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Abstract: *Teucrium polium* L. is a plant with various claims of ethnobotanical use, primarily for inflammatory diseases. Chemical studies have already isolated different types of terpenes from the species, and studies have established the pharmacological potential. The present study evaluated the components of *T. polium* essential oil cultivated in Algerian Saharan Atlas. GC-MS identified the major components as Fenchone (31.25%), 3-Carene (15.77%), cis-Limonene oxide (9.77%), and Myrcene (9.15%). In the in silico prediction, molecules with more than 1% abundance were selected. Regarding Lipinski's rule, all molecules followed the rule. All molecules were found to be toxic in at least one model, with some molecules being non-genotoxic (6, 8, 10, 11, 12, 13), others being non-mutagenic (5, 7, 9, 14). Three molecules were selected that showed the best results in pharmacokinetic and toxicity studies: the molecules that did not present carcinogenic potential (7 - Myrtenal; 9 - Myrtenol; 14 – Verbenol). The molecular target was established and it seems that all three bind to the Nuclear Factor NF-kappa-B. Based on the docking and molecular dynamics results. These molecules have potential as anti-inflammatory agents, with further in vitro and in vivo studies needed to evaluate their activities and toxicity.

Keywords: nuclear factor NF-kappa-B; *Teucrium polium* L; monoterpenes; sesquiterpenes; verbenol; myrtenal; myrtenol

1. Introduction

Teucrium polium L. (Lamiaceae) is found in Europe, North Africa, and Asia. The following medicinal claims are attributed to it: treatment of inflammatory diseases, gastrointestinal disorders, diabetes, rheumatism, indigestion, abdominal pain, colds, and urogenital diseases [1,2].

Chemical studies conducted on *T. polium* oil have identified compounds belonging to the following classes: sesquiterpenes (α- and τ-cadinols), (E)-β-caryophyllene and its oxide forms, neoclerodane diterpenoids, and monoterpenes. The proportions of these chemical constituents vary

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according to the collection site [2,3], and possibly factors such as the time of plant collection, the part used for oil extraction, among others.

The following compounds have already been identified in *T. polium* oil and listed as major components in at least one study: β-caryophyllene [4–8], germacrene D [5], limonene [5,9], p-cymene, 2,4-di-tert-butylphenol [9], α-pinene [6,10], α-thujene, terpinen-4-ol [10], ledol oxide (II), linalyl acetate, β-eudesmol [11], α-cardinol, caryophyllene oxide, epi-α-muurolol, cadalene, longiverbenone, carvacrol [6], 11-acetoxyeudesman-4-α-ol, α-bisabolol [7], β-pinene, α-muurolol, α-cadinol, α-muurolol, α-cardinol, α-cardinol [8], caryophyllene, γ-muurolene, cadinol, α-gurjunene, rosifoliol, 3-carene, γ-muurolene, α-phellandrene [12], carvacrol, torreyol [13], lycopersen, dodecane, 1,5-dimethyl decahydro naphthalene, tridecane [14], myrcene, menthofuran, ocimene, pulegone [15], β-eudesmol [16], β-pinene, limonene, α-phellandrene, linalool, terpinen-4-ol, γ- and δ-cadinenes, cedrol, cedrenol, guaiol. In summary, more than 80 molecules have been identified in *T. polium* oils [17].

The essential oil of *T. polium*, with α-pinene, linalool, and caryophyllene oxide as its major components, demonstrated activity against Gram-positive bacteria (Staphylococcus *aureus* and Staphylococcus *epidermidis*) and Gram-negative bacteria [18]. Essential oils obtained from subspecies also showed activity against Acinetobacter baumannii and Staphylococcus *aureus* [18]

T. polium is known for its antidiabetic effects through various mechanisms, such as increasing insulin secretion and levels, inducing the regeneration of pancreatic β-cells, reducing oxidative damage, promoting glucose uptake in muscle tissues, inhibiting α-amylase activity, and enhancing GLUT-4 translocation [2]. The antidiabetic effect was also observed in male Wistar rats induced with diabetes by STZ injection (60 mg/kg, i.p.) and treated with Teucrium *polium* extract (100, 200, and 400 mg/kg) via daily gavage for 6 weeks. The results showed that the group treated with the extract exhibited reductions in glucose, triglycerides, and serum cholesterol, in addition to attenuating oxidative stress in aortic and cardiac tissues [19]

Due to its antimicrobial and antidiabetic potential, as well as the variation in chemical composition, it is crucial to identify the possible pharmacological markers of the species and their potential mechanisms of action, toxicity, and other aspects. In this context, in silico studies prove to be an important tool for predicting molecular structures and potential mechanisms of action of such compounds, as this type of study allows for the computational simulation of compounds from databases to predict various parameters such as physicochemical, pharmacokinetic, and toxicological properties [20]

This work is based on the analysis of the essential oil (EO) extracted from *T. polium*, with the major molecules selected for investigations related to physicochemical, pharmacokinetic, toxicological predictions, biological activities, and potential targets of action. Subsequently, molecular modeling of the selected compounds was performed.

2. Results

2.1. Characterization of T. polium Essential Oil

Thirty-three chemical compounds were identified representing 92.62% of the *T. polium* essential oil from the aerial parts (Table 1, Figure 1). Generally, total amounts of monoterpene hydrocarbons in the essential oil were higher than in other groups. In the characterization of *T. polium* oil, 14 molecules were identified with concentrations of 1% or greater (Figure 1), with the major compounds being Fenchone (31.25%), 3-Carene (15.77%), Limonene oxide, cis- (9.77%), and Myrcene (9.15%). An additional 10 compounds were present with concentrations of 1% or greater (Supplementary Material).

Table 1. Essential oil composition of aerial parts of *Teucrium polium*.

	RRI	References[a,b]	Compounds	RA (%)
1	946	939-957	Camphene	0.40
2	953	937-953	Verbenene	0.26

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3	1008	997-1027	3-Carene	15.77
4	1009	990-1009	α-Phellandrene	0.75
5	1055	1059-1087	Fenchone	31.25
6	1064	1027-1050	β-Ocimene, (E)-	1.02
7	1089	1089	p-Cymene	0.65
8	1122	1106-1134	α-Campholenal	0.59
9	1132	1122-1144	Limonene oxide, cis-	9.77
10	1140	1140-1175	Myrcene	9.15
11	1146	1146	Verbenol	1.02
12	1150	1110-1150	δ-2-Carene	0.72
13	1160	1121-1158	Pinocarvone	0.91
14	1162	1147-1176	Linalool oxide	0.64
15	1165	1134-1165	cis-Verbenol	0.36
16	1169	1122-1169	3-Carene	0.80
17	1182	1182	cis-Pinocarveol	2.92
18	1186	1159-1191	α -Terpineol	0.46
19	1194	1169-1200	Myrtenol	1.47
20	1195	1171-1206	Myrtenal	2.31
21	1204	1190-1224	Verbenone	0.38
22	1235	1206-1235	Carvone	0.28
23	1254	1259-1284	Bornyl acetate	0.31
24	1270	1270-1302	Terpinen-4-ol acetate	0.54
25	1290	1290-1316	Myrtenyl acetate	0.70
26	1484	1458-1491	Germacrene D	2.56
27	1500	1474-1501	Bicyclogermacrene	1.56
28	1521	1508-1539	δ-Cadinene	1.18
29	1577	1562-1590	Spathulenol	1.47
30	1640	1610-1650	α-Muurolol, epi-	0.43
31	1649	1649-1686	α-Bisabolol	0.34
32	1654	1619-1662	α-Cadinol	0.35
33	1677	1676	(Z)-Nerolidyl acetate	1.30
		Grouped compounds (%)		
		Monoterpene hydrocarbons		43,15
		Oxygenated monoterpenes		43,74
		Sesquiterpenes hydrocarbons		5.73
		Total identified compounds (%)		92.62

RRI: Relative retention indices, RA (≥0.25): Relative area (peak area relative to the total peak area) [82,83].

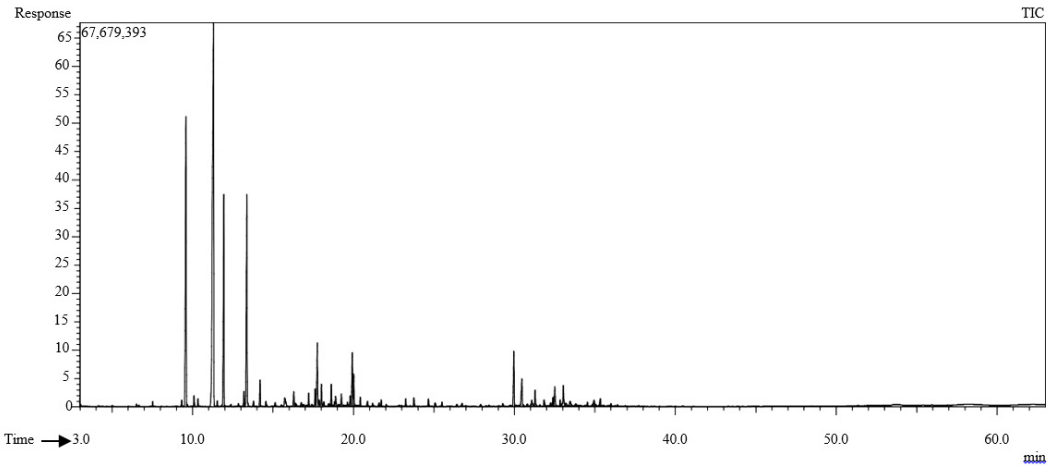

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Figure 1. Gas chromatographic-flame ionization detector (GC-FID) profile of the essential oil of *Teucrium polium*.

