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NATURAL PRODUCT ANALYSIS

Onosma inexspectata and *Onosma armenum* as Novel Sources of Phytochemicals with Determination by High-Performance Liquid Chromatography–Mass Spectrometry (HPLC-MS/MS) with Evaluation of the Antioxidant and Enzyme Inhibitory Capacities

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ABSTRACT

The goal of the current work was to study the phytochemical content of *Onosma inexspectata* Teppner and *O. armenum* DC. The antioxidant properties using six assays and the enzyme inhibitory capacities against α-amylase, α-glucosidase, tyrosinase, and the anticholinesterase were also evaluated. The levels of phenolic compounds were approximately 22 and 28 mg gallic acid equivalent/g for *O. inexspectata* and *O. armenum*, respectively. Twenty-seven compounds were identified and quantified using liquid chromatography–electrospray ionization tandem mass spectrometry. Chlorogenic acid, apigenin 7-glucoside, and luteolin 7-glucoside were the major compounds in *O. inexspectata*. *Onosma armenum* contained an attractive level of hesperidin. The antioxidant assays showed significant differences between the two species, except for the phosphomolybdenum assay. The results of the inhibitory enzyme assays revealed no significant differences between the extracts, except for α-glucosidase inhibition. The present study may underline the possibility to use *O. inexspectata* and *O. armenum* as promising sources of phytochemicals that may serve against many body disorders.

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KEYWORDS
Onosma species; high-performance liquid chromatography–tandem mass spectrometry; electrospray ionization; chlorogenic acid; apigenin 7-glucoside

Introduction

Nowadays, due to the side effects of synthetic molecules, there is a high demand for natural molecules used as antioxidants, colorants and food additives. Plants contain a wide range of these biomolecules, such as phenolics, sterols, carotenoids, and tocopherols. These compounds have demonstrated beneficial capacities against a variety of diseases, such as diabetes and inflammation. Furthermore, many studies have reported that the plant extracts are effective against many key enzymes, which can help to find cures for many health problems, such as acetylcholinesterase for neuro-degenerative complications, tyrosinase for skin diseases, and α-amylase and α-glucosidase for diabetes

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(Pillaiyar, Manickam, and Namasivayam 2017; Beidokhti et al. 2020; Das et al. 2020). These results are in harmony with the traditional use of medicinal plants to treat many illnesses. Indeed, it has been reported that persons who daily consume vegetables present near half the risk of developing complicated diseases (Gescher et al. 1998; Sachdeva, Sachdev, and Sachdeva 2013).

For these reasons, it is important to look for known and unknown species that contain wide varieties of these beneficial molecules. The *Onosma* L. Boraginaceae family grows extensively across Asia. Sarikurkcu, Sahinler, and Tepe (2020) have reported that 150 *Onosma* species have been identified with 95 species in Turkey. Many authors have suggested that *Onosma* species are used traditionally to treat a variety of sicknesses such as fever, abdominal pain, and bronchitis (Mašković et al. 2015; Sarikurkcu, Sahinler, and Tepe 2020). Furthermore, previous studies have reported that *Onosma* species have been used for coloring food stuffs, oils, and medicinal preparations (Ozgen et al. 2006; Kumar, Kumar, and Kishore 2013). In the literature, there are data about phytochemical contents in a few number of *Onosma* species such as *Onosma aucheriana* DC., *O. frutescens* Lam., and *O. Sericea* Willd. (Sarikurkcu, Sahinler, and Tepe 2020), *Onosma gigantea* Lam. (Sarikurkcu et al. 2018), *O. heterophyllum* Griseb (Ozer et al. 2018), and *O. aucheriana* DC. (Mašković et al. 2015). However, to the best of our knowledge, no data are available about *O. inexspectata* Teppner and *O. armenum* DC.

Hence, the purpose of the current work was to identify and quantify the phenolic compounds extracted from *Onosma inexspectata* and *O. armenum* using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS) and to study their antioxidant capacities together with the enzyme inhibitory capacities against acetylcholinesterase, tyrosinase, α-amylase, and α-glucosidase.

Materials and methods

Chemicals

Methanol, ferric chloride, and Folin–Ciocalteu's reagent were purchased from Merck (Darmstadt, Germany). 5,5-Dithio-bis-2-nitrobenzoic acid (DTNB), 1,1-diphenyl-2-picrylhy-drazyl (DPPH), 3,4-dihydroxy-L-phenylala-nine (L-DOPA), tyrosinase, and phenolic standards were purchased from Sigma Chemical (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were of analytical grade.

