

Article

Computational Study of Terpenes from *Schinus molle* L. Fruit Essential Oil as Potential Modulators of Osteoarthritis-Related Inflammatory Pathways: A Network Pharmacology and Molecular Docking Study

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Abstract

This study investigated the effects of terpene compounds from *Schinus molle* fruit essential oil (EO) against osteoarthritis (OA) using integrated network pharmacology, molecular docking, and molecular dynamics. The EO, obtained by steam distillation (yield: 6.95%), was characterized by GC-MS, detecting 55 peaks (98.61% of the total), of which 49 were identified and dominated mainly by monoterpene hydrocarbons (73.93%), with α -phellandrene (20.35%), camphene (11.36%), and Z- β -ocimene (8.69%) as the major constituents. Additionally, seven compounds with favorable ADME profiles and low predicted toxicity were selected for the target prediction. Overlap analysis between compound targets and osteoarthritis-related genes (Os-teoDIP) revealed 171 common genes that triggered inflammatory pathways, including PI3K-Akt, HIF-1, and NOD-like receptor signaling. Protein–protein interaction network analysis identified 11 hub genes, including TLR4, HSP90AA1, PTGS2, and MAPK1. Molecular docking revealed that γ -cadinene exhibited the best binding affinities, particularly against HSP90AA1 (−6.38 kcal/mol) and TLR4 (−5.87 kcal/mol). A 200 ns molecular dynamics simulation confirmed the stability of the γ -cadinene–TLR4 complex through persistent hydrophobic contacts with residues F379, C391, F409, I310, and F377, with contact occupancy of up to 0.95. The receptor backbone RMSD plateaued between 2.5 and 3.5 Å after equilibration, and the ligand remained in the binding pocket throughout the trajectory. These findings suggest that the terpenes of *S. molle* EO, particularly γ -cadinene, may modulate the inflammatory pathways related to OA. However, it is necessary to complement experimental validation in vitro and in vivo.



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Keywords: volatile oil; inflammation; natural products; computational study; toxicity; Lipinski's rule

1. Introduction

Osteoarthritis (OA) is a chronic musculoskeletal disorder that affected more than 500 million people in 2020, representing a leading cause of chronic pain and physical disability, particularly in the elderly population [1,2]. Its prevalence increases with age, obesity, and a sedentary lifestyle, representing a substantial economic burden on public healthcare systems by increasing direct medical costs and reducing productivity [3]. OA mainly affects the knee, hip, and hand joints of the body. Health projections indicate that the global burden of OA will continue to rise in parallel with aging populations and the obesity epidemic because of the association between a high body mass index and OA [4].

The pathogenesis of OA involves a cascade of events including the progressive degradation of articular cartilage, synovial hyperplasia, subchondral bone remodeling, and osteophyte formation [5]. Several pro-inflammatory cytokines, such as interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6), contribute to OA progression, which drives chondrocyte catabolism and extracellular matrix degradation through the upregulation of matrix metalloproteinases (MMPs) [6]. Toll-like receptor 4 (TLR4) has emerged as a key mediator of innate immune activation in OA, triggering NF- κ B-dependent transcription of inflammatory genes in synoviocytes and chondrocytes [7]. Furthermore, phosphoinositide 3-kinase/protein kinase B (PI3K-Akt) and hypoxia-inducible factor 1-alpha (HIF-1 α) have been shown to play crucial roles in chondrocyte survival and metabolic adaptation under hypoxic joint conditions [8,9], whereas cyclooxygenase-2 through its participation in prostaglandin synthesis is involved in pain and inflammation [10]. The mitogen-activated protein kinase (MAPK) signaling pathway is linked to inflammation and chondrocyte death [11]. Currently, the primary pharmacological treatment involves the use of non-steroidal anti-inflammatory drugs and corticosteroids. While these medications offer relief from symptoms, their prolonged use can lead to serious side effects, driving the emerging interest in natural products as safer treatment alternatives.

Several studies have focused on natural products that have been investigated as therapeutic alternatives for OA management, acting through complementary mechanisms. At the cartilage level, natural compounds such as flavonoids (diosmetin, formononetin, luteolin, casticin, and acacetin) alkaloids (α -solanine, chelidone, and vindoline) saponins (hederagenin and timosaponin B-II), and terpenoids (oridonin, oleanolic, astaxanthin, and artemisinin) have been shown to promote cartilage matrix synthesis, increase aggrecan and collagen II, alleviate inflammation by inhibiting PI3K/AKT/mTOR pathway, reduce oxidative stress, and suppress chondrocyte apoptosis. Others, such as curcumin and resveratrol, have shown efficacy comparable to that of conventional NSAIDs in clinical trials [12]. At the immune level, natural products have also been reported to modulate macrophage polarization and the Th17/Treg balance implicated in inflammation associated with OA, although evidence for other immune-cell populations remains at an earlier, exploratory stage [13].

Schinus molle L. (Anacardiaceae Family) is commonly known as molle in Peru. It is native to Argentina, Northeast Brazil, South Brazil, North Chile, Paraguay, Peru, and Uruguay [14]. *S. molle* fruits are found at the ends of branches and are clustered in panicles. Small fruits are drupes characterized by their purplish-red skin that surrounds the seeds. Initially, fruits are bright green, but they turn red as they ripen (Figure 1) [15]. Traditionally, an ancestral beverage is prepared based on the fruits consumed by local populations in Ayacucho, Peru, known as “chicha de molle” (in Spanish) [16]. Fruits contain essential oils, carotenoids, phenols, terpenes, tannins, and other bioactive compounds that contribute to their therapeutic properties [17,18]. Extracts derived from *S. molle* fruits exhibit antimicrobial activity against certain pathogens, indicating their potential utility in treating infections [19]. Furthermore, seeds possess anti-inflammatory and analgesic properties,

rendering them beneficial for the management of pain and inflammation [20]. Some studies have also reported their potential antioxidant [21] and antitumor activities [22].



Figure 1. (A) *Schinus molle* plant collected in Ica, Peru. (B) *Schinus molle* fruits (C) Essential oil (EO) of *Schinus molle* obtained after steam distillation, showing the nearly colorless/transparent oil layer separated from water.

Among natural sources with potential anti-inflammatory activity, *S. molle* fruit essential oil (EO) has gained increasing attention due to its rich terpene composition, particularly monoterpenes and sesquiterpenes, which are known to modulate inflammatory pathways [23]. Previous studies have demonstrated that terpene compounds, such as α -pinene, β -pinene, and sesquiterpenes, inhibit NF- κ B activation, suppress pro-inflammatory cytokine production, and reduce COX-2 expression [24]. Given that TLR4-mediated inflammation and PI3K–Akt signaling are central to OA pathogenesis and that *S. molle* terpenes have demonstrated anti-inflammatory and analgesic properties, its EO represents a promising alternative for multi-target therapeutic strategies against OA. As EOs are not recommended for oral use, this study proposes the development of a topical formulation as a suitable pharmaceutical vehicle for the local delivery of bioactive terpenes.

Network pharmacology focuses on the analysis and interaction of biological networks by integrating pharmacology, bioinformatics, and network analysis to identify potential drug targets and optimize new drug candidates [25,26]. This approach has been successfully applied to investigate the molecular basis of the effects of EOs and plant extracts against inflammatory and degenerative diseases, including OA [27,28]. Therefore, this study aimed to investigate the potential therapeutic mechanisms of *Schinus molle* fruit EO on osteoarthritis through an integrated computational approach combining network pharmacology, molecular docking, and molecular dynamics simulations, to identify key hub targets and elucidate the molecular interactions underlying their predicted anti-inflammatory activity.

2. Materials and Methods

2.1. Plant Material and Essential Oil Obtention

Ripe fruits of *Schinus molle* L. were collected from the Los Aquijes district in the Ica province of Ica, Peru, located approximately 525 m above sea level, with geographical coordinates of 14.096669° S and 75.690831° W. The plant material was harvested in July 2022. Taxonomic identification was based on a voucher specimen previously deposited at the Herbarium of the Universidad Nacional Mayor de San Marcos (Id. 089-USM-MHN-2022). Prior to extraction, the fruits were air-dried for 72 h and semi-ground using a disc mill, a step that disrupts the fruit epicarp/seed structure and facilitates the release of the essential oil that would otherwise remain trapped during distillation. Preliminary pilot extractions monitoring the essential oil volume at 30 min intervals showed that the yield reached a plateau as early as 30 min, with no significant increase observed up to 2 h. Based

on these results, the EO was obtained by steam distillation for 2 h using a semi-industrial stainless-steel distillation unit (chamber capacity: 4 L; Supplementary Figure S1) [29], using approximately 604.3 g of dried, semi-ground fruit, yielding 6.95% (*v/w*, calculated relative to the dry fruit weight) of EO. The obtained EO (42.0 mL) was a nearly colorless and transparent liquid. Residual water was removed using approximately 100 mg of anhydrous sodium sulfate (Na_2SO_4) and filtered. The dried EO was stored at 4 °C until further chromatographic analysis.

