Arabian Journal of Chemistry (2020) **13**, 5027–5039

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

The phenolic contents, antioxidant and anticholinesterase activity of section *Amaracus* (Gled.) Vogel and *Anatolicon* Ietsw. of *Origanum* L. species

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Received 20 November 2019; accepted 28 January 2020
Available online 6 February 2020

KEYWORDS

Origanum;
Amaracus;
Anatolicon;
Essential oil;
Phenolics;
Antioxidant activity;
AChE;
BChE

Abstract *Origanum boissieri* Ietsw., *O. saccatum* P.H.Davis, *O. solymicum* P.H.Davis and *O. ayliniae* Dirmenci & T.Yazıcı belonging to sect. *Amaracus* (Gled.) Vogel, *O. sipyleum* L. and *O. hypericifolium* O.Schwarz & P.H.Davis belonging to sect. *Anatolicon* Ietsw. were analyzed for their chemical composition of essential oil and phenolic components. The essential oil compositions were analysed by using GC-MS and GC-FID. The phenolic contents of the chloroform, acetone, and methanol extracts were analyzed using LC-MS/MS. Antioxidant activities of the extracts were investigated by using three methods; DPPH free radical scavenging activity, β-carotene linoleic acid assays and CUPRAC assays. The essential oil compositions of the section *Amaracus* were found to be as carvacrol type (*O. ayliniae, O. boissieri*) and *p*-cymene type (*O. saccatum, O. solymicum*). In the section of *Anatolicon,* while *O. sipyleum* was found as γ-terpinene type, *O. hypericifolium* was carvacrol type. In the extracts, the most abundant components were determined as flavonoids, coumaric acids and derivatives. Especially rosmarinic acid and penduletin were detected in high

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.arabjc.2020.01.025
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amounts. Among the studied species, extracts of *O. ayliniae* showed quite good activity for all methods. The extracts from all species showed remarkable antioxidant activity. Inhibition capability of the extracts against acetyl and butyrylcholinesterase enzymes (AChE and BChE) were determined. The extracts were found as inactive against AChE. The moderate inhibition capacity observed against BChE.

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1. Introduction

Many species of aromatic plants have been used in the treatment of various diseases as spice among the population since ancient times. Especially in the last two decades, the members of Lamiaceae (Labiatae) family has become important (Baser, 1993; Celep and Dirmenci, 2017). Turkey is respected as an important gene-centre for the Lamiaceae family. The family is represented by 48 genera, 603 species and 782 taxa in Turkey (Celep and Dirmenci, 2017; Yılmaz et al., 2017). The rate of endemism in the family is 44% (Yılmaz et al., 2017).

One of the most used Lamiaceae members is *Origanum* L. The genus *Origanum* comprises 43 species in the world. The species are mainly concentrated in the temperate regions of the Mediterranean and South-West Asia. In Turkey, the genus *Origanum* consist of 21 species (24 taxa), 13 of which are endemic and, 13 hybrids (12 of which are endemic) (Dirmenci et al., 2018a; Dirmenci et al., 2018b; Dirmenci et al., 2019; Yılmaz et al., 2017). In Turkey, endemic species are concentrated within the Mediterranean region (Ietswaart, 1982). Section *Amaracus* (Gled.) Vogel consists of 4 endemic species: *Origanum boissieri* Ietsw. (Taş mercan), *O. saccatum* P.H.Davis (Tahtacı kekiği), *O. solymicum* P.H.Davis (Kuz mercan) and a new species of *O. ayliniae* Dirmenci & T.Yazıcı. Section *Anatolicon* Ietsw. consists of 2 endemic species: *O. sipyleum* L. (Mor mercan) and *O. hypericifolium* O.Schwarz & P.H.Davis (Delik mercan). *Origanum* species have areas of usages in the pharmaceutical and food industry due to the antioxidant (Fotakis et al., 2016; Hajlaoui et al., 2016; Yan et al., 2016), antimicrobial (Hajlaoui et al., 2016), antibacterial (Evrendilek, 2015), cytotoxic (Sivropoulou et al., 1996), antifungal (Manohar et al., 2001), insecticidal (Pavela, 2004) and other biological activities of their essential oil, which rich in phenolic compounds, especially carvacrol.

Due to their biological activities (Yılmaz et al., 2017; Fotakis et al., 2016; Hajlaoui et al., 2016), many phytochemical studies of *Origanum* species have been studied intensively (Yan et al., 2016; Evrendilek, 2015). The studies especially focused on essential oils and their biological activities. Phenolic compounds, especially carvacrol and thymol were determined as the main compounds in the *Origanum* essential oils (Yılmaz et al., 2017; Sezik et al., 1993; Baser et al., 1993a; Baser et al., 1993b). In addition, there are many studies in the literature about phenolic composition of the extracts of *Origanum* species (Ozkan et al., 2007).

In the previous studies, *O. solymicum* (Tumen et al., 1994; Figuérédo et al., 2006), *O. saccatum* (Tümen et al., 1995; Ozcan and Chalchat, 2009) and *O. boissieri* (Baser and Duman, 1998) were detected as *p*-cymene rich. *O. hypericifolium* (Baser et al., 1994; Celik et al., 2010; Ili, 2016) and *O. sipyleum* (Baser et al., 1992) were found carvacrol, *p*-cymene and γ-terpinene rich. Biological activities of essential oil and extracts of the *Origanum* species have also been determined such as antioxidant, antimicrobial, antibacterial, antifungal and antileishmanial activities (Baser et al., 1993a; Ozcan and Chalchat, 2009; Fakir et al., 2015; Dulger, 2006; Nakiboglu et al., 2007; Ozbilgin et al., 2014; Karagöz et al., 2015). The studies especially focused on *Anatolicon* section. The antioxidant activity and total phenolic content of water, ethanol, methanol and acetone extracts (Nakiboglu et al., 2007), antioxidant, antimicrobial and free-radical-scavenging activities of the methanol extract and antileishmanical activity of *O. sipyleum* (Baser et al., 1993a; Ozbilgin et al., 2014; Karagöz et al., 2015) were reported before. There are few studies reporting the activity and phenolics of essential oil of *O. hypericifolium* and *O. saccatum*. Total phenolic content, antioxidant, antimicrobial activity (Celik et al., 2010), antifungal activity (Ocak et al., 2012) of *O. hypericifolium*, and antibacterial and antimicrobial activity of *O. saccatum* were reported in the literature (Ozcan and Chalchat, 2009; Sozmen et al., 2011).

There is only one study in the literature for *Amaracus* section. The antimicrobial activity of *O. solymicum* was investigated previously (Dulger, 2006). *O. ayliniae* is a newly identified species and has been reported to belong to the *Amaracus* section (Dirmenci et al., 2018a). Its chemical components and activities were studied for the first time in this study.

