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NATURAL PRODUCT ANALYSIS

Onosma polyantha vs. *Onosma mollis*: Analysis of Phenolic Compounds Using Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS) and Assessment of the Antioxidant Activity

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ABSTRACT

To our knowledge, this is the first work on the influence of species upon the bioactive molecules, antioxidant properties, and enzyme inhibitory capacities against tyrosinase and α-amylase of *Onosma polyantha* and *O. mollis*. The levels of phenolic compounds were from 6.55 to 10.37 mg gallic acid equivalent/g extract. The concentrations of total flavonoids varied from 2.71 to 10.78 mg quercetin equivalent/g extract. Twenty-five compounds were quantified via liquid chromatography – electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). Significant differences were found between the two species. Chlorogenic acid, rosmarinic acid, hesperidin, and luteolin 7-glucoside were the major compounds in both species. Four antioxidant assays together with two enzyme tests confirmed that *O. polyantha* and *O. mollis* extracts exhibited remarkable antioxidant and enzyme inhibitory capacities. Statistical analyses confirmed that the biological activities depend on the synergism between phenolic compounds and radicals. The results proposed *O. polyantha* and *O. mollis* species as potential sources of bioactive compounds for industrial application.

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Antioxidant capacity; liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS); *Onosma mollis*; *Onosma polyantha*; phenolic compounds

Introduction

The increased accumulation of reactive oxygen species (ROS) can generate oxidative stress, cellular injuries, and chronic illnesses, such as aging and cardiovascular diseases. Indeed, several papers have shown that reactive oxygen species react with proteins, lipids, and DNA and induce damage (Stanely Mainzen Prince and Hemalatha 2018). Some commonly known synthetic antioxidants such as butylated hydroxyltoluene (BHT) and butylated hydroxyanisole (BHA) have been largely used for food conservation. However, many studies have reported the side effects of these compounds (Su et al. 2016).

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For these reasons, studies have been focused on natural antioxidant supplements as alternative against free radicals and their oxidative damages. Indeed, plants contain a high range of bioactive compounds that can be used as bioantioxidants, such as carotenoids, tocopherols, essential oils, and phenolic compounds. These latter are secondary metabolites that are of great importance due to their proved beneficial effects against many illnesses, such as inflammation, nephro- and hepatotoxicity (Tir et al. 2019; Feriani et al. 2020). Furthermore, the inhibitory effects against enzymes, such as tyrosinase and α-amylase, are valuable tools to verify the advantageous importance of plant extracts (Zhang et al. 2017; Tlili, Kirkan, and Sarikurkcu 2019). Indeed, it is well known that the study of the enzyme inhibitory properties of many products is one of the widely useful approaches against many health problems, such as tyrosinase for skin disorders, acetylcholinesterase for neurodegenerative problems, and α-glucosidase and α-amylase for diabetes mellitus (Locatelli et al. 2017).

It has been reported that people who eat daily vegetables have about half the risk of developing degenerative diseases (Gescher et al. 1998). The content of the extracted biomolecules from plants is influenced by various factors, such as plant species and the techniques used (Tlili et al. 2018a; Sarikurkcu, Ozer, and Tlili 2020).

Thus, it is important to discover conventional and nonconventional plants that contain wide ranges of bioactive molecules. *Onosma* L. is the largest genus of Boraginaceae family. The 102 species have been identified in Turkey while the genus has 150 species worldwide (Sarikurkcu et al. 2018). *Onosma* species are used medicinally against many body disorders such as fever, strangury, abdominal pain, and bronchitis and in dietary as food supplements such as adulterant agent in red chili powder (Chakraborti, Raghav, and Lal 2001; Ozgen et al. 2006). Recent studies regarding the phenolic composition and the antioxidant capacity have been reported for some *Onosma* species, such as *O. aucheriana* (Mašković et al. 2015) *O. heterophyllum* (Ozer et al. 2018), and *O. isaurica and O. bracteosa* (Saravanakumar et al. 2019).

