

In silico prediction of pharmacological and toxicological properties of terpenoids in *Centaurea verutum* essential oil

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ARTICLE HISTORY

Received: Feb. 5, 2025

Accepted: Sep. 29, 2025

KEYWORDS

Centaurea verutum,
Terpenoids,
Essential Oil,
SwissADME,
In silico.

Abstract: *Centaurea* is the fourth largest genus within the Asteraceae family, comprising over 600 species distributed worldwide. This study aimed, for the first time, to utilize an *in silico* approach to predict the pharmacological and toxicological properties of terpenoid compounds found in the essential oil of *Centaurea verutum*. Through a comprehensive analysis using Protox, SwissADME, Lipinski's Rule of Five, and biological activity assessments, the study highlights the safety, bioavailability, and therapeutic potential of these compounds. Most of the compounds exhibited low toxicity with LD50 values ranging from 3900 to 5300 mg/kg. The SwissADME analysis suggested good bioavailability for most compounds, although some, like Phytol and β -Farnesene, show low gastrointestinal absorption. Lipinski's rule confirms the drug-likeness of the compounds, with moderate lipophilicity and molecular masses within the optimal range. The biological activity prediction revealed significant antineoplastic, anti-inflammatory, and antieczematic effects, with several compounds, including Caryophyllene oxide, β -Caryophyllene, and Spathulenol, demonstrating potential in cancer therapy and skin conditions. The study also identified compounds with effects on lipid metabolism, mucosal protection, and hormonal regulation. Gene expression analysis revealed that certain compounds could modulate genes related to oxidative stress response and cellular metabolism, further supporting their therapeutic potential. These findings suggest that the essential oil of *Centaurea verutum* holds promise for developing novel pharmaceutical agents for various medical applications, including cancer, inflammation, and metabolic disorders.

INTRODUCTION

Bioactive compounds derived from plants have garnered global attention due to their unique biochemical activities and significant health benefits. Over the past 30 years, plant-derived drugs originating from secondary metabolites have accounted for more than 25% of all newly approved drugs. Furthermore, 50% of commercially successful medicinal components have been developed based on knowledge from plant secondary metabolites (Flores-Holguín *et al.*, 2021). Traditionally, standard drug discovery techniques have been effectively employed to develop state-of-the-art pharmaceuticals. However, the process from lead identification to

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e-ISSN: 2148-6905

clinical evaluation remains both costly and time-consuming. For example, the estimated cost of bringing a new therapeutic drug to market is approximately \$1.8 billion, requiring over 12 years to complete. Worse still, the attrition rate of drug candidates remains as high as 96% (Ojuka *et al.*, 2023).

In recent decades, the pharmaceutical industry has increasingly adopted computer-aided drug design (CADD) techniques to accelerate drug development, aiming to reduce time, costs, and failures, particularly in the final stages. CADD approaches rely on calculated properties and predictive models to identify therapeutic targets and assess safety liabilities (Gomes *et al.*, 2021).

Centaurea is the fourth largest genus within the Asteraceae family, comprising over 600 species distributed worldwide, particularly in Western Asia and the Mediterranean regions (Reda *et al.*, 2021). *Centaurea* species have long been utilized in traditional medicine for treating various ailments such as malaria, diabetes, rheumatism, diarrhea, hemorrhoids, and neurological disorders. Additionally, *Centaurea* spp. have been used to alleviate inflammation, common colds, cough, fever, and ophthalmic disorders (Fattaheian-Dehkordi *et al.*, 2021). The therapeutic potential of *Centaurea* is largely supported by its ability to synthesize a diverse array of specialized metabolites, including flavonoids, sesquiterpene lactones, triterpenes, and lignans. *Centaurea* essential oils exhibit qualitative and quantitative variations influenced by genetic, geographic, climatic, and seasonal factors (Reda *et al.*, 2021).

This study aimed, for the first time, to utilize an *in silico* approach to predict the physicochemical properties, ADMET profiles, pharmacological potentials, cytotoxicity, gene expression profiles, as well as macromolecular targets of the compounds identified in the essential oil of *C. verutum* through gas chromatography–mass spectrometry (GC–MS) analysis.

METHODS

In this study, the focus is exclusively on the terpenoid compounds found in the essential oil of *Centaurea verutum*. These terpenoids were previously identified in our previous study (Joujeh *et al.*, 2018), where the essential oil was extracted from the dried aerial parts of the plant using a Clevenger apparatus and subsequently analysed by gas chromatography coupled with mass spectrometry (GC/MS).

Toxicity Prediction

The toxicity of the phytoconstituents was predicted using ProTox 3.0 (<https://tox.charite.de/protox3/>), an online tool designed to assess the toxicity of small molecules. ProTox 3.0 uses a combination of molecular similarity, fragment propensities, and machine-learning, based on a total of 61 models for the prediction of toxicity endpoints. The chemical names of the compounds, represented by Simplified Molecular Input Line Entry System (SMILES) codes, were acquired from PubChem. The SMILES notations were subsequently used as input to the ProTox 3.0 tool.

The tool provided predictions for the lethal dose, 50% (LD50), and toxicity class for each compound. Toxicity is often expressed in terms of LD50, the dose required to cause death in 50% of test subjects, typically measured in mg/kg of body weight. Toxicity classes are based on the Globally Harmonized System (GHS) for chemical classification and labeling. The LD50 values are categorized as follows:

- Class I: fatal if swallowed ($LD50 \leq 5$ mg/kg)
- Class II: fatal if swallowed ($5 < LD50 \leq 50$ mg/kg)
- Class III: toxic if swallowed ($50 < LD50 \leq 300$ mg/kg)
- Class IV: harmful if swallowed ($300 < LD50 \leq 2000$ mg/kg)
- Class V: may be harmful if swallowed ($2000 < LD50 \leq 5000$ mg/kg)
- Class VI: non-toxic ($LD50 > 5000$ mg/kg) (Banerjee *et al.*, 2024).

Pharmacokinetics Properties (ADMET Profile)

To assess the pharmacokinetic properties of potential compounds, it is essential to understand their absorption, distribution, metabolism, and excretion (ADME) characteristics, which help explain how drugs function within the human body. SMILES notations were used as inputs in the SWISS-ADME tool (<http://www.swissadme.ch/>, accessed on 20 December 2024) (Daina *et al.*, 2017), an online platform designed to predict various drug properties such as ESOL (Log S), GIA, BBB permeant, P-glycoprotein substrate, CYP1A2 inhibitor, CYP3A4 inhibitor, (iLOGP), bioavailability score, and a bioavailability radar was obtained using the SwissADME tool.

Lipinski's Rule of Five (RO5)

The compounds were screened for Lipinski's Rule of Five (RO5) using the Supercomputing facility for bioinformatics and computational biology tool (Lipinski, 2004; Jayaram *et al.*, 2012). This screening process examines parameters such as molecular weight, number of hydrogen bond donors, hydrogen bond acceptors, log P value, and molar refractivity to assess drug-likeness and predict the likelihood of success in in vivo trials.

Pharmacological Properties

The potential biological activities of the compounds were predicted using the PASS online web server (<http://way2drug.com/passonline/>, accessed on 21 December 2024) (Filimonov *et al.*, 2014). This tool relies on two probabilities: Pa, which indicates the likelihood of a compound being active, and Pi, which reflects the likelihood of it being inactive. Both values range from 0 to 1. Only those activities where Pa > 0.8 and Pi < 0.02 were considered, as these thresholds suggest a higher level of predictive accuracy and relevance.

Gene Expression Profiles

The DIGEP Pred online server (<http://www.way2drug.com/ge/>, accessed on 30 December 2024) was utilized to predict the potential effects of the compounds on gene expression. Upregulation refers to a cell's ability to enhance the expression of a particular gene in response to a stimulus, while downregulation is the opposite. The prediction is based on PASS (Prediction of Activity Spectra for Substances) technology, and training sets created on the basis of data on drug-induced changes of gene expression profiles retrieved from Comparative Toxicogenomics Database (CTD), Connectivity Map Database and L1000 Project (Lagunin *et al.*, 2013). Only genes where Pa > 0.85 and Pi < 0.04 were considered.

Cytotoxicity Prediction

The potential cytotoxic effects of the compounds on human tumor cell lines were assessed using CLC-Pred (Cell Line Cytotoxicity Predictor) web service (<http://www.way2drug.com/cell-line/>, accessed on 5 January 2025) (Lagunin *et al.*, 2018). CLC-Pred is an in silico tool designed to predict the cytotoxicity of chemical compounds in both non-transformed and cancerous cell lines, based on their structural formulas. The prediction is based on PASS (Prediction of Activity Spectra for Substances) technology (<https://www.way2drug.com/PASSonline>) and the training set created on the basis of data on cytotoxicity retrieved from ChEMBLdb (version 23) (<https://www.ebi.ac.uk/chembl/db/>). Only cell lines where Pa > 0.3 and Pi < 0.04 were considered.

Macromolecular Targets Prediction

For macromolecular target prediction, SwissTargetPrediction (<http://www.swisstargetprediction.ch/index.php>, accessed on 5 January 2025) was employed to identify the most likely macromolecular targets of the compounds. The prediction method combines 2D and 3D similarity with a database of 370,000 known active molecules on more than 3000 proteins from various species (Daina *et al.*, 2019). The targets with probabilities > 0.04 from the SwissTargetPrediction results were prioritized, as they indicate a stronger predicted interaction between the compound and the target, based on the model's scoring.

RESULTS

Toxicity Prediction

Among the identified compounds, β -Caryophyllene and Germacrene D exhibited the highest LD50 values (5300 mg/kg), marking them as the least toxic within the group (see Table 1). Similarly, compounds including Caryophyllene oxide, 1,5-Epoxyalsalial-4(14)-ene, Beta-Selinene, Salvia-4(14)-en-1-one, Phytol, β -Farnesene, and Nerolidol (LD50: 5000 mg/kg) also demonstrated high safety margins. These values indicate a robust tolerance threshold, which is favorable for formulations requiring minimal toxicity risks.

Spathulenol, α -Bisabolene, and 4,10(14)-Cadinadien-beta-ol presented slightly lower LD50 values, ranging between 3900 and 4390 mg/kg, though they remain well within the low-toxicity classification

Table 1. Prediction of the toxicity of the tested compounds.

Compounds	Molecular Formula	Smile	Predicted Toxicity Class	Predicted LD50 (mg/kg)
Caryophyllene oxide	C15H24O	<chem>C[C@@]12CC[C@@H]3[C@H](CC3(C)C)C(=C)C[C@H]1O2</chem>	5	5000
Spathulenol	C15H24O	<chem>C[C@@]1(CC[C@@H]2[C@@H]1[C@H]3[C@H](C3(C)C)CCC2=C)O</chem>	5	3900
Salvia-4(14)-en-1-one	C15H24O	<chem>CC(C)[C@@H]1CC[C@@]2([C@H]1CC(=C)CCC2=O)C</chem>	5	5000
Beta-Selinene	C15H24	<chem>CC(=C)[C@@H]1CC[C@]2(CCCC(=C)[C@@H]2C1)C</chem>	5	5000
4,10(14)-Cadinadien-beta-ol	C15H24O	<chem>CC1=CC2C(CC1)C(=C)C[C@@H](C2C(C)C)O</chem>	5	4300
1,5-Epoxyalsalial-4(14)-ene	C15H24O	<chem>CC(C)C1CCC2(C1C3C(=C)CCC2O3)C</chem>	5	5000
β -Caryophyllene	C15H24	<chem>C/C1=C\CCC(=C)[C@H]2CC([C@@H]2CC1)(C)C</chem>	5	5300
Germacrene D	C15H24	<chem>C/C1=C\CCC(=C)/C=C/[C@@H](CC1)C(C)C</chem>	5	5300
Phytol	C20H40O	<chem>C[C@@H](CCC[C@@H](C)CCC/C(=C/CO)/C)CC(C)C</chem>	5	5000
Nerolidol	C15H26O	<chem>CC(=CCC/C(=C/CCC(C)(C=C)O)/C)C</chem>	5	5000
β -Farnesene	C15H24	<chem>CC(=CCC/C(=C/CCC(=C)C=C)/C)C</chem>	5	5000
α -bisabolene	C13H18O2	<chem>CC1=CCC(CC1)C(=CCC=C(C)C)C</chem>	5	4390
Carene	C10H16	<chem>CC1=CCC2C(C1)C2(C)C</chem>	5	4800

Pharmacokinetic and Drug Likeness Screening

The SwissADME analysis of the terpenoid compounds from *Centaurea verutum* essential oil provided comprehensive insights into their pharmacokinetic properties (see Table 2). All compounds showed a consistent bioavailability score of 0.55, indicating good potential for oral bioavailability. Most of the compounds were predicted to have high gastrointestinal absorption (GIA), with exceptions such as Beta-Selinene, β -Caryophyllene, Germacrene D, Phytol, and Carene, which displayed low GIA.

