Phytochemical Analysis and Biological Evaluation of Two Endemic Onosma Species (Onosma cappadocica and O. rutilum)

Bulent Kirkan 1, Cengiz Sarikurkc 2*, Arzuhan Sihoglu Tepe 3

1 Suleyman Demirel University, Water Institute, Isparta-Turkey; bulentkirkan@sdu.edu.tr (B.K.);
2 Afyonkarahisar Health Sciences University, Faculty of Pharmacy, TR-03100, Afyonkarahisar-Turkey; sarikurkcu@gmail.com (C.S.);
3 Kilis 7 Aralik University, Vocational High School of Health Services, Department of Pharmacy Services TR-79000, Kilis-Turkey; arzu_han23@hotmail.com (A.S.T.);
* Correspondence: sarikurkcu@gmail.com (C.S.);

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Abstract: Onosma species have been used by the public for centuries to treat various diseases. This study aimed to analyze the chemical composition, antioxidant, and enzyme inhibitory activity potentials of the methanol extracts of O. cappadocica and O. rutilum, endemic to Turkish flora. The chemical compositions of the extracts were determined by using spectrophotometric and chromatographic methods. The biological activities of the extracts were determined by using antioxidant and enzyme inhibitory test systems. It was determined that the extracts contain high amounts of hesperidin, hyperoside, and rosmarinic acid. While O. rutilum exhibited higher activity in phosphomolybdenum, ABTS radical scavenging and ferrous ion chelating assays, reducing power, and DPPH radical scavenging tests resulted in the superiority of O. cappadocica. O. rutilum exhibited higher inhibition activity on all enzymes (acetylcholinesterase, butyrylcholinesterase, tyrosinase, and α-amylase), except for α-glucosidase. It was concluded that the extracts in question could be used as alternative agents in the food, cosmetic and medical industries due to their antioxidant and enzyme inhibitory activities.

Keywords: Onosma cappadocica; Onosma rutilum; antioxidant; enzyme inhibitory; chemical composition.

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1. Introduction

Researchers have particularly focused on redox reactions in both metabolism and foodstuffs. One of the most important reasons for this interest is the oxidation steps of redox reactions. Oxidative metabolism is indispensable for the healthy running of metabolic processes. Free radicals and other reactive oxygen species are produced due to oxidation reactions. Under normal conditions, free radicals produced play critical roles in certain in vivo regulatory systems [1]. When these reactive molecules are produced in excess amounts, antioxidant enzymes such as peroxidases, catalase, and superoxide dismutase try to protect cells from the destructive effects of these molecules. Excessive free radicals affect cellular respiration, disrupt the structure of membrane lipids, oxidize cellular proteins and DNA.

Moreover, these molecules can cause serious disruptions in cellular signal transduction pathways [2,3]. Free radicals can cause unwanted side effects on foods as well as their side
effects on metabolism (e.g., change of color, taste, and odor of foods) [4,5]. It is estimated that nearly half of global fruit and vegetable production is wasted due to oxidation reactions. One of the most important ways to prevent losses due to oxidation reactions is to use antioxidant molecules. Plants have many phytochemicals that show antioxidant activity, and these molecules are extremely important in terms of the sustainability of metabolic reactions and food safety [6].

In addition to their antioxidant activities, Phytochemicals may also have an inhibitory effect on some critical enzymes. Because of this potential, phytochemicals are being investigated extensively in the medical and cosmetic industries to discover new and alternative inhibitory agents. Cholinesterases are enzymes on which these molecules show an inhibitory effect. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are responsible for terminating the physiological effects of acetylcholine (ACh) and butyrylcholine (BCh), respectively. This situation causes rapid weakening of cognitive functions in people with neurodegenerative diseases such as Alzheimer's and dementia [7,8]. Intense efforts are being made to discover new and alternative molecules that have inhibitory effects on AChE and BChE to prevent these diseases' progression [9].

