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Evaluation of Chemical Composition and Biological Activity of Essential Oil of *Origanum vulgare* L. ssp. Glandulosum

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Abstract

The present work focuses on *Origanum vulgare* L. ssp. glandulosum (Desf.), an aromatic and medicinal plant endemic to Algeria and Tunisia. The essential oil was extracted and analyzed by GC and GC/MS, revealing 43 compounds representing 98.55% of the oil, with para-cymene (25.61%), thymol (23.13%), and carvacrol (20.32%) as the main constituents. Antibacterial activity was evaluated through disk diffusion and broth microdilution assays, showing strong inhibitory effects against tested strains. Antioxidant activity, assessed using the DPPH radical scavenging method, showed a moderate potential with an IC_{50} of 461.62 μg/mL, confirming the biological value of this species.

Keywords: *Origanum vulgare* L.; antibacterial activity; antioxidant activity

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1. Introduction

Origanum vulgare L., commonly known as oregano, is an aromatic and medicinal plant belonging to the Lamiaceae family and widely distributed across Mediterranean regions. It includes several subspecies that differ in their chemical composition and biological properties. Among them, *Origanum vulgare* ssp. glandulosum is particularly valued for the richness and diversity of its essential oil. This oil is characterized by high levels of phenolic and monoterpenic compounds, mainly p-cymene, thymol, and carvacrol, which have been consistently reported as dominant constituents in different studies [1,2].

Oregano essential oils are well known for their strong antimicrobial activity. In the case of *O. vulgare* ssp. glandulosum, they have shown remarkable effectiveness against various pathogenic bacteria, including Bacillus species, with inhibition zones ranging from 21.5 to 41 mm and a minimum inhibitory concentration (MIC) of 0.4 mg/mL [1]. The proposed antimicrobial mechanisms involve enzyme inhibition, disruption of efflux pumps, and cytoplasmic membrane damage, ultimately compromising bacterial viability (Soltani et al.) [3].

In addition to their antimicrobial potential, both the essential oils and hydromethanolic extracts of *Origanum vulgare* L. ssp. *glandulosum (Desf.)* exhibit significant antioxidant activity. DPPH radical scavenging assays have demonstrated their ability to neutralize free radicals, thereby confirming their therapeutic relevance by Krimat et al. and-Morshedloo et al. [2,4]. The combination of antimicrobial and antioxidant properties highlights the importance of this plant in traditional medicine and supports its potential applications in pharmaceutical, food, and cosmetic industries.

2. Materials and Methods

Extraction of Essential Oils

In June 2012, flowering *Origanum vulgare* L. ssp. *glandulosum (Desf.)*. plants were collected from Nechmaya (Guelma, northeastern Algeria). The dried aerial parts were used for essential oil extraction by hydrodistillation (Clevenger apparatus, 2 h). The obtained oil was dried over anhydrous sodium sulfate, stored at 4 °C, and its yield was calculated relative to the dry weight of the plant material.

GC/MS

The analyses of the volatile constituents were run on a Hewlett-Packard GC-MS system: Gas Chromatograph Model 7890 coupled to a 5975 Mass Selective Detector; and equipped with a DB5 MS column (20 m × 0.18 mm, 0.18 µm, Agilent Technologies, USA). The injector and detector were operated at 280 and 300 °C, respectively. Oven temperature was programmed (50 °C for 3.2 min, then 50 to 300 °C at 8 °C/min and subsequently, held isothermally for 5 min). As a carrier gas, Helium with a flow rate of 1.0 mL/minute was used.

1 µL of the essential oil diluted in hexane (1/30) was injected in split mode with a ratio of 1:250. The mass spectrometer operated in electron impact mode at 70 eV; and an ionization energy of 1800 V and an ion source temperature, 230 °C. Mass spectra were recorded in scan mode over an *m*/*z* range of 33–550.

The identification of components was based on comparison of retention time of each component (Rt) and their mass spectra with those of Wiley 275 mass spectra and NIST (National Institute of Standards and Technology) libraries and those described by Adams30.

Also, a homemade MS library with the spectra corresponding to pure substances and components of known essential oils was used.

Antimicrobial activity

Microbial strains

The essential oil was tested against three laboratory reference strains obtained from the American Type Culture Collection (ATCC): *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 29212.

Disc diffusion method

Antibacterial activity was assessed using the disc diffusion method. Bacterial suspensions (0.5 McFarland), prepared from young colonies, were inoculated onto the culture medium. Sterile paper discs (6 mm, Whatman) impregnated with 10 µL of essential oil were placed on the agar. The Petri dishes, sealed with parafilm, were kept at 4 °C for 2 h and then incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured (mm) and expressed as mean ± standard deviation. All tests were performed in triplicate [5].

Determination of Minimum Inhibitory Concentrations (MIC)

The minimum inhibitory concentration (MIC) of the essential oil was determined against the sensitive bacterial strains. Inocula, prepared from 12 h broth cultures and adjusted to 0.5 McFarland, were inoculated into 96-well plates containing nutrient broth. The essential oil, dissolved in 10% DMSO, was tested over a concentration range of 0.048 to 50 mg/mL by two-fold serial dilutions. After incubation at 37 °C for 18–24 h, bacterial growth was assessed visually and confirmed by the addition of nitroblue tetrazolium (NBT) [5,6].

