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Phytochemistry and Biological Activities of Essential Oils from *Satureja calamintha* Nepeta

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Abstract: *Satureja calamintha* nepeta (*S. calamintha*) has a history of successful use in the treatment of bacterial and fungal diseases. The present study was designed to investigate the chemical composition and antioxidant and antimicrobial activities of essential oils extracted from wild *S. calamintha* (EOSS) and domesticated *S. calamintha* (EOSD) for comparison purposes. Hydrodistillation was used to extract the essential oils (EOs), while GC/MS was used for chemical analysis. Antioxidant activity was studied using DPPH and FRAP assays. Antifungal activity was performed against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum*), while antibacterial activity was tested against clinically resistant bacteria, namely *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Proteus mirabilis*. By using ab=n in silico approach, the antioxidant and antimicrobial activities of the main compounds of EOSS and EOSD were also investigated. The yields obtained of EOSS and EOSD were 2.80% and 1.95%, respectively, with a dominance of eucalyptol, pulegone and rotundifolone. Concerning the antioxidant power, the IC50 values recorded by the use of the DPPH assay were in the range of 23.03 ± 4.30 and 24.09 ± 4.38 µg/mL for EOSS and EOSD, respectively, while by using the FRAP assay, the EC_{50} values were in the range of 55.38 ± 2.16 and 60.72 ± 7.71 µg/mL for EOSS and EOSD, respectively. Importantly, both essential oils of EOSS and EOSD exhibited good antibacterial activity against all studied bacteria; notably, the inhibition zone ranged from 14 ± 0.00 to 48.67 ± 1.15 mm and the MICs ranged from 0.37 ± 0.00 to 5.96 ± 0.00 µg/mL. Similarly, the studied EOs showed important antifungal activities compared to all the studied fungi, wherein the inhibition percentage ranged from 47.33 ± 1.15 to 89.18 ± 0.75%, while the MICs ranged from 0.18 ± 0.00 to 2.98 ± 0.00 µg/mL. The molecular docking results showed that piperitenone and pulegone strongly inhibited human acetylcholinesterase, whereas (+)-Isomenthone and piperitenone strongly inhibited *S. aureus* nucleoside diphosphate kinase, and *E. coli* beta-ketoacyl-[acyl carrier protein] synthase, respectively. The outcome of this article suggests that EOs of *S. calamintha* can be developed as alternative agents to manage drug-resistant phenomena and free radical issues.

Keywords: antimicrobial; antioxidant; essential oil; molecular docking; *Satureja calamuntha*

1. Introduction

For a long time, aromatic plants have occupied a very important place in the daily life of mankind; they are considered a real source of bioactive compounds, and they are used in the field of agrifood, cosmetics, pharmaceuticals, and perfumery. Among the most important plant families used for therapeutic and food purposes, Lamiaceae can be noted, which possesses about 6900 species and 233 genera, including *Satureja* [1]. The genus *Satureja* is distributed mainly around the Mediterranean basin, Asia, and boreal America [2]. *S. calamintha* is extensively dispersed over the Mediterranean, Asia, and American regions [2]. It is perennial, pubescent, aromatic, and 40–80 cm high. It has flexible and hairy stems that carry opposite and slightly serrated leaves attached to a medium petiole [2]. Native to Europe and the Mediterranean Basin [3], several ailments, including motion sickness, gastrointestinal distress, viral illness, and diarrhea, have long been treated using the genus Satureja [4]. *S. calamintha*. has also been traditionally used in the treatment of various diseases such as indigestion, nausea, diarrhea, cramps, infectious diseases, and muscle pain [4]. *Satureja* extracts have been reported to possess various therapeutic effects. In addition, this plant possesses antiseptic, antioxidant, antibacterial, antifungal, antidiarrheal, anti-inflammatory, and antispasmodic properties [5]. *S. calamintha* is very popular in Morocco and Algeria, where it is used as an odoriferous agent in perfumes and as a powerful disinfectant [6].

The literature has reported the chemical composition of wild *S. calamintha* in Morocco and Algeria [7,8]. These studies concluded that the chemical composition of its essential oil (EO) varies considerably according to the area of collection [7,9,10]

Indeed, several studies have revealed that medicinal and aromatic products have pharmacological properties, including antimicrobial [11,12] and antioxidant properties [13], and have shown that the essential oil of *S. calamintha* has an antimicrobial and antioxidant effect [7,10,14]. The excessive use of wild species by the population leads to a decrease in the abundance of *S. calamintha* nepeta. Notably, the harvest of medicinal plants during flowering and before the seeds germinate leads to a decrease in the regeneration of these plants [15]. Therefore, the process of domestication may serve to protect the plant from extinction. One of the most effective ways to prevent the extinction of useful plants is to cultivate them as houseplants [8,16]. However, domestication has the potential to alter the chemical composition of plants, and as a result, their biological processes may be altered as well. Thus, understanding the effect of domestication is necessary to successfully cultivate such therapeutic species [17].

The present study was designed to Investigate the chemical composition and antioxidant and antimicrobial activities of the essential oils extracted from wild *S. calamintha* (EOSS) and domesticated *S. calamintha* (EOSD. Notably, to the best of our knowledge, this is the first study comparing the chemical composition and the antimicrobial and antioxidant activities of wild and domesticated *S. calamintha* EOs.

2. Materials and Methods

2.1. Plant Material

In this work, the aerial parts (at flowering stage) and seeds (at post-flowering stage) of wild *S. calamintha* nepta were collected in August in the northern region of Morocco, namely Bouchfaa, in the province of Taza (Bouchfaa 34°06′10.2″ N 4°17′15.4″ W), (Figure 1).

2.2. Seed Germination

To perform germination, the seeds of *S. calamintha* nepta were collected from the wild plant at the vegetative stage. The collected seeds were not treated and kept in plastic bags until use. Next, seeds were isolated and sorted manually before being sown in trays and placed in a greenhouse at ambient temperature. Each tray was seeded with 75 seeds with 5 seeds per hole. Humidity was maintained relatively high by watering the trays every day. The cumulative percentage of germination was calculated every day for a month to track the development of the germination. After being germinated, the seeds were

transferred into a greenhouse. The ability of the regenerated plants to recover in their natural habitat was evaluated by transplanting them into the field up to the eight-leaf stage. Before being placed at the herbarium of the University of Fez, Morocco, the specimen underwent botanical identification by a botanist. Prior to extraction, the leaves of the studied plant were air-dried for ten days in the shade at room temperature.