The main reason for the unique activity of aromatic plants is the chemical components which they contain. Not only essential oils but also to determine other secondary metabolites is important. *Origanum* species, which are rich in essential oils, have been studied with many studies, but there are few studies about their phenolic contents (Yılmaz et al., 2017). The objectives of this study are to investigate antioxidant and anticholinesterase activities and to determine the phenolic composition of the extracts obtained from *Origanum* species belonging to sect. *Amaracus* (*O. boissieri, O. saccatum, O. solymicum, O. ayliniae*) and sect. *Anatolicon* (*O. sipyleum, O. hypericifolium*). Furthermore, the composition of the essential oils depends on factors such as year, climate, solar angle, and collection region; the volatile oil compositions of the species of these two sections have been re-examined.

2. Materials and methods

2.1. Plant material

Localities, coordinates and collector numbers of the *Origanum* species are given in 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	Collector Number	Species	Locality	Altitude (m)	Coordinates	Year
OB	TD 4285	*Origanum boissieri*	Mersin: Tarsus, Between Çamlıyayla and Saimdibi, 15th km	1842	N37 22 824 E34 55 510	16.08.2014
OS	TD 4296	*Origanum saccatum*	Alanya: Between Gökbel and Çökelek plateau, 8th km	1372	N36 62 604 E32 33 147	17.08.2014
OSL	TD 4302	*Origanum solymicum*	Antalya: Kemer, Kesme boğazı, under P. brutia, calcareous rocks	104	N36 59 767 E30 49 907	18.08.2014
OA	TD 4435	*Origanum ayliniae*	Aydın: Kuşadası, Dilek Peninsula NationalPark, rocky slopes	1195	N37 39 412 E27 08 575	30.07.2015
OSP	TD 4308	*Origanum sipyleum*	Denizli: Between Serinhisar and Denizli, 5th km	1039	N37 61 968 E29 26 801	19.08.2014
OH	TD 4315	*Origanum hypericifolium*	Denizli: Honaz, Honaz mountain, north slope, on the road of Arpacık plateau, under P.nigra	1268	N37 72 990 E29 26 676	19.08.2014

2.2. Chemicals

Chloroform (Merck), acetone (Merck) and methanol (Merck) were used for the preparation of the extracts. The compounds were used as standards in LC-MS/MS analysis given in the supplementary material. 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), which were stored at −20 °C in glass containers. 100 mg/L curcumin solution was freshly prepared, from which 50 µL was used as an Internal Standard (IS) in all experiments (Çarıkçı et al., 2018; Sagir et al., 2017).

2.3. General

LC-MS/MS experiments were performed by a Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole (Istanbul, Turkey) mass spectrometry, equipped with a SynergyMax C18 column (250 × 2 mm i.d., 5-µm particle size). The compounds used as standards in LC-MS/MS analyses were given in the supplementary material. For the antioxidant and anticholinesterase activity, the absorbance (UV and visible range 230 nm to 750 nm) was measured using a multiplate reader (Beckman Coulter DTX 880 Multimode Detector). 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 × 0.32 m m, with 0.25 µm film thickness) was used with helium as a carrier gas at 1 mL/min flow rate (138 kPa). The detailed procedures were given in the supplementary material.

2.4. Essential oil

The aerial parts of *Origanum* species (100 g of each) which were air-dried in shade, were chopped into small pieces and subjected to hydrodistillation with water for 4 h, using a Clevenger-type apparatus to produce the essential oil. The obtained essential oils were stored in amber vials at 4 °C for further analyses. Essential oil yields of species are 0.51%, 1.13%, 0.65%, 0.60%, 0.73% and 0.26% from *O. boissieri, O. saccatum, O. solymicum, O. ayliniae, O. sipyleum* and *O. hypericifolium,* respectively.

2.5. Preparation of extracts

The air-dried grinded approximately 100 g of plant samples were directly extracted with methanol for 15 days. After filtration and evaporation, they were named M1. Also, another 100 g of the plant was extracted with chloroform (C) for 15 days. After filtration and evaporation, the residue was extracted with acetone (Ac) and methanol (MeOH) for 15 days, respectively. They were named C, Ac, and M2. All the extracts were kept at −20 °C until they were used for experimental studies.

2.6. Determination of antioxidant activity

2.6.1. β-carotene bleaching method

The antioxidant activity was evaluated using β-carotene-linoleic acid model system (Miller, 1971; Yılmaz et al., 2017). β-carotene (0.5 mg) in 1 mL of chloroform was added to 25 µL of linoleic acid, and 200 mg of Tween 40 emulsifier mixture. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen, was through vigorous shaking. A mixture of four thousand microlitres was transferred into different test tubes containing different concentrations of the sample (10, 25, 50 and 100 µg/mL). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The emulsion system was incubated for 2 h at 50 °C. A blank, devoid of β-carotene, was prepared for background subtraction. BHA, BHT and α-tocopherol were used as standard compounds. In the end, IC_{50} values of all samples were calculated.

2.6.2. DPPH free radical scavenging method

The free radical scavenging activity of the extracts was determined spectrophotometrically by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (Blois, 1958; Reddy et al., 2015; Ertas et al., 2015; Sreedhar et al., 2016; Halfon et al., 2019). In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of DPPH in methanol was prepared

and 160 µL of this solution was added to 40 µL of sample solutions in methanol at different concentrations (10, 25, 50 and 100 µg/mL). These tubes were left in the dark for 30 min. The measurements were made at 517 nm. BHA, BHT and α-tocopherol were used as standard compounds. The potentials of samples on DPPH were determined and compared to the standards. In the end, IC_{50} values of all samples were calculated. The reduction in absorbance shows the DPPH free radical scavenging of samples capability.

2.6.3. The CUPRAC method

The reducing capacities of extracts were evaluated using CUPRAC method (Apak et al., 2008; Apak, 2019). Briefly, 1 mM DMF, 10 mM $CuCl_2$, 7.5 mM Neocuproine, 1 M NH_4-CH_3COO (pH 7.0) solution, and distilled water were mixed in volume ratio 1:1:1:0.6. After 180 ul of the mixture was dispersed into the wells, 25 µL diluted compounds (dilution ratio 1:20) in EtOH. The samples were kept for 30 min at 25 °C. The absorbance was measured at 450 nm against a reagent blank. Ethanol was used as a negative control. Curcumin was used as a positive control.

2.6.4. Determination of the anticholinesterase activity

In vitro inhibition of AChE and BChE of the samples was assessed by the spectrophotometric method developed by Ellman, Courtney, Andres and Featherston (Ellman et al., 1961; Yılmaz et al., 2016; Reddy et al., 2015). Activities of AChE and BChE were designated using 5,50-dithiobis (2-nitrobenzoic) acid (DTNB) (Ellman et al., 1961; Yilmaz et al., 2016). The test solutions and 150 mL of 100 mM sodium phosphate buffer (pH 8.0) were mixed with AChE or BChE enzymes solutions. The mixture waited at 25 °C for 15 min. Then, 0.5 mM DTNB was added. The reaction was then initiated by the addition of acetylthiocholine iodide (0.71 mM) or butyrylthiocholinechloride (0.2 mM). The activity was measured at 412 nm. Methanol was used as a solvent to dissolve test compounds and the controls. Inhibition % of AChE or BChE was determined by a comparison of the rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH 8.0) using the formula; $[(E-S)/E] \times 100$ where E is the activity of enzyme without test sample, and S is the activity of enzyme with test sample. Galanthamine (4 mg/mL) was used as a positive control. All tests were conducted in triplicate.

