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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *Ocimum minimum* ESSENTIAL OILS

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The taxonomy of the genus is complex due to hybridization within the genus and large morphological variation. In early records, *Ocimum minimum* is classified as varieties of *O. bacilicum* (*Ocimum basilicum* var. *minimum*) [1].

The essential oil extracted from *Ocimum* (Basil) species is one of the most widespread oils and is used as an aromatic agent in foods and beverages and as fragrances in pharmaceutical and industrial products.

The essential oil from plants has been known to possess biological activity, such as antioxidant, antibacterial, and antifungal.

This popular herb is used as both a fresh and a dried food spice and in traditional medicine. In addition, basil is valued for its pharmaceutical properties, for example, the aromatic oils produced in their leaves [2]. Spices used in different types of food to improve flavour, since ancient times, are well known for their antioxidant properties. It was reported that extracts obtained from spices had antioxidant activities [3]. However, there is no information on 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity, superoxide anion radical scavenging activity, total antioxidant activity using the ferric thiocyanate method, and the reducing power of essential oils. The purpose of this study was to evaluate the chemical composition and antioxidant activity of essential oils.

The composition and relative percentages of the essential oil of two *O. minimum* landraces were elucidated by the aid of GC/MS analysis. The percentage composition of the essential oils is given in Table 1. In total, 47 components were identified in the landraces, accounting for 82.29 and 83.22% in the essential oil of landraces I and II, respectively. Oils of both samples were characterized by high percentages of oxygenated monoterpenes (37.9 and 48.2%, respectively). The main component was linalool in both landraces, although the oils present some differences in the percentages of linalool, with 20.22 and 27.91, respectively. While there is chemical diversity in the essential oil of *Ocimum* species [3], linalool was one of the most common compounds in the oils [4]. The chemical composition of the essential oil of *O. minimum* has been studied. Linalool was the major component in the oil of *O. minimum*, similar to our study. But there are quantitative differences in linalool content between the literature and our studies. According to previous studies [2, 5], linalool varied between 52 and 54% in brush basil essential oil. Contrary to our results, Ozcan and Chalchat [4] stated that geranyl acetate was the major components in the essential oil of *O. minimum* from Turkey. The researcher explained that the differences were due to environmental and genetic factors. 1,8-Cineole was the other important component in both samples (13.38 and 14.65%). 1,8-Cineol is one of the key components used in the classification of the different chemotypes of basil reaching 60% in the literature [4].

The essential oil of *O. minimum* was also characterized by the presence of eugenol (8.37%), germacrene D (3.94–6.04%), γ-cadinene (3.51–5.47%), and δ-cadinene (2.39–4.48%). The components were characterized by previous studies on the essential oil of sweet basil [2, 5, 6].

Antioxidant capacity is widely used as a parameter to characterize food or medicinal plants and their bioactive components. In this study, the antioxidant activity of the essential oils, BHA, BHT, and α-tocopherol has been evaluated in a series of *in vitro* test: 1,1-diphenyl-2-picrylhydrazyl free radical scavenging, ferric thiocyanate method, reducing power, and scavenging of superoxide anion radical-generated non-enzymatic systems.

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TABLE 1. Essential Oil Composition *Ocimum minimum*

Components	Rt	Landraces I (LR-1), %	Landraces II (LR-2), %
Monoterpene hydrocarbons			
β-Pinene	4.85	1.37	1.44
Sabinene	5.24	0.71	0.74
δ-3-Carene	5.93	–	0.93
β-Myrcene	6.50	0.76	0.72
α-Terpinene	6.76	0.16	0.23
γ-Terpinene	8.43	0.32	0.50
cis-β-Ocimene	8.74	2.38	0.93
p-Cymene	9.01	0.27	0.47
α-Terpinolene	9.27	0.39	0.43
Total		6.36	6.39
Oxygenated monoterpenes			
1,8-Cineole	7.65	13.38	14.65
trans-Sabinene hydrate	12.88	0.50	0.65
Camphor	13.72	1.31	0.84
Linalool	14.59	20.22	27.91
α-Terpineole	16.65	2.49	2.50
Geraniol	18.73	–	1.69
Total		37.90	48.24
Sesquiterpene hydrocarbons			
α-Copaene	13.47	0.36	0.28
β-Bourbonene	13.93	0.16	0.09
α-Guaiene	15.18	2.38	1.38
α-Cadinene	15.75	0.24	0.15
trans-β-Farmesene	15.82		0.35
β-Cubebene	16.23	3.23	3.28
trans-β-Caryophyllene	16.49	0.34	–
Germacrene D	16.90	6.04	3.94
Bicyclogermacrene	17.18	0.93	0.47
β-Elemene	17.31	0.16	0.11
δ-Cadinene	17.61	4.48	2.39
Calamenene	18.37	0.33	0.19
trans-β-Ionone	19.90	–	0.08
γ-Cadinene	22.87	5.47	3.51
Total		24.12	16.22
Oxygenated sesquiterpenes			
Nerolidol	21.21	0.50	0.31
δ-Costol	22.08	0.15	0.08
α-Cadinol	22.95	0.16	0.11
Veridiflorol	23.30	0.24	0.19
β-Eudesmol	23.43	0.86	0.66
7-Epi-amiteol	23.68	0.21	0.12
Total		2.12	1.47
Aromatic compounds			
Methyl eugenol	21.69	3.12	2.03
Eugenol	22.79	8.37	8.37
Cavicol	24.65	0.17	0.13
Total		11.66	10.53
Others			
2-Hexenal	7.83		0.08
1-Octanol	14.66		0.14
Hexadecanoic acid	32.87	0.13	0.15

