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Research Article

LC-MS/MS analyses and biological activities of *Onosma sintenisii* and *O. mutabile*

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Abstract: This study was aimed to investigate the chemical compositions, *in vitro* antioxidant and enzyme inhibitory activities of methanol extracts from *O. sintenisii* and *O. mutabile*. Spectrophotometric analyzes showed that the total phenolic and flavonoid content of *O. mutabile* was higher than *O. sintenisii*. Findings from the chromatographic analyzes also confirmed the spectrophotometric analyses. It was determined that *O. mutabile* contains high levels of apigenin 7-glucoside and rosmarinic acid. *O. mutabile* extract exhibited higher activity in all of the antioxidant activity tests. *O. sintenisii* exhibited higher inhibitory activity on other enzymes except for α-amylase. It was understood that there was a close relationship between the antioxidant activities of the extracts and their chemical compositions. However, it was concluded that more detailed tests should be done to determine the phytochemicals responsible for the enzyme inhibitory activities of the extracts in question.

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1. INTRODUCTION

The origin of the word 'onosma' is based on the Latin word 'osma'. The word 'osma' has been used by Latin communities to mean fragrance (Stearn, 1993). Since *Onosma* species are among the plant species that have just begun to be discovered in their biological activities, the number of studies on these species is limited. It has been reported that *Onosma* species characteristically contain some phenolic compounds, alkaloids, and naphthoquinones (Mehrabian *et al.,* 2012). In addition, it has been found that the alkanines and shikonins in *Onosma* species are also found in other members of Boraginaceae and are responsible for interesting biological activities such as wound healing, pain relief, anti-inflammatory, anti-microbial, etc. (Zhou *et al.,* 1992; Kumar *et al.,* 2013).

Antioxidants are essential compounds in the food industry. These agents protect the lipids in foods against oxidation, preventing the formation of toxic oxidation products and the bitterness of the food. Due to these properties, antioxidants extend the shelf life of food and prevent commercial losses (Serafini & Peluso, 2016; Bi *et al.,* 2017). Some synthetic

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antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used in the food industry over the past decades. However, interest in natural antioxidant compounds of plant origin has increased over time due to the researchers' concerns about the side effects of these compounds on health (Surh, 2006; Choi *et al.,* 2014). Oxidative stress also triggers many health problems in organisms, such as cancer, cardiovascular system disorders, and rapid aging (Yashin *et al.,* 2017). Researchers working in both medicine and pharmacy agree that phytochemicals can significantly contribute to the relief of health problems associated with oxidative stress (Yesiloglu *et al.,* 2013; Samah *et al.,* 2017; Yashin *et al.,* 2017).

Alzheimer's disease (AD), a progressive neurodegenerative disease, primarily affects older people. The risk of contracting the disease doubles every five years after the age of 65. Authorities suggest that more than 130 million of the world's population will be in the grip of AD by 2050 (Prince *et al.,* 2016). The most prominent clinical symptom is neuronal loss and a decrease in acetylcholine (ACh) levels in the patients' forebrain, cortex, and hippocampus. In parallel with these molecular changes, cognitive disorders, learning difficulties, and memory loss occurs in patients. Researchers suggest that this is caused by disruption of signal transmission in cholinergic neurons (Selkoe, 1996; de la Torre, 2004; Ferri *et al.,* 2004). Since two main cholinesterases (ChE)s [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)] are responsible for the regulation of ACh level in the brain, the most effective treatment approach in AD is to inhibit the activities of these enzymes (Genç *et al.,* 2016). Progression could be delayed in AD treatment with some ChE inhibitors (tacrine, galantamine, rivastigmine, etc.) used today (Rampa *et al.,* 2001). However, due to the short half-lives, low bioavailability, limited therapeutic efficacy, and toxicity of these compounds, researchers are making intense efforts to discover new ChE inhibitors. Plants are one of the primary sources used for this purpose (Almansour *et al.,* 2020).

