ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 11:5 (2017) 439-455

records of natural products

Screening of Chemical Composition, Antioxidant and Anticholinesterase Activity of Section *Brevifilamentum* of *Origanum* (L.) Species

Hasibe Yılmaz[1], Sema Çarıkçı[2*], Turgut Kılıç[3], Tuncay Dirmenci[4], Turan Arabacı[5] and Ahmet C. Gören[1]

[1] *TUBITAK National Metrology Institute (UME), Department of Chemistry, Gebze-Kocaeli, Türkiye*

[2] *Balıkesir University, Faculty of Sciences and Arts, Department of Chemistry, Campus of Çağış, -Balıkesir, Türkiye*

[3] *Balıkesir University, Necatibey Education Faculty, Department of Science Educations, Balıkesir, Türkiye*

[4]*Balıkesir University, Necatibey Education Faculty, Department of Biology Educations, Balıkesir, Türkiye*

[5]*Inonu University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Malatya, Türkiye*

(Received April 19, 2017; Revised May 17, 2017; Accepted May 25, 2017)

Abstract: Six *Origanum* species, *Origanum acutidens* (Hand. -Mazz.) Ietsw. (OA), *Origanum brevidens* (Bornm.) Dinsm. (OB), *Origanum haussknechtii* Boiss. (OC), *Origanum husnucan-baseri* H.Duman, Aytaç & A.Duran (OHB), *Origanum leptocladum* Boiss. (OL), *Origanum rotundifolium* Boiss. (OR), belonging to sect. *Brevifilamentum* were analyzed for their essential oil and phenolic components. For the essential oil analyses, GC-MS and GC-FID were used. Phenolic contents of the aerial parts of the chloroform, acetone, and methanol extracts were analyzed using LC-MS/MS. Antioxidant activity of the species was investigated by three methods; DPPH free radical scavenging activity, *β*-carotene linoleic acid assays and CUPRAC assays. Also, acetyl and butyrylcholinesterase inhibition of the extracts were investigated. While the essential oil contents of the section *Brevifilamentum* showed difference in chemotype, the phenolic contents were found to be coumaric acids and derivatives. These groups were the most abundant components of the extracts. Especially rosmarinic acid was detected in high amounts in acetone and methanol extracts. OA had the best activity both in antioxidant and anticholinesterase assays.

Keywords: *Origanum*; *Bravifilamentum*; essential oil; phenolics; antioxidant activity; anticholinesterase activity.

[*] Corresponding author: E-Mail: carikci@balikesir.edu.tr; Phone:090-266-6121278, *Fax:*090-266-6121215.

The article was published by ACG Publications
www.acgpubs.org/RNP © Published 06/13/2017 EISSN:1307-6167
DOI: http://doi.org/10.25135/acg.rnp.56.17.04.029

1. Introduction

Aromatic plants are often used in traditional medicine due to their beneficial long-term effects on health for prevention and treatment of different diseases. They have also been used as cooking ingredients for their flavoring quality since ancient times [1]. Many of these plants belong to the family of Lamiaceae (Labiatae), which has a very intense expansion throughout the world; they are predominately distributed around the Mediterranean region, consisting of about 7200 species and 236 genera. According to the recent studies, it is the third largest family in Turkey with 46 genera and about 600 species (ca. 750 taxa), approximately 350 of which are endemic to Turkey and the endemism rate is about 44% [2-6].

One of the most used Lamiaceae members is *Origanum*. They are known as "kekik" in Turkey, “oregano” in the world. *Origanum* has 43 species (51 taxa) and 15 hybrids worldwide [7-12]. Most of its species are distributed in Turkey. *Origanum* comprises 23 species (26 taxa) and eight hybrids in Turkey, 20 of which are endemic. The species are mainly concentrated in the Mediterranean area of Turkey. Section *Brevifilamentum* Ietsw., which is the main material of this study was characterized by leathery leaves, large and nodding spikes and 2-lipped calyces. Section *Brevifilamentum* consists of 6 species, 5 of which are endemic to Turkey: *Origanum acutidens* (Hand. -Mazz.) Ietsw. (endemic), *O. brevidens* (Bornm.) Dinsm. (endemic), *O. haussknechtii* Boiss. (endemic), *O. husnucan-baseri* H.Duman, Aytaç & A.Duran (endemic), *O. leptocladum* Boiss. (endemic) and *O. rotundifolium* Boiss.

The oregano is used as a condiment or herbal tea. Moreover, its essential oil (*Origanum* oil, kekik yağı) and hydrosol (*Origanum* water, kekik suyu) are produced from dried oregano and have been used in traditional medicine to treat some diseases such as gastric ulcers, diarrhea, blood cholesterol, glucose level and pain in rheumatism [13].

Many phytochemical studies have been conducted to investigate the chemical profiles and activities of *Origanum* species. The studies especially focused on essential oil profiles and showed oregano essential oils to be rich in phenolic compounds, like carvacrol and thymol [13, 14]. Additionally, extract of the species includes phenolics such as rosmarinic acid, caffeic acid, ferulic acid, apigenin, luteolin, salvigenin and catechin [15-17]. Biological activities of the species and their extracts have also been described such as antioxidant, antimicrobial, antifungal, antispasmodic, antitumoral, analgesic, antimutagenic, angiogenic, antiparasitic, antiplatelet and anti-elastase [18]. In *Brevifilamentum* section, the studies especially focused on two species, i.e. *O. acutidens* and *O. rotundifolium*. Chemical profile [19-22] and insecticidal [23-29], antioxidant [30-33], antimicrobial and antibacterial [34-36] activities of the essential oils and some extracts were studied. It has been reported that essential oil of *O. acutidens* was found to be insecticidal due to its high concentration of carvacrol. Essential oils of *O. haussknechtii* [37], *O. husnucan-baseri* [38] were also reported to show antibacterial activity [39] and *O. leptocladum* [22, 40-41].

The present study is on identification, quantification and evaluation of antioxidant and anticholinesterase activities of essential oils and phenolic constituents of extracts of six *Origanum* species, belonging to section *Brevifilamentum*. Although there are numerous reports on the essential oils of the species, this is the first report on the analysis of phenolic composition and antioxidant and anticholinesterase activities of the extracts.

2. Materials and Methods

2.1. Plant Material

Collection location, coordinates and time of the *Origanum* species are given in the Table 1. The species were identified by Dr. Tuncay Dirmenci at Balıkesir University. Voucher specimens were deposited at the Herbarium of Faculty of Education, Balıkesir University, Balıkesir, Turkey.

