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ORIGINAL PAPER

Study on some chemical parameters and essential oil compositions in two *Origanum* species grown in Van Region, Turkey

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Abstract

Primary and secondary metabolites, which are natural products produced by plants, are mainly used, either directly or indirectly, in industries. These are active substances, for example etheric oils (essential oils, essences), alkaloids, tannins, bitter substances, etc., that are predominantly involved in plant metabolism. They increase the body's defence system, support the functions of organs and accelerate healing. Thus, they have positive effects on the functions of certain tissues and organs. In this study, some bioactive and biochemical compounds, dry matter, total antioxidant activity, total flavonoid and total phenolic content, macro-micro and heavy metal content, essential oil yield and content in *Origanum onites* and *Origanum dubium* species were determined. It was found that total ash (10.61-12.03%), dry matter (38.24-43.47%), total antioxidant activity (121.41-162.17 µmol TE g^{-1}), total phenolic content (187.04-192.25 mg GAE g^{-1}), total flavonoid content (10.01-17.57 mg QE 100 g^{-1}), chlorophyll amount (23.26-23.30 dx) and NBI (the Nitrogen Balance Index) (13.90-28.23 dx) were higher in *O. dubium* than in *O. onites*. Yield of essential oil was determined between 3.130 and 3.240 in *O. onites* and *O. dubium* species, respectively. Carvacrol (78.24%), γ-terpinene (6.650%) and cymene (3.950%) components were found to be dominant in the essential oil of *O. onites*. On the other hand, the main components of essential oil obtained from *O. dubium* species were carvacrol (22.01%), γ-terpinene (23.55%), cis-sabinene hydrate (13.90%), terpinen-4-ol (8.500%), α-terpinene (4.94%), cymene (4.94%) and trans-sabinene hydrate (3.35%).

Keywords: aromatic plant, biochemical content, etheric oils, medicinal plant, oregano

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INTRODUCTION

Lamiaceae is the third largest family of plants in Turkey, with 46 genera, 782 taxa comprising 603 species and 179 subspecies and varieties, of which 346 taxa (271 species and 75 subspecies and varieties) are endemic. Based on recent results, the *Origanum* genus in Turkey comprises 27 species and 31 taxa. The endemism rate by species and taxon is 67% and 58%, respectively (Celep, Dirmenci 2017).

Turkey has been considered an important place for plant diversity worldwide, especially considering the diversity of the *Origanum* genus (Taşcıoğlu et al. 2018, Can 2019). Many types of oregano are used as a spice in dishes around the world. Among the *Origanum* species, *Origanum onites*, also known as Turkish oregano, is widely consumed (Bozdemir, 2019). In Turkey, this genus is generally used in the treatment of diseases such as headache, dizziness, cough, flu, gastrointestinal disease, bronchitis, high cholesterol, diabetes, abdominal pain, hypertension and toothache (Tepe et al. 2016). The *Origanum* species, commonly known as oregano, is one of the most famous and economically important culinary herbs in the world (Peter 2012). It was observed that *Origanum* species have several biological activities such as antimicrobial, anticancer, antidiabetic, antioxidant, antibacterial, antifungal, antinociceptive and antilipase ones (Quiroga et al. 2013, Walker et al. 2016, Waller et al. 2017). Also, it is extremely important to analyze the elements contained in plants consumed as food or medicine.

Edible plants are an important source of nutrients, including micronutrients and bioactive components. They contribute to the daily requirement supply of some minerals such as iron, potassium and phosphorus, as well as dietary fiber and vitamin C (Guzelsoy et al. 2017, Pinela et al. 2017). Many edible plants are rich in nutrients and vitamins. Also, it is supposed that they are extremely important in terms of balanced nutrition culture, especially in societies with scarce food resources (Çetinkaya, Yıldız 2018).

In addition to the floristic richness of our country, the identification and analysis of *Origanum* species is extremely important. Such studies reveal the safety of plants in terms of consumers' health and provide data for future research.

This study was carried out to determine total ash, dry matter, total antioxidant, total phenolic and total flavonoids content, nitrogen balance index (NBI), chlorophyll, flavonol, anthocyanin, nutrient elements, essential oil composition and yield of *O. dubium* Boiss. and *O. onites* L., which are cultivated in the Van region and widely consumed in Turkey.