The iLOGP values ranged from 2.63 to 4.85, reflecting moderate to high lipophilicity. Compounds like Phytol and β -Farnesene exhibited higher iLOGP values, indicating stronger lipophilic tendencies, which may affect their permeability across biological membranes. In terms of blood-brain barrier (BBB) permeability, most compounds, such as Caryophyllene oxide, Spathulenol, and Nerolidol, were predicted to penetrate the BBB, while others, such as Beta-Selinene and β -Caryophyllene, were not.

The compounds showed no inhibition of CYP1A2 or CYP3A4 enzymes, except Nerolidol and β -Farnesene, which were identified as CYP1A2 inhibitors.

All of the compounds, except for Phytol, were not identified as substrates for P-glycoprotein (P-gp), indicating that they are unlikely to be actively pumped out of cells by this efflux transporter. Phytol, on the other hand, was flagged as a P-gp substrate, indicating it may be actively pumped out of cells, potentially affecting its bioavailability. Water solubility, as indicated by ESOL (Log S), ranged from -3.13 to -5.98, with Phytol being the least soluble.

Table 2. Prediction of the ADMET properties of the tested compounds.

Phytochemicals	ESOL (Log S)	GIA	BBB permeant	P-gp substrate	CYP3A4 inhibitor	CYP1A2 inhibitor	(iLOGP)	Bioavailability score
Caryophyllene oxide	-3.45	High	Yes	No	No	No	3.15	0.55
Spathulenol	-3.17	High	Yes	No	No	No	3.04	0.55
Salvial-4(14)-en-1-one	-3.40	High	Yes	No	No	No	2.87	0.55
Beta-Selinene	-4.47	Low	No	No	No	No	3.31	0.55
4,10(14)Cadinadien-beta-ol	-3.13	High	Yes	No	No	No	3.13	0.55
1,5-Epoxy-salvial-4(14)-ene	-3.52	High	Yes	No	No	No	3.15	0.55
β -Caryophyllene	-3.87	Low	No	No	No	No	3.25	0.55
Germacrene D	-4.03	Low	No	No	No	No	3.31	0.55
Phytol	-5.98	Low	No	Yes	No	No	4.85	0.55
Nerolidol	-3.80	High	Yes	No	No	Yes	3.64	0.55
β -Farnesene	-4.44	Low	No	No	No	Yes	3.86	0.55
α -bisabolene	-4.92	Low	No	No	No	No	3.58	0.55
Carene	-3.44	Low	Yes	No	No	No	2.63	0.55

The radar plots generated from the SwissADME analysis provide an overview of the physicochemical properties of the tested compounds. These properties include lipophilicity (LIPO), size, polarity (POLAR), solubility (INSOLU), saturation (INSATU), and flexibility (FLEX). Each compound exhibits a unique profile, reflecting its potential for bioavailability and drug-likeness. For instance, Phytol shows high flexibility and lipophilicity, whereas compounds such as Salvial-(14)-en-1-one and Caryophyllene oxide demonstrated a balanced profile (see [Figure 1](#)).

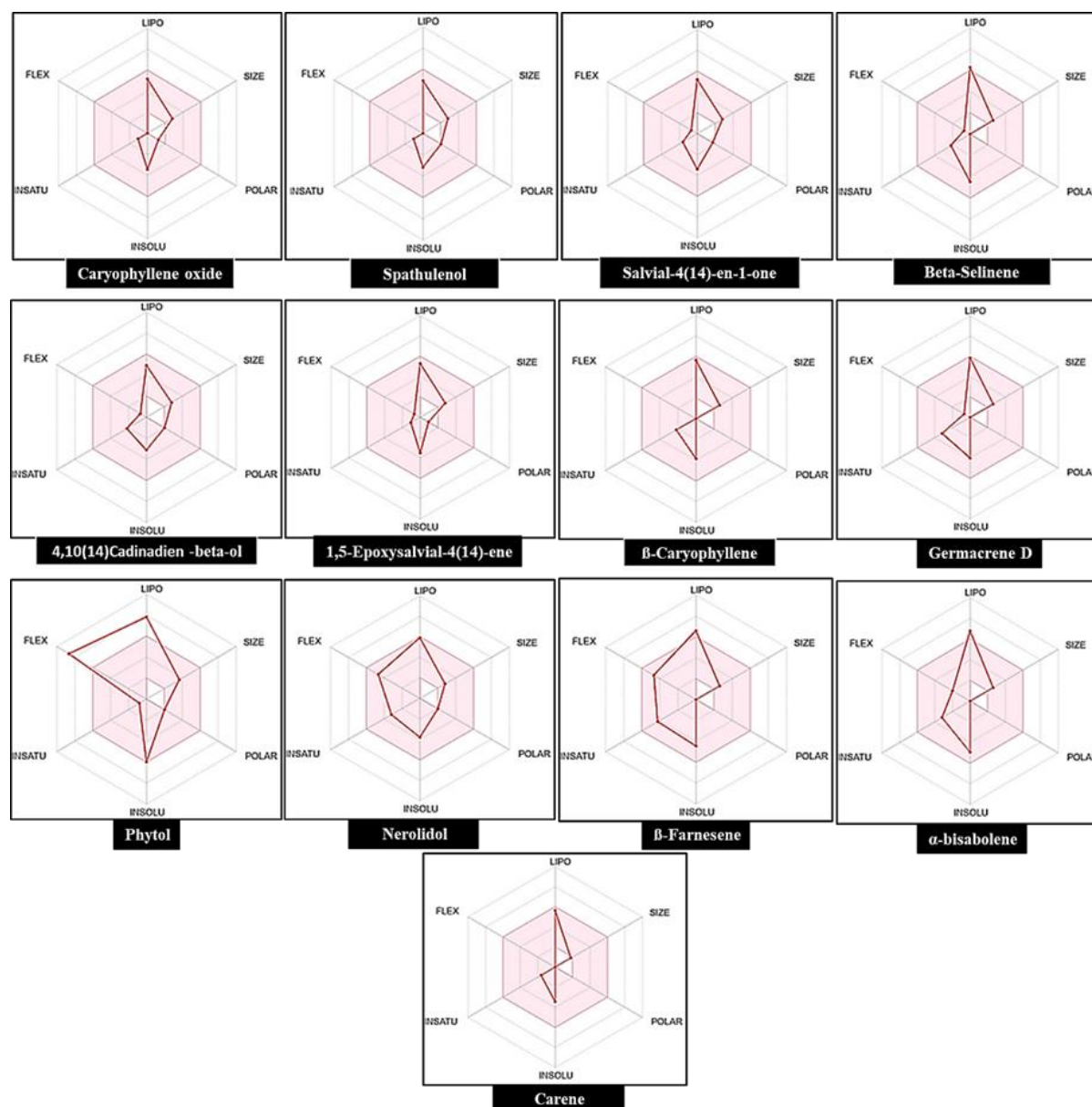


Figure 1. Radar plots of the tested compounds.

LIPO: lipophilicity, SIZE: size, POLAR: polarity, INSOLU: insolubility, INSATU: unsaturation, FLEX: flexibility.

Lipinski's Rule of Five

The evaluation of the Lipinski's Rule of Five parameters for the terpenoid compounds in *Centaurea verutum* essential oil revealed a variety of molecular characteristics that influence their drug-like properties (see Table 3).

- **Molecular Mass:** The majority of compounds have molecular masses between 204 and 220 Da. However, Phytol stands out with a higher molecular mass of 296 Da.
- **LogP (Lipophilicity):** The LogP values indicate moderate lipophilicity for the majority of compounds. The LogP values range from 2.99 (Carene) to 6.36 (Phytol). These values suggest that most compounds should be able to cross biological membranes effectively, with Phytol being the most lipophilic compound, which could imply a greater affinity for lipid environments.
- **Hydrogen Bond Acceptors (HBA) and Donors (HBD):** The hydrogen bonding capabilities of the compounds are also within acceptable limits. Most compounds have 0-1 hydrogen bond acceptor (HBA), which aligns with the Lipinski rule that suggests a maximum of 10 HBAs for good drug-likeness. Some compounds such as Phytol, Spathulenol, 4,10(14)-

Cadinadien-beta-ol, and Nerolidol have 1 HBDs, while the others have 0 HBDs, which also aligns with the Lipinski rule.

- **Molar Refractivity:** The molar refractivity values range from 43.75 (Carene) to 95.56 (Phytol), with most compounds around 66-68. These values suggest good balance in size and polarity, which is essential for efficient absorption and distribution.

Table 3. The analysis results of lipinski rules of five.

Phytochemicals	Mass	Hydrogen bond donor	Hydrogen bond acceptor	LOGP	Molar refractivity
Rules	Less than 500 Da	Less than 5	Less than 10	Less than 5	between 40-130
Caryophyllene oxide	220	0	1	3.93	66.26
Spathulenol	220	1	1	3.38	65.97
Salvial-4(14)-en-1-one	220	0	1	3.98	67.15
Beta-Selinene	204	0	0	4.72	66.74
4,10(14)Cadinadien -beta-ol	220	1	1	3.55	68.06
1,5-Epoxysalvial-4(14)-ene	220	0	1	3.79	66.19
β -Caryophyllene	204	0	0	4.72	66.74
Germacrene D	204	0	0	4.89	68.83
Phytol	296	1	1	6.36	95.56
Nerolidol	222	1	1	4.39	72.47
β -Farnesene	204	0	0	5.20	70.99
α -bisabolene	204	0	0	5.03	68.90
Carene	136	0	0	2.99	43.75

Pharmacological Properties

The biological activity profiles of the evaluated terpenoid compounds highlighted several shared pharmacological properties (see Table 4). A notable number of compounds, including Caryophyllene oxide, Spathulenol, Salvial-4(14)-en-1-one, Beta-selinene, 4,10(14)-Cadinadien-beta-ol, 1,5-Epoxysalvial-4(14)-ene, β -Caryophyllene, Germacrene D, β -Farnesene and α -Bisabolene, exhibited significant antieczematic activity ($Pa > 0.8$). This consistent finding suggests that these terpenoids may modulate inflammatory pathways associated with eczema. Several terpenoids, including Caryophyllene oxide, Salvial-4(14)-en-1-one, Beta-selinene, 4,10(14)-Cadinadien-beta-ol, 1,5-Epoxysalvial-4(14)-ene, β -Caryophyllene, Germacrene D and β -Farnesene, demonstrated antineoplastic activity ($Pa > 0.8$). These compounds also exhibited related activities such as apoptosis agonism (Caryophyllene oxide, Beta-selinene, β -Caryophyllene) and inhibition of HIF1A expression (Caryophyllene oxide). This convergence of activities supports their potential role in cancer treatment, targeting mechanisms such as tumor cell survival, hypoxia adaptation, and uncontrolled proliferation. Compounds like Beta-selinene, Phytol, β -Farnesene, and α -Bisabolene showed retinol dehydrogenase inhibitory activity, implicating their involvement in vitamin A metabolism and potential applications in dermatological disorders. Phytol, Nerolidol, and β -Farnesene demonstrated activities related to lipid metabolism, including lipid metabolism regulation, prenyl-diphosphatase inhibition, and fatty-acyl-CoA synthase inhibition. These properties suggest that these terpenoids may play a role in modulating isoprenoid biosynthesis, relevant for cellular signaling, membrane structure maintenance, antimicrobial defense, and addressing metabolic disorders. Nerolidol, Phytol and β -Farnesene exhibited strong mucomembranous protector activity. This activity is indicative of their ability to maintain the integrity of mucosal barriers, which could be beneficial in inflammatory bowel diseases, respiratory conditions, or infections affecting mucosal surfaces. Compounds like Beta-selinene, 4,10(14)-Cadinadien-beta-ol, and Phytol demonstrated a wide array of activities, indicating their potential as multitarget agents. Compounds such as Beta-selinene, 4,10(14)-Cadinadien-

beta-ol, Germacrene D, and α -Bisabolene exhibited carminative activity ($Pa > 0.8$). This property highlights their potential for alleviating gastrointestinal discomfort, by relaxing smooth muscles or reducing gas accumulation in the digestive tract. Certain terpenoids, including Beta-selinene, 4,10(14)-Cadinadien-beta-ol, α -Bisabolene, and Carene, were identified as substrates for specific cytochrome P450 (CYP) enzymes. This suggests these compounds could play a role in modulating drug metabolism. Salvial-4(14)-en-1-one, Phytol, and Carene exhibited inhibition of testosterone 17beta-dehydrogenase, indicating potential utility in hormonal regulation or conditions associated with androgen excess, such as polycystic ovary syndrome (PCOS) or prostate hyperplasia.