Today, phytochemicals are also under scrutiny for their tyrosinase inhibitory activities. Excessive tyrosinase activity, which catalyzes the melanogenesis process, causes browning of foods and thus deterioration of their flavor (Sasaki & Yoshizaki, 2002; Fattahifar *et al.,* 2018). Tyrosinase hyperactivity also causes excessive accumulation of melanin in skin cells. Inhibition of this enzyme prevents browning in fruits and vegetables and provides skin whitening in organisms (Pillaiyar *et al.,* 2017). Therefore, tyrosinase inhibitors are among the favorite agents of the cosmetic industry. However, the cytotoxic and mutagenic properties of some synthetic tyrosinase inhibitors used today are of concern to health authorities (Baurin *et al.,* 2002). Therefore, there is a need for new tyrosinase inhibitors that do not have harmful side effects on the body (Guo *et al.,* 2020).

Diabetes is one of the most common diseases that afflict human beings and are common around the world. Since diabetes treatment is costly and complex, it can sometimes create difficulties for the functioning of medical systems (King *et al.,* 1998; Kameswararao *et al.,* 2003). The most effective way to prevent the increase in blood sugar level, especially immediately after meals (postprandial hyperglycemia), is to inhibit carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) (Balan *et al.,* 2017). Researchers suggest that plants are rich sources of α-amylase and α-glucosidase inhibitors (Chokki *et al.,* 2020).

In this study, it was aimed to investigate the chemical compositions, antioxidant activities, and inhibitory activities of the methanol (MeOH) extracts obtained from the aerial parts of two *Onosma* species (*O. sintenisii* Hausskn. ex Bornm., *O. mutabile* Boiss. & Hausskn.) distributed naturally in Turkey on AChE, BChE, tyrosinase, α-amylase, and α-glucosidase.

2. MATERIAL and METHODS

2.1. Plant Material

The aerial parts of *O. sintenisii* (635 m., 37° 31' 13" N 30° 52' 26" E, herbarium number: OC. 5039) and *O. mutabile* (1550 m., 38° 24' 49.05" N 36° 27' 21.96" E, herbarium number: OC.5040) were collected from Todurge lake, Hafik, Sivas-Turkey, and Ayranlik village, Sariz, Kayseri-Turkey in 2019, respectively. The plants were identified and deposited by Dr. Olcay CEYLAN from the Department of Biology, Mugla Sitki Kocman University, Mugla-Turkey.

2.2. Preparation of The Extracts

Methanol extracts of both plants were prepared by maceration. Extract yields of *O. sintenisii* and *O. mutabile* were measured as 13.51% and 3.96% (w/w), respectively. Details of the extraction procedure can be found in the supplementary file.

2.3. Determination of The Phenolic Compositions of The Extracts

The chemical compositions of *Onosma* extracts were determined qualitatively and quantitatively using spectrophotometric and chromatographic methods (Zengin *et al.,* 2015; Cittan & Çelik, 2018). Experimental details for determining chemical composition are provided in the supplementary file.

2.4. Antioxidant and Enzyme Inhibition Capacity

Phosphomolybdenum, radical scavenging, reducing power, and ferrous ion chelating assays were used to determine the antioxidant activities of the extracts. (Apak *et al.,* 2006; Tepe *et al.,* 2011; Zengin *et al.,* 2015). On the other hand, inhibitory activities of the extracts on AChE, BChE, tyrosinase, α-amylase, and α-glucosidase were also performed by following the methods specified in the literature (Ozer *et al.,* 2018). Details on the tests performed can be found in the supplementary file.

2.5. Statistical Analysis

Details of the statistical analyzes applied to the data obtained from the tests are given in the supplementary file.

3. RESULTS

3.1. Chemical Compositions of The Extracts

Total phenolic and flavonoid contents of the MeOH extract obtained from *O. sintenisii* and *O. mutabile* are given in Figure 1. As in many studies published previously by our research group, the amounts of phenolics in the extracts were higher than the amounts of flavonoids in the current study. When the species are compared with each other, it is seen that both phenolic and flavonoid contents of *O. mutabile* are higher than *O. sintenisii*. Total phenolic and flavonoid contents of O. mutabile were 38.95 mg GAEs/g and 25.49 mg QEs/g, respectively.

