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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF THE ESSENTIAL OILS OF *Teucrium orientale* var. *orientale* AND *Teucrium orientale* var. *puberulens*

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Finding healing power in plants is a traditional and ancient concept. However, since the advent of potent synthetic antibiotics in the 1950s, the use of plant derivatives as antimicrobials has diminished. In recent years the essential oils and extracts of many plant species have become popular, and attempts to characterize their bioactive principles have gained momentum in many pharmaceutical and food-processing applications [1].

The preservative effect of many plant species and herbs suggests the presence of antioxidative and antimicrobial constituents. A number of phenolic compounds with strong antioxidant and antimicrobial activities have been identified in these plants, especially those belonging to the Lamiaceae family, and are of interest to food manufacturers as consumers move towards functional foods with specific health effects [2]. The essential oils produced by different plant species are much more acceptable to the end consumers than synthetic substances, and they do not cause bacterial resistance, mainly because they are present in a wide spectrum of compounds [3].

The genus *Teucrium*, which belongs to the family Lamiaceae, includes more than 300 species widespread all around the world. There are 27 species (39 taxa) in the flora of Turkey, eight of which are endemic [4]. The reported oils were mainly represented by β-caryophyllene and germacrene D [5–7] and by caryophyllene oxide and α-pinene [7]. *Teucrium* species are bitter, astringent, and antirheumatic herbs that reduce inflammation and stimulate the digestion, and they have been used as herbal medicines for coughs and asthma since ancient times [2]. In Lebanon, an infusion of the flowers of *Teucrium orientale* is used in folk medicine as hypoglycemic, vermifuge, and antipyretic, and to treat stomach and intestinal problems [8]. *Teucrium* species are used in Yemeni folk medicine as antispasmodic and insect repellent [9]. In Sardinia, in Baronia of Siniscola, it has been used in the past to cure malaria [10]. *Teucrium* species have been used as a stimulant, tonic, diaphoretic, and appetizer, and against stomach pains and diabetes in Turkish folk medicine [11]. *T. orientale* L., named "Kirve otu" in Anatolia, is widespread in the dry and stony places of Turkey [4, 11].

The purpose of this work is to investigate the chemical composition and antimicrobial activity of the essential oils from the two *Teucrium* species collected in Turkey: *T. orientale* var. *orientale* and *T. orientale* var. *puberulens*.

Chemical Composition of the Essential Oils. Water-distillation of dried aerial parts of *Teucrium orientale* var. *orientale* yielded 0.04% (v/w) of a pale yellow oil, while that of *Teucrium orientale* var. *puberulens* yielded 0.03% (v/w) of a light yellowish oil. About 60 constituents (95.8% of the total oil) and 42 constituents (89.4% of the total oil) were identified by means of GC-MS analysis of the essential oils from *T. orientale* var. *orientale*, and *T. orientale* var. *puberulens* (Table 1). The major components of *T. orientale* var. *orientale* and *T. orientale* var. *puberulens* were β-caryophyllene (15.3–19.0%), germacrene D (14.2–12.8%), and caryophyllene oxide (14.0–19.0%).

The abundance of β-caryophyllene (19%) and germacrene D (12.8%) in the essential oil of *T. orientale* var. *puberulens* was similar to that of a previous report [12], with slight changes in concentrations. However, *T. orientale* var. *puberulens* oil from different localities (Siran-Gumushane) in Turkey was characterized by a high content of 2-methylcumarone (20.0%) [12].

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TABLE 1. Composition of the *T. orientale* var. *orientale* and *T. orientale* var. *puberulens* Essential Oils, %