MATERIALS AND METHODS

Plant materials

The study material consisted of samples of *Origanum* species grown in the Medicinal and Aromatic Plants Garden of Van Yuzuncu Yil University. Two *Origanum* species, *O. dubium* Boiss. and *O. onites* L., were grown in different gardens in order to determine the adaptation ability. These species are widely consumed in Turkey both as a spice and a medicinal plant.

Dry matter was determined by drying the samples at 105°C for 24 h in an oven. For the total ash (inorganic matter) determination, an Electrical Muffle furnace set at 550°C was used. The mineral constituents of the plant samples were investigated as follows: first, the dried samples were ashed in a furnace with hydrochloric acid and nitric acid (AR) (AOAC 2000). Then, distilled water (50 ml) were added to samples in a volumetric flask. All assays were performed triplicate and the standard materials were being utilized for chemical analyses. Atomic Absorption Spectrometry (AAS) was used to determine K, Ca, Mg, Fe contents. ICP-OES (Inductively coupled plasma-Optical emission spectrometer) by Thermo Scientific was also used to determine other microelements and heavy metals (Mn, Zn, Cu, Ni, As, Cd, Co, Cr and Pb). This technique (ICP-OES) involves the stimulation of a sample by argon plasma heated to 10,000 K by electromagnetic induction and the determination of the excited elements according to the specific wavelengths they emit. The plasma is obtained electromagnetically by exciting argon gas in the induction coils with a radio frequency (RF) generator. This event occurs when the hot plasma ionizes the incoming gas and the process goes on continuously.

The content of total phenolic compounds was measured according to Obanda Owuor (1997) method. The antioxidant activity was also determined based on the Antioxidant Power (FRAP) (Iron (III) antioxidant power reduction) method (Benzie Strain 1996) followed by readings of the absorbance at 593 nm, and antioxidant activity values were recorded as Trolox equivalent (TE) mg^{-1}. The total flavonoid content was determined according to the method developed by Quettier-Deleu et al. (2000) with some modifications. The total amount of flavonoids was measured at 415 nm and calculated in mg quercetin equivalent (QE) 100 g^{-1} DM by using the calibration curve according to standard quercetin.

The nitrogen balance index (NBI), chlorophyll, flavonol and anthocyanin contents were measured on the leaf samples using Dualex scientific+ (FORCE-A, France) before harvesting.

The essential oils were isolated from air-dried plant material by hydrodistillation for 3 h, using a Clevenger-type apparatus (Karik et al. 2018). The oils were dried over anhydrous sodium sulphate and stored at +4°C in the dark.

The essential oil composition was analyzed by gas chromatography (Agilent 5975C) coupled to a flame ionization detector and mass spectrometry (Agilent 5975C) using a capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 µm). Essential oils were diluted in a 1:50 ratio with hexane. A GC-MS/FID analysis was carried out at the split mode of 50:1. The injection volume and temperature were adjusted as 1 µl and 250°C, respectively. Helium (99.9%) was the carrier gas passed at a constant flow rate of 1 ml min. MS spectra were monitored between 35 and 450 amu and the ionization mode used was the electronic impact at 70 eV. Identification of compounds: the relative percentage of the components was calculated from GC-FID peak areas, and the components were identified by Wiley 7n, Nist 05 and Flavour and Fragrance Natural and Synthetic Compounds (ver. 1.3) Libraries.

The data obtained were statistically analyzed using COSTAT software. All analyses carried out in the study were performed in three replications, and standard deviations were determined.

RESULTS AND DISCUSSION

In the study, the total ash content of *O. onites* and *O. dubium* species were determined as 10.61% and 12.03%, and the dry matter amounts were determined as 38.24% and 43.47%, respectively (Figure 1). Tunçtürk et al. (2018) determined total ash amount and dry matter in *Malva sylvestris*, *Falcaria vulgaris* and *Chenopodium botrys* as 13.00-19.26%, 7.00-23.18% and 20.84-18.32%, respectively. In another study, Tunçtürk et al. (2017) reported that the dry matter and ash in ten wild plants growing in Eastern Anatolia were 11.23-20.80%, 4.33- 20.7%, respectively.