The chemical standards of gallic acid, chlorogenic acid, rosmarinic acid, sinapic acid, pyrocatechol, 2,5-dihydroxybenzoi acid, (p)-catechin, 4-hydroxybenzoic acid, epicatechin, syringic acid, vanillin, pinoresinol, p-coumaric acid, taxifolin, quercetin, caffeic acid, ferulic acid, luteolin, apigenin, and 2-hydroxycinnamic acid were obtained from Sigma-Aldrich (St. Louis, MO). Vanillic acid, kaempferol, hesperidin, luteolin 7-glucoside, apigenin 7-glucoside, 3,4-dihydroxyphenylacetic acid, eriodictyol, and 3-hydroxybenzoic acid were purchased from Fluka (St. Louis, MO).

Plant material and extract preparation

The aerial parts of *O. inexspectata* Teppner (1050 m., 37°47'4.56"N 30°07'32.16"E, Herbarium number: OC.5042) and *Onosma armenum* DC. (15 m., 36°31'54.12"N

30°32'1.32"E, Herbarium number: OC.5044) were collected from Burdur and Antalya, Turkey, in 2019. Samples were collected from 8 to 13 plants, mixed and a representative sample was employed for further analysis. The plants were collected, identified, and deposited by Dr. Olcay Ceylan from the Department of Biology, Mugla Sitki Kocman University, Mugla-Turkey.

The samples were dried out of direct sunlight and dehumidified in an airy environment. 5 g of each *Onosma* species was macerated for 24 h using 100 mL of methanol. This process was repeated, and the extracts were combined and concentrated under low pressure. The yields of *O. inexspectata* and *O. armenum* were 17.03% and 6.13% (w/w), respectively.

First, the extracts were re-dissolved in methanol and stock solutions were prepared at a concentration of 10 mg/mL. These stock solutions were diluted with methanol as necessary before LC-MS/MS analysis and biological activity assays.

Phenolic and flavonoid determination

Sample solution (0.25 mL) and 1 mL of diluted Folin–Ciocalteu reagent (1:9) were mixed vigorously. After adding 0.75 mL of Na_2CO_3 (1%), the mixture was allowed to stand at room temperature. The absorbance was measured at 760 nm (Slinkard and Singleton 1977). The total phenolic content was expressed as equivalents of gallic acid from the calibration relationship: $A = 0.0327$ gallic acid (μg) $-$ 0.0025 (R^2: 0.9998).

To assess the total flavonoids, the sample solution was mixed with 2% of aluminum trichloride in methanol (v/v), and the mixture was incubated 10 min at room temperature. A blank was prepared by mixing the sample solution with methanol without $AlCl_3$ (v/v). The absorbance was measured at 415 nm (Arvouet-Grand et al. 1994). The total flavonoid level was expressed as equivalents of quercetin using the calibration relationship: $A = 0.0305$ quercetin (μg) + 0.0042 (R^2: 0.9993).

Liquid chromatography–electrospray tandem mass spectrometry

The quantities of the bioactive compounds in the extracts of *O. inexspectata* and *O. armenum* were estimated using LC–ESI–MS/MS (Cittan and Çelik 2018). An Agilent Technologies 1260 Infinity liquid chromatography system, a 6420 Triple Quad mass spectrometer, and a Poroshell 120 EC-C18 (100 mm × 4.6 mm I.D., 2.7 μm) column were used. The mobile phases were: (A) formic acid solution (0.1%, v/v) and (B) methanol. The gradient was: 0.00 min 2% B, 3.00 min 2% B, 6.00 min 25% B, 10.00 min 50% B, 14.00 min 95% B, 17.00 min 95% B, and 17.50 min 2% B. 25 °C was the temperature of the column. The injection volume and the flow rate were 2.0 μL and 0.4 mL/min.

The LC system was interfaced to the tandem mass spectrometer through an electrospray ionization (ESI) source. The electrospray source of the MS was operated in the negative and positive multiple reaction monitoring (MRM) modes. The gas temperature, the gas flow, the capillary voltage, and the nebulizer pressure were 300 °C, 11 L/min, −3.5 kV, and 40 psi, respectively. An authentic standard solution was analyzed using the same conditions and was used to identify the analyte peaks. An LC–ESI–MS/MS MRM

