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Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS) Identification of Phytochemicals and the Effects of Solvents on Phenolic Constituents, Antioxidant Capacity, Skin-Whitening and anti-Diabetic Activity of *Onosma mitis*

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ABSTRACT

To the best of our knowledge, this is the first study on the effect of solvent (water, methanol, and ethyl acetate) upon phenolics extracted from *Onosma mitis*. The antioxidant capacities together with the enzyme inhibitory properties toward tyrosinase and α-amylase were also assessed. The yields of the extract were 2.62, 10.93, and 21.68% for ethyl acetate, methanol, and water, respectively. The concentrations of phenolic compounds were from 11.89 (ethyl acetate) to 2.66 mg gallic acid equivalent/g extract (methanol). Twenty-five molecules were identified and quantified by liquid chromatography – electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). Significant differences were present between the extracts; methanol presented the highest number of compounds (25) while ethyl acetate provided the lowest (18). Chlorogenic acid, luteolin 7-glucoside, rosmarinic acid, and apigenin 7-glucoside were the major compounds regardless of solvent. Antioxidant and inhibitory enzyme assays showed that O. *mitis* exhibited interesting biological properties. The results illustrate the use of O. *mitis* as a promising source of biomolecules for industrial applications.

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Introduction

Recently, due to the increasing demand for phenolics as natural antioxidants and food stabilizers, many studies have focused on the extraction of these biomolecules. Indeed, the solvent is crucial for extraction due to its influence upon the quantity and quality. In fact, the extract influences on the diversity of the compounds therefore affects the biological results. Various solvents, such as water, acetone, methanol, ethanol,

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chloroform, and ethyl acetate, have been used to extract phytochemicals (Azabou et al. 2020; Sarikurkcu, Ozer, and Tlili 2020; Ismail et al. 2019; Mejri et al. 2014). Although the solvent type is key in the extraction of phenolics, no precise system has been recommended for these bioantioxidants. However, regardless of solvent, plant extracts presented diverse biological activities, including antioxidant, anti-diabetic, antimicrobial, and anti-inflammatory properties (Mejri et al. 2014; Prabakaran et al. 2018; Sarikurkcu, Ozer, and Tlili 2019; Ren et al. 2020).

Onosma L. is in the Boraginaceae family widely distributed across the Asia. More than 150 *Onosma* species have been identified, including 95 in Turkey, 29 in China, and 8 in Pakistan (Sarikurkcu, Sahinler, et al. 2020). The origin of the name of the species is Latin 'osma', which means "smell" in Greek (Stearn 1993). Previous studies have reported that *Onosma* species are used traditionally against many illnesses such as abdominal pain, fever, and bronchitis (Mašković et al. 2015; Sarikurkcu, Sahinler, et al. 2020). Furthermore, *Onosma* is used as food additive as an adulterant agent in red chili powder (Ozgen et al. 2006). Recent studies have been reported for the phenolic and the antioxidant properties of *Onosma* species, such as *O. aucheriana* (Mašković et al. 2015), *O. heterophyllum* (Ozer et al. 2018), and *O. pulchra* (Sarikurkcu, Sahinler, et al. 2020) *O. aucheriana*, *O. frutescens*, and *O. Sericea* (Sarikurkcu, Sahinler, and Tepe 2020). But to our knowledge, no data is avaliable in the literature regarding *Onosma mitis*.

Hence, the goal of the present work was to use liquid chromatography - electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS) as a sensitive, high-throughput, rapid, and accurate method to characterize phenolic extracts from the aerial parts of *O. mitis*. The high-throughput screening employed provides a clear picture of the bioactive compounds in the targeted *Onosma* species. The knowledge of the biomelcules in *Onosma* species may guide the potential discovery of drugs from these species. Hence, the influence of solvent upon the antioxidant activity and the enzyme inhibitory properties were targeted.

Materials and methods

Chemicals

Methanol, Folin–Ciocalteu's reagent, and ferric chloride were purchased from Merck (Darmstadt, Germany). 3,4-Dihydroxy-L-phenylala-nine (L-DOPA), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), 1,1-diphenyl-2-picrylhy-drazyl (DPPH), 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 rosmarinic acid, gallic acid, pyrocatechol, (þ)-catechin, 2,5-dihydroxybenzoi acid, chlorogenic acid, 4-hydroxybenzoic acid, epicatechin, syringic acid, vanillin, taxifolin, quercetin, sinapic acid, p-coumaric acid, luteolin,caffeic acid, ferulic acid, 2-hydroxycinnamic acid, pinoresinol, and apigenin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Vanillic acid, hesperidin, luteolin 7-glucoside, 3,4-dihydroxyphenylacetic acid, kaempferol, apigenin 7-glucoside, 3-hydroxybenzoic acid, and eriodictyol, were purchased from Fluka (St. Louis, MO, USA).

