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Phenolic Compound Analysis and Antioxidant Properties of *O. Alborosea*

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ABSTRACT

The *Onosma* genus has been used as a treatment among the public for a long time. In this study, the content analysis and some biological activities of the plant *O. Alborosea* Fisch. & C.A.Mey. ssp. (*O. alborosea*) used in traditional medicine in our country were investigated. For this purpose, methanol and water extracts of the plant were taken, and the total phenolic substance amount of each extract was determined by HPLC device. FRAP, CUPRAC reduction methods, DPPH, and ABTS radical scavenging methods were used to determine antioxidant activities. According to the results of our study, water and methanol extracts showed lower activity than standard antioxidants. It can be said that the reason for this is related to the ratio of the phenolic amount they contain. Since no antioxidant studies were found when the literature search related to the current plant species was conducted, it is thought that our study will guide other studies to be carried out with this plant species and is important in terms of protecting our plants, which are our genetic treasure, and transferring them to future generations.

Keywords: Antioxidant, Free Radical, Muş, Phenolic Substance, *O. Alborosea*.

O. Alborosea Bitkisinin Fenolik Bileşen Analizi ve Antioksidan Özellikleri

ÖZET

Onosma bitkisi uzun uzun zamandan beri halk arasında tedavi edici olarak kullanılmaktadır. Çalışmamızda ülkemizde geleneksel tıpta kullanılan *O. Alborosea* Fisch. &C.A.Mey. ssp.'nın (*O. alborosea*) bitkisinin içerik analizi ve bazı biyolojik aktiviteleri araştırıldı. Bu amaçla, bitkinin metanol ve su ekstraktları alınarak, her bir ekstraktın toplam fenolik madde miktarı HPLC cihazı aracılığıyla belirlendi. Antioksidan aktivitelerini belirlemek için FRAP, CUPRAC indirgeme yöntemleri ile DPPH, ABTS radikali süpürme yöntemleri kullanıldı. Çalışmamızın sonuçlarına göre, su ve metanol ekstraktlarının standart antioksidanlara göre daha düşük aktivite gösterdikleri tespit edildi. Bunun sebebinin, içerdikleri fenolik miktarının oranıyla ilişkili olduğu söylenebilir. Mevcut bitki türüyle alakalı literatür taraması yapıldığında özellikle antioksidan çalışmalara rastlanmadığından çalışmamızın bu bitki türü ile yapılacak başka çalışmalara rehperlik edeceği, genetik hazinemiz olan bitkilerimizin korunması ve nesillere aktarılması açısından önem arz ettiği düşünülmektedir.

Anahtar Kelimeler: Antioksidan, Fenolik Madde, Muş, *O. Alborosea*, Serbest Radikal.

INTRODUCTION

Oxidative stress caused by excessive accumulation of free radicals causes various diseases in the body. Therefore, antioxidants play a vital role in eliminating oxidative stress and restoring the balance [1, 2]. In addition to enzymes, hormones and metabolites in the body, dietary antioxidants are important compounds in establishing this balance. Due to the side effects of synthetic antioxidants, plants are preferred by researchers for antioxidant defense. The presence of secondary compounds such as natural phenolic compounds, flavonoids, terpenes, and organic acids in plants is the main reason for these preferences [3, 4, 5].

The largest member of the Boraginaceae family, which includes important genera, is *Onosma* L. There are more than 100 species of this genus in Turkey and about half of them are endemic [5]. It has been reported that *Onosma* genus, which is used for medicinal purposes among people, is preferred in the treatment of abdominal pain, wound healing, fever and burns [6, 7]. This plant genus, which is also used for food purposes, has also been used in food coloring and PCR analyses [8, 9, 10]. This plant, which has versatile benefits, has also attracted great interest by researchers. As a result of the studies, it was reported that this plant genus is antioxidant, antibacterial, anticancer, anti-inflammatory and wound healing [10]. *O. Alborosea*, described by the Russian botanist Friedrich Ernst Ludwig Fischer and the German-born Russian botanist Carl Anton von Meyer, is known as a rock sucker in Turkey [11]. It is distributed in rocky areas and can be distinguished by different color transformations in its flowers.

