Article

Not peer-reviewed version

Essential Oils from Wild Albanian Lamiaceae: GC-MS Profiling, Biological Activity, and Enhanced Delivery via Nanoencapsulation

Elton Basha *, Erjon Mamoçi, Aniket Sharma, Entela Hodaj-Çeliku, Sanije Zejnelhoxha, Mădălina L. Medeleanu, Sonia A. Socaci, Bledar Bisha

Posted Date: 16 July 2025

doi: 10.20944/preprints202507.1209.v1

Keywords: *Origanum vulgare* L. subsp. hirtum; *Thymus capitatus*; *Satureja montana*; antioxidant; antimicrobial; natural food preservatives

 doi:10.20944/preprints202507.1209.v1

Article

Essential Oils from Wild Albanian Lamiaceae: GC-MS Profiling, Biological Activity, and Enhanced Delivery via Nanoencapsulation

Elton Basha [1,*], Erjon Mamoçi [2], Aniket Sharma [3], Entela Hodaj-Çeliku [4], Sanije Zejnelhoxha [2], Mădălina L. Medeleanu [5], Sonia A. Socaci [5] and Bledar Bisha [3]

[1] Department of Agri-Food Technology, Agricultural University of Tirana, Tirana, Albania
[2] Department of Food Science and Biotechnology, Agricultural University of Tirana, Tirana, Albania
[3] Department of Animal Science, University of Wyoming, Wyoming, United States of America
[4] Department of Chemistry, Agricultural University of Tirana, Tirana, Albania
[5] Department of Food Science, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Cluj-Napoca, Romania
* Correspondence: ebasha@ubt.edu.al

Abstract

The growing demand for natural preservatives has driven interest in essential oils (EOs) from medicinal and aromatic plants. This study examines the potential of EOs from six wild populations of Albanian *Lamiaceae*, specifically *Origanum vulgare* subsp. *hirtum*, *Thymus capitatus*, and *Satureja montana* species, to be utilized for food conservation, among other possible uses. The EOs were extracted by hydrodistillation, and their chemical profiles were analysed through GC-MS. DPPH and ABTS assays were performed to evaluate antioxidant activity. The antimicrobial efficacy of the oils was assessed using the broth microdilution method against six common foodborne pathogens: *Salmonella enterica* serovar Enteritidis, *Escherichia coli*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Micrococcus luteus*, and one fungus, *Candida albicans*. The most potent EOs in terms of yield and biological activity, resulting from *O. vulgare* subsp. *hirtum* and *T. capitatus*, were encapsulated in oil-in-water emulsions, which were characterized for particle size and zeta potential. The results show that the populations of *O.vulgare* subsp. *hirtum* and *T. capitatus* taken in the study belong to carvacrol chemotypes and their EOs show strong antioxidant activity and are effective against all tested microorganisms. Nanoemulsions prepared with these EOs showed promising stability, indicating their potential as natural preservatives in food applications.

Keywords: *Origanum vulgare* L. subsp. *hirtum*; *Thymus capitatus*; *Satureja montana*; antioxidant; antimicrobial; natural food preservatives

1. Introduction

The demand for natural preservatives that can replace artificial additives in food products is increasing due to growing focus over food safety and shelf-life enhancement [1,2]. Although synthetic preservatives are effective, they often provoke health and toxicity concerns, leading customers to prefer more natural food additives. This has led to a renewed interest in plant-based natural preservatives, especially essential oils (EOs) [2]. Essential oils are complex mixtures of volatile organic compounds that are extracted from plants and possess a variety of bioactive qualities, such as antioxidant, antimicrobial, and anticarcinogenic effects [3]. Among the many plant families, the *Lamiaceae* family which includes medicinal and aromatic plants (MAPs) like *Origanum*, *Thymus*, and *Satureja* has been found to contain a substantial amount of essential oils with strong biological activity, which suggests that they could be used as natural food preservatives [4].

 doi:10.20944/preprints202507.1209.v1

Carvacrol and thymol, two phenolic compounds possessing significant antibacterial and antioxidant properties, are prevalent in essential oils derived from *Lamiaceae* species, including *O. vulgare*, *T. capitatus* (syn. *Thymbra capitata*), and *S. montana* [5]. These compounds are suitable for food preservation applications, as they have been shown to inhibit lipid oxidation and microbiological proliferation [6].

One of the primary functions of EOs in food preservation is their antioxidant action, preventing oxidation of lipids and other food constituents, which can lead to rancidity and nutrient degradation. Phenolic substances in EOs, which can act as hydrogen donors and neutralize free radicals, are mainly responsible for their antioxidant properties [3]. EOs from *Origanum*, *Thymus*, and *Satureja* species have been demonstrated in multiple studies to possess antioxidant properties, effectively scavenging free radicals and protecting food from oxidative damage [7,5]. The robust antioxidant capacity of *T. capitatus* and *O. vulgare* essential oils has been evidenced by their notable DPPH and ABTS radical scavenging activities. [8]. Likewise, *S. montana* essential oils have shown antioxidant properties comparable to synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) [9]. The antioxidant qualities of *Origanum* and *Thymus* species are largely attributed to their high concentrations of carvacrol and thymol, with carvacrol being especially potent because of its capacity to donate hydrogen atoms in order to neutralize free radicals [10].

Additionally, EOs have well-established antimicrobial profile and have been studied for their ability to stop the growth of bacteria and fungi. It is thought that EOs antimicrobial properties stem from their capacity to denature proteins, damage cell membranes, and obstruct microbial metabolism [11]. Strong activity against a variety of foodborne pathogens, such as *S.* Enteritidis, *E. coli*, *P. aeruginosa*, and *C. albicans*, has been shown by essential oils derived from *Lamiaceae* species, especially *O. vulgare* and *T. capitatus* [12,13]. In similar fashion as for the antioxidant properties, the principal bioactive compounds in *Origanum* and *Thymus* essential oils responsible for their antibacterial effects are thymol and carvacrol. Thymol has been shown to possess bactericidal properties against both Gram-negative and Gram-positive bacteria, including *Salmonella enterica* and *Listeria monocytogenes* [14]. Thymol is a multipurpose substance for food preservation because it has antifungal as well as antibacterial qualities [15]. These EOs broad-spectrum antimicrobial activity has prompted research into using them as natural food preservatives because they can control the growth of pathogens and spoilage microorganisms that cause foodborne illnesses.

Even though EOs show significant potential as natural preservatives, their application in food systems is usually limited by their volatility, inadequate solubility, and low stability under various environmental conditions, such as temperature and light. Encapsulation has been proposed and explored to enhance the stability, bioavailability, and controlled release of essential oils in food matrices in order to overcome these challenges. By adding active ingredients to nanoscale delivery systems like nanoemulsions, preservation of essential oils from deterioration and their functional qualities are enhanced [16].

Oil-in-water nanoemulsions have emerged as an effective delivery system for essential oils, as they can encapsulate hydrophobic compounds like EOs, protecting them from oxidation and enhancing their stability during storage [17]. Since the smaller droplet sizes improve the dispersion of essential oils in food products, fostering better interaction with microorganisms and enhancing their antimicrobial and antioxidant effects, nanoemulsions not only offer stability but also the benefit of increased bioavailability [18]. *Origanum*, *Thymus*, and *Satureja* essential oils have been successfully encapsulated in nanoemulsions in a number of studies, indicating their potential as useful food preservation ingredients [19-21].

Because of its varied climate and flora, the territory of Albania is very rich in different MAPs. The country's natural landscapes host extensive wild populations of *Origanum*, *Thymus*, and *Satureja* species, traditionally utilized for culinary and medicinal applications. Albanian *Lamiaceae* species are an appealing subject for investigation owing to their potential in food preservation, with their biodiversity and optimal growing conditions [22]. Despite the growing number of studies on the biological activities of these plants, the literature still lacks information regarding the distinctive

 doi:10.20944/preprints202507.1209.v1

chemical profiles, antioxidant, and antibacterial properties of Albanian varieties of *Origanum*, *Thymus*, and *Satureja*. This work aims to fill this knowledge gap by examining the chemical composition of EOs from these species sourced from various regions of Albania, assessing their bioactive properties, and exploring the viability of their nanoencapsulation for enhanced stability and efficacy in food systems and potential use as natural food preservatives.