2.7. Statistical analysis

Statistical analyses were used to evaluate antioxidant activity results by One-way ANOVA test. (GraphPad, Software 8.3.0). $p < 0.05$ was taken as the minimum level of significance.

3. Results and discussion

3.1. Essential oil

A total of 61 different compounds were identified, constituting 97.8–100.0% of the total oil. 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. *O. boissieri* and *O. hypericifolium* were found to be rich in oxygenated monoterpenes. The main compound of the essential oil of *O. boissieri* and *O. hypericifolium* was carvacrol (30.1%, 68.8%, respectively). Other main compounds were determined as *p*-cymene (29.8%) and cis-*β*-terpineol (10.2%) for *O. boissieri,* borneol (9.2%) and (*Z*)-caryophyllene (5.4%) for *O. hypericifolium*. In the previous study, *O. boissieri* was found to be rich in *p*-cymene (42.8%) and carvacrol (17.57%) (Baser and Duman, 1998). The chemical composition of the *O. hypericifolium* essential oil was investigated by several studies. For the essential oil obtain, two different methods were used: steam distillation (SD) and direct thermal desorption (DTD). The determined major compounds were as follow: *p*-cymene and carvacrol (Celik et al., 2010), *p*-cymene (37.26%), thymol (11.86%) and borneol (10.26%) (Fakir et al., 2015), in the fruit and flower parts *p*-cymene (34.33%), carvacrol (21.76%) and thymol (19.54%) (Ocak et al., 2012). In the study, which aimed to determine of the difference in the oil composition of development stages of the *O. hypericifolium,* carvacrol (64.33%) found to be the major component of the oil when collected before flowering, whereas *p*-cymene (36.10–47.75%) was the major component when collected while in full flowering (Baser et al., 1994). Unlike these studies, it was reported that thymol (59.3%) was found to the main compound of *O. hypericifolium* (Figuérédo et al., 2006). *O. ayliniae* was dedected as oxygenated monoterpene rich and main compound was carvacrol (53.7%), with carvacrol methyl ether (14.4%) and *p*-cymene (13.9%). This is the first study of the essential oil composition of *O. ayliniae*. *O. saccatum, O. solymicum* and *O. sipyleum* were detected as monoterpene hydrocarbons rich. *p*-Cymene (37.9%, 29.6%, respectively), carvacrol (21.6%, 15.2%, respectively) and γ-terpinene (12.5%, 12.7%, respectively) were the main compounds of the essential oils of *O. saccatum* and *O. solymicum*. In different studies, essential oil of *O. saccatum* was characterized by its high content of *p*-cymene (Tümen et al., 1995; Ozcan and Chalchat, 2009; Sozmen et al., 2011). In the oil of *O. solymicum*, the major constituent was indentified as *p*-cymene (53.07%) (Tumen et al., 1994). *O. sipyleum* was found monoterpene hydrocarbon rich and the main compounds were detected as γ-terpinene (28.7%), *p*-cymene (21.6%) and carvacrol (21.2%). In the previous study, the oils of *O. sipyleum* collected from four different locations were investigated and, γ-terpinene (10.80–26.60%), *p*-cymene (3.76–36.60%), thymol methylether (trace-19.90%), carvacrol methylether (0.41–10.20%), thymol (0.23–7.30%) and carvacrol (0.82–12.20%) were determined as main compounds (Baser et al., 1992).

In the present study, the essential oil composition of *Amaracus* and *Anatolicon* section has been analyzed to have different chemotypes. This study demonstrated the presence of *O. boissieri, O. ayliniae* and *O. hypericifolium* in carvacrol type, which is known for its antioxidant, antimicrobial (Mathela et al., 2010), antifungal (Vinciguerra et al., 2019) and acaricidal (Cetin et al., 2010) activities. *O. saccatum* and *O. solymicum* were reported as *p*-cymene type, which is known for its antioxidant (Oliveira et al., 2015), acetylcholinesterase activity (Miyazawa and Yamafuji 2006) and antifungal (Kordali et al., 2008; Mirzania et al., 2018), phytotoxic and

Table 2 Essential oil composition of section *Amaracus* and *Anatolicon*.

			Amaracus				*Anatolicon*	
No	Compounds	KI*	OB**	OS**	OSL**	OA**	OSP**	OH**
	Hydrocarbons and derivatives							
1	3-methyl nonane	971	–	0.1	3.6	–	0.6	t
2	1-octen-3-ol	979		1.4	0.5	–	0.4	1.4
3	3-octanol	991	–	0.1	0.2	–	0.1	0.1
4	2-methyl decane	1063		–	0.5	–	0.2	0.6
5	undecane	1100	–	–	8.0	–	0.3	0.1
	% identified		**–**	**1.6**	**12.8**	**–**	**1.6**	**2.2**
	Monoterpene hydrocarbons							
6	α-thujene	930	–	**–**	0.4	–	0.7	–
7	α-pinene	939	–	0.1	1.5	–	0.3	–
8	camphene	954	0.3	–	1.1	–	t	–
9	sabinene	975	–	–	0.5	–	–	–
10	*β*-pinene	979	–	0.2	0.6	–	3.3	–
11	α-phellandrene	1003	–	t	0.1	–	0.1	–
12	α-terpinene	1017	–	0.4	1.2	–	1.6	t
13	***p*-cymene**	**1025**	**29.8**	**37.9**	**29.6**	**13.9**	**21.6**	1.6
14	limonene	1029	–	0.1	0.6	–	0.5	t
15	(E)-*β* -ocimene	1050	1.7	–	–	1.7	0.4	–
16	**γ-terpinene**	1060	0.2	**12.5**	**12.7**	–	**28.7**	1.3
	% identified		**32.0**	**51.2**	**48.3**	**15.6**	**57.2**	**2.9**
	Oxygenated monoterpenes							
17	sabinene hydrate-cis	1070	0.1	–	–	0.8	–	–
18	α-terpinolene	1089	–	–	0.3	–	0.1	0.1
19	pinene hydrate	1123	0.6	–	–	–	–	–
20	terpineol	1134	0.9	–	–	–	–	–
21	**cis-*β*-terpineol**	1144	**10.2**	–	–	–	–	–
22	camphor	1146	6.4	–	0.2	0.5	–	–
23	menth-3-en-8-ol	1150	2.6	–	–	–	–	–
24	menthone	1153	0.4	–	–	0.1	–	–
25	trans-*β*-terpineol	1163	0.5	–	–	0.1	–	–
26	borneol	1169	–	1.8	9.0	–	1.8	**9.2**
27	4-terpineol	1177	–	2.2	0.8	–	0.6	1.3
28	α-terpineol	1189	–	5.4	1.2	–	0.3	1.0
29	myrtenol	1196	–	0.3	0.2	–	–	0.1
30	carveol-cis	1229	–	–	–	0.7	–	–
31	carvone	1243	–	1.8	–	–	2.5	–
32	carvacrol methyl ether	1245	2.6	–	–	**14.4**	–	–
33	bornyl acetate	1289	–	0.2	0.3	–	–	–
34	thymol	1290	2.5	–	–	–	–	–
35	cymen-7-ol	1291	–	2.6	–	–	–	0.3
36	terpinene-7-al	1291	0.6	–	–	–	–	–
37	carvacrol, ethyl ether	1298	0.6	–	–	–	–	–
38	**carvacrol**	**1299**	**30.1**	**21.6**	**15.2**	**53.7**	**21.2**	**68.8**
	% identified		**58.1**	**35.9**	**28.4**	**70.3**	**26.5**	**80.8**
	Sesquiterpene hydrocarbons							
37	δ-elemene	1338	2.1	–	–	3.1	–	–
38	α-cubebene	1351	0.2	–	–	–	–	–
39	α-ylangene	1375	–	–	–	0.2	–	–
40	α-copaene	1377	–	–	–	–	1.1	–
41	*β*-bourbonene	1388	0.4	–	–	–	–	0.2
42	*β*-elemene	1391	–	0.2	–	–	–	–
43	(Z)-caryophyllene	1409	–	4.3	3.9	–	2.5	**5.4**
44	α-gurjunene	1410	–	0.1	–	–	–	–
45	aromadendrene	1441	2.0	–	–	5.6	–	–
46	α-humulene	1455	0.2	0.7	0.3	–	0.3	0.3
47	E-*β*-farnesene	1457	0.2	0.1	–	–	–	–
48	allo-aromadendrene	1460	1.0	–	–	2.1	–	–
49	τ-muurolene	1480	–	–	1.3	–	1.9	2.4
50	germacrene D	1485	–	–	1.3	–	4.0	0.4
51	α-cadinene	1539	–	–	0.1	–	0.8	0.2