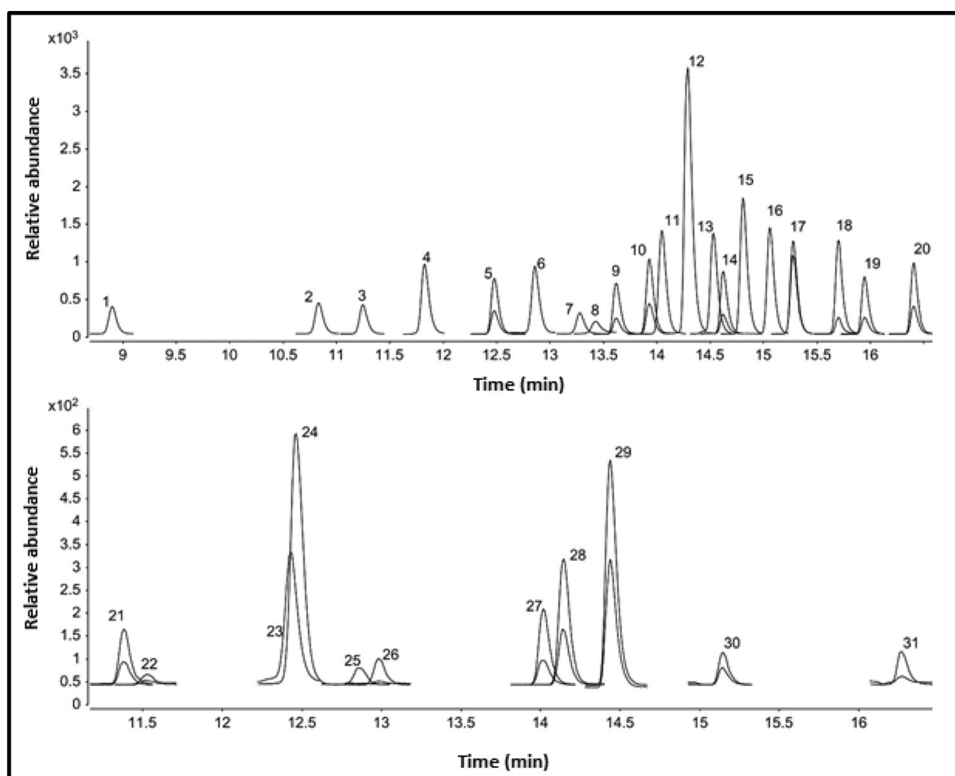

Figure 1. LC–ESI–MS/MS MRM chromatograms of phenolic compounds. 1–31 represent the chromatograms of gallic acid, protocatechuic acid, 3,4-dihydroxyphenylacetic acid, chlorogenic acid, (−)-epicatechin, caffeic acid, 3-hydroxybenzoic acid, vanillin, verbascoside, taxifolin, p-coumaric acid, luteolin 7-glucoside, hyperoside, rosmarinic acid, apigenin 7-glucoside, 2-hydroxycinnamic acid, eriodictyol, quercetin, luteolin, apigenin, (+)-catechin, pyrocatechol, 2,5-dihydroxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, sinapic acid, ferulic acid, hesperidin, pinoresinol and kaempferol, respectively.

chromatogram and analytical characteristics of 31 standard phenolic compounds are provided in Figure 1 and Table 1.

Antioxidant capacities and biological activities

The antioxidant activities of *O. inexspectata* and *O. armenum* extracts were assessed using the following tests: phosphomolybdenum, CUPRAC, FRAP, DPPH, ABTS, and ferrous ion chelating (Hatano et al. 1988; Re et al. 1999; Apak et al. 2006) with some modifications (Tepe et al. 2011; Zengin et al. 2015; Kocak et al. 2016). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and ethylenediaminetetraacetic acid (EDTA) were used as positive references.

The inhibitory capacities of the extracts toward α-amylase were estimated using the Caraway-Somogyi iodine/potassium iodide (IKI) method (Yang et al. 2012) with some modifications (Sarikurkcu et al. 2018). Briefly, 25 μL of the extract and 50 μL of α-amylase solution in phosphate buffer (pH 6.9 with 6 mM sodium chloride) were mixed and the solution was allowed to stand for 10 min at 37 °C. Next, 50 μL of starch solution

Table 1. Analytical figures of merit for the phenolics in the *O. inexspectata* and *O. armenum* methanolic extracts.