Table 1. List of the *Origanum* species with locality, altitude and collection time**.**

Code	Herbarium no	Species	Locality and Coordinates	Altitude (m)	Year
OA	T.A. 2825	*Origanum acutidens*	Malatya: Between Gündüzbey and Kozluk 3rd km, rocky slopes	1000	2013
OB	T.D. 4270	*Origanum brevidens*	Osmaniye : Between Yarpuz and Yağlıpınar; N 37.01064 E 36.27651	1310	2014
OH	T.A. 2824	*Origanum haussknechtii*	Erzincan: Between Kemaliye and Arapkir, 15th-20th km, calcareous rocks N 39.153170 E 38.620290	1097	2013
OHB	T.D. 4298	*Origanum husnucan-baseri*	Between Alanya and Gökbel plateau, 43rd km; Kuşyuvası area, tunnels N 36.55894 E 32.34865	1352	2014
OL	T.D. 4290	*Origanum leptocladum*.	Karaman: Between Ermenek and Kazancı, Görmeli village, above 1st km; N 36.54299 E 32.96344	885	2014
OR	T.D. 3943	*Origanum rotundifolium*	On the road of Artvin – Ardanuç N 41.193790 E 41.867840	600-700	2013

2.2. Chemicals

Chloroform, acetone, and methanol were used for the preparation of the extracts. Following compounds were used as standards in LC-MS/MS analysis: fumaric acid (99%, Sigma-Aldrich), pyrogallol (98%, Sigma-Aldrich), rutin (94%, Sigma-Aldrich), chlorogenic acid (95%, Sigma-Aldrich), gallic acid (99 %, Merck), syringic acid (95%, Sigma-Aldrich), (*E*)-ferulic acid (99%, Sigma-Aldrich), caffeic acid (98%, Sigma-Aldrich), pelargonin chloride (98%, Sigma-Aldrich), quercitrin (97%, Sigma-Aldrich), salicylic acid (99 %, Sigma-Aldrich), *p*-coumaric acid (98 %, Sigma-Aldrich), luteolin-7-*O*-glu (99%, AppliChem), rosmarinic acid (96%, Sigma-Aldrich), pyrogallol (98 %, Sigma-Aldrich), apigenin (95 %, Sigma-Aldrich), kaempferol (96 %, Sigma-Aldrich) and isorhamnetin (98 %, Extra Synthese, Genay-France). Stock solutions were prepared as 10 mg/L in methanol. HPLC grade methanol was purchased from Merck (Darmstadt, Germany). Calibration solutions were prepared in methanol in a linear range. Dilutions were performed using automatic pipettes and glass volumetric flasks (A class). 100 mg/L curcumin solution was freshly prepared, from which 50 µL was used as an Internal Standard (IS) in all experiments.

2.3. Essential oil

The air-dried aerial parts of *Origanum* species (100 g of each) were subjected to hydro distillation with water for 4 h, using a Clevenger-type apparatus to produce the essential oil. Essential oil yields of species are 0.81, 1.29, 0.70, 0.62, 0.76 and 0.16 from OA, OB, OH, OHB, OL, OR, respectively.

2.4. Gas chromatography–mass spectrometry (GC-MS) and GC– flame ionization detector (GC-FID) conditions

GC-MS was conducted on Thermo Electron Trace 2000 GC model gas chromatography and Thermo Scientific TSQ GC-MS/MS. A Phenomenex DB5 fused silica column (30 m x 0.32 mm, with 0.25 µm film thickness) was used with helium as a carrier gas at 1mL/min flow rate (138 kPa). The GC temperature program was set as follows; 150 ^{0}C hold for 5 min, ramp to 250 ^{0}C at 3 ^{0}C/min and hold for 10 min. The temperature of the MS transfer line was set to 230 ^{0}C. Using scan mode, a mass range from 50 to 650 *m/z*. The GC oven temperature was kept at 60 ^{0}C for 10 min and programmed to 220 ^{0}C at a rate of 4 ^{0}C/min and then kept constant at 220 ^{0}C for 15 min. The split ratio was adjusted to 1:20, the injection volume was 0.1 mL and EI/MS was recorded at 70 eV ionization energy. Mass range was *m/z* 35–500 amu. A homologous series of *n*-alkanes was used as a reference in the calculation of Kovats Indices (KI). Identification of the compounds was based on the comparison of their Kovats Indices and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as the literature data [42-44]. GC-FID performed using Thermo Electron Trace GC-FID detector and same GC program was used as stated above.

2.5. Preparation of Extracts

Approximately 100 grams of shade-dried powdered plant samples were extracted with methanol (M) for 15 days. After filtration and evaporation, they named **M1** for each plant samples. Also another 100 gram of the plant was extracted with chloroform (C) for 15 days. After filtration and evaporation, the residuary plant was extracted with acetone (Ac) and methanol for 15 days in the same way. They were named **C**, **Ac**, and **M2** for each species.

2.6. Liquid Chromatography-Mass spectrometry

LC-MS/MS experiments were performed by a Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole (Istanbul, Turkey) mass spectrometry equipped with a Synergy Max C18 column (250 x 2 mm i.d., 5mm particle size). The mobile phase was composed of water (A, 0.1 % formic acid) in methanol (B, 0.1 % formic acid), the gradient programme of which was 0-1.00 minute 55 % A and 45 % B, 1.01-20.00 minutes 100 % B and finally 20.01-23.00 55 % A and 45 % B. The flow rate of the mobile phase was 0.25 mL/min, and the column temperature was set to 30 oC. The injection volume was 10 mL.

The best mobile phase solution was determined to be a gradient of acidified methanol and water system. Triple quadrupole mass spectrometry was used due to its fragmented ion stability [45]. The optimum ESI parameters were determined as 2.40 mTorr CID gas pressure, 5000.00 V ESI needle voltage, 600.00 V ESI shield voltage, 300.00 °C drying gas temperature, 50.00 °C API housing temperature, 55 psi Nebulizer gas pressure and 40.00 psi drying gas pressure. A detailed information on the experiment parameters is given in supporting information.

2.7. Preparation of test solution for LC-MS/MS

To fifty mg of each extract in round bottom flask was added 4 mL of the ethanol-water mixture (50:50 v/v). In order to obtain a good solubility, the flask was refluxed on a water bath until a clear solution was obtained. They were then transferred into a 5 mL of volumetric flask and diluted to volume. A portion of 1 mL of this stock solution was transferred into a 5 mL of another volumetric flask, and 50 mL of curcumin solution was added as internal standard and diluted to the volume with methanol and mixed. The solution was filtered through a 0.45 µm Millipore Millex-HV filter and the final solution (1 mL) was transferred into a capped auto sampler vial and 10 mL of sample was injected to LC for each run. The samples in the auto sampler were kept at 15 ^{0}C during the experiment.

2.8. Validation of experiments and uncertainty evaluation

Curcumin was used as an internal standard in validation experiments of all of the compounds. The validation parameters were determined to be linearity, repeatability, LOD (limit of detection) and LOQ (limit of quantification) experiments. The linearity for each compound for the reported method was determined by analyzing standard solution. The linearity ranges of each compound are given in Table 2 in supporting information. The correlation coefficients (r^2) were found to be ≥0.99. Linear regression equations of the reported compounds are also presented in supporting information. Detailed procedures of uncertainty evaluation were reported previously in the literature [45-47].

2.9. Activity

Inhibitory activities of acetyl- and butyrylcholinesterase were measured by a slightly modified spectrophotometric method, developed by Ellman, Courtney, Andres and Featherston [48-50]. Acetylthiocholine iodide and butyryl thiocholine iodide were used as substrates of the reaction, and DTNB method was applied for the measurement of the anticholinesterase activity [48-49]. The antioxidant activities were measured based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, β-carotene linoleic acid assays and cupric (Cu^{2+}) ion reducing power assay (CUPRAC) [50-56]. The detailed procedure of the biological activities is given in Supplemental Material.

3. Results and Discussion

3.1. Essential Oil

The aerial parts of the six *Origanum* species belonging to section *Brevifilamentum* were analyzed by using GC-MS and GC-FID, 74 components of which were accounted for 94.1-99.8% of the total oil composition. The components were classified into 6 classes based on their chemical structures: hydrocarbons and derivatives, monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and phenolic compounds. Essential oil compositions of the species are given in Table 2.