DPPH Radical Scavenging Capacity Test

The DPPH radical scavenging capacity of essential oils was evaluated according to Takao et al. [7]. with calculation of IC_{50} and the antioxidant activity index (AAI) (Scherer and Godoy, 2009). Stock solutions (2000 μg/mL in methanol) were diluted (1000–7.81 μg/mL). Each dilution (2 mL) was mixed with 2 mL of DPPH solution (80 μg/mL), incubated for 30 min in the dark, and the absorbance was measured at 517 nm. Methanol + DPPH served as the negative control, and ascorbic acid as the positive control [8].

$$\text{Antioxidant activity (\%)} = 100 \times [(\text{A control} - \text{A sample})/\text{A control}]$$

IC_{50}: concentration required to reduce 50% of the DPPH radical (calculated from the inhibition percentage vs. concentration curve).

$$\text{AAI} = [\text{Final DPPH concentration } (\mu g/mL)]/[IC_{50}\ (\mu g/mL)]$$

3. Results and Discussions

The studied essential oil contains 43 identified compounds representing 98.54% of the total, as detailed in Table 1. The composition dominated by p-cymene (25.6%), γ-terpinene (16.6%), thymol (23.1%), and carvacrol (20.3%), which together account for nearly 86% of the composition. This profile, characteristic of the thymol/carvacrol chemotype, confers strong antimicrobial, antifungal, and antioxidant activities, enhanced by the synergy between phenols and their monoterpene precursors.

Table 1. Chemical composition of Origanum vulgare L. ssp. glandulosum (Desf.) essential oil.

	RT	Compounds	% FID	KI
1	5,78	Alpha-Thujene	0.895	924
2	5,92	Alpha-Pinene	0.716	932
3	6,25	Camphene	0.12	946
4	6,71	Sabinene	0.018	696
5	6,8	Beta-Pinene	0.154	974
6	6,87	3-Octen-ol	0.52	974
7	6,97	3-Octanone	0.161	979
8	7,04	Myrcene	1.494	988
9	7,35	Alpha-Phellandrene	0.188	1002
10	7,38	Delta-3-Carene	0.072	1008
11	7,55	Alpha-Terpinene	1.787	1014
12	7,72	P-Cymene	25.615	1020
13	7,77	Limonene	0.403	1024
14	7,8	Beta-Phéllandrene	0.207	1025
15	7,83	Eucalyptol	0.05	1031
16	7,89	(Z)-Beta-Ocimene	0.074	1032
17	8,08	(E)-Beta-Ocimene	0.06	1044
18	8,31	Gamma-Terpinene	16.612	1054
19	8,5	Cis-Hydrate de Sabinene	0.197	1065
20	8,74	Terpinolene	0.065	1086
21	8,83	Para-Cymenene	0.066	1089
22	8,99	Linalol	0.87	1095
23	9,05	Trans- Sabinene Hydrate	0.12	1098
24	9,78	Camphor	0.012	1141
25	10,17	Borneol	0.214	1165
26	10,3	Terpinen-4-ol	0.311	1174
27	10,39	Para-Cymen-8-ol	0.216	1179
28	10,53	Alpha-Terpineol	0.386	1186
29	10,99	Thymol methyl-Ether	0.051	1232
30	11,12	Carvacrol methyl ether	0.18	1241

31	11,71	Thymol Isomer MW 150	0.163	1258
32	11,87	Thymol	23.129	1289
33	11,99	Carvacrol	20.321	1298
34	13,58	Beta-Caryophyllene	0.848	1417
35	13,75	Thymohydroquinone	0.487	1553
36	14,02	Alpha-Humulene	0.047	1452
37	14,62	Beta-Bisabolene	0.283	1505
38	14,7	Gamma-Cadinene	0.018	1513
39	14,75	Delta-Cadinene	0.025	1522
40	14,81	Beta-Sesquiphellandrene	0.539	1521
41	14,99	(E)-Alpha-Bisabolene	0.422	1529
42	15,46	Spathulenol	0.02	1577
43	15,54	Caryophyllene Oxide	0.41	1582
	Total		98.546	

Research on Origanum essential oils reveals significant chemical variability across species and populations. Nabti et al. demonstrated that Algerian *Origanum vulgare* L. ssp. *glandulosum (Desf.)* contains highly variable concentrations of thymol (15.2–56.4%), carvacrol (2.8–59.6%), γ-terpinene (9.9–21.8%), and p-cymene (8.5–13.9%) depending on the population studied [9]. The essential oils showed potent antibacterial activity against multidrug-resistant uropathogenic *E. coli* strains. Zinno et al. characterized *Origanum vulgare* L. ssp. *glandulosum (Desf.)* genotypes belonging to carvacrol and thymol chemotypes, finding that the carvacrol genotype had a less complex chemical profile with higher levels of the most active compound [10]. Sharifi-Rad et al. noted that major Origanum essential oil components include terpenes, phenols, and flavonoids, with predominant carvacrol and thymol occurrence [11]. Napoli et al. confirmed thymol-type biotypes in Sicilian oregano, with thymol, γ-terpinene, and p-cymene as main components, showing significant compositional variations across harvest dates and years [12].