2. Results and Discussion

2.1. Yield of Extracted EOs

EO yield (Table 1), expressed as a percentage of dry plant material, is a critical parameter in assessing a plant's industrial potential for EO production. *O. vulgaris* subsp. *hirtum*, commonly known as Greek oregano, demonstrated the highest EO yields among the studied species. The essential oil of *O. vulgare* subsp. *hirtum* had a pale-yellow color with a medium yield of 3.945 % (v/w), a medium percentage compared to other studies of wild populations from Albania [23, 24]. These results are consistent with previous studies confirming *O. vulgare* subsp. *hirtum* has superior EO yields compared to other subspecies and the yield of the population taken in study, if cultivated, could be improved with appropiate agronomical practices [25,26,27]. High yields in this species are often associated with rich contents of carvacrol and thymol, compounds known for their strong antimicrobial activity [28]. The high yield makes this plant a promising candidate for cultivation and commercial applications in food preservation, pharmaceuticals, and aromatherapy.

Table 1. Yield of extracted Eos.

Species	Plant Code	Yield of aerial parts (%*)	Yield (%*) of leaves and flowers without stems
O. vulgaris subsp. *hirtum*	OV-L	4.06	6.19
O. vulgaris subsp. *hirtum*	OV-P	3.83	6.11
T. capitatus	TC-M	1.66	3.00
T. capitatus	TC-L	0.75	2.30
S. montana	SM-B	0.39	0.80
S. montana	SM-S	0.73	0.79

*mL of EO per 100 grams of dried plant material.

While *T. capitatus* produced moderate yields with a medium of 1.205 % (v/w), and sample TC-M nearly doubled the yield of TC-L. EOs resulted in a pale-yellow color. The variability in EO percentage is commonly attributed to edaphoclimatic factors, as well as plant age and genotype [29]. The authors couldn't identify reports of *Thymus capitatus* EO yield from Albania. Similar yields have been reported in North African regions, typically ranging from 0.5% to 2.0% [30]. While the yield is lower than *O. vulgaris, T. capitatus* oils are often rich in phenolic monoterpenes like thymol and carvacrol, providing potent antimicrobial and antioxidant effects that justify its use despite moderate yields [31].

S. montana samples displayed the lowest EO yields in this study, with a medium yield of 0.56% (v/w) where the essential oil had a pale-yellow color. SM-S had a considerably higher EO yield compared to SM-B which would make it more promising for industrial applications. However, these values fall within the range reported in prior reports from Albania and other countries where EO yields for this species are typically between 0.25% and 1.69%, depending on location and genotype [22,32-34]. Despite the lower oil output, *S. montana* essential oil is often high in components associated with strong biological activities [35]. Therefore, despite lower yields, the species might hold significance in specialised applications where quality outweighs quantity.

 doi:10.20944/preprints202507.1209.v1

2.2. Quantitative and Qualitative Analysis of the EOs

GC-MS analysis of the *O. vulgare* subsp. *hirtum* EOs identified in total 26 different components, counting for 96.68% and 97.37% of the EOs of OV-P and OV-L samples respectively. The most abundant compound was Carvacrol, followed by γ-Terpinene, p-Cymene, and Thymol counting on average for 77.2%, 5.6%, 3.42% and 1.28% respectively (Table 2). These results are in line with another report of *O. vulgare* subsp. *hirtum* from south Albania where carvacrol composed 79.8% of the EOs [24]. The EOs of *O. vulgaris* subsp. *hirtum* is clearly a carvacrol-type, which is consistent with previous chemotaxonomic classifications of Greek oregano [36]. Carvacrol is well known for strong antioxidant, antimicrobial and also anti-inflammatory [37]

Both oils are very similar in chemical composition with minor differences which are unlikely to significantly alter the overall bioactivity profile.

Table 2. Composition of the essential oils of *O. vulgare* subsp. *hirtum.*

Compounds [a]	AI [b]	OV-P	OV-L	ID [c]
α-Thujene	924	1.16	0.97	AI, MS
α-Pinene	931	0.95	0.75	AI, MS, Co-GC
Camphene	945	0.54	0.66	AI, MS
1-Octen-3-ol	983	0.6	0.6	AI, MS
β-Myrcene	990	1.5	1.5	AI, MS, Co-GC
δ-2-Carene	1003	0.2	0.14	AI, MS
α-Phellandrene	1005	0.2	0.2	AI, MS
α-Terpinene	1016	0.98	1.03	AI, MS
p-Cymene	1025	3.8	3.04	AI, MS, Co-GC
Sylvestrene	1029	0.3	0.26	AI, MS
Eucalyptol	1030	0.36	nd	AI, MS
trans-Ocimene	1038	0.1	0.08	AI, MS
cis-Ocimene	1050	0.1	0.06	AI, MS
γ-Terpinene	1059	5.8	5.4	AI, MS, Co-GC
trans-Sabinenehydrate	1070	0.46	0.34	AI, MS
Terpinolene	1085	0.1	0.07	AI, MS
Linalool	1102	0.1	nd	AI, MS, Co-GC
α-Thujone	1104	0.1	0.07	AI, MS
Camphor	1143	0.15	0.13	AI, MS
Terpinen-4-ol	1181	0.15	0.09	AI, MS, Co-GC
Thymol	1294	2.32	0.23	AI, MS, Co-GC
Carvacrol	1304	74.6	79.8	AI, MS
α-Ylangene	1371	0.1	0.05	AI, MS
β-Caryophyllene	1417	1.1	1.2	AI, MS, Co-GC
α-Humulene	1454	0.16	0.2	AI, MS, Co-GC
Caryophyllene oxide	1582	0.75	0.5	AI, MS, Co-GC
Total (%)		96.68	97.37	

[a]Compounds listed in order of elution from an HP-5 MS capillary column; [b] AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C23); [c] Identification method: AI=Arithmetic Index, MS=mass spectrum, Co-GC=Coinjection with authentic compound, nd=not detected. Concentrations below 0.05% are marked as tr (traces).

GC-MS analysis of the *T. capitatus* EOs identified in total 19 different components, counting for 93.65% and 91.6% of the EOs of TC-M and TC-L samples respectively. The most abundant compound was Carvacrol, followed by p-Cymene, and γ-Terpinene, counting on average for 75.25%, 4.48% and 3.37% respectively (Table 3). Consistency in major components indicates genetic stability, despite environmental variability of these two populations.

 doi:10.20944/preprints202507.1209.v1

T. capitatus samples clearly fits the carvacrol chemotype, similar to *Origanum* oils, and confirms its high bioactivity profile effective against Gram-positive, Gram-negative bacteria [38] and fungi [39]. The high carvacrol levels exceed many commercial standards, placing these oils among the most potent natural antimicrobial agents in the *Lamiaceae* family.

Table 3. Composition of the essential oils of *T. capitatus.*

Compounds [a]	AI [b]	TC-M	TC-L	ID [c]
α-Pinene	931	1.3	1.3	AI, MS, Co-GC
β-Pinene	973	0.2	0.4	AI, MS, Co-GC
Octen-3-ol	983	0.2	0.8	AI, MS
β-Myrcene	992	0.1	1.3	AI, MS, Co-GC
α-terpinene	931	0.9	1.0	AI, MS, Co-GC
p-Cymene	1024	4.4	4.56	AI, MS, Co-GC
γ-terpinene	1055	2.8	3.94	AI, MS, Co-GC
cis-Sabinenehydrate	1067	0.4	0.2	AI, MS
Linalool	1101	0.9	0.5	AI, MS, Co-GC
Borneol	1164	0.9	0.7	AI, MS, Co-GC
4-carvomenthenol	1185	0.7	0.5	AI, MS, Co-GC
o-cymen-5-ol	1280	0.2	0.2	AI, MS, Co-GC
2-isopropyl-5-methyl-phenol	1295	0.3	0.2	AI, MS, Co-GC
Carvacrol	1304	77.7	72.8	AI, MS
5-isopropyl-2-methyl phenol	1358	nd	0.3	AI, MS
2-isopropyl-5-methyl-phenyl acetate	1377	0.1	nd	AI, MS
Caryophyllene	1419	1.95	2.2	AI, MS, Co-GC
Spathulenol	1578	0.1	0.2	AI, MS
Carryophyllene oxide	1583	0.5	0.5	AI, MS, Co-GC
Total (%)		93.65	91.6	

[a]Compounds listed in order of elution from an HP-5 MS capillary column; [b] AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C23); [c] Identification method: AI=Arithmetic Index, MS=mass spectrum, Co-GC=Coinjection with authentic compound, nd=not detected. Concentrations below 0.05% are marked as tr (traces).