In this study, phenolic components of methanol and water extracts of *O. Alborosea* species collected in Muş province were determined by HPLC. Ferrous ion

reducing power (FRAP), cupric ion reducing power (CUPRAC), 1,1-Diphenyl 2-picryl hydrazyl (DPPH) radical scavenging and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging methods were used to determine the antioxidant capacity of the extracts. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ascorbic acid (AA) were preferred as standard antioxidants in antioxidant studies. Working with different extracts of the plant contributes to the richness of the research. However, since we did not have enough plant tissue, we preferred to work with only methanol and water extracts of the plant. Since no antioxidant studies were found in the literature on the present plant species, it is thought that this study is important in terms of guiding future studies and protecting our plants, which are our genetic treasure, and transferring them to generations.

MATERIAL and METHOD

Plant Collection And Species Identification

O. Alborosea was collected in East Anatolia, Muş, Çavuş Mountain (38° 44′ 17- 41° 29′ 29), and the species was determined by Murat Aydın ŞANDA of the Molecular Biology and Genetics Department of Muş Alparslan University. The chemical processes were carried out in the laboratories of Muş Alparslan University.

Preparation of Extracts

The plants were collected during the growing season and dried. After species identification, the water and methanol extracts of the plant were obtained using a Soxhlet apparatus. For the extracts, about 50 g of plant samples were dissolved in 300 mL of methanol and water, respectively. The extracts were filtered and lyophilized in the laboratories of the Molecular Biology and Genetics Department of Muş Alparslan University.

Phenolic Compound Analysis by HPLC

17 different standards (ascorbic acid, myricetin, quercetin, apigenin, abscisic acid, kaempferol, curcumin, catechol, vanillin, rosemarinic acid, caffeic acid, gallic acid, 4-hydroxybenzoic acid, cinnamic acid, 3,4-dihydroxybenzoic acid, salicylic acid and trans-p-coumaric acid) were weighed to a total concentration of 10 mg/ml and placed in 50 ml volumetric flasks. Then 1% acetic acid and acetonitrile (1/9) were mixed and added to the standards, followed by the addition of methanol in the same ratio to prepare the stock solution necessary to dissolve the standards. After dilution of the stock solutions, the concentration of the extracts was diluted to 20 mg/ml using the stock solution used in the standard. Finally, the filtered extracts were injected into the HPLC [12]. HPLC analysis was performed by using the Agilent Technologies 1260 Infinity II HPLC (Agilent, USA). The HPLC configuration consisted of a 1260 DAD WR detector (272 nm, 280 nm and 310 nm wavelength), 1260 Quat Pump VL pump (1.0 mL/min flow rate), 1260 Vial sampler (20 µL injected) and G7130A column furnace (28°C). The analytical column used for the analysis was ACE 5 C18 (250 x 4.6 mm id).

Antioxidant Assays

Reducing Power of Fe^{+3} Ions

The reducing power of Fe^{+3} ions to Fe^{+2} ions was determined by Oyaizu method [13]. The volume of the samples (25, 50 and 100 µg/ml) taken in tubes was completed to 200 µl with distilled water. After adding 500 µl each of buffer solution (pH: 6.6) and $K_3Fe(CN)_6$ to each tube, it was incubated for twenty minutes (50 ^oC). Then 500 µl of trichloroacetic acid (TCA) was added to the mixture. After centrifugation, 500 µl of the supernatant was taken and the same amount of distilled water and 100 µl of $FeCl_3$ were added. Absorbances were measured at 700 nm in a micro-volume spectrophotometer.

Cu^{+2} Ions Reduction Power By CUPRAC Method

After taking different concentrations of extracts and standards into test tubes, their total volumes were made up to 1 ml with distilled water. 0.25 ml each of $CuCl_2$, ethanolic neocuprin and acetate buffer were added. After thirty minutes of incubation, the absorbance values of the samples at 450 nm were recorded [14].