Table 4. Bioactivity prediction of the tested compounds.

N	Compounds	Pa	Pi	Activity
1	Caryophyllene oxide	0.950	0.004	Antineoplastic
		0.836	0.006	Apoptosis agonist
		0.810	0.011	HIF1A expression inhibitor
		0.812	0.016	Antieczematic
2	Spathulenol	0.826	0.013	Antieczematic
5	Salvial-4(14)-en-1-one	0.892	0.005	Antineoplastic
		0.883	0.006	Antieczematic
		0.825	0.019	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
		0.935	0.003	Antieczematic
6	β -Selinene	0.936	0.004	Antineoplastic
		0.894	0.004	Dermatologic
		0.870	0.001	Retinol dehydrogenase inhibitor
		0.833	0.006	Apoptosis agonist
		0.833	0.008	CYP2C substrate
		0.826	0.002	Transcription factor stimulant
		0.826	0.002	Transcription factor NF kappa B stimulant
		0.824	0.003	CYP2C11 substrate
		0.818	0.004	Antipsoriatic
		0.806	0.004	Carminative
8	4,10(14)Cadinadien -beta-ol	0.933	0.003	Antieczematic
		0.887	0.005	Antineoplastic
		0.845	0.003	Carminative
		0.834	0.028	CYP2C12 substrate
		0.816	0.009	Prostaglandin-E2 9-reductase inhibitor
10	1,5-Epoxy-salvial-4(14)-ene	0.897	0.005	Antineoplastic
		0.879	0.006	Antieczematic
11	β -Caryophyllene	0.915	0.005	Antineoplastic
		0.897	0.005	Antieczematic
		0.847	0.005	Apoptosis agonist
13	Germacrene D	0.915	0.004	Antieczematic
		0.885	0.002	Carminative
		0.817	0.010	Antineoplastic
15	Phytol	0.911	0.002	Prenyl-diphosphatase inhibitor
		0.907	0.001	Retinol dehydrogenase inhibitor
		0.905	0.005	Ubiquinol-cytochrome-c reductase inhibitor
		0.893	0.007	Phobic disorders treatment
		0.885	0.002	Undecaprenyl-phosphate mannosyltransferase inhibitor
		0.862	0.012	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
		0.852	0.009	Mucomembranous protector
		0.845	0.012	Acrocylindropepsin inhibitor
		0.845	0.012	Saccharopepsin inhibitor
		0.845	0.012	Chymosin inhibitor

16	Nerolidol	0.835	0.001	Plastoquinol-plastocyanin reductase inhibitor
		0.828	0.005	Lipid metabolism regulator
		0.821	0.016	Polyporopepsin inhibitor
		0.817	0.014	Sugar-phosphatase inhibitor
		0.804	0.017	Alkenylglycerophosphocholine hydrolase inhibitor
		0.814	0.030	Aspulvinone dimethylallyltransferase inhibitor
		0.983	0.002	Mucomembranous protector
		0.916	0.003	Cell adhesion molecule inhibitor
		0.909	0.002	Prenyl-diphosphatase inhibitor
		0.875	0.002	Undecaprenyl-phosphate mannosyltransferase inhibitor
		0.865	0.001	BRAF expression inhibitor
		0.861	0.004	Lipid metabolism regulator
		0.867	0.016	Aspulvinone dimethylallyltransferase inhibitor
		0.843	0.004	Antisecretoric
		0.835	0.002	Ecdysone 20-monooxygenase inhibitor
17	β -Farnesene	0.831	0.004	Fatty-acyl-CoA synthase inhibitor
		0.811	0.008	GST A substrate
		0.800	0.007	Antiinflammatory
		0.804	0.013	Beta-adrenergic receptor kinase inhibitor
		0.804	0.013	G-protein-coupled receptor kinase inhibitor
		0.952	0.003	Mucomembranous protector
		0.937	0.001	Prenyl-diphosphatase inhibitor
		0.913	0.001	Undecaprenyl-phosphate mannosyltransferase inhibitor
		0.910	0.006	Aspulvinone dimethylallyltransferase inhibitor
		0.898	0.002	Fatty-acyl-CoA synthase inhibitor
		0.892	0.003	CYP2E1 inhibitor
		0.884	0.005	Antineoplastic
20	α -bisabolene	0.875	0.004	Antineoplastic (breast cancer)
		0.843	0.001	Retinol dehydrogenase inhibitor
		0.824	0.002	BRAF expression inhibitor
		0.814	0.001	2,3-Oxidosqualene-lanosterol cyclase inhibitor
		0.816	0.015	Antieczematic
22	Carene	0.920	0.004	Antieczematic
		0.895	0.001	Retinol dehydrogenase inhibitor
		0.867	0.003	Carminative
		0.815	0.017	CYP2J substrate
		0.878	0.009	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
		0.860	0.009	CYP2J substrate
		0.863	0.021	CYP2C12 substrate
		0.815	0.005	Antidyskinetic

Gene Expression Profiles

The results showed that certain compounds have a broad impact on specific genes, with multiple compounds influencing the expression of the same gene (see [Table 5](#)), highlighting the potential for these compounds to have overlapping biological effects.

Several compounds showed an upregulatory effect on the expression of Forkhead Box O1 (FOXO1) gene, such as Salvial-4(14)-en-1-one, β -Selinene, and Germacrene D. FOXO1 is an important regulator of oxidative stress response, cell proliferation, cell death, and metabolism (Zhao *et al.*, 2023). It plays an essential role in regulating cellular homeostasis, especially under oxidative stress conditions. The modulation of FOXO1 expression may indicate a compound's ability to influence cellular resilience to oxidative damage, which is important for reducing age-related cell damage and promoting cellular repair mechanisms. Similarly, Carene, Germacrene D, 4,10(14)-Cadinadien-beta-ol, 1,5-Epoxy-salvial-4(14)-ene, Salvial-4(14)-en-1-one, β -Selinene, β -Caryophyllene, and Spathulenol showed an upregulatory effect on the expression

of Protein Phosphatase 1 Catalytic Subunit Beta (PPP1CB). This gene encodes a catalytic subunit of protein phosphatase 1, which plays a significant role in various cellular processes by regulating signal transduction, gene expression, cell cycle progression, and metabolism (Mestareehi and Abu-Farsakh, 2024). In contrast, Caryophyllene oxide, Spathulenol, 4,10(14)Cadinadien-beta-ol, β -Selinene and β -Caryophyllene showed a downregulatory effect on the expression of Sestrin 1 (SESN1) gene. SESN1 is a stress-responsive protein that plays a critical role in cellular homeostasis and protection against stress-induced damage, including oxidative stress and DNA damage (Sun *et al.*, 2023). 4,10(14)Cadinadien-beta-ol, 1,5-Epoxysalvial-4(14)-ene, β -Selinene, Salvial-4(14)-en-1-one, Germacrene D, β -Caryophyllene, and Spathulenol showed a downregulatory effect on the expression of Thymosin Beta-15A (TMSB15A) gene. TMSB15A is part of the thymosin beta family; it belongs to the group of WASP-homolog 2-domain binding proteins that are required for the repeated depolymerization of actin filaments during cellular movements (Samaha *et al.*, 2020).

Table 5. Probable effects of the tested compounds on the expression of some genes: mRNA-based prediction result.

Compounds	Pa	Pi	Genes (Up Regulation)	Pa	Pi	Genes (Down Regulation)
Caryophyllene oxide	0.879	0.019	WT1	0.929	0.008	NSF
				0.904	0.009	SESN1
				0.860	0.023	MYNN
Spathulenol	0.904	0.013	NUCB2	0.892	0.014	SESN1
	0.871	0.009	ALDOA	0.902	0.037	TMSB15A
	0.885	0.031	FOXO1	0.887	0.035	TMEM194A
	0.880	0.033	LGALS9	0.872	0.028	NAP1L1
	0.877	0.034	PPP1CB	0.877	0.037	GART
	0.879	0.037	CACNG4	0.871	0.034	CTSL2
	0.879	0.037	CHRNE	0.871	0.034	GPR19
	0.874	0.033	COL22A1	0.871	0.034	LFNG
	0.874	0.033	KLK13	0.871	0.034	MIR20B
	0.862	0.022	FOXO4	0.871	0.034	NUP54
	0.871	0.034	CNN2	0.871	0.034	PRR16
	0.871	0.034	DYSF	0.871	0.034	SP4
	0.871	0.034	FAM110C	0.862	0.031	ASPH
	0.871	0.034	MYO5B	0.855	0.025	TNPO2
	0.871	0.034	PPFIBP2	0.866	0.036	CBX2
	0.862	0.026	PLXNA2			
	0.853	0.022	EPB41L3			
	0.866	0.036	TMEM56			
	0.838	0.019	APPBP2			
	0.851	0.038	CREB3L1			
	0.851	0.038	RNASEL			
Salvial-4(14)-en-1-one	0.935	0.013	FOXO1	0.915	0.029	TMSB15A
	0.896	0.005	KLK7	0.896	0.030	TMEM194A
	0.915	0.025	CACNG4	0.886	0.027	CTSL2
	0.915	0.025	CHRNE	0.886	0.027	GPR19
	0.890	0.005	TRIOBP	0.886	0.027	LFNG
	0.898	0.025	PPP1CB	0.886	0.027	MIR20B
	0.893	0.025	COL22A1	0.886	0.027	NUP54
	0.893	0.025	KLK13	0.886	0.027	PRR16
	0.885	0.026	CREB3L1	0.886	0.027	SP4
	0.885	0.026	RNASEL	0.886	0.032	GART
	0.886	0.027	CNN2	0.883	0.029	ADORA2B
	0.886	0.027	DYSF	0.883	0.029	CDCA4
	0.886	0.027	FAM110C	0.883	0.029	CENPJ
	0.886	0.027	MYO5B	0.883	0.029	DNAJC9
	0.886	0.027	PPFIBP2	0.883	0.029	DONSON
	0.880	0.024	DNAJB14	0.883	0.029	HAUS8