Plant material

The aerial parts of *Onosma mitis* Boiss. & Heldr. were collected from the Korkuteli-Denizli Highway, Korkuteli, Antalya-Turkey on May 2018 (1440 m., 37°02'41"N; 30°04'34"E). Dr. Olcay Ceylan, a senior taxonomist from the Department of Biology, Mugla Sitki Kocman University, Mugla-Turkey, carried out the taxonomical identification of the plant material. The plant material was deposited at the herbarium of Mugla Sitki Kocman University (Voucher Number: OC.5035). The aerial parts of the plant were used to obtain aqueous, ethyl acetate, and methanol extracts.

Extraction

The aerial parts (5 g) of plant species were macerated for 24 hours to separately prepare the ethyl acetate and methanol extracts. The extracts were then concentrated under vacuum to remove the solvent. To obtain the water extract, the plant material (5 g) was mixed with 100 ml of boiling water (1:20), shaken at 150 rpm for 15 min, and lyophilized (Sarikurkcu et al. 2018). The yields of ethyl acetate, methanol, and water extracts were 2.62, 10.93, and 21.68% (w/w), respectively.

Phenolic and flavonoid determination

To estimate the content of total phenolics, 0.25 mL of sample solution were mixed with 1 ml of diluted Folin-Ciocalteu reagent (1:9), shaken vigorously, and 0.75 mL of Na_2CO_3 (1%) were added. The absorbance was read at 760 nm after 2 h at room temperature. Total phenolic content was expressed as equivalents of gallic acid (Sarikurkcu, Ozer, and Tlili 2019).

For the determination of total flavonoids, 1 mL of sample solution was mixed with the same volume of 2% aluminum trichloride in methanol. The blank was prepared by adding 1 mL sample solution to 1 mL methanol without $AlCl_3$. The absorbance was read at 415 nm after 10 min incubation at room temperature. Total flavonoid content was expressed as equivalents of quercetin (Sarikurkcu, Ozer, and Tlili 2019).

Identification of phenolics by liquid chromatography – electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS)

The bioactive compounds in the extracts of *Onosma mitis* were determined by LC–ESI–MS/MS (Cittan and Çelik, 2018). An Agilent 1260 Infinity liquid chromatograph with a 6420 Triple Quad mass spectrometer was used. A Poroshell 120 EC-C18 (100 mm × 4.6 mm I.D., 2.7 μm) column was employed for the separation. Formic acid solution (0.1%, v/v) and methanol were the mobile phase (A and B, respectively). The gradient profile was set to: 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 injection volume was 2.0 μl and the flow rate 0.4 ml/min.

The tandem mass spectrometer was interfaced to the LC system via an electrospray ionization (ESI) source. The electrospray source of the MS was operated in the negative

and positive multiple reaction monitoring (MRM) modes. The interface conditions included a gas temperature of 300 °C, a gas flow of 11 l/min, and a capillary voltage of −3.5 kV. 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.

Screening of biological activity

The antioxidant activities of the ethyl acetate, methanol, and water extracts of *O. mitis* were analyzed by the phosphomolybdenum, CUPRAC, FRAP, DPPH, ABTS, and ferrous ion chelating (Apak et al. 2006; Kocak et al. 2016; Tepe et al. 2011; Zengin et al. 2015) assays. In addition, skin-whitening and anti-diabetic activities of extracts were investigated by tyrosinase and α-amylase inhibitory activity measurements (Sarikurkcu et al. 2018).

Statistical analysis

The results were expressed as mean ± standard deviation. SPSS v. 22.0 software was used for statistical calculations. Tukey's honestly significant difference post hoc test at 0.05 and ANOVA (one-way analysis of variance) tests were used to identify statistically significant difference. Furthermore, the relative antioxidant capacity index was determined to convert the results into a comparable form. Since characterization of the contribution of phytochemicals to antioxidant activity provides better results, the phenolic and flavonoid contents were integrated in the calculations (Sun and Tanumihardjo 2007).

Results and discussion

Phytochemical compositions of the extracts

The presence of bioactive compounds of plant species or plant tissues is affected by the environmental conditions, the extraction solvents, and analytical technique (Sinan et al. 2020; Chigayo et al. 2016; Özcan, Tzakou, and Couladis 2009). In order to estimate the phytochemical composition of *Onosma mitis* using water, methanol, and ethyl acetate, the total phenolics and total flavonoids were determined spectrophotometrically (Figure 1). Methanol provided the best results for total phenolics (20.66 mg GAEs/g) and flavonoids (27.79 mg QEs/g). Water extract occupied the second position (18.65 mg GAEs/g and 18.42 mg QEs/g for total phenolics and flavonoids, respectively). The poorest extract was ethyl acetate with 11.89 mg GAEs/g for total phenolics and 6.49 mg QEs/g for total flavonoids. Previous studies have reported that methanol yielded a higher phenolic content compared to water (Sinan et al. 2020; Chigayo et al. 2016) and ethyl acetate (Barbouchi et al. 2020). However, water has been suggested to be more efficient for phenolics than methanol (Barbouchi et al. 2020). The observed differences were due to the polarity of the solvents and the degree of solubility of the compounds. İn general, polar solvents give higher extraction yields. The higher efficiency for methanol was due to the nature of the individual molecules that were subsequently determined.