2.2. Determination of EO Composition

The volatile constituents of the *S. molle* EO were analyzed using a gas chromatography–mass spectrometry (GC–MS) system consisting of a Trace 1310 gas chromatograph coupled to an ISQ 7000 single-quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The separation was performed on a non-polar capillary column (5% phenyl-methylpolysiloxane; 30 m $\times$ 0.25 mm i.d., 0.20 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). Samples were injected in split mode (40:1), with 1 μL of EO diluted in cyclohexane (1:100, *v/v*). The identification of volatile compounds was based on a comparison of their mass spectra with the NIST 23 Mass Spectral Library and the calculated linear retention indices (LRI). The LRI values were calculated according to the Van den Dool and Kratz equation [30], using a homologous series of n-alkanes (C9–C25, Sigma-Aldrich, St. Louis, MO, USA) that were analyzed under identical chromatographic conditions. The analysis was performed in triplicate and the results are expressed as the mean $\pm$ standard deviation (SD) of the relative peak area (%), calculated from the GC-FID chromatogram without the use of correction factors, and do not represent mass or molar concentrations. The raw GC-MS data are provided in Supplementary Table S1.

2.3. Prediction of ADME and Toxicological Properties

For ADME and toxicological studies, only those compounds identified in the EO at concentrations above 1% were selected. This group was then subjected to thorough assessments, including ADME (absorption, distribution, metabolism, and excretion) and toxicological property analyses. These detailed evaluations aimed to ensure safety and determine the pharmacological properties. The SwissADME [31] and ProTox-II [32] databases were used (<http://www.swissadme.ch>, http://tox.charite.de/protox_II accessed on 15 March 2026), considering parameters such as Lipinski, Ghose, Veber, Egan, and Muegge, as well as LD50 (mg/kg), Toxicity Class, Hepatotoxicity, Mutagenicity, and Carcinogenicity.

2.4. Identification of Potential Targets for Bioactive Compounds from *Schinus molle* and Genes Associated with Osteoarthritis

The therapeutic targets of the bioactive compounds were identified using the SwissTargetPrediction (<https://www.swisstargetprediction.ch>, accessed on 15 March 2026) [33] and SuperPred 3.0 (<https://prediction.charite.de/index.php>, accessed on 15 March 2026) [34] platforms. For SwissTargetPrediction, a probability cutoff greater than zero was used, whereas for SuperPred, a cutoff of greater than 50% probability was applied. Additionally, for SuperPred, the results were converted to gene symbols using the UniProt database (<https://www.uniprot.org> accessed on 15 March 2026) [35].

The OsteoDIP database (<https://ophid.utoronto.ca/OsteoDIP> accessed on 15 March 2026) [36] was used to identify genes related to osteoarthritis. The selection of available subjects on the platform was completed. The next step involved using a Venn diagram to match the plant bioactive compounds with the genes associated with osteoarthritis.

2.5. Analysis of Gene Ontology and Metabolic Pathway Enrichment

Gene Ontology and KEGG metabolic pathway enrichment analyses were performed on the plant's therapeutic targets and osteoarthritis genes using the ShinyGO 0.85 platform (<https://bioinformatics.sdstate.edu/go> accessed on 17 March 2026) [37]. The analysis was carried out with an FDR cutoff of 0.05, and redundant pathways were removed. The top 10 gene ontology pathways and top 20 KEGG metabolic pathways were selected for further analyses. The results were visualized using the SRplot platform (<http://www.bioinformatics.com.cn/srplot>) accessed on 17 March 2026 [38].

2.6. Construction and Analysis of the Protein–Protein Interaction Network

A protein–protein interaction network was constructed based on the common genes using Cytoscape 3.10.4 software [39], with a confidence threshold of 0.7 and restricted to *Homo sapiens*. Subsequently, the network was analyzed, and hub genes were identified. This analysis employed the CytoHubba 0.1 application [40] and the Degree, MCC, Betweenness, and Bottleneck algorithms, selecting the top 25 genes for each algorithm.

2.7. Molecular Docking

The hub genes identified were analyzed using the bioactive compounds of *Schinus molle* using molecular docking analysis. For this analysis, Autodock Vina version 1.2.7 was used [41]. Proteins were downloaded from the RCSB PDB [42] server and prepared by dehydration, removal of monoatomic ions, and the addition of hydrogens. The co-crystallized ligands in the protein structures were used as references to generate a grid box, which was set with a margin of 5 Å. The terpenes were downloaded from PubChem [43] in SDF format and subjected to a 5000-step minimization before docking. The results were visually analyzed using the Biovia Discovery Studio software 2026.

2.8. Molecular Dynamics

Molecular dynamics simulations were carried out with YASARA (v 25.1.13) using the AMBER14 force field. The simulated system was built from the TLR4–MD2 ectodomain complex; both the TLR4 receptor chain and the MD2 co-receptor chain were retained, along with the docked γ -cadinene ligand. The complex was solvated with TIP3P water in a cubic simulation cell of $100 \times 95 \times 60$ Å, providing an adequate margin between the solute and its periodic images. Concentrations of Na^+ and Cl^- were added to neutralize the system and adjust the ionic strength to 0.154 M NaCl. A screenshot of the completely solvated system is provided in Figure S2, and the corresponding trajectory movie is provided as Video S1 (Supplementary Materials). The final system consisted of 56,042 atoms: 6655 from the protein, distributed across 416 residues; a ligand with 41 atoms; plus water molecules and equilibrium counterions. A 200 ns simulation was performed with the md_run.mcr script under physiological conditions (pH 7.4, 310 K, constant pressure, water density 0.997 g/mL), with periodic boundary conditions imposed throughout. A total of 401 snapshots were collected, and backbone and ligand RMSD, radius of gyration, secondary structure content, per-residue RMSF, principal component analysis, and ligand–receptor interaction occupancies were computed with the md_analyze.mcr script.

Prior to the production run, the solvated system was subjected to steepest-descent energy minimization to reduce steric clashes. Equilibration was integrated into the trajectory rather than being performed as a separate stage: the temperature was maintained at 310 K by velocity rescaling, and the pressure was controlled by cell rescaling to preserve a water density of 0.997 g/mL, both applied from the first integration step. The system reached equilibrium within the first ~40 ns, as indicated by the stabilization of the backbone RMSD and the radius of gyration; this initial segment was excluded from the stability

analysis, and all conclusions regarding the γ -cadinene–TLR4 interaction were drawn from the equilibrated portion of the trajectory (>40 ns).

3. Results

3.1. Determination of the Chemical Composition of *S. molle* Fruits EO

The *S. molle* EO was characterized using GC-MS analysis, and 55 peaks were detected (Figure 2 and Figure S3 and Table S1) representing 98.61% of the total composition, of which 49 compounds were identified and six remained unidentified (Table 1 and Figure 3). The most abundant class was the monoterpene hydrocarbons (73.93% of the EO), followed by the sesquiterpene hydrocarbons (18.19%), oxygenated sesquiterpenes (2.64%) and oxygenated monoterpenes (0.84%). The other minor unidentified components accounted for 3.00% of the total composition. The main components of the EO were α -phellandrene (20.35%), camphene (11.36%), α -pinene (8.12%), Z- β -ocimene (8.69%), β -phellandrene (7.66%), and β -pinene (6.24%). These six compounds accounted for approximately 62% of the total EO, confirming the dominance of monoterpenes. In contrast, most of the other components were found in trace or minor quantities (<1.0%).