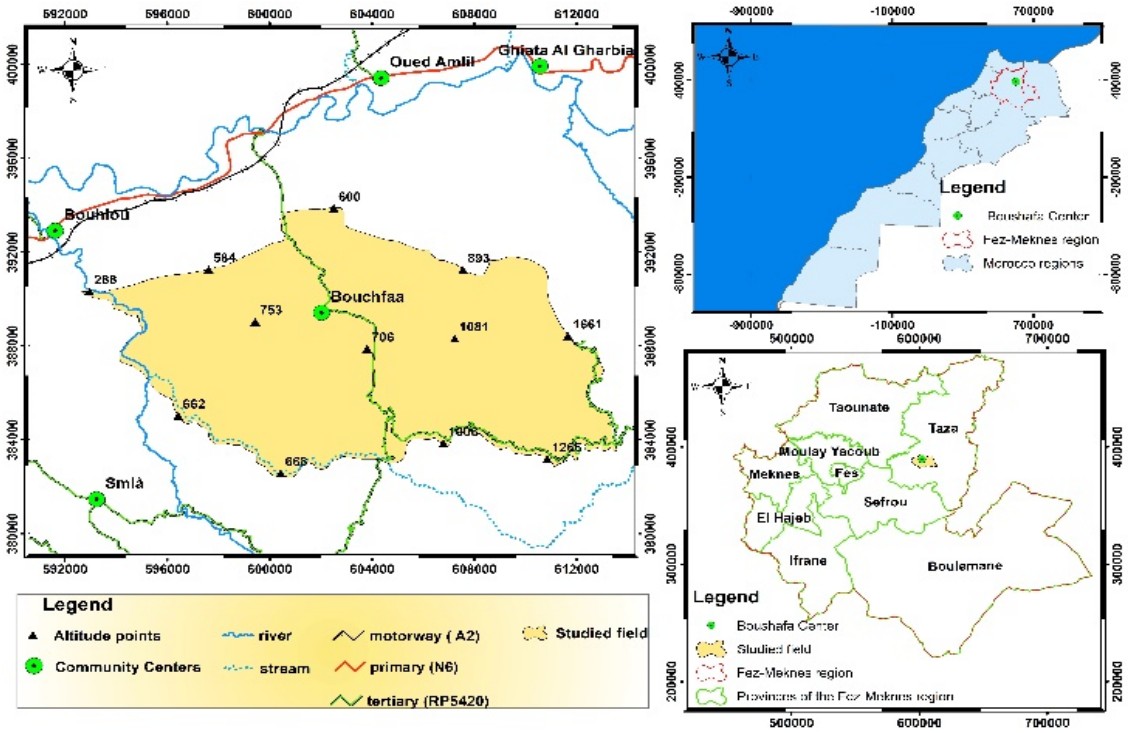


Figure 1. Geographical map of the commune of Bouchfaa in the Taza province. The map was prepared using QGIS software.

2.3. Extraction of Essential Oils

The *S. calamintha* (wild and cultivated varieties) was collected in the Taza region, Morocco, in 2019 before being identified by the botanist Professor Amina Bari under the voucher specimen (T/Bou.Sc.2019), which has been deposited in the herbarium of the Faculty of Sciences Dhar El-Mahraz, Sidi Mohamed Ben Abdellah University, Fez, Morocco. Next, in a round-bottomed flask, 200 g of finely cut aerial parts was soaked in 750 mL of distilled water before being extracted using hydro-dilatation for 4 h. The resulting EOs were stored at 4 °C until further use. The aerial parts of the studied plant were shade-dried. Next, in a round-bottomed flask, 200 g of finely cut aerial parts was soaked in 750 mL of distilled water prior to extraction by using hydro-dilation for 2 h. The EOs obtained were stored at 4 °C until eventual use.

2.4. GC–MS Analysis of Essential Oils

The identification of the different chemical compounds contained in the essential oils was performed using gas chromatography–mass spectrometry (Manufacturer: Agilent Technologies, Santa Clara, CA, USA). Briefly, the separation of individual compounds was conducted with the use of a GC Column HP-5MS at 30 m, 0.250 mm and 0.250 µm. Helium was employed as a carrier at 0.9 mL/s. The oven temperature was raised from 60 to 300 °C/min for 20 min. The injection temperature was 250 °C and the temperature of the interface was 260 °C. The identification of each separate chemical compound was carried out on the basis of its mass spectra compared to those in the NIST database [18].

2.5. Antioxydant Activity

2.5.1. DPPH Test

Analysis of the free radical scavenging activity of DPPH was performed according to the protocol described by Wang et al. [19], with small modifications. Briefly, in test tubes, 1.5 mL of a solution of DPPH solubilized in methanol was added to 0.2 mL of each concentration of the EOs samples (0.1 to 2 mg/mL). The obtained mixture was vigorously shaken prior to incubation in the darkness for 30 min at ambient temperature before the absorbance was measured at 517 nm. The percentage % of inhibition was calculated using the following formula:

$$\%\text{Antioxidant activity} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad (1)$$

where "A control" is the absorbance of the control and "OD sample" is the absorbance of the test. Notably, quercetin was used as a positive control and IC_{50} was calculated by plotting the percent inhibition versus oil concentration.

2.5.2. FRAP Test

The reducing power test was performed as described by Moattar [20], with some modifications. In brief, 2.5 mL of the essential oil from each wild and domesticated *S. calamintha* plant was mixed with 2.50 mL of phosphate buffer (pH 6.60) and 2.50 mL of potassium ferricyanide (1%). After incubation at 50 °C for 20 min, 2.5 mL of 10% $C_2HCl_3O_2$ was added to the medium prior to centrifugation for 10 min at 3000 rpm. Subsequently, 2.50 mL of the obtained solution was combined with 2.5 mL of water and 0.50 mL of $FeCl_3$ (0.1%). The quercetin was used as a positive control and the IC_{50} was determined by plotting the absorbance against the corresponding sample concentration.

2.6. Antimicrobial Activity of Essential Oils of S. calamintha

2.6.1. Disk Diffusion Test

The bacterial strains used in the experiment, including *Staphylococcus aureus* ATCC-6633, *Escherichia coli* K-12, *Bacillus subtilis* DSM-6333, and *Proteus mirabilis* ATCC-29906, were inoculated onto Petri plates containing Mueller–Hinton agar media at a density of 1–5 × 10^6 CFU/mL. Next, paper discs (6 mm in diameter) were soaked in 20 µL of EOs of wild and domesticated *S. calamintha* before being placed on the inoculated dishes. Afterwards, all bacterial stains were incubated at 37 °C for 24 h prior to determining the inhibition zone diameters.

The fungi used in the experiment, including *A. niger*, *A. flavus*, and *F. oxysporum*, were incubated with *C. albicans* and were inoculated onto Petri plates containing Potato Dextrose Agar (PDA) media. Next, paper discs (6 mm in diameter) were soaked with 20 µL of EOs of wild and domesticated *S. calamintha* before being placed on the inoculated dishes. Afterwards *A. niger*, *A. flavus*, and *F. oxysporum* were incubated at 30 °C, while *C. albicans* was incubated at 37 °C for seven days prior to determining the inhibition zone diameters [21,22].