R_t (min)	Phenolic	Calibration relationship	R^2	LOD (μg/L)	LOQ (μg/L)
8.891	Gallic acid	$y = 4.82x - 26.48$	0.9988	1.46	4.88
10.818	Protocatechuic acid	$y = 5.65x - 9.99$	0.9990	1.17	3.88
11.224	3,4-Dihydroxyphenylacetic acid	$y = 5.13x - 12.39$	0.9990	1.35	4.51
11.369	(+)-Catechin	$y = 1.45x + 1.95$	0.9974	3.96	13.20
11.506	Pyrocatechol	$y = 0.11x - 0.52$	0.9916	9.62	32.08
11.802	Chlorogenic acid	$y = 12.14x + 32.34$	0.9995	0.55	1.82
12.412	2,5-Dihydroxybenzoic acid	$y = 3.79x - 14.12$	0.9980	2.12	7.08
12.439	4-Hydroxybenzoic acid	$y = 7.62x + 22.79$	0.9996	1.72	5.72
12.458	(−)-Epicatechin	$y = 9.11x - 9.99$	0.9971	1.85	6.18
12.841	Caffeic acid	$y = 11.09x + 16.73$	0.9997	3.15	10.50
12.843	Vanillic acid	$y = 0.49x - 1.61$	0.9968	2.56	8.54
12.963	Syringic acid	$y = 0.74x - 1.54$	0.9975	3.75	12.50
13.259	3-Hydroxybenzoic acid	$y = 3.69x - 12.29$	0.9991	1.86	6.20
13.397	Vanillin	$y = 2.02x + 135.49$	0.9926	15.23	50.77
13.589	Verbascoside	$y = 8.59x - 28.05$	0.9988	0.82	2.75
13.909	Taxifolin	$y = 12.32x + 9.98$	0.9993	1.82	6.05
13.992	Sinapic acid	$y = 2.09x - 6.79$	0.9974	2.64	8.78
14.022	p-Coumaric acid	$y = 17.51x + 53.73$	0.9997	1.93	6.44
14.120	Ferulic acid	$y = 3.32x - 4.30$	0.9992	1.43	4.76
14.266	Luteolin-7-glucoside	$y = 45.25x + 156.48$	0.9996	0.45	1.51
14.412	Hesperidin	$y = 5.98x + 0.42$	0.9993	1.73	5.77
14.506	Hyperoside	$y = 16.32x - 1.26$	0.9998	0.99	3.31
14.600	Rosmarinic acid	$y = 9.82x - 17.98$	0.9989	0.57	1.89
14.781	Apigenin-7-glucoside	$y = 21.33x - 31.69$	0.9983	0.41	1.35
15.031	2-Hydroxycinnamic acid	$y = 16.72x - 26.94$	0.9996	0.61	2.03
15.118	Pinoresinol	$y = 0.80x - 2.69$	0.9966	3.94	13.12
15.247	Eriodictyol	$y = 14.24x - 0.50$	0.9998	0.80	2.68
15.668	Quercetin	$y = 14.68x - 18.25$	0.9997	1.23	4.10
15.923	Luteolin	$y = 8.96x + 26.80$	0.9992	1.34	4.46
16.236	Kaempferol	$y = 0.82x - 3.06$	0.9959	3.30	10.99
16.382	Apigenin	$y = 11.29x + 38.05$	0.9987	0.96	3.20

Note: R_t, retention time. LOD and LOQ: limit of detection and limit of quantification.

(0.05%) were added to initiate the reaction and the mixture was incubated at 37 °C for 10 min. To stop the reaction, 25 μL of HCl (1 M) were added. Lastly, 100 μL iodine-potassium iodide solution was added and the absorbance was determined at 630 nm. Acarbose was used as positive reference.

α-Glucosidase inhibitory activity was measured by a literature method (Palanisamy et al. 2011) with some modifications (Sarikurkcu et al. 2018). To estimate the inhibitory properties of the extracts against α-glucosidase, the following solution was prepared: 50 μL of sample, 50 μL of glutathione, 50 μL of α-glucosidase in phosphate buffer (pH 6.8) and 50 μL of PNPG. The mixture was incubated at 37 °C for 15 min. 50 μL of sodium carbonate (0.2 M) were added to stop the reaction and the absorbance was measured at 400 nm. Acarbose was used as the positive control.

To assess the ability of the extracts to inhibit tyrosinase activity, the method using L-DOPA as substrate was used as previously reported (Erdogan Orhan et al. 2012) with small modifications (Sarikurkcu et al. 2018). Kojic acid was used as positive reference. Briefly, 25 μL of the extract were mixed with 40 μL of tyrosinase solution and incubated at 25 °C for 15 min. 40 μL of L-DOPA were added to initiate the reaction. The absorbance was measured at 492 nm after incubation at 25 °C for 10 min.

