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Determination of Antioxidant Capacities of Extracts of *Sorbus subfusca* (ledeb. ex. nordm.) boiss

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Highlights:

- Water (WESS) and ethyl alcohol (EESS) lyophilized extracts showed significant antioxidant potential
- The high antioxidant capacity of *Sorbus subfusca* suggests its potential in natural product-based antioxidants
- Total phenolic compounds measured at 43.5 µg GAE/mg extract for WESS and 43.0 µg GAE/mg extract for EESS

Keywords:

- Antioxidant
- Phenolic content,
- Reducing capacity
- Rosacese,
- *Sorbus subfusca*

ABSTRACT:

Sorbus subfusca (ledeb. ex. nordm.) boiss. (*Sorbus subfusca*) belongs to the Rosacese family. It is commonly referred to as highland rowan. It is an endemic species found only in the eastern Black Sea Region of Turkey and in a few countries on the Asian Continent. Both water (WESS) and ethyl alcohol (EESS) lyophilized forms were used as extracts. Different reducing capacity methods and radical scavenging activity methods were used to study the antioxidant activities of the extracts. Total phenolic compounds were calculated as 43.5 (WESS) and 43.0 (EESS) µg GAE/mg extract. This value is an indication that it can take place in plants with high phenolic content. Peroxidation inhibition percentages of linoleic acid emulsion at 20 µg mL^{-1} concentration for WESS and EESS; WESS was calculated as 70.93% and EESS as 82.63%. The high antioxidant capacity of *Sorbus subfusca*, an endemic species, brings up the preference of natural products as antioxidants. It is thought that these studies will draw a new path to the literature, especially alternative medicine and pharmacological studies.

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INTRODUCTION

12 species and 17 taxa of *Sorbus* grow naturally in our country. 12 species and 17 taxon of *Sorbus* grow naturally in our country. *Sorbus* species are used in many areas. *Sorbus species are used in many areas.* In landscaping, its leaves are used with care because of their aesthetics. *Sorbus* has also found a place for itself in the pharmacology sector. In addition to these properties, it can be used against some diseases (Gültekin, Gülcü, Çelik, Gürlevik, & Öztürk, 2007).

Sorbus subfusca mostly grows at altitudes between 1200 and 2400 m (Akkemik, 2018). *Sorbus subfusca* is 2-6 m tall and has a densely branched structure. Within the genus *Sorbus*, the subfusca species is the last to bloom. The fruits of this species are dark red in color and elliptical in shape. These elliptical fruits are 10-13 mm long (Gökşin, 1982).

Oxygen consumption inherent in cell growth leads to the formation of a number of reactive oxygen species (ROS) (Topal, 2020; Türkan, Huyut, Basbugan, & Gülçin, 2020). High levels of ROS may occur as a result of endogenous metabolic reactions in the human body (Türkeş, 2019).

If ROS is not excreted effectively by cellular components, it leads to disease conditions and is involved in more than 100 diseases (Mutlu et al., 2023; Topal & Gülçin, 2022). ROS causes cell death and oxidizes biomolecules by leading up to tissue demage (Topal, Ozturk Sarıkaya, & Topal, 2021). ROS can form from all aerobic organisms and readily react with many biomolecules, including proteins, lipoproteins, lipids, and DNA (Han, Yılmaz, & Gülçin, 2018; Sarikaya & Gulcin, 2013; Topal, Öztürk Sarıkaya , & Topal, 2021).

Alzheimer's disease is a neurodegenerative disease that causes memory deficit and some behavioral disorders in daily life (M. Topal, 2019). The brain is one of the organs affected by unstable redox states and oxidative damage by overproduction of ROS. This oxidative stress causes the irregular functioning of the endogenous antioxidant system, which plays a critical role in Alzheimer's pathology (Özaslan et al., 2022; F. Topal, 2019b).

Recently, the possible toxicity and undesirable effects of synthetic antioxidants have been considered. Thus, the increasing popularity of natural food additives has increased the interest in natural antioxidants and decreased the interest in synthetic antioxidants. In addition, studies on natural additives have gained momentum strongly, as they do not pose any health risks to consumers (Göçer, Akıncıoğlu, Öztaşkın, Göksu, & Gülçin, 2013; F. Topal, 2019a).

Plants and other natural resources, as a result of studies; has always served humanity as a medical resource (Gulcin, 2020; Zehiroglu & Ozturk Sarikaya, 2019). Rosaceae family, of which *Sorbus subfusca* is a member, is used in medicinal drugs; It is also known to be aromatic. For this reason, it has an important place in terms of economy (Ekin, Gokbulut, Aydin, Donmez, & Orhan, 2016; Kalkman, 2004).