2.6.2. Determination of the Minimum Inhibitory Concentration (MIC)

The determination of the minimum inhibitory concentration of wild and domesticated *S. calamintha* EOs against the four bacterial and four fungal strains was performed using the microdilution method, as described by Sarker et al. [23]. Briefly, a micro-dilution was performed by diluting the sample by a factor of 2 in each well, with the exception of the last well, which acted as the positive control for growth. After 24 h of incubation for the bacteria, 48 h for *C. albicans* and 7 days for *A. niger*, *A. flavus* and *F. oxysporium* were incubated at 37 °C and 30 °C, respectively. The MIC was determined by applying the colorimetric method (TTC 0.2% (*w*/*v*)) [24,25]

2.7. In Silico Molecular Docking of Antioxidant and Antimicrobial Activities of EO of Wild and Domesticated S. calamintha

In this molecular docking study, the inhibition of human acetylcholinesterase (AChE) was chosen to assess the antioxidant activity [26]; meanwhile, *S. aureus* nucleoside diphosphate kinase and *E. coli* beta-ketoacyl-[acyl carrier protein] synthase were chosen to examine the antibacterial activity [27,28].

The primary chemicals found in the essential oils of *S. calamintha*, both wild and domesticated, were acquired in SDF format from the PubChem database. They were then produced using the OPLS3 force field and using the LigPrep tool in the Schrödinger Software Maestro 11.5 software. After ionization at pH values of 7.0 and 2.0, each ligand can yield up to 32 stereoisomers. Using the PDB IDs 4EY7, 1FJ4, and 3Q8U, respectively, the three-dimensional crystal structures of AChE, *E. coli* beta-ketoacyl-[acyl carrier protein] synthase, and S. aureus nucleoside diphosphate kinase were retrieved from the protein data bank in PDB format.

Schrödinger-Maestro v11.5's Protein Preparation Wizard was used to create and polish each structure. The OPLS3 force field was used to minimize the structure. Volumetric spacing was accomplished using a receptor grid that was set to 20 × 20 × 20. In Glide of Schrödinger-Maestro v 11.5 SP, flexible ligand docking was performed [29].

2.8. Statistical Analysis

All results obtained were presented as triplicate experiment means ± standard deviation. The significant difference between means was examined with the use of analysis of variances (two-way ANOVA). In addition, Tukey's multiple range tests at $p < 0.05$ were performed using GraphPad Prism 8.0.1.

3. Results and Discussion

3.1. Phytochemical Characterization of EOs

The yields of essential oil of the wild and domesticated *S. calamintha* obtained in the present study were 2.80% and 1.95%, respectively. Other studies conducted in Morocco and Algeria on the wild plant reported that the yields of the essential oils of *S. calamintha* were 0.082% and 1.3%, respectively [7,30]. The EOs of *S. calamintha* studied in the present work showed chemical variability, identified via GC–MS (Figure 2 and Table 1). *S. calamintha* EO contained 42 identified compounds, of which the major compounds were eucalyptol (23.10%), pulegone (12.44%), rotundifolone (9.68%), spathulenol (6.52%), piperitone oxide (5.37%), menthol (4.58%) and isomenthone (4.40%). Meanwhile, the domesticated *S. calamintha* EO had 36 compounds, which were identified via GC–MS. The latter was also found to be rich in eucalyptol (22.23%), pulegone (12%), rotundifolone (10.49%), spathulenol (7.59%) and menthol (6.04%). The two EOs shared almost similar compounds, with a slight difference in concentrations (Table 1). This difference may be related to several environmental factors, such as climate, soil, rain and exposure. Studies conducted in Morocco reported that EOs from many *S. calaminta* nepta samples mainly comprised borneol (34.520%), α-campholenic aldehyde (14.260%), cedren-13-ol (6.450%) and manoyloxide (3.780%) [30]. Meanwhile, other works mentioned that pulegone (39.5%), neo-menthol (33%) and isomenthone (19.6%) were dominant in the EOs of *S. calamintha* from Algeria [9]. Rossi et al. and Couladis also recorded that pulegone (49–41%) and menthone (21.5–32%) dominate in the EOs of *S. calamintha* from Corsica and Greece, respectively [23,24].

Table 1. Phytochemicals contained in the wild and domesticated *S. calamintha*.

Peak	Compounds	RI		EOSS		EOSD	
		CT	LT	R.T	Area (%)	R.T	Area (%)
1	α-Pinene	939	939	7.885	1.21	7.894	0.90
2	Sabinene	970	975	9.020	0.49	9.025	0.39

Table 1. *Cont.*

Peak	Compounds	RI		EOSS		EOSD	
		CT	LT	R.T	Area (%)	R.T	Area (%)
3	β-Pinene	979	979	9.176	2.35	9.181	1.57
4	o-Cymene	1023	1026	-	-	10.563	0.31
5	Limonene	1026	1029	10.712	3.39	10.728	1.41
6	Eucalyptol	1030	1031	10.799	22.23	10.824	23.10
7	Linalool	1096	1096	-	-	12.798	0.25
8	(+)-Isomenthone	1163	1162	-	-	14.513	4.40
9	Isomintlactone	2422	2422	22.348	0.61	-	-
10	Mintlactone	1310	1314	23.736	2.37	-	-
11	Menthol	1171	1171	14.921	6.04	14.940	3.52
12	Borneol	1165	1169	14.995	2.63	15.010	1.76
13	Trans-Isopulegone	1590	1596	15.074	1.37	15.082	1.76
14	Terpinen-4-Ol	1172	1177	15.236	0.35	15.245	0.40
15	Carvone oxide	1260	1263	16.295	2.14	16.297	2.20
16	Exo-2-Hydroxycineole	991	991	-	-	16.585	0.26
17	Pulegone	1237	1237	16.891	12.00	16.928	12.44
18	Piperitone Oxide	1255	1256	-	-	17.340	5.37
12	4-Hydroxy-2,6,6-trimethyl-1-cyclohexenecarboxylic acid	1699	1698	-	-	17.521	0.71
20	Piperitenone	1362	1368	17.745	0.58	17.748	0.26
21	Isophytol acetate	2215	2218	-	-	18.165	0.26
22	Sabina ketone	1154	1159	-	-	18.250	0.32
23	Thymol	1290	1290	18.531	3.06	18.319	2.15
24	Carvacrol	1299	1299	18.295	2.51	18.560	2.03
25	2,6,6-Trimethylbicylo [3.1.1]hept-2-ene	942	945	-	-	18.641	0.31
26	2-Oxabicyclo[2.2.2]octan-6-one, 1,3,3-trimethyl-	1031	1031	-	-	18.712	0.29
27	γ-Diosphenol	1105	1107	-	-	19.247	0.31
28	Piperitenone	1342	1343	19.653	3.18	19.669	2.16
29	Rotundifolone	1458	1459	20.236	10.49	20.288	9.68
30	2-Butylcyclopentanone	1128	1128	-	-	20.350	0.22
31	Tetrahydroactinidiolide	1284	1288	-	-	20.441	0.36
32	5-Hepten-3-yn-2-ol,6-methyl-5-(1-methylethyl)-	1458	1460	-	-	21.806	0.37
33	Menthofurolactone	1353	1353	19.890	2.40	19.916	2.98
34	4-(2-Methyl-cyclohex-1-enyl)-but-3-en-2-one	786	786	-	-	23.615	1.01
35	Mintlactone	1310	1314	24.518	1.35	24.531	0.76
36	2-(2-Methyl-propenyl)-cyclohexanone	1158	1158	-	-	24.965	0.41
37	Peperinic acid	1380	1380	-	-	25.216	0.37