One of the biological potentials of phytochemicals that attracts the attention of researchers is tyrosinase inhibitory activity. Some phenolic compounds have been proven to show strong tyrosinase inhibitory activity [10,11]. Tyrosinase is a critical enzyme involved in the synthesis of melanin. In the excessive synthesis of this pigment, dermatological disorders such as hyperpigmentation, age spots, melasma, etc., occur on the skin [12]. Tyrosinase is also responsible for the enzymatic browning of plant foods [13]. Scientists pay special attention to phytochemicals to treat dermatological disorders in the medical field and their skin whitening potential in cosmetics [14].

In addition to the biological activity potentials given above, phytochemicals can also show inhibitory activity on α-amylase and α-glucosidase. Many studies in the literature show that some secondary metabolites of plant origin can effectively inhibit α-amylase and α-glucosidase [15-23]. Therefore, to prevent hyperglycemia, researchers consider the inhibitory activity of these molecules on the enzymes in question as an up-to-date and effective treatment approach [24].

In previous studies conducted by our research group, it has been proven that some members of Onosma have promising biological activity potentials [25-33]. The purpose of this study was to expose the chemical composition, antioxidant, and enzyme inhibitory activity potentials of two Onosma species [O. cappadocica (Siehe ex Riedl), O. rutilum (Hub.-Mor.)] endemic to Turkish flora.

2. Materials and Methods

2.1. Plant material.

The aerial parts of Onosma cappadocica Siehe ex Riedl (1020 m., 37°33'15"N 31°18'29"E, Herbarium number: OC.5052) and Onosma rutilum Hub.-Mor. (820 m., 36°20'10"N 33°44'12"E, Herbarium number: OC.5053) were collected from Asagiyaylabel village, Sutculer-Isparta and Balandız plateau, Silifke-Mersin-Turkey, respectively.
2.2. Determination of the phenolic compositions of the extracts.

Total phenolic and flavonoid contents of the extracts were determined according to Zengin et al. [34]. Additionally, analytical parameters recommended by Cittan and Çelik [35] were followed in LC-ESI-MS/MS analyzes.

2.3. Biological activity.

The extracts' antioxidant and enzyme inhibitory activities were determined by following the methods given in the literature [26,34,36-40].

2.4. Statistical analysis.

In the statistical analysis applied to the data obtained from the present study, the method used by Zengin et al. [34] was followed.

3. Results and Discussion

3.1. Chemical composition.

Amounts of total phenolic and flavonoid compounds of MeOH extracts isolated from O. cappadocica, and O. rutilum are given in Figure 1. According to the data in the figure, O. cappadocica was found to be richer in both phenolic and flavonoid compounds than O. rutilum. Amounts of total phenolic and flavonoid compounds in O. cappadocica were determined as 61.91 mg TEs/g extract and 29.96 mg QEs/g extract, respectively, while these values were 57.70 mg TEs/g extract and 18.16 mg QEs/g extract for O. rutilum, respectively. As stated in the introduction, our research group has carried out some studies on the chemical composition of various Onosma species [25-33]. In these studies, it was determined that the phenolic and flavonoid contents of Onosma species (O. sieheana, O. stenoloba, O. frutescens, O. aucheriana, O. sericea, O. pulchra, O. ambigens, O. polyantha, O. mollis, O. tauricum) were 6.55-69.06 mg TEs/g extract and 65.57 mg QEs/g extract, respectively. The data obtained from the present study were found to be compatible with the phenolic and flavonoid contents of other Onosma species in the literature.