In this study, terpenes were selected, including 4 monoterpenes (3-Carene, Figure 2A; Myrcene, Figure 4A; β-Ocimene, Figure 13A, (E)-; Verbenol, Figure 14A), 5 monoterpenoids (Fenchone, Figure 1A; Limonene oxide, cis-, Figure 3A; Cis-Pinocarveol, Figure 5A; Myrtenal, Figure 7A; Myrtenol, Figure 9A), 3 sesquiterpenes (Germacrene D, Figure 6A; Bicyclogermacrene, Figure 8A; δ-Cadinene, Figure 12A), and 2 sesquiterpenoids (Spathulenol, Figure 10A; (Z)-Nerolidyl acetate, Figure 11A).

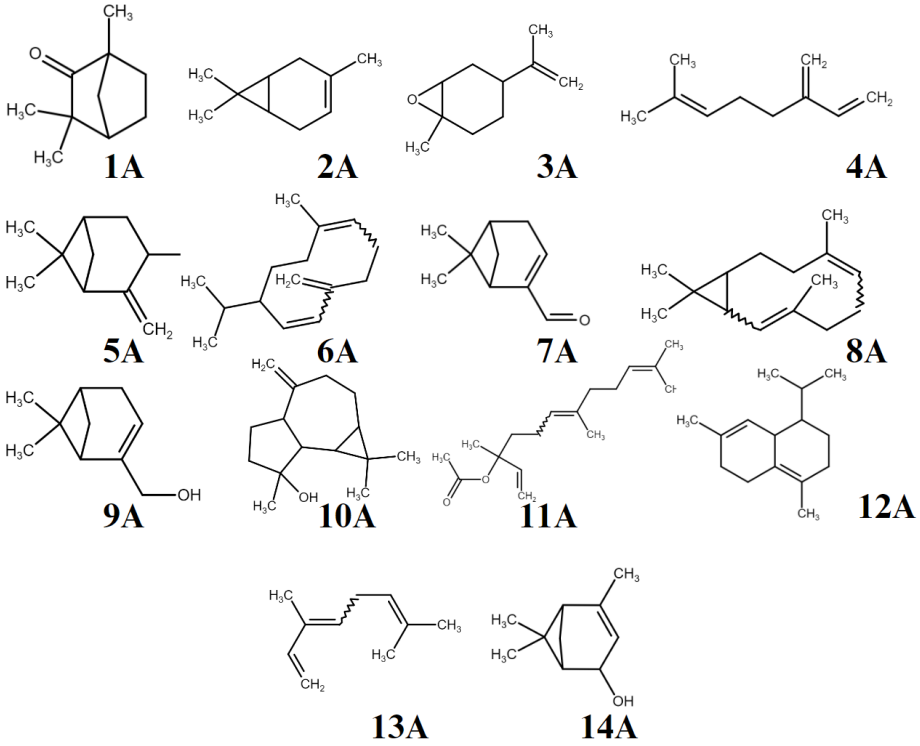

Figure 2. Molecules found from *T. Polium* essential oil: 1 - Fenchone; 2 - 3-Carene; 3 - Limonene oxide, cis-; 4 - Myrcene; 5 - cis-Pinocarveol; 6 - Germacrene D; 7 - Myrtenal; 8 - Bicyclogermacrene; 9 - Myrtenol; 10 - Spathulenol; 11 - (Z)-Nerolidyl acetate; 12 - δ-Cadinene; 13 - β-Ocimene, (E)-; 14 - Verbenol.

2.2. Predictions of Physicochemical, Pharmacokinetic, and Toxicity Aspects

No molecule violated the Lipinski rule with adaptation; however, it is worth noting that all exhibited very low polar surface areas (0 to 26.3 Å) and reduced numbers of hydrogen bond acceptors and donors (Table 2).

Table 2. Prediction of physicochemical properties.

Molecules	MM	LogP	TPSA	nHBA	nHBD
1	152.237	2.402	17.07	1	0
2	136.238	2.999	0.00	0	0
3	152.237	2.520	12.53	1	0
4	136.238	3.475	0.00	0	0
5	152.237	1.970	20.23	1	1
6	204.357	4.891	0.00	0	0
7	150.221	2.178	17.07	1	0
8	204.357	4.725	0.00	0	0
9	152.237	1.971	20.23	1	1
10	220.356	3.386	20.23	1	1

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11	264.409	4.967	26.30	2	0
12	204.357	4.725	0.00	0	0
13	136.238	3.475	0.00	0	0
14	152.237	1.970	20.23	1	1

Lipinski's rule: LogP - oil-water partition coefficient ≤ 5; TPSA: topological polar surface area ≤140 Å; nHBA: number of hydrogen bond acceptors ≤ 10; nHBD: number of hydrogen bond donor groups ≤ 5; MM - molecular mass ≤ 500D (Lipinski, 2004). 1 - Fenchone; 2 - 3-Carene; 3 - Limonene oxide, cis-; 4 - Myrcene; 5 - cis-Pinocarveol; 6 - Germacrene D; 7 - Myrtenal; 8 - Bicyclogermacrene; 9 - Myrtenol; 10 - Spathulenol; 11 - (Z)-Nerolidyl acetate; 12 - δ-Cadinene; 13 - β-Ocimene, (E)-; 14 - Verbenol.

Analysis of the pharmacokinetic parameters suggests that all molecules have moderate permeability in Caco-2 cells, moderate to high permeability in MDCK cells, and high intestinal absorption. Some molecules appear to have low potential for binding to plasma proteins and moderate distribution to the central nervous system (CNS) (3 and 7), while others, despite high plasma protein binding, seem to have a high potential for distribution to the CNS (4, 6, 8, 11, 12, 13, and 14). All molecules undergo phase 1 metabolism by CYP3A4 and inhibit at least one CYP enzyme (Table 3).

Table 3. Prediction of pharmacokinetic properties.

	Absorption			Distribution		Metabolism	
Molecules	**MDCK**	**Caco 2**	**HIA**	PP	BBB	CYP Inibition	CYP phase 1
1	M	M	H	H	M	2C9,3A4	3A4
2	H	M	H	H	H	2C9	3A4
3	H	M	H	L	M	2C9,3A4	W 3A4
4	H	M	H	H	H	2C9,3A4	3A4
5	M	M	H	L	H	2C9,3A4	W 3A4
6	M	M	H	H	H	2C9,2C19	3A4
7	H	M	H	L	M	2C9	W 3A4
8	M	M	H	H	H	2C9	3A4
9	H	M	H	L	H	2C9	W 3A4
10	H	M	H	L	H	2C9,3A4	3A4
11	M	M	H	H	H	2C19,2C9,3A4	3A4
12	M	M	H	H	H	2C19,2C9	3A4
13	M	M	H	H	H	2C19,2C9	3A4
14	H	M	H	H	H	2C9	W 3A4

BBB: blood-brain barrier; CYP: cytochrome P450; HIA: human intestinal absorption, S*: strongly; F*: freely; NO: not observed; W: weakly; H: high; L: low; M: medium; 1 - Fenchone; 2 - 3-Carene; 3 - Limonene oxide, cis-; 4 - Myrcene; 5 - cis-Pinocarveol; 6 - Germacrene D; 7 - Myrtenal; 8 - Bicyclogermacrene; 9 - Myrtenol; 10 - Spathulenol; 11 - (Z)-Nerolidyl acetate; 12 - δ-Cadinene; 13 - β-Ocimene, (E)-; 14 - Verbenol.

All molecules were shown to be toxic to some marine organisms; however, the molecules that appear to have no mutagenic potential (6, 8, 10, 11, 12, and 13) were carcinogenic to mice and rats (6, 8, 11, 12, and 13) or only to rats (10). On the other hand, the molecules that were not carcinogenic to any animal species (5, 7, 9, and 14) showed mutagenic potential (Table 4). Considering all the evaluated toxicities, it can be suggested that, despite their mutagenic potential, molecules 5, 7, 9, and 14 are the most promising.

Table 4. Prediction of toxicity.