However, to our knowledge, no results are available about *O. polyantha* and *O. mollis*. Hence, the aim of the current study was to identify the phenolic compounds from the aerial parts of *O. polyantha* and *O. mollis* using liquid chromatography-electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS). Furthermore, the effects of species on antioxidant activity and the enzyme inhibitory capacity were assessed.

Materials and methods

Chemicals

Gallic acid, (+)-catechin, pyrocatechol, chlorogenic acid, 2,5-dihydroxybenzoic acid, 4-hydroxybenzoic acid, (−)-epicatechin, caffeic acid, syringic acid, vanillin, taxifolin, sinapic acid, p-coumaric acid, ferulic acid, rosmarinic acid, 2-hydroxycinnamic acid, pinoresinol, quercetin, luteolin, and apigenin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Vanillic acid, 3-hydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, apigenin 7-glucoside, luteolin 7-glucoside, hesperidin, eriodictyol, and kaempferol were obtained from Fluka (St. Louis, MO, USA). Verbascoside, protocatechuic acid, and hyperoside were purchased from HWI Analytik (Ruelzheim, Germany). Methanol and

formic acid of HPLC grade were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany), respectively.

Ultra-pure water (18 mΩ) was obtained from a Milli-Q water purification system (Millipore). All other chemicals were of analytical grade.

Plant material

The aerial parts of *Onosma polyantha* DC. (1367 m., 38°44'20"N 37°14'31"E, Herbarium number: OC.5030) and *Onosma mollis* DC. (1380 m., 38°45'27"N 37°14'06"E, Herbarium number: OC.5032) were collected from Sugul Valley, Gurun, Sivas-Turkey on 23 June 2019. The plants were identified and deposited by Dr. Olcay Ceylan from the Department of Biology, Mugla Sitki Kocman University, Mugla-Turkey.

Preparation of the extracts

The samples were air dried in darkness and 5 g of the ground samples were macerated for 24 h in 100 mL of MeOH. The maceration process was repeated and the extracts obtained were mixed and concentrated under low pressure. The final extracts were stored at +4 °C until further use. The yields of *O. polyantha* and *O. mollis* were 17.03% and 6.13%, respectively.

Determination of the phenolic compositions of the extracts

The phytochemical compositions of *O. polyantha* and *O. mollis* methanol extracts were determined using spectrophotometric together with chromatographic methods.

The total quantities of the total phenolic compounds and flavonoids were assessed spectrophotometrically (Zengin et al. 2015a).

For total phenolic content, 0.25 mL of sample solution was mixed with Folin-Ciocalteu reagent (1 ml, 1:9). After 3 min, 0.75 mL of Na_2CO_3 solution (1%) were added and the absorbance was measured at 760 nm after 2 h incubation at room temperature. The results are reported as equivalents of gallic acid.

For total flavonoid content, 1 mL of sample solution was mixed with the same volume of $AlCl_3$ (2%) in methanol. Similarly, a blank was prepared by adding sample solution (1 ml) to methanol (1 ml) without $AlCl_3$. After 10 min incubation at room temperature, the sample and blank absorbance were measured at 415 nm. Absorbance of the blank was subtracted from that of the sample. Results are shown as equivalents of quercetin.

Chromatographic measurements were carried out using an Agilent Technologies 1260 Infinity liquid chromatography system hyphenated to a 6420 Triple Quad mass spectrometer. Chromatographic separation of phytochemicals was performed using a Poroshell 120 EC-C18 (100 mm × 4.6 mm I.D., 2.7 μm) column. LC–ESI–MS/MS analyzes were performed following the analytical conditions cited by Sarikurkcu, Ozer, and Tlili (2020).

The mobile phase was made up from solvent A (0.1%, v/v formic acid solution) and solvent B (methanol). The gradient profile was set as follows: 0.00 min 2% B eluent, 3.00 min 2% B eluent, 6.00 min 25% B eluent, 10.00 min 50% B eluent, 14.00 min 95% B

eluent, 17.00 min 95% B and 17.50 min 2% B eluent. The column temperature was maintained at 25 °C. The flow rate was 0.4 mL min^{-1} and the injection volume was 2.0 μL.