(*continued on next page*)

Table 2 (*continued*)

			Amaracus				*Anatolicon*	
No	Compounds	KI*	OB**	OS**	OSL**	OA**	OSP**	OH**
	% identified		**6.1**	**5.4**	**6.9**	**11.0**	**10.6**	**8.9**
	Oxygenated sesquiterpenes							
52	spathulenol	1578	3.4	–	1.3	2.5	1.8	1.2
53	caryophyllene oxide	1583	0.1	3.0	2.1	0.1	0.5	1.7
54	α-cadinol	1654	0.3	–	–	–	–	–
55	ledol	1590	–	0.2	–	–	–	0.1
56	viridiflorol	1593	–	–	–	–	0.2	t
57	α-cadinol	1660	–	0.1	–	–	–	0.1
58	valeranone	1675	–	0.3	0.2	–	0.2	0.1
59	α-bisabolol	1686	–	0.1	t	–	0.2	–
	% identified		**3.8**	**3.7**	**3.6**	**2.6**	**2.9**	**3.2**
	Total (%)		**100.0**	**97.8**	**100.0**	**99.5**	**98.8**	**98.0**

* KI Kovats indices.
** OB: *O. boissieri,* OS: *O. saccatum,* OSL: *O. solymicum,* OA: *O. ayliniae,* OSP: *O. sipyleum,* OH: *O. hypericifolium.*

insecticidal properties (Kordali et al., 2008). O. *O. sipyleum* was reported as γ-terpinene type, which is known for its acetylcholinesterase (Miyazawa and Yamafuji 2006) and acaricidal (Cetin et al., 2010) activities.

3.2. Phenolic contents

The phenolic contents were analyzed under four groups; flavonoids and derivatives, coumaric acid and derivatives, simple phenolics and others and dicarboxylic acid (Fig. 2). Identified compounds and their quantities are given in Tables 3–6.

While the main phenolic components of the methanol extracts (M1 and M2) and the acetone extracts of species were shown differ in chemical structure, C extracts were determined rich in flavonoids and derivatives. Rosmarinic acid, penduletin, salvigenin, fumaric acid, kaempferol, gallic acid and pyrogallol are analyzed as most common compounds in the extracts. Main compounds of C extracts were analyzed as penduletin and salvigenin. Methanol extracts (M1 and M2) and Ac extracts were rich in rosmarinic acid. M2 extracts of *O. boissieri*, *O. solymicum* and *O. sipyleum*, Ac extracts of *O. saccatum*, *O. ayliniae* and *O. hypericifolium* were determined as rich in phenolic compounds. Especially Ac extract of *O. saccatum* was the richest (4092.48 mg/kg). *O. saccatum* and *O. solymicum* were found as the richest species, whereas *O. ayliniae* was the poorest in terms of phenolic compounds.

Rosmarinic acid was determined as the main components of most of the extracts: M1, Ac and M2 extracts of *O. boissieri*; M1, C and M2 extracts of *O. saccatum*; M1 and Ac extracts of *O. solymicum*; Ac and M2 extracts of *O. sipyleum*. There are few studies reporting the activity and phenolics of various extracts of *O. sipyleum*. Ozkan et al. (2007) reported the presence of apigenin, carvacrol, hesperidin, naringenin, rutin and vitexin in *O. sipyleum* methanol extract. Total phenolic contents, DPPH, OH radicals scavenging and total antioxidant capacities of *O. sipyleum* were investigated (Nakiboglu et al., 2007). Total phenolic content, antioxidan activity of water, methanol and chloroform extracts of *O. sipyleum, O. hypericifolium, O. majorana* and *O. onites* were reported in the literature (Semiz et al., 2018). Also, Zengin et al. (2019) reported that *O. sipyleum* can be considered as a good source of phenolic compounds such as rosmarinic acid and phlorizin. The C extracts of *O. boissieri*, *O. saccatum* and *O. hypericifolium*, Ac and M2 extracts of *O. ayliniae* were found to be rich in a flavonoid derivatives penduletin. Another flavonoid derivatives salvigenin was determined as the main compound for the C extracts of *O. solymicum*, *O. ayliniae* and *O. sipyleum*, kaempferol was determined as a major compound for Ac extracts of *O. saccatum* and *O. sipyleum*. While fumaric acid was determined as the main compounds for M1 extracts of *O. sipyleum* and *O. hypericifolium*, gallic acid was determined in M2 extract of *O. solymicum*.

3.3. Activity

The antioxidant activities were determined mainly using three methods; DPPH free radical scavenging activity, *β*-carotene linoleic acid assays and CUPRAC assays. In the DPPH and *β*-carotene linoleic acid assays, the activities were determined at four concentrations: 10, 25, 50 and 100 μg/mL. BHA, BHT and α-tocopherol were used as standards. The results are given as 50% inhibition concentrations (IC_{50}) in Table 7. In the CUPRAC, $TEAC_{CUPRAC}$ values of the extracts were calculated by using curcumin as a reference. $TEAC_{CUPRAC}$ of curcumin was found as 0.9 mmol TR g^{-1} (Fig. 1).