GC-MS analysis of the *S. montana* EOs identified in total 47 different components, counting for 98.33% and 80.83% of the EOs of SM-B and SM-S samples respectively. The most abundant compound was Thymol, followed by, p-Cymene, Carvacrol methyl ether and γ-Terpinene counting on average for 40.65%, 10.35%, 5.35% and 5.05% respectively (Table 4). Thymol was markedly higher in SM-B sample with almost the double of SM-S concentration.

S. montana oils display thymol chemotype, particularly in SM-B. This population presents the highest thymol concentration compared to other reports from Albania [22,32,40] making it a valuable candidate for cultivation and industrial applications. Thymol is associated with potent antiseptic and antibacterial properties [41] which could be suitable for food preservation. The higher thymol content in SM-B indicates a stronger bioactivity potential compared to SM-S. However, SM-S shows greater chemical diversity, possibly due to genetic biodiversity from ecological and geographic variability [22].

Table 4. Composition of the essential oils of *S. montana.*

Compounds [a]	AI [b]	SM-B	SM-S	ID [c]
α-Thujene	926	1.3	1.4	AI, MS
α-Pinene	931	0.9	0.7	AI, MS, Co-GC
Camphene	945	0.1	0.8	AI, MS
β-Pinene	973	0.1	0.1	AI, MS, Co-GC

 doi:10.20944/preprints202507.1209.v1

Octen-3-ol	983	nd	0.2	AI, MS
β-Myrcene	992	1.4	1.0	AI, MS, Co-GC
α-Phellandrene	1003	1.03	0.9	AI, MS
δ-2-Carene	1008	0.2	0.3	AI, MS
δ-3-Carene	1015	1.3	1.4	AI, MS, Co-GC
p-Cymene	1024	8.9	11.8	AI, MS, Co-GC
Limonene	1027	0.6	0.9	AI, MS
Eucalyptol	1029	0.3	0.4	AI, MS
trans-Ocimene	1040	0.3	0.8	AI, MS
cis-Ocimene	1050	0.13	0.2	AI, MS
γ-Terpinene	1059	4.7	5.4	AI, MS, Co-GC
cis-Sabinenehydrate	1067	1.5	4.2	AI, MS
Terpinolene	1087	0.2	0.3	AI, MS
trans-Sabinenehydrate	1098	1.4	0.1	AI, MS
Linalool	1101	3.3	0.5	AI, MS, Co-GC
α-Thujone	1104	0.94	0.1	AI, MS
β-Thujone	1116	0.04	tr	AI, MS
cis-p-Menth-2-en-1-ol	1122	0.2	tr	AI, MS
Camphor	1143	0.3	0.3	AI, MS
Borneol	1164	2.4	2.8	AI, MS, Co-GC
δ-Terpineol	1169	nd	0.7	AI, MS
Terpinene-4-ol	1176	1.98	3.2	AI, MS, Co-GC
p-Cymen-8-ol	1187	0.3	0.1	AI, MS
α-Terpineol	1191	0.04	0.2	AI, MS
Thymol methyl ether	1236	1.98	0.1	AI, MS
Carvacrol methyl ether	1244	5.2	5.5	AI, MS
Bornyl acetate	1286	0.04	nd	AI, MS, Co-GC
Thymol	1294	52.8	28.5	AI, MS, Co-GC
Carvacrol	1304	2.5	1.2	AI, MS
Thymyl acetate	1356	0.45	0.5	AI, MS
α-Copaene	1375	0.1	0.1	AI, MS
β-Burbonene	1384	0.1	0.2	AI, MS
β-Caryophyllene	1419	nd	2.3	AI, MS, Co-GC
β-Copaene	1428	nd	0.2	AI, MS
γ-Elemene	1434	nd	0.6	AI, MS
Aromadendrene	1438	nd	0.5	AI, MS
Myltayl-4(12)-ene	1443	nd	nd	AI, MS
α-Carryophyllene	1453	0.6	0.2	AI, MS, Co-GC
Allo-Aromadendrene	1460	0.7	0.2	AI, MS
Dauca-5,8-diene	1474	nd	0.55	AI, MS
γ-Muurolene	1477	nd	0.25	AI, MS
Spathulenol	1578	nd	0.13	AI, MS
Carryophyllene oxide	1583	nd	1.0	AI, MS, Co-GC
Total (%)		98.33	80.83	

[a]Compounds listed in order of elution from an HP-5 MS capillary column; [b] AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C23); [c] Identification method: AI=Arithmetic Index, MS=mass spectrum, Co-GC=Coinjection with authentic compound, nd=not detected. Concentrations below 0.05% are marked as tr (traces).

All three species contain biosynthetic precursors (γ-terpinene, p-cymene), showing enzymatic direction toward either carvacrol or thymol pathways [42]. Based on carvacrol/thymol content, *O. vulgaris* subsp. *hirtum* and *T. capitatus* are stronger antimicrobials, while *S. montana* shows slightly broader compositional complexity but lower potency. Oils rich in carvacrol (above 70%) are ideal for

 doi:10.20944/preprints202507.1209.v1

pharmaceutical and preservative uses. *S. montana* could appeal to niche markets that favor thymol-based formulations with milder olfactory characteristics.

2.3. Antioxidant Activity

Antioxidant activity of the EOs expressed as IC_{50} for DPPH and ABTS assays are reported in Table 5. Lower IC_{50} values (μg/mL) indicate stronger antioxidant activity.

Table 5. Antioxidant activity of EOs expressed as IC50 values (μg/mL). .

EOs FROM SAMPLES	DPPH μg/mL	ABTS μg/mL
OV-L	530 ± 8	110 ± 10
OV-P	600 ± 12	120 ± 8
TC-M	530 ± 9	180 ± 9
TC-L	570 ± 8	220 ±13
SM-B	1200 ± 5	460 ± 27
SM-S	820 ± 4	500 ± 14

OV-L and OV-P EOs exhibited the lowest IC_{50} values, especially in the ABTS assay, indicating the strongest antioxidant capacity among all tested oils. This correlates strongly with their high carvacrol content (74.6–79.8%), a phenolic monoterpene known for effective free radical scavenging, hydrogen donation ability due to its hydroxyl group and lipophilic character enhancing membrane interaction [43]. *T. capitatus* EOs, also rich in carvacrol (72.8–77.7%), showed similarly high antioxidant performance, although slightly less potent than *O. vulgaris*, possibly due to marginally lower carvacrol levels and total phenolics.

S. montana EOs demonstrated weaker antioxidant activity, especially SM-B with the highest DPPH IC_{50} (1200 μg/mL). Despite having high thymol content (52.8% in SM-B, 28.5% in SM-S), the antioxidant potency was significantly lower than carvacrol-rich oils. Although thymol is also a phenolic compound, it is generally less effective than carvacrol in radical scavenging due to differences in redox potential, steric hindrance from methyl groups [44], electronic resonance stabilization of the phenoxyl radical.

The ABTS assay proved more sensitive across all oils, in accordance with what is generally reported in other works [45].

Oxidative degradation of food lipids leads to rancidity, loss of flavor, discoloration, and decrease of nutritional value. Essential oils rich in carvacrol (e.g., O. *vulgaris, T. capitatus*) showed strong antioxidant activity [46], indicating their potential as natural alternatives to synthetic antioxidants like BHT and BHA. Their ability to scavenge both lipophilic (DPPH) and hydrophilic (ABTS) radicals broadens their applicability in emulsions, fats, meat products, and oils. While *S. montana* EOs were less effective as antioxidants, their aromatic and antimicrobial properties may still be valuable as sensory enhancers and microbial inhibitors, especially in combination with other preservatives.

2.4. Antimicrobial Activity

Table 6 shows the antimicrobial activity of the six EOs using the broth microdilution method in accordance with CLSI guidelines [47].

Table 6. Minimum Inhibitory Concentration of essential oils against six pathogens.

EO from sample	MIC (mg/ml)					
	E. coli ATCC 10535	*S.* Enteritidis ATCC 49223	*P. aeruginosa* ATCC 9027	*M. luteus* ATCC 10240	*S. maltophilia* ATCC 13637	*C. albicans* ATCC 10231
OV-L	0.312	1.250	1.250	0.312	0.156	0.312
OV-P	0.625	0.625	1.250	0.625	0.156	0.312
TC-M	0.312	0.625	2.5	0.625	0.156	0.156
TC-L	0.625	1.250	NO MIC	0.625	0.312	0.156

 doi:10.20944/preprints202507.1209.v1

SM-B	0.625	1.250	NO MIC	0.625	1.250	0.312
SM-S	1.250	2.5	2.5	0.625	0.625	0.625

MICs were determined and ranged from 0.15 to 2.5 mg/mL. *C. albicans* and *S. maltophilia* were the most susceptible organisms overall, while *P. aeruginosa* was the most resistant. *P. aeruginosa* is known for its resistance mechanisms, including low outer membrane permeability and active efflux systems, which limit the efficacy of hydrophobic substances like EOs [48].