Molecules	**Alga**	**Daphnia**	**Medaka fish**	**Minnow fish**	**Ames**	**Carcino Rato/Cam***
1	T	NT	VT	VT	TA1535_10RLI	N/P
2	T	NT	VT	VT	TA100_10RLI	N/P

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3	T	NT	VT	VT	TA1535_10RL; 100_10RLI;1535_NA	P/P
4	T	T	VT	VT	TA1535_NA	P/N
5	T	NT	VT	VT	TA100_10RLI; 1535_NA	N/N
6	T	T	VT	VT	N	P/P
7	T	NT	VT	VT	TA1535_10RLI; 100_10RLI	N/N
8	T	T	VT	VT	N	P/P
9	T	NT	VT	VT	TA1535_10RLI; 100_10RLI	N/N
10	T	T	VT	VT	N	P/N
11	T	T	VT	VT	N	P/P
12	T	T	VT	VT	N	P/P
13	T	T	VT	VT	N	P/P
14	T	NT	VT	VT	TA1535_10RLI; TA100_10RLI	N/N

T: toxic; NT: non-toxic; N: negative; P: positive. Parameters: Algae - < 1 mg/L toxic; > 1 mg/L non-toxic (Costa, et al., 2008); Daphnia Test: < 0.22 μg/mL Toxic; > 0.22 μg/mL - non-toxic (Guilhermino, et al., 2000); Test on Medaka and Minnow fish: < 1 mg/L - very toxic; 1- 10 mg/L- toxic; 10-100 mg/L- harmful and > 100 mg/L- extremely toxic (Zuncker,1985), Carcino Rat/mice*= carcinogenicity in rat/mice. T-toxic, NT-non-toxic, VT-very toxic, N-negative, P-positive. 1 - Fenchone; 2 - 3-Carene; 3 - Limonene oxide, cis-; 4 - Myrcene; 5 - cis-Pinocarveol; 6 - Germacrene D; 7 - Myrtenal; 8 - Bicyclogermacrene; 9 - Myrtenol; 10 - Spathulenol; 11 - (Z)-Nerolidyl acetate; 12 - δ-Cadinene; 13 - β-Ocimene, (E)-; 14 - Verbenol.

Another aspect evaluated is the potential acute oral toxicity of the molecule, with the highest LD50 found for compound 11 (Class VI). Other molecules exhibited an LD50 greater than 2000 mg/kg (1, 2, 7, 8, 10, 12, 13, and 14). Possible side effects of these molecules were also assessed, with no events reported for 3, 5, 6, 12, and 13 (Table 5).

Table 5. Prediction of oral toxicity.

Molecules	LD50 (mg/kg)	Toxicity class	Side effects
1	3087	V	I
2	2799	V	I/T
3	1447	IV	-
4	2561	V	I/T
5	1971	IV	-
6	1471	IV	-
7	2448	V	I
8	2766	V	I/T/M
9	1736	IV	I
10	3278	V	I/T
11	5923	VI	T
12	2090	V	-
13	2652	V	-
14	2280	V	I

LD50 - lethal dose 50%. NO - nothing observed. I - Irritant, T - Tumorigenic, M - Mutagenicity. Category I: 1< LD50≤ 5mg/kg - Extremely Toxic; Category II: 5 < LD50 ≤50mg/kg- Highly Toxic; Category III: 50 < LD50 ≤ 300 mg/kg - Moderately Toxic; Category IV: 300 < LD50 ≤ 2,000mg/kg - Low Toxic; Category V: 2000 < LD50 ≤ 5,000 Unlikely to Cause Acute Damage; Category VI: DL50>5000 No damage. Source: ABNT NBR, 2009; RDC No. 294, 2019. 1 - Fenchone; 2 - 3-Carene; 3 - Limonene oxide, cis-; 4 - Myrcene; 5 - cis-Pinocarveol; 6 - Germacrene D; 7 - Myrtenal; 8 - Bicyclogermacrene; 9 - Myrtenol; 10 - Spathulenol; 11 - (Z)-Nerolidyl acetate; 12 - δ-Cadinene; 13 - β-Ocimene, (E)-; 14 - Verbenol.

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2.3. Predictions of Potential Molecular Targets of Compounds in T. polium Essential Oil

Based on the studies of physicochemical predictions, pharmacokinetics, and toxicity, it can be suggested that the most promising molecules are 7, 9, and 14. Subsequently, targets with potential for biological activity related to cancer (Nuclear Factor NF-kappa-B p105 subunit) were identified with a correction and precision probability greater than 90%, and the PDB (Protein Data Bank) code (1SVC) for docking was obtained through the online server, as shown in Table 6.

Table 6. Molecular Target Assessment.

Molecules	Probability	Prediction accuracy	Target Name	PDB
7	91.76%	96.09%	NF-kappa-B	1SVC
9	96.52%	96.09%	NF-kappa-B	1SVC
14	92.39%	96.09%	NF-kappa-B	1SVC

PDB: Protein Data Bank; NF-kappa-B: Nuclear factor NF-kappa-B p105 subunit; 7 - Myrtenal; 9 - Myrtenol; 14 – Verbenol.

2.3. Docking Molecular Simulation

Docking was performed on the Nuclear Factor NF-kappa-B protein with Myrtenal, Myrtenol, and Verbenol, showing only favorable interactions for all molecules, with van der Waals interactions predominating (Figure 3).

The compound Myrtenal formed hydrogen bonds with residues Arg57, Arg59, and Gly141, and Alkyl interactions with residues Pro65 and Val115. Myrtenol interacted with hydrogen bonds with residues Tyr60 and Val61, Pi-Alkyl interactions with Arg59 and Val115, and Alkyl interactions with residues Phe56 and His67. Verbenol formed hydrogen bond interactions with residues Pro65 and Gly68, Pi-Alkyl interactions with Arg59, and Alkyl interactions with Phe56, Val115, and Ile142 (Figure 3).

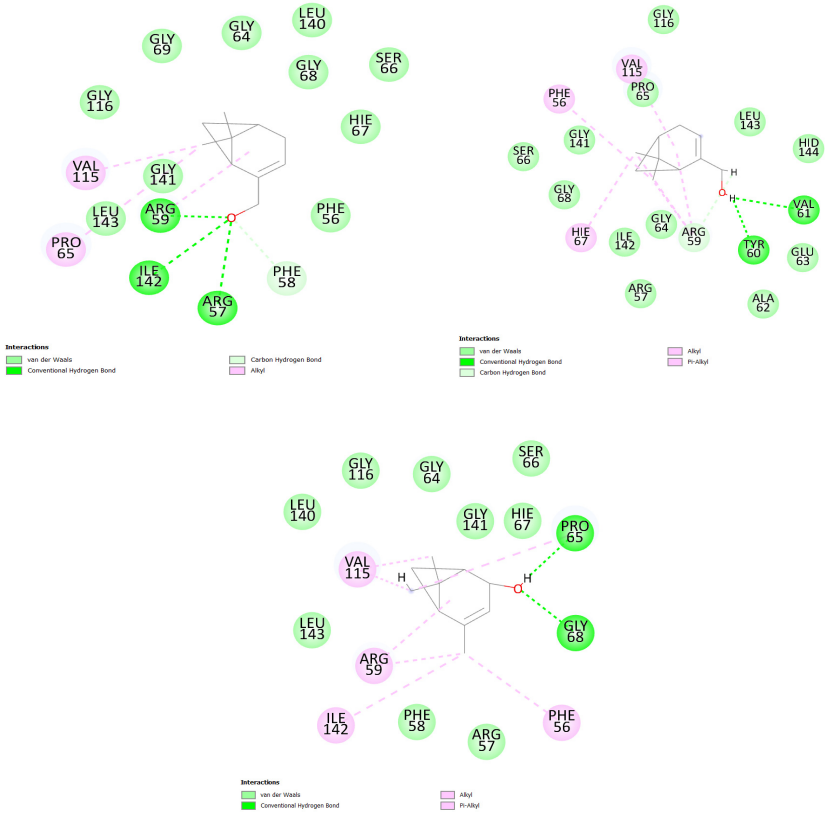

Figure 3. Representation of 2D interactions of molecules myrtenal, myrtenol, verbenol and proteín Nuclear fator NF- kappa-B. Image generated with Discovery Studio 3.5 Visualizer.

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2.4. Molecular Dynamics

The RMSD graph (Figure 4A) illustrates the structural stability of the NF-κB protein in its unbound form (Apo) and when complexed with Myrtenal, Myrtenol, and Verbenol over 200 ns of simulation. The average RMSD values for the protein in the Apo, Myrtenal, Myrtenol, and Verbenol forms were 5.39 Å, 4.11 Å, 5.44 Å, and 7.29 Å, respectively (Figure 4A). The Myrtenal compound exhibited greater stability and less fluctuation compared to Myrtenol and Verbenol, with a value close to that of the Apo protein, indicating that this compound is dynamically more efficient in stabilizing the protein. Detailed RMSD results for the protein and ligands are available in (Figure S1) of the supplementary material.