The essential oil composition of *Brevifilamentum* section has been analyzed to have different chemotypes. OA was found to be rich in monoterpene hydrocarbons with the main compound of *m*-cymene (39.2%). Other compounds are *allo*-aromadendrene (24.8%) and aromadendrene (11.8%). In the previous studies, OA was reported as carvacrol type [19,28]. OB was found to be rich in phenolic compounds, and its main compounds were identified as carvacrol (72.1%), carvone (9.1%) and borneol (7.1%). Carvacrol constitutes the major part of the oil, and the rest of the essential oil is rich in oxygenated monoterpenes. To the best of our knowledge, there has been no report on the essential oil composition of OB, and the present study is the first study. OH and OL were rich in sesquiterpene hydrocarbons (35.7% and 34.8%). Main compounds of the essential oil of OH and OL were aromadendrene (23.7%) and τ-murolene (20.1%), respectively.

Other main compounds were determined as carvacrol (16.2%) and *allo*-aromadendrene (15.2%) for OH, and *p*-cymene (17.1%) and borneol (16%) for OL. In the previous study, OH was found to be rich in *p*-cymene [37] and OL was reported in three different studies as *p*-cymene [40], carvacrol [41] and thymol/carvacrol type [22]. Another member of this section, OHB is rich in oxygenated monoterpenoids and its main compounds were *cis*-β-terpineol (24.3%), menth-3-en-8-ol (22.6%) and menthone (13.1%). This species was previously reported as monoterpenoid-rich and the main compounds were borneol, α-terpineol and *trans*-sabinene hydrate [38]. The last member of the section, OR was rich in oxygenated sesquiterpenoids, and its main compound was α-cadinol (27.8%). Other main compounds were found to be isopulegyl acetate (19.8%), which has an oxygenated monoterpene structure and limonene oxide (15.3%). It was reported in the literature that OR volatile oil was rich in monoterpenoids, and the main compound was *cis*-sabinene hydrate [20].

Previous studies showed that, the essential oils of the species had different chemical contents. The differences in the chemical composition of the oils might be related to local, climatic and seasonal differences [13, 20].

Table 2. Essential oil composition of Section *Brevifilamentum*

Compounds	KI	OA	OB	OH	OHB	OL	OR
Hydrocarbons and derivatives							
3-methyl-nonane	971	-	-	-	-	t	-
1-octen-3-ol	979	-	0.4	-	-	0.6	-
3-octanol	991	-	0.4	-	-	0.2	-
2-methyl-decane	1063	-	1.0	-	-	-	-
undecane	1100	-	0.3	-	-	0.2	-
menth-3-en-8-ol	1150	-	-	-	22.6	-	-
geranyl butyrate	1564	-	-	4.5	-	-	-
% identified		**-**	**2.1**	**4.5**	**22.6**	**1.0**	**-**
Monoterpene hydrocarbons							
α-thujene	930	-	-	-	t	-	-
α-pinene	939	-	-	-	0.3	t	-
camphene	954	-	-	-	0.6	0.2	-
sabinene	975	-	-	-	0.1	-	-
β-pinene	979	0.9	-	-	0.1	0.1	-
2-carene	1002	1.3	-	0.9	-	-	-
α-phellandrene	1003	-	-	-	0.1	-	-
terpinene	1017	-	-	-	0.6	0.1	-
p-cymene	1025	-	1.3	-	3.0	17.1	-
limonene	1029	0.3	-	-	-	0.1	-
β-phellandrene	1030	-	-	-	0.2	-	-
(*Z*)-β-ocimene	1037	-	-	-	-	t	-
τ-terpinene	1048	3.4	-	1.9	-	-	-
(*E*)-β-ocimene	1050	-	-	-	3.8	-	-
γ-terpinene	1060	1.4	-	-	0.6	3.7	-
***m*-cymene**	**1085**	**39.2**	-	0.6	-	-	-
% identified		**46.5**	**1.3**	**3.4**	**9.4**	**21.3**	**-**

Oxygenated monoterpenes							
eucalyptol	1031	1.9	-	2.1	-	t	-
cis-sabinene hydrate	1070	-	-	-	1.6	-	-
α-terpinolene	1089	0.5	-	-	-	0.5	-
trans-sabinene hydrate	1098	-	-	-	0.4	-	-
pinene hydrate	1123	-	-	-	1.0	-	-
limonene oxide	1137	0.1	-	-	-	-	15.3
trans-pinocarveol	1139	0.4	-	-	-	-	-
***cis*-β-terpineol**	1144	0.3	-	-	**24.3**	-	-
camphor	1146	-	0.1	-	-	0.8	-
menthone	1153	-	-	-	13.1	-	-
trans-β-terpineol	1163	-	-	-	0.3	-	-
borneol	1169	-	7.1	-	-	16.0	-
4-terpineol	1177	-	0.5	-	-	1.3	-
p-cymene-8-ol	1180	-	-	-	1.4	-	-
α-terpineol	1189	-	3.4	-	-	5.5	-
dihydrocarvone	1190	0.4	-	1.9	-	-	-
myrtenol	1196	-	0.2	-	0.3	0.3	-
linalyl formate	1216	4.6	-	-	-	-	-
carveol-cis	1229	-	-	-	0.8	-	-
carvone	1243	-	9.1	-	-	-	-
carvacrol methyl ether	1245	-	-	-	0.6	-	-
linalyl acetate	1257	1.2	-	-	-	-	-
p-cymen-7-ol	1287	-	-	-	-	2.3	-
bornyl acetate	1289	-	-	-	-	1.6	-
carvacrol ethyl ether	1298	-	-	-	0.8	-	-
isopulegyl acetate	1335	-	-	-	-	-	19.8
carvacryl acetate	1367	0.2	-	-	-	-	-
% identified		**9.6**	**20.4**	**4.0**	**44.6**	**28.3**	**35.1**
Sesquiterpene hydrocarbons							
α-copaene	1377	-	-	-	1.2	-	-
β-bourbonene	1388	-	-	-	2.4	1.7	-
(*Z*)-caryophyllene	1409	-	1.6	-	-	5.1	-
aromadendrene	1437	11.8	-	**23.7**	8.4	-	15.7
α-himachalene	1451	0.2	-	12.0	-	-	-
α-humulene	1455	-	-	-	2.3	-	-
(*E*)-β-farnesene	1457	-	-	-	2.9	-	-
allo-aromadendrene	1458	24.8	-	15.2	0.5	-	5.0
germacrene-D	1485	-	0.3	-	0.6	6.6	-
τ-muurolene	1480	-	0.6	-	-	**20.1**	-
γ-cadinene	1514	0.3	-	-	-	0.9	-
α-cadinene	1539	0.5	0.1	-	-	0.4	-
% identified		**37.6**	**2.6**	**50.9**	**18.3**	**34.8**	**20.7**

Oxygenated sesquiterpenes							
bisabolene epoxide	1504	-	-	3.3	-	-	5.6
spathulenol	1572	-	0.8	-	1.6	8.5	-
caryophyllene oxide	1583	-	0.6	4.2	-	4.0	-
viridiflorol	1593	-	-	-	-	0.4	10.0
α-cadinol	1660	1.1	-	7.6	-	0.7	**27.8**
% identified		**1.1**	**1.4**	**15.1**	**1.6**	**13.6**	**43.4**
Phenolic compounds							
tymol	1290	1.2	-	-	t	-	-
carvacrol	1299	2.8	**72.1**	16.2	3.3	-	-
% identified		**4.0**	**72.1**	**16.2**	**3.3**	**-**	**-**
Total (%)		**98.8**	**99.8**	**94.1**	**99.8**	**99.0**	**99.2**

3.2. Phenolic Contents

Analysis of the phenolic components and quantity of the extracts of the species, which were prepared through (i) extraction of the shade-dried powdered plant with methanol (M1) and chloroform (C), (ii) extraction of the residual plant with acetone (Ac) and methanol (M2), were conducted using LC-MS/MS technique. Phenolic compounds were analyzed under three groups; (i) flavonoids and derivatives, (ii) coumaric acid and derivatives and (iii) simple phenolics and others. Compounds and their respective amounts per type are given in Tables 3-6. Methanol (M1 and M2) extracts, prepared by both techniques were found to be rich in quantity. These are followed by Ac and C respectively. Considering the chemical structures of the phenolic substances, it is obvious that they better dissolved in polar solvents.