To evaluate the free radical scavenging effectiveness of extracts of species, DPPH method was used. Methanol (M1 and M2) and acetone (Ac) extracts of both section have good antioxidant activity for all tested methods. As shown in Table 7, among the studied species, all of the extracts of *O. ayliniae* exhibited a significant activity for *β*-carotene and DPPH methods when compared to that of standard antioxidants. In particular, *O. ayliniae* M1 extract exhibited a remarkable DPPH free radical scavenging activity. IC_{50} values for the radical scavenging activity of *O. ayliniae* M1 extract were found to be 7.63 μg/mL. Also, free radical scavenging activity of *O. ayliniae* extracts were compared to those of BHA, BHT and α-tocopherol. On the other hand, IC_{50} values for BHA, BHT and α-tocopherol were found to be 9.53 μg/mL, 11.04 μg/mL, 12.50 μg/mL, respectively. These results indicated that the free radical scavenging effect of *O. ayliniae* M1 extract was higher than those of BHA, BHT and

Table 3 Phenolic contents of the M1 extracts.

	Amaracus				*Anatolicon*	
	OB*	OS*	OSL*	OA*	OSP*	OH*
Flavonoids and derivatives						
Kaempferol	7.91 ± 0.56	119.09 ± 8.41	65.44 ± 4.62	14.9 ± 1.05	150.61 ± 10.6	208.75 ± 14.73
Salvigenin	–	104.83 ± 7.13	16.64 ± 1.13	97.06 ± 6.61	47.36 ± 3.22	29.11 ± 1.98
Penduletin	22.11 ± 2.24	169.52 ± 17.19	3.5 ± 0.36	103.77 ± 10.5	–	273.75 ± 27.75
Isorhamnetin	–	41.59 ± 3.67	–	–	–	–
Quercetin	–	13.47 ± 1.79	12.59 ± 1.67	–	–	11.88 ± 1.58
Quercetagetin-3,6-dimethylether	14.33 ± 2.68	52.54 ± 9.84	1.63 ± 0.3	–	–	3.19 ± 0.6
Quercitrin	–	–	–	–	22.91 ± 1.46	–
Luteolin	–	12.27 ± 3.15	5.02 ± 1.29	–	19.74 ± 5.07	40.26 ± 10.34
Luteolin-7-O-glucoside	–	–	9.6 ± 0.98	–	21.63 ± 2.2	–
Luteolin-5-O-glucoside	–	–	–	–	134.15 ± 8.63	–
Rutin	2.54 ± 0.17	15.02 ± 0.98	10.35 ± 0.68	–	1.79 ± 0.12	5.28 ± 0.35
Pelargonin	–	–	–	50.65 ± 5.15	–	–
Total (mg/kg dried herba)	46.89	528.33	124.77	266.38	398.19	572.22
Coumaric acids and derivatives						
Caffeic acid	71.62 ± 14.17	78.98 ± 15.63	99.45 ± 19.68	–	112.33 ± 22.2	79.36 ± 15.71
(*E*)-Ferulic acid	70.18 ± 4.9	158.83 ± 11.1	143.73 ± 10.04	187.43 ± 13.1	130.43 ± 9.11	123.15 ± 8.61
Chlorogenic acid	13.09 ± 1.81	123.39 ± 17.09	70.07 ± 9.7	166.29 ± 23.0	11.12 ± 1.54	15.79 ± 2.19
Rosmarinic acid	**1358.25 ± 104.15**	**1462.53 ± 112.1**	**2020.01 ± 154.8**	–	–	–
Syringic acid	25.19 ± 1.7	–	–	–	–	–
Total (mg/kg dried herba)	**1538.33**	**1823.73**	**2333.26**	353.72	253.88	218.3
Simple phenolics and others						
Gallic acid	5.67 ± 0.39	7.24 ± 0.5	7.79 ± 0.54	–	5.15 ± 0.36	5.81 ± 0.4
Pyrogallol	–	29.95 ± 1.99	31.21 ± 2.08	**690.73 ± 45.9**	12.71 ± 0.85	27.5 ± 1.83
Total (mg/kg dried herba)	5.67	37.19	39	690.73	17.86	33.31
Dicarboxylic acid						
Fumaric acid	34.58 ± 2.4	189.08 ± 13.11	201.59 ± 13.98	–	**216.91 ± 15.0**	**258.25 ± 17.91**
Total (mg/kg dried herba)	34.58 ± 2.4	189.08 ± 13.11	201.59 ± 13.98	–	**216.91 ± 15.0**	**258.25 ± 17.91**
	1625.47	**2578.33**	**2698.62**	**1310.83**	**886.84**	**1082.08**

* OB: *O. boissieri*, OS: *O. saccatum*, OSL: *O. solymicum*, OA: *O. ayliniae*, OSP: *O. sipyleum*, OH: *O. hypericifolium*.

Table 4 Phenolic content of the C extracts.

	Amaracus			***Anatolicon***	
	OB*	**OS***	**OSL***	**OA***	**OSP***
Flavonoids and derivatives					
Kaempferol	–	15.58 ± 1.1	–	–	–
Salvigenin	25.28 ± 1.72	240.62 ± 16.38	**17.03 ± 1.16**	**398.51 ± 27.12**	**18.79 ± 1.28**
Penduletin	**62.73 ± 6.36**	**418.7 ± 42.45**	9.94 ± 1.01	206.54 ± 20.94	12.48 ± 1.27
Isorhamnetin	–	85.98 ± 7.59	–	56.37 ± 4.11	–
Quercetin	–	–	–	10.25 ± 1.02	–
Quercetagetin-3,6-dimethylether	35.53 ± 6.65	94.26 ± 17.65	–	26.33 ± 5.22	–
Total (mg/kg dried herba)	123.54	855.14	26.97	698.00	31.27
Coumaric acids and derivatives					
Caffeic acid	9.27 ± 1.83	8.41 ± 1.66	5.95 ± 1.18	–	5.71 ± 1.13
Chlorogenic acid	6.8 ± 0.94	7.78 ± 1.08	6.79 ± 0.94	–	8.00 ± 1.11
Syringic acid	25.19 ± 1.7	–	–	–	–
Rosmarinic acid	3.72 ± 0.29	4.16 ± 0.32	4.7 ± 0.36	–	3.62 ± 0.28
Total (mg/kg dried herba)	44.98	20.35	17.44	–	17.33
Simple phenolics and others					
Gallic acid	5.67 ± 0.39	–	–	–	–
Total(mg/kg dried herba)	5.67	–	–	–	–
Dicarboxylic acid					
Fumaric acid	34.58 ± 2.4	–	–	–	–
Total (mg/kg dried herba)	34.58 ± 2.4	–	–	–	–
	208.77	**875.49**	**44.41**	**698.00**	**48.60**

* OB: *O. boissieri*, OS: *O. saccatum*, OSL: *O. solymicum*, OA: *O. ayliniae*, OSP: *O. sipyleum*, OH: *O. hypericifolium*.