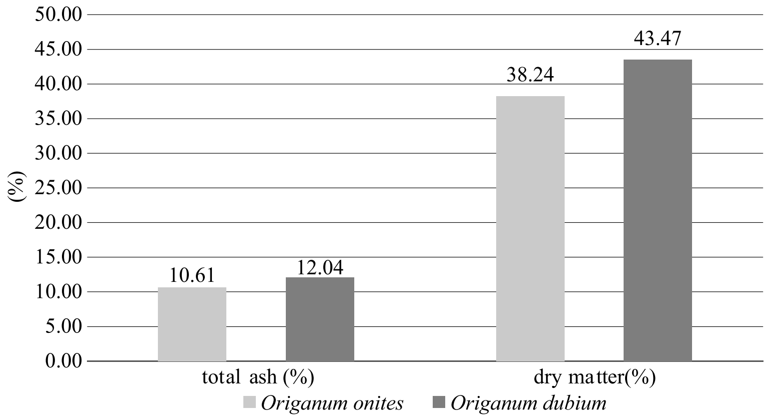

Fig. 1. The total ash and dry matter content of *O. onites* and *O. dubium* species

Total antioxidant, phenolic and flavonoid contents in the *O. onites* species was lower than in the *O. dubium* species. Studies have shown that different species and varieties of the same species present significant differences in the content of flavonoids, phenolic and antioxidant activities. Plants are rich sources of bioactive components. Phenolic compounds, which have radical scavenging ability, contain one or more hydroxyl groups attached to a ring (Mitsuda et al. 1996). Medicinal plants naturally produce some secondary metabolites, such as flavonoids, which can be used for various purposes (Al-osaj 2016). Flavonoids are consumed in large quantities in a daily diet. Ercetin et al. (2012) showed that the antioxidant properties are in correlation with the total flavonoid content in *Calendula officinalis*. Kindlovits et al. (2016) reported that the phenol content of *Achillea collina* ranged from 139 to 220 mg GAE 100 g^{-1}, which is correlated with our results (Figure 2).

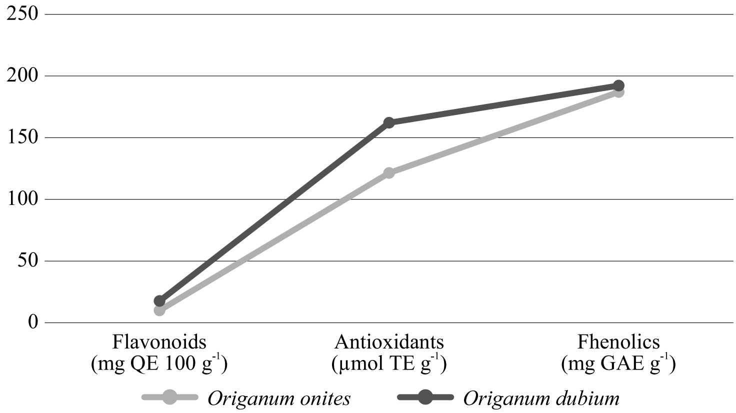

Fig. 2. Total antioxidant, total phenolic and total flavonoid content of the *Origanum* species

The total phenolic content in *O. acutidens* was 142.78 mg mL^{-1} (Fidan et al. 2018), and ranged between 21.34-231.55 µg mg^{-1} (Oke-Altuntas et al. 2018). It was observed that the total phenolic content is higher in the species which are important for health and often used as study material. Yılmaz et al. (2019) determined the total antioxidant content of two species from the family Lamiaceae (*Thymbra spicata* 91.65 mg TE and *O. onites* 160.35 mg TE). In our study, this value was determined as 121.41 and 162.17 mg TE in *O. onites* and *O. dubium* species, respectively.

Yolcu (2024) examined total carotenoid content, total phenolic content, and antioxidant activity (CUPRAC and FRAP) parameters in control group of *Origanum vulgare* L. ssp. *hirtum* and determined the values of 0.35 mg g^{-1} GAE, 0.81 mg g^{-1}, 4.73 mM g^{-1} TE and 0.80 mM g^{-1} AAE respectively.

In the measurements made with the Dualex device, it was determined that the amount of chlorophyll (23.26 Dualex index), anthocyanin content (0.100 Dualex index) and flavonol (1.706 Dualex index) in *O. onites* species was higher than *O. dubium*, and the NBI (13.90 Dualex index) was lower (Table 1). Dordas (2009) found the chlorophyll content in *O. onites* as 32.9-37.2. It was found that the chlorophyll content determined in our study was low compared to the other studies. It is supposed that the genetic structure, different species and cultivated conditions may be responsible for this difference.