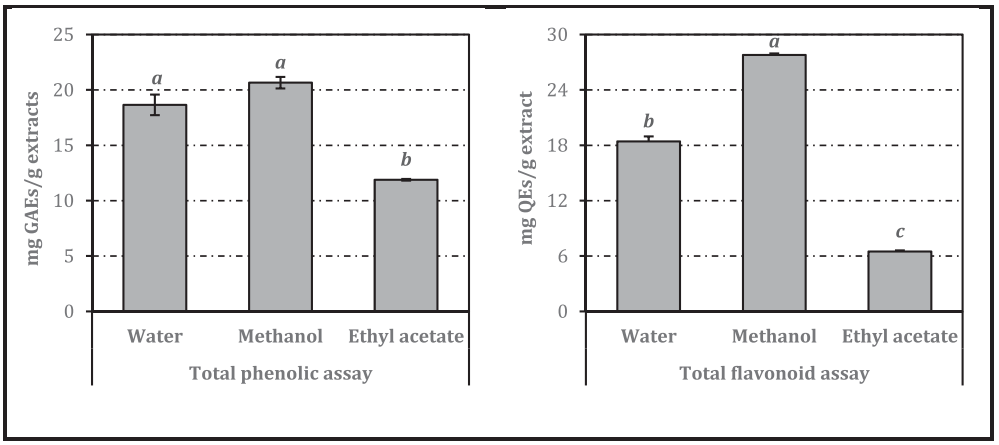

Figure 1. Total phenolics and flavonoids of *O. mitis* extracts. The total phenolics and flavonoids were determined by Folin–Ciocalteu's and aluminum trichloride assays and results are presented as the gallic acid equivalent (GAE) and quiercetin equivalent (QE), respectively. Values with the same superscripts are not different from the honestly significant difference after Tukey's hoc test at the 5% significance level.

Twenty-five compounds were determined in the extracts (Table 1) by LC–ESI–MS/MS (Figure 2). Furthermore, the chromatographic analysis corroborated the above measurements. LC–ESI–MS/MS showed that the methanol extract had the richest quantitative and qualitative composition, followed by the aqueous and ethyl acetate extracts. The total content of the methanol extract was 17331.16 µg/g extract. Twenty-three compounds were identified in the water extract for a total of 5306.49 µg/g extract. The ethyl acetate extract provided eighteen molecules with a total of 3476.21 µg/g extract.

The primary compounds in the methanol extract were luteolin 7-glucoside (3035 µg/g extract) followed by apigenin 7-glucoside (1459 µg/g extract). Three other compounds were also identified in an appreciable concentrations: chlorogenic acid (175 µg/g extract), apigenin (153 µg/g extract), and hesperidin (123 µg/g extract). Luteolin, *p*-coumaric acid, and caffeic acid were present at71.59, 62.96, and 50.97 µg/g extract, respectively. The lowest values were observed for quercetin, gallic acid, and eriodictyol (0.96, 0.95, and 0.49 µg/g extract, respectively). In the water extract, the major component was rosmarinic acid (5209 µg/g extract), followed by luteolin 7-glucoside (3847 µg/g extract), apigenin 7-glucoside (3142 µg/g extract), and chlorogenic acid (3136 µg/g extract). Hesperidin and hyperoside were also identified at high levels (621.93 and 526.78 µg/g extract, respectively). 4-Hydroxybenzoic acid and caffeic acid ware present at 357.25 and 168.89 µg/g extract, respectively. The lowest value was for eriodictyol at 0.57 µg/g extract. In the ethyl acetate extract, the concentrations were lower than in the others. The major compounds were luteolin 7-glucoside and rosmarinic acid (976 and 892 µg/g extract, respectively). The levels of apigenin 7-glucoside, chlorogenic acid, and hyperoside were 447.31, 153.55, and 130.68 µg/g extract, respectively. The concentrations of quercetin and gallic acid were higher than in the other extracts (18.64 and 14.60 µg/g extract, respectively). The observed differences between extracts were due to the nature of the solvent and the samples (Ballesteros-Vivas et al. 2019; Venkatachalam et al. 2020). Since there was no literature data available about *O. mitis*, the results were

Table 1. Concentration (μg/g extract) of selected phenolics in *O. mitis* extracts[1].

