N. Engl. J. Med.*, **367**, 495 (2012); https://doi.org/10.1056/NEJMoa1109071
30. S. Showkat, D. Dharumadurai and T.S. Kumar, *Microb. Pathog.*, **198**, 107104 (2025); https://doi.org/10.1016/j.micpath.2024.107104
31. B.C. Evans, C.E. Nelson, S.S. Yu, K.R. Beavers, A.J. Kim, H. Li, H.M. Nelson, T.D. Giorgio and C.L. Duvall, *J. Vis. Exp.*, **2013**, e50166 (2013); https://doi.org/10.3791/50166
32. F. Ben Mefteh, A. Daoud, A. Chenari Bouket, B. Thissera, Y. Kadri, H. Cherif-Silini, M. Eshelli, F.N. Alenezi, A. Vallat, T. Oszako, A. Kadri, J.M. Ros-García, M.E. Rateb, N. Gharsallah and L. Belbahri, *Int. J. Mol. Sci.*, **19**, 1986 (2018); https://doi.org/10.3390/ijms19071986