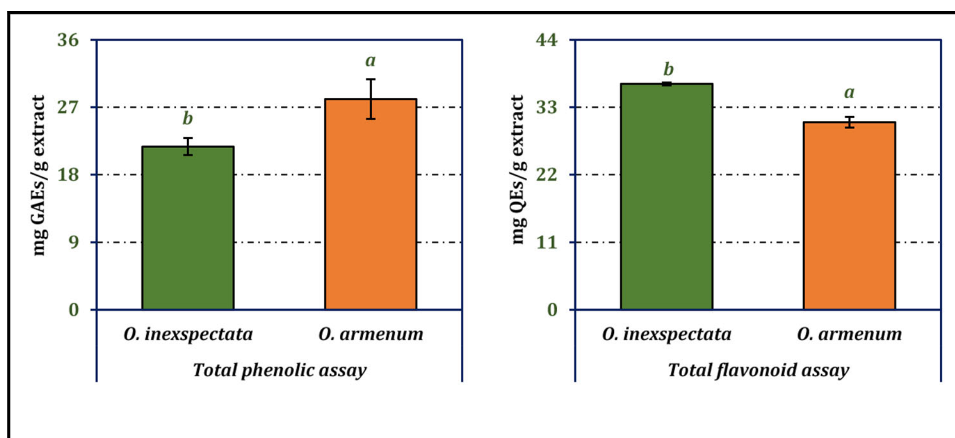

Figure 2. Total phenolic and flavonoid contents of *O. inexspectata* and *O. armenum* methanolic extracts QEs and GAEs are quercetin and gallic acid equivalents, respectively. Values indicated by the same superscripts are not different from each other according to the Student's t-test at the 5% significance level.

Ellman's method was used to estimate the cholinesterase inhibitory capacity of the extract (Ellman et al. 1961), and galanthamine was used as the positive control. A mixture of 50 μL of extract, 125 μL of DTNB and 25 μL of AChE in Tris-HCl buffer (pH 8.0) was incubated at 25 °C for 15 min. To initiate the reaction, 25 μL of acetylthiocholine iodide (ATCI) were added. After 10 min of incubation at 25 °C, the absorbance was measured at 405 nm.

Statistical analysis

The results were presented as the mean of three repetitions and standard deviation. ANOVA (one-way analysis of variance) by Tukey's honestly significant difference post hoc test and Student's *t*-test with $\alpha = 0.05$ (SPSS v. 22.0) were applied to identify statistical differences between the measurements.

Results and discussion

Phytochemical compositions of the extracts

The difference among the bioactive compounds content of plant species may be due to genetic factors, environmental conditions, and the techniques used (Yang et al. 2018). In order to evaluate the phytochemical content of *O. inexspectata* and *O. armenum*, the total phenolics and total flavonoids were determined. To the best of our knowledge, this is the first study to characterize the phytochemical contents of these species (Figure 2). It was clear that *O. armenum* exhibited the higher level of total phenolics (28.09 ± 2.65 mg GAEs/g) and *O. inexspectata* contained the higher quantity of flavonoids (36.80 ± 0.23 mg QEs/g).

To further explore the phytochemical composition, LC–ESI–MS/MS was used (Figure 3) and twenty-seven bioactive compounds were identified (Table 2), including 13 phenolic acids and 10 flavonoids. The highest level of phenolic acids was detected in *O. armenum* extract due to the chlorogenic acid (ca. 36,970 μg/g) compared to *O.*

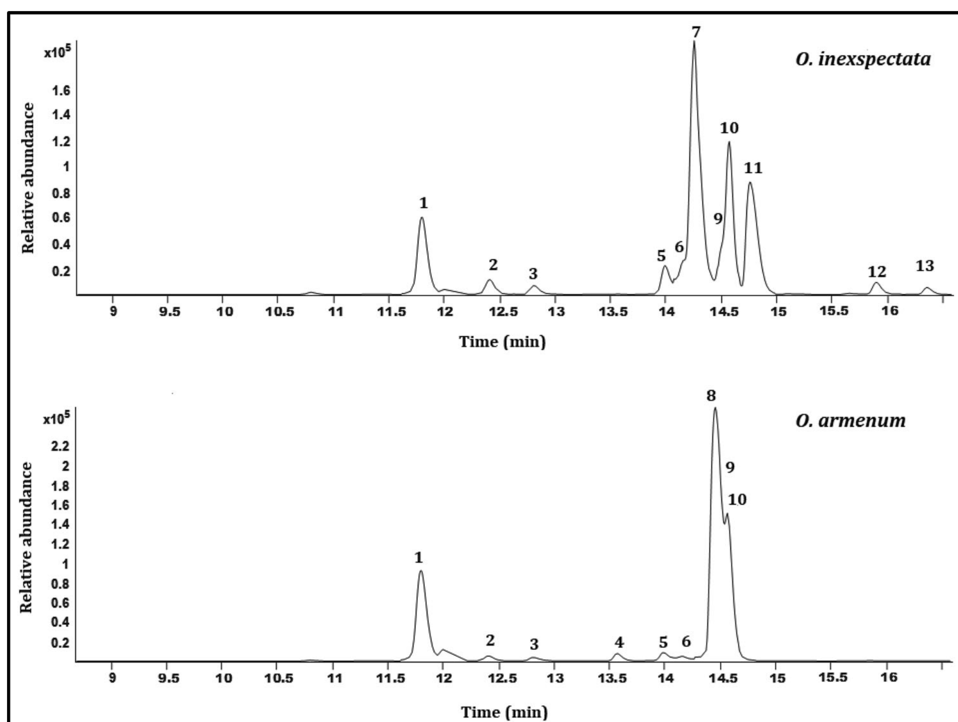

Figure 3. LC–ESI–MS/MS chromatograms of *O. inexspectata* and *O. armenum* methanolic extracts: (1) chlorogenic acid, (2) 4-hydroxybenzoic acid, (3) vanillic acid, (4) verbascoside, (5) p-coumaric acid, (6) ferulic acid, (7) luteolin-7-glucoside, (8) hesperidin, (9) hyperoside, (10) rosmarinic acid, (11) apigenin-7-glucoside, (12) luteolin, and (13) apigenin.