Compound	RRI	A, %	B, %	Compound	RRI	A, %	B, %
1-Octen-3-ol	1515	0.3	0.2	*E*-β-Damascenone	1833	0.2	0.3
E,Z-2,4-Heptadienal	1534	0.8	N.d	*cis*-Calamene	1842	0.1	N.d
Cyclosativene	1548	0.2	N.d	Geranyl acetone	1853	0.4	0.2
α-Copaene	1558	1.3	0.6	α-Calacorene	1904	0.5	0.2
E,E-2,4-Heptadienal	1562	0.1	0.1	Cubebol	1918	0.4	0.5
α-Bourbonene	1578	0.1	0.2	γ-Calacorene	1934	0.2	N.d
β-Bourbonene	1584	2.7	3.5	Isocaryophyllene oxide	1947	0.7	1.1
Benzaldehyde	1592	0.1	N.d	Caryophyllene oxide	1955	14.0	19.0
β-Cububene	1600	0.1	0.2	Salvial-4(14)-en-1-one	1971	0.3	N.d
β-Terpineol	1603	N.d	0.9	Norbourbonanone	1975	0.3	0.4
Linalool	1604	0.9	N.d	*E*-Nerolidol	1979	0.2	N.d
Longifolene	1633	N.d	0.5	Ledol	1982	0.4	N.d
trans-α-Bergamotene	1642	0.1	N.d	Humulene epoxide II	1992	1.6	1.9
β-Cubebene	1650	0.4	0.7	Cubenol	2004	0.5	N.d
β-Caryophyllene	1657	15.3	19.0	Globulol	2010	0.3	N.d
δ-Muurolene	1692	0.2	N.d	Hexahydrofarnesyl acetone	2037	0.6	0.8
allo-Aromadendrene	1695	0.5	0.4	Spathulenol	2042	6.4	5.3
Pulegone	1697	0.6	0.7	Cedrol	2055	0.6	0.4
trans-β-Farnesene	1706	1.3	1.2	Nonanoic acid	2060	0.3	0.2
α-Humulene	1716	2.4	2.8	*nor*-Copaanone	2064	0.4	N.d
γ-Muurolene	1730	0.5	0.3	τ-Cadinol	2071	0.6	0.5
α-Terpineol	1734	0.3	N.d	τ-Muurolol	2081	0.7	0.5
Germacrene D	1749	14.2	12.8	δ-Cadinol	2087	0.7	N.d
α-Muurolene	1754	0.1	N.d	Carvacrol	2094	0.2	0.2
Z,E-α-Farnesene	1757	N.d	0.8	α-Cadinol	2109	0.9	0.7
β-Bisabolene	1758	2.2	N.d	Decanoic acid	2125	0.6	0.4
Bicyclogermacrene	1767	3.6	2.9	Caryophyllenol II	2207	N.d	2.1
δ-Cadinene	1783	2.1	0.9	Dodecanoic acid	2268	0.6	0.8
γ-Cadinene	1786	0.3	0.1	Phytol	2401	0.5	1.0
β-Sesquiphellandrene	1792	0.1	N.d	Tetradecanoic acid	2480	0.8	N.d
Methyl acetylsalicylate	1802	Tr.	N.d	Hexadecanoic acid	2761	3.1	4.0
Cadina-1,4-diene	1804	0.1	N.d	Total		95.8	89.4
(*E,E*)-2,4-Decadienal	1824	0.1	0.1				

A: *T. orientale* var. *orientale* essential oil; B: *T. orientale* var. *puberulens* essential oil.
RRI: relative retention indices; Tr.: trace (< 0.1%). N.d: not detected.

In addition, caryophyllene oxide (19.0%) and spathulenol (5.3%), which appeared as major constituents in our study, were absent in the previous studies from Turkey. In 1990, the oils of six *Teucrium* species from the Iberian peninsula and the Balearic Islands were characterized by high contents of aristolene, β-caryophyllene, α-humulene, alloaromadendrene, caryophyllene epoxide, and spathulenol [13]. Caryophyllene oxide, linalool, and β-caryophyllene were also identified as major compounds in the oil of *T. orientale* L: spp. *orientale* collected from Fars Province, Iran [14]. We have already reported β-caryophyllene (15.3–19.0%), germacrene D (14.2–12.8%), and caryophyllene oxide (14.0–19.0%) as the main compounds in both *T. orientale* var. *orientale* and *T. orientale* var. *puberulens*. As a result of this finding, the chemical composition of *T. orientale* var. *orientale* and *T. orientale* var. *puberulens* essential oils is compatible with the previous findings.