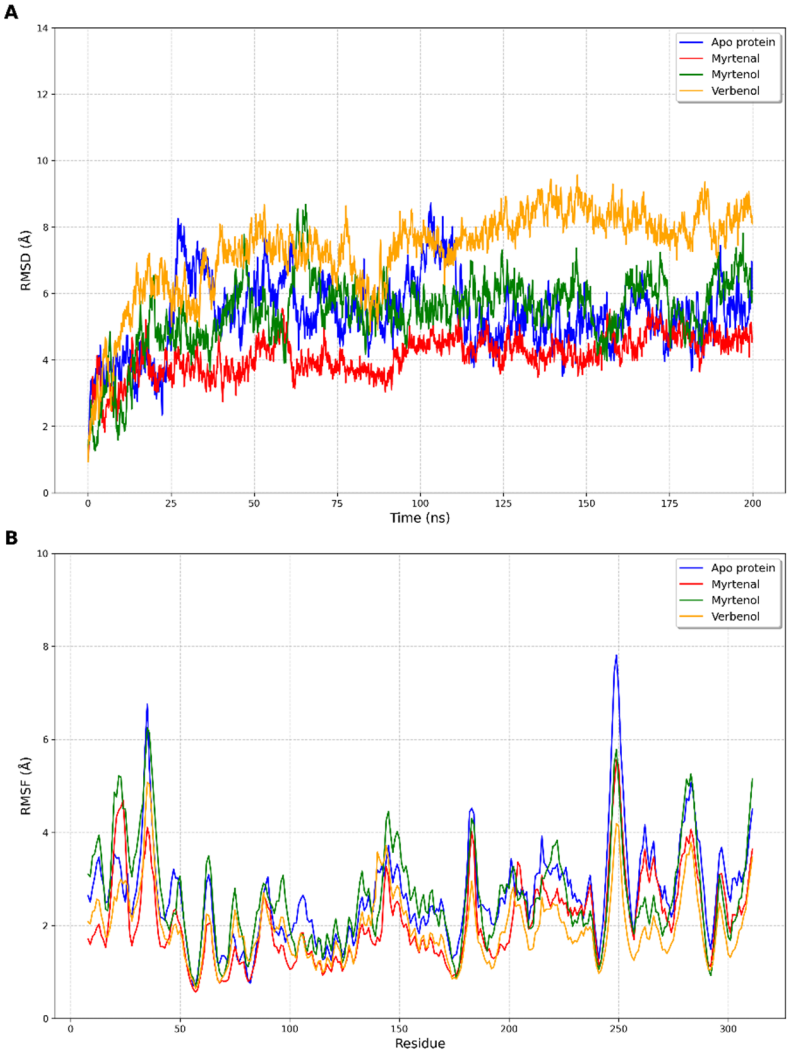

Figure 4. Chart of RMSD (A) and RSMF (B) of the Apo form of the protein Nuclear factor NF-kappa-B and complexed with Myrtenal, Myrtenol and Verbenol.

The RMSF graph (Figure 4B) illustrates the average residual fluctuations over time for each residue of the protein in its different forms. It is observed that the largest fluctuations are particularly pronounced in specific residues, especially between residues 32-37 and residues 246-253, corresponding to loop regions, which are more flexible. In the region where the ligand is accommodated, between residues 16-26, there is a lower fluctuation in the complex with Myrtenal, which is consistent with the RMSD data. Notably, the complex with Myrtenol shows the highest fluctuations in several regions of the protein, corroborating the RMSD observation that this ligand induces greater structural instability. The fluctuations observed in the complexes with Myrtenal and Verbenol are comparable and smaller than those with Myrtenol, suggesting that these ligands have a lesser impact on the protein's dynamics.

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The greater instability observed with Myrtenol may be associated with a weaker or less specific binding to the active site, or the induction of larger conformational adjustments in the protein to accommodate the ligand. In contrast, the relatively stable behavior of the protein in complexes with Myrtenal and Verbenol suggests that these ligands are more compatible with the active site, resulting in smaller conformational fluctuations. These data are crucial for understanding the structure-function relationship and can guide future studies in the chemical modification of these ligands to enhance their efficacy and specificity.

2.5. MM-GBSA Binding Energies

The binding energies (ΔGbind) were calculated for the Myrtenal-1SVC, Myrtenol-1SVC, and Verbenol-1SVC complexes using the MM-GBSA method. The components of the interaction energies, including van der Waals energies (ΔEvdw), electrostatic energies (ΔEele), polar solvation free energy (ΔGGB), and apolar solvation free energy (ΔGSA), were analyzed for each complex (Table 7).

Table 7. Binding energies and their components calculated by MM-GBSA (in kcal/mol).

Complex	Δ*E*vdw	Δ*E*ele	ΔG_{GB}	ΔG_{SA}	ΔG_{bind}
Myrtenal-1SVC	− 8.92 ± 2.99	− 98.53 ± 8.44	81.12 ± 5.85	− 107.46 ± 7.33	− 26.33 ± 3.57
Myrtenol-1SVC	− 5.59 ± 2.89	− 58.77 ± 8.62	40.73 ± 5.94	− 58.37 ± 7.47	− 17.64 ± 3.65
Verbenol-1SVC	− 17.01 ± 2.59	− 81.45 ± 8.51	76.32 ± 7.24	− 98.46 ± 8.28	− 22.14 ± 3.36

The results show that the Myrtenal-1SVC complex exhibited the most favorable binding energy (ΔGbind = −26.33 ± 0.11 kcal/mol), followed by Verbenol-1SVC (ΔGbind = −22.14 ± 0.10 kcal/mol) and Myrtenol-1SVC (ΔGbind = −17.64 ± 0.10 kcal/mol). These values indicate that Myrtenal forms the most stable complex with the 1SVC protein, which is consistent with the lower conformational fluctuations observed in the RMSD and RMSF data, suggesting a strong interaction of this compound with the protein's interaction site.

The analysis of the energetic components reveals that, in all complexes, electrostatic energy (ΔEele) plays a predominant role in stabilizing the ligand-protein interactions, especially for Myrtenal-1SVC, which showed the most negative ΔEele value (−98.53 ± 0.26 kcal/mol). However, this strong electrostatic contribution is partially offset by the polar solvation free energy (ΔGGB), which is higher for Myrtenal, indicating that the electrostatic interactions are strongly solvated.

The MM-GBSA analysis results reinforce the observations made in the RMSD and RMSF analyses. Myrtenal, which had the highest free binding energy (− 26.33 ± 3.57 kcal/mol), also induced the smallest structural fluctuations, suggesting a combination of strong interaction and dynamic conformational adjustment. Verbenol, which showed a free binding energy (–22.14 ± 3.36 kcal/mol), provided better conformational stability than Myrtenol, as observed in the RMSD and RMSF analyses.

3. Discussion

The essential oil obtained from *T. polium* was subjected to GC-MS analysis, revealing the major constituents as Fenchone (31.25%), 3-Carene (15.77%), Limonene oxide, cis- (9.77%), and Myrcene (9.15%). When comparing these results to other studies, it is observed that other metabolites such as β-caryophyllene [3], limonene [10], ledene oxide II [11], α-cardinol [21], carvacrol [6], and β-pinene were the major constituents [22]. Studies on the environmental impact on the composition of T. polium oil are still scarce; however, it is known that factors such as altitude, water availability, macro and micronutrients in the soil, relative air temperature, and soil pH directly affect the chemical profile of plants [23]

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Myrcene was reported in previous studies as the major component of the essential oil of *T. polium* [24–28]. Myrcene was found to be the major compound in our study, too. However, the main constituents of the essential oils of the aerial parts were oxygenated monoterpenes and monoterpene hydrocarbons, which were in good agreement with the previous reports [24,29–32].

On the other hand, Germacrene D was detected as major compound in the essential oils of *T. polium* samples from different regions [28,30,33–35]. Similarly, Germacrene D was detected as the main compound in our study. While Fenchone, 3-Carene and Limonene oxide, cis- were found to be the main compounds in our study, they were minor or absent in essential oils of Teucrium [10,33,36]. Therefore, environmental factors, the plant part used in the extraction process, and the collection time can influence the chemical composition of the essential oil.

All selected molecules adhered to Lipinski's rule and appear to exhibit high intestinal absorption. However, only molecules 2, 4, 5, 6, 8-14 distribute into the CNS. Adhering to Lipinski's rule is crucial for drug candidates as it indicates that the drug will be well absorbed in the gastrointestinal tract and adequately distributed throughout the body, allowing for oral administration [37–39]

All molecules seem to be metabolized by CYP3A4, but they inhibit CYP and sometimes more than one CYP. Molecules that inhibit CYP can interfere with the metabolism of other drugs, necessitating dose adjustments. Another evaluated parameter was toxicity, with 8, 10, 11, 12, 13 not being mutagenic, while 7, 9, and 14 were not carcinogenic. Unfortunately, no compound was devoid of toxicity; however, all compounds had an LD50 > 1400 mg/kg. Therefore, repeated-dose toxicity studies, in vivo genotoxicity, and in vivo carcinogenicity studies are important for understanding toxic effects and potential mechanisms.