α-tocopherol. Lower IC_{50} value indicates higher radical scavenging activity. *O ayliniae* M1 extract consisted of pyrogallol, ferulic acid and chlorogenic acid as dominant compounds. According to recent reports, pyrogallol showed effective radical scavenger activity (Ozturk Sarikaya, 2015). In general, free radical scavenging and antioxidant activities of the phenolic compounds depend on the number of hydroxyl groups (–OH) and their positions on the aromatic rings (Ahmad et al., 2018; Tian and Liu, 2018; Phương et al., 2018; Lan et al., 2018). The Chloroform (C) extracts of *O. boissieri*, *O. saccatum*, *O. solymicum*, *O. sipyleum* and *O. hypericifolium* were showed lowest activities. IC_{50} values for DPPH free radical scavenging activities for *O. boissieri, O. saccatum, O. solymicum, O. sipyleum* and *O. hypericifolium* C extracts were found to be 91.62 μg/mL, 91.76 μg/mL, 96.92 μg/mL, 95.03 μg/mL and 94.30 μg/mL, respectively. It could be concluded that weak activity observed in that species is associated with a low amount of phenolic compounds.

O. ayliniae M1 and M2 extracts showed great lipid peroxidation inhibition in the β-carotene-linoleic acid system (IC_{50} 7.95 μg/mL, 7.99 μg/mL, respectively). IC_{50} values of BHA and BHT were found to be 6.12 μg/mL; 6.35 μg/mL and 6.13 μg/mL; 6.47 μg/mL, respectively. None of the tested extracts showed greater antioxidant activity than BHA or BHT. On the other hand, IC_{50} values for α-tocopherol were found to be 9.47 μg/mL and 9.11 μg/mL. The results show that *O. ayliniae* M1 and M2 extracts exhibited higher activities than the α-tocopherol. The lower inhibition value was found in C extracts.

Cu^{2+} reducing ability (CUPRAC method) is frequently used to determine the reducing powers of curcumin and M1, C, Ac and M2 extracts of *Origanum* species (Fig. 1). In CUPRAC method, same as other methods, *O. ayliniae* extracts have better activity than the other studied species as well as curcumin, which was used as a standard compound. Cu^{2+} reducing powers of the *O. ayliniae* extracts decreased as follows: M1 (2.74 mmol TR g^{-1}), M2 (2.62 mmol TR g^{-1}), Ac (1.59 mmol TR g^{-1}), C (1.27 mmol TR g^{-1}) and curcumin (0.9 mmol TR g^{-1}). Additionally, rosmarinic acid-rich extracts M1 and M2 of the species had the best activity.

Acetylcholinesterase (AChE) enzyme plays an important role of the cholinergic system in the central and peripheral nervous system (Gülçin et al., 2019). Acetylcholine (ACh) as a neurotransmitter decreases due to the decline in acetyltransferase

Table 5 Phenolic contents of the Ac extracts.

	Amaracus				*Anatolicon*	
	OB*	OS*	OSL*	OA*	OSP*	OH*
Flavonoids and derivatives						
Kaempferol	49.68 ± 3.51	**1307.04 ± 92.25**	420.71 ± 29.69	10.59 ± 0.75	646.58 ± 45.64	**648.93 ± 45.8**
Kaempferol-3-rutinoside	–	21.73 ± 1.96	3.66 ± 0.33	–	2.51 ± 0.23	8.3 ± 0.75
Salvigenin	–	173.9 ± 11.83	45.26 ± 3.08	246.39 ± 16.7	–	–
Penduletin	17.15 ± 1.74	378.86 ± 38.41	15.53 ± 1.57	**403.86 ± 40.9**	20.33 ± 2.06	478.88 ± 48.5
Isorhamnetin		105.85 ± 9.34	10.79 ± 0.95	–	–	–
Quercetin	27.84 ± 3.7	281.61 ± 37.44	173.82 ± 23.11	–	–	135.46 ± 18.0
Quercetagetin-3,6-dimethylether	16.74 ± 3.14	81.92 ± 15.34	14,63 ± 2.74	58.36 ± 8.59	–	5.63 ± 1.05
Isoquercetin	–	–	1.91 ± 0.55	–	–	–
Luteolin	2.65 ± 0.68	337.74 ± 86.75	94.86 ± 24.37	3.58 ± 0.15	180.73 ± 46.42	185.34 ± 47.6
Luteolin-7-O-glucoside	–	1.7 ± 0.17	7.19 ± 0.73	–	6.68 ± 0.68	–
Apigenin	–	–	–	10.38 ± 0.89	–	–
Rutin	1.49 ± 0.1	114.44 ± 7.5	2.21 ± 0.14	–	2.00 ± 0.13	2.33 ± 0.15
Pelargonin	–	–	–	88.59 ± 9.02	–	–
Total (mg/kg dried herba)	115.55	2804.79	790.57	821.75	858.83	1464.87
Coumaric acids and derivatives						
p-Coumaric acid	–	5.97 ± 0.92	–	–	4.84 ± 0.74	–
Caffeic acid	46.78 ± 9.26	77.79 ± 15.39	73.4 ± 14.53	–	100.8 ± 19.95	27.8 ± 5.5
(E)-Ferulic acid	–	2.82 ± 0.2	3.28 ± 0.23	–	8.56 ± 0.6	–
Chlorogenic acid	6.91 ± 0.96	–	7.45 ± 1.03	–	–	6.84 ± 0.95
Rosmarinic acid	**403.00 ± 30.9**	1295.34 ± 99.33	**967.42 ± 74.18**	–	**1760.8 ± 135.0**	175.84 ± 13.5
Total (mg/kg dried herba)	456.69	1381.92	1051.55	–	1875.00	210.48
Simple phenolics and others						
Gallic acid	6.05 ± 0.42	7.54 ± 0.52	7.95 ± 0.55	–	7.42 ± 0.51	5.1 ± 0.35
Ellagic acid	7.91 ± 0.53	–	–	–	–	14.37 ± 0.96
Vanillin	–	–	–	–	7.87 ± 0.72	–
Total (mg/kg dried herba)	13,96	7,54	7.95	–	15,29	19.47
Dicarboxylic acid						
Fumaric acid	27.09 ± 1.88	4.08 ± 0.28	–	–	59.91 ± 4.15	–
Total (mg/kg dried herba)	27.09 ± 1.88	4.08 ± 0.28	–	–	59.91 ± 4.15	–
	613.29	**4092.48**	**1850.07**	**821.75**	**2809.00**	**1694.82**

* OB: *O. boissieri*, OS: *O. saccatum*, OSL: *O. solymicum*, OA: *O. ayliniae*, OSP: *O. sipyleum*, OH: *O. hypericifolium*.

Table 6 Phenolic contents of the M2 extracts.