	0.878	0.022	PDZD2	0.883	0.029	MCMBP
	0.886	0.030	LGALS9	0.883	0.029	NUP155
	0.883	0.029	PFKFB2	0.883	0.029	PARP2
	0.883	0.029	SH3BGRL	0.883	0.029	RMI1
	0.883	0.029	TRIM13	0.883	0.029	SCARA3
	0.877	0.028	KCNH2	0.883	0.029	SLC16A14
	0.877	0.028	PLLP	0.883	0.029	SUV39H2
	0.877	0.028	SLC25A4	0.877	0.028	MAST4
	0.877	0.028	ZYX	0.877	0.028	MSH5
	0.880	0.032	TMEM56	0.877	0.028	TREX1
	0.838	0.021	NUDT11	0.880	0.032	CBX2
	0.840	0.026	FOXO4	0.876	0.034	RPS19
	0.838	0.025	CD68	0.865	0.027	GEMIN4
				0.868	0.031	PPIH
				0.865	0.030	ASPH
				0.869	0.034	C9ORF40
	0.908	0.028	CACNG4	0.933	0.004	MYNN
	0.908	0.028	CHRNE	0.921	0.005	SESN1
	0.891	0.026	COL22A1	0.912	0.031	TMSB15A
	0.891	0.026	KLK13	0.888	0.034	TMEM194A
	0.889	0.029	PPP1CB	0.882	0.029	CTSL2
	0.882	0.029	CNN2	0.882	0.029	GPR19
	0.882	0.029	DYSF	0.882	0.029	LFNG
	0.882	0.029	FAM110C	0.882	0.029	MIR20B
	0.882	0.029	MYO5B	0.882	0.029	NUP54
	0.882	0.029	PPFIBP2	0.882	0.029	PRR16
	0.884	0.032	FOXO1	0.882	0.029	SP4
	0.874	0.023	PDZD2	0.858	0.013	EFNB2
	0.881	0.032	LGALS9	0.878	0.036	GART
	0.869	0.021	FOXO4	0.874	0.033	CBX2
	0.874	0.030	CREB3L1	0.873	0.033	ADORA2B
	0.874	0.030	RNASEL	0.873	0.033	CDCA4
	0.874	0.033	TMEM56	0.873	0.033	CENPJ
	0.873	0.033	PFKFB2	0.873	0.033	DNAJC9
	0.873	0.033	SH3BGRL	0.873	0.033	DONSON
	0.873	0.033	TRIM13	0.873	0.033	HAUS8
	0.863	0.026	PLXNA2	0.873	0.033	MCMBP
	0.866	0.031	KCNH2	0.873	0.033	NUP155
	0.866	0.031	PLLP	0.873	0.033	PARP2
	0.866	0.031	SLC25A4	0.873	0.033	RMI1
	0.866	0.031	ZYX	0.873	0.033	SCARA3
	0.860	0.032	DNAJB14	0.873	0.033	SLC16A14
				0.873	0.033	SUV39H2
				0.845	0.006	WDR77
				0.866	0.031	MAST4
				0.866	0.031	MSH5
				0.866	0.031	TREX1
				0.858	0.026	HIG2
				0.861	0.031	ASPH
				0.859	0.031	NAP1L1
4,10(14)Cadinadien - beta-ol	0.939	0.008	PPP1CB	0.918	0.013	PPIH
	0.917	0.010	DNAJB14	0.914	0.030	TMSB15A
	0.909	0.027	CACNG4	0.889	0.027	ADORA2B
	0.909	0.027	CHRNE	0.889	0.027	CDCA4
	0.896	0.017	NUCB2	0.889	0.027	CENPJ
	0.889	0.027	PFKFB2	0.889	0.027	DNAJC9
	0.889	0.027	SH3BGRL	0.889	0.027	DONSON
	0.889	0.027	TRIM13	0.889	0.027	HAUS8
	0.890	0.029	FOXO1	0.889	0.027	MCMBP
	0.884	0.029	COL22A1	0.889	0.027	NUP155

1,5-Epoxysalvial-4(14)-ene	0.884	0.029	KLK13	0.889	0.027	PARP2
	0.881	0.029	CNN2	0.889	0.027	RMI1
	0.881	0.029	DYSF	0.889	0.027	SCARA3
	0.881	0.029	FAM110C	0.889	0.027	SLC16A14
	0.881	0.029	MYO5B	0.889	0.027	SUV39H2
	0.881	0.029	PPFIBP2	0.891	0.032	TMEM194A
	0.878	0.029	CREB3L1	0.886	0.033	GART
	0.878	0.029	RNASEL	0.881	0.029	CTSL2
	0.881	0.032	LGALS9	0.881	0.029	GPR19
	0.875	0.029	KCNH2	0.881	0.029	LFNG
	0.875	0.029	PLLP	0.881	0.029	MIR20B
	0.875	0.029	SLC25A4	0.881	0.029	NUP54
	0.875	0.029	ZYX	0.881	0.029	PRR16
	0.869	0.025	PLXNA2	0.881	0.029	SP4
	0.876	0.033	TMEM56	0.881	0.029	C9ORF40
	0.864	0.030	SQLE	0.875	0.029	MAST4
	0.855	0.023	FOXO4	0.875	0.029	MSH5
				0.875	0.029	TREX1
				0.876	0.033	CBX2
				0.875	0.034	RPS19
				0.864	0.024	SESN1
				0.861	0.030	NAP1L1
				0.861	0.031	ASPH
				0.859	0.029	GEMIN4
β-Caryophyllene	0.931	0.010	PFKFB2	0.931	0.010	ADORA2B
	0.931	0.010	SH3BGR1	0.931	0.010	CDCA4
	0.931	0.010	TRIM13	0.931	0.010	CENPJ
	0.923	0.020	CACNG4	0.931	0.010	DNAJC9
	0.923	0.020	CHRNE	0.931	0.010	DONSON
	0.908	0.021	PPP1CB	0.931	0.010	HAUS8
	0.905	0.020	COL22A1	0.931	0.010	MCMBP
	0.905	0.020	KLK13	0.931	0.010	NUP155
	0.894	0.024	CNN2	0.931	0.010	PARP2
	0.894	0.024	DYSF	0.931	0.010	RMI1
	0.894	0.024	FAM110C	0.931	0.010	SCARA3
	0.894	0.024	MYO5B	0.931	0.010	SLC16A14
	0.894	0.024	PPFIBP2	0.931	0.010	SUV39H2
	0.895	0.026	FOXO1	0.923	0.013	C9ORF40
	0.894	0.026	LGALS9	0.924	0.024	TMSB15A
	0.891	0.024	CREB3L1	0.901	0.027	TMEM194A
	0.891	0.024	RNASEL	0.894	0.024	CTSL2
	0.884	0.018	FOXO4	0.894	0.024	GPR19
	0.885	0.020	PDZD2	0.894	0.024	LFNG
	0.888	0.025	SQLE	0.894	0.024	MIR20B
	0.885	0.022	DNAJB14	0.894	0.024	NUP54
	0.888	0.025	KCNH2	0.894	0.024	PRR16
	0.888	0.025	PLLP	0.894	0.024	SP4
	0.888	0.025	SLC25A4	0.888	0.025	MAST4
	0.888	0.025	ZYX	0.888	0.025	MSH5
	0.884	0.022	PLXNA2	0.888	0.025	TREX1
	0.888	0.029	TMEM56	0.892	0.029	GART
	0.858	0.020	FAM49A	0.888	0.029	CBX2
	0.855	0.020	ABAT	0.884	0.027	BARD1
	0.855	0.020	ERMP1	0.886	0.030	RPS19
	0.855	0.020	MSX2	0.881	0.026	PPIH
	0.848	0.019	NUDT11	0.876	0.025	ASPH
	0.850	0.021	CD68	0.873	0.024	GEMIN4
	0.851	0.034	NUCB2	0.864	0.024	TNPO2
	0.897	0.016	FOXO4	0.923	0.005	SESN1
	0.897	0.026	PPP1CB	0.893	0.013	MYNN

	0.854	0.039	COL22A1	0.856	0.035	PPIH
	0.854	0.039	KLK13			
	0.912	0.019	PPP1CB	0.882	0.026	PPIH
	0.877	0.025	DNAJB14	0.877	0.039	TMEM194A
	0.885	0.039	IL4R	0.863	0.030	NAP1L1
	0.877	0.037	CHRNE	0.852	0.040	CTSL2
	0.860	0.026	PLXNA2	0.852	0.040	GPR19
Germacrene D	0.866	0.036	COL22A1	0.852	0.040	LFNG
	0.866	0.036	KLK13	0.852	0.040	MIR20B
	0.852	0.040	CNN2	0.852	0.040	SP4
	0.852	0.040	DYSF			
	0.852	0.040	FAM110C			
	0.852	0.040	MYO5B			
	0.852	0.040	PPFIBP2			
	0.892	0.008	OS9	0.855	0.006	NADK
Phytol	0.858	0.008	TPI1	0.862	0.024	TNPO2
	0.859	0.014	HSPCB			
	0.977	0.004	GCLC	0.856	0.031	NAP1L1
Nerolidol	0.860	0.026	PLXNA2			
	0.850	0.033	SQLE			
β -Farnesene	0.909	0.016	GCLC	-	-	-
α -bisabolene	-	-	-	-	-	-
Carene	0.865	0.038	PPP1CB	0.866	0.030	SEC16A

Cytotoxicity Prediction

The analysis of the compounds revealed distinct patterns of activity across different cell lines (see Table 6). Caryophyllene oxide, Beta-Selinene, Beta- Caryophyllene, Beta-Farnesene, and 1,5-Epoxy-salvial-4(14)-ene showed significant effects on leukemia cell lines such as HL-60 and NALM-6. These compounds may share molecular targets linked to leukemia-specific pathways. Further studies could assess whether these compounds act as direct inhibitors of key leukemia-promoting enzymes or signaling pathways (e.g., STAT3, JAK/PI3K). Caryophyllene oxide, 4,10(14)-Cadinadien-beta-ol, Beta- Caryophyllene, and Beta-Farnesene demonstrated notable activity on melanoma-related lines, especially SK-MEL-2. Beta-Farnesene was effective against glioblastoma lines, including SNB-75 and SF-295, indicating the potential for targeted brain tumor therapies. Several compounds, including Caryophyllene oxide, Beta-Selinene, Germacrene D, α -bisabolene, Carene and Beta-Caryophyllene, exhibited moderate effects on pancreatic carcinoma (PANC-1), suggesting limited but noteworthy potential. While these compounds may not be highly potent, they could serve as adjuvants in combination therapies. Further investigation into the synergistic or combinatory effects of the essential oil components is warranted.

Table 6. Cytotoxicity probabilities of the tested compounds on some tumor cell lines.

Compound	Pa	Pi	Cell line	Cell line full name	Tissue	Tumor type
Caryophyllene oxide	0.647	0.012	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.589	0.022	NCI-H838	Non-small cell lung cancer. 3 stage	Lung	Carcinoma
	0.532	0.017	SK-MEL-2	Melanoma	Skin	Melanoma
	0.520	0.025	SK-MEL-1	Metastatic melanoma	Skin	Melanoma
	0.437	0.010	NALM-6	Adult B acute lymphoblastic leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.444	0.036	NCI-H187	Small cell lung carcinoma	Lung	Carcinoma
	0.438	0.035	A2058	Melanoma	Skin	Melanoma
	0.364	0.023	8505C	Thyroid gland undifferentiated (anaplastic) carcinoma	Thyroid	Carcinoma
	0.370	0.039	RPMI-8226	Multiple myeloma	Haematopoietic and lymphoid tissue	Myeloma
	0.353	0.035	T98G	Glioblastoma	Brain	Carcinoma
	0.335	0.023	PANC-1	Pancreatic carcinoma	Pancreas	Carcinoma
	0.320	0.011	SMMC-7721	Hepatocellular carcinoma	Liver	Carcinoma
Spathulenol	0.331	0.029	MKN-7	Gastric carcinoma	Stomach	Carcinoma
	0.401	0.031	NALM-6	Adult B acute lymphoblastic leukemia	Haematopoietic and lymphoid tissue	Leukemia
Salvial-4(14)-en-1-one	0.472	0.004	NALM-6	Adult B acute lymphoblastic leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.341	0.037	H9	T-lymphoid	Haematopoietic and lymphoid tissue	Leukemia
	0.314	0.038	MKN-7	Gastric carcinoma	Stomach	Carcinoma
	0.527	0.024	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
Beta-Selinene	0.440	0.008	8505C	Thyroid gland undifferentiated (anaplastic) carcinoma	Thyroid	Carcinoma
	0.425	0.009	SW480	Colon adenocarcinoma	Colon	Adenocarcinoma
	0.419	0.007	PANC-1	Pancreatic carcinoma	Pancreas	Carcinoma
	0.409	0.023	NALM-6	Adult B acute lymphoblastic leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.393	0.013	DLD-1	Colon adenocarcinoma	Colon	Adenocarcinoma
	0.351	0.032	H9	T-lymphoid	Haematopoietic and lymphoid tissue	Leukemia
	0.525	0.017	SK-MEL-2	Melanoma	Skin	Melanoma
4,10(14)-Cadinadien -beta-ol	0.436	0.010	NALM-6	Adult B acute lymphoblastic leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.304	0.003	1A9	Ovarian adenocarcinoma	Ovary	Adenocarcinoma
	0.320	0.035	MKN-7	Gastric carcinoma	Stomach	Carcinoma
	0.528	0.004	DLD-1	Colon adenocarcinoma	Colon	Adenocarcinoma
1,5-Epoxy salvial-4(14)-ene	0.539	0.023	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.441	0.009	NALM-6	Adult B acute lymphoblastic leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.331	0.029	MKN-7	Gastric carcinoma	Stomach	Carcinoma
	0.337	0.037	8505C	Thyroid gland undifferentiated (anaplastic) carcinoma	Thyroid	Carcinoma
β-Caryophyllene	0.506	0.020	SK-MEL-2	Melanoma	Skin	Melanoma
	0.500	0.027	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia