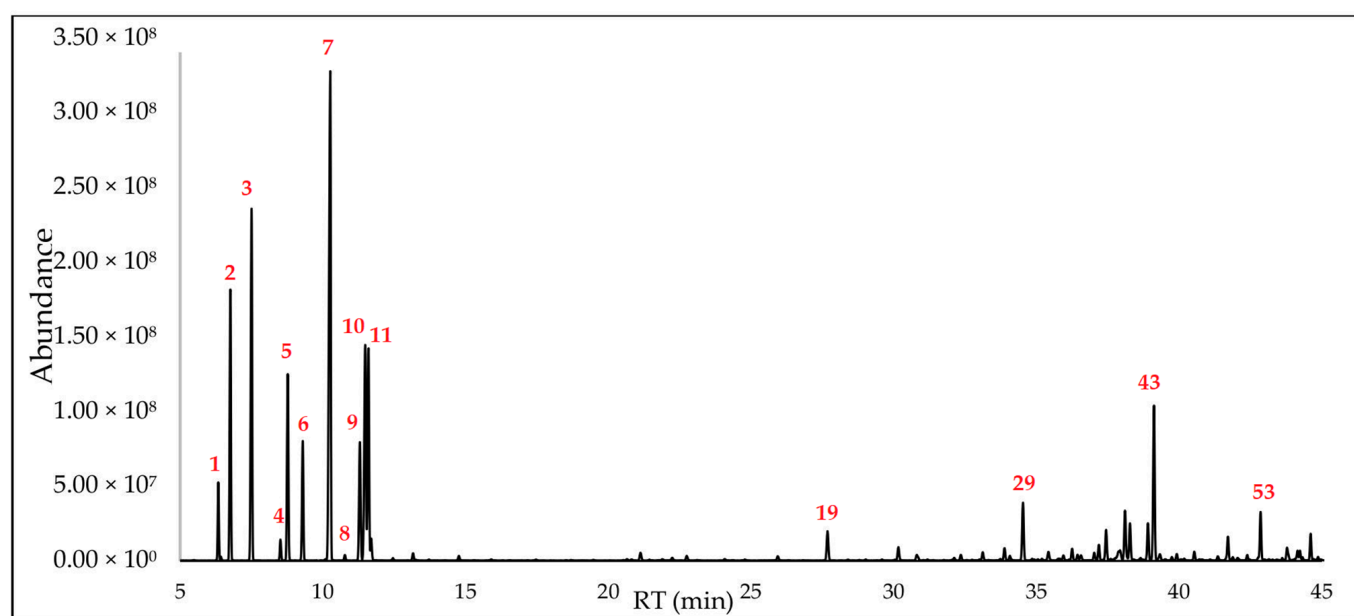


Figure 2. Chromatogram of chemical volatile constituents of *S. molle* fruit EO. Numbered peaks correspond to Table 1.

Table 1. Chemical composition of *S. molle* EO identified using GC-MS.

#	RT	Chemical Constituent	Molecular Formula	LRI ^a	LRI ^b	%	S.D.
1	6.33	α -Thujene	C ₁₀ H ₁₆	922	924	2.07	0.81
2	6.76	α -Pinene	C ₁₀ H ₁₆	932	932	8.12	0.12
3	7.50	Camphene	C ₁₀ H ₁₆	950	946	11.36	1.91
4	8.51	Sabinene	C ₁₀ H ₁₆	973	969	0.63	0.12
5	8.77	β -Pinene	C ₁₀ H ₁₆	979	974	6.24	3.12
6	9.30	Myrcene	C ₁₀ H ₁₆	991	988	3.86	0.21
7	10.26	α -Phellandrene	C ₁₀ H ₁₆	1019	1002	20.35	2.12
8	10.77	α -Terpinene	C ₁₀ H ₁₆	1028	1014	0.17	0.12

Table 1. Cont.

#	RT	Chemical Constituent	Molecular Formula	LRI ^a	LRI ^b	%	S.D.
9	11.30	ρ -Cymene	C ₁₀ H ₁₄	1037	1020	4.37	0.81
10	11.48	Z- β -Ocimene	C ₁₀ H ₁₆	1040	1032	8.69	1.12
11	11.60	β -Phellandrene	C ₁₀ H ₁₆	1042	1025	7.66	2.71
12	11.69	1,8-Cineole	C ₁₀ H ₁₈ O	1044	1031	0.40	0.21
13	13.16	γ -Terpinene	C ₁₀ H ₁₆	1069	1054	0.24	0.11
14	14.77	Terpinolene	C ₁₀ H ₁₆	1097	1086	0.17	0.09
15	21.13	Terpinen-4-ol	C ₁₀ H ₁₈ O	1187	1174	0.32	0.03
16	22.25	α -Terpineol	C ₁₀ H ₁₈ O	1204	1186	0.12	0.02
17	22.75	Not identified	-	1212	-	0.19	0.14
18	25.94	Not identified	-	1262	-	0.17	0.12
19	27.69	Isobornyl acetate	C ₁₂ H ₂₀ O ₂	1289	1283	1.17	0.64
20	30.17	Not identified	-	1334	-	0.51	0.01
21	30.81	Not identified	-	1346	1328	0.29	0.16
22	32.12	2,6-dimethoxy-Phenol	C ₈ H ₁₀ O ₃	1361	1346	0.13	0.05
23	32.36	α -Copaene	C ₁₅ H ₂₄	1376	1374	0.21	0.18
24	33.01	β -Cubebene	C ₁₅ H ₂₄	1388	1387	t	0.02
25	33.13	β -Elemene	C ₁₅ H ₂₄	1390	1389	0.32	0.24
26	33.74	Not identified	-	1402	-	t	0.01
27	33.89	α -Gurjunene	C ₁₅ H ₂₄	1406	1409	0.46	0.33
28	34.08	Not identified	-	1410	1409	0.18	0.09
29	34.54	E-Caryophyllene	C ₁₅ H ₂₄	1420	1417	2.19	1.01
30	35.43	Aromadendrene	C ₁₅ H ₂₄	1439	1439	0.35	0.29
31	35.82	cis-Muurola-3,5-diene	C ₁₅ H ₂₄	1448	1448	0.12	0.07
32	35.95	trans-Muurola-3,5-diene	C ₁₅ H ₂₄	1451	1451	0.19	0.09
33	36.26	α -Humulene	C ₁₅ H ₂₄	1457	1452	0.40	0.31
34	36.45	cis-Cadina-1(6),4-diene	C ₁₅ H ₂₄	1461	1461	0.21	0.12
35	36.57	cis-Muurola-4(14),5-diene	C ₁₅ H ₂₄	1464	1465	0.21	0.14
36	37.04	trans-Cadina-1(6),4-diene	C ₁₅ H ₂₄	1474	1475	0.29	0.19
37	37.20	γ -Muurolene	C ₁₅ H ₂₄	1478	1478	0.55	0.42
38	37.45	Germacrene D	C ₁₅ H ₂₄	1483	1480	1.08	0.81
39	37.94	trans-Muurola-4(14),5-diene	C ₁₅ H ₂₄	1494	1493	0.76	0.63
40	38.11	Bicyclogermacrene	C ₁₅ H ₂₄	1498	1500	1.92	0.93
41	38.29	α -Muurolene	C ₁₅ H ₂₄	1502	1500	1.32	0.54
42	38.92	δ -Amorphene	C ₁₅ H ₂₄	1517	1511	1.32	0.68
43	39.13	γ -Cadinene	C ₁₅ H ₂₄	1522	1513	5.64	1.82
44	39.33	trans-Calamenene	C ₁₅ H ₂₂ O	1527	1521	0.28	0.04
45	39.75	trans-Cadina-1,4-diene	C ₁₅ H ₂₄	1537	1533	0.11	0.10
46	39.93	α -Cadinene	C ₁₅ H ₂₄	1541	1537	0.22	0.03
47	40.54	Elemol	C ₁₅ H ₂₆ O	1556	1548	0.31	0.21
48	41.36	Palustrol	C ₁₅ H ₂₆ O	1576	1567	0.16	0.12
49	41.77	Spathulenol	C ₁₅ H ₂₄ O	1586	1577	0.92	0.77
50	41.89	Caryophyllene oxide	C ₁₅ H ₂₄ O	1589	1589	0.10	0.06
51	42.06	Globulol	C ₁₅ H ₂₆ O	1593	1590	0.11	0.05
52	42.40	Viridiflorol	C ₁₅ H ₂₆ O	1601	1592	0.17	0.10
53	42.86	Dill apiole	C ₁₂ H ₁₄ O ₄	1615	1620	1.70	0.54
54	43.79	γ -Eudesmol	C ₁₅ H ₂₆ O	1643	1630	0.55	0.47
55	44.16	α -Cadinol	C ₁₅ H ₂₆ O	1654	1652	0.31	0.09
Monoterpene hydrocarbons (%)						73.93	
Oxygenated monoterpenes (%)						0.84	
Sesquiterpene hydrocarbons (%)						18.19	
Oxygenated sesquiterpenes (%)						2.64	
Others (%)						3.00	
Total Identified (%)						98.61	

RT retention time; LRI^a: linear retention index obtained from the database [44]; and LRI^b: linear retention index calculated against n-alkanes C9–C25; % relative peak area (GC-FID/MS), not mass or molar concentration.