Table 1. *Cont.*

Peak	Compounds	RI		EOSS		EOSD	
		CT	LT	R.T	Area (%)	R.T	Area (%)
38	Spathulenol	1577	1578	25.963	7.59	25.994	5.52
39	Caryophyllene Epoxide	1466	1466	26.145	1.50	26.162	1.27
40	2-Pentanone,4-hydroxy-4-methyl	847	847	5.312	0.35	-	-
41	l-Menthone	1160	1162	14.507	2.26	-	-
42	Menthofuran	1164	1164	14.742	0.47	-	-
43	(1S)-1,3,3-trimethylnorbornan-2-ol	1160	1160	15.652	0.85	15.658	1.43
44	Isobornyl formate	1239	1239	16.651	0.31	-	-
45	Carvone oxide	1263	1263	17.313	1.93	-	-
46	7,11-Epoxymegastigma-5(6)-en-9-one	1312	1312	17.998	0.43	-	-
47	Cyclohexasiloxane, dodecamethyl-	1350	1349	18.709	0.35	-	-
48	1-Isopropenyl-4-methyl-1,2-cyclohexanediol	1090	1090	19.790	0.42	-	-
49	Indene-1,7(4H)-dione,3a,7a-dihydro-5-methyl-	1590	1590	20.155	0.47	-	-
50	3H-Naphtho[2,3-b]furan-2-one,4-hydroxy-4a,5-dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydro-	2014	2016	22.041	0.30	-	-
51	4-(2-Methyl-cyclohex-1-enyl)-but-3-en-2-one	780	786	23.605	0.29	-	-
52	2,4-Bis-(tert.-butyl-)-phenol	1513	1513	23.917	0.36	-	-
53	Dinitropentamethylenetetramine	1874	1876	43.160	0.33	-	-
54	3,6-Dimethyl-5,6,7,7a-tetrahydrobenzofuran	1380	1380	-	-	14.751	0.64
55	Isoborneol	1160	1160	-	-	15.010	1.76
56	Mintlactone	1314	1314	-	-	23.755	1.42
57	3,6-Dimethyl-4,5,6,7-tetrahydrobenzofuran-2(3H)-one	1380	1380	-	-	22.373	1.93
	Monoterpene hydrocarbons			7.44		4.83	
	Oxygenated monoterpenes			79.17		82.74	
	Sesquiterpene hydrocarbons			-		-	
	Oxygenated sesquiterpenes			9.72		7.43	
	Others			2.63		3.97	
	Total identified (%)			98.96		99.97	

EOSS: *S. calamintha* wild essential oil; EOSD: *S. calamintha* domesticated essential oil; "-": absence; RI: retention index; CT: calculate; LT: literature.

3.2. Antioxidant Activity

The antioxidant effect of the wild and domesticated *S. calamintha* EOs was evaluated using DPPH and FRAP assays. The results are given as IC_{50} values and percent of inhibition (Table 2 and Figure 3). Figure 3A shows that all the three curves are quite similar, including the fact that DPPH inhibition increases sharply at doses ranging from 0 to 500 µg/mL, before stabilizing at higher concentrations. Analysis of the curves (Figure 3A) shows that the efficiency of free radical neutralization for the wild and domestic species is about the same, with the highest level of inhibition occurring at concentrations of 1000 µg/mL. The IC_{50} values indicate a very small difference between the wild and domes-

tic *S. calamintha* ($p < 0.05$ vs. quercetin). The IC_{50} values are 23.03 ± 4.306, 24.096 ± 5.26, and 17.733 ± 0.1788 µg/mL for the wild *S. calamintha* EO, the domesticated *S. calamintha* EO, and quercetin, respectively. Figure 3B shows the iron-reducing/antioxidant power of the two species and the absorbance values of quercetin at ʎ= 700 as a function of concentration. From Figure 3B, it is evident that the absorbance increases proportionally as the concentration increases. The IC_{50}, which represents the concentration of EO required to achieve an absorbance of 0.5, was obtained from the plot of the absorbance recorded at 700.00 nm versus the corresponding concentration of EO. A significant difference was also recorded between the two species: wild-type *S. calamintha* (55.382 ± 2.160 µg/mL) and domesticated *S. calamintha* (60.720 ± 7.710 µg/mL) compared to the positive control (28.414 ± 0.060 µg/mL).