After analyzing the pharmacokinetic studies and toxicities, molecules without carcinogenic potential were selected (7 - Myrtenal; 9 - Myrtenol; 14 - Verbenol). Myrtenal exhibited antihyperglycemic effects, reducing blood glucose levels and hemoglobin A1C, and aiding in weight recovery [40].Derivatives of Myrtenal have shown activity against various cell lines[40–43]

Other activities related to Myrtenal derivatives include: anxiolytic [44]; antiviral [44]; antifungal [45]; and analgesic [46]. Another selected molecule was Myrtenol, which inhibits biofilm formation and virulence in drug-resistant Acinetobacter *baumannii*. Myrtenol improved the susceptibility of BP-AB to the antibiotic's amikacin, piperacillin/tazobactam, cefoperazone/sulbactam, and ceftazidime. This molecule regulates the expression of biofilm-associated genes in the BP-AB strain, and qPCR analysis reduced the expression levels of bfmR, bap, csuA/B, and ompA in groups D, E, and F compared to groups A, B, and C. A non-significant reduction in bfmR, bap, csuA/B, and ompA levels was also found in groups A, B, and C. The genes bfmR, bap, csuA/B, and ompA are key regulators of the transition from biofilm formation to maturation in the BP-AB strain [47]. Myrtenol protects against myocardial ischemia-reperfusion injury through antioxidant and anti-apoptotic mechanisms [48]Verbenol exhibited anti-ischemic and anti-inflammatory properties [49]

To identify the potential target involved in the biological activity of Myrtenal, Myrtenol, and Verbenol, prediction studies were conducted, suggesting that all three bind to Nuclear Factor NF-kappa-B, a family of transcription factors involved in inflammation, immunity, cell proliferation, differentiation, and survival [50]In recent years, the presence and activation of Nuclear Factor NF-kappa-B in different types of cancer has been highlighted, as well as the importance of developing inhibitors that act directly on Nuclear Factor NF-kappa-B [51]. The possibility of therapeutically targeting this factor allows for a significant advance in tumor destruction during treatment, thereby enhancing antitumor activity[52]

It is worth highlighting the medicinal importance of Teucrium species, which have been used since ancient times in the Mediterranean region for treating gastrointestinal issues and maintaining healthy endocrine gland function, as well as for treating malaria and severe dermatological disorders. However, studies evaluating their activity are scarce. Evaluations of the essential oils and ethanolic extracts of Teucrium *polium* and Teucrium *parviflorum* have shown that the extracts exhibited antioxidant, anti-butyrylcholinesterase, anti-tyrosinase, and anti-urease activities through in vitro

 doi:10.20944/preprints202411.0589.v1

and in silico assays[53]It is noteworthy that T. *polium* oil demonstrated moderate antioxidant potential [54]

An in vivo study with the ethanolic extract of T. *polium* demonstrated the plant's anti-inflammatory potential at concentrations of 50 mg/kg, 100 mg/kg, and 150 mg/kg, leading to a reduction in paw edema in rats [55]. When correlating this result with prediction studies, the regulation of NF-κB activity is crucial to prevent chronic inflammation, meaning that substances with anti-inflammatory activity can suppress NF-κB activation or interfere with its translocation to the nucleus, reducing the expression of inflammatory genes [51] In addition to its involvement in the inflammatory process, NF-kappa-B (NF-κB) is involved in cell proliferation, apoptosis (programmed cell death), stress response, and other aspects relevant to cancer development and progression [52].

It should be noted that the chronic inflammation process favors mutations, uncontrolled cell proliferation, and resistance to apoptosis, all of which are processes that can facilitate carcinogenesis [56].Furthermore, NF-κB induces the production of vascular endothelial growth factor (VEGF) and regulates molecules involved in cell mobility and tissue invasion, such as matrix metalloproteinases (MMPs) [57,58]Considering this, it can be suggested that these molecules hold promise as antitumor and anti-inflammatory agents, and in vitro and in vivo studies are necessary to determine the best therapeutic use of these molecules.

4. Materials and Methods

4.1. Chemical Studies

4.1.1. Plant Material, and Extraction of the Essential Oil

The aerial parts of *T.polium L.* (Lamiaceae) were collected in April 2023, from the Laghouat city (located in the south part of the Algerian Saharan Atlas), the GPS coordinates were (33°47'59'' N 2°51'54'' E). The plant material was taxonomically identified by the botanical survey, and its voucher specimen (LBAS Tp/04/23) was deposited in the Herbarium of the Laboratory of Biological and Agricultural Sciences, University of Amar Telidji, Laghouat, Algeria. After drying, and grinding the plant, 100 g of powder were mixed with 1.5 L of distilled water in a round-bottomed flask and placed in a Clevenger type apparatus for hydrodistillation. After 3 hours, the essential oil is recuperated and stored in a sealed vial at 4 °C until analysis.

4.1.2. Chromatographic Analysis

For analysis of essential oil, Shimadzu GCMS QP 2010 ULTRA with RXİ-5MS capillary column (30 m × 0.25 mm inner diameter, film thickness 0.25 μm) was used. The percentage composition of the essential oil was written by calculating Gas Chromatography-Flame Ionization Detection (GC/FID) peaks.

RXİ-5MS capillary column (30 m × 0.25 mmi. d., film thickness 0.25 μm) was used with helium as the carrier gas. The injector temperature was 250°C, and the split flow was 1 ml/min. The GC oven temperature was kept at 40°C for 3 min and programmed to 240°C at a rate of 4°C/min and then kept constant at 240°C for 53 min. For chemical component identification, Wiley and NIST electronic libraries were used [59,60]

4.2. In Silico Evaluation

The molecules were drawn using the Marvin Js online program (https://marvinjs-demo.chemaxon.com/latest/demo.html), and for the determination of physicochemical properties, the online server Home-ADMElab was used (https://admet.scbdd.com) [61]. The Lipinski's Rule of Five or "Rule of Five" was considered [37]. For pharmacokinetic and toxicity predictions, the PreADMET program (version 2.0, Copyright © 2005 – 2017) was used, which considers pharmacokinetic properties (A – absorption; D – Distribution; M – Metabolism/Biotransformation; E – Excretion) and evaluation of toxicity parameters (T – Toxicity; PREADMET, 2020).

For the assessment of toxicity in marine organisms, the criteria used were as follows: for toxicity in algae [62]; for Daphnia sp [63]; for Medaka [64]; and for [62] .The mutagenicity risk was assessed

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by the Ames test with the following strains of *Samonella Typhimurium:* TA100-10RLI and TA 100-NA mutation in His G46e plasmid pKM101 without S9; TA1535- 10RLI and TA1535-NA mutation in His G46 [65]

The carcinogenic potential of the compounds was evaluated in rats and mice and referred to as: (+) carcinogenic and (–) non-carcinogenic. To predict acute oral toxicity (lethal dose 50%- LD_{50}), the online software PROTOX II was used [66] considering the classification from I to VI, according to ABNT NBR 14725-2 (2019). Adverse events that may occur with the use of the molecule were also evaluated.

4.3. Molecular Target and Docking

Based on the results obtained from the in-silico studies, particularly regarding carcinogenicity and mutagenicity, the molecules were selected for docking. Initially, these molecules were submitted to the Superpred Webserver [67], a server used to predict the molecular target with potential interaction with the investigated ligands com potential related to cancer. The only target that showed relevance for the investigated biological activity was Nuclear Factor NF-kappa-B, obtained from the Protein Data Bank (PDB ID 1SVC), as the compounds with this target achieved scores for therapeutic activity interaction (≥90% binding probability and ≥90% prediction accuracy). Other targets, such as DNA-(apurinic or apyrimidinic site) lyase and the LSD1/CoREST complex, were not used because, despite potential therapeutic activity, they showed binding probability and prediction accuracy below 90%.

Initially, the molecular structures of Myrtenal, Myrtenol, and Verbenol were retrieved from the PubChem database and optimized using the DFT/B3LYP/cc-pVDZ quantum method with the Gaussian 09 program. The crystallographic structure of the Nuclear Factor NF-kappa-B p105 enzyme was obtained from the Protein Data Bank (PDB ID: 1SVC) [68]. This PDB structure consists of 364 amino acids, corresponding to residues 2 to 365 of the full 968-amino-acid sequence [69]. Among the 968 residues, the domain spanning amino acids 42 to 367, known as the Rel Homology Domain (RHD), binds to DNA at the major groove and is responsible for the transcriptional activity of the protein. Therefore, this region represents a potential binding site for small molecules aimed at inhibiting DNA transcription and was selected as the protein's binding site, as proposed in the study [70]

Molecular docking was performed using the Molegro Virtual Docker (MVD) version 5.5 program [71]. The center of the sphere was defined with coordinates x: 40.37, y: 27.49, and z: 44.60, with a radius of 12 Å. The scoring function used was the MolDock Score. Analysis of intermolecular interactions was carried out using the Discovery Studio Visualizer (Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, version 2021, San Diego: Dassault Systèmes, 2021).

4.4. Molecular Dynamics

To gain further insights into the dynamic behavior and intermolecular interactions, the protein in its unbound form (Apo) and in complex with Myrtenal, Myrtenol, and Verbenol were subjected to molecular dynamics (MD) simulations using the GPU-accelerated Amber22 software [72]. The restrained electrostatic potential (RESP) procedure was used to calculate the atomic charges of the ligands using the Gaussian 09 program at the HF/6-31G* theory level [73]. The structures of the protein and the ligands were treated using the amber force field ff14SB and the general amber force field (GAFF), respectively [74,75].

The protonation states of the amino acid residues were calculated at pH 7.4 using the PDB2PQR server [76]. A TIP3P water box with a 12 Å radius was used to solvate the systems, and counterions were added to neutralize the system's charges. To neutralize the systems and maintain a physiological concentration (0.15 M), Na+ and Cl− ions were added [77].