ROS formation is more evident during mitochondrial respiration. To reduce this situation, the need for antioxidant molecules increases (Apak et al., 2022). In view of this, it is important to examine potential antioxidant plants, fruits and vegetables and to determine their antioxidant properties. It was aimed to investigate the antioxidant capacities of *Sorbus subfusca*, which is found only in one region in our country and is very open to research. By evaluating the literature, it is thought that natural antioxidant foods can be supported by looking at the antioxidant capacities of *Sorbus subfusca*.

MATERIALS AND METHODS

Materials and Methods

Preparation of plant extracts

Sorbus subfusca fruit (0.5 kg) was collected from Çaykara region of Trabzon province of Turkey during September. Plants, Erzincan Binali Yıldırım University, Faculty of Arts and Sciences, Department of Biology, was diagnosed by Dr. Ali Kandemir. Herbarium registration number (Kandemir 11307).

Extraction procedures

To prepare the water-soluble extract (WESS) from 50 grams of Sorbus subfusca fruit, the fruit was first crushed in a blender and then mixed with 100 mL of water. This mixture was left overnight at room temperature on a magnetic stirrer. The resulting solution was filtered through cheese-cloth and Whatman No.1 paper. The filtrate was then transferred to a flask and placed in a deep freezer. Subsequently, it underwent lyophilization to obtain a dry extract.

For the ethanolic extract (EESS) determination, 100 mL of ethanol was added to the same 50-gram sample and left for 12 hours. Afterward, the mixture was filtered using Whatman No. 1 paper, and the filtrate was collected. Ethanol was removed from the collected filtrate using a rotary evaporator at 40°C until a dry extract was obtained. Both extracts were stored in a plastic bottle at -20°C until further use.

Total flavonoid and phenolic contents

To quantify the phenolic content using the Folin-Ciocalteu method (Kalın, Gülçin, & Gören, 2015), 500 µL of Folin-Ciocalteu reagent was added to the mixture, followed by the addition of 1.5 mL of 2% Na_2CO_3 after 3 minutes. The samples were then stirred at room temperature for 2 hours in a shaker. Subsequently, the absorbance of the samples was measured at 760 nm against a blank composed of distilled water. The amount of gallic acid equivalent (GAE) corresponding to the absorbance values was determined using an equation derived from a prepared standard curve.

For the determination of total flavonoids in the extracts, the method outlined by Park et al. (1997) was employed. Specifically, 750 µL of highland mountain ash extract was added to a vezin cup and transferred to a test tube. The extract was then diluted with 4550 µL of an ethanol solution containing 100 µL (1 M) of CH_3COOK and 100 µL (10%) $Al(NO_3)_3$ solutions. The mixture was thoroughly mixed using a vortex and incubated at room temperature for 40 minutes. Following incubation, the absorbance at 415 nm was recorded. Quercetin served as the standard for determining the total flavonoid concentration, expressed as microgram quercetin equivalent (QE), calculated from the equation obtained from the standard quercetin plot.

Reducing ability assay

For the Ferric Reducing Antioxidant Power (FRAP) method (F. Topal *et al.,* 2016), 2.25 mL of 20 mM $FeCl_3$ solution and 2.25 mL of FRAP reagent were combined to reach a final volume of 5000 µL. The prepared tubes were vortexed thoroughly. After a 10-minute incubation period, the absorbances were measured at 593 nm.

For the Cupric Reducing Antioxidant Capacity (CUPRAC) method (Apak, Güçlü, Özyürek, Esin Karademir, & Erçağ, 2006), $7.5x10^{-3}$ M neocuprine, 0.01 M $CuCl_2$ solution and 1 M CH_3COONH_4 buffer were added to tubes containing both EESS and WESS. The absorbance of the mixture was assessed at 450 nm.

Additionally, for iron reduction using the Oyaizu method, a solution with pH 6.6 and 0.2 mM phosphate buffer containing 1% $K_3Fe(CN)_6$ was added to tubes containing EESS and WESS. The mixture was then incubated at 50°C for 20 minutes. Subsequently, 10% trichloroacetic acid (TCA) and 0.1% $FeCl_3$ were added to the mixture, and the absorbance was measured at 700 nm (Oyaizu, 1986).

Radical scavenging assay

0.5 mL of a stock solution of DPPH was utilized for assessing DPPH free radical scavenging activity. Various concentrations of EESS and WESS extracts were subsequently introduced. The absorbance was then measured at 517 nm (Gülçin et al. 2011).

For the DMPD radical scavenging activity assay, $DMPD^{\cdot+}$ was generated. Different concentrations of EESS and WESS were added to the assay tubes, followed by the addition of 1 L of $DMPD^{\cdot+}$ solution. After incubating for 50 minutes in the absence of light, absorbance was measured at 505 nm, following the methodology described by Hendek Ertop & Öztürk Sarikaya in 2017.