O. vulgaris subsp. *hirtum* EOs showed strong activity (MIC ≤ 0.625 mg/mL) *against E. coli, M. luteus, S. maltophilia*, and *C. albicans*. Likely due to high carvacrol content (74.6–79.8%), a phenolic monoterpene known to disrupt bacterial membranes and induce leakage of cytoplasmic contents [13,48]. Slight differences in MIC values between OV-L and OV-P EOs may reflect minor differences in thymol content or minor components.

In *T. capitatus* sample TC-M showed broad-spectrum activity, with very low MICs against *C. albicans* (0.156 mg/mL) and *E. coli* (0.312 mg/mL). TC-L had no inhibitory effect on *P. aeruginosa*, indicating possible influence of lower carvacrol content (4.9% less) and other minor constituents. Still, their antimicrobial potency, is explained through their high carvacrol content (72.8–77.7%).

S. montana samples demonstrated weakest antimicrobial activity, with the highest MICs levels on all tested strains compared to the other two species taken in study. Lower efficacy is consistent with lower carvacrol content and higher relative content of thymol and monoterpenes with weaker antimicrobial effects (e.g., borneol, p-cymene).

Carvacrol is generally reported to have stronger antimicrobial activity than thymol, although both are potent and structurally similar phenolic compounds [49].

The variation in antimicrobial activity between the essential oils of the same species can be attributed to small differences in chemical composition where the interaction among major and minor compounds could have synergistic or antagonistic interactions which explain the differences in MICs [50].

To our knowledge this is the first report on the activity of the EO from *S. montana* on the multidrug resistant *S. maltophilia* bacteria with MIC doses of 1.25 and 0.625 mg/ml, SM-B and SM-S respectively.

EOs are generally recognized as safe (GRAS) when used at appropriate concentrations. In the context of targeting spoilage and pathogenic microorganisms, EOs from *O. vulgaris* and *T. capitatus*, due to their low MICs and broad-spectrum efficacy, are suitable for preserving perishable foods such as minced meats and sausages, ready-to-eat salads or dips, dairy products etc. [51,52] . The strong aroma and flavour of carvacrol-rich oils may require dose optimization, combination with other hurdles (e.g., refrigeration, mild heat, or acids) [53] or better incorporation in delivery systems, such as edible films and coatings [54], or nanoemulsions [55] for improved dispersion in aqueous food systems, to enhance efficacy and minimize sensory impacts.

As encapsulation and nanoemulsion technologies can improve stability, solubility, and controlled release, minimizing flavor impact while preserving efficacy, the most promising EOs were tested for incorporation in nanoemulsion delivery system.

2.5. EOs Nanoemulsions

To be ideal candidates for incorporation into nanoemulsions, EOs should meet the following: high yield – to ensure feasibility and cost-effectiveness, strong antioxidant activity – to prevent oxidative deterioration, potent antimicrobial activity – to inhibit or eliminate foodborne pathogens, chemical stability and compatibility with emulsifiers and food matrices.

From the plant species taken in our study, *O. vulgaris* subps. *hirtum* and *T. capitatus* resulted the most prominent, respectively represented by EOs of samples OV-L and TC-M which performed better. Specifically, OV-L had a slightly higher yield, and slightly stronger antioxidant and antimicrobial activity compared to OV-P. TC-M, in general performed better then TC-L sample (Table 7). Nevertheless, high EO yields in *O. vulgaris* subps. *hirtum* (3.8–4.1%) ensure scalability and

 doi:10.20944/preprints202507.1209.v1

economic viability. Although yield is lower in *T. capitatus* samples, its chemical richness in carvacrol, potent antifungal and antibacterial activity, and strong antioxidant action justify its use in targeted nanoemulsion formulations, especially for high-value foods.

Table 7. Comparison of yield, composition and biological activity of the EOs from MAPs plants taken in study.

EO Sample	Yield (%)	Key Active Components	Antioxidant Activity (DPPH/ABTS, µg/mL)	Antimicrobial Spectrum
OV-L	**4.06**	**Carvacrol (74.6%)**, thymol	530/**110**	Broad: strong
OV-P	3.83	**Carvacrol (79.8%)**, lower thymol	600/120	Broad, strong
TC-M	1.66	Carvacrol (77.7%), p-cymene	**530/180**	Broad, strong
TC-L	0.75	Carvacrol (72.8%)	570/220	Moderate; inactive against *P. aeruginosa*
SM-B	0.39	Thymol (52.8%), low carvacrol	1200/460	Weak, inactive against *P. aeruginosa*
SM-S	0.73	Thymol (28.5%), carvacrol (1.2%)	820/500	Weakest overall

Results from DLS (Dynamic Light Scattering) of nanoemulsions incorporating OV-L and TC-M EOs, for the particle size and Z-potential which are key parameters influencing the stability, bioavailability, and functionality of EO nanoemulsions in food systems are shown in Table 8.

Table 8. Essential oil nanoemulsions characterisation.

SAMPLE	Particle size (nano meters)	Z-potential
OV-L	132.4 ±15.3	-11.2 ± 2.8 mV
TC-M	191.8± 17.2	-9.6 ± 0.5 mV

According to literature, emulsion droplets with size <200 nm are ideal for improved optical clarity, enhanced bioavailability and penetration into microbial membranes and greater colloidal stability due to reduced gravitational separation [56]. OV-L EOs nanoemulsion particle size was in optimal nano-range (<200 nm) with a mean size of 132.5 nm which indicates well-dispersed and finely emulsified droplets, while also TC-M EOs nanoemulsion was in nano-range even though at the upper end limit.

Zeta potential may act as a partial indicator of the physical stability of the emulsion created. The higher the absolute value of Zeta potential, the more stable is an emulsion. To ensure the formation of a robust energy barrier against the coalescence of dispersed droplets, it has been advised to attain high absolute zeta potential values (exceeding ±30 mV) in most prepared emulsions [57]. Nevertheless, analyzing zeta potential for a nanoemulsion stabilized with Tween 80 is not always essential for understanding stability — and its interpretive value is limited — because Tween 80 provides primarily steric rather than electrostatic stabilization by forming a hydrated, bulky layer around droplets, preventing coalescence through physical hindrance rather than electrostatic repulsion. For example, Tan, T.B. et al. [58] reported that no increase in particle size was seen for the Tween 80-stabilized nanodispersion, even when zeta potentials were brought near 0 mV, due to the non-ionic characteristics of the Tween 80 emulsifier, which imparts stability via steric hindrance.

In summary it can be deduced that both nanoemulsion formulations were good but might need co-stabilizers for an extended shelf life. OV-L EO nanoemulsion offer the best size characteristics for transparent or colloidally stable food systems (e.g., beverages, edible films), though stabilization can be further optimised. TC-M EO emulsions are adequate for use in short-term preservation, active packaging, or surface treatments where long-term droplet stability is less critical.

 doi:10.20944/preprints202507.1209.v1

3. Materials and Methods

3.1. Plant Material

Six plants, two of each concerned species, *O. vulgaris* subsp. *hirtum*, *T. capitatus* and *S. montana* were collected in August 2023, as they are known to accumulate higher amounts of essential oil when exposed to high radiation and temperature [29,59,60].

Information on the collection place of these populations is shown in Table 9.

Table 9. Plant samples.

Species	Plant Code	Collection date	Altitude* (m.a.s.l.)	Location	Coordinates	
					Latitude (N)	Longitude (E)
O. vulgaris subsp. *hirtum*	OV-L	13.08.2023	~55	Lukovë	39°97′88′′	19°91′29′′
O. vulgaris subsp. *hirtum*	OV-P	4.08.2023	~1200	Qafë Pishë	40°25′92′′	19°79′11′
T. capitatus	TC-M	4.08.2023	~54	Mallakastër	41°58′47′′	20°13′12′′
T. capitatus	TC-L	13.08.2023	~179	Lukovë	39°98′19′′	19°91′71′′
S. montana	SM-B	4.08.2023	~1500	Bego Mauntain	40°25′44′′	19°78′86′′
S. montana	SM-S	19.08.2023	~724	Dajti Mountain	41°36′90′′	19°94′20′′

*m.a.s.l. - meters above sea level.