The tandem mass spectrometer was interfaced to the LC system via an ESI source. The electrospray source of the MS was operated in negative and positive multiple reaction monitoring (MRM) mode and the interface conditions were as follows: capillary voltage of −3.5 kV, gas temperature of 300 °C and gas flow of 11 L min^{-1}. The nebulizer pressure was 40 psi. The peaks of the analytes were identified by comparing the retention times, together with the monitoring ion pairs in an authentic standard solution.

Antioxidant activity and enzyme inhibitory capacity

To evaluate the total antioxidant activities of the extracts, the phosphomolybdenum assay was used (Zengin et al. 2015a). Sample solution (0.2 mL) was combined with 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance was measured at 695 nm after 90 min incubation at 95 °C.

Furthermore, the $DPPH^{\cdot}$ (1,1-diphenyl-2-picrylhydrazyl) and $ABTS^{+}$ [2,2-azino-bis (3-ethylbenzothiazloine-6-sulphonic acid) radical cation], were used to assess the radical scavenging capacity of the extracts (Zengin et al. 2015b). For DPPH radical scavenging activity, 1 mL of sample solution was mixed with 4 mL of a 0.004% methanol solution of DPPH. The absorbance was measured at 517 nm after 30 min incubation at room temperature in dark.

For the ABTS cation radical scavenging activity, the $ABTS^{+}$ radical cation was directly produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 12 to 16 h in dark at the room temperature. Prior to beginning the assay, ABTS solution was diluted with methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. Next, 1 mL of sample solution was mixed with 2 mL of ABTS solution. The absorbance was measured at 734 nm after 7 min incubation at room temperature.

Although the method described by Tepe et al. (2011) was used in the ferrous ion chelating assay. Briefly, 2 mL of sample solution were added to $FeCl_2$ solution (0.05 mL, 2 mM). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Similarly, a blank was prepared by adding sample solution (2 mL) to $FeCl_2$ solution (0.05 mL, 2 mM) and water (0.2 mL) without ferrozine. Then, the sample and blank absorbance were measured at 562 nm after 10 min incubation at room temperature.

To estimate the reducing power capacity [cupric ion reducing (CUPRAC), and ferric reducing antioxidant power (FRAP)], the methods used by Apak et al. (2006) and Kocak et al. (2016) were applied.

For the CUPRAC assay, 0.5 mL of sample solution were added to a premixed reaction mixture containing $CuCl_2$ (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM) and ammonium acetate buffer (1 mL, 1 M, pH 7.0). Similarly, a blank was prepared by adding 0.5 mL of sample solution to a premixed reaction mixture (3 mL) without $CuCl_2$. The absorbance was subsequently measured at 450 nm after 30 min incubation at room temperature.

For the FRAP assay, 0.1 mL of sample solution were added to a premixed FRAP reagent (2 ml) containing acetate buffer (0.3 M, pH 3.6), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (10 mM) in 40 mM HCl and ferric chloride (20 mM) in a ratio of 10:1:1 (v/v/v). The absorbance was measured at 593 nm after 30 min incubation at room temperature.

The antioxidant activities of the extracts in each protocol were determined using the relationships obtained from the calibration graphs of the standard antioxidants.

Additionally, the enzyme inhibitory capacities vs. tyrosinase and α-amylase were assessed (Sarikurkcu, Ozer, and Tlili 2020). Tyrosinase inhibitory activity was measured using a modified dopachrome method with levodopa as the substrate. The 25 µL of sample solution were mixed with tyrosinase solution (40 µL) and phosphate buffer (100 µL, pH 6.8) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of levodopa (40 µL). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (tyrosinase) solution. The sample and blank absorbance were measured at 492 nm after 10 min incubation at 25 °C. The results are provided in kojic acid equivalents.