inexspectata (ca. 21,360 μg/g). Apart from this molecule, *O. inexspectata* contained the highest levels of vanillic acid (1094 ± 53 μg/g), 4-hydroxybenzoic acid (1507 ± 37 μg/g), *p*-coumaric acid (1103 ± 31 μg/g), and ferulic acid (ca. 950 μg/g). Furthermore, 3-hydroxybenzoic acid was only detected in the *O. inexspectata* extract. It was also remarkable that the levels of rosmarinic acid were similar in the two species. Similarly, the highest concentration of flavonoids was found in *O. armenum* due to the hesperidin level (ca. 248,000 μg/g). Apart from this molecule, the levels of the all detected flavonoids were higher in *O. inexspectata*, especially apigenin 7-glucoside (ca. 26,400 μg/g), hyperoside (ca. 5990 μg/g), and luteolin (ca. 1330 μg/g). It was also remarkable that taxifolin was detected only in *O. inexspectata*.

However, *O. inexspectata* was characterized by high levels of other compounds, such as pinoresinol (ca. 800 μg/g) compared to *O. armenum* (63.19 ± 4.59 μg/g). On the other hand, the highest level of verbascoside was observed in *O. armenum* extract (ca. 764 μg/g).

The observed differences among samples were due to the species characteristics (Ballesteros-Vivas et al. 2019). Since there was no data in the literature about the phytochemicals in *O. inexspectata* and *O. armenum*, these results were compared to those for other *Onosma* species. In *O. aucheriana*, rosmarinic acid at 71,332 μg/g, luteolin 7-glucoside at 24,408 μg/g, and apigenin 7-glucoside at 20,432 μg/g were the primary compounds. In *O. pulchra*, rosmarinic acid at 18,561 μg/g extract and apigenin 7-glucoside at 16,884 μg/g were the main components. *O. frutescens* contained rosmarinic acid at

Table 2. Concentrations (μg/g extract) of phenolics in *O. inexspectata* and *O. armenum* methanolic extracts.

Phenolic	*O. inexspectata*	*O. armenum*
Phenolic acids		
Gallic acid	12.44 ± 0.23[a]	14.2 ± 0.3[b]
Protocatechuic acid	271.9 ± 10.2[b]	123 ± 1.04[a]
Chlorogenic acid	21,360 ± 210[a]	36,970 ± 80[b]
2,5-Dihydroxybenzoic acid	131.3 ± 1.9[b]	61.02 ± 0.6[a]
4-Hydroxybenzoic acid	1507 ± 37[b]	644 ± 16[a]
Caffeic acid	364.2 ± 5.2[b]	196 ± 3[a]
Vanillic acid	1094 ± 53[b]	594 ± 37[a]
Syringic acid	62.6 ± 4.2[a]	54 ± 0.1[a]
3-Hydroxybenzoic acid	11.24 ± 1.4	Not detected
Sinapic acid	85.35 ± 4.58[b]	30 ± 1[a]
p-Coumaric acid	1103 ± 31[b]	357 ± 13[a]
Ferulic acid	952.5 ± 6.7[b]	407 ± 21[a]
Rosmarinic acid	5867 ± 5[a]	5693 ± 107[a]
Flavonoids		
Taxifolin	2.64 ± 0.1	Not detected
Luteolin 7-glucoside	19812 ± 781[b]	29.04 ± 3[a]
Hesperidin	60.32 ± 1.32[a]	248,000 ± 200[b]
Hyperoside	5992 ± 406[b]	3048 ± 20[a]
Apigenin 7-glucoside	26,400 ± 1000[b]	45 ± 5.25[a]
Eriodictyol	4.42 ± 0.3[b]	0.20 ± 0.01[a]
Quercetin	57.20 ± 2.3[b]	13.12 ± 0.1[a]
Luteolin	1332 ± 60[b]	21.41 ± 2[a]
Kaempferol	120.4 ± 0.1[b]	5.77 ± 0.23[a]
Apigenin	445.4 ± 8.5[b]	10.53 ± 0.1[a]
Others		
3,4-Dihydroxyphenylacetic acid	8.23 ± 0.02[b]	5.92 ± 0.32[a]
Vanillin	67.1 ± 3.3[b]	45 ± 1.32[a]
Verbascoside	29.8 ± 2.2[a]	764 ± 0.8[b]
Pinoresinol	801 ± 10 [b]	63.2 ± 4.6[a]

Note: The data indicated by the same superscripts within the same row are not different from each other according to the Student's *t*-test at 5% significance level.