Compound	Ethyl acetate	Methanol	Water
Gallic acid	14.60 ± 0.22^{a}	4.13 ± 0.16^{b}	0.95 ± 0.01^{c}
Protocatechuic acid	36.71 ± 0.96^{a}	38.41 ± 0.20^{a}	1.50 ± 0.04^{b}
3,4-Dihydroxyphenylacetic acid	Not detected	2.16 ± 0.10	2.78 ± 0.05
Chlorogenic acid	153.55 ± 1.40^{c}	3136.6 ± 2.3^{a}	175.20 ± 1.69^{b}
2,5-Dihydroxybenzoic acid	Not detected	29.76 ± 0.31	8.88 ± 0.46
4-Hydroxybenzoic acid	385.25 ± 3.40^{a}	357.25 ± 4.09^{b}	12.72 ± 0.57^{c}
Caffeic acid	90.16 ± 1.18^{b}	168.89 ± 2.42^{a}	50.97 ± 0.37^{c}
Vanillic acid	Not detected	18.18 ± 0.56	8.31 ± 0.05
Syringic acid	Not detected	5.40 ± 0.49	2.93 ± 0.42
Vanillin	Not detected	7.70 ± 0.78	Not detected
Verbascoside	19.88 ± 3.32	2.97 ± 0.06	Not detected
Sinapic acid	5.53 ± 1.47^{b}	17.97 ± 0.51^{a}	1.74 ± 0.09^{c}
p-Coumaric acid	84.93 ± 2.40^{a}	61.40 ± 0.39^{b}	62.96 ± 0.36^{b}
Ferulic acid	57.20 ± 3.11^{a}	35.64 ± 1.25^{b}	19.76 ± 0.29^{c}
Luteolin 7-glucoside	976.68 ± 31.64^{c}	3847.8 ± 46.5^{a}	3035.4 ± 15.9^{b}
Hesperidin	38.29 ± 1.73^{c}	621.93 ± 7.73^{a}	123.91 ± 2.14^{b}
Hyperoside	130.68 ± 2.19^{b}	526.78 ± 8.13^{a}	69.75 ± 3.22^{c}
Rosmarinic acid	892.16 ± 107.76^{b}	$5209.\ 5 \pm 52.3^{a}$	26.43 ± 1.30^{c}
Apigenin 7-glucoside	447.31 ± 9.37^{c}	3142.1 ± 38.4^{a}	1459.9 ± 1.3^{b}
Pinoresinol	Not detected	7.89 ± 0.56	13.78 ± 0.20
Eriodictyol	Not detected	0.57 ± 0.01	0.49 ± 0.07
Quercetin	18.64 ± 0.45^{a}	3.78 ± 0.01^{b}	0.96 ± 0.02^{c}
Luteolin	50.66 ± 1.16^{b}	48.61 ± 0.42^{b}	71.59 ± 5.63^{a}
Kaempferol	36.45 ± 2.61^{a}	7.93 ± 0.17^{b}	2.06 ± 0.23^{b}
Apigenin	29.53 ± 1.00^{b}	25.86 ± 0.22^{b}	153.48 ± 7.57^{a}

[1]The values indicated by the same superscripts within the same row are not different according to the Tukey's honestly significant difference post hoc test at 5% significance level.

compared to measurements of other *Onosma* species. Sarikurkcu, Sahinler, and Tepe (2020) reported that the major compounds in methanol extract of *O. aucheriana*, *O. frutescens*, and *O. sericea* were rosmarinic acid (71,332 μg/g extract), hyperoside (15,682 μg/g extract), and apigenin 7-glucoside (22707 μg/g extract). The main compound in the methanol and aqueous extracts of *O. pulchra* was rosmarinic acid (18561 and 2517 μg/g extract) and in ethyl acetate extract was apigenin 7-glucoside at 1808 μg/g extract (Sarikurkcu, Sahinler, et al. 2020). In addition to solvent effects, the differences may due to genetic differences.

Antioxidant activity

Antioxidant capacities are commonly used during the measurement of the concentration necessary to provide the maximal effect (Gmax) or 50% of the maximum response/inhibition (EC_{50}/IC_{50}) which represents the minimum concentration required to cause a significant effect (Chen, Bertin, and Froldi 2013; de Torre et al. 2019). Indeed, antioxidants neutralize the harmful effects of free radicals on cell membranes through mechanisms that include single electron transfer (SET) and the ability to chelate transition metals (Prior, Wu, and Schaich 2005; Brewer 2011). SET assays involve scavenging effects compared to 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), cupric-ion reducing antioxidant capacity (CUPRAC), and ferric reducing ability of plasma (FRAP) (Shahidi and Zhong 2015). The results of the antioxidant capacity of the extracts of the current study are shown in