In addition to the spectrophotometric analyses applied to the extracts, quantitative chromatographic analyses were also performed to determine the concentrations of the compounds in Table 1 in the extracts. Analyzes showed that both extracts were significantly higher in apigenin 7-glucoside and luteolin 7-glucoside, the flavonoid glycosides, apigenin, a flavonoid aglycone, rosmarinic acid, and pinoresinol. *O. mutabile* was richer in these compounds than *O. sintenisii*. This finding was found to be consistent with those obtained from spectrophotometric analyses. The concentrations of apigenin 7-glucoside, rosmarinic acid, luteolin 7-glucoside, pinoresinol and apigenin in *O. mutabile* were 112284.57, 47562.37, 8446.38, 5005.55 and 3114.73 µg/g, respectively. On the other hand, both extracts did not contain (+)-catechin, pyrocatechin, (-)-epicatechin, verbascoside, taxifolin, 2-hydroxycinnamic acid, and kaempferol.

Figure 1. Antioxidant capacities, total phenolics and flavonoids contents of *O. sintenisii* and *O. mutabile* extracts [GAEs, QEs, TEs, EDTAEs: Gallic acid, quercetin, trolox, and ethylenediaminetetraacetic acid (disodium salt) equivalents]. There is no statistical difference between the values marked with the same superscripts on the bars.