The tested essential oil exhibited strong broad-spectrum antibacterial activity, with large inhibition zones against *E. coli*, *S. aureus*, and *E. faecalis*, and low MIC values (0.78–1.56 mg/mL), as presented in Table 2, indicating high efficacy at very low concentrations. This activity is mainly attributed to phenolic compounds, particularly thymol and carvacrol, which are known to disrupt membrane integrity and inhibit biofilm formation. Comparable findings have been reported for *Origanum vulgare* L. ssp. *glandulosum (Desf.)* essential oils, which demonstrated significant activity against both multidrug-resistant and reference strains of *E. coli* and *S. aureus* (Nabti et al.; Bouaouina et al.; Atanasova-Stamova et al.) [12–14]. Notably, the nanoencapsulation of *Origanum vulgare* L. ssp. *glandulosum (Desf.)* oil, despite reducing the levels of major volatiles, preserved its antibacterial and antibiofilm properties [12]. Overall, essential oils rich in thymol and carvacrol show great promise as natural antimicrobial agents effective against a wide range of bacteria, including multidrug-resistant strains, and may serve as potential alternatives to conventional antibiotics.

Table 2. Antimicrobial activities of *Origanum vulgare* L. ssp. *glandulosum (Desf.)* essential oil.

Test Microorganisms	IZ	MIC
Escherichia coli ATCC 25922	49.66 ± 1.25	0.78
Staphylococcus aureus ATCC 25923	51.83 ± 2.56	1.56
Enterococcus faecalis ATCC 29212	51.83 ± 2.56	0.78

IZ = Inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 5 µL of essential oil. MIC = Minimal inhibitory concentrations as (mg/mL).

The studied essential oil exhibited moderate antioxidant activity with an IC_{50} value of 461.62 μg/mL, much higher than that of ascorbic acid (25.59 μg/mL), as shown in Table 3, indicating a relatively weak radical scavenging capacity. This activity is mainly attributed to phenolic monoterpenes (thymol, carvacrol), while the predominance of non-phenolic constituents explains the limited efficacy observed. These findings are consistent with previous reports showing that the antioxidant potential of Origanum essential oils strongly depends on their phenolic content: Quintana Quispe et al. reported high antioxidant activity in Peruvian oregano, Santos et al. confirmed the effectiveness of Brazilian *Origanum vulgare* L. ssp. *glandulosum (Desf.)* using the ABTS assay, and Guo et al. (2021) demonstrated that carvacrol-rich oils exhibited radical scavenging activity comparable to synthetic BHT [15–17]. Moreover, Ridaoui et al. (2024) highlighted the major contribution of isolated carvacrol to antioxidant activity. Despite its moderate efficacy, the tested oil retains significant biological interest, particularly due to the possible synergy between its antioxidant and antimicrobial properties [18].

In Table 3, the essential oil of Origanum vulgare ssp. glandulosum showed moderate antioxidant activity (IC_{50} = 461.62 μg/mL), much lower than that of vitamin C (25.59 μg/mL). This limited efficiency may be due to the high proportion of p-cymene, a weakly active compound, despite the presence of major phenolic constituents such as thymol and carvacrol.

Table 3. Antioxidant activities of *Origanum vulgare* L. ssp. *glandulosum (Desf.)*. essential oil.

Sample	IC50
Essential oil	461.62
Vitamine C	25.59

In comparison, other chemotypes rich in thymol or carvacrol exhibited much lower IC_{50} values, such as 106.6 μg/mL Gruľová et al. or 1.8 ± 0.8 mg/mL Laothaweerungsawat et al. [19,20]. Antioxidant activity also varies with environmental factors, particularly altitude Goyal et al. Moreover, subspecies such as *O. vulgare* subsp. hirtum and *O. vulgare* subsp. vulgare showed similar antioxidant potential despite their different chemical compositions, suggesting a synergistic effect among constituents Kosakowska et al. [22]. Overall, the studied essential oil exhibits moderate antioxidant potential, which depends on chemical composition and interactions between its components.

4. Conclusions

The essential oil of *Origanum vulgare* L. ssp. *glandulosum (Desf.)* was found to be rich in phenolic compounds, mainly thymol and carvacrol, supported by their monoterpene precursors such as p-cymene and γ-terpinene. This chemical profile confers a strong broad-spectrum antibacterial activity, demonstrated by large inhibition zones and low MIC values, as well as a moderate antioxidant activity, attributable to its phenolic constituents. Although less potent than ascorbic acid in radical scavenging, the oil retains significant biological value due to the synergy between its antimicrobial and antioxidant properties. These findings confirm the importance of this endemic plant as a natural source of bioactive agents and highlight its potential applications in the pharmaceutical, food, and cosmetic industries as a sustainable alternative to synthetic molecules.

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