Table 1

NBI, chlorophyll, anthocyanin and flavonol contents of the *Origanum* species

Specification	***O. onites***	***O. dubium***
NBI	13.90	28.23
Cholorophyll	23.26	23.30
Anthocyanin	0.100	0.080
Flavonol	1.706	0.990

NBI – nitrogen balance index

In the study, the nutrient contents of *O. onites* and *O. dubium* species were determined 26.80-31.56 g kg^{-1} in potassium (K) concentration, 18.20-16.07 g kg^{-1} in calcium (Ca) concentration, 5.201-3.576 g kg^{-1} in magnesium (Mg) concentration, 798.07-639.23 mg kg^{-1} in iron (Fe) concentration, 46.50-30.50 mg kg^{-1} in zinc (Zn) concentration, 12.48-12.73 mg kg^{-1} in copper (Cu) concentration and 55.29-54.36 mg kg^{-1} in manganese (Mn) concentration, respectively (Figure 3). In other studies, the potassium value determined as 0.35-0.88% in *O. onites* L. (Gönüz, Özörgücü 1999) and 2.43% in *A. millefolium* L. (Saraç et al. 2021). Also, the Ca content was determined as 1.17-3.30% (Erdoğan Bayram 2018) and 1.00% (Baydar, Erdal 2004) in *O. onites* L., and was determined as 4243.07 (Fırat et al. 2020) ppm in *O. acutidens*. The amount of Mg in oregano was determined by Fırat et al.

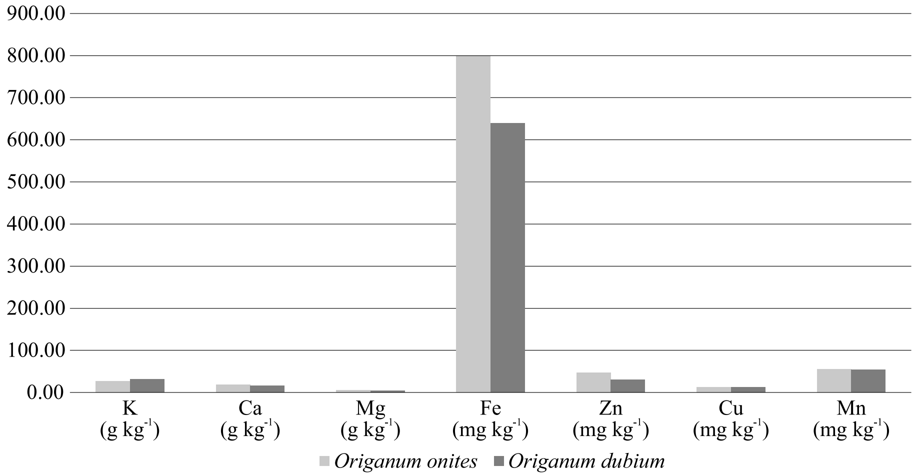

Fig. 3. Macro- and micronutrient contents of the *Origanum* species

(2020) as 1844.41 ppm and by Erdoğan Bayram (2018) as 0.27-0.35%. The iron amounts in *O. onites* L. were also determined as 51-335 ppm (Erdoğan Bayram 2018) and 77.75 ppm (Baydar, Erdal 2004). In other studies, the iron content was detected as 360.4 mg kg^{-1} in *A. millefolium* L. (Saraç et al. 2021), 144.7 mg kg^{-1} in *Polygonum cognatum* Meissn. (Saraç et al. 2018) and 225.8 mg kg^{-1} in *Rumex crispus* L. (Dastan, Sarac 2018). The zinc amounts were reported between 14-45 ppm (Erdoğan Bayram 2018) and 63.75 ppm (Baydar, Erdal 2004) in *O. onites* L., 47.6 mg kg^{-1} in *A. millefolium* L. (Saraç et al. 2021) and 40.3 mg kg^{-1} in *R. crispus* L. (Saraç et al. 2018). The Mn content in *O. onites* was determined as 59-85 ppm (Erdogan Bayram 2018) and 49.00 ppm (Baydar, Erdal 2004).