	0.396	0.009	PANC-1	Pancreatic carcinoma	Pancreas	Carcinoma
	0.402	0.030	NALM-6	Adult B acute lymphoblastic leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.465	0.005	NALM-6	Adult B acute lymphoblastic leukemia	Haematopoietic and lymphoid tissue	Leukemia
Germacrene D	0.327	0.031	MKN-7	Gastric carcinoma	Stomach	Carcinoma
	0.317	0.029	PANC-1	Pancreatic carcinoma	Pancreas	Carcinoma
Phytol	0.357	0.020	MKN-7	Gastric carcinoma	Stomach	Carcinoma
Nerolidol	0.340	0.002	U-937/GTB	Histiocytic lymphoma	Blood	Lymphoma
	0.319	0.035	MKN-7	Gastric carcinoma	Stomach	Carcinoma
	0.665	0.007	SF-295	Glioblastoma	Brain	Glioblastoma
	0.503	0.020	SK-MEL-2	Melanoma	Skin	Melanoma
	0.489	0.019	SR	Adult immunoblastic lymphoma	Haematopoietic and lymphoid tissue	Lymphoma
	0.470	0.017	LOX IMVI	Melanoma	Skin	Melanoma
	0.470	0.028	SK-OV-3	Ovarian carcinoma	Ovary	Carcinoma
	0.470	0.034	PC-3	Prostate carcinoma	Prostate	Carcinoma
β-Farnesene	0.427	0.021	RPMI-8226	Multiple myeloma	Haematopoietic and lymphoid tissue	Myeloma
	0.434	0.039	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.392	0.002	U-937/GTB	Histiocytic lymphoma	Blood	Lymphoma
	0.397	0.035	NALM-6	Adult B acute lymphoblastic leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.381	0.025	RXF 393	Renal carcinoma	Kidney	Carcinoma
	0.381	0.038	SNB-75	Glioblastoma	Nervous system	Glioblastoma
	0.338	0.032	HOS	Osteosarcoma	Bone	Sarcoma
	0.324	0.033	MKN-7	Gastric carcinoma	Stomach	Carcinoma
	0.500	0.020	786-0	Renal carcinoma	Kidney	Carcinoma
	0.451	0.023	U-251	Glioma	Brain	Glioma
	0.429	0.031	HCC 2998	Colon adenocarcinoma	Colon	Adenocarcinoma
	0.414	0.031	UO-31	Renal carcinoma	Kidney	Carcinoma
α-bisabolene	0.404	0.027	SK-MEL-5	Melanoma	Skin	Melanoma
	0.378	0.026	LOX IMVI	Melanoma	Skin	Melanoma
	0.319	0.029	PANC-1	Pancreatic carcinoma	Pancreas	Carcinoma
	0.312	0.039	MKN-7	Gastric carcinoma	Stomach	Carcinoma
	0.559	0.037	Hs 683	Oligodendroglioma	Brain	Glioma
	0.448	0.019	HOP-18	Non-small cell lung carcinoma	Lung	Carcinoma
Carene	0.332	0.010	SMMC-7721	Hepatocellular carcinoma	Liver	Carcinoma
	0.332	0.024	PANC-1	Pancreatic carcinoma	Pancreas	Carcinoma
	0.334	0.028	MKN-7	Gastric carcinoma	Stomach	Carcinoma

Macromolecular Targets Prediction

The study conducted on the potential targets of various compounds found in the essential oil of *Centaurea verutum* using the SwissTargetPrediction platform provided detailed insights into the target classes of these compounds. While several compounds exhibited low to moderate probabilities with various protein targets, β -Caryophyllene showed higher probabilities of binding to key receptors and enzymes, which may suggest their relevance in the treatment of a range of conditions, particularly related to inflammation, metabolic disorders, and neurological conditions. The interaction of β -Caryophyllene with PPARA and CNR2 is particularly noteworthy, as both receptors are involved in lipid metabolism and inflammatory pathways. Additionally, the interaction with CNR2, a receptor in the endocannabinoid system, suggests that β -Caryophyllene could have potential anti-inflammatory and neuroprotective effects.

Notably, Caryophyllene oxide exhibited a range of interactions with several targets, including Squalene monooxygenase (SQLE) and Lanosterol synthase (LSS), both enzymes, with relatively low probabilities of 0.06. It also showed interaction with electrochemical transporters like Serotonin transporter (SLC6A4) and Dopamine transporter (SLC6A3), with a similar low probability of 0.06. Spathulenol, another compound, interacted with UDP-glucuronosyltransferase 2B7 (UGT2B7) and 11-beta-hydroxysteroid dehydrogenase 1 (HSD11B1), both enzymes, with probabilities of 0.12 and 0.06, respectively. Additionally, it showed some interaction with other enzymes like Indoleamine 2,3-dioxygenase (IDO1) and Cyclooxygenase-1 (PTGS1), which are involved in metabolic processes and inflammatory responses. For Salvial-4(14)-en-1-one, targets included several proteases such as Cathepsin K (CTSK) and Epoxide hydratase (EPHX2), as well as enzymes involved in cellular regulation like Poly [ADP-ribose] polymerase-1 (PARP1). 4,10(14)-Cadinadien-beta-ol showed interactions with several nuclear receptors, such as LXR-alpha, Androgen Receptor, Estrogen receptor alpha, Estrogen receptor beta, Nuclear receptor ROR-alpha, Peroxisome proliferator-activated receptor alpha, Mineralocorticoid receptor. 1,5-Epoxysalvial-4(14)-ene primarily interacted with serotonin and dopamine transporters, indicating its potential neurological effects. On the other hand, β -Caryophyllene emerged as one of the more promising compounds, showing strong interaction with Peroxisome proliferator-activated receptor alpha (PPARA) and Cannabinoid receptor 2 (CNR2) with probabilities of 0.69, indicating potential therapeutic roles in metabolic and neuroinflammatory conditions. Germacrene D also displayed affinity with similar targets, including PPARA and CNR2. Phytol interacted with enzymes like UDP-glucuronosyltransferase 2B7 (UGT2B7) and nuclear receptors like Androgen Receptor (AR) with probabilities of 0.09, suggesting moderate potential for therapeutic applications. Nerolidol demonstrated a strong interaction with Squalene monooxygenase (SQLE), an enzyme critical in sterol biosynthesis, with a binding value of 0.11 ([Table 7](#), [Appendix to table No.7](#) and [Figure 2](#)).

Table 7. Macromolecular targets prediction of the tested compounds.

Compounds	Target	Common name	Uniprot ID	Target class	Probability	Known actives (3D/2D)
Caryophyllene oxide	Squalene monooxygenase (by homology)	SQLE	Q14534	Enzyme	0.06	0 / 1
	Lanosterol synthase	LSS	P48449	Enzyme	0.06	0 / 8
	Serotonin transporter	SLC6A4	P31645	Electrochemical transporter	0.06	17 / 0
	Dopamine transporter	SLC6A3	Q01959	Electrochemical transporter	0.06	30 / 0
Spathulenol	UDPglucuronosyltransferase 2B7	UGT2B7	P16662	Enzyme	0.12	27 / 22
	11-beta-hydroxysteroid dehydrogenase 1	HSD11B1	P28845	Enzyme	0.06	52 / 18
	Indoleamine 2,3-dioxygenase	IDO1	P14902	Enzyme	0.06	6 / 0
	Cyclooxygenase-1	PTGS1	P23219	Oxidoreductase	0.06	1 / 1
	LXR-alpha	NR1H3	Q13133	Nuclear receptor	0.06	1 / 18
	Progesterone receptor	PGR	P06401	Nuclear receptor	0.06	41 / 0
	Dopamine transporter (by homology)	SLC6A3	Q01959	Electrochemical transporter	0.06	51 / 0
	Isoprenylcysteine carboxyl methyltransferase	ICMT	O60725	Enzyme	0.06	55 / 0
	Cathepsin K	CTSK	P43235	Protease	0.06	69 / 0
	11-beta-hydroxysteroid dehydrogenase 1	HSD11B1	P28845	Enzyme	0.06	145 / 17
Salvial-4(14)-en-1-one	Alcohol dehydrogenase alpha chain	ADH1A	P07327	Oxidoreductase	0.06	12 / 0
	Epoxide hydratase (by homology)	EPHX2	P34913	Protease	0.06	64 / 0
	Poly [ADP-ribose] polymerase-1	PARP1	P09874	Enzyme	0.06	27 / 0
	Melatonin receptor 1A	MTNR1A	P48039	Family A G protein-coupled receptor	0.06	91 / 0
	Melatonin receptor 1B	MTNR1B	P49286	Family A G protein-coupled receptor	0.06	66 / 0
	Zinc finger protein GLI2	GLI2	P10070	Transcription factor	0.06	1 / 0
	Zinc finger protein GLI1	GLI1	P08151	Transcription factor	0.06	1 / 0
	Muscarinic acetylcholine receptor M2	CHRM2	P08172	Family A G protein-coupled receptor	0.06	23 / 0
	Muscarinic acetylcholine receptor M1	CHRM1	P11229	Family A G protein-coupled receptor	0.06	55 / 0
	Epoxide hydrolase 1	EPHX1	P07099	Protease	0.06	57 / 0
	Cathepsin L	CTSL	P07711	Protease	0.06	24 / 0
	Cathepsin (B and K)	CTSB	P07858	Protease	0.06	30 / 0
	P2X purinoceptor 7	P2RX7	Q99572	Ligand-gated ion channel	0.06	70 / 0
	Metabotropic glutamate receptor 5	GRM5	P41594	Family C G protein-coupled receptor	0.06	40 / 0
	Alcohol dehydrogenase gamma chain	ADH1C	P00326	Oxidoreductase	0.06	4 / 0
	Translocator protein (by homology)	TSPO	P30536	Membrane receptor	0.06	85 / 0
	MAP kinase p38 alpha	MAPK14	Q16539	Kinase	0.06	20 / 0
	Norepinephrine transporter	SLC6A2	P23975	Electrochemical transporter	0.06	26 / 0
	Polyadenylate-binding protein 1	PABPC1	P11940	Unclassified protein	0.06	7 / 0