Antimicrobial Activity. The *in vitro* antimicrobial tests of the essential oils from the two *Teucrium* species in question resulted in a range of growth inhibition patterns against pathogenic microorganisms (Table 2). The results of the antimicrobial assays indicated that *Enterococcus faecalis* and *Staphylococcus aureus* were inhibited by the oil of *T. orientale* var. *orientale* and *T. orientale* var. *puberulens* moderately with a MIC value of 100 and 50 μg/mL, respectively. Both *Teucrium* oils were also found to possess anticandidal activity against *Candida albicans*, with MIC values of 50 and 25 μg/mL, and against *Candida tropicalis*, with MIC values of 25 and 12.5 μg/mL, respectively.

In conclusion, *T. orientale* var. *orientale* and *T. orientale* var. *puberulens* essential oils possess significant anticandidal activity but moderate antibacterial activity.

TABLE 2. Antimicrobial Activity of *Teucrium* Species Essential Oils, MIC in μg/mL

Microorganisms	Source	*T. orientale* var. *orientale*	*T. orientale* var. *puberulens*	Standard agent
Enterococcus faecalis	ATCC 29212	100	50	0.78[a]
Staphylococcus aureus	ATCC 29213	100	50	0.39[a]
Escherichia coli	ATCC 25922	400	400	3.12[a]
Pseudomonas aeruginosa	ATCC 27853	400	400	>75[a]
Candida albicans	Ege Univ.(TR)	50	25	12.5[b]
Candida tropicalis	Ege Univ.(TR)	25	12.5	12.5[b]

[a]Ampicilin; [b]flucanozole.

Plant Material. The aerial parts (leaves and flowers) of *T. orientale* var. *orientale* (voucher No. BY 16804) and *T. orientale* var. *puberulens* (voucher No. BY 16805) were collected from Erzurum Province (Turkey), between Erzurum and Ispir from 1500 m altitude in 27.06.2008. The voucher specimens of these plants have been deposited at the Herbarium of Balikesir University in Balikesir, Turkey.

Extraction of the Essential Oil. Air-dried parts of the plants were submitted for 3 h to water distillation using a Clevenger apparatus to produce the essential oils in a yield of 0.04% and 0.03% (v/w) based on the dry weight of the samples from *T. orientale* var. *orientale* and *T. orientale* var. *puberulens*, respectively. The oils were dried over anhydrous sodium sulfate and, after filtration, stored at +4°C until tested and analyzed.

Antimicrobial Screening. Antimicrobial activities of the the essential oils were determined by the agar dilution procedure outlined by the Clinical and Laboratory Standards Institute [15, 16]. Minimal inhibitory concentrations for each compound were investigated against the standard bacterial strains *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 and the yeasts *Candida albicans* and *Candida tropicalis* obtained from American Type Culture Collection (Rockville, MD.) and the Department of Microbiology, Faculty of Medicine, Ege University (Turkey). Bacterial strains were subcultured on Muller Hinton Broth (HiMedia Laboratories Pvt. Ltd. Mumbai, India), and yeasts strains on RPMI 1640 broth (Sigma-Aldrich Chemie GmbH Taufkirchen, Germany). Their turbidities matched that of McFarland No. 0.5 turbidity standard [17]. The stock solution of the essential oils was prepared in dimethyl sulfoxide (DMSO), which had no effect on the microorganisms in the concentrations studied. All of the dilutions were done with distilled water. The concentrations of the tested compounds were 800, 400, 200, 100, 50, 25, 12.5, and 6.25 μg/mL. Ampicilin (FAKO, Istanbul, Turkey) and fluconazole (FAKO, Istanbul, Turkey) were used as standard antimicrobial agents. A loopful (0.01 mL) of the standardized inoculum of the bacteria and yeasts (10^6 CFUs/mL) was spread over the surface of the agar plates. All the inoculated plates were incubated at 35°C, and the results were evaluated after 16–20 h of incubation for bacteria and 48 h for yeasts. The lowest concentration of the compounds that prevented visible growth was considered to be the minimal inhibitory concentration (MIC).

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