Each solvated system was minimized in four stages: (i) ions and water molecules; (ii) hydrogen atoms; (iii) water molecules and hydrogen atoms; and (iv) the entire system. All steps were performed using 5000 steps with the steepest descent method and 5000 additional steps with the conjugate gradient algorithm. Subsequently, each system was heated for 200 ps to 300 K under constant volume

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with positional restraints on the solute. An unrestrained equilibration step of 1 ns under constant pressure was performed. Langevin dynamics was employed to control the temperature (300 K) with a collision frequency of 2 ps–1. The SHAKE algorithm [77] was used to restrain bond lengths involving hydrogen atoms, while the Particle Mesh Ewald (PME) method [78] was employed to handle long-range electrostatic interactions. A 10 Å cutoff was applied for non-bonded interactions.

Finally, 200 ns of production was conducted without positional restraints at a constant temperature of 300 K. The pressure was controlled by a Berendsen barostat. The structural analysis of each system was performed by calculating the root-mean-square deviations (RMSD) and the root-mean-square fluctuations (RMSF) of the backbone atoms of the protein.

4.5. MM-GBSA Binding Free Energy Calculation

To estimate the binding free energy (ΔG_bind) of the compounds Myrtenal, Myrtenol, and Verbenol with the Nuclear Factor NF-kappa-B p105 protein, we used the MM-GBSA method implemented in AmberTools23 [79]. The calculations utilized the final 10 ns (1000 frames) of the MD simulation trajectories. Established literature provides detailed descriptions of the MM-GBSA equations [80,81].

5. Conclusions

Based on docking and molecular dynamics results, it can be suggested that the most promising compounds are Myrtenal and Myrtenol. The results obtained in this study allowed for the realization of chemical studies, that is, the prioritization of molecules that should be isolated from the oil and identified. Once isolated, in vitro assays are planned, as well as studies on cytotoxicity, genotoxicity, mutagenicity, and mechanisms of cell death. After analyzing the results, the active molecule with the lowest toxic potential will undergo studies to evaluate its possible mechanisms of action. Subsequently, structural modification studies will be conducted to increase the inhibition potential and reduce toxicity.

The final stage of the pharmacological studies will involve in vivo studies (toxicity and activity) to establish dose-response correlation. If the pharmacological potential is confirmed, product development is expected. In summary, the essential oil from T. *polium*, due to its composition, appears to be highly promising as an anti-inflammatory and antitumor agent.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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References

1. Abdollahi, M.; Karimpour, H.; Monsef-Esfehani, H.R. Antinociceptive Effects of Teucrium Polium L. Total Extract and Essential Oil in Mouse Writhing Test. Pharmacol Res 2003, 48, 31–35, doi:https://doi.org/10.1016/S1043-6618(03)00059-8.
2. Bahramikia, S.; Yazdanparast, R. Phytochemistry and Medicinal Properties of Teucrium Polium L. (Lamiaceae). *Phytotherapy Research* 2012, *26*, 1581–1593, doi:https://doi.org/10.1002/ptr.4617.
3. Bahramikia, S.; Hemmati Hassan Gavyar, P.; Yazdanparast, R. Teucrium Polium L: An Updated Review of Phytochemicals and Biological Activities. *Avicenna J Phytomed* 2022, *12*, 224–240, doi:10.22038/ajp.2021.19155.
4. Vokou, D. and B.; Jean-M Volatile Constituents of Teucrium Polium. *J Nat Prod* 1985, *48*, 498–499.
5. Bezić, N.; Vuko, E.; Dunkić, V.; Ruščić, M.; Blažević, I.; Burčul, F. Antiphytoviral Activity of Sesquiterpene-Rich Essential Oils from Four Croatian Teucrium Species. *Molecules* 2011, *16*, 8119–8129, doi:10.3390/molecules16098119.
6. Essid, R.; Rahali, F.Z.; Msaada, K.; Sghair, I.; Hammami, M.; Bouratbine, A.; Aoun, K.; Limam, F. Antileishmanial and Cytotoxic Potential of Essential Oils from Medicinal Plants in Northern Tunisia. *Ind Crops Prod* 2015, *77*, 795–802, doi:10.1016/J.INDCROP.2015.09.049.
7. Sayyad, R.; Farahmandfar, R. Influence of Teucrium Polium L. Essential Oil on the Oxidative Stability of Canola Oil during Storage. *J Food Sci Technol* 2017, *54*, 3073–3081, doi:10.1007/s13197-017-2743-0.
8. Masoudi, S. Volatile Constituents from Different Parts of Three Lamiacea Herbs from Iran. *Iran J Pharm Res* 2018, *17*, 365.
9. Vahdani, M.; Faridi, P.; Zarshenas, M.M.; Javadpour, S.; Abolhassanzadeh, Z.; Moradi, N.; Bakzadeh, Z.; Karmostaji, A.; Mohagheghzadeh, A.; Ghasemi, Y. Major Compounds and Antimicrobial Activity of Essential Oils from Five Iranian Endemic Medicinal Plants. *Pharmacognosy Journal* 2011, *3*, 48–53, doi:10.5530/PJ.2011.22.10.
10. Djabou, N.; Lorenzi, V.; Guinoiseau, E.; Andreani, S.; Giuliani, M.C.; Desjobert, J.M.; Bolla, J.M.; Costa, J.; Berti, L.; Luciani, A.; et al. Phytochemical Composition of Corsican Teucrium Essential Oils and Antibacterial Activity against Foodborne or Toxi-Infectious Pathogens. *Food Control* 2013, *30*, 354–363, doi:10.1016/J.FOODCONT.2012.06.025.
11. Hussain, J.; Rehman, N.U.; Al-Harrasi, A.; Ali, L.; Khan, A.L.; Albroumi, M.A. Essential Oil Composition and Nutrient Analysis of Selected Medicinal Plants in Sultanate of Oman. *Asian Pac J Trop Dis* 2013, *3*, 421–428, doi:10.1016/S2222-1808(13)60095-X.
12. El Atki, Y.; Aouam, I.; El kamari, F.; Taroq, A.; Lyoussi, B.; Taleb, M.; Abdellaoui, A. Total Phenolic and Flavonoid Contents and Antioxidant Activities of Extracts from Teucrium Polium Growing Wild in Morocco. *Mater Today Proc* 2019, *13*, 777–783, doi:10.1016/J.MATPR.2019.04.040.
13. Fitsiou, E.; Pappa, A. Anticancer Activity of Essential Oils and Other Extracts from Aromatic Plants Grown in Greece. *Antioxidants* 2019, *8*, doi:10.3390/antiox8080290.
14. Ebadollahi, A.; Taghinezhad, E. Modeling and Optimization of the Insecticidal Effects of Teucrium Polium L. Essential Oil against Red Flour Beetle (Tribolium Castaneum Herbst) Using Response Surface Methodology. *Information Processing in Agriculture* 2020, *7*, 286–293, doi:https://doi.org/10.1016/j.inpa.2019.08.004.
15. Wassel, G.M.; Ahmed, S.S. On the Essential Oil of Teucrium Polium L. 1974.
16. Ghiglione, C.; Lemordant, D.; Gast, M. The Chemical Composition of Teucrium Polium Ssp. Cylindricum. Characterization of Alkanes and of Beta-Eudesmol. 1976.
17. Cozzani, S.; Muselli, A.; Desjobert, J.-M.; Bernardini, A.-F.; Tomi, F.; Casanova, J. Chemical Composition of Essential Oil of Teucrium Polium Subsp. Capitatum (L.) from Corsica. *Flavour Fragr J* 2005, *20*, 436–441, doi:https://doi.org/10.1002/ffj.1463.
18. Moghtader, M.; Salari, H.; Farahmand, A. Anti-Bacterial Effects of the Essential Oil of Teucrium Polium L. on Human Pathogenic Bacteria. *Iranian Journal of Medical Microbiology* 2013, *7*.
19. Zabihi, N.A.; Mousavi, S.M.; Mahmoudabady, M.; Soukhtanloo, M.; Sohrabi, F.; Niazmand, S. Teucrium Polium L. Improves Blood Glucose and Lipids and Ameliorates Oxidative Stress in Heart and Aorta of Diabetic Rats. *Int J Prev Med* 2018, *9*.
20. Shaker, B.; Ahmad, S.; Lee, J.; Jung, C.; Na, D. In Silico Methods and Tools for Drug Discovery. *Comput Biol Med* 2021, *137*, 104851, doi:https://doi.org/10.1016/j.compbiomed.2021.104851.
21. Khani, A.; Heydarian, M. Fumigant and Repellent Properties of Sesquiterpene-Rich Essential Oil from Teucrium Polium Subsp. Capitatum (L.). *Asian Pac J Trop Med* 2014, *7*, 956–961, doi:10.1016/S1995-7645(14)60169-3.
22. Ben Othman, M.; Bel Hadj Salah-Fatnassi, K.; Ncibi, S.; Elaissi, A.; Zourgui, L. Antimicrobial Activity of Essential Oil and Aqueous and Ethanol Extracts of Teucrium Polium L. Subsp. Gabesianum (L.H.) from Tunisia. *Physiology and Molecular Biology of Plants* 2017, *23*, 723–729, doi:10.1007/s12298-017-0444-9.