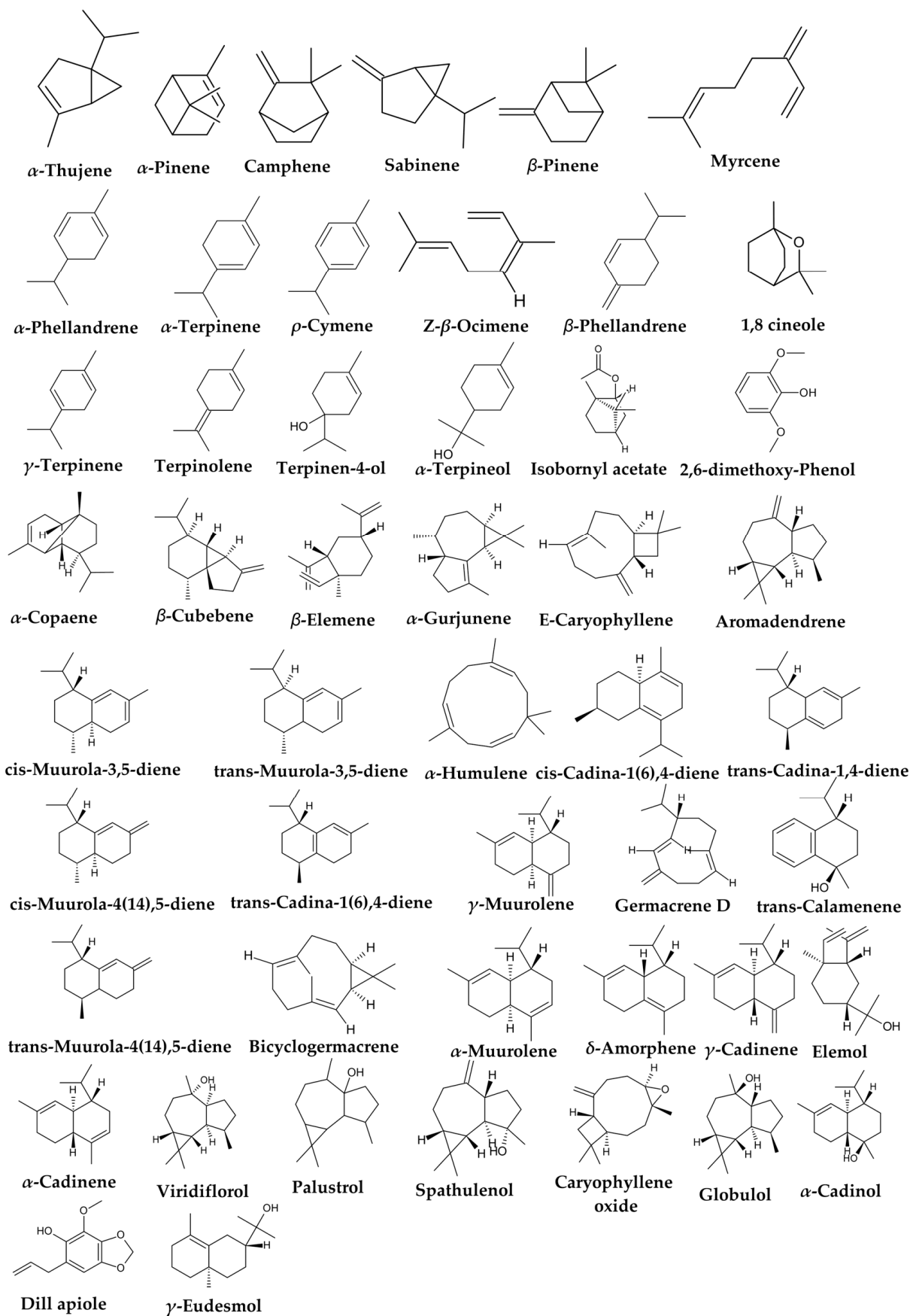


Figure 3. The chemical volatile constituents of *S. molle* fruit EO.

3.2. ADME Properties and Predicted Toxicity Profiles of Chosen *Schinus molle* Compounds

SwissADME and ProTox-II screening of the volatile constituents of *S. molle* showed a generally favorable drug-likeness and safety profile. Most compounds met multiple drug-likeness filters, especially the Ghose and Muegge rules, with few violations of the Veber and Egan filters. All molecules fell within toxicity classes 3, 5, and 6, with high predicted LD₅₀ values, indicating low acute toxicity. In contrast, Z-β-ocimene had a lower LD₅₀ (113 mg/kg, class 3), suggesting a higher predicted toxicity than the other compounds. The predicted probabilities of hepatotoxicity, mutagenicity, and carcinogenicity were low, and all were inactive for these endpoints (Table 2). These data support the selection of these volatiles as promising candidates for further target and pathway analysis.

Table 2. ADME properties and predicted toxicity profiles of selected *Schinus molle* constituents.

Molecule	Lipinski *	Ghose *	Veber *	Egan *	Muegge *	LD ₅₀ (mg/kg)	Toxicity Class	Hepatotoxicity	Mutagenicity	Carcinogenicity
α-Pinene	1	1	0	0	2	3700	5	Inactive (0.86)	Inactive (0.93)	Inactive (0.60)
Camphene	1	1	0	0	2	5000	5	Inactive (0.79)	Inactive (0.1)	Inactive (0.56)
β-Pinene	1	1	0	0	2	4700	5	Inactive (0.80)	Inactive (0.95)	Inactive (0.66)
α-Phellandrene	0	1	0	0	2	5700	6	Inactive (0.86)	Inactive (0.92)	Inactive (0.52)
Z-β-Ocimene	0	1	0	0	2	113	3	Inactive (0.83)	Inactive (0.90)	Inactive (0.51)
β-Phellandrene	0	1	0	0	2	5000	5	Inactive (0.76)	Inactive (0.93)	Inactive (0.65)
γ-Cadinene	1	0	0	0	1	4400	5	Inactive (0.84)	Inactive (0.69)	Inactive (0.76)

* Number of violations.

3.3. Functional Enrichment of *Schinus molle*–Osteoarthritis Overlapping Targets

To explore how *Schinus molle* constituents might interact with osteoarthritis, we predicted protein targets using SwissTargetPrediction and SuperPred, and then compiled OA-related genes from OsteoDIP. The Venn diagram showed 171 overlapping genes, indicating potential candidate nodes at the interaction point of compound activity and disease.

Using 171 common genes between the predicted *Schinus molle* targets and osteoarthritis-related genes, we performed Gene Ontology enrichment analysis (Figure 4). The overlapping targets mainly relate to inflammatory and stress-related processes, such as the inflammatory response, cellular responses to oxygen- and nitrogen-containing compounds, and regulation of multicellular and cellular communication. At the cellular component level, enriched terms included the plasma membrane and synaptic regions, such as the cell surface, synapse, plasma membrane region, and plasma membrane-bound cell projection, suggesting that many targets are located at or near the cell membrane interface. Most genes involved in catalytic activities and binding, especially protein kinase activity, phosphotransferase activity, and binding to nucleotides, nucleoside phosphates, and heterocyclic compounds, are involved in kinase-mediated signaling and ligand–receptor interactions.

KEGG pathway analysis placed these targets within higher-order signaling networks (Figure 5). The 171 common genes were enriched in pathways related to Environmental Information Processing, such as neuroactive ligand–receptor interaction, PI3K–Akt, HIF-1, calcium, and sphingolipid signaling, which modulate cell survival, metabolism, and stress adaptation. Enrichment was also observed for NOD-like receptor, chemokine signaling, neutrophil extracellular trap formation, platelet activation, Th17 cell differentiation, and neurotrophin signaling, linking immune regulation, inflammation, and neuro–immune crosstalk. In the human disease category, targets were mapped to pathways in cancer, lipid metabolism and atherosclerosis, human cytomegalovirus infection, various cancer-related pathways, and AGE–RAGE signaling in diabetic complications, suggesting that the *Schinus molle*–osteoarthritis interface is connected to broader pathophysiological networks beyond joint degeneration.