Inhibitory activity on α-amylase was performed using the Caraway-Somogyi iodine/potassium iodide (IKI) method. The 25 µL of sample solution were mixed with α-amylase solution (50 µL) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96-well micro plate and incubated for 10 min at 37 °C. After preincubation, the reaction was initiated by the addition of starch solution (50 µL, 0.05%). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme solution (α-amylase). The reaction mixture was incubated 10 min at 37 °C. The reaction was then stopped with the addition of HCl (25 µL, 1 M) followed by the introduction of iodine-potassium iodide solution (100 µL). The sample and blank absorbance were measured at 630 nm. Absorbance of the blank was subtracted from that of the sample. The results are provided in acarbose equivalents.

Statistical analysis

The results are shown as mean value ± 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 version 22.0) were applied to characterize the statistical significance among the data.

Results and discussion

Phenolic compounds

To our knowledge, this is the first study regarding the phenolic compounds from the aerial parts of *Onosma polyantha* and *O. mollis*. The results presented in Figure 1 show that total phenolic compounds and total flavonoids content differed significantly between the two species. *Onosma polyantha* presented the higher flavonoid level (10.78 mg QEs/g extract) while the highest total phenolics were present in *O. mollis* (10.37 mg GAEs/g extract). The observed differences were probably due to the effect of species as has been reported previously (Tlili et al. 2018b; Saravanakumar et al. 2019).

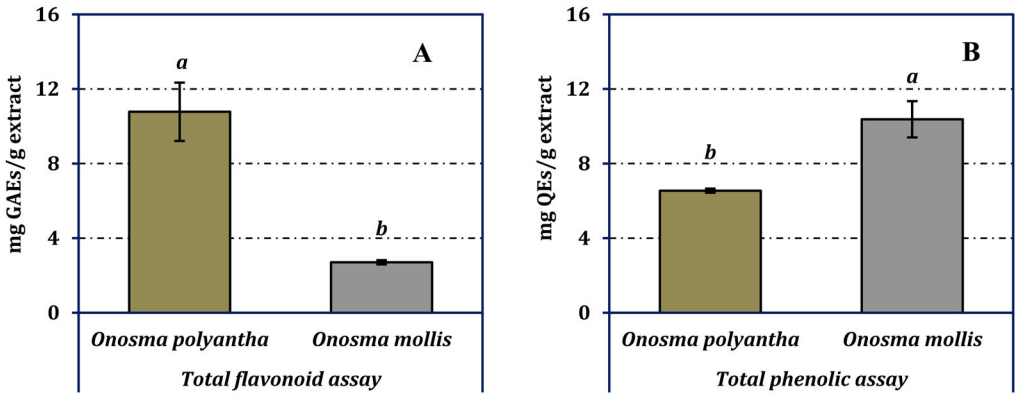

Figure 1. (A) Total phenolic and (B) flavonoid contents of *Onosma polyantha* and *O. mollis* methanol extracts. QEs and GAEs represent the quercetin and gallic acid equivalents, respectively. Values indicated by the same superscripts are not different from each other according to Student's *t*-test at 5% significance level.

Since we did not find information in the literature about the phenolic compounds in these two species, a comparative with other *Onosma* species was performed. Indeed, the contents of total phenolic compounds in this study were lower than those reported for *O. isaurica* and *O. bracteosa* (Saravanakumar et al. 2019) and similar to those present in *O. heterophyllum* (Ozer et al. 2018). The total flavonoids levels were higher than those reported in *O. isaurica* and *O. heterophyllum*. Regardless of species, results of this study showed that *O. polyantha* and *O. mollis* were an important source of phenolic compounds when compared to other medicinal species, such as capers (Yahia et al. 2020), *Periploca laevigata* (Tlili et al. 2018a), and *Symphytum anatolicum* (Sarikurkcu, Ozer, and Tlili 2019).

The liquid chromatography coupled with electrospray tandem mass spectrometry-mass chromatograms of *O. polyantha* and *O. mollis* extracts are shown in Figure 2. The major detected compounds were presented in Figure 3. Indeed, 25 phenolic compounds were identified in *O. polyantha* and *O. mollis* extracts and significant differences were detected between the compositions of each extract (Table 1). Among them and in ascending order, the major compounds in *O. polyantha* were luteolin 7-glucoside (ca. 1460 µg/g extract), rosmarinic acid (ca. 1590 µg/g extract), and chlorogenic acid (ca.5750 µg/g extract).