DPPH Radical Scavenging Assay

Ethanol was added to the extracts (25 µg/µl, 50 µg/µl and 100 µg/µl) in test tubes to a final volume of 600 µl. After 200 µL of DPPH solution was added, the reaction was allowed to incubate for about 30 minutes to complete the reaction. Absorbance values at 517 nm were measured with the help of a spectrophotometer [15]. Radical scavenging activities of the extracts were calculated according to the following equation.

DPPH radical scavenging activity (%): (Ac-As/Ac)*100

Ac: Absorbance value of control, As: Absorbance value of samples.

ABTS Radical Scavenging Assay

ABTS solution was diluted with phosphate buffer until the absorbance of the solution reached 0.750 ± 0.025 at 734 nm. After the total volume of the samples was made up to 200 ml with water, 1 mL of $ABTS^{+\cdot}$ the solution was added. After 30 minutes of incubation, the absorbance values of the extracts were measured at 734 nm [16]. Radical scavenging activities of the extracts were calculated according to the following equation.

ABTS radical scavenging activity (%): (Ac-As/Ac)*100

RESULTS and DISCUSSION

Phenolic Substance Analysis

The phenolic compound composition of *O. alborosea* analyzed by HPLC was evaluated with phenolics introduced as standards. The results showed that the water and methanol extracts of the plant were poor in terms of phenolic content, but the total phenolic content of both extracts was similar (Table 1). According to the results, ascorbic acid (3.15 ng/µL) and catechol (2.24 ng/µL) were found in the water extract, while quercetin (3.21 ng/µL), cinnamic acid (0.99 ng/µL) and ascorbic acid (0.69 ng/µL) were found in the methanol extract.

Table 1. Phenolic content of water and methanol extracts of *O. alborosea* (ng/µL)

Phenolics	***O. alborosea* -water**	***O. alborosea* -methanol**
Ascorbic acid	3,15041±0.24	0,69218±0.06
Gallic acid	-	-
3, 4-dihydroxybenzoic acid	-	-
4- hydroxybenzoic acid	-	-
Trans-p coumaric acid	-	-
Myricetin	-	-
Abscisic acid	-	-
Quercetin	-	3,21354±0.26
Apigenin	-	-
Kaemferol	-	-
Curcumin	-	-
Catechol	2,24031±0.11	-
Vanillin	-	-
Caffeic acid	-	-
Cinnamic acid	-	0,99032±0.07
Rosemarinic acid	-	-
Salicylic acid	-	-

In the literature review, it was observed that *Onosma* genus contains various bioactive components such as phenolics, flavonoids, terpenes, and alkaloids. *Wazir et al.* found that the crude extract of *O. hispidium* was particularly rich in alkaloids, flavonoids, glycosides and phenols [17]. *Saravanakumar et al.* reported that the extracts of *O. bracteosa* and *O. isaurica* were very rich in phenolics and flavonoids. *O. bracteosa* species contains 2,5-dihydroxybenzoic acid, gallic acid, protocatechuic acid, 3,4-dihydroxyphenylacetic acid, chlorogenic acid, 4 hydroxybenzoic acid, vanillic acid, syringic acid, 3-hydroxybenzoic acid, vanillin, p-coumaric acid, ferulic acid, luteolin 7-glucoside, hesperidin, hyperoside, apigenin 7-glucoside, pinoresinol, eriodictyol and quercetin compounds were found in *O. isaurica*. Caffeic acid, sinapic acid and rosmarinic acid were higher in *O. isaurica*, while caffeic acid, sinapic acid and rosmarinic acid were higher in *O. isaurica* [18]. In another study, total phenolic and flavonoid analyses of *O. gracilis* and *O. oreodoxa* extracts showed that *O. oreodoxa* extract had richer phenol and flavonoid content than *O. gracilis* extract [19]. *Kirkan et al.* found that *Onosma trapezuntea* extract contained higher total phenolics (24.08 mg GAE/g extract) and flavonoids (14.51 mg QE/g) compared to *O. rigidum* (10.63 mg GAE/g and 7.42 mg QE/g). Total flavonoid levels constituted more than 50% of total phenolics in both species [10]. When the results of this study are compared with the studies in the literature, there are differences in the content and amounts of phenolic compounds. This may be due to different species, geographical differences, climate change, methods used and the use of different solvents.