The species identification for each population was carried out by the Genetics and Plant Breeding Laboratory, Agricultural University of Tirana, Albania, and the herbarium specimen vouchers were deposited in the same laboratory. The plant materials were dried in a well ventilated, shaded area, at room temperature about 25 °C and relative humidity around 50%. Once dried, stems were separated from leaves and flowers.

3.2. EOs Extraction

The EOs were extracted by hydrodistillation using a Clevenger apparatus [61]. One hundred grams of dried plant material were minced finely and put into a one-liter flask with 500 mL of distilled water. Distillation went on for three hours at a rate of three millilitres per minute. The oil yield was calculated as a percentage of volume by weight (% v/w) relative to the dry weight of the plant material. The obtained EOs were stored at 4 °C prior to analysis.

3.3. Reagents and Microbial Strains

Antioxidant radical screening reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)] were purchased from Alfa Aesar (Massachusetts, United States). Methanol was secured from VWR International (Fontenay-sous-Bois, France) and ethanol from Merk KGaA (Darmstadt, Germany).

The bacterial American type culture collection strains *S. enterica* serovar Enteritidis (ATCC:49223), *E. coli* (ATCC:10535), *P. aeruginosa* (ATCC:9027), *S. maltophilia* (ATCC:13637), *M. luteus* (ATCC:10240), along with one fungal isolate *C. albicans* (ATCC:10231) were procured from Microbiologics, Inc., (Minnesota United States), 96-well plates were secured from Corning Inc. (New York, United States).Blood agar medium and Muller Hinton Broth were procured from Remel Inc, (California, United States), 0.5 Polymer McFarland Standard from Thermo Fisher Scientific (Massachusetts, United States) and Dimethylsulfoxide (DMSO) from Sigma-Aldrich (Missouri, United States).

 doi:10.20944/preprints202507.1209.v1

3.4. Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry EOs analyses were performed on a Shimadzu GC-2010-GCMSQP2010 system operating at 70 eV. The temperature program was from 60 °C to 250 °C, at a rate of 5 °C/min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection volume of each sample was 1 µL. Retention times for all compounds were determined according to Van den Dool and Kratz, 1963 [62], using n-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of NIST21 and NIST107 (National Institute of Standards and Technology, USA), Massada, 1976 [63] and by comparison of their retention indices with literature data Adams, 2007 [64]. Component relative concentrations were calculated based on GC peak areas without using correction factors. EOs were often subjected to co-chromatography with authentic compounds (Fluka, Sigma).

3.5. Free radical Scavenging Activity

The free radical scavenging activity of the essential oils was measured in vitro using DPPH and ABTS assays according to Brand-Williams et al., (1995) [65] and to Re et al., (1998) [66] respectively. The stock solution for the DPPH assay was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and stored at 20°C. The working solution was obtained by diluting the DPPH stock solution with methanol to achieve an absorbance of about 0.98±0.02 at 517 nm using a spectrophotometer (Biochrom Ltd. Libra S22). The stock solution for the ABTS assay was prepared by dissolving ABTS in water at a concentration of 7 mM. The ABTS radical cation ($ABTS^{\bullet+}$) was generated by mixing the ABTS stock solution with potassium persulfate at a final concentration of 2.45 mM, followed by incubation in the dark at room temperature for 12–16 h. The working solution was then prepared by diluting the ABTS stock with ethanol until the absorbance reached approximately 0.70 ± 0.02 at 734 nm, as determined using the spectrophotometer.

Three mL aliquots of each solution were combined with 77 µL of the sample at 6 concentrations (50, 100, 200, 5000, 1000, 2000 µg/mL), then thoroughly mixed. For the DPPH assay, mixtures were incubated in darkness at room temperature for 30 min., then the absorbance was measured at 517 nm. For the ABTS assay the absorbance was recorded at 734 nm after 5-6 minutes of reaction in similar conditions. The controls were prepared as above but without essential oil. The activity was evaluated based on the percentage of the DPPH and ABTS radicals removed as the following equation:

Scavenging activity in % = {(absorbance of control - absorbance of sample)/(absorbance of control)} x 100. The result was calculated as the concentration of essential oil that inhibits 50% of the free radical (Inhibition Concentration - IC_{50}).

3.6. Evaluation of Antimicrobial Activity by Micro-Dilution Broth Method

The antimicrobial activities of the EOs were evaluated against a panel of clinical and food-borne pathogens using standard American Type Culture Collection strains (ATCC), five bacteria along with one fungal isolate. Stock cultures were maintained at 4 °C and subcultured immediately before use. Prior to EO treatment, bacterial strains were incubated at 37 °C and the fungal isolate at 28 °C, each for 18–20 h on blood agar, ensuring cultures were in optimal growth phase.

The MIC (Minimum Inhibitory Concentration) of each EO was determined using a broth microdilution method in 96-well plate following the Clinical & Laboratory Standards Institute (CLSI) protocols (47). In brief, bacterial suspensions were adjusted to a final concentration of 10^5 CFU/mL cells standardized by 0.5 McFarland in Muller Hinton Broth (MHB) media. EO stock solutions were prepared at 100 mg/ml of by dissolving them in DMSO. From this stock, a working solution of 5 mg/ml was subsequently diluted in MHB media, ensuring the final DMSO concentration is less than 5%. Serial two-fold dilutions were performed to achieve EO concentrations ranging from 5.0 to 0.0097 mg/mL in the microplate wells. Finally, 100 µL of the bacterial suspension was added to each well. The plate setup included the 11th column as the media control (negative control), and the 12th column containing bacteria and media (positive control). Additionally, rows D and E were

 doi:10.20944/preprints202507.1209.v1

designated for solvent controls, with row D with only EO (solvent control) and row E for DMSO (concentration used to dissolve EO) and bacteria served as DMSO control. The plates were incubated at 37 °C for 24 h. MIC was determined using Tecan I-control software (Infinite M Plex TECAN) to measure the Optical Density (OD) at 600nm compared with the positive control. Everything was kept constant for determining antifungal activity except incubation, which was done for 45-48h, with OD determined at 530nm. MIC was determined as the lowest concentration of EO that inhibited the visible growth of the tested microorganism [67].

3.7. Encapsulation of EOs

The oregano and thyme EOs nanoemulsions were prepared by using the method previously described by Bodea, Cătunescu, Palop, Fernandez, and Garre, (2023) [68] with some modifications. Specifically, a total oil phase was formed with 2 mL oregano EO and 1.35 mL sunflower oil as carrier oil. A coarse emulsion was first produced by mixing the oil phase and 2.5 mL Tween 80, followed by combining with deionised water added dropwise under continuous stirring at room temperature. The emulsion formed after 4.15 mL of water was added. For the thyme EO nanoemulsion the constituents were in slight different concentrations: 1 mL of EO, 1.0125 mL of sunflower oil, and 1.875 mL of Tween 80. The coarse emulsion was homogenised by ultrasonification (USF) for 15 minutes at 100% amplitude to obtain the functionalized nanoemulsion. The maximum temperature of the samples during sonication was kept at 25ºC.

Nanoemulsions were formed using water, sunflower oil, which are all recognised as GRAS, and Tween 80, a permitted food additive, by the Food and Drug Administration of USA. Due to its safety profile, Tween 80 (T80), sometimes referred to as Polysorbate 80, is widely used and has been approved by the European Food Safety Authority (EFSA) for use in food items [69]. Nanoemulsion were immediately stored at refrigeration and analysed after two weeks.

The average droplet size and zeta potential were determined by dynamic light scattering (DLS) (Zetasizer Nano, Malvern).

3.8. Statistical Analysis

All experiments were performed in triplicate and the standard deviation calculated. The IC_{50} value was determined by simple linear regression, using the percentage inhibition data over the linear dose range. The standard deviation of the IC_{50} was calculated by error propagation, based on the standard errors of the slope and intercept. The analyses were performed with GraphPad Prism software 10.5.0.