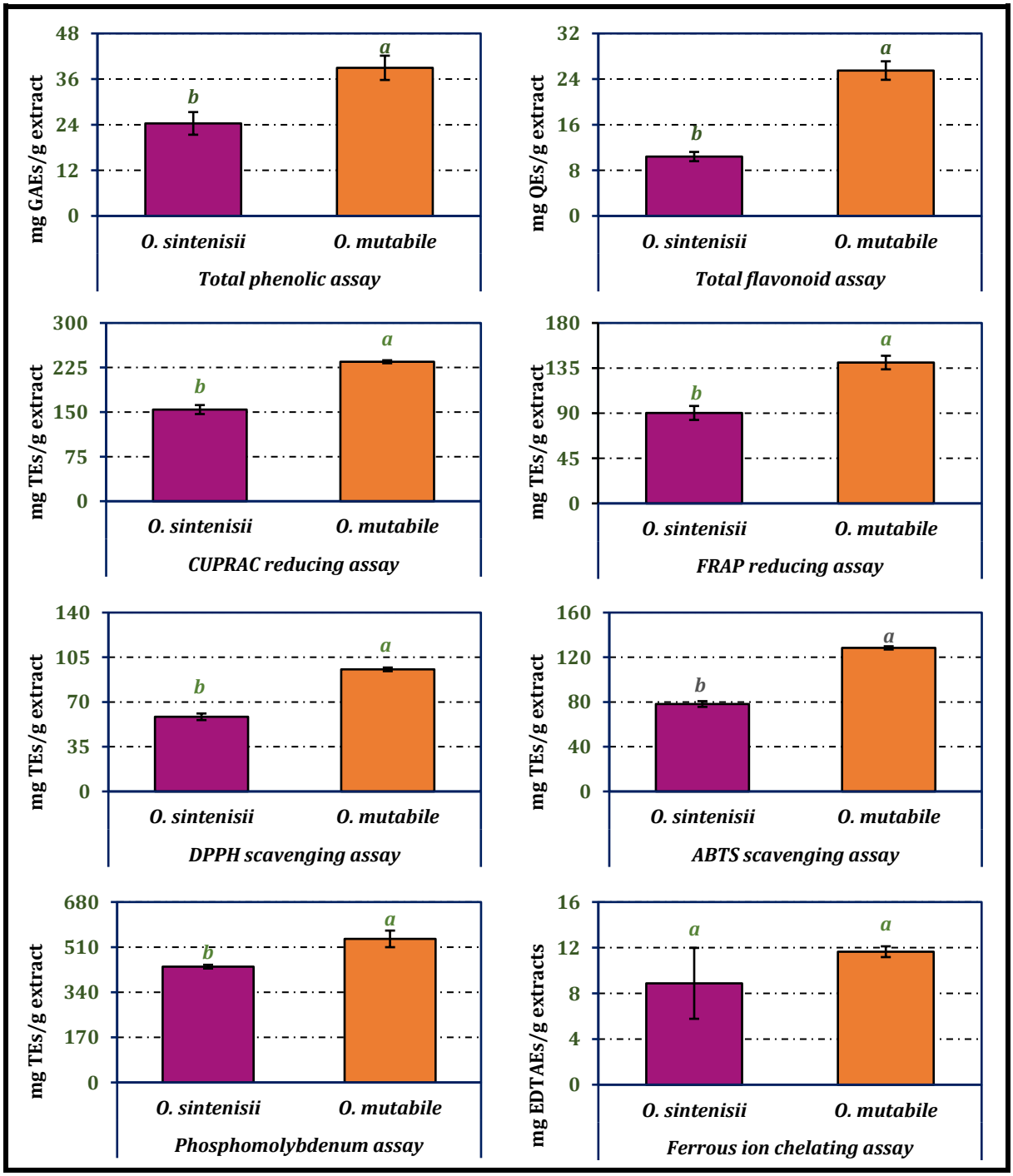


Table 1. Concentration (µg/g extract) of selected phytochemicals in *O. sintenisii* and *O. mutabile* extracts.

Compound	*O. sintenisii*	*O. mutabile*
Gallic acid	2.43 ± 0.02^b	11.55 ± 0.45^a
Protocatechuic acid	66.04 ± 1.00^b	117.27 ± 7.70^a
3,4-Dihydroxyphenylacetic acid	3.33 ± 0.24	nd
(+)-Catechin	nd	nd
Pyrocatechol	nd	nd
Chlorogenic acid	4990.28 ± 55.61^a	34.66 ± 2.19^b
2,5-Dihydroxybenzoic acid	15.07 ± 0.53^b	325.71 ± 11.11^a
4-Hydroxybenzoic acid	241.21 ± 0.74^b	1105.12 ± 3.44^a
(-)-Epicatechin	nd	nd
Caffeic acid	256.38 ± 10.30^b	833.43 ± 18.75^a
Vanillic acid	171.82 ± 1.48^b	901.90 ± 49.30^a
Syringic acid	12.02 ± 0.93^b	49.52 ± 0.65^a
3-Hydroxybenzoic acid	nd	13.01 ± 0.25
Vanillin	27.49 ± 0.69^b	81.04 ± 2.20^a
Verbascoside	nd	nd
Taxifolin	nd	nd
Sinapic acid	4.81 ± 0.44^b	73.62 ± 0.63^a
p-Coumaric acid	30.62 ± 4.04^b	221.51 ± 7.93^a
Ferulic acid	117.98 ± 0.22^b	474.74 ± 8.33^a
Luteolin 7-glucoside	2346.37 ± 1.36^b	8446.38 ± 137.54^a
Hesperidin	71.73 ± 1.80^b	226.16 ± 2.90^a
Hyperoside	8.89 ± 0.26^b	664.89 ± 9.32^a
Rosmarinic acid	20610.01 ± 113.72^b	47562.37 ± 127.59^a
Apigenin 7-glucoside	3253.85 ± 67.76^b	112284.57 ± 2262.44^a
2-Hydroxycinnamic acid	nd	nd
Pinoresinol	61.74 ± 0.63^b	5005.55 ± 527.22^a
Eriodictyol	0.17 ± 0.01^b	0.28 ± 0.03^a
Quercetin	1.93 ± 0.08^b	5.85 ± 0.16^a
Luteolin	300.81 ± 9.73^b	442.61 ± 33.42^a
Kaempferol	nd	nd
Apigenin	585.48 ± 3.18^b	3114.73 ± 22.44^a

There is no statistical difference between values marked with the same superscripts on the same row. nd, not detected.