However, in *O. mollis,* the primary molecules were hesperidin (ca.2524 µg/g extract) and rosmarinic acid (ca. 3166 µg/g extract). The level of chlorogenic acid in *O. mollis* (ca. 691 µg/g) was 8.31-fold lower than the value in *O. polyantha*. The levels of apigenin 7-glucoside (ca. 592 µg/g), apigenin (ca. 728 µg/g), luteolin (ca. 424 µg/g), and 4-hydroxybenzoic acid (ca. 560 µg/g) in *O. polyantha* extract were 23.93-, 13.07-, 2.46-, and 2.06-fold, respectively, higher than those detected in *O. mollis* extract (ca. 22 µg/g, ca. 55 µg/g, ca. 172 µg/g and ca. 271 µg/g, respectively).

On the other hand, the levels of caffeic acid (ca. 126 µg/g), vanillic acid (ca. 213 µg/g), protocatechuic acid (ca. 95 µg/g) and pinoresinol (677 µg/g) in *O. mollis* were 1.99-, 1.68-, 1.36-, and 1.28-fold, respectively, higher than those observed in *O. polyantha*. 3,4-Dihydroxyphenylacetic acid was detected only in *O. mollis* (5.72 µg/g). İt was also remarkable that the levels of sinapic acid, quercetin, verbascoside, hyperoside, and 3-

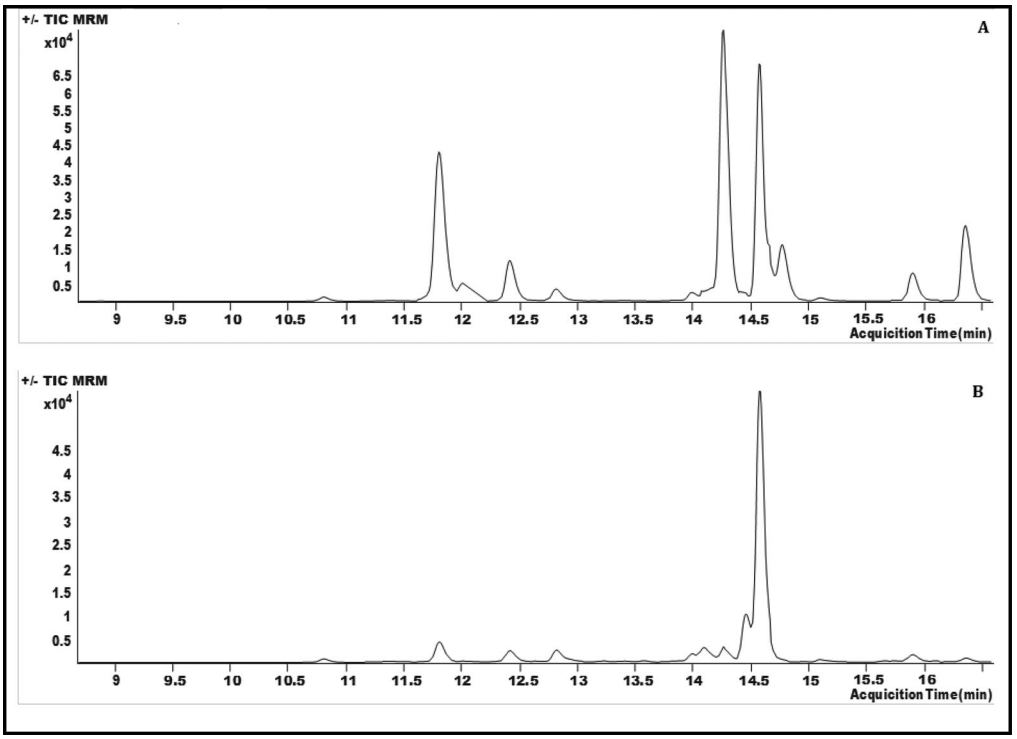

Figure 2. Liquid chromatography electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS) chromatograms of the methanol extracts of (A) *Onosma polyantha* and (B) *O. mollis*.

hydroxybenzoic acid were highly present in *O. mollis* (14.96 µg/g, 19.19 µg/g, 20.84 µg/g, 47.96 µg/g, and 52.88 µg/g, respectively) when compared to *O. polyantha* extract (1.94 µg/g, 1.72 µg/g, 1.67 µg/g, 2.48 µg/g, and 3.73 µg/g, respectively). Eriodictyol presented the lowest levels in the species (0.17 and 0.42 µg/g in *O. polyantha* and *O. mollis*, respectively).