4. Conclusions

This work investigated on the potential of essential oils (EOs) from wild Albanian *Lamiaceae* species, *O. vulgare* subsp. *hirtum*, *T. capitatus*, and *S. Montana*, as natural preservatives for food applications. The main bioactive compounds in these oils were carvacrol and thymol, both of which are wildly known for their strong antioxidant and antimicrobial activities. The extracted EOs exhibited high antioxidant activity, as evidenced by their low IC_{50} values in the DPPH and ABTS assays, confirming their ability to scavenge free radicals and prevent oxidative damage. Additionally, the antimicrobial efficacy of the EOs against six foodborne pathogens indicated that the oils possessed broad-spectrum antimicrobial activity, including Gram-negative, Gram-positive bacteria, and fungi. These findings underline the potential of these EOs as natural preservatives.

Oil-in-water nanoemulsions were formed to enhance the stability and delivery of the EOs. The formed nanoemulsions exhibited promising physical characteristics, including small droplet sizes, indicating the potential for prolonged release of the bioactive compounds. However, further investigation is required to assess their long-term stability and practicality in food applications. The commercial viability of these encapsulated oils will depend on the ability to comprehend how they behave at various temperatures, storage conditions, and during food processing.

 doi:10.20944/preprints202507.1209.v1

In conclusion, the results of this study indicate the high potential of EOs from Albanian *O. vulgare* subsp. *hirtum*, and *T. capitatus* as natural preservatives in the food industry. Considering their strong antioxidant and antimicrobial activities as well as their successful nanoencapsulation, they can be effective substitutes for synthetic preservatives which are increasingly viewed as undesirable by consumers. Additionally, the nanoemulsions obtained in this study can be utilized as functional ingredients in food formulations, providing a practical way to add essential oils to foods without changing the organoleptic characteristics.

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

Author Contributions: Conceptualization, E.B. and E.M.; methodology, E.M. and B.B.; validation, B.B., S.S. and E.M.; formal analysis, E.H.Ç., A.S. and M.M.; investigation, E.B.; data curation, S.Z.; writing—original draft preparation, E.B. and S.Z.; writing—review and editing, B.B., S.S. and M.M.; project administration, E.B.; funding acquisition, E.M. All authors have read and agreed to the published version of the manuscript.

Funding: This publication was made possible with the financial support of National Agency for Scientific Research, Technology and Innovation (NASRI), Albania. Its content is the responsibility of the author, the opinion expressed in it is not necessarily the opinion of NASRI.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are thankful to Mr. Besnik Koçi from BioBes sh.p.k. for the organization and support in the expeditions for collection of plant materials. The authors are also grateful to Prof. Dr. Alban Ibraliu for species identification and backing for EOs extraction.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Teshome, E.; Forsido, S.F.; Rupasinghe, H.P.V.; Keyata, E.O. Potentials of Natural Preservatives to Enhance Food Safety and Shelf Life: A Review. Sci. World J. 2022, 2022, 9901018. https://doi.org/10.1155/2022/9901018
2. Dong, H.; Xu, Y.; Zhang, Q.; Li, H.; Chen, L. Activity and Safety Evaluation of Natural Preservatives. Food Res. Int. 2024, 190, 114548. https://doi.org/10.1016/j.foodres.2024.114548
3. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological Effects of Essential Oils—A Review. Food Chem. Toxicol. 2008, 46, 446–475. https://doi.org/10.1016/j.fct.2007.09.106.
4. Sun, J.; Sun, P.; Kang, C.; Zhang, L.; Guo, L.; Kou, Y. Chemical Composition and Biological Activities of Essential Oils from Six Lamiaceae Folk Medicinal Plants. Front. Plant Sci. 2022, 13, 919294. https://doi.org/10.3389/fpls.2022.919294
5. Chorianopoulos, N.; Kalpoutzakis, E.; Aligiannis, N.; Mitaku, S.; Nychas, G.-J.E.; Haroutounian, S.A. Essential Oils of Satureja, Origanum, and Thymus Species: Chemical Composition and Antibacterial Activities Against Foodborne Pathogens. J. Agric. Food Chem. 2004, 52, 8261–8267. https://doi.org/10.1021/jf049113i
6. Ozogul, Y.; Karsli, G.T.; Yazgan, H.; Ozogul, F.; Regenstein, J.M. Enhanced Pathogen Control Through Thymol and Carvacrol Nanoemulsions: A Microfluidization Approach. Food Bioprocess Technol. 2025, 18, 5377–5387. https://doi.org/10.1007/s11947-025-03759-z.
7. Sarac, N.; Ugur, A. Antimicrobial Activities of the Essential Oils of Origanum onites L., Origanum vulgare L. Subspecies hirtum (Link) Ietswaart, Satureja thymbra L., and Thymus cilicicus Boiss. & Bal. Growing Wild in Turkey. J. Med. Food 2008, 11(3), 568–573. https://doi.org/10.1089/jmf.2006.105.

 doi:10.20944/preprints202507.1209.v1

8. Baratta, M.T.; Dorman, H.J.D.; Deans, S.G.; Figueiredo, A.C.; Barroso, J.G.; Ruberto, G. Antimicrobial and Antioxidant Properties of Some Commercial Essential Oils. Flavour Fragr. J. 1998, 13, 235–244. https://doi.org/10.1002/(SICI)1099-1026(1998070)13:4<235::AID-FFJ733>3.0.CO;2-T.
9. Stoilova, I.; Bail, S.; Buchbauer, G.; Stoyanova, A.; Krastanov, A.; Staneva, D. Chemical Composition, Olfactory Evaluation and Antioxidant Effects of the Essential Oil of Satureja montana L. Nat. Prod. Commun. 2008, 3(7). https://doi.org/10.1177/1934578X0800300701.
10. Chroho, M.; Rouphael, Y.; Petropoulos, S.A.; Bouissane, L. Carvacrol and Thymol Content Affects the Antioxidant and Antibacterial Activity of Origanum compactum and Thymus zygis Essential Oils. Antibiotics 2024, 13, 139. https://doi.org/10.3390/antibiotics13020139
11. Nazzaro, F.; Fratianni, F.; De Martino, L.; Coppola, R.; De Feo, V. Effect of Essential Oils on Pathogenic Bacteria. Pharmaceuticals 2013, 6, 1451–1474. https://doi.org/10.3390/ph6121451.
12. D'Amato, S.; Rossi, C.; Maggio, F.; Valbonetti, L.; Savini, V.; Paparella, A.; Serio, A. Antilisterial Effectiveness of Origanum vulgare var. hirtum and Coridothymus capitatus Essential Oils and Hydrolates Alone and in Combination. Foods 2024, 13, 860. https://doi.org/10.3390/foods13060860.
13. Burt, S. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods—A Review. Int. J. Food Microbiol. 2004, 94, 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022.
14. Friedman, M.; Henika, P.R.; Mandrell, R.E. Bactericidal Activities of Plant Essential Oils and Some of Their Isolated Constituents Against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica. J. Food Prot. 2002, 65, 1545–1560. https://doi.org/10.4315/0362-028X-65.10.1545.
15. de Castro, R.D.; de Souza, T.M.; Bezerra, L.M.; Ferreira, G.L.; Costa, E.M.; Cavalcanti, A.L. Antifungal Activity and Mode of Action of Thymol and Its Synergism with Nystatin Against Candida Species Involved with Infections in the Oral Cavity: An In Vitro Study. BMC Complement. Altern. Med. 2015, 15, 417. https://doi.org/10.1186/s12906-015-0947-2.
16. McClements, D.J. Nanoemulsions versus Microemulsions: Terminology, Differences, and Similarities. Soft Matter 2012, 8, 1719–1729. https://doi.org/10.1039/C2SM06903B.
17. Maurya, A.; Singh, V.K.; Das, S.; Prasad, J.; Kedia, A.; Upadhyay, N.; Dubey, N.K.; Dwivedy, A.K. Essential Oil Nanoemulsion as Eco-Friendly and Safe Preservative: Bioefficacy Against Microbial Food Deterioration and Toxin Secretion, Mode of Action, and Future Opportunities. Front. Microbiol. 2021, 12, 751062. https://doi.org/10.3389/fmicb.2021.751062.
18. Singh, I.R.; Pulikkal, A.K. Preparation, Stability and Biological Activity of Essential Oil-Based Nanoemulsions: A Comprehensive Review. OpenNano 2022, 8, 100066. https://doi.org/10.1016/j.onano.2022.100066.
19. Enayatifard, R.; Akbari, J.; Babaei, A.; Rostamkalaei, S.S.; Hashemi, S.M.H.; Habibi, E. AnTC-Microbial Potential of Nano-Emulsion Form of Essential Oil Obtained from Aerial Parts of Origanum vulgare L. as Food Additive. Adv. Pharm. Bull. 2021, 11, 327–334. https://doi.org/10.34172/apb.2021.028.
20. Jayari, A.; Donsì, F.; Ferrari, G.; Maaroufi, A. Nanoencapsulation of Thyme Essential Oils: Formulation, Characterization, Storage Stability, and Biological Activity. Foods 2022, 11, 1858. https://doi.org/10.3390/foods11131858.
21. Rinaldi, F.; Maurizi, L.; Conte, A.L.; Marazzato, M.; Maccelli, A.; Crestoni, M.E.; Hanieh, P.N.; Forte, J.; Conte, M.P.; Zagaglia, C.; Longhi, C.; Marianecci, C.; Ammendolia, M.G.; Carafa, M. Nanoemulsions of Satureja montana Essential Oil: Antimicrobial and Antibiofilm Activity Against Avian Escherichia coli Strains. Pharmaceutics 2021, 13, 134. https://doi.org/10.3390/pharmaceutics13020134.
22. Ibraliu, A.; Mi, X.; Elezi, F.; Sala, F. Analysis of Essential Oils of Three Wild Medicinal Plants in Albania. J. Med. Plants Res. 2011, 5(1), 58–62.
23. Elezi, F.; Plaku, F.; Ibraliu, A.; Stefkov, G.; Karapandzova, M.; Kulevanova, S.; Aliu, S. Genetic Variation of Oregano (Origanum vulgare L.) for Etheric Oil in Albania. Agric. Sci. 2013, 4, 449–454. https://doi.org/10.4236/as.2013.49060.
24. Hodaj-Çeliku, E.; Tsiftsoglou, O.; Shuka, L.; Abazi, S.; Hadjipavlou-Litina, D.; Lazari, D. Antioxidant Activity and Chemical Composition of Essential Oils of Some Aromatic and Medicinal Plants from Albania. Nat. Prod. Commun. 2017, 12(5). https://doi.org/10.1177/1934578X1701200525.