3.2. Antioxidant Activities of The Extracts

The antioxidant activities of the extracts are given in Figure 1 in terms of positive control equivalents and Table 2 IC_{50} or EC_{50}. To elucidate the antioxidant activity potential of the extracts, various methods in which different antioxidant activity mechanisms were tested were used together. Thus, the extracts' total antioxidant and radical scavenging activities, chelating, and reducing powers were documented. In all test systems, *O. mutabile* exhibited higher activity

than *O. sintenisii*. *O. mutabile*'s activity values in reducing power (CUPRAC and FRAP), radical scavenging (DPPH and ABTS), phosphomolybdenum, and ferrous ion chelating assays were 234.71, 140.53, 95.56, 128.42, 541.13 mg TEs/g, and 11.65 mg EDTAEs/g, respectively. The extracts exhibited more potent activity in the CUPRAC test than they did in the FRAP test. Although the activity values were close to each other, the ABTS radical scavenging activities of the extracts were higher than the DPPH scavenging activities. The main reason why *O. mutabile* exhibits higher activity than *O. sintenisii* is thought to be closely related to its phytochemical composition. Because the concentration of the significant components given in Table 1 was higher in *O. mutabile*.

Table 2. Antioxidant capacities of standards and *O. sintenisii* and *O. mutabile* extracts.

Assays	*O. sintenisii*	*O. mutabile*	Trolox	EDTA
1	2.54 ± 0.04^{c}	2.05 ± 0.12^{b}	1.09 ± 0.04^{a}	-
2	1.78 ± 0.09^{c}	1.17 ± 0.01^{b}	0.29 ± 0.04^{a}	-
3	1.11 ± 0.08^{c}	0.71 ± 0.03^{b}	0.10 ± 0.01^{a}	-
4	4.27 ± 0.19^{c}	2.61 ± 0.04^{b}	0.27 ± 0.04^{a}	-
5	3.75 ± 0.10^{c}	2.22 ± 0.07^{b}	0.33 ± 0.05^{a}	-
6	6.44 ± 2.48^{b}	4.44 ± 0.19^{ab}	-	0.05 ± 0.003^{a}

1: Phosphomolybdenum (EC50: mg/mL), 2: CUPRAC reducing power (EC50: mg/mL), 3: FRAP reducing power (EC50: mg/mL), 4: DPPH radical scavenging (IC50: mg/mL), 5: ABTS radical scavenging (IC50: mg/mL), 6: Ferrous ion chelating (IC50: mg/mL). There is no statistical difference between values marked with the same superscripts on the same row.

3.3. Enzyme Inhibitory Activities of The Extracts

In the current study, ChEs, α-amylase, α-glucosidase, and tyrosinase inhibitory activity tests were applied to determine the anti-Alzheimer's, anti-diabetic and skin-whitening activities, in addition to the antioxidant activities of the extracts. Results are given in Figure 2 in terms of positive control equivalent and Table 3 in terms of IC_{50}.

Table 3. Enzyme inhibitory capacities of standards and *O. sintenisii* and *O. mutabile* extracts.

Assays	*O. sintenisii*	*O. mutabile*	Galanthamine	Kojic acid	Acarbose
1	1.11 ± 0.03^{b}	1.37 ± 0.07^{c}	0.0036 ± 0.0004^{a}	-	-
2	2.92 ± 0.12^{b}	8.63 ± 0.43^{c}	0.0057 ± 0.0004^{a}	-	-
3	2.30 ± 0.0^{b}	2.30 ± 0.07^{b}	-	0.30 ± 0.04^{a}	-
4	3.13 ± 0.14^{b}	2.67 ± 0.09^{b}	-	-	1.10 ± 0.14^{a}
5	1.02 ± 0.01^{a}	2.54 ± 0.02^{c}	-	-	1.67 ± 0.07^{b}