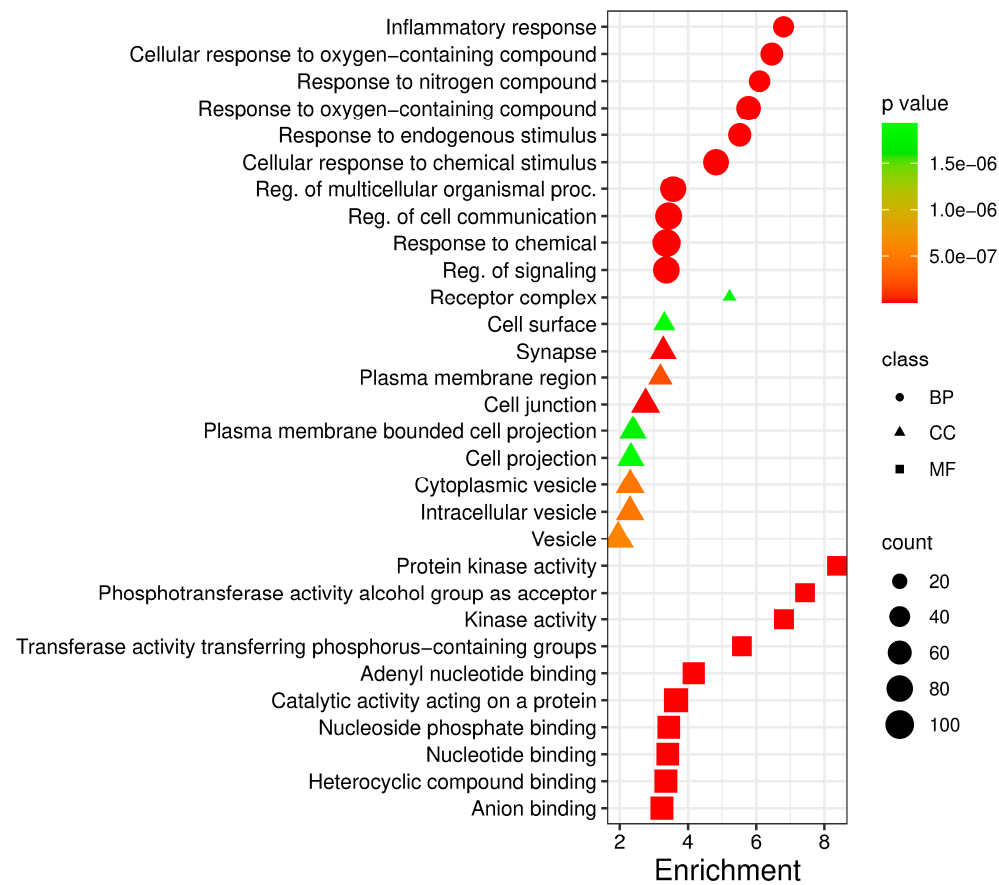


Figure 4. Gene Ontology enrichment analysis of the 171 overlapping targets between *Schinus molle* constituents and osteoarthritis genes, showing the most significantly enriched biological processes, cellular components, and molecular functions ordered by enrichment score.

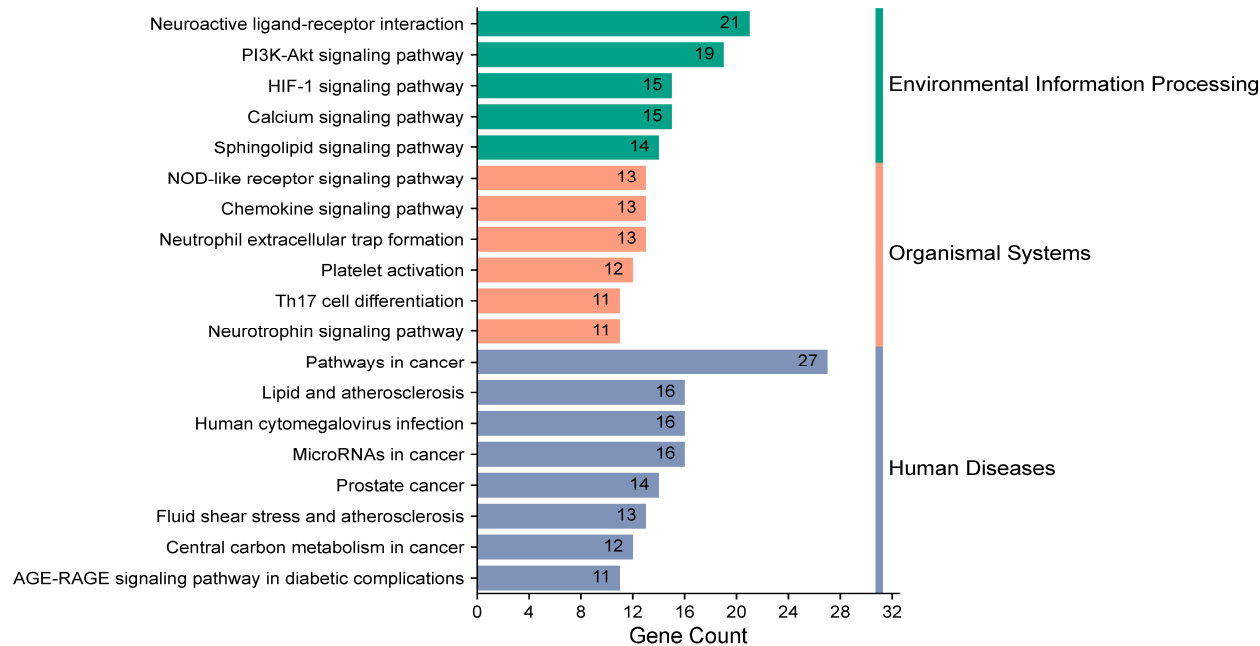


Figure 5. KEGG pathway enrichment analysis of the 171 common targets highlighted the top signaling and disease-related pathways classified into Environmental Information Processing, Organismal Systems, and Human Diseases according to gene count.

3.4. Identification of Hub Targets in the *Schinus molle*–Osteoarthritis PPI Network

A PPI network of the 171 overlapping genes was constructed and visualized using Cytoscape (Figure 6A). It contained 169 nodes (two genes were not identified) and 314 edges with an average of 5.51 neighbors per node, indicating moderate connectivity. The network had a path length of 3.33, a clustering coefficient of 0.35 and a density of 0.052, indicating a small-world topology with non-random clustering and sparse connections. It had a diameter of 8, radius of 4, heterogeneity of 1.004, centralization of 0.247, and 47 connected components, indicating partial fragmentation into modules. In addition, we used the CytoHubba plugin to rank the nodes by degree, MCC, betweenness and bottleneck to identify the key targets in this interactome. Venn analysis of the top 25 nodes from each metric identified an 11-gene core that was consistently prioritized across algorithms and acts as a key mediator of the influence of *Schinus molle* on osteoarthritis signaling networks (Figure 6B).

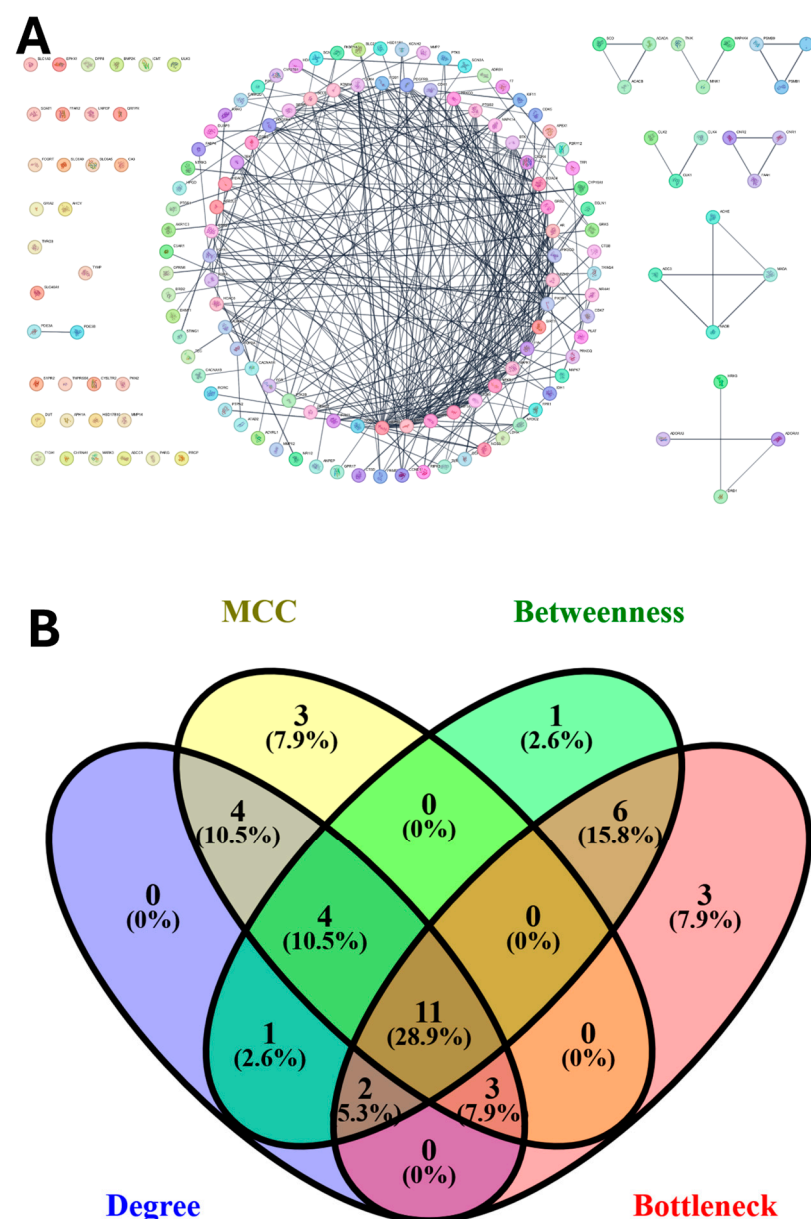


Figure 6. (A) Protein–protein interaction network constructed from overlapping targets between *Schinus molle* constituents and OA-related genes. (B) Venn diagram summarizing the top 25 hub genes identified by CytoHubba using four centrality indices, and highlighting their overlap.