Methanol (M1 and M2) and Ac extracts are very rich in coumaric acid and its derivatives. The most common compounds found in extracts are (*E*)-ferulic, caffeic and rosmarinic acids. Especially rosmarinic acid was found to be rich in Ac extracts of all members of *Brevifilamentum* section. Presence of rosmarinic acid in Lamiaceae members was reported in different studies [16, 54-55]. Flavonoids and their derivatives commonly exist in C extract, salvigenin and penduletin, which were reported previously in *Origanum* species [15], are the compounds of all types found in this extract. OH and OA are the richest species in terms of phenolic compounds. Based on the number of compounds, however, OA is the richest type with 24 types of different phenolic compounds, whereas OL type has the lowest number of compounds with 18 different compounds.

The most abundant compounds in the extracts are as follows: in M1 extract: rosmarinic acid for OA, OH, OL and OR, fumaric acid for OB and (*E*)-ferulic acid for OHB. In M2 extracts: rosmarinic acid for OA, OL and OR, gallic acid for OB, (*E*)-ferulic acid for OH, luteolin for OHB. In Ac extracts: rosmarinic acid was found to be the main compound of OA, OB, OH, OHB and OR, whereas kaempferol was found to be the main compound of OL species. In the poorest C extract, the main compound for OA and OH is quercetagetin-3,6-dimethylether, salvigenin for OB and OHB and penduletin for OL and OR.

3.3. Activity

The antioxidant activities were determined applying DPPH free radical scavenging activity, β-carotene linoleic acid assays and CUPRAC assays. Inhibition of lipid peroxidation and DPPH free radical scavenging effect were determined at 10, 25, 50, and 100 μg/mL.

Antioxidant activity results revealed that *Brevifilamentum* section has good antioxidant capacity for all applied three methods. For the CUPRAC method, OB showed the best activity (Figure 1). M2 extracts of OB had better activity than curcumin, which was used as standard. For especially

the methanol extracts having 50 and 100 μg/mL concentrations, DPPH and β-carotene methods showed higher activities, and OA was found to be the one with the highest activity, which was followed by OB and OH, especially with M2 extracts. The results are given in the Figure 2.

The anticholinesterase activities of M1, M2, C and Ac extracts were determined at 200 μg/mL, and galantamine was used as a standard compound. It was observed that only OB inhibited AChE enzyme slightly. For BChE enzyme, except OH, inhibition was not observed M2 extracts of the species. The species with the highest BChE inhibition is OA (Table 7).

It could be concluded that high activity observed in that species is associated with high amount of phenolic compounds. In C extracts, species with high amounts of quercetagetin-3.6-dimethylether and salvigenin have better BChE inhibition values. Direct methanol (M1) and Ac extracts of these types have moderate BChE enzyme inhibition capacity.

Table 3. Phenolic contents of the M1 extracts

Species	OA	OB	OH	OHB	OL	OR
Flavonoids and derivatives						
Kaempferol	21024.29±1483.89	262.88±18.55	167.2±11.8	20.74±1.46	63.09±4.45	217.11±15.32
Kaempferol-3-rutinoside	130.74±11.82	-	2.83±0.26	-	-	0.23±0.02
Salvigenin	204.12±13.89	-	0.47±0.03	-	-	106.44±7.24
Penduletin	1339.08±135.75	10.42±1.06	46.2±4.68	-	6.61±0.67	0.17±0.02
Isorhamnetin	-	-	-	-	-	10.44±0.92
Quercetin	7.18±0.95	4.56±0.61	-	-	-	-
Quercetagetin-3,6-dimethylether	-	4.02±0.75	355.18±66.5	-	-	156.31±29.27
Isoquercetin	621.16±178.23	0.67±0.19	6.53±1.87	-	-	-
Quercitrin	-	-	-	-	-	-
Luteolin	8858.06±2275.32	62.28±16	56.27±14.45	-	6.47±1.66	83.83±21.53
Luteolin-7-*O*-Glucoside	2177.97±221.64	10.27±1.04	14.17±1.44	-	-	17.84±1.82
Luteolin-5-*O*-Glucoside	1707.99±109.89	-	35.59±2.29	-	-	0.31±0.02
Apigenin	5164.75±416.06	-	68.4±5.51	-	-	-
Rutin	1569.63±102.81	15.53±1.02	31.29±2.05	1.55±0.1	2.78±0.18	78.41±5.14
Coumaric Acids and derivatives						
p-Coumaric acid	709.28±109.19	-	-	-	-	2.40±0.37
Caffeic acid	6126.98±1212.46	174.78±34.59	190.06±37.61	45.86±9.08	44.69±8.84	191.50±37.9
(*E*)-Ferulic acid	12150.65±849.07	134.55±9.4	353.19±24.68	96.42±6.74	57.56±4.02	134.96±9.43
Chlorogenic acid	-	11.13±1.54	-	10.45±1.45	11.55±1.6	11.81±1.64
Rosmarinic acid	102800±7882.62	-	2092.79±160.47	27.47±2.11	1556.15±119.32	3520.48±269.95
Simple Phenolics and others						
Fumaric acid	24053.6±1668.09	369.43±25.62	165.59±11.48	37.07±2.57	126.31±8.76	1827.03±126.7
Gallic acid	-	7.00±0.49	-	7.99±0.55	5.85±0.41	-
Pyrogallol	657.8±43.77	50.02±3.33	15.1±1.01	27.51±1.83	-	32.97±2.19
Ellagic acid	-	-	-	-	-	-
Vanillin	-	-	-	-	-	-
Syringic acid	746.23±50.25	47.39±3.19	12.52±0.84	-	-	-
Salicylic acid	990.02±399.68	-	67.05±27.07	-	-	18.30±7.39
p-OH benzoic acid	-	-	-	-	-	-
Total (mg/kg dried herba)	**191039.53**	**1164.93**	**3680.43**	**275.06**	**1881.06**	**6410.54**