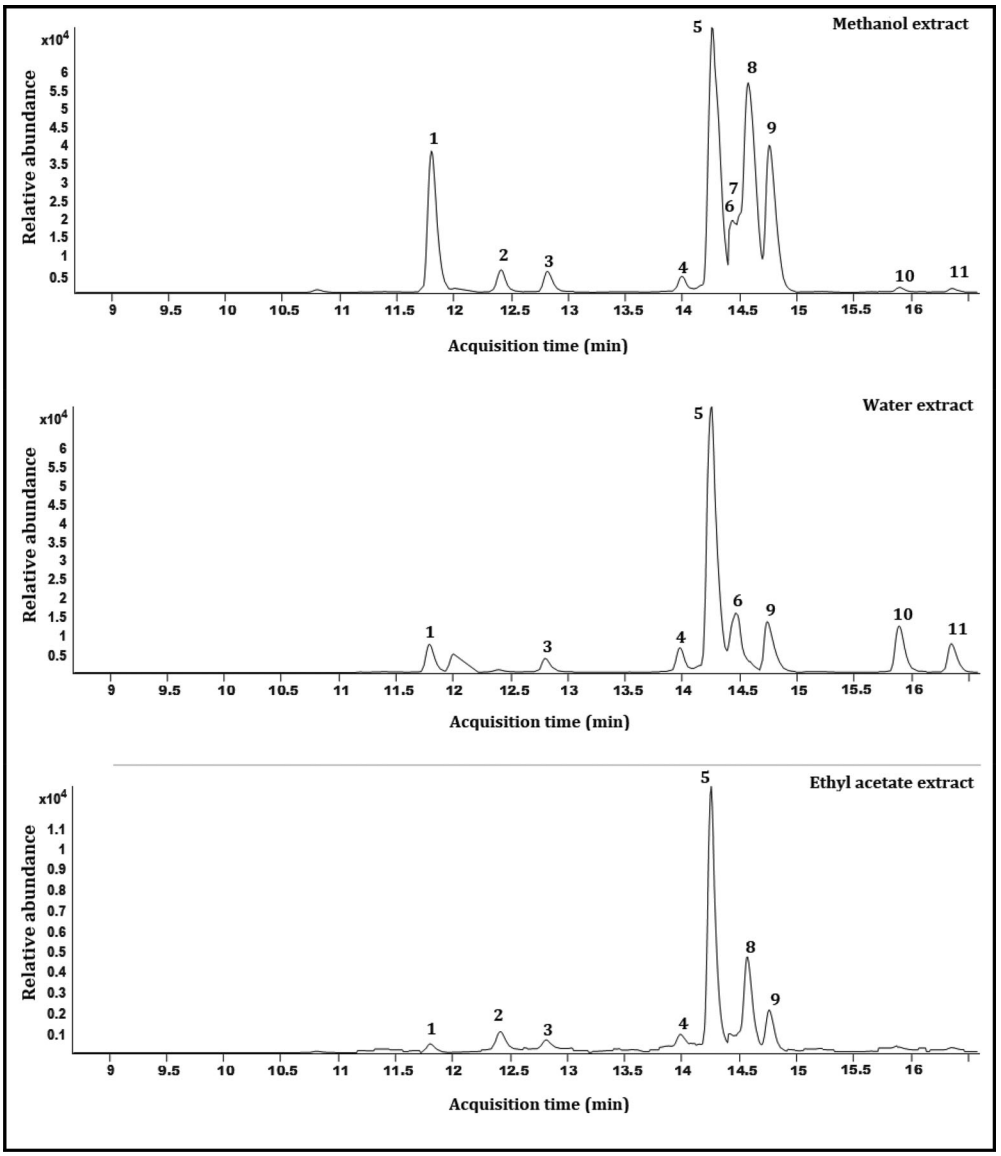

Figure 2. LC–ESI–MS/MS chromatograms of *O. mitis* extracts. Peak identification: (1) chlorogenic acid, (2) 4-hydroxybenzoic acid, (3) caffeic acid, (4) *p*-coumaric acid, (5) luteolin-7-glucoside, (6) hesperidin, (7) hyperoside, (8) rosmarinic acid, (9) apigenin-7-glucoside, (10) luteolin, and (11) apigenin.

Table 2 and Figure 3. The antioxidant activities were in harmony with the phytochemical content and the ethyl acetate extract exhibited the lowest antioxidant capacities. For the DDPH, ABTS and FRAP assays, there was no significant differences between the antioxidant activities of the methanol and water extracts. The aqueous extract showed the highest activities for the CUPRAC and ferrous ion chelating assays, whereas methanol exhibited the highest activity for the phosphomolybdenum assay.

The differences were probably due to the diverse biomolecules in the solvent extracts as reported previously (Dube, Meyer, and Marnewick 2017; Boulila et al. 2015). No

Table 2. Antioxidant activity of *O. mitis* extracts.[1]

Sample	DPPH scavenging[2]	ABTS scavenging[2]	CUPRAC reducing[3]	FRAP reducing[3]	Phosphomolybdenum[3]	Ferrous ion chelating[2]	Tyrosinase inhibition[2]	α-Amylase inhibition[2]
Ethyl acetate	22.69 ± 2.85^{b}	11.50 ± 1.37^{c}	4.41 ± 0.05^{e}	1.71 ± 0.01^{c}	3.10 ± 0.06^{c}	2.45 ± 0.11^{c}	2.27 ± 0.12^{b}	2.32 ± 0.10^{b}
Methanol	4.86 ± 0.11^{a}	3.41 ± 0.33^{b}	2.58 ± 0.01^{d}	1.15 ± 0.02^{b}	2.15 ± 0.01^{b}	4.30 ± 0.15^{d}	2.60 ± 0.15^{b}	5.42 ± 0.02^{c}
Water	4.23 ± 0.16^{a}	2.76 ± 0.13^{b}	2.40 ± 0.04^{c}	1.25 ± 0.13^{b}	3.01 ± 0.27^{c}	1.12 ± 0.02^{b}	4.49 ± 0.43^{c}	16.73 ± 0.27^{d}
Trolox	0.27 ± 0.02^{a}	0.29 ± 0.01^{a}	0.27 ± 0.01^{b}	0.09 ± 0.01^{a}	1.05 ± 0.07^{a}	Not determined	Not determined	Not determined
BHA[4]	0.25 ± 0.01^{a}	0.18 ± 0.01^{a}	0.14 ± 0.01^{a}	0.09 ± 0.01^{a}	Not determined	Not determined	Not determined	Not determined
BHT[4]	1.15 ± 0.07^{a}	0.29 ± 0.01^{a}	0.19 ± 0.01^{ab}	0.15 ± 0.01^{a}	Not determined	Not determined	Not determined	Not determined
EDTA[4]	Not determined	Not determined	Not determined	Not determined	Not determined	0.053 ± 0.004^{a}	Not determined	Not determined
Kojic acid	Not determined	Not determined	Not determined	Not determined	Not determined	Not determined	0.27 ± 0.01^{a}	Not determined
Acarbose	Not determined	Not determined	Not determined	Not determined	Not determined	Not determined	Not determined	1.03 ± 0.04^{a}