3.5. Molecular Docking Validation of *S. molle* Constituents Against Hub Osteoarthritis Targets

We validated the predicted network interactions by molecular docking of seven *S. molle* compounds with 11 key targets (Figure 7). Each compound–target pair was docked to generate ten poses, and the best pose was selected. The binding affinities ranged from -3.0 to below -6.0 kcal/mol, with most values between -4.0 and -5.5 kcal/mol. The heat-shock proteins HSP90AA1 and HSP90AB1 exhibited strong affinities, notably for γ -cadinene (-6.38 and -6.06 kcal/mol, respectively). TLR4 also showed favorable binding, particularly with γ -cadinene (-5.87 kcal/mol), followed by α -phellandrene and camphene. CXCR4 was also bound to γ -cadinene. PTGS2 and MAPK1 showed moderate to strong binding affinities for various terpenes. The transcription factors AR, EZH2, HIF1A, HDAC4, and STAT3 exhibited weaker interactions, with scores ranging from -3.5 to -5.0 kcal/mol. Among all compound–target pairs, γ -cadinene showed the most favorable and consistent binding affinities across the hub targets. Although HSP90AA1 and HSP90AB1 yielded marginally stronger scores, TLR4 was prioritized for molecular dynamics on mechanistic grounds: it is a central mediator of innate immune activation in osteoarthritis, driving NF- κ B-dependent transcription of inflammatory genes in chondrocytes and synoviocytes, and it ranked among the network hub genes. Among the immunologically central receptors, γ -cadinene bound TLR4 most favorably. The 2D interaction diagrams for γ -cadinene with all 11 hub targets, illustrating this trend in binding stability, are provided in Figure S4 (Supplementary Materials).

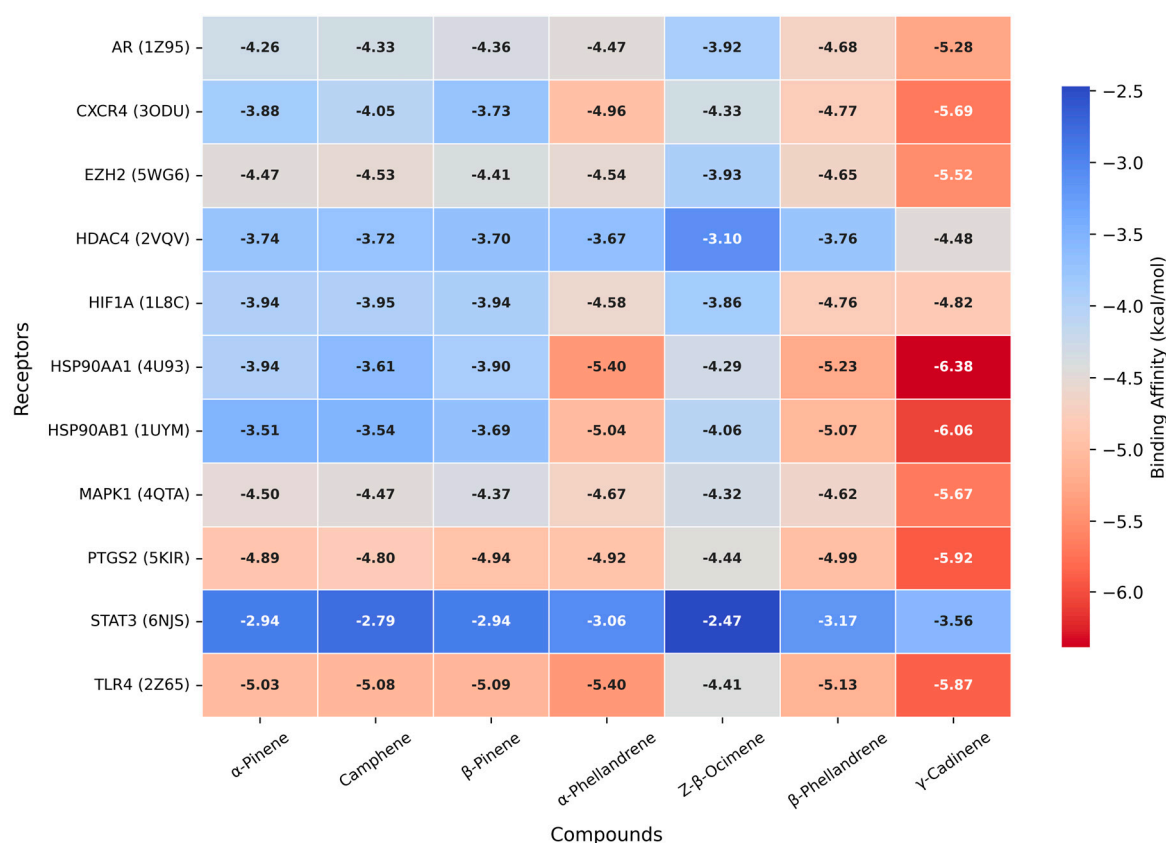


Figure 7. Binding affinity heatmap of molecular docking between *S. molle* compounds and hub targets. The best-scoring pose is shown. Dark red indicates stronger predicted binding.

To visualize the binding mode of the favorable docking score, we analyzed the 3D and 2D interaction profiles of the γ -cadinene–TLR4 complex. In the 3D view (Figure 8A), γ -cadinene fits into a hydrophobic pocket of TLR4, contacting the surrounding structure. The 2D diagram showed mainly hydrophobic contacts (purple) with residues Ile

C:32, Ile C:52, Ile C:61, Val C:48, Phe C:119, and Phe C:121 (Figure 8B). The aromatic and aliphatic side chains around the sesquiterpene scaffold highlight the lipophilic nature of γ -cadinene, which explains its strong affinity and motivates molecular dynamics to assess binding stability.

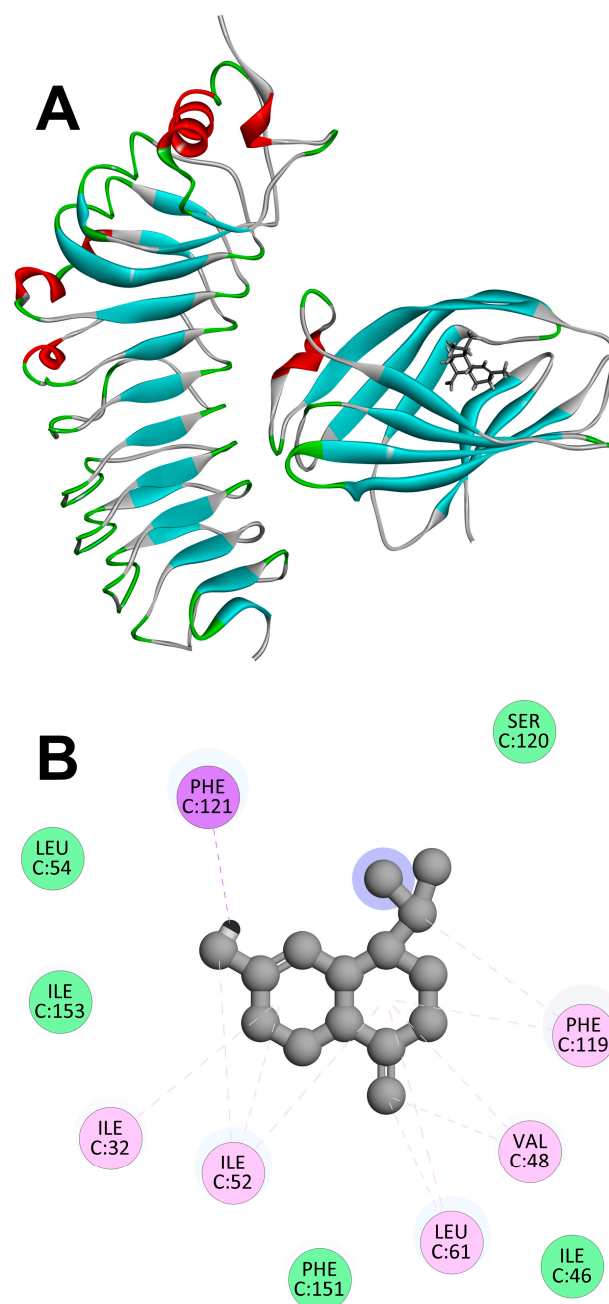


Figure 8. Interaction profiles of γ -cadinene bound to TLR4. (A) The 3D view shows the position of the ligand within TLR4. (B) A 2D diagram illustrating the surrounding residues: purple for hydrophobic interactions and green for polar interactions.