Table 4. Phenolic content of the M2 extracts

Compounds	OA	OB	OH	OHB	OL	OR
Flavonoids and derivatives						
Kaempferol	253.07±17.86	-	162300±11455.09	-	177.01±12.49	57.88±4.09
Kaempferol-3-rutinoside	-	-	12174.51±1100.24	-	-	-
Salvigenin	-	-	648.93±44.16	-	-	-
Penduletin	-	-	4366.06±442.62	-	-	-
Isorhamnetin	-	-	592.15±52.26	-	-	-
Quercetin		-	2104.05±279.72	-	-	-
Quercetagetin-3,6-dimethylether	24.05±4.50	-	76700±14360.91	-	-	0.52±0.1
Isoquercetin	6.69±1.92	-	5749.28±1649.65	-	-	-
Quercitrin	31.58±2.01	-	-	-	-	-
Luteolin	95.9±24.63	-	79350±20382.18	430.84±110.7	36.85±9.46	16.00±4.11
Luteolin-7-*O*-Glucoside	50.18±5.11	-	30083.67±3061.44	207.93±21.16	6.64±0.68	2.48±0.25
Luteolin-5-*O*-Glucoside	22.29±1.43	-	58400±3757.47	-	-	-
Apigenin	23.72±1.91	-	33201.13±2674.60	-	-	-
Rutin	26.6±1.74	-	14892.73±975.44	-	6.54±0.43	4.16±0.27
Coumaric Acids and derivatives						
p-Coumaric acid	-	323.90±64.1	4931.1±759.08	-	-	-
Caffeic acid	119.58±23.66	-	190450±37687.79	154.49±30.57	125.75±24.89	118.46±23.4
(*E*)-Ferulic acid	422.47±29.52	-	399000±27881.52	-	123.61±8.64	92.74±6.48
Chlorogenic acid	37.49±5.19	123.21±17.06	11027.18±1527.00	52.2±7.23	9.68±1.34	22.06±3.05
Rosmarinic acid	2494.15±191.25	-	-	-	2105.32±161.43	2634.67±22.02
Simple Phenolics and others						
Fumaric acid	538.13±37.32	-	117500±8148.5	-	290.83±20.17	1031.18±71.51
Gallic acid	-	487.27±33.79	-	333.56±23.13	6.24±0.43	-
Pyrogallol	21.06±1.40	-	24833.91±1652.56	-	22.03±1.47	26.03±1.73
Ellagic acid	-	-	-	-	-	-
Vanillin	-	-	-	-	-	-
Syringic acid	10.9±0.73	-	19628.58±1321.70	-	9.58±0.65	-
Salicylic acid	21.43±8.65	-	106550±43015.10	-	-	48.75±19.68
p-OH benzoic acid	135.99±10.80	-	32429.73±2576.46	-	-	151.56±12.04
Total (mg/kg dried herba)	**4335.28**	**934.38**	**1386912.28**	**1179.02**	**2920.08**	**4206.49**

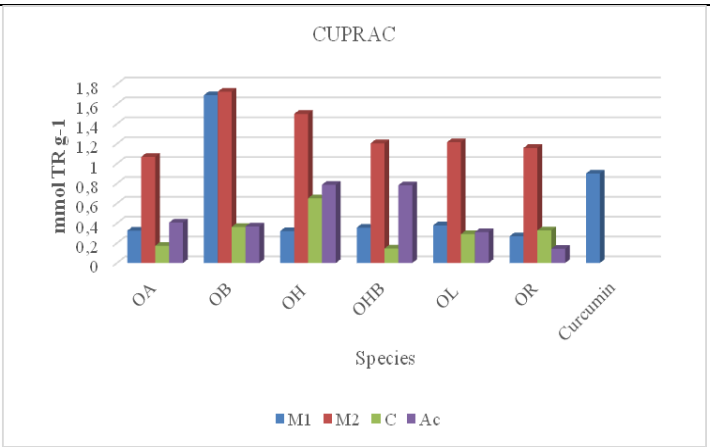

Figure 1. Cu^{2+} reducing power (CUPRAC) assay of the extracts and curcumin

Table 5. Phenolic content of the C extracts

Compounds	OA	OB	OH	OHB	OL	OR
Flavonoids and derivatives						
Kaempferol	-	-	18.42±1.3	-	-	-
Kaempferol-3-rutinoside	-	-	-	-	-	-
Salvigenin	4.41±0.3	12.06±0.82	-	18.16±1.24	11.06±0.75	69.84±4.75
Penduletin	20.56±2.08	4.80±0.49	14.6±1.48	9.43±0.96	81.83±8.3	93.35±9.46
Isorhamnetin	-	-	-	-	-	-
Quercetin	-	-	-	-	-	-
Quercetagetin-3.6-dimethylether	38.82±7.27	-	133.11±24.92	-	-	87.42±16.37
Isoquercetin	-	-	-	-	-	-
Quercitrin	-	-	-	-	-	-
Luteolin	-	-	-	-	-	-
Luteolin-7-*O*-Glucoside	-	-	-	-	-	-
Luteolin-5-*O*-Glucoside	-	-	-	-	-	-
Apigenin	-	-	9.64±0.78	-	-	-
Rutin	-	-	-	-	-	-
Coumaric Acids and derivatives						
p-Coumaric acid	-	7.91±1.56	-	-	-	-
Caffeic acid	-	-	-	6.24±1.23	12.05±2.38	-
(*E*)-Ferulic acid	-	-	-	-	-	-
Chlorogenic acid	-	6.83±0.95	-	10.18±1.41	7.8±1.08	-
Rosmarinic acid	-	3.37±0.26	-	3.53±0.27	3.63±0.28	-
Simple Phenolics and others						
Fumaric acid	-	-	-	-	-	-
Gallic acid	-	-	-	-	-	-
Pyrogallol	-	-	-	-	-	-
Ellagic acid	-	-	-	-	-	-
Vanillin	-	-	-	4.7±0.43	6.33±0.58	-
Syringic acid	-	-	-	-	-	-
Salicylic acid	-	-	-	-	-	-
p-OH benzoic acid	-	-	-	4.15±0.33	5.49±0.44	-
Total (mg/kg dried herba)	**63.79**	**34.97**	**175.77**	**56.39**	**128.19**	**250.61**

Table 6. Phenolic content of the Ac extracts

Compounds	OA	OB	OH	OHB	OL	OR
Flavonoids and derivatives						
Kaempferol	349.4±24.66	1149.62±81.14	85.38±6.03	170.9±12.06	577.63±40.77	65.64±4.63
Kaempferol-3-rutinoside	3.83±0.35	27.32±2.47	-	-	2.49±0.23	-
Salvigenin	-	-	-	10.24±0.7	-	16.08±1.09
Penduletin	4.17±0.42	44.86±4.55	18.8±1.91	12.26±1.24	54.7±5.55	43.55±4.41
Isorhamnetin	-	71.15±6.28	-	5.5±0.49	-	-
Quercetin	3.48 ±0.46	79.15±10.52	-	3.03±0.4	7.9±1.05	-
Quercetagetin-3.6-dimethylether	10.25±1.92	31.16±5.83	179.49±33.61	-	-	45.53±8.53
Isoquercetin	-	-	-	-	-	-
Quercitrin	-	-	-	-	-	-
Luteolin	134.77±34.62	329.99±84.76	22.36±5.74	58.34±14.99	129.97±33.38	10.51±2.70
Luteolin-7-*O*-Glucoside	4.71±0.48	31.71±3.23	-	-	-	-
Luteolin-5-*O*-Glucoside	-	8.84±0.57	-	-	-	-
Apigenin	67.05±5.40	-	32.56±2.62	-	-	21.19±1.71
Rutin	4.93±0.32	9.24±0.61	16.18±1.06	8.21±0.54	1.59±0.1	1.45±0.09
Coumaric Acids and derivatives						
p-Coumaric acid	-	96.55±19.11	-	14.53±2.24	-	-
Caffeic acid	50.22±9.94	-	65.62±12.99	59.7±11.81	32.39±6.41	21.05±4.17
(*E*)-Ferulic acid	25.86±1.81	-	27.69±1.94	46.92±3.28	-	9.02±0.63
Chlorogenic acid	-	7.16±0.99	-	8.18±1.13	6.78±0.94	-
Rosmarinic acid	574.02±44.02	1404.50±107.7	1056.84±81.04	1002.22±76.85	322.06±24.7	78.67±6.03
Simple Phenolics and others						
Fumaric acid	-	-	-	4.27±0.3	-	-
Gallic acid	-	8.78±0.61	-	12.09±0.84	5.40±0.37	-
Pyrogallol	-	-	-	-	-	-
Ellagic acid	-	9.82±0.66	-	-	-	-
Vanillin	8.5±0.78	-	-	46.38±4.24	22.33±2.04	-
Syringic acid	9.49± 0.64	10.88±0.73	-	18.04±1.21	1.25±0.08	-
Salicylic acid	-	-	-	-	-	-
p-OH benzoic acid	-	-	-	47.05±3.74	22.83±1.81	-
Total (mg/kg dried herba)	**1250.68**	**3320.73**	**1504.92**	**1527.86**	**1187.32**	**312.69**