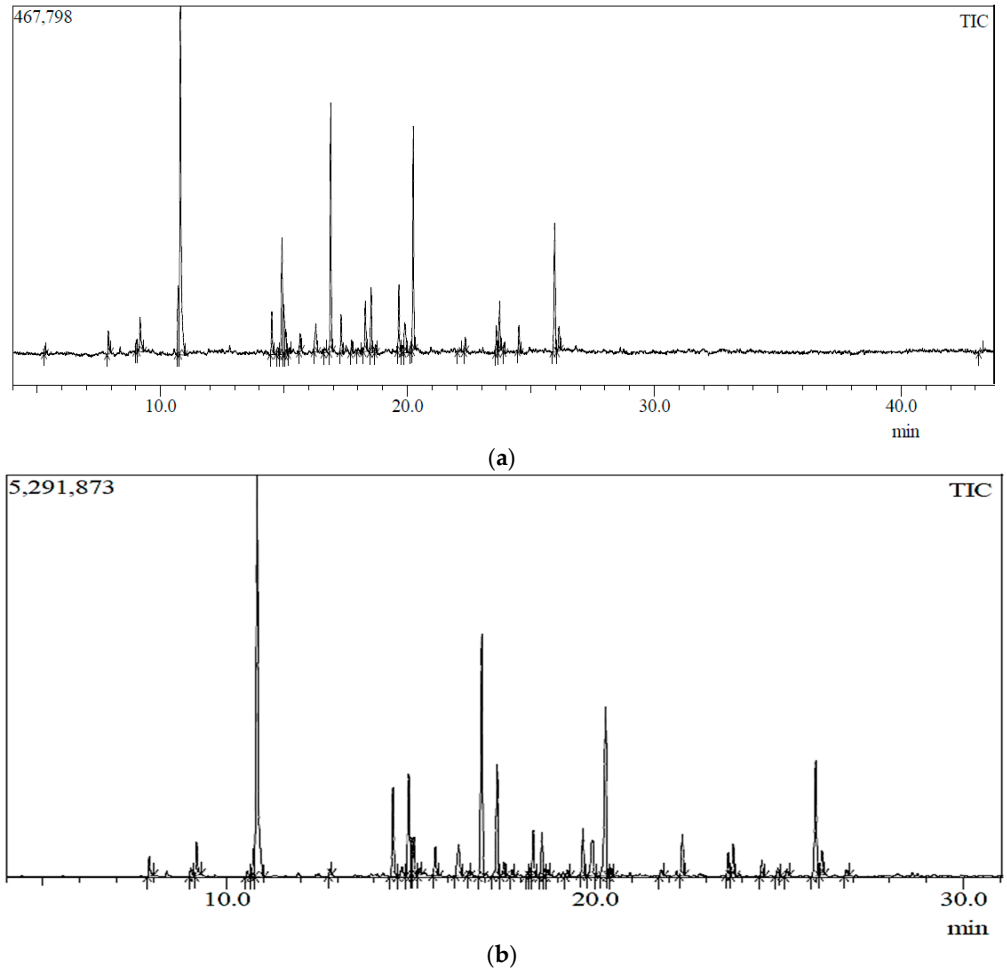
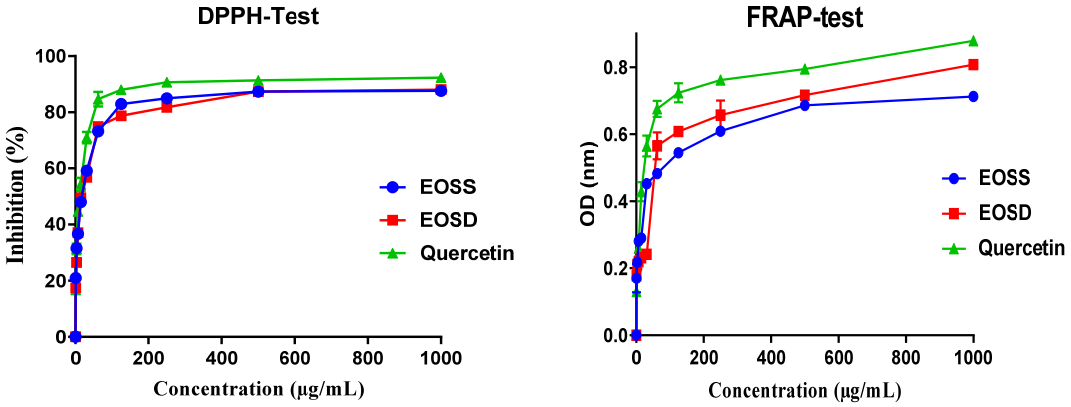


Figure 2. GC–MS chromatograms of essential oils; EOSS (**a**) and EOSD (**b**).

Table 2. Antioxidant power of EOSS and EOSD determined via the use of DPPH and FRAP assays.

	IC_{50} (µg/mL)	
	DPPH	**FRAP**
EOSS	23.030 ± 4.306 [a]	55.382 ± 2.16 [a]
EOSD	24.096 ± 4.381 [a]	60.720 ± 7.71 [a]
Quercetin	17.733 ± 1.788 [b]	28.414 ± 0.06 [b]

EOSS: wild *S. calamintha* EOs; EOSD: domesticated *S. calamintha* EOs. Row values with the same letters indicate a significant difference according to multiple Tukey tests at $p < 0.05$.



(**A**) (**B**)

Figure 3. Percentage of DPPH inhibition (**A**) and iron-reducing power (**B**).

A work conducted in Morocco on the *S. calamintha* EO showed the scavenging capacity of DPPH (22.01 ± 3.13%) [31]. Babajafari et al. [5] revealed that different species of Satureja exert antioxidant, lipid peroxidation-inhibiting and anti-inflammatory activities. In addition, Bouzidi et al. [7] showed that *S. calamintha* oil has a significant effect on the inhibition of the free radicals produced by DPPH with an IC_{50} of 31.25 µg/mL. However, the antioxidant activity could be due to the presence of polyphenolic compounds. Labiod et al. [32] revealed that pulegone has remarkable antioxidant properties.

3.3. Antibacterial Activity

The inhibition zone diameters and MIC values of the wild and domesticated *S. calamintha* EOs against the tested pathogenic bacterial strains are presented in Table 3 and Figure 4, respectively. When compared to the antibiotic streptomycin sulfate, both EOs showed promising antibacterial action against all the bacteria used in the present study, and a more pronounced effect was recorded against *E. coli* K12, with inhibition diameters of 25.67 ± 0.58 and 48.67 ± 1.15 mm and MIC values of 1.49 ± 0.00 and 0.373 ± 0.00 µg/mL for the domesticated and wild *S. calamintha* EOs, respectively. However, the domesticated and wild *S. calamintha* EOs showed the lowest activity against *S. aureus* ATCC6633, with inhibition diameters of 14 ± 0.00 and 12.67 ± 0.58 mm and MIC values of 5.96 ± 0.00 and 5.96 ± 0.00 µg/mL, respectively.