	Amaracus				*Anatolicon*	
	OB*	OS*	OSL*	OA*	OSP*	OH*
Flavonoids and derivatives						
Kaempferol	56.5 ± 3.99	99.48 ± 7.02	685.87 ± 48.41	8.56 ± 0.6	114.06 ± 8.05	–
Salvigenin	–	–	–	158.31 ± 10.77	–	–
Penduletin	–	14.37 ± 1.46	–	**239.61 ± 24.29**	–	–
Quercetin	8.95 ± 1.19	4.66 ± 0.62	–	–	24.41 ± 1.56	–
Quercetagetin-3,6-dimethylether	–	5.16 ± 0.97	–	–	–	–
Luteolin	5.55 ± 1.42	16.09 ± 4.13	–	–	30.23 ± 7.76	–
Luteolin-7-*O*-glucoside	–	4.27 ± 0.43	–	–	62.88 ± 6.4	–
Luteolin-5-*O*-glucoside	–	–	–	–	36.92 ± 2.38	–
Rutin	6.22 ± 0.41	142.22 ± 9.32	–	–	–	–
Pelargonin	–	–	–	53.6 ± 5.45	–	–
Total (mg/kg dried herba)	77.22	286.25	685.87	460.08	268.50	–
Coumaric acids and derivatives						
Caffeic acid	218.59 ± 43.26	122.23 ± 24.19	–	–	135.21 ± 26.76	–
(*E*)-Ferulic acid	182.95 ± 12.78	210.18 ± 14.69	–	99.69 ± 6.97	136.57 ± 9.54	–
Chlorogenic acid	10.83 ± 1.5	224.61 ± 31.1	437.23 ± 60.55	50.17 ± 6.95	9.23 ± 1.28	–
Rosmarinic acid	**2085.33 ± 159**	**2421.83 ± 185**	–	–	**2495.11 ± 191.32**	–
Syringic acid	222.3 ± 14.97	–	–	–	–	–
Total (mg/kg dried herba)	2720.00	2978.85	437.23	149.86	2776.12	–
Simple phenolics and others						
Gallic acid	–	7.02 ± 0.49	**1046.31 ± 72.5**	5.67 ± 0.39	5.12 ± 0.36	–
Pyrogallol	20.23 ± 1.35	17.23 ± 1.15	–	–	13.62 ± 0.91	–
Total (mg/kg dried herba)	20.23	24.25	1046.31	5.67	18.74	
Dicarboxylic acid						
Fumaric acid	218.87 ± 15.1	268.38 ± 18.61	–	–	216.14 ± 14.99	–
Total (mg/kg dried herba)	218.87 ± 15.1	268.38 ± 18.61	–	–	216.14 ± 14.99	–
	3036.32	**3557.73**	**2169.41**	**615.61**	**3044.62**	**–**

* OB: *O. boissieri,* OS: *O. saccatum,* OSL: *O. solymicum,* OA: *O. ayliniae,* OSP: *O. sipyleum,* OH: *O. hypericifolium.*

Table 7 DPPH free radical scavenging activity and lipid peroxidation of the extracts, BHA, BHT and α-tocopherol (IC_{50} μg/mL).

	M1		C		Ac		M2	
	β-carotene	DPPH	*β*-carotene	DPPH	*β*-carotene	DPPH	*β*-carotene	DPPH
OB*	31.23 ± 3.01	91.99 ± 6.78	91.66 ± 3.00	91.62 ± 13.27	40.58 ± 17.95	84.79 ± 1.30	12.64 ± 1.04	29.83 ± 1.10
OS*	9.72 ± 0.53	32.64 ± 5.46	91.37 ± 8.69	91.76 ± 1.60	15.39 ± 4.81	47.27 ± 11.04	12.33 ± 0.83	20.11 ± 2.49
OSL*	9.19 ± 0.46	27.53 ± 4.49	89.12 ± 0.82	96.92 ± 2.11	10.19 ± 1.01	42.22 ± 2.31	8.68 ± 0.27	11.64 ± 0.49
OA*	7.95 ± 7.95	7.63 ± 0.17	13.61 ± 3.35	38.86 ± 0.80	9.64 ± 0.76	29.58 ± 3.13	7.99 ± 0.97	9.59 ± 0.67
OSP*	13.59 ± 3.98	20.12 ± 4.39	88.05 ± 7.73	95.03 ± 2.95	35.19 ± 5.75	42.39 ± 3.36	8.38 ± 0.31	18.99 ± 0.50
OH*	9.13 ± 1.41	30.45 ± 0.54	89.56 ± 2.65	94.30 ± 2.82	18.83 ± 1.92	38.29 ± 2.03	9.01 ± 0.22	31.32 ± 2.77
BHA	6.12 ± 0.07	10.14 ± 0.81	6.13 ± 0.08	11.86 ± 0.20	6.14 ± 0.05	9.59 ± 0.66	6.13 ± 0.10	9.53 ± 0.30
BHT	6.35 ± 0.29	11.42 ± 2.49	6.33 ± 0.09	11.05 ± 0.75	6.39 ± 0.07	11.57 ± 1.63	6.47 ± 0.13	11.04 ± 0.18
α-Tocopherol	9.47 ± 1.78	12.93 ± 2.99	9.29 ± 0.09	12.44 ± 0.65	9.27 ± 0.96	12.56 ± 0.71	9.11 ± 0.21	12.50 ± 0.08

IC_{50} values are mean ± SD (n = 3).

* OB: *O. boissieri,* OS: *O. saccatum,* OSL: *O. solymicum,* OA: *O. ayliniae,* OSP: *O. sipyleum,* OH: *O. hypericifolium.*

activity and choline (Ch). It was reported that the reduction of ACh and BCh levels in hippocampus and cortex in the brain is the most remarkable biochemical change in Alzhemer Diease (AD) patients. As a result of this, one of the treatment approaches for AD is inhibition of AChE and BChE enzymes that break down ACh and BCh (Gülçin et al., 2019; Taslimi et al., 2020). Inhibitors of AChE, such as galanthamine, are used frequently to treat the symptoms of AD (Loizzo et al., 2009), which hydrolyses the acetylcholine compound involved in the communication between synapses in the nervous system. The less specific BChE has recently been a focus of research, because BChE concentration stays the same, or is even up-regulated, while AChE is dramatically down-regulated in the brains of patients suffering from AD. AChE inhibitors had a

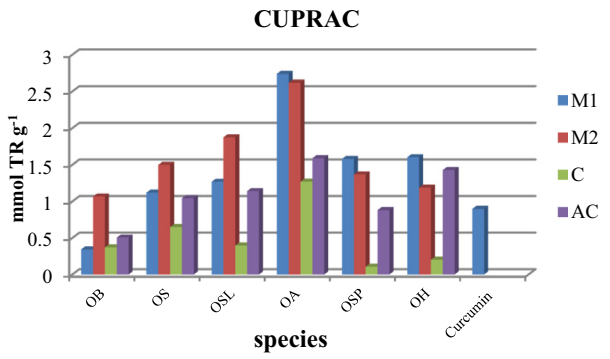


Fig. 1 Cu^{2+} ion reducing power (CUPRAC) assay of the extracts and curcumin.

common usage in medicine, especially for the treatment of AD. They have been used in clinical trials, including natural substances. Phenolic compounds had been also recognized as AChE inhibitors and promising lead compounds for AD. Therefore, finding new AChE and BChE sources is very important and one of the best sources is plants.

Anticholinesterase activities of extracts of species were determined at 200 µg/mL and galanthamine was used as a standard compound. The results are given in the Table 8.