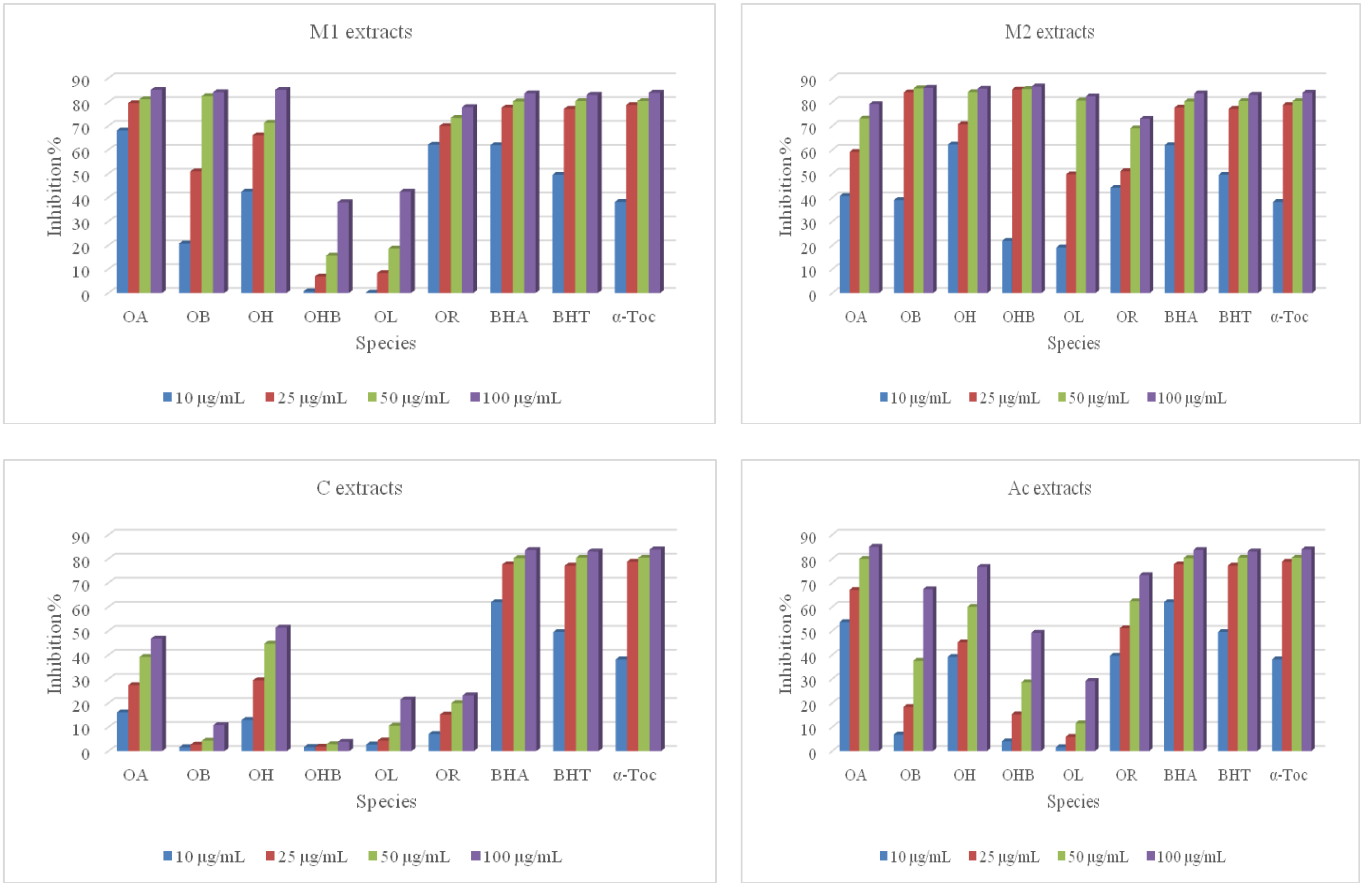

Figure 2. Antioxidant Activities of the extracts; DPPH free radical scavenging activity of the extracts and α-Toc, BHA and BHT

Table 7. Anticholinesterase Activity of the the extracts

	Inhibition% (200µg/mL)							
	AChE				**BChE**			
	M1	**C**	**Ac**	**M2**	**M1**	**C**	**Ac**	**M2**
OA	0	0	0	0	52.67±0.22	50.64±0.21	45.93±0.86	0
OB	0	33.48±1.23	23.33±0.45	5.59±0.29	14.88±0.35	35.20±0.93	49.63±0.66	0
OH	0	0	0	0	41.08±0.79	38.90±1.12	31.47±0.58	9.52±0.48
OHB	0	0	0	0	23.14±0.43	33.49±1.65	31.91±0.63	0
OL	0	0	0	0	8.44±0.16	50.93±1.35	47.97±0.62	0
OR	0	0	0	0	23.98±0.63	36.22±0.82	37.64±0.93	0
Control *	80.24±0.28	82.10±0.51	82.10±0.51	80.24±0.28	80.78±1.22	82.05±0.48	82.05±0.48	80.78±1.22

* Galantamine used as a control.

Figure 3. Inhibition (%) of lipid peroxidation of the extracts and α-Toc, BHA and BHT

In conclusion, we examined and reported the main essential oil, phenolic components and antioxidant and anticholinesterase activity of methanol, chloroform and acetone extracts of six *Origanum* species belonging to sect. *Brevifilamentum*, five of which are endemic to Turkey. Although there are numerous reports on the volatile compositions of these species, there is no report available on the phenolic composition. To the best of our knowledge, the phenolic compositions and activities of the extracts are reported for the first time. It has been shown that the oils of sect. *Brevifilamentum* are different types. However, when the phenolic compounds are considered, they are found to be rich in coumaric acid and its derivatives. Especially rosmarinic acid was the main compound of this section. Besides, the species which have rosmarinic acid in a high proportion showed the best activity. This study supported that *Origanum* species is very important species which are commonly used as spices, tea and alternative medicinal drug for many purposes throughout the world.

Acknowledgments

This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) [grant number 113Z225].