A similar composition has been reported for *O. bracteosa* species by Saravanakumar et al. (2019) who suggested that the major compounds were chlorogenic acid, rosmarinic acid, and luteolin 7-glucoside. Kirkan et al. (2018) reported that among the 12 detected compounds in *O. tauricum,* the major components were chlorogenic acid and rosmarinic acid, but the values were lowest than those detected in the current work. Ozer et al. (2018) suggested that rosmarinic acid is the most prevalent compound among the 12 detected in *O. heterophyllum*.

Sarikurkcu et al. (2018) reported than of the 11 detected compounds in *O. gigantea,* the primary component was rosmarinic acid. When compared to medicinal species such as *Rosmarinus officinalis*, *Thymus vulgaris*, and *Camellia sinensis* (Tlili and Sarikurkcu, 2020) the phenolic compounds detected in *O. polyantha* and *O. mollis* extracts clarified in part the use of these species in traditional medicine (Chakraborti, Raghav, and Lal 2001; Ozgen et al. 2006) and encourages the potential use of their extract as food additives. The results also explained the beneficial effects recently reported for some *Onosma* species, such as the anti-inflammatory, antinociceptive, and antimicrobial activities (Tosun et al. 2008; Katanić Stanković et al. 2020).

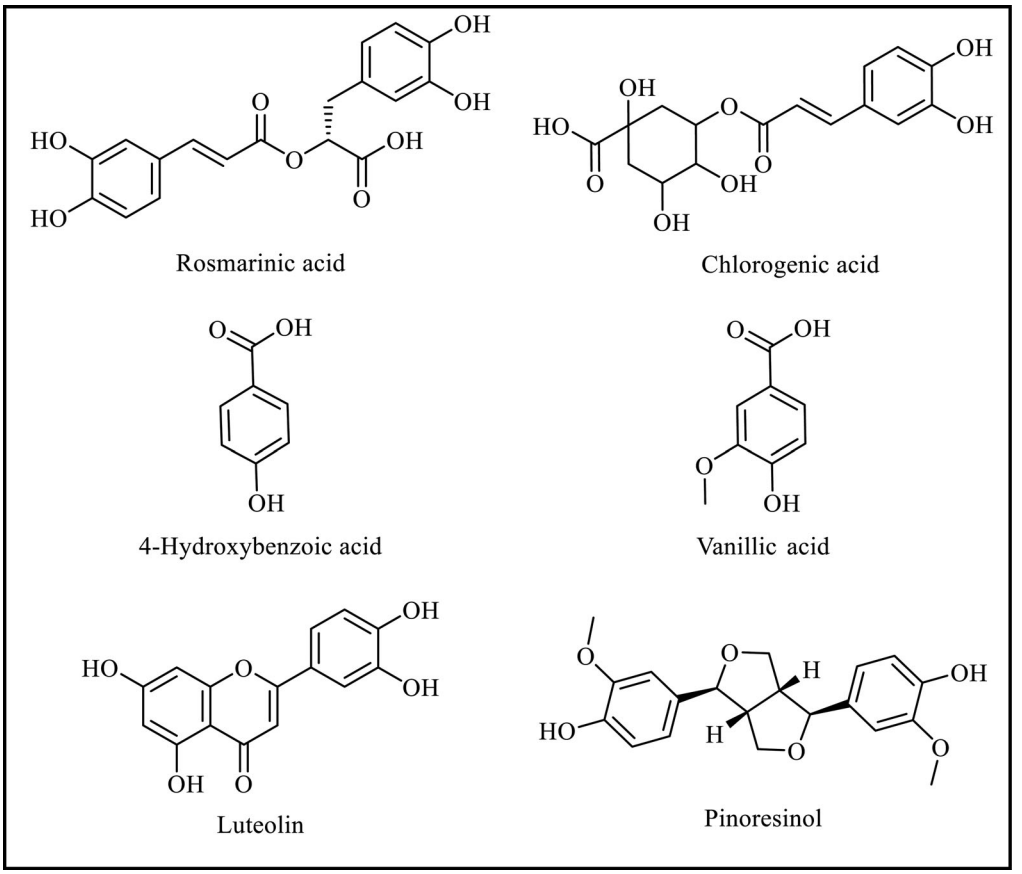

Figure 3. Primary molecules identified by LC-ESI-MS/MS in *Onosma polyantha* and *O. mollis*.

Antioxidant capacity and enzyme inhibitory effects

Synthetic or natural antioxidants play an important role in the reduction of reactive oxygen species-induced oxidative stress (Deng et al. 2014) and therefore, the determination of the phenolic compounds in plants extract allows the characterization of their beneficial effects. In the current study, six assays were used to calculate the antioxidant activity of *O. polyantha* and *O. mollis* extracts (Table 2). Results showed that all extracts exhibited antioxidant capacities, which encourages the use of these species as sources of phenolic compounds for the food and pharmaceutical industries. Despite the observed differences in the composition between the two species, the