[1]Values indicated by the same superscripts within the same column are not different according to the Tukey's honestly significant difference post hoc test at 5% significance level.
[2]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.
[3]EC_{50} (mg/mL): Effective concentration at which the absorbance was 0.5 for phosphomolybdenum, CUPRAC and FRAP reducing assays.
[4]BHA, BHT, and EDTA: Butylated hydroxyanisole, butylated hydroxytoluene, and ethylenediaminetetraacetic acid (disodium salt), respectively.

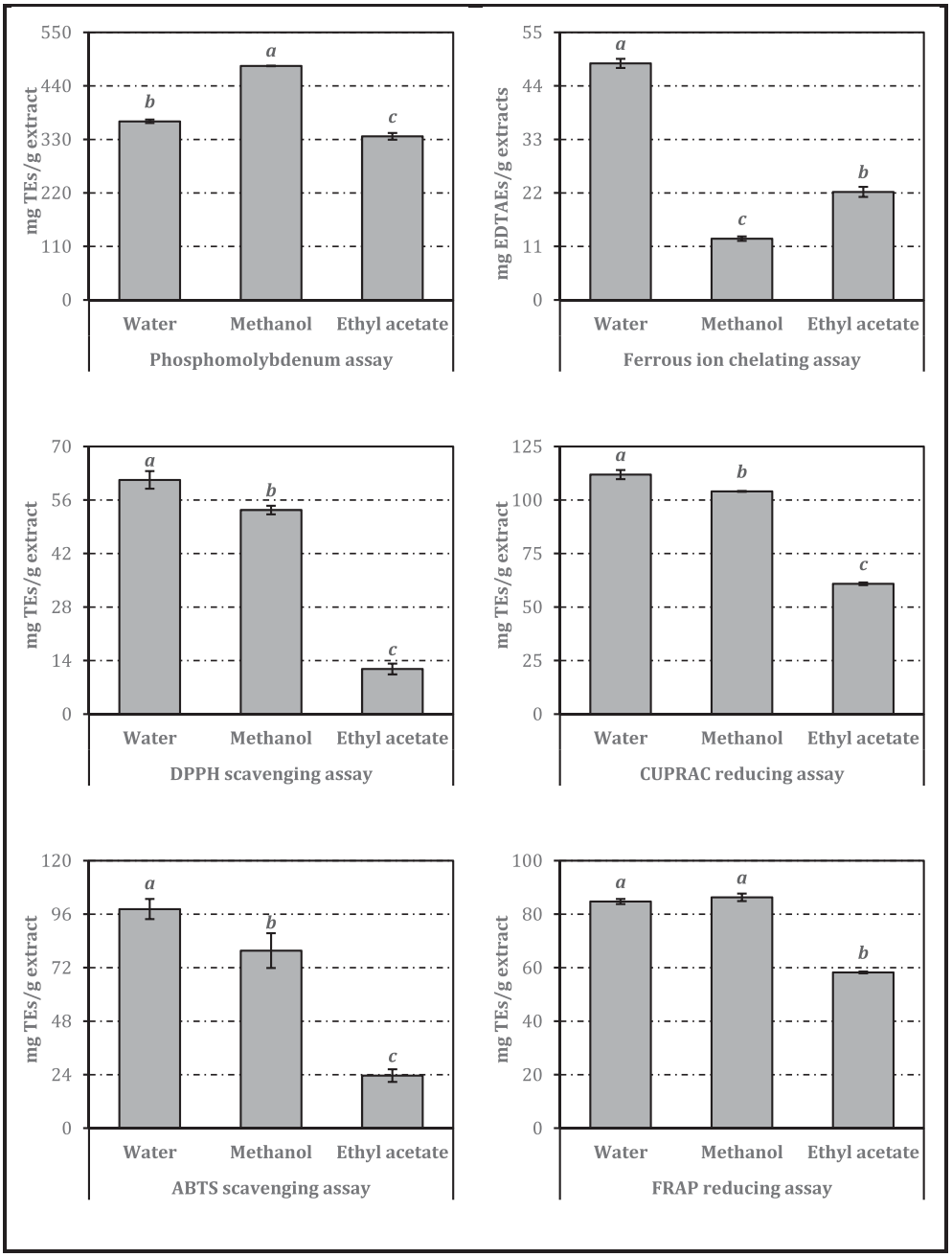

Figure 3. Antioxidant activity of *O. mitis* extracts. TEs and EDTAEs are trolox and ethylenediaminetetraacetic acid (disodium salt) equivalents, respectively. Values indicated by the same superscripts are not different from the honestly significant difference after Tukey's hoc test at the 5% significance level.

specific *in vitro* antioxidant assay reflects the actual antioxidant properties (Apak et al. 2013). Nevertheless, these methods provide an estimate of the total phenolic content/antioxidant capacity in plant extracts (Granato et al. 2016).