3.6. Molecular Dynamics Simulation Validates the Stability of the γ -Cadinene–TLR4 Complex

A 200 ns molecular dynamics simulation was performed to test whether γ -cadinene maintained a stable interaction with TLR4 beyond the static docking pose. After approximately 40 ns, the TLR4 backbone stabilized, with C α RMSD values between 2.5 and 3.5 Å for the remainder of the trajectory and a returned to approximately 2.5 Å over the final 20 ns (Figure 9A). This range is consistent with the expected flexibility of a large extracellular

receptor domain and does not indicate any structural instability. The radius of gyration remained near 25 Å, and the secondary structure content was preserved throughout the simulation, confirming that the receptor did not unfold.

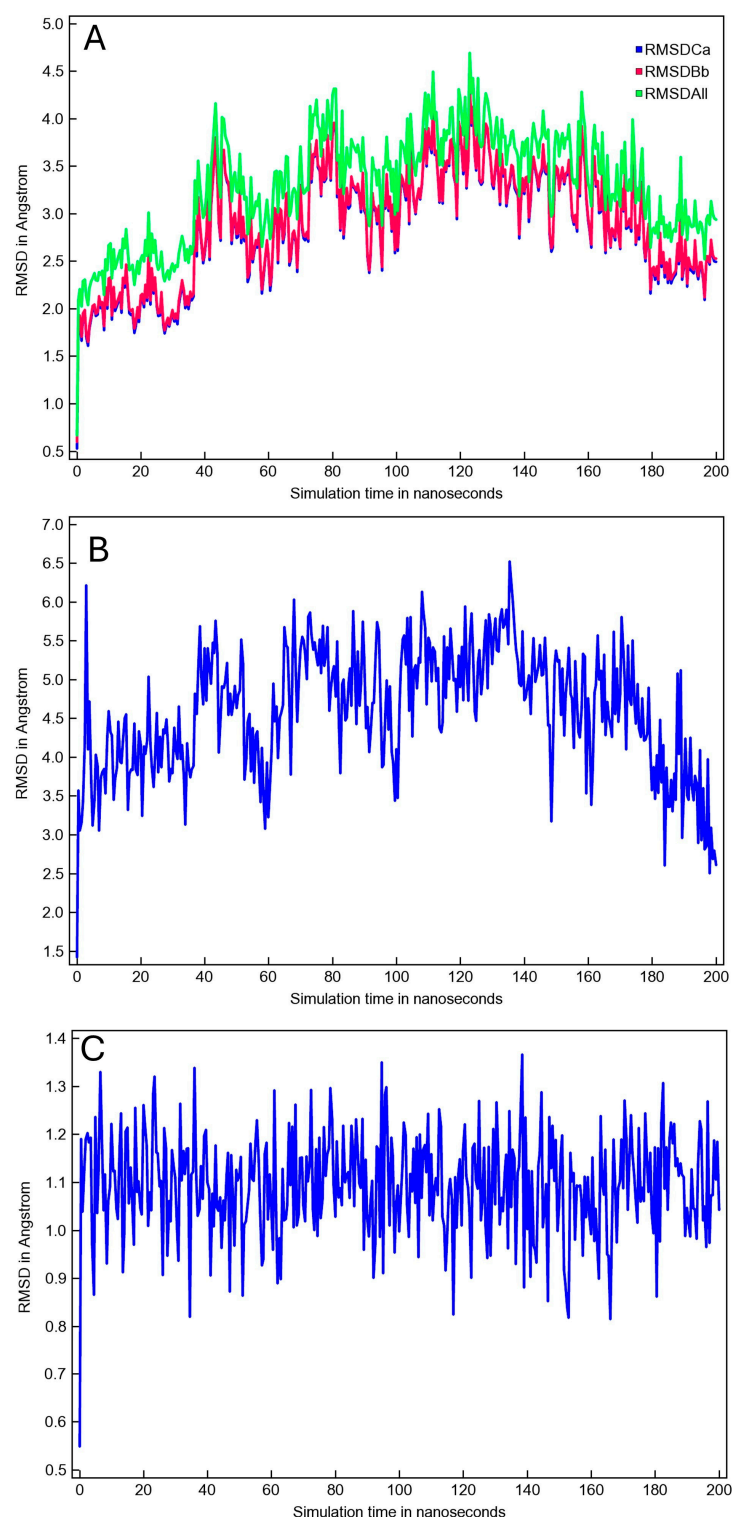


Figure 9. Molecular dynamics analysis of the γ -cadinene-TLR4 complex for over 200 ns. **(A)** Solute RMSD from the reference structure: C α (blue), backbone (red), and all heavy atoms (green). **(B)** Ligand displacement RMSD after superposing the receptor, showing the positional sampling of γ -cadinene within the binding pocket. **(C)** Ligand conformation RMSD after superposition on the ligand, showing its internal conformational stability.

Additionally, γ -cadinene remained within the binding pocket for the entire 200 ns. The ligand displacement RMSD fluctuated between 3.5 and 6.0 Å, reached a transient maximum near 6.5 Å around 135 ns, and decreased to 2.5–3.0 Å over the last 20 ns (Figure 9B), reflecting positional sampling within the hydrophobic pocket rather than detachment. The ligand conformation RMSD remained below 1.3 Å throughout (Figure 9C), showing that the sesquiterpene behaved as a rigid body without internal conformational changes.

The interface between γ -cadinene and TLR4 was entirely hydrophobic; no hydrogen bonds were observed, which is consistent with the nonpolar nature of the sesquiterpene. The residues that interacted most with γ -cadinene throughout the trajectory were F409, C391, F379, I310, F377, L336, L319, and I290, all of which formed hydrophobic contacts with occupancies of 0.88 or higher (Table 3). These residues differed from those identified in the docking pose (Ile32, Val48, Ile52, Ile61, Phe119, and Phe121). This shift is expected because docking places the ligand in a rigid conformation of the crystallized receptor, whereas the simulation allows TLR4 to relax, the hydrophobic pocket to rearrange, and γ -cadinene to migrate toward a more energetically favorable site within the same domain, as reflected by the rise and subsequent stabilization of the ligand displacement RMSD (Figure 8B). Both sites share the same hydrophobic and aromatic-rich character. Therefore, the simulation refines rather than contradicts the docking result, defining the binding site occupied by γ -cadinene in the solvated, flexible receptor.

Table 3. Hydrophobic contacts between γ -cadinene and TLR4 residues, ranked by occupancy over the 200 ns trajectory. Occupancy is the fraction of snapshots in which the contact was present.

TLR4 Residue	Contact Type	Occupancy
F409	Hydrophobic	0.95
C391	Hydrophobic	0.95
F379	Hydrophobic	0.93
I310	Hydrophobic	0.93
F377	Hydrophobic	0.92
L336	Hydrophobic	0.91
L319	Hydrophobic	0.88
I290	Hydrophobic	0.88

4. Discussion

The *S. molle* EO yield obtained in this study (6.95%, *v/w*, relative to dry fruit weight) was higher than that reported by Atti dos Santos et al. for *S. molle* fruits from Brazil using Clevenger-based steam distillation on dried fruit for 1 h (0.49%, *v/w*) [45], which represents the most directly comparable study in terms of units and plant material basis. Other studies report yields in different units or using fresh fruit material, which limits comparisons. For instance, Volpini-Klein et al. reported 1.8% $\pm$ 0.3% (hydrodistillation) and 0.8% $\pm$ 0.1% (steam distillation) from fresh fruits [46]. Pérez-López et al. reported approximately 2.3% of the colorless oil and 1.0% of the yellowish oil (*w/w*) from fresh fruit using a 4 h Clevenger hydrodistillation [47]. Additionally, the *S. molle* fruits used in our study were obtained from arid zones and high-temperature conditions, characteristic of desert weather, which likely differs from the collection conditions reported by Huaman et al. [48], who found a yield of 3.6% (*w/w*) obtained by steam distillation for 3 h from samples collected from Canta, Lima, Peru. Therefore, environmental factors such as temperature, humidity, soil composition, season of collection, and solar exposure during fruit development, as well as the type of apparatus and its operational parameters used in the extraction process, might influence the extraction yield.