results did not show significant differences in the antioxidant activity except for total antioxidant capacity, which confirms that the antioxidant properties are due to the relationship between phenolic compounds and also between phenolic compounds and oxidants as has been previously reported (Tlili, Kirkan, and Sarikurkcu 2019; Sarikurkcu, Ozer, and Tlili 2020).

In addition to the antioxidant capacity, it has been reported that plant extracts with α-amylase and tyrosinase inhibitory capacities are considered to be alternative sources for pharmaceutical industries (Kim and Uyama 2005; Aslan et al. 2010). The results of the inhibitory effect of *O. polyantha* and *O. mollis* extracts toward the two enzymes are shown in Table 2.

Table 1. Concentration (μg/g extract) of the identified bioactive compounds in the methanolic extracts from *Onosma polyantha* and *O. mollis*.

Compounds	*O. polyantha*	*O. mollis*
Gallic acid	2.82 ± 0.04^{b}	4.90 ± 0.02^{a}
Protocatechuic acid	56.51 ± 1.50^{b}	95.21 ± 0.07^{a}
3,4-Dihydroxyphenylacetic acid	Not detected	5.72 ± 0.04
Chlorogenic acid	5749.8 ± 175.7^{a}	691.37 ± 27.79^{b}
2,5-Dihydroxybenzoic acid	13.46 ± 0.58^{b}	26.55 ± 2.48^{a}
4-Hydroxybenzoic acid	560.10 ± 1.20^{a}	271.56 ± 1.10^{b}
Caffeic acid	63.36 ± 0.08^{b}	126.02 ± 0.28^{a}
Vanillic acid	155.56 ± 13.26^{b}	213.10 ± 11.93^{a}
Syringic acid	8.86 ± 0.62^{b}	22.08 ± 1.03^{a}
3-Hydroxybenzoic acid	3.73 ± 0.12^{b}	58.88 ± 1.01^{a}
Vanillin	16.55 ± 0.22^{b}	42.12 ± 3.61^{a}
Verbascoside	1.67 ± 0.01^{b}	20.84 ± 0.13^{a}
Sinapic acid	1.94 ± 0.35^{b}	14.96 ± 0.81^{a}
p-Coumaric acid	35.14 ± 0.49^{a}	52.40 ± 6.76^{a}
Ferulic acid	96.98 ± 0.86^{b}	504.66 ± 19.52^{a}
Luteolin 7-glucoside	1460.3 ± 24.9^{a}	47.87 ± 4.59^{b}
Hesperidin	18.52 ± 2.15^{b}	2524.5 ± 76.6^{a}
Hyperoside	2.48 ± 0.83^{b}	47.96 ± 1.00^{a}
Rosmarinic acid	1589.4 ± 76.7^{b}	3166.5 ± 58.2^{a}
Apigenin 7-glucoside	592.78 ± 1.01^{a}	22.13 ± 0.87^{b}
Pinoresinol	527.52 ± 7.71^{b}	677.00 ± 10.14^{a}
Eriodictyol	0.17 ± 0.02^{b}	0.42 ± 0.07^{a}
Quercetin	1.72 ± 0.22^{b}	19.19 ± 0.30^{a}
Luteolin	424.78 ± 0.79^{a}	172.52 ± 5.87^{b}
Apigenin	728.41 ± 35.93^{a}	55.70 ± 3.13^{b}