Commonly detected elements in *O. onites* and *O. dubium* species are indicated in the Figure 4. The maximum limit values of heavy metals deter-

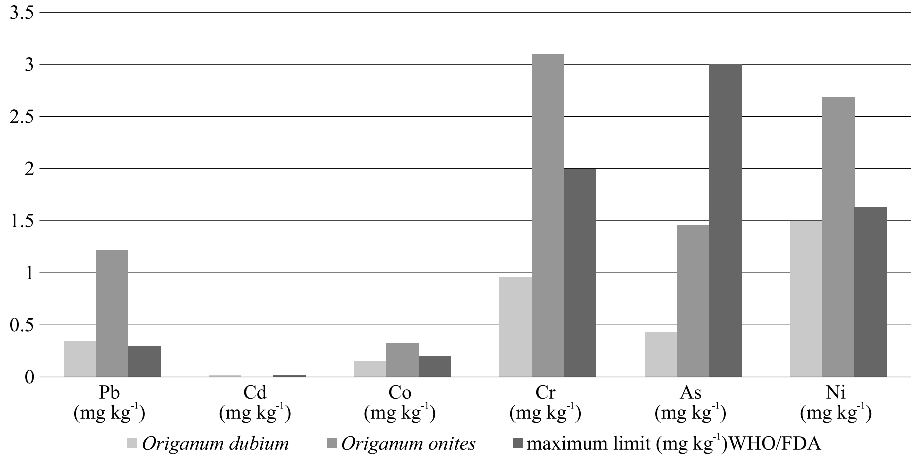

Fig. 4. The content of heavy metals in *O. onites*, *O. dubium* and WHO/FDA maximum limit

mined according to the food codex and shown in the Figure 4. The amounts of heavy metals detected in the current study vary when compared to the maximum allowable values determined by the WHO/FDA. Except for Pb, other elements were found to be low in *O. dubium* species. In *O. onites*, it was observed that the heavy metal contents were slightly above the tolerable threshold value.

Senkal at al. (2019) showed that the heavy metal content in the aerial parts of *Salvia virgata* was determined as follows: Al > Ni > Cr > Co > Cd. The limit values for Cd, Cr, and Ni determined by the WHO/FDA are 0.3, 0.02, and 1.63 ppm, respectively (Lone et al. 2003). The amounts of the three metals detected in the current study were lower than the maximum permissible values determined by the WHO/ FDA. The results of other studies were found to be compatible with our results. *O. onites* and *O. dubium* are used as tea, spice or nutrient due to the high amount of elements such as iron (Fe), calcium (Ca), potassium (K) and magnesium (Mg), as seen in the Figure 4.

The consumption of this species is thought to have a positive effect on human health.

The presence of heavy metals in food is a global problem. The maximum level of Cd in the analyzed beverages was 3.2 μg, and that of Pb was 82.6 μg kg^{-1} in coffee. The tolerable level of intake of Cd (TWI) and Pb (Benchmark dose lower confidence limit: BMDL) with the analyzed beverages did not exceed 2.5%. However, special note should be taken of Pb, as the level of this metal was higher than that of Cd, and for beverages with a higher weight per serving, the intake of Pb can exceed consumer-safe levels if they are consumed on a regular basis (Winiarska-Mieczan et al. 2023). In our study, it was determined that Cd levels in *O. dubium* and *O. ortines* species were below the tolerable threshold value. It was concluded that *O. onites* species had high Pb concentration and caution should be taken about its consumption.

It was observed that *O. onites* species had higher essential oil content compared to *O. dubium*. The essential oil yield was determined as 3.240% in *O. onites* and 3.130% in *O. dubium* species. The essential oil yield in *O. vulgare* subsp. *hirtum* L. plant was determined as 2.59-4.59% in the first year and 2.12-3.78% in the second year (Sancaktaroğlu, Bayram 2011). Similarly, essential oil yield has been reported as 2.1-3.4% in İzmir oregano (*O. onites*) (Ceylan 1996), 0.1-0.3% in *O.vulgare* ssp. *vulgare* (Kokkini et al.

Table 2

The essential oil components (%) of *O. onites*

RI	RT	Essential oil compounds	Amount of essential oil compounds (%)
1010	10.811	*α*-pinene	0.370
1013	10.938	*α*-thujene	0.720
1148	16.419	myrcene	1.660
1165	17.183	*α*-terpinene	1.670
1230	20.025	*γ*-terpinene	6.650
1255	21.105	cymene	3.950
1448	28.575	*trans*-sabinene hydrate	0.430
1523	31.137	linalool	0.260
1532	31.416	*cis*-sabinene hydrate	0.250
1586	33.151	terpinen-4-ol	2.220
1678	35.945	*α*-terpineol	0.180
1682	36.041	borneol	0.750
1706	36.755	*β*-bisabolene	1.430
1980	44.09	caryophyllene oxide	0.200
2175	48.056	thymol	0.300
2206	48.78	carvacrol	78.24
Essential oil yield (% of dry weight): % 3.24			

RT – retention time, RI – retention index (Kovat's)

1989), 2.22-3.58% in *O. onites* (Can et al. 2021). The essential oil yield obtained in our study was at the average level. There are 24 main components obtained in the essential oil of *O. dubium* species and 16 main components in *O. onites* species (Tables 2, 3). The main components of essential oil obtained from *O. dubium* species were carvacrol (22.01%), *γ*-terpinene (23.55%), cis-sabinene hydrate (13.90%), terpinen-4-ol (8.500%), *α*-terpinene (4.940%) and cymene (4.940%).