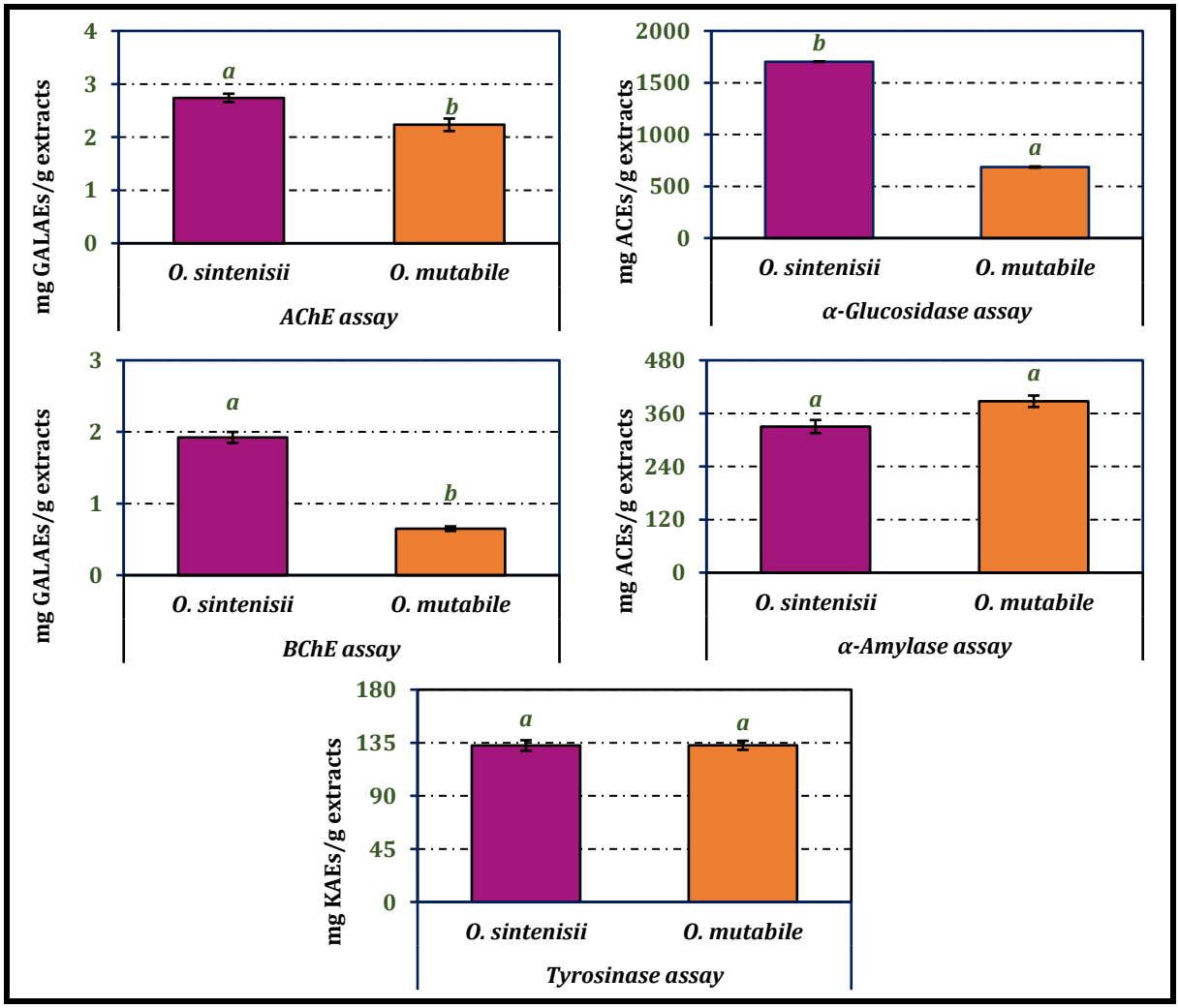
1: AChE inhibition (IC50: mg/mL), 2: BChE inhibition (IC50: mg/mL), 3: Tyrosinase inhibition (IC50: mg/mL), 4: α-Amylase inhibition (IC50: mg/mL), 5: α-Glucosidase inhibition (IC50: mg/mL). There is no statistical difference between values marked with the same superscripts on the same row.

According to the data in Figure 2 and Table 3, the extracts exhibited higher inhibitory activity on AChE than on BChE. The ChE inhibitory activity of *O. sintenisii* was higher than that of *O. mutabile*. The AChE and BChE inhibitory activity of *O. sintenisii* were 2.74 and 1.92 mg GALAEs/g, respectively, while *O. mutabile* exhibited 2.23 and 0.65 mg GALAEs/g inhibitory activity on the enzymes in question. In both test systems, the inhibitory activities of the extracts were statistically different from each other.