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

ORCID
Ahmet C Goren: 0000-0002-5470-130X

References

[1] T. Baytop (1984). Türkiye'de bitkiler ile tedavi, İstanbul Üni. Yay. İstanbul-Türkiye. (In Turkish)
[2] A. Güner, S. Aslan, T. Ekim, M. Vural and M. Babaç (2012). Türkiye bitkileri listesi (damarlı bitkiler). Nezahat Gökyiğit botanik bahçesi ve flora araştırmaları derneği yayını. İstanbul. (In Turkish)
[3] T. Özcan, T. Dirmenci, F. Coşkun, E. Akçiçek and Ö. Güner (2015). A new species of *Teucrium* sect. *Scordium* (Lamiaceae) from SE of Turkey, *Turk. J. Bot.* **39**, 310-317.
[4] M. Vural, H. Duman, T. Dirmenci and T. Özcan. (2015). A new species of *Teucrium* sect. *Stachyobotrys* (Lamiaceae) from the south of Turkey, *Turk. J. Bot.* **39**, 318-324.
[5] H. Duman and T. Dirmenci (2017). A new species of *Micromeria* (Lamiaceae) from Köyceğiz (Muğla, southwest of Turkey), *Turk. J. Bot.* DOI: 10.3906/bot-1612-49 (in press)
[6] F. Celep and T. Dirmenci (2017). Systematic and Bio-Geographic Overview of Lamiaceae in Turkey. *in Proceedings of International Symposium on Advances in Lamiaceae Science, Antalya, Turkey.*
[7] [cited 15/04/2017]. Royal Botanic Gardens-Kew [Web Page] 2017; Available from: http:// http://www.kew.org/
[8] J. H. Ietswaart (1980). A taxonomic revision of the genus *Origanum* (Labiatae). Leiden Botanical Series. No. 4, Leiden University Press, Netherlands.
[9] T. Dirmenci, T. Yazıcı, T. Arabacı, S. Çelenk, T. Özcan and E. Dündar (2016). A new hybrid of *Origanum* L.: *O.* × *dumanii* Dirmenci, Arabacı & Yazıcı. *in Proceedings of SEAB, Antalya, Turkey.*
[10] T. Dirmenci, T. Yazıcı, T. Arabacı, S. Çelenk, T. Özcan and E. Dündar (2016). A new hybrid of *Origanum* L.: *O.* × *bilgilii* Dirmenci, Yazıcı & Arabacı, *in Proceedings of SEAB, Antalya, Turkey.*
[11] T. Dirmenci, T. Yazıcı, T. Arabacı, S. Çelenk, T. Özcan and E. Dündar (2016). A new species of *Origanum* L.: *O. ayliniae* Dirmenci, Akçiçek &Yazıcı. *in Proceedings of SEAB, Antalya, Turkey.*
[12] T. Dirmenci, T. Yazıcı, T. Arabacı, S. Çelenk, T. Özcan and E. Dündar (2016). A new hybrid of *Origanum* L.: *O.* × *adae* Dirmenci, Yazıcı & Akçiçek, *in Proceedings of SEAB, Antalya, Turkey.*
[13] K. H. C. Başer (2002). The *Turkish Origanum Species. In: Oregano. The Genera Origanum and Lippia,* (Ed. SE Kintzios). Taylor and Francis, London.
[14] P. K. Joshi, N. Joshi, G. Tewari and S. Tandon (2015). Chemical, biological and pharmacological aspects of *Origanum* species: A brief review, *J. Indian. Chem. Soc.* **92**, 1603-1615.
[15] G. Özkan and M. M. Özcan (2014). Some phenolic compounds of extracts obtained from *Origanum* species growing in Turkey, *Environ. Monit. Asses.* **186**, 4947-4957.
[16] A. Basli, J. C. Delaunay, E. Pedrot, S. Bernillon, K. Madani, J. P. Monti, J. M. Merillon, M. Chibane and R. Tristan (2014). New cyclolignans from *Origanum glandulosum* active against b-amyloid aggregation, *Rec. Nat. Prod.* **8(3)**, 208-216.
[17] S. Gülsoy (2012). Evaluation of essential oils and phenolic compounds of some *Origanum* (Labiatae/Lamiaceae) taxonomy, *Asian J. Chem.* **24(6)**, 2479.
[18] S. Chishti, A. Z. Kaloo and P. Sultan (2013). Medicinal impotance of genus *Origanum*: A review, *J. Pharmacognosy Phytother.* **5 (10),** 170-177.
[19] K. H. C. Baser, G. Tümen and H. Duman (1997). The essential oil of *Origanum acutidens* (Hand.-Mazz.) Iestwaart, *J.Essent. Oil Res.* **9**, 91-92.
[20] K. H. C. Baser, T. Özek and G. Tümen (1995). The essential oil of *Origanum rotundifolium* Boiss., *J. Essent. Oil Res*. **7**, 95-96.
[21] G. Figueredo, M. M. Ozcan, J. C. Chalchat, Y. Bagci, P. Chalard, F. Al Juhaimis and S. Dogu (2015). Chemical composition of essential oil from aerial parts and fruits of *Hyssopus officinalis* L., *Origanum acutidens*, *Prangos uechritzii*, H-hircinum L. and *Hypericum polyphyllum* subsp *polyphyllum*, *Zeitschrift fur Arznei-& Gewurzpflanzen*, **20(4)**, 185-191
[22] G. Figuérédo, J. C. Chalchat and B. Pasquier (2006). Studies of Mediterranean oregano populations IX: chemical composition of essential oils of seven species of oregano of various origins, *J. Essent. Oil Res.* **18(4)**, 411-415.
[23] S. Kordali, B. Emsen and E. Yıldırım (2013). Fumigant toxicity of essential oils from fifteen plant species against Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae), *Egyp. J. Biolog. Pest. Cont*. **23(2)**, 241-246.
[24] O. Caglar, T. Calmasur, I. Aslan and O. Kaya (2007). Insecticidal effect of essential oil of *Origanum acutidens* against several stored product pests, *Fresen. Environ. Bull.* **16(11)**, 1395-1400.

[25] M. Kesdek, S. Kordali, A. Usanmaz and S. Ercisli (2015). The toxicity of essential oils of some plant species against adults of colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), *Comptes rendus de l'Académie bulgare des Sciences*. **68(1)**, 127-136.

[26] M. Kesdek, N. Bayrak, S. Kordali, A. Usanmaz, G. Contuk and S. Ercisli (2013). Larvicidal Effects of some essential oils against larvae of the pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller)(Lepidoptera: Thaumetopoeidae), *Egyp. J. of Biolog. Pest Cont.* **23(2)**, 201-207.

[27] T. Gokturk, S. Kordali, O. Calmasur and G. Tozlu (2011). Insecticidal effects of essential plant oils against larvae of great spruce bark beetle, *Dendroctonus micans* (Kugelann)(Coleoptera: Curculionidae: Scolytinae), *Fres. Environ. Bull.* **20**, 2365-2370.

[28] S. Kordali, A. Cakir, H. Ozer, R. Cakmakci, M. Kesdek and E. Mete (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene, *Bioresour. Technol.* **99(18)**, 8788-8795.

[29] E. Tozlu, A. Cakir, S. Kordali, G. Tozlu, H. Ozer and T. A. Akcin (2011). Chemical compositions and insecticidal effects of essential oils isolated from *Achillea gypsicola*, *Satureja hortensis*, *Origanum acutidens* and *Hypericum scabrum* against broadbean weevil (*Bruchus dentipes*), *Sci. Horticult.* **130(1)**, 9-17.