In *O. onites* species, it was determined that essential oils consisted of carvacrol (78.24%), *γ*-terpinene (6.650%), cymene (3.950%), terpinen-4-ol (2.220%), *α*-terpinene (1.1.670%), myrcene (1.660%), and *α*- *β*-bisabolene

Table 3

The essential oil ratio and components ratio (%) of *O. dubium*

RI	RT	Essential oil compounds	Amount of essential oil compounds (%)
1010	10.806	*α*-pinene	0.640
1013	10.93	*α*-thujene	1.780
1094	14.056	*β*-pinene	0.260
1108	14.63	sabinene	2.820
1148	16.413	myrcene	2.010
1165	17.179	*α*-terpinene	4.940
1185	18.047	limonene	1.070
1195	18.49	*β*-phellandrene	0.940
1230	20.042	*γ*-terpinene	23.55
1255	21.102	cymene	4.940
1266	21.571	*α*-terpinolene	1.050
1448	28.571	*trans*-sabinene hydrate	3.350
1523	31.136	linalool	0.710
1532	31.42	*cis*-sabinene hydrate	13.90
1536	31.51	linalyl acetate	0.520
1532	31.874	*cis*-sabinene hydrate	0.570
1566	32.491	bornyl acetate	0.330
1586	33.161	terpinen-4-ol	8.500
1609	33.87	*cis*-*p*-mentha-2-en-1-ol	0.210
1678	35.941	*α*-terpineol	2.980
1682	36.041	borneol	0.420
1706	36.757	*β*-bisabolene	0.370
1722	37.188	bicyclogermacrene	1.000
2206	48.773	carvacrol	22.01
		unidentified	1.140
Essential oil yield (% of dry weight): % 3.13			

RT – retention time, RI – retention index (Kovat's)

(1.430%). As a result of the study, it was seen that the essential oil components differed according to the species. The main component of both species was identified to be carvacrol. The results were similar or higher than findings of other researchers who reported that carvacrol is the main component in different *Origanum* species, and the carvacrol ratio is 42.5% (Baser et al. 1993), 42.46% (Tulmen, Baser 1993), 49.70-69.89% (Wakim et al. 2013), 66.99% in stem and 58.39-64.53% in leaves (Özel, Tekin 2021). Besides, it was found to be lower than the findings of some researchers 82.8-84.6% (Novak et al. 2010) and 78.4% (Farhat et al. 2012). This may be due to differences in growing conditions, genotype differences and harvest time differences.

CONCLUSIONS

Nowadays, medicinal and aromatic plants are widely used as spices, herbal teas, food supplements and food additives. It is frequently preferred because of its antioxidant properties, richness of nutrients, and its use as aroma and colorant. Origanum species, which are widely used worldwide especially in Turkey, are also important medicinal and aromatic plants. For this reason, it is important to know the nutrient and biochemical contents, essential oil ratio and components of the species. In this study total ash, dry matter, total antioxidant, total phenolic and total flavonoids content, nitrogen balance index, chlorophyll, anthocyanin, macro and micro nutrient content, essential oil content and composition of *O. dubium* and *O. onites* species were determined. Results showed that the species are rich in plant nutritional value and biochemical components. In addition, it is supposed that these species can be an extremely important alternative food source for a balanced nutrition culture, especially in societies with scarce food resources. Considering the metals contained in the species, it was determined that *O. onites* was higher than the maximum limit value determined by WHO/FDA. It was concluded that the daily consumption of the species should be kept within certain limits. Based on the determined component and essential oil content, *O. onites* may be preferred in oregano production because of its high carvacrol content of 78.24%.

Authors' contribution

E.S – investigation, resources, writing–original draft, R.T. – conceptualization, methodology, validation, L.N. – investigation, resources, writing–original draft, O.C. – methodology, formal analysis, M.T. – writing – review and editing, visualization, supervision. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors ensure that they have neither professional nor financial connections related to the manuscript sent to the Editorial Board.

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