Results of LC–ESI–MS/MS analysis performed to determine the amounts of certain phytochemicals in the extracts are given in Table 1. According to the data in the table, more than half of the phytochemicals were found to be higher in O. cappadocica extract. A similar finding is also valid for hesperidin, hyperoside, and rosmarinic acid, which were considered major compounds. The amounts of the compounds in question in the MeOH extract of O. cappadocica were 21079.32, 18659.12, and 103943.08 μg/g extract, respectively. The amounts of the same compounds in O. rutilum extract were found to be 2979.77, 1719.82, and 29186.28 μg/g extract, respectively. In addition to these findings, the amount of chlorogenic acid in O. rutilum extract was also significantly high (5627.89 μg/g extract). The aforementioned compounds were also found in high amounts in some Onosma species in the literature. It has been reported that hesperidin, hyperoside, and rosmarinic acid are found in high amounts in O. ambigens [30], O. aucheriana, O. frutescens, O. sericea [32], O. gigantea [28], O. polyantha, O. mollis [33], O. pulchra [29], O. sieheana, O. stenoloba [31] and O. tauricum var. tauricum [25]. Therefore, the data obtained from the present study were found to be compatible with the literature data.
Figure 1. Antioxidant activities, total phenolics, and flavonoids of the samples [GAEs: Gallic acid equivalent, QEs: quercetin equivalent, TEs: trolox equivalent, EDTAEs: ethylenediaminetetraacetic acid (disodium salt) equivalent]. Values marked with the different superscripts were different statistically.

3.2. Antioxidant activity.

To determine the antioxidant activities of the extracts, phosphomolybdenum, reducing power (CUPRAC and FRAP), radical scavenging (on DPPH and ABTS radicals), and ferrous ion chelating activity assays were applied. The data obtained from each test are presented in Figure 1 and Table 2 in terms of positive control equivalent and IC\textsubscript{50}, respectively.

Phosphomolybdenum assay, in which the total antioxidant activities of the extracts were determined, resulted in the superiority of \textit{O. rutilum} extract. In this test system, while the IC\textsubscript{50} value of \textit{O. rutilum} was 1.37 mg/mL that of \textit{O. cappadocica} remained at 1.39 mg/mL.
Table 1. Concentration (µg/g extract) of selected phytochemicals in the samples.

| Compounds                   | O. microcarpum | O. nana   |
|-----------------------------|----------------|-----------|
| Gallic acid                 | 11.93±0.26     | 17.02±0.59 |
| Protocatechuic acid         | 199.37±1.81    | 186.88±0.28 |
| 3,4-Dihydroxyphenylacetic acid | 12.33±0.15   | 9.81±1.02  |
| (+)-Catechin               | nd             | nd        |
| Pyrocatechol               | nd             | nd        |
| Chlorogenic acid           | 650.22±8.73    | 5627.89±43.75 |
| 2,5-Dihydroxybenzoic acid | 296.03±8.92    | 206.46±11.45 |
| 4-Hydroxybenzoic acid      | 589.05±8.78    | 480.34±44.92 |
| (+)-Epicatechin            | nd             | nd        |
| Caffeic acid               | 1580.27±9.35   | 588.61±40.27 |
| Pyrocatechol               | nd             | nd        |
| Protocatechol              | 707.26±113.10  | 499.75±54.38 |
| Syringic acid              | 46.90±1.41     | 124.33±1.52 |
| 3-Hydroxybenzoic acid      | 8.66±0.22      | 7.56±0.74  |
| Vanillin                   | 58.49±0.84     | 78.97±1.28  |
| Verhacoside                | nd             | 25.19±6.47  |
| Taxifolin                  | 6.79±0.34      | 11.08±0.14  |
| Sinapic acid               | 10.46±0.52     | 35.64±0.87  |
| p-Coumaric acid           | 119.88±0.52    | 199.43±1.54 |
| Ferulic acid               | 395.28±5.55    | 215.43±30.57 |
| Luteolin 7-glucoside       | 257.89±6.40    | 41.76±0.14  |
| Hesperidin                 | 21079.32±55.58 | 2979.77±20.90 |
| Hyperoside                 | 18659.12±209.11 | 1719.82±556.38 |
| Rosmarinic acid            | 103943.08±114.18 | 29186.28±3873.77 |
| Apigenin 7-glucoside       | 183.53±0.12    | 34.41±1.22  |
| 2-Hydroxyxycinnamic acid   | nd             | nd        |
| Pinoresinol                | 110.89±7.23    | 91.89±12.02 |
| Eriodictyol                | nd             | nd        |
| Quercetin                  | 15.56±0.27     | 13.54±0.15  |
| Luteolin                   | 56.67±2.93     | 628.05±23.10 |
| Kaempferol                 | 30.11±2.03     | 28.70±1.52  |
| Apigenin                   | 14.27±0.25     | 20.10±1.45  |