	Indoleamine 2,3-dioxygenase	IDO1	P14902	Enzyme	0.06	8 / 0
	Thymidylate synthase	TYMS	P04818	Transferase	0.06	3 / 0
	Quinone reductase 2	NQO2	P16083	Enzyme	0.06	12 / 0
Beta-Selinene	-	-	-	-	-	-
	LXR-alpha	NR1H3	Q13133	Nuclear receptor	0.12	2 / 20
	Androgen Receptor	AR	P10275	Nuclear receptor	0.11	76 / 76
	Cytochrome P450 19A1	CYP19A1	P11511	Cytochrome P450	0.07	78 / 108
	Serotonin transporter	SLC6A4	P31645	Electrochemical transporter	0.06	17 / 5
	Cytochrome P450 17A1	CYP17A1	P05093	Cytochrome P450	0.06	16 / 40
	Estrogen receptor alpha	ESR1	P03372	Nuclear receptor	0.06	2 / 40
	Estrogen receptor beta	ESR2	Q92731	Nuclear receptor	0.06	4 / 48
	Testis-specific androgen-binding protein	SHBG	P04278	Secreted protein	0.06	13 / 40
	Sterol regulatory element-binding protein 2	SREBF2	Q12772	Unclassified protein	0.06	0 / 1
	Niemann-Pick C1-like protein 1	NPC1L1	Q9UHC9	Other membrane protein	0.06	0 / 11
	Butyrylcholinesterase	BCHE	P06276	Hydrolase	0.06	18 / 2
	Protein-tyrosine phosphatase 1B	PTPN1	P18031	Phosphatase	0.06	2 / 53
	Squalene monooxygenase	SQLE	Q14534	Enzyme	0.06	0 / 4
	Acetylcholinesterase	ACHE	P22303	Hydrolase	0.06	27 / 1
	HMG-CoA reductase	HMGCR	P04035	Oxidoreductase	0.06	13 / 23
4,10(14)-Cadinadien-beta-ol	Muscarinic acetylcholine receptor M2	CHRM2	P08172	Family A G protein-coupled receptor	0.06	13 / 2
	Nuclear receptor ROR-gamma	RORC	P51449	Nuclear receptor	0.06	1 / 9
	Norepinephrine transporter	SLC6A2	P23975	Electrochemical transporter	0.06	10 / 2
	11-beta-hydroxysteroid dehydrogenase 1	HSD11B1	P28845	Enzyme	0.06	67 / 19
	Nuclear receptor ROR-alpha	RORA	P35398	Nuclear receptor	0.06	0 / 3
	Transient receptor potential cation channel subfamily M member 8	TRPM8	Q7Z2W7	Voltage-gated ion channel	0.06	1 / 0
	UDP-glucuronosyltransferase 2B7	UGT2B7	P16662	Enzyme	0.06	28 / 6
	Vesicular acetylcholine transporter	SLC18A3	Q16572	Electrochemical transporter	0.06	2 / 0
	Peroxisome proliferator-activated receptor alpha	PPARA	Q07869	Nuclear receptor	0.06	0 / 11
	Peroxisome proliferator-activated receptor delta	PPARD	Q03181	Nuclear receptor	0.06	0 / 8
	Polyadenylate-binding protein 1	PABPC1	P11940	Unclassified protein	0.06	7 / 0
	Cytochrome P450 51	CYP51A1	Q16850	Cytochrome P450	0.06	1 / 2
	Corticosteroid binding globulin	SERPINA6	P08185	Secreted protein	0.06	3 / 14
	Carboxylesterase 2	CES2	O00748	Enzyme	0.06	0 / 10
	Receptor-type tyrosine-protein phosphatase F (LAR)	PTPRF	P10586	Membrane receptor	0.06	0 / 1
	T-cell protein-tyrosine phosphatase	PTPN2	P17706	Phosphatase	0.06	0 / 16

	Phospholipase A2 group 1B	PLA2G1B	P04054	Enzyme	0.06	0 / 1
	Low molecular weight phosphotyrosine protein phosphatase	ACP1	P24666	Phosphatase	0.06	0 / 1
	Glucose-6-phosphate 1-dehydrogenase	G6PD	P11413	Enzyme	0.06	0 / 2
	Aldo-keto reductase family 1 member B10	AKR1B10	O60218	Enzyme	0.06	0 / 6
	Cytochrome P450 2C19	CYP2C19	P33261	Cytochrome P450	0.06	3 / 2
	Isoprenylcysteine carboxyl methyltransferase	ICMT	O60725	Enzyme	0.06	54 / 0
	Mineralocorticoid receptor	NR3C2	P08235	Nuclear receptor	0.06	27 / 5
	Lysosomal acid lipase/cholesteryl ester hydrolase	LIPA	P38571	Enzyme	0.06	4 / 0
	Estradiol 17-beta-dehydrogenase 3	HSD17B3	P37058	Enzyme	0.06	1 / 0
	Ileal bile acid transporter	SLC10A2	Q12908	Electrochemical transporter	0.06	20 / 0
	DNA topoisomerase I	TOP1	P11387	Isomerase	0.06	0 / 2
	Potassium-transporting ATPase alpha chain 2	ATP12A	P54707	Primary active transporter	0.06	0 / 2
	Protein-tyrosine phosphatase 1C	PTPN6	P29350	Phosphatase	0.06	0 / 1
1,5-Epoxyalvial-4(14)-ene	Serotonin transporter	SLC6A4	P31645	Electrochemical transporter	0.06	16 / 0
	Dopamine transporter	SLC6A3	Q01959	Electrochemical transporter	0.06	29 / 0
	Norepinephrine transporter	SLC6A2	P23975	Electrochemical transporter	0.06	3 / 0
	Thrombin	F2	P00734	Protease	0.06	0 / 6
	Trypsin I	PRSS1	P07477	Protease	0.06	0 / 5
	Chymotrypsin C	CTRC	Q99895	Protease	0.06	0 / 3
	Cathepsin K	CTSK	P43235	Protease	0.06	10 / 0
	Cathepsin L	CTSL	P07711	Protease	0.06	3 / 0
β-Caryophyllene	Cathepsin (B and K)	CTSB	P07858	Protease	0.06	3 / 0
	Peroxisome roliferatoractivated receptor alpha	PPARA	Q07869	Nuclear receptor	0.69	1/6
Germacrene D	Cannabinoid receptor 2	CNR2	P34972	Family A G protein-coupled receptor	0.69	1/1
	Peroxisome proliferatoractivated receptor alpha	PPARA	Q07869	Nuclear receptor	0.05	1 / 3
Phytol	Cannabinoid receptor 2	CNR2	P34972	Family A G protein-coupled receptor	0.05	1 / 1
	UDP-glucuronosyltransferase 2B7	UGT2B7	P16662	Enzyme	0.09	1 / 8
	Dual specificity phosphatase Cdc25A	CDC25A	P30304	Phosphatase	0.09	8 / 1
	Dual specificity phosphatase Cdc25B	CDC25B	P30305	Phosphatase	0.09	8 / 1
	Androgen Receptor	AR	P10275	Nuclear receptor	0.09	40 / 26
	Glycine receptor subunit alpha-1	GLRA1	P23415	Ligand-gated ion channel	0.09	1 / 0
	Nuclear receptor ROR-gamma	RORC	P51449	Nuclear receptor	0.09	15 / 9
	Nuclear receptor ROR-alpha	RORA	P35398	Nuclear receptor	0.09	3 / 3
	Isoprenylcysteine carboxyl methyltransferase	ICMT	O60725	Enzyme	0.09	2 / 0
	Anti-estrogen binding site (AEBS) (by homology)	DHCR7	Q9UBM7	Enzyme	0.09	2 / 0
	Smoothed homolog	SMO	Q99835	Frizzled family G protein-coupled receptor	0.09	19 / 0

	Niemann-Pick C1-like protein 1	NPC1L1	Q9UHC9	Other membrane protein	0.09	18 / 4
	Protein kinase C alpha	PRKCA	P17252	Kinase	0.09	23 / 0
	p53-binding protein Mdm-2	MDM2	Q00987	Other nuclear protein	0.09	46 / 0
	LXR-beta	NR1H2	P55055	Nuclear receptor	0.09	7 / 0
	Cannabinoid receptor 2	CNR2	P34972	Family A G protein-coupled receptor	0.09	116 / 1
	11-beta-hydroxysteroid dehydrogenase 2	HSD11B2	P80365	Enzyme	0.09	6 / 0
	Progesterone receptor	PGR	P06401	Nuclear receptor	0.09	5 / 0
	C-X-C chemokine receptor type 3	CXCR3	P49682	Family A G protein-coupled receptor	0.09	5 / 0
	Monoglyceride lipase	MGLL	Q99685	Enzyme	0.09	6 / 0
	Dopamine D2 receptor	DRD2	P14416	Family A G protein-coupled receptor	0.09	90 / 0
	Squalene monooxygenase	SQLE	Q14534	Enzyme	0.11	0 / 5
	Beta-secretase 1	BACE1	P56817	Protease	0.06	223 / 0
	Period circadian protein homolog 2	PER2	O15055	Unclassified protein	0.06	39 / 0
	Progesterone receptor	PGR	P06401	Nuclear receptor	0.06	158 / 0
	Estradiol 17-beta-dehydrogenase 2	HSD17B2	P37059	Enzyme	0.06	71 / 0
	Indoleamine 2,3-dioxygenase	IDO1	P14902	Enzyme	0.06	12 / 0
	Glucagon receptor	GCGR	P47871	Family B G protein-coupled receptor	0.06	47 / 0
	Metabotropic glutamate receptor 5	GRM5	P41594	Family C G protein-coupled receptor	0.06	124 / 0
		PSEN2	P49810			
		PSENEN	Q9NZ42			
		NCSTN	Q92542			
		APH1A	Q96BI3			
		PSEN1	P49768			
		APH1B	Q8WW43			
	Gamma-secretase			Protease	0.06	164 / 0
	Glucocorticoid receptor	NR3C1	P04150	Nuclear receptor	0.06	249 / 0
	Dopamine transporter (by homology)	SLC6A3	Q01959	Electrochemical transporter	0.06	233 / 0
	Histamine H3 receptor	HRH3	Q9Y5N1	Family A G protein-coupled receptor	0.06	15 / 0
	Ileal bile acid transporter	SLC10A2	Q12908	Electrochemical transporter	0.06	79 / 0
	Calcium sensing receptor	CASR	P41180	Family C G protein-coupled receptor	0.06	20 / 0
	Phosphodiesterase 2A	PDE2A	O00408	Phosphodiesterase	0.06	87 / 0
	Phosphodiesterase 10A	PDE10A	Q9Y233	Phosphodiesterase	0.06	522 / 0
	Nociceptin receptor	OPRL1	P41146	Family A G protein-coupled receptor	0.06	38 / 0
	Cytochrome P450 2C9	CYP2C9	P11712	Cytochrome P450	0.06	10 / 0
	Cytochrome P450 3A4	CYP3A4	P08684	Cytochrome P450	0.06	3 / 0
	LXR-alpha	NR1H3	Q13133	Nuclear receptor	0.06	7 / 12
	Tyrosine-protein kinase JAK3	JAK3	P52333	Kinase	0.06	101 / 0
	Neuropeptide Y receptor type 5	NPY5R	Q15761	Family A G protein-coupled receptor	0.06	85 / 0

Nerolidol*

	Vasopressin V1a receptor	AVPR1A	P37288	Family A G protein-coupled receptor	0.06	37 / 0
	c-Jun N-terminal kinase 1	MAPK8	P45983	Kinase	0.06	80 / 0
	Protein kinase C delta	PRKCD	Q05655	Kinase	0.06	48 / 0
	Tubulin--tyrosine ligase	TTL	Q8NG68	Enzyme	0.06	4 / 0
	Protein farnesyltransferase	FNTA FNTB	P49354 P49356	Enzyme	0.06	158 / 2
	HERG	KCNH2	Q12809	Voltage-gated ion channel	0.06	80 / 0
	Proteinase-activated receptor 1	F2R	P25116	Family A G protein-coupled receptor	0.06	28 / 0
	Vanilloid receptor	TRPV1	Q8NER1	Voltage-gated ion channel	0.06	110 / 0
	Polyadenylate-binding protein 1	PABPC1	P11940	Unclassified protein	0.06	8 / 0
	MAP kinase p38 alpha (by homology)	MAPK14	Q16539	Kinase	0.06	237 / 0
	C-C chemokine receptor type 1	CCR1	P32246	Family A G protein-coupled receptor	0.06	88 / 0
	Voltage-gated potassium channel subunit Kv1.5	KCNA5	P22460	Voltage-gated ion channel	0.06	105 / 0
	Voltage-gated potassium channel subunit Kv1.3	KCNA3	P22001	Voltage-gated ion channel	0.06	30 / 0
	Liver glycogen phosphorylase	PYGL	P06737	Enzyme	0.06	45 / 0
	Metabotropic glutamate receptor 2	GRM2	Q14416	Family C G protein-coupled receptor	0.06	9 / 0
	Geranylgeranyl transferase type I beta subunit	PGGT1B	P53609	Enzyme	0.06	21 / 0
	Dual specificity phosphatase Cdc25A	CDC25A	P30304	Phosphatase	0.06	16 / 0
	Cytochrome P450 11B1	CYP11B1	P15538	Cytochrome P450	0.06	26 / 0
	Cytochrome P450 11B2	CYP11B2	P19099	Cytochrome P450	0.06	26 / 0
	Bombesin receptor subtype-3	BRS3	P32247	Family A G protein-coupled receptor	0.06	23 / 0
β-Farnesene	-	-	-	-	-	-
α-bisabolene	-	-	-	-	-	-
Carene	Peroxisome proliferatoractivated receptor alpha	PPARA	Q07869	Nuclear receptor	0.06	1 / 6
	Cannabinoid receptor 2	CNR2	P34972	Family A G protein-coupled receptor	0.06	1 / 1

* Detailed data for nerolidol are presented in the supplementary material due to space limitations.