Antioxidant Activities

Ferrous ions (Fe^{+3}) reduction analysis (FRAP) can be defined as an electron transfer. When the FRAP results of the study were evaluated, it was observed that the Fe^{+3} ion reduction capacities of the extracts generally increased with the increase in concentration (**Figure 1**). While water extract showed better performance than methanol extract, BHT, BHA and AA standard antioxidants showed the strongest effect, respectively.
Excess iron, one of the most important metals for the organism, can cause several undesirable side effects. Excess Fe^{+2} ions in the environment can be converted to hydroxyl radical, a very dangerous free radical, by the Fenton reaction [20]. Therefore, FRAP is one of the most preferred methods for researchers. In our research, we found that studies on the reducing power of the genus *Onosma* were quite limited. *Saravanakumar et al.* found that the activities of *O. isaurica* and *O. bracteosa* extracts were equivalent to 85.76 ± 4.34 mg and 88.98 ± 6.62 mg trolox acid, respectively, according to FRAP results [18].

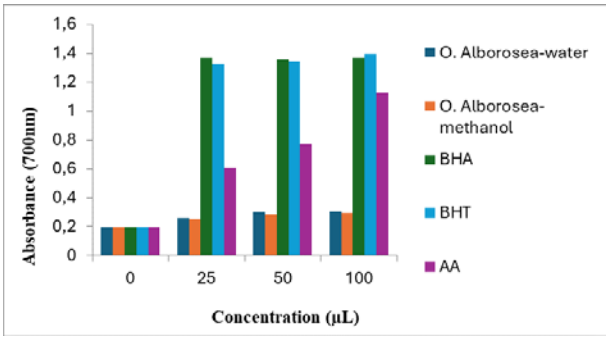

Figure 1. Comparison of ferrous ion reducing capacity of water and methanol extracts of *O. alborosea* with standard antioxidants (BHA, BHT and AA)

In one study, *O. Trapezuntea* was reported to have a stronger reducing capacity than *O. Rigidum* [10], while in another study [21] *O. Sericea* was reported to be a stronger reductant than *O. Stenoloba*. When the studies were evaluated in general, it was reported that species rich in secondary compounds showed stronger performance.

The results of CUPRAC assay showed that water and methanol extracts had activities close to each other and standard antioxidants. It was also found that the reducing power of the extracts and standard antioxidants increased with the increase in concentration (**Figure 2**).

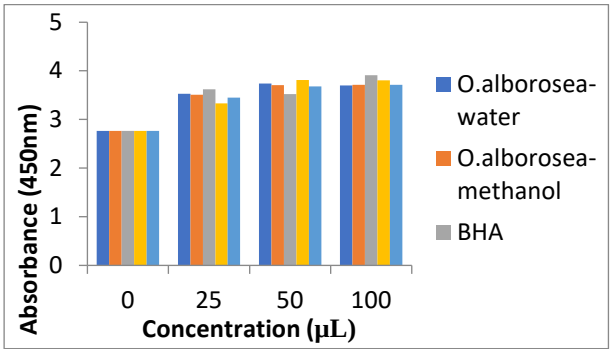

Figure 2. Comparison of cupric ion reducing capacity of water and methanol extracts of *O. alborosea* with standard antioxidants (BHA, BHT and AA)

In the literature, it has been reported that the species-rich in phenolic and flavonoids have stronger performance in studies on the CUPRAC method of *Onosma* genus [10, 21, 22]. This evaluation is consistent with the results of our study.

DPPH and ABTS methods were preferred for the radical scavenging activities of the study. These methods are the most preferred methods by researchers because they are cheap, fast and reliable [23, 24]. According to the results of DPPH radical scavenging activity, BHA showed the highest activity while water extract showed the lowest activity. The activity of the extracts and standard antioxidants generally increased with increasing concentration (Figure 5). At the highest concentration (100 µg/mL), the activities of the samples were in the following order: BHA (88.57%) > BHT (84.89%) > AA (64.61%) > Methanol extract (34.50%) > Water extract (25.08%).