1 Within the same row, values marked with different superscripts were statistically different. nd, not detected.

O. cappadocica showed higher activity than O. rutilum in CUPRAC and FRAP tests, in which the reducing powers of the extracts were analyzed. In these tests, O. cappadocica exhibited 0.63 and 0.39 mg/mL activity, respectively. The activity value of O. rutilum in CUPRAC and FRAP tests was 0.70 and 0.43 mg/mL, respectively. None of the extracts showed as high reducing power as trolox.

The extracts exhibited different activity profiles in DPPH and ABTS radical scavenging activity tests. While O. cappadocica showed higher activity in DPPH radical scavenging assay ($IC_{50} = 1.36$ mg/mL), ABTS radical scavenging assay resulted in the superiority of O. rutilum extract ($IC_{50} = 1.19$ mg/mL). DPPH and ABTS radical scavenging activities of trolox were 0.27 and 0.31 mg/mL, respectively.

In the current study, the last test system applied to determine the antioxidant activities of the extracts is ferrous ion chelating ability. O. rutilum extract was more effective than O. cappadocica in chelating potential. Chelating capacity of O. rutilum was 4.83 mg/mL, while that of O. cappadocica was 5.11 mg/mL.

There is no data in the literature regarding the antioxidant activity of neither O. cappadocica nor O. rutilum. However, the data obtained from the studies performed by our research group on other Onosma species showed that Onosma genus members stand out in terms of their significant antioxidant activities [25-33]. The data obtained from the present study were compatible with the literature data.
In addition to comparing the data obtained from the present study with other *Onosma* species, it is also useful to refer to the studies on the contribution of the compounds found to be high in the extracts to antioxidant activity. In a study investigating the antioxidant activities of the pulps of two Brazilian passion fruits, it was reported that the test materials exhibited significant antioxidant activity, and according to the results of principal component analysis (PCA), this activity may be closely related to some phytochemicals, including hesperidin [41]. In another study carried out by Lin *et al.* [42], the effect of increasing the amount of flavonoid in *Moringa oleifera* leaves on antioxidant activity was investigated. In the study in question, it was reported that the antioxidant activity of the leaf extract obtained using the ultrasonic-assisted extraction (UAE) technique was higher than the extracts obtained by conventional methods and contained a higher amount of hyperoside (10.26 mg/g dry weight). Rosmarinic acid is one of the most studied phytochemicals in the literature regarding its antioxidant activity potential [43-45]. Therefore, there is no doubt about the contribution of phenolic acid in question to the antioxidant activity of the extract.
3.3. Enzyme inhibitory activity.

The inhibitor activities of MeOH extracts obtained from *O. cappadocica* and *O. rutilum* on AChE, BChE, tyrosinase, α-amylase, and α-glucosidase are given in Table 2 terms of IC$_{50}$ and Figure 2 in terms of positive control equivalents.