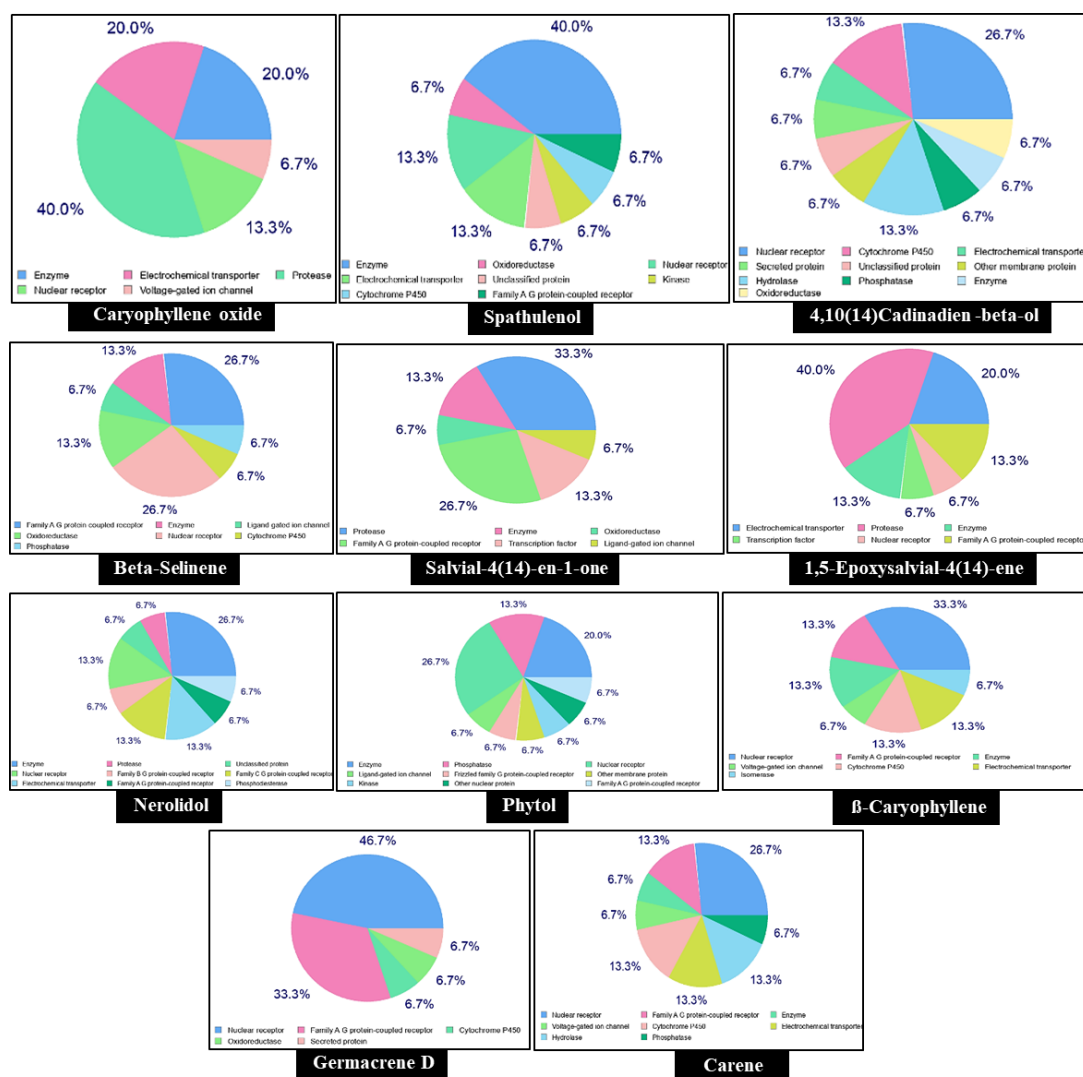


Figure 2. The predicted macromolecular targets of the tested compounds.

DISCUSSION and CONCLUSION

The analysis of the terpenoid constituents in *Centaurea verutum* essential oil revealed a consistent pattern of low toxicity across all identified compounds. Each compound falls within toxicity class 5, indicating minimal toxicological concern, with predicted LD50 values ranging between 3900 and 5300 mg/kg. These results highlight the overall safety profile of the essential oil, supporting its potential for safe applications.

The SwissADME analysis revealed promising pharmacokinetic profiles for the majority of the terpenoid compounds. Compounds such as Caryophyllene oxide and Spathulenol stand out due to their high GIA, moderate lipophilicity, and BBB permeability, making them suitable candidates for applications that require systemic or central nervous system (CNS) activity. Their solubility levels further suggest moderate aqueous solubility, which supports potential oral formulations.

In contrast, compounds like Phytol and β-Farnesene exhibited low GIA and low solubility, with high lipophilicity (iLOGP >3.8). These characteristics could limit their bioavailability and distribution, necessitating modifications such as the use of delivery systems or derivatives to enhance their pharmacokinetic properties. The predicted CYP1A2 inhibition by Nerolidol and β-Farnesene may also warrant caution, as such interactions could influence drug metabolism and clearance in clinical applications. Overall, the analyzed terpenoids demonstrated favorable bioavailability and safety profiles, with some variations in GIA, lipophilicity, and BBB permeability.

The compounds from *Centaurea verutum* essential oil largely conform to Lipinski's Rule of Five, which is used to predict drug-likeness. All compounds fall within the acceptable range of Molar Refractivity (40-130), indicating they possess favorable molecular properties for bioactivity. The LogP values indicate moderate lipophilicity for the majority of compounds. However, a LogP value of 6.36 for Phytol could suggest strong membrane permeability, but it may also indicate a potential for poor aqueous solubility and limited bioavailability, especially for oral administration. The remaining compounds exhibit LogP values within the optimal range for crossing cell membranes without significant accumulation in lipid-rich tissues. The hydrogen bond acceptor and donor values are within the acceptable range for all compounds, supporting the idea that these molecules can interact efficiently with biological targets without excessive interactions that might hinder absorption or distribution. The majority of compounds have molecular masses between 204 and 220 Da, which is consistent with the ideal range under Lipinski's rule (less than 500 Da), ideal for ensuring proper absorption and systemic distribution. From a pharmacological perspective, the PASS analysis revealed significant biological activities, including anti-inflammatory, antineoplastic, and metabolic regulatory properties. The presence of antieczematic and antineoplastic activities in multiple compounds suggests a potential role in dermatological and oncological treatments. Furthermore, the ability of some terpenoids to regulate lipid metabolism and modulate mucosal protection supports their potential use in metabolic and gastrointestinal disorders. Gene expression analysis provided insight into the molecular mechanisms underlying the bioactivity of these compounds. The upregulation of FOXO1 by several terpenoids indicates a role in oxidative stress resilience and cellular homeostasis, while the downregulation of SESN1 suggests modulation of stress response pathways. Additionally, interactions with leukemia and melanoma cell lines reinforce the anticancer potential of these compounds, warranting further in vitro and in vivo investigations to explore their precise mechanisms of action. The target prediction analysis identified key molecular targets, particularly for β -Caryophyllene, which showed significant interactions with PPARA and CNR2, implicating potential anti-inflammatory and metabolic benefits. The ability of various terpenoids to interact with nuclear receptors, enzymes, and transporters highlights their broad pharmacological potential, paving the way for further studies on their therapeutic applications. Despite the promising results, many of the compounds showed low probabilities, indicating that further research, including experimental validation, is necessary to confirm their efficacy and potential for drug development.

The findings underscore the pharmacological relevance of *Centaurea verutum* essential oil, with multiple compounds exhibiting promising safety, pharmacokinetic properties, drug-likeness, and biological activities. With antineoplastic, antieczematic, cytotoxic effects on several tumor cell lines, and other therapeutic potentials, these compounds are prime candidates for further development into pharmaceuticals, particularly in the realms of cancer therapy, dermatological treatment, and metabolic disorders. Previous studies have highlighted the diverse biological effects of essential oils derived from various *Centaurea* species. The essential oil obtained from *Centaurea alexanderina* has shown promising anti-hyperglycemic, antioxidant, and antimicrobial activities (Abdallah *et al.*, 2025). In the case of *Centaurea pterocaula*, its essential oil demonstrated notable anti-inflammatory properties by inhibiting the 5-lipoxygenase enzyme, with an IC₅₀ of 86.22 μ g/mL. Additionally, it exhibited significant activity against DPPH radicals and α -amylase, with IC₅₀ values of 83.05 and 79.66 μ g/mL, respectively (Sen *et al.*, 2021). A sesquiterpene lactone compound, 17-epichlorohyssopifolin A, isolated from *Centaurea pseudosinica*, showed considerable cytotoxic effects against three human cancer cell lines (HepG2, PC-3, and HT-29) and induced G1 phase cell cycle arrest in HepG2 and HT-29 cells (Alamri *et al.*, 2023). Furthermore, essential oil from *Centaurea bimorpha* demonstrated effective antibacterial, antifungal, antibiofilm, and anticoagulant activities (Melliti *et al.*, 2025).

The gene expression and target interaction data further suggest a broad therapeutic scope, supporting multitarget treatment strategies. Future studies should focus on experimental

validation of these properties, including in vivo toxicity assessments, mechanistic studies on specific molecular targets, and formulation strategies to enhance bioavailability. Additionally, the potential synergistic effects of these compounds should be explored to maximize their therapeutic efficacy.

Acknowledgments

This manuscript is supported by University of Aleppo, Syria.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Data Availability Statement

Research data is available and can be sent with e-mail upon request.

Authorship Contribution Statement

Ruba Joujeh: Conceived the ideas, participated in the experimental part, discussed the data, revised and edited the manuscript for final preparation, and supervised the project. **Dima Joujeh:** Conceived the ideas, carried out the experimental part, discussed the data, and wrote the original draft of the manuscript. Both authors read and approved the final manuscript.