23. Kutchan, T.M. Ecological Arsenal and Developmental Dispatcher. The Paradigm of Secondary Metabolism PLANTS PRODUCE A LARGE NUMBER OF CHEMICALS OF DIVERSE STRUCTURE AND CLASS; 2001; Vol. 125;.
24. Cakir, A.; Duru, M.E.; Harmandar, M.; Ciriminna, R.; Passannanti, S. Volatile Constituents of Teucrium Polium L. from Turkey. *Journal of essential oil Research* 1998, *10*, 113–115.
25. Maizi, Y.; Meddah, B.; Tir Touil Meddah, A.; Gabaldon Hernandez, J.A. Seasonal Variation in Essential Oil Content, Chemical Composition and Antioxidant Activity of Teucrium Polium L. Growing in Mascara (North West of Algeria). *Journal of Applied Biotechnology Reports* 2019, *6*, 151–157.
26. Reaisi, Z.; Yadegari, M.; Shirmardia, H.A. Effects of Phenological Stage and Elevation on Phytochemical Characteristics of Essential Oil of Teucrium Polium L. and Teucrium Orientale L. *Int J Hortic Sci Technol* 2019, *6*, 89–99.
27. Sabzeghabaie, A.; Asgarpanah, J. Essential Oil Composition of Teucrium Polium L. Fruits. *Journal of EssEntial oil rEsEarch* 2016, *28*, 77–80.
28. Boulila, A.; Béjaoui, A.; Messaoud, C.; Boussaid, M. Variation of Volatiles in Tunisian Populations of Teucrium Polium L.(Lamiaceae). *Chem Biodivers* 2008, *5*, 1389–1400.
29. Sadeghi, H.; Jamalpoor, S.; Shirzadi, M.H. Variability in Essential Oil of Teucrium Polium L. of Different Latitudinal Populations. *Ind Crops Prod* 2014, *54*, 130–134.
30. Belmekki, N.; Bendimerad, N.; Bekhechi, C.; Fernandez, X. Chemical Analysis and Antimicrobial Activity of Teucrium Polium L. Essential Oil from Western Algeria. *J Med Plants Res* 2013, *7*, 897–902.
31. Gülsoy Toplan, G.; Göger, F.; Taşkin, T.; Ecevit-Genç, G.; Civaş, A.; Işcan, G.; Kürkçüoğlu, M.; Mat, A.; Başer, K.H.C. Phytochemical Composition and Pharmacological Activities of Teucrium Polium L. Collected from Eastern Turkey. *Turk J Chem* 2022, *46*, 269–282, doi:10.3906/kim-2107-13.
32. Ghavam, M.; Markabi, F.S. Evaluation of Yield, Chemical Profile, and Antimicrobial Activity of Teucrium Polium L. Essential Oil Used in Iranian Folk Medicine. *Appl Biochem Biotechnol* 2024, 1–17.
33. Donmez, I.E. Volatile Oil Composition of Teucrium Species of Natural and Cultivated Origin in the Lake District of Turkey. 2022.
34. Aburjai, T.; Hudaib, M.; Cavrini, V. Composition of the Essential Oil from Jordanian Germander (Teucrium Polium L.). *Journal of Essential Oil Research* 2006, *18*, 97–99.
35. Lograda, T.; Messaoud, R.; Chalard, P. Chemical Analysis and Antimicrobial Activity of Teucrium Polium L. Essential Oil from Eastern Algeria; 2014;
36. Catinella, G.; Badalamenti, N.; Ilardi, V.; Rosselli, S.; De Martino, L.; Bruno, M. The Essential Oil Compositions of Three Teucrium Taxa Growing Wild in Sicily: HCA and PCA Analyses. *Molecules* 2021, *26*, 643.
37. Lipinski, C.A. Lead-and Drug-like Compounds: The Rule-of-Five Revolution. *Drug Discov Today Technol* 2004, *1*, 337–341.
38. Dolabela, M.F.; Silva, A.R.P. da; Ohashi, L.H.; Bastos, M.L.C.; Silva, M.C.M. da; Vale, V.V. Estudo in Silico Das Atividades de Triterpenos e Iridoides Isolados de Himatanthus Articulatus (Vahl) Woodson. 2018.
39. de Barros, R.C.; Araujo da Costa, R.; Farias, S.D.P.; de Albuquerque, K.C.O.; Marinho, A.M.R.; Campos, M.B.; Marinho, P.S.B.; Dolabela, M.F. In Silico Studies on Leishmanicide Activity of Limonoids and Fatty Acids from Carapa Guianensis Aubl. *Front Chem* 2024, *12*, 1394126.
40. Rathinam, A.; Pari, L. Myrtenal Ameliorates Hyperglycemia by Enhancing GLUT2 through Akt in the Skeletal Muscle and Liver of Diabetic Rats. *Chem Biol Interact* 2016, *256*, 161–166, doi:10.1016/J.CBI.2016.07.009.
41. Wang, J.; Sintim, H.O. Dialkylamino-2, 4-dihydroxybenzoic Acids as Easily Synthesized Analogues of Platensimycin and Platencin with Comparable Antibacterial Properties. *Chemistry–A European Journal* 2011, *12*, 3352–3357.
42. V Suslov, E.; Yu Ponomarev, K.; D Rogachev, A.; A Pokrovsky, M.; G Pokrovsky, A.; B Pykhtina, M.; B Beklemishev, A.; V Korchagina, D.; P Volcho, K.; F Salakhutdinov, N. Compounds Combining Aminoadamantane and Monoterpene Moieties: Cytotoxicity and Mutagenic Effects. *Med Chem (Los Angeles)* 2015, *11*, 629–635.
43. Luzina, O.; Filimonov, A.; Zakharenko, A.; Chepanova, A.; Zakharova, O.; Ilina, E.; Dyrkheeva, N.; Likhatskaya, G.; Salakhutdinov, N.; Lavrik, O. Usnic Acid Conjugates with Monoterpenoids as Potent Tyrosyl-DNA Phosphodiesterase 1 Inhibitors. *J Nat Prod* 2020, *83*, 2320–2329, doi:10.1021/acs.jnatprod.9b01089.
44. Kapitsa, I.G.; Suslov, E. V; Teplov, G. V; Korchagina, D. V; Komarova, N.I.; Volcho, K.P.; Voronina, T.A.; Shevela, A.I.; Salakhutdinov, N.F. Synthesis and Anxiolytic Activity of 2-Aminoadamantane Derivatives Containing Monoterpene Fragments. *Pharm Chem J* 2012, *46*, 263–265.
45. Li-Zhulanov, N.S.; Zaikova, N.P.; Sari, S.; Gülmez, D.; Sabuncuoğlu, S.; Ozadali-Sari, K.; Arikan-Akdagli, S.; Nefedov, A.A.; Rybalova, T. V; Volcho, K.P. Rational Design of New Monoterpene-Containing Azoles and Their Antifungal Activity. *Antibiotics* 2023, *12*, 818.