130,481 μg/g, hyperoside at 15,682 μg/g, and hesperidin at 14,312 μg/g were the major compounds. The major compounds of *O. sericea* were pigenin 7-glucoside at 22,707 μg/g, luteolin 7-glucoside at 21,948 μg/g, and rosmarinic acid at 21,467 μg/g. It has been suggested that regardless of species, the main compound is rosmarinic acid (Sarikurkcu et al. 2018; Sarikurkcu, Sahinler, and Tepe 2020; Tlili et al. 2021). Together with the genetic effects, the observed differences were also due to the specific response of the species to the variations of biotic and abiotic conditions. Indeed, previous studies have demonstrated that environmental factors modify the composition of phytochemicals in individual plants within the same population (Escobar-Bravo, Klinkhamer, and Leiss 2017). Furthermore, it has been reported that same species may respond differently to environmental effects by producing more phenolic acids than flavonoids due to ultraviolet radiation and the synthesis of higher level of flavonoids than phenolic acids when exposed to natural ultraviolet light (Hashiba, Iwashina, and Matsumoto 2006).

Antioxidant capacity

Since there was no specific *in vitro* antioxidant test that reflects all antioxidant properties, many assays are needed to compare the bioactive content and antioxidant capacity of the plant extract (Granato et al. 2018). The results of the antioxidant activities of *O.*

Table 3. Antioxidant activities of *O. inexspectata* and *O. armenum* methanolic extracts.

Assays	*O. inexspectata*	*O. armenum*	Trolox	BHA	BHT	EDTA
Phosphomolybdenum (EC_{50}: mg/mL)	2.07 ± 0.07^{c}	2.27 ± 0.18^{c}	1.05 ± 0.07^{b}	0.31 ± 0.02^{a}	0.40 ± 0.01^{a}	Not determined
CUPRAC reducing (EC_{50}: mg/mL)	1.63 ± 0.01^{d}	1.08 ± 0.02^{c}	0.27 ± 0.01^{b}	0.13 ± 0.01^{a}	0.17 ± 0.01^{a}	Not determined
FRAP reducing (EC_{50}: mg/mL)	1.04 ± 0.01^{d}	0.70 ± 0.01^{c}	0.10 ± 0.004^{a}	0.09 ± 0.004^{a}	0.19 ± 0.007^{b}	Not determined
DPPH radical (IC_{50}: mg/mL)	3.77 ± 0.10^{d}	2.03 ± 0.04^{c}	0.25 ± 0.02^{a}	0.21 ± 0.02^{a}	0.99 ± 0.02^{b}	Not determined
ABTS radical (IC_{50}: mg/mL)	2.68 ± 0.04^{c}	1.97 ± 0.13^{b}	0.30 ± 0.02^{a}	0.20 ± 0.01^{a}	0.29 ± 0.01^{a}	Not determined
Ferrous ion chelating (IC_{50}: mg/mL)	2.01 ± 0.09^{a}	6.51 ± 0.97^{b}	Not determined	Not determined	Not determined	0.05 ± 0.002^{a}

Note: The data indicated by the same superscripts within the same row are not different from each other according to the Tukey's honestly significant difference post hoc test at 5% significance level. EC_{50} (mg/mL), effective concentration at which the absorbance was 0.5 for reducing power and phosphomolybdenum assays. IC_{50} (mg/mL), inhibition concentration at which 50% of the DPPH and ABTS radicals were scavenged, and the ferrous ion-ferrozine complex were inhibited. BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) and EDTA (ethylenediaminetetraacetic acid) were used as positive controls.

inexspectata and *O. armenum* extracts for six assays are shown in Table 3. Except for the phosphomolybdenum (EC_{50}: 2.07 ± 0.07 mg/mL and 2.27 ± 0.18 mg/mL for *O. inexspectata* and *O. armenum*, respectively) and ferrous ion chelating (IC50: 2.01 ± 0.09 mg/mL and 6.51 ± 0.97 mg/mL for *O. inexspectata* and *O. armenum*, respectively) assays, the highest antioxidant activities were in the *O. armenum* extract (1.08 ± 0.02 mg/mL, 0.7 ± 0.01 mg/mL, 2.03 ± 0.04 mg/mL, and 1.97 ± 0.13 mg/mL for CUPRAC, FRAP, DPPH, and ABTS, respectively). The results show similar results for the synthetic antioxidants (BHA, BHT, and EDTA). Previous studies have reported that other *Onosma* species showed antioxidant properties (Ozer et al. 2018; Sarikurkcu et al. 2018; Sarikurkcu, Sahinler, and Tepe 2020; Tlili et al. 2021). The observed differences may be due to the efficiency of the bioactive compounds against free radicals (Djeridane et al. 2006). Furthermore, these differences may be also due to the synergism/antagonism among the antioxidants in the extracts (Granato et al. 2018).