Total antioxidant activity was determinated in the linoleic acid emulsion system by the ferric thiocyanate method [7]. The total antioxidant activity of the essential oil and the reference compounds such BHA, BHT, and α-tocopherol was determined by the ferric thiocyanate method and increased steadily with increasing concentration. The essential oils and standard compounds exhibited effective antioxidant activity. At 50 μg/mL concentration, the effects of essential oil, BHA, and BHT on lipid peroxidation of linoleic acid emulsion expressed as percentage inhibition was 94.8, 97.5, 97.1, and 98.5%, respectively, and greater than that of α-tocopherol (70.4%) at the same concentration.

The percentage inhibition of peroxidation in the linoleic acid system by 25 and 50 μg/mL concentrations of essential oil was found to be 73.8, 94.8, 90.8, and 95.0% respectively. On the other hand, the percentage inhibition of 50 μg/mL concentrations of BHA, BHT, and α-tocopherol was found to be 97.1, 98.5, and 70.4% respectively.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing power of the essential oil and standards (BHT and α-tocopherol) were determined using the potassium ferricyanide reduction method. For measurement of the reductive ability, the Fe^{3+}-Fe^{2+} transformation was investigated in the presence of essential oil using the method of Oyaizu [8]. Like the antioxidant activity, the reducing power of essential oils, BHT, and α-tocopherol increased with increasing concentration. At different concentrations, LR-1 and LR-2 showed higher activities than the control, and these differences were statistically significant ($P<0.05$). The reducing power of essential oil and standard compounds exhibited the following order: BHT > α-tocopherol > essential oils (LR-2>LR-1).

The antioxidant activities of the essential oil and standard antioxidants such as BHT were determined using the DPPH method. DPPH is widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances in food systems [9, 10].

DPPH free radical scavenging is an accepted mechanism by which antioxidants act in inhibiting lipid oxidation; scavenging of DPPH radical was used in this work. The method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. The results of analysis show a significant decrease ($P<0.05$) in the concentration of DPPH radical due to the scavenging ability of essential oils (LR-1 and LR-2), and standard BHT was used as reference radical scavengers. The antioxidant properties of plant extracts should be evaluated in a variety of model systems using different indices to determine the effectiveness of such antioxidant materials. The results obtained in this study clearly showed that both essential oils (LR-1 and LR-2) have powerful antioxidant activity against various antioxidant systems *in vitro*; moreover, these extracts can be used as easily accessible sources of natural antioxidants and as possible food supplements or in pharmaceutical applications. It can also be used in stabilizing food against oxidative deterioration.

Seeds of the brush basil (*O. minimum* L.*)* were collected from two different location on Turkey. One of them is from Denizli (LR-1) and another Gaziantep (LR-2). The plants were grown under identical (same environmental and soil conditions) conditions. Seeds were sown on a medium (1:1:1 washed sand, horse manure, and field soil) in greenhouse conditions on April 5, 2006. Seedlings were grown until the 3–5 leaf stage. The seedlings were transplanted into pilots in the Gaziosmanpasa University Experimental Research Station on June 2, 2006. The plants were harvested at the full blooming stage and dried at 35°C for essential oil distillation. GC–MS analysis was performed using an Agilent system model 6890 with a model 5973 mass selective detector equipped with HP-Innowax fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm). For GC–MS detection, an electron ionization system, with ionization energy of 70 eV, was used. Helium was the carrier gas, at a flow rate of 1.3 mL/min. The oven temperature programming was the same with GC analysis. Injector and MS transfer line temperatures were set at 220°C and 250°C, respectively. Diluted samples (1/100 in chloroform, v/v) of 2.0 μL were injected in the split/splitless (5:1 split) mode. Identification of oil components was accomplished based on comparison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILEY and NIST database/ChemStation data system).

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