O. sintenisii showed higher activity in both α-amylase, and α-glucosidase inhibitory activity tests performed to reveal the anti-diabetic activity potential of the extracts. The extracts were more effective on α-glucosidase than α-amylase. The α-amylase and α-glucosidase inhibitory

activities of *O. sintenisii* were 330.08 and 1702.44 mg ACEs/g, respectively. On the other hand, the inhibitory activity of *O. mutabile* on these enzymes was determined as 387.38 and 686.04 mg ACEs/g, respectively. As can be understood from the findings, the inhibitory activities of the extracts were statistically different from each other.

Figure 2. Enzyme inhibitory capacities of *O. sintenisii* and *O. mutabile* extracts (GALAEs: galanthamine equivalent, KAEs: kojic acid equivalent, ACEs: acarbose equivalent). There is no statistical difference between the values marked with the same superscripts on the bars.



In the case of tyrosinase inhibitory activity assay, it was understood that the skin whitening activity potentials of the extracts were almost equal to each other. The activity potentials of *O. sintenisii* and *O. mutabile* were 132.66 and 132.82 mg KAEs/g, respectively. This finding means that the tyrosinase inhibitory activity of both extracts is statistically indistinguishable from each other.

4. DISCUSSION and CONCLUSION

Researchers have begun to focus on the chemical composition and biological activities of *Onosma* species in recent years. Therefore, there is no sufficient data in the literature regarding the chemical composition and activity potential of many *Onosma* species. This also applies to the *Onosma* species analyzed in the current study. In the literature, there are studies on pollen and/or nutlet morphologies of *O. sintenisii* and *O. mutabile* (Akcin, 2007; Binzet, 2011). However, the researchers revealed some phytochemicals such as alkaloids, naphthoquinones,

alkannins, and shikonins, which are characteristic of this genus (Zhou *et al.,* 1992; Mehrabian *et al.,* 2012; Kumar *et al.,* 2013). However, the phytochemicals documented in detail above in *O. sintenisii* and *O. mutabile* have been brought to the literature for the first time with the present study.

As stated in Section 3.1, there are no reports in the literature regarding the antioxidant activities of *Onosma* species analyzed in the current study. However, from the data presented in Table 1, it is possible to infer which major compounds may contribute to the antioxidant activity of *O. mutabile*. Some researchers have reported that extracts rich in some flavonoid glycosides, such as apigenin 7-glucoside and luteolin 7-glucoside, exhibit remarkable antioxidant activities (Pavlenko-Badnaoui *et al.,* 2021; Salamatullah *et al.,* 2021). In addition, in some studies conducted by our research group on the antioxidant activities of some other *Onosma* species, antioxidant activities of extracts rich in these compounds were found to be high (Sarikurkcu *et al.,* 2020a, 2020b; Sarikurkcu *et al.,* 2020c; Sarikurkcu *et al.,* 2020d). Literature data confirm that rosmarinic acid can also contribute significantly to antioxidant activity (Tzima *et al.,* 2021; Wang *et al.,* 2021; Zhuang *et al.,* 2021). There are also some reports in the literature that pinoresinol or some derivatives of this compound, or some extracts containing high amounts of this compound, alleviate the oxidative stress suppression and the severity of the symptoms developing accordingly (Youssef *et al.,* 2020; Lei *et al.,* 2021). The same is also true for apigenin, a flavonoid aglycone. In a study by Wu *et al.* (2021), it was reported that apigenin ameliorates doxorubicin-induced renal injury via inhibition of oxidative stress and inflammation. The literature data above confirm that the compounds in question may have contributed significantly to the antioxidant activity of *O. mutabile*.