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

Table 2. Biological activities of the methanolic extracts from *O. polyantha* and *O. mollis*.

Assays	*O. polyantha*	*O. mollis*	BHA	Trolox	EDTA	Acarbose	Kojic acid
FRAP reducing (EC_{50}: mg/mL)	2.36 ± 0.29^{b}	2.53 ± 0.58^{b}	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}	–	–	–
CUPRAC reducing (EC_{50}: mg/mL)	3.96 ± 0.29^{b}	4.38 ± 0.23^{b}	0.13 ± 0.01^{a}	0.28 ± 0.02^{a}	–	–	–
Phosphomolybdenum (EC_{50}: mg/mL)	2.74 ± 0.16^{b}	3.95 ± 0.36^{c}	0.31 ± 0.01^{a}	1.05 ± 0.08^{a}	–	–	–
ABTS radical (IC_{50}: mg/mL)	6.97 ± 1.43^{b}	8.73 ± 0.45^{b}	0.19 ± 0.01^{a}	0.31 ± 0.02^{a}	–	–	–
DPPH radical (IC_{50}: mg/mL)	14.75 ± 1.49^{b}	14.22 ± 1.16^{b}	0.20 ± 0.01^{a}	0.25 ± 0.01^{a}	–	–	–
Ferrous ion chelating (IC_{50}: mg/mL)	2.04 ± 0.01^{c}	1.95 ± 0.02^{b}	–	–	0.05 ± 0.003^{a}	–	–
α-Amylase inhibition (IC_{50}: mg/mL)	2.99 ± 0.03^{b}	2.94 ± 0.01^{b}	–	–	–	0.97 ± 0.03^{a}	–
Tyrosinase inhibition (IC_{50}: mg/mL)	2.10 ± 0.03^{b}	2.18 ± 0.01^{b}	–	–	–		0.30 ± 0.01^{a}

The results indicated by the same superscripts within the same row are not different from each other according to Tukey's honestly significant difference post hoc test at the 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, the α-amylase and tyrosinase activities were inhibited, and the ferrous ion-ferrozine complex were inhibited.

BHA and EDTA, butylated hydroxyanisole, and ethylenediaminetetraacetic acid (disodium salt), respectively.

- not determined.

When compared to the inhibitory effect of kojic acid and acarbose as standard compounds with inhibitory effect against tyrosinase and α-amylase enzymes, respectively, it was clear that the extracts exhibited enzyme inhibitory capacities. The results show the

absence of significant differences between the two extracts, which confirmed the role of the synergism/antagonism cited above. Other authors have reported the enzyme inhibitory effects of *Onosma* species, such as *O. gigantea* (Sarikurkcu, Ozer, and Tlili 2018), *O. heterophyllum* (Ozer et al. 2018), *O. isaurica and O. bracteosa* (Saravanakumar et al. 2019)

Conclusions

To the best of our knowledge, there has been no previous study characterizing the phytochemical content of *Onosma polyantha* and *O. mollis*. This work targeted the effect of species on the phenolic compounds content together with the biological activity. Twenty-five phenolic compounds were identified and the major compounds differ among the two species.

All extracts showed interesting antioxidant and enzyme inhibitory activities. The absence of significant difference in the *in vitro* studies confirmed the interactions between phenolic compounds. The results highlighted the potential use of *O. polyantha* and *O. mollis* species as a source of bioactive molecules for food and pharmaceutical industries. More studies, including *in vivo* assays, are required to more explore these species.

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