The M1 and Ac extracts of *O. hypericifolium* showed moderate AChE (51.32 ± 2.69% and 49.80 ± 0.53%, respectively) and BChE (54.91 ± 0.85% and 62.80 ± 0.55%, respectively) inhibitory activity. In contrast, the M1, C and Ac extracts of *O. boissieri, O. saccatum, O. solymicum* and *O. sipyleum* was only exhibited activity against butyrylcholinesterase enzyme. M2 extract of *O. solymicum* showed mild butyrylcholinesterase inhibitory activity (8.55 ± 0.40%), while M2 extracts of other species exhibited no activity. Orhan et al., (2007) reported that there was no correlation between acetylcholinesterase and butyrylcholinesterase enzyme inhibition and phenolic contents. They had reported that some of these compounds are not inhibitor for AChE and BChE. Rather than phenolic acids, flavonoid derivatives such as quercetin, genistein, luteolin-7-*O*-rutinoside were found to be more effective inhibitors (Orhan et al., 2009; Jung and Park, 2007). Structural requirements of flavonoids as AChE and BChE inhibitors have been investigated (Orhan et al., 2007; Panche et al., 2016). It was reported that catechol moiety on ring B and this moiety has positive effects on the enzyme-inhibiting activities of quercetin contributing to its binding with the enzyme (Orhan et al., 2007; Orhan et al., 2009). Amongst the tested extracts, the Ac and direct methanol (M1) extracts of *O. hypericifolium* were shown to have the best acetylcholinesterase (54.91 ± 0.85%) and butyrylcholinesterase (62.08 ± 0.55%) inhibitory activity, which might be due to high flavonoid content of M1 and Ac extracts of *O. hypericifolium*. These results are consistent with the literature.

4. Conclusion

All member of the section of *Amaracus* and *Anatolicon* of *Origanum* are endemic species for Turkey. Also, *O. ayliniae* was just identified recently and added to sect. *Amaracus*. In the present study, essential oil and phenolic composition of the methanol, chloroform and acetone extracts of both sections

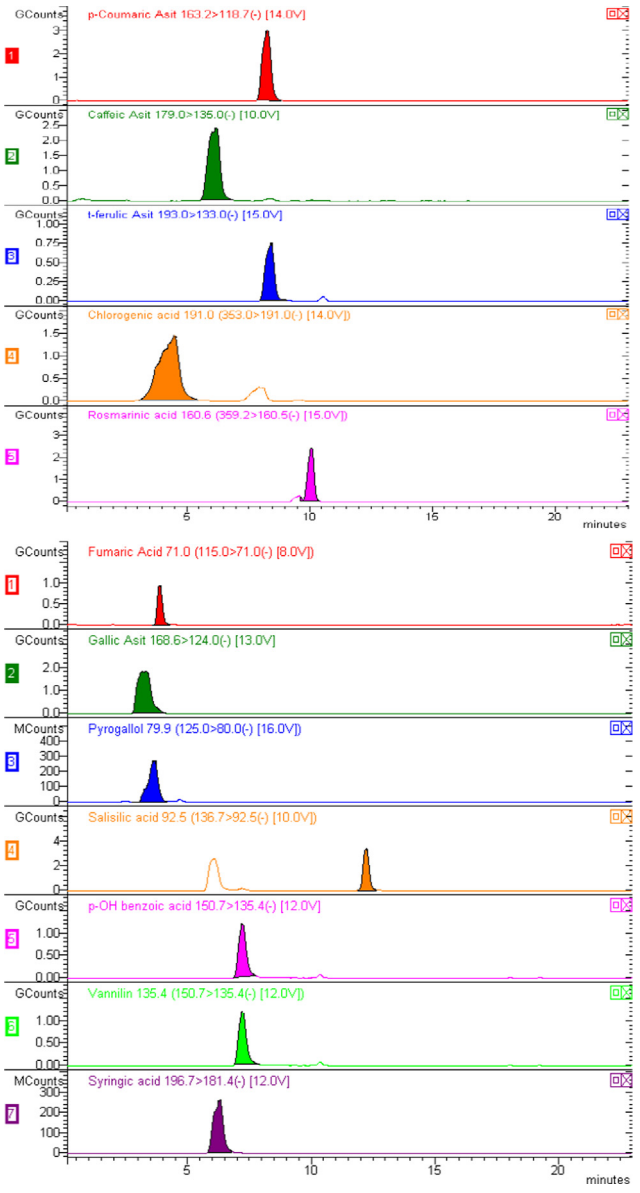


Fig. 2 Standards chromatogram of secondary metabolites (Phenolics and Others) by LC-MS/MS (5 mg/L).

were investigated. Also, the antioxidant and anticholinesterase activities of the extracts were determined. There are numerous reports on the chemical composition of essential oil of the species. In our study, it was found that the oil composition of the sect. *Amaracus* and sect. *Anatolicon* were different chemotypes, and also, our findings are consistent with the literature. It can be said that the differences in the chemical composition of essential oils depend on climatic, geographic conditions, harvest period, distillation time and distillation technique. To the best of our knowledge, this is the first report on the phenolic composition and anticholinesterase activities of the species. A considerable qualitative and quantitative variation was observed in the phenolic compounds of extracts of the species. Rosmarinic acid, penduletin, salvigenin, fumaric acid, kaempferol, gallic acid and pyrogallol were determined as the main phenolic compounds of the species. The richest extracts in

Table 8 Anticholinesterase activity of the the extracts.

	AChE % Inhibition (200 μg/mL)				BChE % Inhibition (200 μg/mL)			
	M1	C	Ac	M2	M1	C	Ac	M2
OB**	0	0	0	0	28.96 ± 0.65	46.69 ± 0.63	31.14 ± 0.58	0
OS**	0	0	0	0	31.46 ± 1.10	37.39 ± 1.20	59.42 ± 0.25	0
OSL**	0	0	0	0	3.00 ± 0.50	49.35 ± 0.82	48.76 ± 0.86	8.55 ± 0.40
OSP**	0	0	0	0	37.34 ± 0.37	35.59 ± 0.38	21.09 ± 0.21	0
OH**	51.32 ± 2.69	0	49.81 ± 0.53	0	54.91 ± 0.85	33.79 ± 0.86	62.08 ± 0.55	0
Galanthamine*	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

* Positive control.
** OB: *O. boissieri,* OS: *O. saccatum,* OSL: *O. solymicum,* OA: *O. ayliniae,* OSP: *O. sipyleum,* OH: *O. hypericifolium.*

terms of phenolic compounds were Ac M1 and M2. Studies on phenolic compounds have shown that these compounds are quite good antioxidant chemicals. Therefore, extracts rich in phenolic compounds have been shown good antioxidant activity. Specifically, it was determined that M1 and M2 extracts which rich in phenolic compounds showed good antioxidant properties. In anticholinesterase activities; inhibition against the AChE enzyme was determined only for the extract of Ac and M1 of the *O. hypericifolium*, while the BChE enzyme was inhibited moderately by the all studied extracts. Therefore, it can be said that the extracts of these species having weak anticholinesterase effect while having a good antioxidant effect. This study supported that *Origanum* species are very important natural herbal products which are commonly used as an alternative to antioxidants in the pharmaceutical and food industry.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2020.01.025.

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