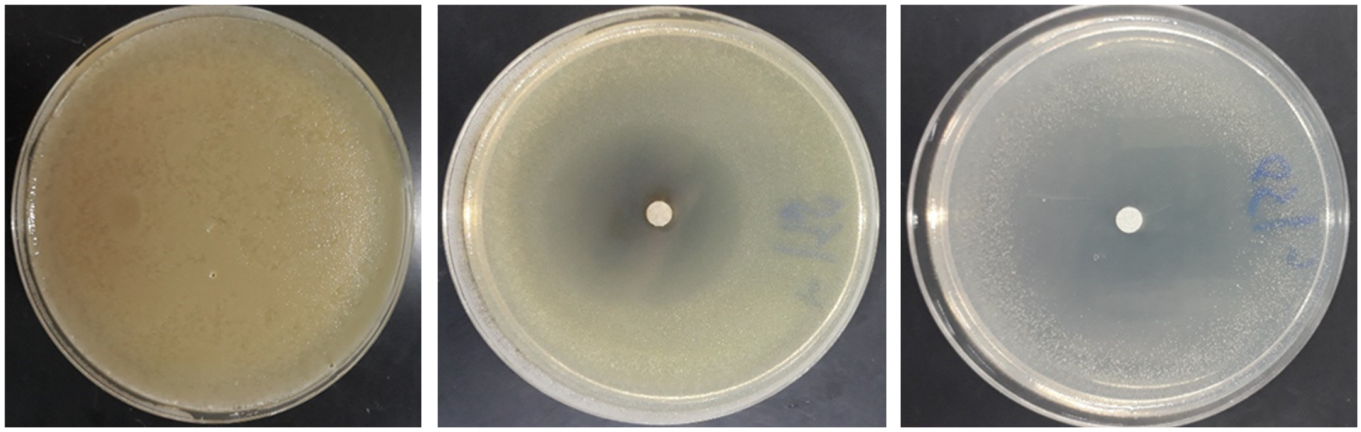
Table 3. Antibacterial activity and MIC of EOSS and EOSD.

		EODS	EOSS	Streptomycin
Staphylococcus aureus ATCC6633	Diameter of inhibition (mm)	14 ± 0.00 ^{a}	12.67 ± 0.58 ^{b}	11 ± 1.00 ^{c}
	MIC (µg/mL)	5.96 ± 0.00 ^{a}	5.96 ± 0.00 ^{a}	1.56 ± 0.00 ^{b}
Escherichia coli K12	Diameter of inhibition (mm)	25.67 ± 0.58 ^{a}	48.67 ± 1.15 ^{b}	0.00 ± 0.00 ^{c}
	MIC (µg/mL)	1.49 ± 0.00 ^{a}	0.373 ± 0.00 ^{b}	-
Bacillus subtilis DSM6333	Diameter of inhibition (mm)	18.67 ± 1.15 ^{a}	25.67 ± 0.58 ^{b}	0.00 ± 0.00 ^{c}
	MIC (µg/mL)	2.98 ± 0.00 ^{a}	2.98 ± 0.00 ^{a}	-

Table 3. *Cont.*

		EODS	EOSS	Streptomycin
Proteus mirabilis ATCC29906	Diameter of inhibition (mm)	26.00 ± 1.73 [a]	30.67 ± 1.15 [b]	0.00 ± 0.00 [c]
	MIC (µg/mL)	1.49 ± 0.00 [a]	1.49 ± 0.00 [a]	-

EOSS: wild *S. calamintha* EOs; EOSD: domesticated *S. calamintha* EOs. Means (±SD, $n = 3$) with similar letters in the same line indicate a significant difference according to Tukey's multiple tests at $p < 0.05$, MIC: minimum inhibitory concentration.



E. coli K12 Control EOSD against *E. coli* K12 EOSS against *E. coli* K12

Figure 4. Antibacterial activity of EOSS and EOSD against *E. coli* K12.

The promising antibacterial activity of the domesticated and wild *S. calamintha* EOs against all pathogenic bacterial strains used in the experiment may be mainly due to the presence of bioactive compounds such as d-limonene, eucalyptol, pulegone, and thymol, which were reported to have anti-bacterial effects [25]. Eucalyptol exhibited antibacterial activity against several pathogenic bacteria, as reported in earlier work [33]. These findings are in line with those reported by Boudjema et al. [14], who demonstrated eucalyptol's potent antibacterial activities against *B. cereus* ATCC10876, *E. faecalis* ATCC49452, *Listeria innocua* CLIP74915, methicillin-resistant *S. aureus* (MRSA) ATCC43300, *S. aureus* ATCC25923, *E. coli* ATCC25922, *K. pneumoniae* ATCC700603, *Salmonella enterica* ATCC43972 and *S. typhimurium* ATCC13311. Our results are consistent with the findings reported by Bouzidi and Kemieg, who showed that the EOs from *S. calamintha* are highly antibacterial against the strains of *S. aureus* and *P. aeruginosa* used in the study (ATCC29273 and ATCC27853, respectively) [14].

3.4. Antifungal Activity

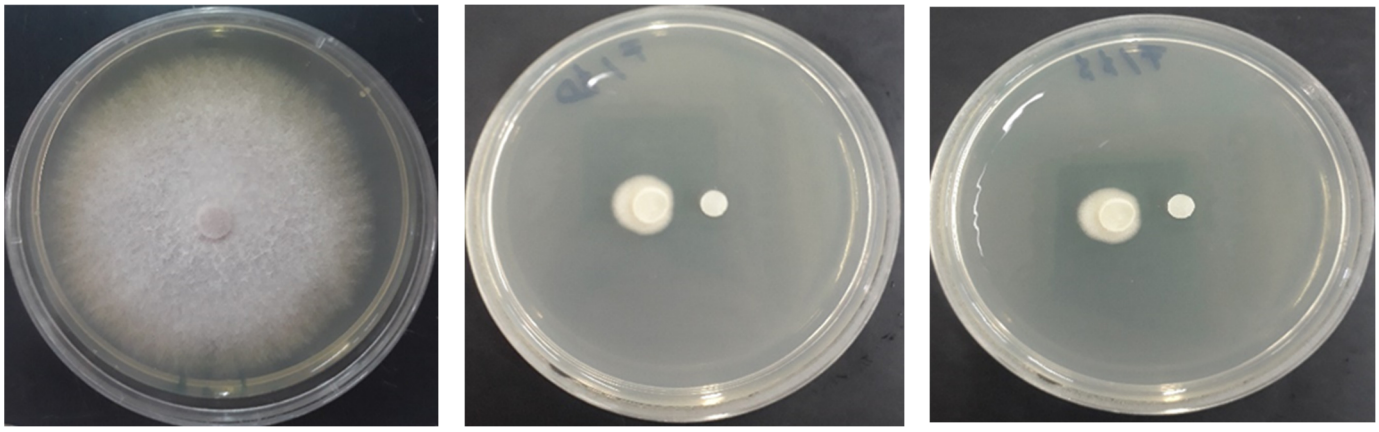
The inhibition percentages and MIC values of the wild and domesticated *S. calamintha* EOs against pathogenic strains tested using the disk diffusion method are displayed in Table 4 and Figure 5. From this Table, it can be observed that the domesticated and wild *S. calamintha* EOs induced good antifungal activity, with inhibition percentages of 89.18 ± 0.75 and 86.58 ± 0.76% and MIC values of 1.49 ± 0.00 and 2.98 ± 0.00 µg/mL against *F. oxysporum*, respectively. With regard to their fungicidal and fungistatic effects, the domesticated and wild *S. calamintha* EOs exhibited a fungicidal effect against all tested fungi strains. The promising antifungal activity of the domesticated and wild *S. calamintha* EOs against the tested pathogenic fungal strains may be due to the richness of EOs in bioactive molecules such as d-limonene, eucalyptol, pulegone, and thymol. These bioactive chemicals have been shown in several studies to significantly inhibit the growth of pathogenic fungi, especially in the study by Saghrouchni et al. [34], who reported that thymol has significant antifungal activity mainly against *F. oxysporum*. Pulegone has been reported as a natural effective compound against *C. albicans* growth [35]. Much research has been dedicated to