 doi:10.20944/preprints202507.1209.v1

25. Giannoulis, K.D.; Kamvoukou, C.-A.; Gougoulias, N.; Wogiatzi, E. Irrigation and Nitrogen Application Affect Greek Oregano (Origanum vulgare ssp. hirtum) Dry Biomass, Essential Oil Yield and Composition. Ind. Crops Prod. 2020, 150, 112392. https://doi.org/10.1016/j.indcrop.2020.112392.
26. Kokkini, S. Taxonomy, Diversity and Distribution of Origanum Species. In Proceedings of the IPGRI International Workshop on Oregano; Padulosi, S., Ed.; CIHEAM Valenzano: Bari, Italy, 1997; pp. 122–132.
27. Baser, K. Hüsnü Can. "Aromatic biodiversity among the flowering plant taxa of Turkey" *Pure and Applied Chemistry*, vol. 74, no. 4, 2002, pp. 527-545. https://doi.org/10.1351/pac200274040527
28. Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T. and Arsenakis, M. (1998) Antifungal activities of Origanum vulgare subsp. hirtum, Mentha spicata, Lavandula angustifolia and Salvia fruticosa Essential Oils against Human Pathogenic Fungi. Journal of Agricultural and Food Chemistry, 46, 1738-1745. http://dx.doi.org/10.1021/jf9708296
29. Mugao, L.G. Factors Influencing Yield, Chemical Composition and Efficacy of Essential Oils. Int. J. Multidiscip. Res. Growth Eval. 2024, 5(4), 169–178. https://doi.org/10.54660/.IJMRGE.2024.5.4.169-178.
30. Hedhili, L., Romdhane, M., Abderrabba, A., Planche, H. and Cherif, I. (2002), Variability in essential oil composition of Tunisian Thymus capitatus (L.) Hoffmanns. et Link.. Flavour Fragr. J., 17: 26-28. https://doi.org/10.1002/ffj.1029
31. Tagnaout, I.; Zerkani, H.; Hadi, N.; El Moumen, B.; El Makhoukhi, F.; Bouhrim, M.; Al-Salahi, R.; Nasr, F.A.; Mechchate, H.; Zair, T. Chemical Composition, Antioxidant and Antibacterial Activities of Thymus broussonetii Boiss and Thymus capitatus (L.) Hoffmann and Link Essential Oils. Plants 2022, 11, 954. https://doi.org/10.3390/plants11070954.
32. Hajdari, A.; Elezaj, R.; Mulla, D.; Mustafa, B.; Quave, C.L.; Novak, J. Chemical Composition of the Essential Oil, Total Phenolics, Total Flavonoids and Antioxidant Activity of Methanolic Extracts of Satureja montana L. Rec. Nat. Prod. 2016, 10(6), 750–760. http://www.acgpubs.org/RNP/2016/Volume10/Issue%201/88-RNP-EO_1601-%20002.pdf
33. Said-Al Ahl, H.A.H.; Kačániova, M.; Mahmoud, A.A.; Hikal, W.M.; Čmiková, N.; Szczepanek, M.; Błaszczyk, K.; Al-Balawi, S.M.; Bianchi, A.; Smaoui, S.; et al. Phytochemical Characterization and Biological Activities of Essential Oil from Satureja montana L., a Medicinal Plant Grown under the Influence of Fertilization and Planting Dates. Biology 2024, 13, 328. https://doi.org/10.3390/biology13050328
34. Zawiślak,G. and Nurzyńska-Wierdak,R., 20193193386, English, Journal article, Poland, doi:10.24326/asphc.2017.5.16, 2545-1405 1644-0692, 16, (5), Lublin, Acta Scientiarum Polonorum - Hortorum Cultus, (159–168), Wydawnictwo Akademii Rolniczej w Lublinie, Variation in winter savory (Satureja montana L.) yield and essential oil production as affected by different plant density and number of harvests., (2017).
35. Serrano, C.; Matos, O.; Teixeira, B.; Ramos, C.; Neng, N.; Nogueira, J.; Nunes, M.L.; Marques, A. Antioxidant and Antimicrobial Activity of Satureja montana L. Extracts. J. Sci. Food Agric. 2011, 91, 1554–1560. https://doi.org/10.1002/jsfa.4347.
36. Kokkini, S.; Karousou, R.; Vokou, D. Pattern of Geographic Variations of Origanum vulgare Trichomes and Essential Oil Content in Greece. Biochem. Syst. Ecol. 1994, 22(5), 517–528. https://doi.org/10.1016/0305-1978(94)90046-9.
37. Mączka, W.; Twardawska, M.; Grabarczyk, M.; Wińska, K. Carvacrol—A Natural Phenolic Compound with Antimicrobial Properties. Antibiotics 2023, 12, 824. https://doi.org/10.3390/antibiotics12050824
38. Maniki, E.; Kostoglou, D.; Paterakis, N.; Nikolaou, A.; Kourkoutas, Y.; Papachristoforou, A.; Giaouris, E. Chemical Composition, Antioxidant, and Antibiofilm Properties of Essential Oil from Thymus capitatus Plants Organically Cultured on the Greek Island of Lemnos. Molecules 2023, 28, 1154. https://doi.org/10.3390/molecules28031154.
39. Salgueiro, L.; Pinto, E.; Gonçalves, M.J.; Pina-Vaz, C.; Cavaleiro, C.; Rodrigues, A.G.; Palmeira, A.; Tavares, C.; Costa-de-Oliveira, S.; Martinez-de-Oliveira, J. Chemical Composition and Antifungal Activity of the Essential Oil of Thymbra capitata. Planta Med. 2004, 70, 572–575. https://doi.org/10.1055/s-2004-827162.
40. Salihila, J.; Nuro, A. Chemical Characterisation of Essential Oil for Satureja montana Population from Burreli Area, Albania. Madridge J. Anal. Sci. Instrum. 2018, 4(1), 88–91.