46. Ponomarev, K.; Morozova, E.; Pavlova, A.; Suslov, E.; Korchagina, D.; Nefedov, A.; Tolstikova, T.; Volcho, K.; Salakhutdinov, N. Synthesis and Analgesic Activity of Amines Combining Diazaadamantane and Monoterpene Fragments. *Med Chem (Los Angeles)* 2017, *13*, 773–779.
47. Liu, L.; Liu, B.; Li, L.; He, M.-X.; Zhou, X.-D.; Li, Q. Myrtenol Inhibits Biofilm Formation and Virulence in the Drug-Resistant Acinetobacter Baumannii: Insights into the Molecular Mechanisms. *Infect Drug Resist* 2022, 5137–5148.
48. de Britto, R.M.; da Silva-Neto, J.A.; Mesquita, T.R.R.; de Vasconcelos, C.M.L.; de Almeida, G.K.M.; de Jesus, I.C.G.; Dos Santos, P.H.; Souza, D.S.; Miguel-dos-Santos, R.; de Sá, L.A. Myrtenol Protects against Myocardial Ischemia-Reperfusion Injury through Antioxidant and Anti-Apoptotic Dependent Mechanisms. *Food and Chemical Toxicology* 2018, *111*, 557–566.
49. Choi, I.-Y.; Lim, J.H.; Hwang, S.; Lee, J.-C.; Cho, G.-S.; Kim, W.-K. Anti-Ischemic and Anti-Inflammatory Activity of (S)-Cis-Verbenol. *Free Radic Res* 2010, *44*, 541–551.
50. Oeckinghaus, A.; Ghosh, S. The NF-KB Family of Transcription Factors and Its Regulation. *Cold Spring Harb Perspect Biol* 2009, *1*, a000034.
51. Betzler, A.C.; Theodoraki, M.N.; Schuler, P.J.; Döscher, J.; Laban, S.; Hoffmann, T.K.; Brunner, C. NF-KB and Its Role in Checkpoint Control. *Int J Mol Sci* 2020, *21*.
52. Li, M.; Liu, P.; Wang, B.; Zhou, J.; Yang, J. Inhibition of Nuclear Factor Kappa B as a Therapeutic Target for Lung Cancer. *Altern Ther Health Med* 2022, *28*.
53. Ersoy, E.; Süvari, G.; Ercan, S.; Eroğlu Özkan, E.; Karahan, S.; Aygün Tuncay, E.; Yeşil Cantürk, Y.; Mataracı Kara, E.; Zengin, G.; Boğa, M. Towards a Better Understanding of Commonly Used Medicinal Plants from Turkiye: Detailed Phytochemical Screening and Biological Activity Studies of Two Teucrium L. Species with in Vitro and in Silico Approach. *J Ethnopharmacol* 2023, *312*, 116482, doi:https://doi.org/10.1016/j.jep.2023.116482.
54. Chabane, S.; Boudjelal, A.; Napoli, E.; Benkhaled, A.; Ruberto, G. Phytochemical Composition, Antioxidant and Wound Healing Activities of Teucrium Polium Subsp. Capitatum (L.) Briq. Essential Oil. *Journal of Essential Oil Research* 2021, *33*, 143–151, doi:10.1080/10412905.2020.1842260.
55. Al-Naemi, H.A.; Alasmar, R.M.; Al-Ghanim, K. Alcoholic Extracts of Teucrium Polium Exhibit Remarkable Anti-Inflammatory Activity: In Vivo Study. *Biomolecules and Biomedicine* 2024, *24*, 82–88, doi:10.17305/bb.2023.9239.
56. Wen, Y.; Zhu, Y.; Zhang, C.; Yang, X.; Gao, Y.; Li, M.; Yang, H.; Liu, T.; Tang, H. Chronic Inflammation, Cancer Development and Immunotherapy. *Front Pharmacol* 2022, *13*.
57. Kaneko, T.; Myo Zaw, S.Y.; Sueyama, Y.; Katsube, K. ichi; Kaneko, R.; Nör, J.E.; Okiji, T. Inhibition of Nuclear Factor Kappa B Prevents the Development of Experimental Periapical Lesions. *J Endod* 2019, *45*, 168–173, doi:10.1016/j.joen.2018.10.006.
58. Alharbi, K.S.; Fuloria, N.K.; Fuloria, S.; Rahman, S.B.; Al-Malki, W.H.; Javed Shaikh, M.A.; Thangavelu, L.; Singh, S.K.; Rama Raju Allam, V.S.; Jha, N.K.; et al. Nuclear Factor-Kappa B and Its Role in Inflammatory Lung Disease. *Chem Biol Interact* 2021, *345*, 109568, doi:10.1016/J.CBI.2021.109568.
59. McLafferty, F.W.; Stauffer, D.B.; Stenhagen, E.; Heller, S.R. The Wiley/NBS Registry of Mass Spectral Data. *(No Title)* 1989.
60. PJ, L. NIST Standard Reference Database Number 69. *NIST Chemistry WebBook* 2003.
61. Dong, J.; Wang, N.-N.; Yao, Z.-J.; Zhang, L.; Cheng, Y.; Ouyang, D.; Lu, A.-P.; Cao, D.-S. ADMETlab: A Platform for Systematic ADMET Evaluation Based on a Comprehensively Collected ADMET Database. *J Cheminform* 2018, *10*, 1–11.
62. Costa, C.R.; Olivi, P.; Botta, C.M.R.; Espindola, E.L.G. A Toxicidade Em Ambientes Aquáticos: Discussão e Métodos de Avaliação. *Quim Nova* 2008, *31*, 1820–1830.
63. Guilhermino, L.; Diamantino, T.; Carolina Silva, M.; Soares, A.M.V.M. Acute Toxicity Test with Daphnia Magna: An Alternative to Mammals in the Prescreening of Chemical Toxicity? *Ecotoxicol Environ Saf* 2000, *46*, 357–362, doi:10.1006/EESA.2000.1916.
64. Zucker, E. Hazard Evaluation Division Standard Evaluation Procedure: Acute Toxicity Test for Freshwater Fish; US Environmental Protection Agency, Office of Pesticide Programs, 1985;
65. Ames, B.N.; McCann, J.; Yamasaki, E. Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test. *Mutat. Res.;(Netherlands)* 1975, *31*.
66. Drwal, M.N.; Banerjee, P.; Dunkel, M.; Wettig, M.R.; Preissner, R. ProTox: A Web Server for the in Silico Prediction of Rodent Oral Toxicity. *Nucleic Acids Res* 2014, *42*, doi:10.1093/nar/gku401.
67. Nickel, J.; Gohlke, B.-O.; Erehman, J.; Banerjee, P.; Rong, W.W.; Goede, A.; Dunkel, M.; Preissner, R. SuperPred: Update on Drug Classification and Target Prediction. *Nucleic Acids Res* 2014, *42*, W26–W31, doi:10.1093/nar/gku477.
68. Müller, C.W.; Rey, F.A.; Sodeoka, M.; Verdine, G.L.; Harrison, S.C. Structure of the NF-KB P50 Homodimer Bound to DNA. *Nature* 1995, *373*, 311–317, doi:10.1038/373311a0.
69. Ruch, P.; Teodoro, D.; Consortium, U. *Uniprot*; 2021;

 doi:10.20944/preprints202411.0589.v1

70. Jain, P.; Sudandiradoss, C. Andrographolide-Based Potential Anti-Inflammatory Transcription Inhibitors against Nuclear Factor NF-Kappa-B P50 Subunit (NF-KB P50): An Integrated Molecular and Quantum Mechanical Approach. *3 Biotech* 2023, *13*, doi:10.1007/s13205-022-03431-9.
71. Bitencourt-Ferreira, G.; de Azevedo, W.F. Molegro Virtual Docker for Docking. *Docking screens for drug discovery* 2019, 149–167.
72. Case, D.A.; Aktulga, H.M.; Belfon, K.; Ben-Shalom, I.; Brozell, S.R.; Cerutti, D.S.; Cheatham III, T.E.; Cruzeiro, V.W.D.; Darden, T.A.; Duke, R.E. *Amber 2021*; University of California, San Francisco, 2021;
73. Frisch, M.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G.A. Gaussian 09, Gaussian. *Inc., Wallingford CT* 2009, *121*, 150–166.
74. Wang, J.; Wolf, R.M.; Caldwell, J.W.; Kollman, P.A.; Case, D.A. Development and Testing of a General Amber Force Field. *J Comput Chem* 2004, *25*, 1157–1174.
75. Maier, J.A.; Martinez, C.; Kasavajhala, K.; Wickstrom, L.; Hauser, K.E.; Simmerling, C. Ff14SB: Improving the Accuracy of Protein Side Chain and Backbone Parameters from Ff99SB. *J Chem Theory Comput* 2015, *11*, 3696–3713, doi:10.1021/acs.jctc.5b00255.
76. Dolinsky, T.J.; Czodrowski, P.; Li, H.; Nielsen, J.E.; Jensen, J.H.; Klebe, G.; Baker, N.A. PDB2PQR: Expanding and Upgrading Automated Preparation of Biomolecular Structures for Molecular Simulations. *Nucleic Acids Res* 2007, *35*, W522–W525, doi:10.1093/nar/gkm276.
77. Jorgensen, W.L.; Chandrasekhar, J.; Madura, J.D.; Impey, R.W.; Klein, M.L. Comparison of Simple Potential Functions for Simulating Liquid Water. *J Chem Phys* 1983, *79*, 926–935, doi:10.1063/1.445869.
78. Darden, T.; York, D.; Pedersen, L. Particle Mesh Ewald: An N· Log (N) Method for Ewald Sums in Large Systems. *J Chem Phys* 1993, *98*, 10089–10092.
79. Case, D.A.; Aktulga, H.M.; Belfon, K.; Cerutti, D.S.; Cisneros, G.A.; Cruzeiro, V.W.D.; Forouzesh, N.; Giese, T.J.; Götz, A.W.; Gohlke, H. AmberTools. *J Chem Inf Model* 2023, *63*, 6183–6191.
80. Genheden, S.; Ryde, U. The MM/PBSA and MM/GBSA Methods to Estimate Ligand-Binding Affinities. *Expert Opin Drug Discov* 2015, *10*, 449–461.
81. da Costa, R.A.; da Rocha, J.A.P.; Pinheiro, A.S.; Da Costa, A. do S.S.; da Rocha, E.C.M.; Josino, L.P.C.; da Silva Gonçalves, A.; e Lima, A.H.L.; Brasil, D.S.B. In Silico Identification of Novel Allosteric Inhibitors of Dengue Virus NS2B/NS3 Serine Protease. *Journal of the Serbian Chemical Society* 2022, *87*, 693–706.
82. Adams, Robert P. 2017. "Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 5 Online Ed." *Gruver, TX USA: Texensis Publishing*.
83. Babushok, V I, P J Linstrom, and I G Zenkevich. 2011. "Retention Indices for Frequently Reported Compounds of Plant Essential Oils." *Journal of Physical and Chemical Reference Data* 40 (4).