the management of pathogenic fungi via the use of various kinds of bioactive compounds, either of natural or synthetic origin. The present results regarding the antifungal activity of the EOs are in agreement with the study by Boudjema et al. [14], which showed that the *S. calamintha* EOs had significant antifungal activities against *C. tropicalis* DIV13-Z087D0VS, *C.tropicalis* DIV13-Z087B0VS, *C. albicans* ATCC-1024, *A. niger* and *A. flavus*. In addition, this study reported a low MIC (more potent), in the order of 0.500% (*v*/*v*), against *C. albicans*. Medjdoub and co-authors investigated the action of *S. calamintha* nepeta EOs on three fungal strains, including *A. flavus*, *A. parasiticus*, and *A. ochraceus*, and revealed that all tested molds were killed with doses of 1/100 and 1/250 (*v*/*v*) after 7 days of incubation [36].

Table 4. Antifungal activity and MIC of EOSS and EOSD.

		EOSD	EOSS	Fluconazol
C. albicans ATCC-10231	Diameter of inhibition (mm)	47.33 ± 1.15 [a]	40.00 ± 0.00 [b]	0.00 ± 0.00 [c]
	MIC (µg/mL)	0.186 ± 0.00 [a]	0.373 ± 0.00 [b]	-
A. niger MTCC-282	Percentage of inhibition (%)	67.19 ± 0.00 [a]	29.68 ± 2.71 [b]	18.46 ± 2.02 [c]
	MIC (µg/mL)	1.49 ± 0.00 [a]	0.373 ± 0.00 [b]	7.125 ± 0.00 [c]
A. flavus MTCC-9606	Percentage of inhibition (%)	41.27 ± 1.37	0.00 ± 0.00	0.00 ± 0.00
	MIC (µg/mL)	0.746 ± 0.00	-	-
F. oxysporum MTCC-9913	Percentage of inhibition (%)	89.18 ± 0.75 [a]	86.58 ± 0.76 [a]	30.77 ± 0.58 [b]
	MIC (µg/mL)	1.49 ± 0.00 [a]	2.98 ± 0.00 [b]	3.125 ± 0.00 [b]

Row values with different letters indicate a significant difference according to Tukey's multiple tests at $p < 0.05$, MIC: minimum inhibitory concentration. Means (±SD, $n = 3$) with.



F. oxysporum MTCC-9913 Control

SD EOs against *F. oxysporum* MTCC-9913

SS EOs against *F. oxysporum* MTCC-9913

Figure 5. Antifungal activity of EOSS and EOSD against *F. oxysporum* MTCC-9913.

3.5. In Silico Molecular Docking of Antioxidant and Antimicrobial Activities of EOs of Wild and Domesticated S. calamintha

Piperitenone is a natural compound found in various herbs such as peppermint and spearmint. It belongs to the class of monoterpenoids and possesses several biological activities. Several studies have investigated the antioxidant properties of piperitenone. In terms of antioxidant activity, it was found to have a strong scavenging effect against free radicals, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radicals [37,38]. These studies showed that piperitenone's antioxidant activity was dose-dependent and increased with increasing concentrations.

Pulegone is another natural organic compound found in various plants. It has been reported to exhibit antioxidant properties due to its ability to scavenge free radicals and prevent oxidative damage. Roy A. et al. investigated the protective effect of pulegone against oxidative stress and inflammation in vitro and in vivo. The authors found that pulegone significantly increased the expression of antioxidant enzymes via the Nrf2 pathway and reduced the expression of pro-inflammatory cytokines via the NF-κB pathway, indicating its potent antioxidant and anti-inflammatory effects [39]. In addition, pulegone exhibited potent antioxidant activity in various assays, including scavenging free radicals and reducing lipid peroxidation [40].

In antioxidant activity, the inhibition of acetylcholinesterase plays a key role [29]. The powerful inhibitory effects of piperitenone, pulegone, and piperitone oxide (identified only in domesticated *S. calamintha*) were demonstrated by the molecular docking of the EO of wild and domesticated *S. calamintha* in the active site of acetylcholinesterase. These compounds had glide scores of −7.682, −7.647, and −7.01 Kcal/mol, respectively, and a glide energy of −22.946, −22.115, and −23.687 Kcal/mol, respectively (Table 5). The 2D and 3D viewers of wild and domesticated *S. calamintha* docked in the active site of acetylcholinesterase showed that the pulegone established one hydrogen bond with residue PHE 295 (Figure 6).

Table 5. Docking results of EOSS and EOSD in active site of acetylcholinesterase (4EY7), *S. aureus* nucleoside diphosphate kinase (PDB: 3Q8U), and *E. coli* beta-ketoacyl-[acyl carrier protein] synthase (PDB: 1FJ4).

	Antioxidant Effect		**Antimicrobial Effect**			
	PDB ID: 4EY7		**PDB ID: 1FJ4**		**PDB ID: 3Q8U**	
	Glide Score (Kcal/mol)	**Glide Energy (Kcal/mol)**	**Glide Score (Kcal/mol)**	**Glide Energy (Kcal/mol)**	**Glide Score (Kcal/mol)**	**Glide Energy (Kcal/mol)**
Eucalyptol	−5.58	−24.743	−5.7	−21.613	−4.172	−14.205
(+)-Isomenthone	−6.49	−22.919	−6.717	−19.745	−4.522	−16.974
MENTHOL	−6.722	−21.106	−6.855	−18.662	−5.392	−18.125
Pulegone	−7.647	−22.115	−7.099	−24.36	−5.067	−18.027
Piperitone Oxide	−7.01	−23.687	−6.399	−22.343	−5.479	−21.285
Piperitenone	−7.682	−22.946	−7.112	−22.296	−4.497	−18.218
Rotundifolone	−5.91	−26.979	−7.104	−27.481	−4.771	−19.301
Spathulenol	−7.011	−26.214	−5.982	−20.041	−4.795	−17.187