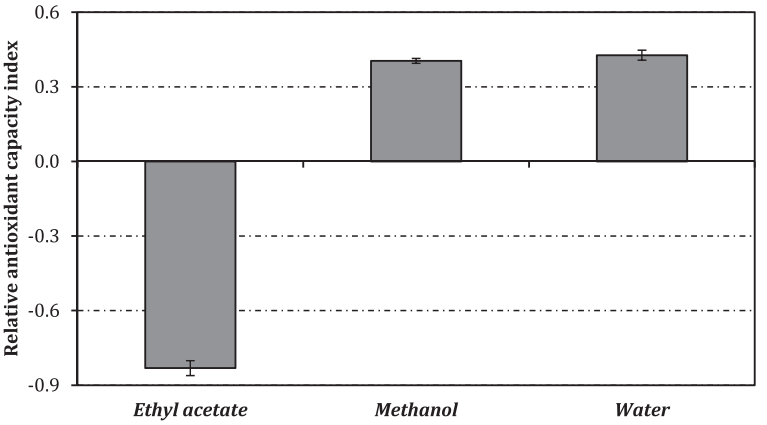

Figure 4. Relative antioxidant capacity indices of the *O. mitis* extracts.

Table 3. Correlations among phenolics and antioxidant assays.[1]

	Phosphomolybdenum	DPPH	ABTS	CUPRAC	FRAP	Ferrous ion chelating	Tyrosinase	α-Amylase
Relative antioxidant capacity index	0.651	0.992	0.976	0.993	0.997[2]	0.279		
Tyrosinase	0.071	−0.777	−0.833[2]	−0.780	−0.653	−0.852[2]		
α-Amylase	−0.389	−0.983[3]	−0.989[3]	−0.983[3]	−0.929[3]	−0.555	0.880[2]	
Total flavonoid	0.923[3]	0.823[2]	0.762	0.827[2]	0.918[3]	−0.189	−0.308	−0.711
Total phenolic	0.807	0.922[3]	0.871[2]	0.924[3]	0.972[3]	0.040	−0.521	−0.849[2]
Chlorogenic acid	0.980[3]	0.371	0.282	0.377	0.547	−0.701	0.259	−0.203
Luteolin 7-glucoside	0.843[2]	0.908[2]	0.863[2]	0.911[2]	0.973[3]	−0.015	−0.471	−0.823[2]
Rosmarinic acid	0.936[3]	0.216	0.125	0.221	0.405	−0.807	0.411	−0.041
Apigenin 7-glucoside	0.983[3]	0.685	0.609	0.689	0.814[2]	−0.392	−0.106	−0.547

[1]Pearson Correlation Coefficients between the parameters.
[2]Significant at $p < 0.05$.
[3]Significant at $p < 0.01$.

Due to the variety of the antioxidants assays, it is difficult to propose a specific method or to compare results between studies (MacDonald-Wicks, Wood, and Garg 2006; Granato et al. 2018). The relative antioxidant capacity index has been used as a reference to choose a more accurate measurement of the extract antioxidant status (Sun and Tanumihardjo 2007). To compare the measurements, the relative antioxidant capacity index values of the extracts were determined as shown in Figure 4. Table 3 and Figure 5 show the correlation coefficients between the relative antioxidant capacity index and the antioxidant capacity. Figure 4 demonstrates that water and methanol extracts presented the highest antioxidant activity (relative antioxidant capacity indices of 0.49 and 0.48), followed by the ethyl acetate extract (relative antioxidant capacity index of −0.97). High correlations were observed between FRAP, CUPRAC, and ABTS/DPPH radical scavenging and relative antioxidant capacity index (0.997, 0.993, 0.976 and 0.992, respectively). Furthermore, for molecules at the highest levels in the extracts (chlorogenic acid, luteolin 7-glucoside, rosmarinic acid, and apigenin 7-glucoside), rosmarinic acid provided the highest negative correlation with the ferrous ion chelating assay that may explain the high activity for the water extract that contained the lowest level of this compound. Chlorogenic acid showed the highest correlation with the