| Activity (IC$_{50}$: mg/mL) | *O. cappadocica* | *O. rutilum* | Trolox | EDTA | Galanthamine | Kojic acid | Acarbose |
|----------------------------|------------------|--------------|--------|------|--------------|-----------|----------|
| A                           | 1.39±0.03$^b$    | 1.37±0.02$^b$ | -      | -    | -            | -         | -        |
| B                           | 0.63±0.01$^b$    | 0.70±0.01$^c$ | -      | -    | -            | -         | -        |
| C                           | 0.39±0.02$^b$    | 0.43±0.03$^b$ | 0.27±0.01$^a$ | -  | -            | -         | -        |
| D                           | 1.36±0.01$^b$    | 1.52±0.10$^b$ | 0.27±0.03$^a$ | -  | -            | -         | -        |
| E                           | 1.25±0.02$^b$    | 1.19±0.02$^b$ | 0.31±0.02$^a$ | -  | -            | -         | -        |
| F                           | 5.11±0.78$^b$    | 4.83±0.29$^b$ | -      | 0.05±0.004$^a$ | -         | -         | -        |
| G                           | 1.28±0.06$^b$    | 1.18±0.01$^b$ | -      | -    | 0.0033±0.0003$^a$ | -         | -        |
| H                           | 3.55±0.04$^c$    | 2.24±0.21$^b$ | -      | -    | 0.0056±0.0002$^a$ | -         | -        |
| I                           | 2.59±0.16$^b$    | 2.24±0.01$^b$ | -      | -    | 0.30±0.02$^a$ | -         | -        |
| J                           | 3.14±0.31$^b$    | 2.78±0.15$^b$ | -      | -    | -            | 0.98±0.03$^c$ | -        |
| K                           | 1.01±0.01$^a$    | 1.44±0.01$^b$ | -      | -    | -            | -         | 1.75±0.04$^a$ |

Table 2. Biological activities of the samples 1.

1 Within the same row, values marked with different superscripts were statistically different. A: Phosphomolybdenum, B: CUPRAC reducing, C: FRAP reducing, D: DPPH radical, E: ABTS radical, F: Ferrous ion chelating, G: AChE inhibition, H: BChE inhibition, I: Tyrosinase inhibition, J: α-Amylase inhibition, K: α-Glucosidase inhibition

The extracts exhibited stronger inhibitory activity on AChE than BChE. The extract of *O. rutilum* exhibited higher inhibitory activity than *O. cappadocica* in both cholinesterase inhibitor activity tests. Inhibitory activities of *O. rutilum* on AChE and BChE were determined as 1.18 and 2.24 mg/mL, respectively. The inhibitory activity of *O. cappadocica* on the enzymes in question was 1.28 and 3.55 mg/mL, respectively.

The extracts exhibited a similar activity profile on tyrosinase. As in the cholinesterase inhibitor activity tests, *O. rutilum* exhibited stronger inhibitory activity on tyrosinase than *O. cappadocica*. In this test system, the tyrosinase inhibitory activity of *O. cappadocica* was 2.24 mg/mL, while that of *O. rutilum* was 2.59 mg/mL.

While *O. rutilum* showed higher activity (IC$_{50}$ = 2.78 mg/mL) in α-amylase inhibitory activity test, it was found that *O. cappadocica* was more effective in α-glucosidase inhibitory activity assay (IC$_{50}$ = 1.01 mg / mL). While extracts were not as effective as acarbose in α-amylase inhibitor activity test, both extracts were found to be more effective than positive control agents in α-glucosidase inhibitory activity test.

There are no reports in the literature regarding the inhibitory activities of *O. cappadocica* and *O. rutilum* on the enzymes mentioned above. Therefore, the data presented here is the first record for the literature. However, as stated in the sections above, data published by our research group on the inhibitory activities of some other *Onosma* species on these enzymes show that these species may be new and alternative agents for the medical and cosmetic industries [25-33]. However, none of the *Onosma* species in the literature exhibited a high α-glucosidase inhibitory activity as in the current study. Therefore, the *Onosma* species analyzed in the present study stand out among other *Onosma* species regarding their potential inhibitory effects on digestive enzymes.
4. Conclusions

In this study, chemical composition, antioxidant, and enzyme inhibitory activities of the MeOH extracts of two endemic Onosma species (O. cappadocica and O. rutilum) were investigated. Both extracts exhibited significant antioxidant activity in phosphomolybdenum and reducing power assays compared to trolox. The extracts did not exhibit as high inhibitory activity on cholinesterases, tyrosinase, and α-amylase as galanthamine, kojic acid, or acarbose. In contrast, they exhibited higher activity on α-glucosidase than acarbose. Therefore, it has been concluded that the plant above materials can be used as alternative sources in preventing postprandial hyperglycemia. However, further quantitative techniques such as bioactivity-guided fractionation are needed to determine the phytochemical(s) responsible for activity in these extracts.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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