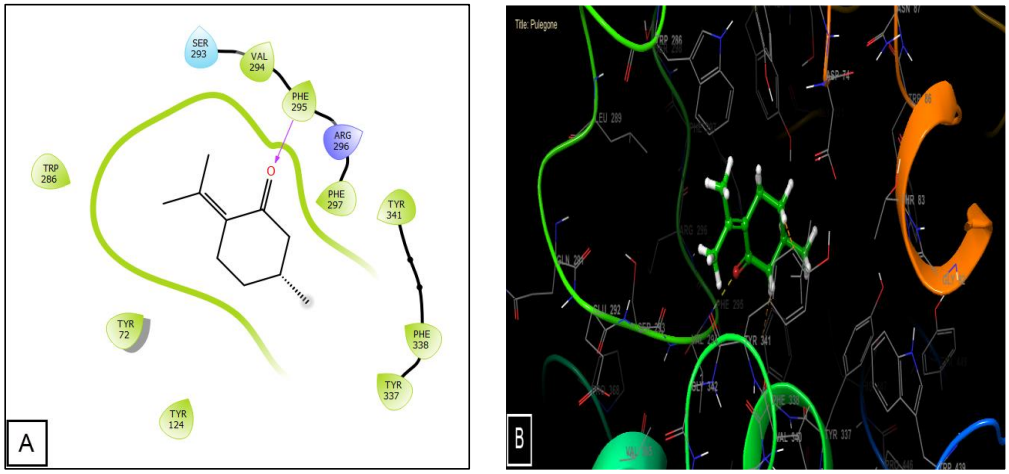


Figure 6. *Cont.*

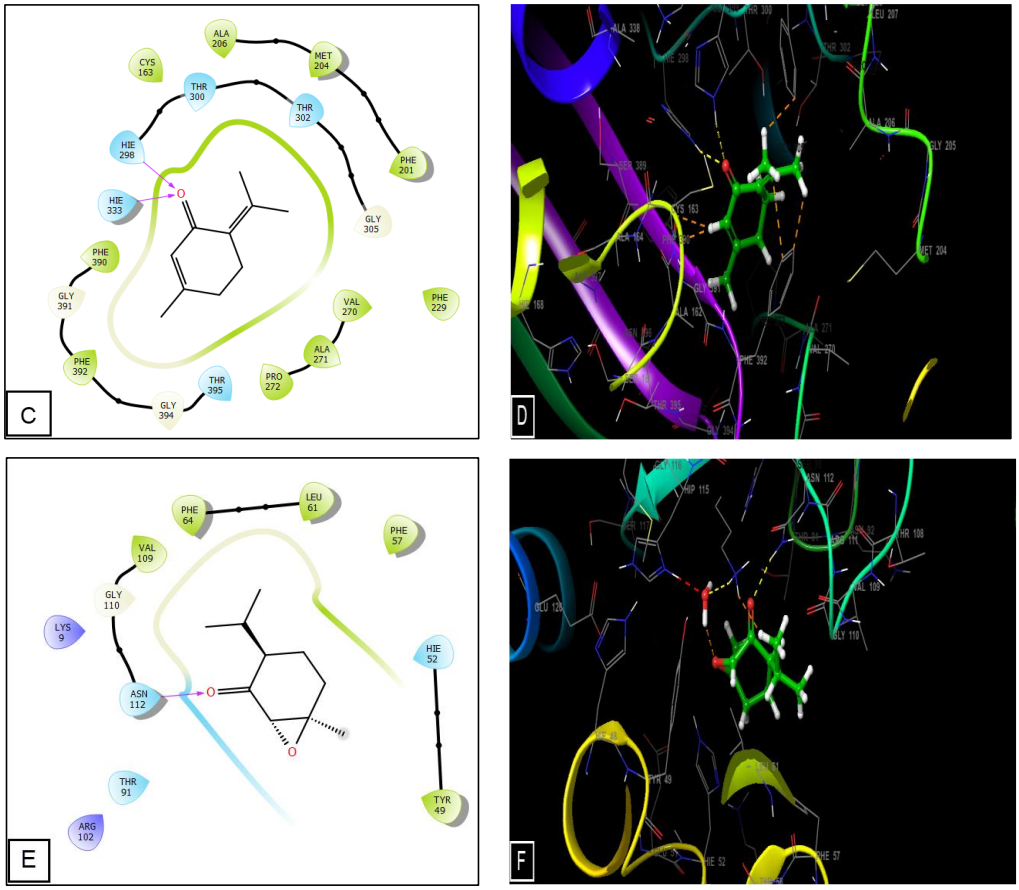


Figure 6. The 2D and 3D diagrams of ligands interactions with the active site. (**A**,**B**) Pulegone interactions with the active site of 4EY7; (**C**,**D**) Piperitenone interactions with the active site of 1FJ4; (**E**,**F**) Piperitone Oxide interactions with the active site of 3Q8U.

In addition to its antioxidant effects, piperitenone has also been shown to have antimicrobial activity against various microorganisms. The authors found that piperitenone oxide (a derivative of piperitenone that is one of the main compounds of the plants studied) exhibited potent antimicrobial activity against a range of bacteria and fungi, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* [41].

Pulegone is a monoterpene ketone found in various essential oils. The antimicrobial properties of pulegone have been extensively studied in vitro, and several mechanisms of action have been proposed. One study demonstrated that pulegone has antibacterial activity against Gram-positive bacteria, including *Staphylococcus aureus* and *Streptococcus pyogenes*, and Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa* [42].

Piperitenone is the most effective molecule against *E. coli* beta-ketoacyl-[acyl carrier protein] synthase (1FJ4), according to our in silico analysis of the antimicrobial activity of the wild and domesticated *S. calamintha* EOs. It has a glide score and glide energy of −7.112 and −22.296 Kcal/mol. Rotundifolone comes in second with a glide score of −7.104 Kcal/mol. The piperitone oxide is the most active compound in the *S. aureus* nucleoside diphosphate kinase (3Q8U), with a glide score and glide energy of −5.479 and −21.285 Kcal/mol, respectively.

Furthermore, 2D and 3D viewers showed that piperitenone established two hydrogen bonds with the residues HIE 298 and HIE 333 in the active site of 1FJ4. Meanwhile, the piperitone oxide established one hydrogen bond with residue ASN 112 in the active site of 3Q8U.

4. Conclusions

S. calamintha is a plant widely used by the local population for its numerous therapeutic properties. The domestication of *S. calamintha* is a simple solution to preserving this plant from extinction. Therefore, we evaluated the effect of domestication on its chemical composition and biological activities. The present study aimed to investigate the chemical composition and antioxidant and antibacterial properties of essential oils extracted from *S. calamintha*. The results showed that the oils of *S. calamintha* are rich in eucalyptol (23.10–22.23%), pulegone (12.44–12%) and rotundifolone (9.68–10.49%), with slight differences in the chemical composition; nevertheless, both essential oils showed almost similar antioxidant and antibacterial effects against clinically important strains. Therefore, domesticating *S. calamintha* could be used as an important alternative so as to preserve such a plant without compromising its biological activities.

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