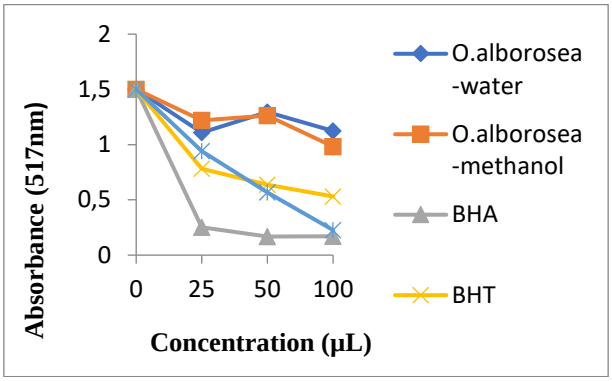

Figure 3. DPPH radical scavenging activity results of extracts and standard antioxidants

According to the ABTS results of the study, AA showed the highest activity while methanol extract showed the lowest activity. The activity of the extracts and standard antioxidants generally increased with increasing concentration (Figure 4). At the highest concentration (100 µg/mL), the activities of the samples were in the following order: AA (93.10%) > BHA (92.50%) > BHT (84.52%) > Water extract (41.62%) > Methanol extract (36.14%).

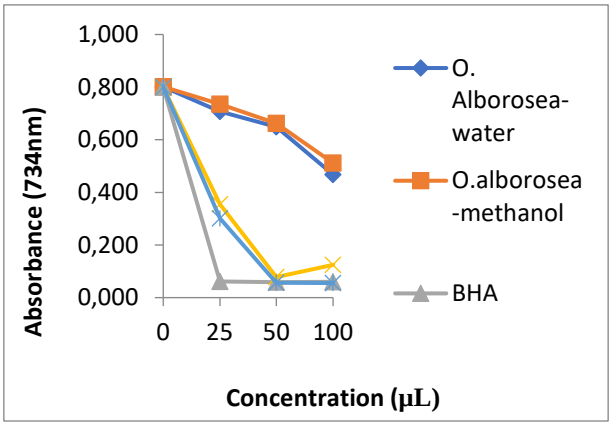

Figure 4. ABTS radical scavenging activity results of extracts and standard antioxidants

According to the study by *Özer et al.*, water extract of *O. heterophyllum* scavenged DPPH radicals most strongly. This was followed by methanol and ethyl acetate, respectively. It was also observed that all three extracts strongly scavenged ABTS radicals, while the weakest activity was observed in ethyl acetate extract [25]. Other studies have also reported that *Onosma* genus is a strong radical scavenger due to the richness of secondary compound contents [10, 19, 21, 22].

The results vary depending on the type of plant, solvent used, climatic conditions and geographical location.

CONCLUSION

Plants rich in phenolic compounds, which are widely used for medicinal purposes, are antioxidant organisms that act as electron donors to scavenge free radicals. In this study, water and methanol extracts of *O. alborosea* collected in Muş province were prepared after species identification. Phenolic content and quantity were determined by HPLC. Four different in vitro methods were used to determine the antioxidant capacity of the extracts. As a result of the chemical content analysis of the plant, ascorbic acid (3.15 ng/µL) and catechol (2.24 ng/µL) were found in the water extract and quercetin (3.21 ng/µL), cinnamic acid (0.99 ng/µL) and ascorbic acid (0.69 ng/µL) in the methanol extract. When the antioxidant results were evaluated, it was observed that the extracts scavenged DPPH and ABTS radicals weaker than standard antioxidants. At the same time, the extracts significantly reduced Fe^{+3} and Cu^{+2} ions. The activities of the samples were generally found to increase with increasing absorbance. The lack of research on the antioxidant activity of *O. alborosea* in the literature makes this study even more important. We hope that this study will shed light on future research on this species.

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