Chromatographic analysis of the *S. molle* fruit EO mainly revealed the presence of monoterpene hydrocarbons representing 73.93%, followed by sesquiterpene hydrocar-

bons with 18.19%. Among these compounds, α -phellandrene (20.35%) and camphene (11.36%) were the most abundant, followed by *Z*- β -ocimene (8.69%), α -pinene (8.12%), β -phellandrene (7.66%), and β -pinene (6.24%). This dominance of monoterpene composition is consistent with chemical profiles previously described for *S. molle* EOs obtained of fruits across different geographic origins, and its variability associated with environmental and ecological factors [49]. In our study, α -phellandrene was found as the most abundant component and this finding is like EOs from other regions. The EO from Chile was primarily composed of α -phellandrene (34.02%), along with β -myrcene (23.87%), limonene (13.99%), and β -phellandrene (7.01%) [50]. Similarly, EOs from North Cyprus exhibited similar compositions, with α -phellandrene ranging between 31.6% and 36.3%, limonene (11.4–13.5%), and β -phellandrene (9.9–12.2%) [51]. EOs from Brazil showed more variation based on the collection site, those from preserved regions were abundant in terpinen-4-ol (28.61%), α -pinene (15.26%), and γ -terpinene (12.45%), whereas EOs from Urban areas had a higher abundance of limonene (39.12%) and sabinene (35.70%) [52], indicating that environmental factors might significantly affect the terpene profile of this species.

Moreover, camphene was the second most abundant compound in the present study (11.36%), and its relatively high proportion is noteworthy. Although camphene has been reported as a minor component in several *S. molle* EOs from South America, its elevated abundance in our sample may reflect the geographic and climatic particularities of the collection site in Ica, Peru, located at approximately 525 m.a.s.l. under arid and high-temperature conditions characteristic of the Peruvian coastal desert. Furthermore, this compound has demonstrated relevant biological activities, including antioxidant, antibacterial, antifungal, anticancer, and anti-inflammatory properties [53], which may contribute to the bioactivity of the EO.

EO characterized in this study is related to the α -phellandrene chemotype, as described in a recent systematic review by Radice et al. [54], which reported that this chemotype co-occurring with β -phellandrene, limonene, and myrcene is the most pharmacologically documented compound, being associated with antimicrobial, antinociceptive, anti-inflammatory, anticancer, neuroprotective, and wound-healing activities. Although less abundant (18.19%), the sesquiterpene fraction is noteworthy with γ -cadinene (5.64%) and bicyclogermacrene (1.92%) as the main representatives, which have shown promising bioactivity in previous studies [54].

The favorable ADME and toxicity profiles observed for the seven selected terpenes are consistent with *S. molle* EO constituents reported in the literature. Martins et al. demonstrated that *S. molle* fruit EO from Portugal exhibited low acute toxicity in Swiss mice at doses up to 2000 mg/kg, with no clinical signs of toxicity or organ alterations [21]. The compliance of most compounds with the Ghose and Muegge drug-likeness filters, combined with their low predicted hepatotoxicity, mutagenicity, and carcinogenicity, suggests that these terpenes possess physicochemical properties seemed to promising molecules as anti-inflammatory agents. Notably, the low number of rule violations observed for multiple drug-likeness criteria is particularly relevant for the development of new topical formulations, where systemic exposure is limited and local bioavailability is the primary pharmacokinetic concern.

Network pharmacology analysis revealed 171 common targets between the predicted targets of *S. molle* terpenes and OA-related genes from the OsteoDIP database, suggesting a broad multi-target interaction. Gene Ontology enrichment highlighted biological processes primarily associated with inflammatory and stress-related responses, while at the molecular function level, protein kinase activity and nucleotide binding were predominant, indicating that these terpenes may modulate kinase-driven signaling cascades that are central to OA pathogenesis. KEGG pathway analysis further placed these common targets within

key inflammatory and survival networks, including the PI3K–Akt, HIF-1, and NOD-like receptor signaling pathways, which have been previously implicated in chondrocyte catabolism, synovial inflammation, and cartilage degradation in OA [8,9]. This pattern is consistent with a previous study by Shi et al., which investigated frankincense and myrrh in knee osteoarthritis and similarly identified MAPK1, PI3K–Akt, NF- κ B, and TNF signaling as key pathways modulated by natural compounds, with MAPK1 and PTGS2 emerging as central hub targets [55].

The hub genes identified through network pharmacology analysis were supported by molecular docking results. Notably, TLR4 and HSP90AA1 demonstrated moderate binding affinities for γ -cadinene (−5.87 and −6.38 kcal/mol, respectively), indicating that this sesquiterpene might concurrently disrupt innate immune activation and chaperone-mediated inflammatory stabilization in OA joints. The interaction between γ -cadinene and TLR4 was confirmed by molecular dynamics simulation, which showed persistent hydrophobic contacts with F409, C391, F379, I310, and F377 (occupancies ≥ 0.92) throughout the 200 ns trajectory, supporting the stability and biological feasibility of this association. PTGS2 and MAPK1, also identified as hub genes, exhibited moderate binding affinities for various *S. molle* terpenes. MAPK1 activation has been linked to the promotion of mitochondrial-mediated apoptosis in chondrocytes by elevating the Bax/Bcl-2 ratio and caspase-3 production, whereas prostaglandins derived from PTGS2 expedite cartilage matrix degradation and chondrocyte death [56]. This suggests that the anti-inflammatory effect of the EO may not depend on a single compound–target interaction but rather on the collective modulation of these pathways by several terpene constituents acting in concert. The multi-target profile observed in our study is typical of EO-based therapeutic strategies and offers a pharmacological advantage over single-target approaches in complex diseases such as OA.

Finally, the main limitations of this study are as follows. The results presented are computational and require experimental validation through in vitro and in vivo studies to confirm the biological activities and therapeutic relevance of these compounds. Future studies should prioritize in vitro assays using chondrocyte models, followed by in vivo validation in established OA animal models, to confirm the predicted anti-inflammatory effects of *S. molle* terpenes, particularly γ -cadinene, on TLR4-mediated OA pathogenesis.

5. Conclusions

The *S. molle* EO contained 55 volatile compounds, with α -phellandrene, camphene, and *Z*- β -ocimene as the most abundant terpenes. Seven bioactive terpenes with favorable ADME profiles and low predicted toxicity were identified as promising candidates. Network pharmacology analysis revealed 171 common targets between *S. molle* constituents and OA-related genes, primarily enriched in inflammatory signaling pathways, such as PI3K–Akt, HIF-1, and NOD-like receptor signaling. From the protein–protein interaction network, 11 hub genes were identified, with TLR4, HSP90AA1, PTGS2, and MAPK1 standing out as key nodes. Molecular docking indicated that γ -cadinene exhibited the most favorable binding profiles across multiple targets, and molecular dynamics simulations confirmed the stability of the γ -cadinene–TLR4 complex over 200 ns, sustained by persistent hydrophobic interactions. These findings suggest that *S. molle* terpenes, particularly γ -cadinene, may exert anti-inflammatory effects relevant to osteoarthritis through the modulation of TLR4-mediated signaling. However, these results are preliminary, and experimental validation through in vitro and in vivo studies is necessary to confirm the biological activity and therapeutic relevance of these compounds.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/biophysica6040075/s1>, Figure S1. Semi-industrial stainless-steel steam distillation unit (chamber capacity: 4 L) used for essential oil extraction from *S. molle* fruits. Figure S2. Ray-traced representation of the complete simulated system. The TLR4–MD2 receptor (cartoon) with the bound γ -cadinene ligand is immersed in a cubic cell of TIP3P water with Na^+ and Cl^- counterions. Periodic boundary conditions were applied; atoms extending beyond the cell are wrapped to the opposite side. Figure S3: Mass spectrum of Major Components of EO; Figure S4: Two-dimensional ligand–receptor interaction profiles of γ -cadinene with the 11 osteoarthritis-related hub targets, ranked by predicted binding affinity. Video S1: Molecular dynamics trajectory (200 ns) of the γ -cadinene–TLR4 complex. Table S1: Raw GC-MS chromatographic data (retention time and intensity values).

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