Enzyme inhibitory properties

Currently there is interest regarding the use of plant extracts against many key metabolic enzymes in order to identify new drugs. The results of this study show for the first time that the anti-diabetic (α-amylase and α-glucosidase inhibitory capacity), the skin-whitening (Tyrosinase inhibitory activity) and anti-cholinesterase potential of *O. inexspectata* and *O. armenum* extracts. Indeed, one possible mechanism to control plasma glucose level in diabetes is the inhibition of the activities of key enzymes that are involved in the digestion of dietary starch into glucose, such as α-glucosidase and α-amylase (Agada et al. 2020). It has been reported that cholinesterase blockers have therapeutic uses against Alzheimer's disease, ataxia, senile dementia, myasthenia gravis and Parkinson's disease (Das et al. 2020). Furthermore, it has been suggested that the majority of the existing cholinesterase inhibitors affect AChE and/or BChE enzymes (Das et al. 2020). The inhibitors of tyrosinase are largely used to treat epidermal hyperpigmentation in humans (Pillaiyar, Manickam, and Namasivayam 2017).

Table 4. Enzyme inhibition activities of *O. inexspectata* and *O. armenum* methanolic extracts.

Assays	*O. inexspectata*	*O. armenum*	Galanthamine	Acarbose	Kojic acid
α-Amylase inhibition (IC_{50}: mg/mL)	3.78 ± 0.34^b	3.85 ± 0.06^b	Not determined	0.96 ± 0.04^a	Not determined
α-Glucosidase inhibition (IC_{50}: mg/mL)	3.76 ± 0.09^c	1.03 ± 0.03^a	Not determined	1.78 ± 0.04^b	Not determined
Tyrosinase inhibition (IC_{50}: mg/mL)	2.18 ± 0.05^b	2.12 ± 0.07^b	Not determined	Not determined	0.31 ± 0.01^a
AChE inhibition (IC_{50}: mg/mL)	1.40 ± 0.05^b	1.31 ± 0.01^b	0.003 ± 0.0001^a	Not determined	Not determined
BChE inhibition (IC_{50}: mg/mL)	>10	>10	0.006 ± 0.0001	Not determined	Not determined

Note: The data indicated by the same superscripts within the same row are not different from each other according to the Tukey's honestly significant difference post hoc test at the 5% significance level. IC_{50} (mg/mL), inhibition concentration at which 50% of the enzymes activities were inhibited. Galanthamine, acarbose, and kojic acid were used as standard references.

The results of *in vitro* enzymes inhibition activities of samples and the standards (Galanthamine, acarbose, and kojic acid) are reported as the IC_{50} values (Table 4). The results show that the two species exhibited inhibitory properties against the tested enzymes. There were no significant differences between the two species except for α-glucosidase inhibition where *O. armenum* was more effective (IC_{50}: 1.03 ± 0.03 mg/mL and 3.76 ± 0.09 mg/mL for *O. armenum* and *O. inexspectata*, respectively). It was also interesting to note that the *O. armenum* extract was more effective against α- glucosidase than acarbose (IC_{50}; 1.78 ± 0.04 mg/mL). These results were similar to those reported in the literature (Sarikurkcu et al. 2018; Sarikurkcu, Sahinler, and Tepe 2020; Tlili et al. 2021) and indicated that the aerial tissue extracts of *O. inexspectata* and *O. armenum* may be new significant enzyme inhibitors.

Conclusions

O. inexspectata and *O. armenum* were investigated for their inhibitory effects against α-amylase, α-glucosidase, tyrosinase and cholinesterase as key metabolic enzymes. The extracts showed inhibitory capacities against the targeted enzymes. The major detected compounds were chlorogenic acid, apigenin 7-glucoside, and hyperoside. The presence of total phenolic compounds and flavonoids in *Onosma* extracts together with the antioxidant properties and enzyme inhibitory capacities of the extracts suggests valuable effects against oxidative stress. Consequently, the *Onosma* extracts are important sources for additional pharmaceutical studies. Therefore, supplementary work should be done focusing upon the individual phenolics inf these species and their antioxidant and enzyme inhibitory properties.

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Disclosure statement

The authors confirm that there are no known conflicts of interest.

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