There are no studies in the literature on the ChE inhibitory activity of *O. sintenisii* and *O. mutabile*. However, based on the data in Table 1, it is possible to know the compounds that contribute to the ChE inhibitory activity of *O. sintenisii*. According to the data in the table, rosmarinic acid is found in high amounts in *O. sintenisii* extract. Some reports in the literature show that this compound or extracts containing high amounts of rosmarinic acid show significant ChE inhibitory activity. Asghari *et al.* (2019) reported that the MeOH extract obtained from *Echium amoenum* showed significant inhibitory activity on both ChEs. The researchers suggested that the plant extract in question contained high amounts of rosmarinic acid and that the compound contributing to the activity was probably rosmarinic acid. In another study by Georgy & Maher (2017), it was reported that rosmarinic acid reduced doxorubicin-induced ChE activity. There are also some studies in the literature that chlorogenic acid has ChE inhibitory activity. In a study investigating the effects of chlorogenic and caffeic acids on systolic blood pressure, angiotensin-1-converting enzyme (ACE), and CHEs in cyclosporine-induced hypertensive rats, it was reported that chlorogenic acid significantly reduced the activity of both ChEs (Agunloye *et al.,* 2019). These findings are thought to be extremely useful in establishing a relationship between the phytochemical compositions of the extracts and their enzyme inhibitory activities.

In an *in silico* study investigating the inhibitory activities of certain flavonoids and phenolic acids on α-amylase and α-glucosidase, it was reported that rosmarinic acid exhibited an IC_{50} value equivalent to acarbose (Tolmie *et al.,* 2021). McCue & Shetty (2004) also obtained findings supporting these results. According to these researchers, rosmarinic acid has an *in vitro* inhibitory effect on porcine pancreatic amylase. Some reports in the literature show that some extracts were containing chlorogenic acid as a major compound exhibit significant inhibitory activity on digestive enzymes (Chokki *et al.,* 2020; Liu *et al.,* 2020a; Liu *et al.,* 2020b;Si *et al.,* 2020). These findings support those from the present study.

As stated in the above section, tyrosinase inhibitory activity of the extracts analyzed in the present study was brought to the literature for the first time with this study. In line with the data

in Figure 2 and Table 3, since it was understood that both extracts showed similar inhibitory activity on tyrosinase, it is helpful to examine the contribution of the primary compounds found in both extracts to the activity. Apigenin 7-glucoside, a flavonoid glucoside, and rosmarinic acid, a phenolic acid, are significant compounds in both extracts. In a study investigating the tyrosinase inhibitory activities of some compounds isolated from *Lepechinia meninii*, rosmarinic acid inhibited the monophenolase and diphenolase activities of tyrosinase at a rate of 4.14 and 8.59 µM, respectively (Crespo *et al.,* 2019). The data presented in the study reported by Lin *et al.* (2011) also supports the literature data above. These researchers suggested that rosmarinic acid inhibits tyrosinase in a non-competitive manner. There are also some reports in the literature that apigenin 7-glucoside may show tyrosinase inhibitory activity. In an *in silico* study by Istifli *et al.* (2021), it was stated that the binding energy of apigenin 7-glucoside to tyrosinase is vital and can be a potential tyrosinase inhibitory agent. It is thought that the literature mentioned above findings may help to establish the relationship between chemical composition and tyrosinase inhibitory activity in the current study.

This study documented the chemical compositions, antioxidant and enzyme inhibitory activities of *O. sintenisii* and *O. mutabile*. The results obtained from the antioxidant activity tests revealed that the activity in question depends on the chemical composition of the extracts. However, in enzyme inhibitory activity tests, an activity profile different from the antioxidant activities of the extracts was determined. Although there are some reports in the literature that the compounds found in high amounts in extracts may contribute to the inhibitory activities of the extracts on these enzymes, it is thought that more detailed tests should be done to detect bioactive phytochemicals.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

Authorship contribution statement

Mehmet Sabih OZER: Methodology, Resources, Visualization, Software, Formal Analysis. **Kemal Erdem SENCAN:** Investigation, Resources, Validation, and Writing -original draft. **Cengiz SARIKURKCU:** Methodology, Formal Analysis, Software. **Bektas TEPE:** Investigation, Resources, Validation, and Writing -original draft.

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