 doi:10.20944/preprints202507.1209.v1

41. Marchese, A.; Orhan, I.E.; Daglia, M.; Barbieri, R.; Di Lorenzo, A.; Nabavi, S.F.; Gortzi, O.; Izadi, M.; Nabavi, S.M. Antibacterial and Antifungal Activities of Thymol: A Brief Review of the Literature. Food Chem. 2016, 210, 402–414. https://doi.org/10.1016/j.foodchem.2016.04.111
42. Krause, S.T.; Liao, P.; Crocoll, C.; Boachon, B.; Förster, C.; Leidecker, F.; Wiese, N.; Zhao, D.; Wood, J.C.; Buell, C.R.; Gershenzon, J.; Dudareva, N.; Degenhardt, J. The Biosynthesis of Thymol, Carvacrol, and Thymohydroquinone in Lamiaceae Proceeds via Cytochrome P450s and a Short-Chain Dehydrogenase. Proc. Natl. Acad. Sci. USA 2021, 118, e2110092118. https://doi.org/10.1073/pnas.2110092118
43. Yanishlieva, N.V.; Marinova, E.M.; Gordon, M.H.; Raneva, V.G. Antioxidant Activity and Mechanism of Action of Thymol and Carvacrol in Two Lipid Systems. Food Chem. 1999, 64, 59–66. https://doi.org/10.1016/S0308-8146(98)00086-7
44. Mastelić, J.; Jerković, I.; Blažević, I.; Poljak-Blaži, M.; Borović, S.; Ivancić-Baće, I.; Smrečki, V.; Zarković, N.; Brčić-Kostić, K.; Vikić-Topić, D.; Müller, N. Comparative Study on the Antioxidant and Biological Activities of Carvacrol, Thymol, and Eugenol Derivatives. J. Agric. Food Chem. 2008, 56(11), 3989–3996. https://doi.org/10.1021/jf073272v
45. Olszowy, M.; Dawidowicz, A.L. Essential Oils as Antioxidants: Their Evaluation by DPPH, ABTS, FRAP, CUPRAC, and β-Carotene Bleaching Methods. Monatsh. Chem. 2016, 147, 2083–2091. https://doi.org/10.1007/s00706-016-1837-0
46. Ebhohimen, I.E.; Okolie, N.P.; Okpeku, M.; Unweator, M.; Adeleke, V.T.; Edemhanria, L. Evaluation of the Antioxidant Properties of Carvacrol as a Prospective Replacement for Crude Essential Oils and Synthetic Antioxidants in Food Storage. Molecules 2023, 28, 1315. https://doi.org/10.3390/molecules28031315
47. M100 Ed34 | Performance Standards for Antimicrobial Susceptibility Testing, 34th Edition. Clinical & Laboratory Standards Institute.
48. Ultee, A.; Kets, E.P.W.; Smid, E.J. Mechanisms of Antimicrobial Action of Thymol and Carvacrol in Chemicals? J. Antimicrob. Chemother. 2004, 54, 386–390. https://doi.org/10.1093/jac/dki
49. Gavaric, N.; Smole Mozina, S.; Kladar, N.V.; Bozin, B. Chemical Profile, Antioxidant and Antibacterial Activity of Thyme and Oregano Essential Oils, Thymol and Carvacrol and Their Possible Synergism. J. Essent. Oil Bear. Plants 2015, 18(4), 1013–1021. https://doi.org/10.1080/0972060X.2014.971069
50. Soulaimani, B. Comprehensive Review of the Combined Antimicrobial Activity of Essential Oil Mixtures and Synergism with Conventional Antimicrobials. Nat. Prod. Commun. 2025, 20(3), 1–22. https://doi.org/10.1177/1934578X251328241
51. Santos, M.I.S.; Marques, C.; Mota, J.; Pedroso, L.; Lima, A. Applications of Essential Oils as Antibacterial Agents in Minimally Processed Fruits and Vegetables—A Review. Microorganisms 2022, 10, 760. https://doi.org/10.3390/microorganisms10040760
52. Holley, R.A.; Patel, D. Improvement in Shelf-Life and Safety of Perishable Foods by Plant Essential Oils and Smoke Antimicrobials. Food Microbiol. 2005, 22, 273–292. https://doi.org/10.1016/j.fm.2004.08.006
53. Kabir, M.N.; Aras, S.; Allison, A.; Adhikari, J.; Chowdhury, S.; Fouladkhah, A. Interactions of Carvacrol, Caprylic Acid, Habituation, and Mild Heat for Pressure-Based Inactivation of O157 and Non-O157 Serogroups of Shiga Toxin-Producing Escherichia coli in Acidic Environment. Microorganisms 2019, 7, 145. https://doi.org/10.3390/microorganisms7050145
54. Zaharioudakis, K.; Salmas, C.E.; Andritsos, N.D.; Kollia, E.; Leontiou, A.; Karabagias, V.K.; Karydis-Messinis, A.; Moschovas, D.; Zafeiropoulos, N.E.; Avgeropoulos, A.; Proestos, C.; Giannakas, A.E. Carvacrol, Citral, Eugenol and Cinnamaldehyde Casein-Based Edible Nanoemulsions as Novel Sustainable Active Coatings for Fresh Pork Tenderloin Meat Preservation. Front. Food Sci. Technol. 2024, 4, 1400224. https://doi.org/10.3389/frfst.2024.1400224
55. de Souza, R.L.; Dantas, A.G.B.; de Oliveira Melo, C.; Felício, I.M.; Oliveira, E.E. Nanotechnology as a Tool to Improve the Biological Activity of Carvacrol: A Review. J. Drug Deliv. Sci. Technol. 2022, 76, 103834. https://doi.org/10.1016/j.jddst.2022.103834
56. McClements, D.J.; Rao, J. Food-Grade Nanoemulsions: Formulation, Fabrication, Properties, Performance, Biological Fate, and Potential Toxicity. Crit. Rev. Food Sci. Nutr. 2011, 51, 285–330. https://doi.org/10.1080/10408398.2011.559558.

 doi:10.20944/preprints202507.1209.v1

57. Zhao, Y.; Wang, C.; Chow, A.H.; Ren, K.; Gong, T.; Zhang, Z.; Zheng, Y. Self-Nanoemulsifying Drug Delivery System (SNEDDS) for Oral Delivery of Zedoary Essential Oil: Formulation and Bioavailability Studies. Int. J. Pharm. 2010, 383, 170–177. https://doi.org/10.1016/j.ijpharm.2009.08.035
58. Tan, T.B.; Yussof, N.S.; Abas, F.; Mirhosseini, H.; Nehdi, I.A.; Tan, C.P. Stability Evaluation of Lutein Nanodispersions Prepared via Solvent Displacement Method: The Effect of Emulsifiers with Different Stabilizing Mechanisms. Food Chem. 2016, 205, 155–162. https://doi.org/10.1016/j.foodchem.2016.03.008
59. Tibaldi, G.; Fontana, E.; Nicola, S. Growing Conditions and Postharvest Management Can Affect the Essential Oil of Origanum vulgare L. ssp. hirtum (Link) Ietswaart. Ind. Crops Prod. 2011, 34(3), 1516–1522. https://doi.org/10.1016/j.indcrop.2011.05.008.
60. Dodoš, T.; Novaković, J.; Vujisić, L.; Marin, P.D.; Rajčević, N. Geographic Variability of Winter Savory Essential Oil. *Ind. Crops Prod.* **2024**, *210*, 118167. https://doi.org/10.1016/j.indcrop.2024.118167
61. Council of Europe. European Pharmacopoeia, 10th ed.; Council of Europe: Strasbourg, France, 2020; Method 2.8.12—Essential Oils.
62. Van den Dool, H.; Kratz, P.D. A Generalization of the Retention Index System Including Linear Temperature Programmed Gas–Liquid Partition Chromatography. J. Chromatogr. 1963, 11, 463–471.
63. Massada, Y. Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry; John Wiley & Sons: New York, NY, USA, 1976.
64. Adams, R.P. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th ed.; Allured Publishing Corporation: Carol Stream, IL, USA, 2007.
65. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT—Food Sci. Technol. 1995, 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.
66. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. Free Radic. Biol. Med. 1999, 26, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.
67. Puškárová, A.; Bučková, M.; Kraková, L.; Pangallo, D.; Kozics, K. The Antibacterial and Antifungal Activity of Six Essential Oils and Their Cyto/Genotoxicity to Human HEL 12469 Cells. Sci. Rep. 2017, 7, 8211. https://doi.org/10.1038/s41598-017-08524-2.
68. Bodea, I.M.; Cătunescu, G.M.; Palop, A.; Fernandez, P.S.; Garre, A. Washing Solutions Containing Nanoemulsified Essential Oils as a Potential Substitute of Commercial Washes for the Decontamination of Escherichia coli O157:H7 on Cherry Tomatoes. LWT 2023, 190, 115549. https://doi.org/10.1016/j.lwt.2023.115549.
69. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of polyoxyethylene (20) sorbitan monooleate as a feed additive for all animal species. EFSA Journal 2016; 14(3):4443, 3 pp. doi:10.2903/j.efsa.2016.4443.