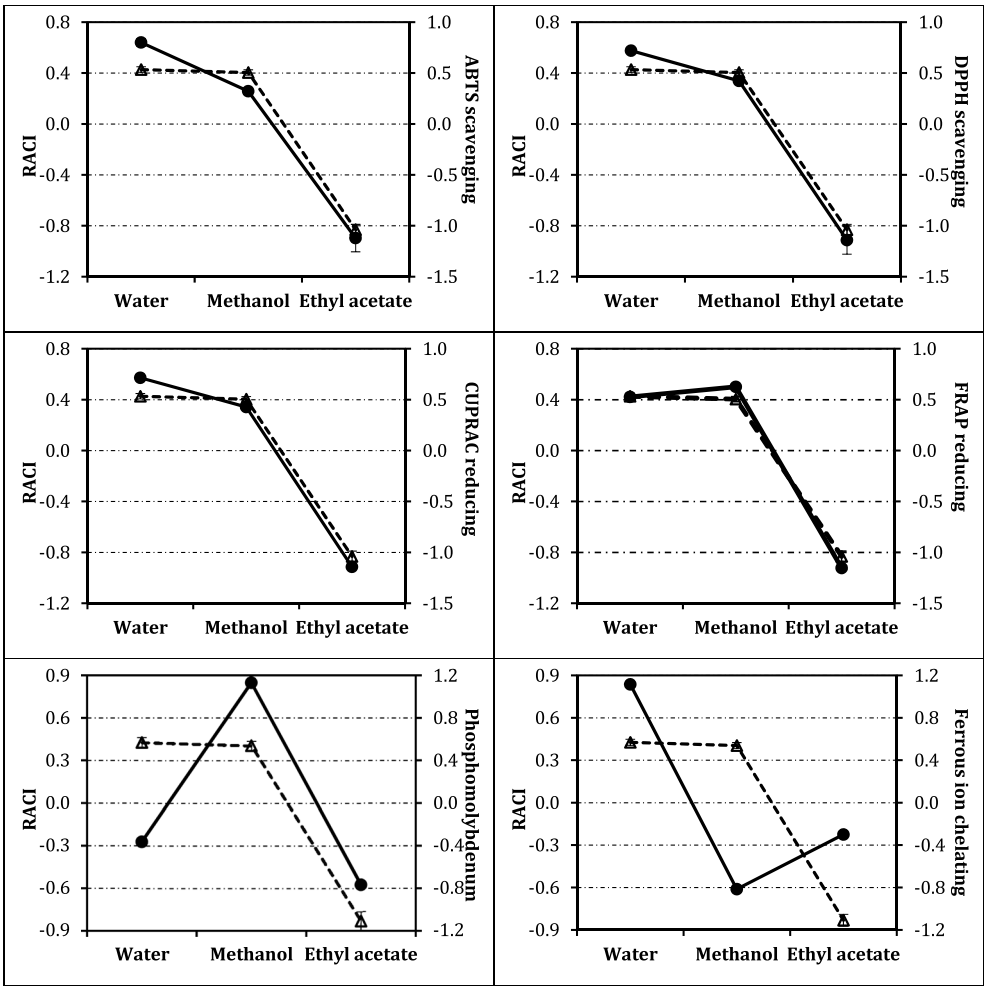

Figure 5. Relative antioxidant capacity indices (dashed lines with triangles) and antioxidant activity (solid lines with circles) of the *O. mitis* extracts.

phosphomolybdenum assay that explained the high activity of methanol extract that contained its highest concentration.

Enzyme inhibitory activity

Table 2 shows the skin-whitening (Tyrosinase inhibitory activity) and the anti-diabetic (α-amylase inhibitory activity) potential of *Onosma mitis* extracts. In contrary to the results above, the highest enzyme inhibition was observed with the ethyl acetate extract (IC_{50}; 2.27 and 2.32 mg/ml, for tyrosinase inhibitory and α-amylase inhibitory activities, respectively). The methanol extract was second (IC_{50} 2.6 and 5.42 mg/ml, for tyrosinase inhibitory and α-amylase inhibitory activities, respectively) followed by the water extract which exhibited the lowest α-amylase inhibitory activity with an IC_{50} of 16.73 mg/ml. The results were contrary to those for *O. pulchra* by Sarikurkcu, Sahinler, et al. (2020) who reported high correlation between chlorogenic acid, luteolin 7-glucoside,

rosmarinic acid, and apigenin 7-glucoside and tyrosinase inhibitory activity. The current results were also not in accord with those for other species such as *Bubonium imbricatum* (Aghraz et al. 2018) and *Echium amoenum* (Asghari et al. 2019). Indeed, the current study supports that the biological activity of the extract does not depend exclusively on the quantities of major compounds but also upon their synergism/antagonism as has been previously reported (Huang et al. 2017; Popa et al. 2012; Wei et al. 2006). The statistical analyses revealed that the activities of all extracts were different from each other. Nevertheless, none of the extracts exhibited an inhibitory activity higher than kojic acid (IC_{50}; 0.27 mg/ml) or acarbose (IC_{50}; 1.03 mg/ml) for tyrosinase inhibitory and α-amylase inhibitory activities, respectively.

Conclusions

There has been no previous study regarding the effect of solvent on phytochemicals isolated from *Onosma mitis*. This work characterized the influence of water, methanol, and ethyl acetate upon the phenolic composition and the biological activities. Twenty-five compounds were identified that varied with the solvent. All extracts presented remarkable antioxidant and enzyme inhibitory capacities. The results underlined the potential use of O. *mitis* species as a source of bioactive molecules in the pharmaceutical and food industries.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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