[30] I. Goze, A. Alim, S. A. Cetinus, A. Cetin, N. Durmus, A.T. Atas and N. Vural (2010). In vitro antimicrobial, antioxidant, and antispasmodic activities and the composition of the essential oil of *Origanum acutidens* (Hand.-Mazz.) Ietswaart, *J. Med. Food*. **13(3)**, 705-709.

[31] I. Goze, A. Alim, A. S. Tepe, M. Sokmen, K. Sevgi and B. Tepe (2009). Screening of the antioxidant activity of essential oil and various extracts of *Origanum rotundifolium* Boiss. from Turkey, *J. Med. Plants Res.* **3(4)**, 246-254.

[32] M. Sökmen, J. Serkedjieva, D. Daferera, M. Gulluce, M. Polissiou, B. Tepe, H. A. Akpulat, F. Sahin and A. Sokmen (2004). In vitro antioxidant, antimicrobial and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*, *J. Agri. Food Chem.* **52(11)**, 3309-3312.

[33] M. Kurşat, I. Emre, Ö. Yılmaz and P. Erecevit (2011). Antioxidant and antimicrobial activity in the seeds of *Origanum vulgare* L. subsp. *gracile* (C. Koch) Ietswaart and *Origanum acutidens* (Hand. -Mazz.) Ietswaart from Turkey, *Grasas Y. Aceites*. **62(4)**, 410-417.

[34] B. Cetin, S. Cakmakci and R. Cakmakci (2011). The investigation of antimicrobial activity of thyme and oregano essential oils, *Turk. J. Agri. Forest.* **35(2)**, 145-154.

[35] F. Dadasoglu, T. Aydin, R. Kotan, A. Cakir, H. Ozer, S. Kordali, R. Cakmakci, N. Dikbas and E. Mete (2011). Antibacterial activities of extracts and essential oils of three *Origanum* species against plant pathogenic bacteria and their potential use as seed disinfectants, *J. Plant Pathol.* **93(2)**, 271-282.

[36] B. Cosge, A. Turker, A. Ipek and B. Gurbuz (2009). Chemical compositions and antibacterial activities of the essential oils from aerial parts and corollas of *Origanum acutidens* (Hand.-Mazz.) Ietswaart, an endemic species to Turkey, *Molecules* **14(5)**, 1702-1712.

[37] K. H. C. Baser, M. Kürkçüoglu and G. Tümen (1998). Composition of the essential oil of *Origanum haussknechtii* Boiss., *J. Essent.Oil Res.***10**, 227-228.

[38] K. H. C. Baser, M. Kürkçüoglu, H. Duman and Z. Aytaç (1998). Composition of the essential oil of *Origanum husnucan-baseri* H.Duman, Z.Aytaç et A.Duran, a new species from Turkey, *J.Essent. Oil Res*. **10**, 419-421.

[39] B. Uysal, F. Sozmen, E. O. Kose, I. Gokhan Deniz and B. S. Oksal (2010). Solvent-free microwave extraction and hydrodistillation of essential oils from endemic *Origanum husnucanbaseri* H. Duman, Aytaç & A. Duran: comparison of antibacterial activity and contents, *Nat. Prod. Res.* **24(17)**, 1654-1663.

[40] K. H. C. Baser, N. Ermin, T. Özek, B. Demirçakmak, G. Tümen and H. Duman (1996). Essential oils of *Thymbra sintenisii* Bornm. et Aznav. subsp. *isaurica* P.H.Davis and *Origanum leptocladum* Boiss., *J.Essent. Oil Res.* **8**, 675-676.

[41] N.Tanker, F.Ilisulu, M.Koyuncu and M.Coskun (1986). Phytochemical screening of plants from the Ermenek-Mut-Gülnar (Turkey) area. III Labiatae, Int. J. Crude Drug Res. **24 (4)**, 177-182.

[42] A. C. Goren, F. Piozzi, E. Akcicek, T. Kılıç, S. Çarıkçı, E. Mozioğlu and W. N. Setzer (2011). Essential oil composition of twenty-two *Stachys* species (mountain tea) and their biological activities, *Phytochem. Lett.* **4**, 448-453.

[43] S. D. Hatipoglu, N. Zorlu, T. Dirmenci, A. C. Goren, T. Ozturk and G. Topcu (2016). Determination of volatile organic compounds in forty-five *Salvia* species by thermal desorption-GC-MS technique, *Rec. Nat. Prod.* **10**, 659-700.

[44] R. P. Adams (2007). Identification of essential oil components by gas chromatography/mass Spectrometry. 4th Ed., Allured Publishing Corp., Carol Stream, Illinois.
[45] A. C. Gören, S. Çıkrıkçı, M. Çergel and G. Bilsel (2009). Rapid quantitation of curcumin in turmeric via NMR and LC-tandem mass spectrometry, *Food Chem.* **113**, 1239-1242.
[46] A. C. Goren, G. Bilsel and M. Bilsel (2007). Rapid and simultaneous determination of 25-OH-vitamin D2 and D3 in human serum by LC/MS/MS: validation and uncertainty assessment, *J. Chem. Metrol.* **1**, 1-10.
[47] P. Kalin, I. Gulcin and A. C. Goren (2015). Antioxidant activity and polyphenol content of Cranberries (*Vaccinium macrocarpon*), *Rec. Nat. Prod.* **9**, 496-502.
[48] G. L. Ellman, K. D. Courtney, V. Andres and R. M. Featherston (1961). A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharma*. **7,** 88-95.
[49] A. Yilmaz, M. Boga and G. Topçu (2016). Novel terpenoids with potential anti-alzheimer activity from *Nepeta obtusicrena*., *Rec. Nat. Prod.* **10(5)**, 530-541.
[50] N.B. Reddy, C.S. Sundar, S.H. Jayaprakash, G.Mohan, P.V.Reddy and C.S.Reddy (2016). Synthesis and antioxidant activity of dioxazaphosphinin-2-ones, *Org.Commun.* 8, 17-23
[51] M. S. Blois (1958). Antioxidant determinations by the use of a stable free radical, *Nature* **181**, 1199-1200.
[52] A. Ertas, A. C. Gören, N. Hasimi, V. Tolan and U. Kolak (2015). Evaluation of antioxidant, cholinesterase inhibitory and antimicrobial properties of *Mentha longifolia* subsp. *noeana* and its secondary metabolites, *Rec. Nat. Prod.* **9(1)**, 105-115.
[53] N.B. Reddy, C.S. Sundar, S.H. Jayaprakash, G. Mohan, P.V. Reddy and C.S. Reddy (2015). Synthesis and antioxidant activity of dioxazaphosphinin-2-ones, *Org. Commun.* **8**, 17-23.
[54] R. Apak, K. Güçlü, M. Özyürek and S. E. Karademir (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay, *Microchim. Acta*. **160**, 413-419.
[55] B. Sreedhar, T.V. Reddy, C.N. Raju and G.V.S. Reddy (2016). Design, synthesis, characterization and bioassay of novel carboxamide derivatives of celecoxib, *Org. Commun.* **9**, 54-64
[56] D. Benedec, D. Hanganu, I. Oniga, B. Tiperciuc, N. K. Olah, O. Raita, C. Bischin, R. Silaghi-Dumitrescu and L. Vlase (2015). Assessment of rosmarinic acid content in six Lamiaceae species extracts and their antioxidant and antimicrobial potential, *Pak. J. Pharm. Sci.* **28**, 2297-2303.