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APPENDIX

Appendix to table No. 7

Nerolidol	p53-binding protein Mdm-2	MDM2	Q00987	Other nuclear protein	0.06	236 / 0
	Acyl-protein thioesterase 2	LYPLA2	O95372	Enzyme	0.06	2 / 0
	Squalene synthetase	FDFT1	P37268	Enzyme	0.06	16 / 0
	LXR-beta	NR1H2	P55055	Nuclear receptor	0.06	5 / 0
	Protein-tyrosine phosphatase 1B	PTPN1	P18031	Phosphatase	0.06	38 / 25
	Cyclooxygenase-2	PTGS2	P35354	Oxidoreductase	0.06	119 / 0
	Vasopressin V2 receptor (by homology)	AVPR2	P30518	Family A G protein-coupled receptor	0.06	20 / 0
	Oxytocin receptor (by homology)	OXTR	P30559	Family A G protein-coupled receptor	0.06	60 / 0
	Muscarinic acetylcholine receptor M1	CHRM1	P11229	Family A G protein-coupled receptor	0.06	116 / 0
	Muscarinic acetylcholine receptor M3	CHRM3	P20309	Family A G protein-coupled receptor	0.06	101 / 0
	Vascular endothelial growth factor receptor 2	KDR	P35968	Kinase	0.06	285 / 0
	Nitric-oxide synthase, brain	NOS1	P29475	Enzyme	0.06	7 / 0
	Aldo-keto-reductase family 1 member C3	AKR1C3	P42330	Enzyme	0.06	28 / 0
	Interleukin-6 receptor subunit beta	IL6ST	P40189	Membrane receptor	0.06	5 / 0
	Sodium channel protein type IX alpha subunit	SCN9A	Q15858	Voltage-gated ion channel	0.06	102 / 0
	Neuropeptide Y receptor type 2	NPY2R	P49146	Family A G protein-coupled receptor	0.06	3 / 0
	Pregnane X receptor	NR1I2	O75469	Nuclear receptor	0.06	4 / 0
	Transient receptor potential cation channel subfamily A member 1	TRPA1	O75762	Voltage-gated ion channel	0.06	8 / 0
	Lysosomal Pro-X carboxypeptidase	PRCP	P42785	Protease	0.06	17 / 0
	Isoprenylcysteine carboxyl methyltransferase	ICMT	O60725	Enzyme	0.06	62 / 0
	FK506-binding protein 1A	FKBP1A	P62942	Isomerase	0.06	20 / 0
	Monoacylglycerol lipase ABHD6	ABHD6	Q9BV23	Enzyme	0.06	6 / 0
	Eukaryotic translation initiation factor 2-alpha kinase 1	EIF2AK1	Q9BQI3	Kinase	0.06	19 / 0
	Geranylgeranyl transferase type I	PGGT1B FNTA	P53609 P49354	Enzyme	0.06	51 / 0
	Epoxide hydratase	EPHX2	P34913	Protease	0.06	90 / 0
	Muscarinic acetylcholine receptor M4	CHRM4	P08173	Family A G protein-coupled receptor	0.06	42 / 0
	Muscarinic acetylcholine receptor M5	CHRM5	P08912	Family A G protein-coupled receptor	0.06	38 / 0
	C5a anaphylatoxin chemotactic receptor	C5AR1	P21730	Family A G protein-coupled receptor	0.06	40 / 0
	Sigma opioid receptor	SIGMAR1	Q99720	Membrane receptor	0.06	128 / 0

Hexokinase type IV	GCK	P35557	Enzyme	0.06	56 / 0
GABA-A receptor; alpha-3/beta-3/gamma-2	GABRB3 GABRA3 GABRG2	P28472 P34903 P18507	Ligand-gated ion channel	0.06	19 / 0
GABA-A receptor; alpha-1/beta-3/gamma-2	GABRB3 GABRG2 GABRA1	P28472 P18507 P14867	Ligand-gated ion channel	0.06	19 / 0
GABA-A receptor; alpha-5/beta-3/gamma-2	GABRB3 GABRG2 GABRA5	P28472 P18507 P31644	Ligand-gated ion channel	0.06	23 / 0
GABA-A receptor; alpha-2/beta-3/gamma-2	GABRA2 GABRB3 GABRG2	P47869 P28472 P18507	Ligand-gated ion channel	0.06	17 / 0
Macrophage colony stimulating factor receptor	CSF1R	P07333	Kinase	0.06	63 / 0
Stem cell growth factor receptor	KIT	P10721	Kinase	0.06	67 / 0
GABA-B receptor	GABBR2 GABBR1	O75899 Q9UBS5	Family C G protein-coupled receptor	0.06	1 / 0
Tyrosine-protein kinase JAK1	JAK1	P23458	Kinase	0.06	103 / 0
Tyrosine-protein kinase JAK2	JAK2	O60674	Kinase	0.06	171 / 0
PI3-kinase p110-delta subunit	PIK3CD	O00329	Enzyme	0.06	197 / 0
Acyl-protein thioesterase 1	LYPLA1	O75608	Enzyme	0.06	1 / 0
DNA-dependent protein kinase	PRKDC	P78527	Kinase	0.06	143 / 0
Estradiol 17-beta-dehydrogenase 3	HSD17B3	P37058	Enzyme	0.06	20 / 0
Squalene monooxygenase	SQLE	Q14534	Enzyme	0.11	0 / 5
Beta-secretase 1	BACE1	P56817	Protease	0.06	223 / 0
Period circadian protein homolog 2	PER2	O15055	Unclassified protein	0.06	39 / 0
Progesterone receptor	PGR	P06401	Nuclear receptor	0.06	158 / 0
Estradiol 17-beta-dehydrogenase 2	HSD17B2	P37059	Enzyme	0.06	71 / 0
Indoleamine 2,3-dioxygenase	IDO1	P14902	Enzyme	0.06	12 / 0
Glucagon receptor	GCCR	P47871	Family B G protein-coupled receptor	0.06	47 / 0
Metabotropic glutamate receptor 5	GRM5	P41594	Family C G protein-coupled receptor	0.06	124 / 0
Gamma-secretase	PSEN2 PSENEN NCSTN	P49810 Q9NZ42 Q92542	Protease	0.06	164 / 0

		APH1A PSEN1 APH1B	Q96BI3 P49768 Q8WW43			
Glucocorticoid receptor		NR3C1	P04150	Nuclear receptor	0.06	249 / 0
Dopamine transporter (by homology)		SLC6A3	Q01959	Electrochemical transporter	0.06	233 / 0
Histamine H3 receptor		HRH3	Q9Y5N1	Family A G protein-coupled receptor	0.06	15 / 0
Ileal bile acid transporter		SLC10A2	Q12908	Electrochemical transporter	0.06	79 / 0
Calcium sensing receptor		CASR	P41180	Family C G protein-coupled receptor	0.06	20 / 0
Phosphodiesterase 2A		PDE2A	O00408	Phosphodiesterase	0.06	87 / 0
Phosphodiesterase 10A		PDE10A	Q9Y233	Phosphodiesterase	0.06	522 / 0
Nociceptin receptor		OPRL1	P41146	Family A G protein-coupled receptor	0.06	38 / 0
Cytochrome P450 2C9		CYP2C9	P11712	Cytochrome P450	0.06	10 / 0
Cytochrome P450 3A4		CYP3A4	P08684	Cytochrome P450	0.06	3 / 0
LXR-alpha		NR1H3	Q13133	Nuclear receptor	0.06	7 / 12
Tyrosine-protein kinase JAK3		JAK3	P52333	Kinase	0.06	101 / 0
Neuropeptide Y receptor type 5		NPY5R	Q15761	Family A G protein-coupled receptor	0.06	85 / 0
Vasopressin V1a receptor		AVPR1A	P37288	Family A G protein-coupled receptor	0.06	37 / 0
c-Jun N-terminal kinase 1		MAPK8	P45983	Kinase	0.06	80 / 0
Protein kinase C delta		PRKCD	Q05655	Kinase	0.06	48 / 0
Tubulin--tyrosine ligase		TTL	Q8NG68	Enzyme	0.06	4 / 0
Protein farnesyltransferase		FNTA FNTB	P49354 P49356	Enzyme	0.06	158 / 2
HERG		KCNH2	Q12809	Voltage-gated ion channel	0.06	80 / 0
Proteinase-activated receptor 1		F2R	P25116	Family A G protein-coupled receptor	0.06	28 / 0
Vanilloid receptor		TRPV1	Q8NER1	Voltage-gated ion channel	0.06	110 / 0
Polyadenylate-binding protein 1		PABPC1	P11940	Unclassified protein	0.06	8 / 0
MAP kinase p38 alpha (by homology)		MAPK14	Q16539	Kinase	0.06	237 / 0
C-C chemokine receptor type 1		CCR1	P32246	Family A G protein-coupled receptor	0.06	88 / 0
Voltage-gated potassium channel subunit Kv1.5		KCNA5	P22460	Voltage-gated ion channel	0.06	105 / 0
Voltage-gated potassium channel subunit Kv1.3		KCNA3	P22001	Voltage-gated ion channel	0.06	30 / 0
Liver glycogen phosphorylase		PYGL	P06737	Enzyme	0.06	45 / 0
Metabotropic glutamate receptor 2		GRM2	Q14416	Family C G protein-coupled receptor	0.06	9 / 0
Geranylgeranyl transferase type I beta subunit		PGGT1B	P53609	Enzyme	0.06	21 / 0
Dual specificity phosphatase Cdc25A		CDC25A	P30304	Phosphatase	0.06	16 / 0

Cytochrome P450 11B1	CYP11B1	P15538	Cytochrome P450	0.06	26 / 0
Cytochrome P450 11B2	CYP11B2	P19099	Cytochrome P450	0.06	26 / 0
Bombesin receptor subtype-3	BRS3	P32247	Family A G protein-coupled receptor	0.06	23 / 0
p53-binding protein Mdm-2	MDM2	Q00987	Other nuclear protein	0.06	236 / 0
Acyl-protein thioesterase 2	LYPLA2	O95372	Enzyme	0.06	2 / 0
Squalene synthetase	FDFT1	P37268	Enzyme	0.06	16 / 0
LXR-beta	NR1H2	P55055	Nuclear receptor	0.06	5 / 0
Protein-tyrosine phosphatase 1B	PTPN1	P18031	Phosphatase	0.06	38 / 25
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Vascular endothelial growth factor receptor 2	KDR	P35968	Kinase	0.06	285 / 0
Nitric-oxide synthase, brain	NOS1	P29475	Enzyme	0.06	7 / 0
Aldo-keto-reductase family 1 member C3	AKR1C3	P42330	Enzyme	0.06	28 / 0
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Sodium channel protein type IX alpha subunit	SCN9A	Q15858	Voltage-gated ion channel	0.06	102 / 0
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Pregnane X receptor	NR1I2	O75469	Nuclear receptor	0.06	4 / 0
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Isoprenylcysteine carboxyl methyltransferase	ICMT	O60725	Enzyme	0.06	62 / 0
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Eukaryotic translation initiation factor 2-alpha kinase 1	EIF2AK1	Q9BQI3	Kinase	0.06	19 / 0
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Hexokinase type IV	GCK	P35557	Enzyme	0.06	56 / 0
GABA-A receptor; alpha-3/beta-3/gamma-2	GABRB3 GABRA3 GABRG2	P28472 P34903 P18507	Ligand-gated ion channel	0.06	19 / 0
GABA-A receptor; alpha-1/beta-3/gamma-2	GABRB3 GABRG2 GABRA1	P28472 P18507 P14867	Ligand-gated ion channel	0.06	19 / 0
GABA-A receptor; alpha-5/beta-3/gamma-2	GABRB3 GABRG2 GABRA5	P28472 P18507 P31644	Ligand-gated ion channel	0.06	23 / 0
GABA-A receptor; alpha-2/beta-3/gamma-2	GABRA2 GABRB3 GABRG2	P47869 P28472 P18507	Ligand-gated ion channel	0.06	17 / 0
Macrophage colony stimulating factor receptor	CSF1R	P07333	Kinase	0.06	63 / 0
Stem cell growth factor receptor	KIT	P10721	Kinase	0.06	67 / 0
GABA-B receptor	GABBR2 GABBR1	O75899 Q9UBS5	Family C G protein-coupled receptor	0.06	1 / 0
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Tyrosine-protein kinase JAK2	JAK2	O60674	Kinase	0.06	171 / 0
PI3-kinase p110-delta subunit	PIK3CD	O00329	Enzyme	0.06	197 / 0
Acyl-protein thioesterase 1	LYPLA1	O75608	Enzyme	0.06	1 / 0
DNA-dependent protein kinase	PRKDC	P78527	Kinase	0.06	143 / 0
Estradiol 17-beta-dehydrogenase 3	HSD17B3	P37058	Enzyme	0.06	20 / 0