In the assessment of ABTS radical scavenging activity, a 7 mM ABTS radical solution was prepared using persulfate solution. Various concentrations of EESS and WESS were added to achieve a final volume of 1.5 mL, with ethanol serving as the solvent. Subsequently, 0.5 mL of ABTS radical solution was added, and the mixture was allowed to incubate in darkness for thirty minutes. Absorbances were then recorded at 734 nm, as per the protocol detailed by Kalın et al. in 2020 (Kalın et al., 2020).

Total antioxidant activity

The thiocyanate method was used for this method (Yen & Chen, 1995). Buffer solution and stock solutions were added. Then, 2.5 mL of linoleic acid emulsion was added to them. Absorbance was measured at 500 nm every 12 hours at 37°C in the dark.

RESULTS AND DISCUSSION

Antioxidants are crucial substances that directly scavenge reactive oxygen species (ROS) or indirectly regulate antioxidant defenses, inhibiting ROS production. These compounds play a vital role in extending the shelf life of various products by delaying lipid peroxidation, a significant source of deterioration during storage or processing (Özler, Topal, Topal, & Öztürk Sarıkaya, 2023). They've become indispensable food additives, preserving sensory and nutritional properties without any adverse effects. Ideally, food-grade antioxidants should be cost-effective, potent at low concentrations, non-toxic, and possess stable structures, with neutral odor and taste. Ease of incorporation into products and good solubility are also advantageous (Topal, 2020).

Moreover, antioxidants offer protective benefits to the human body against free radicals and ROS-induced damage, potentially slowing the progression of chronic diseases. The quest for natural, safe antioxidant sources has surged, with plant-derived antioxidants garnering significant research attention. Incorporating antioxidants into foods helps thwart radical chain reactions of oxidation (Gulcin, 2020; Gülcin, 2012).

The plant extracts are listed in Table 1 and with standart antioxidants compared. High absorbance values indicate high reducing capacity.

Comparison was made between the absorbance values corresponding to the same concentration (20 μg mL^{-1}) of each standard antioxidant, WESS, and EESS. The comparison revealed the relative reduction capacities of ferrous ions (Fe^{3+}) among WESS, EESS, and standard antioxidants as follows: BHA (1.986±0.039) > Trolox (1.426±0.037) > BHT (1.286±0.050) > α-Tocopherol (0.710±0.023) > EESS (0.199±0.025) > WESS (0.128±0.014).

Furthermore, the reduction activities of WESS and EESS were compared with standard antioxidants using the FRAP method, resulting in the following ranking: BHA (1.941±0.039) > Trolox (1.821±0.086) > α-Tocopherol (1.191±0.058) > BHT (0.915±0.121) > EESS (0.354±0.030) > WESS (0.313±0.027).

When the reduction activities of cupric ions (Cu^{2+}) were compared between WESS and EESS, an increasing order was observed as follows: BHA (2.141±0.017) > BHT (1.166±0.118) > Trolox (0.974±0.080) > α-Tocopherol (0.688±0.065) > EESS (0.245±0.013) > WESS (0.180±0.001).

Tablo 1. Absorbance Values of Reduction Studies

Antioxidants	FRAP (593 nm)	Cu^{2+}(450 nm)	Fe^{3+}(700 nm)
BHT	0.915±0.121	1.166±0.118	1.286±0.050
BHA	1.941±0.039	2.141±0.017	1.986±0.039
Trolox	1.821±0.086	0.974±0.080	1.426±0.037
α-Tocopherol	1.191±0.058	0.688±0.065	0.710±0.023
WESS	0.313±0.027	0.180±0.001	0.128±0.014
EESS	0.354±0.030	0.245±0.013	0.199±0.025

Since there was a direct proportional relationship observed between concentration and reduction capacities in all three determination methods, it can be said that the three methods are interrelated. When evaluated separately in each method, it was determined that the antioxidant activities of the utilized WESS and EESS used were lower than those of the standard antioxidants.

When DPPH free radical scavenging determination was evaluated, IC_{50} (μg mL^{-1}) values for WESS and EESS, DPPH free radical scavenging activity are given in Table 2. Accordingly, it is as follows; Trolox (4.98) > BHA (7.70) > α-Tocopherol (10.83) > BHT (43.31) > EESS (138.60) > WESS (173.25).

When the results obtained were compared with some studies, WESS and EESS were found to have high IC_{50} values. High IC_{50} values indicate low antioxidant activity. The IC_{50} values related to DPPH free radical scavenging activity of lyophilized water extracts of gooseberry fruit and leaves were found to be for gooseberry fruit (36.47 μg mL^{-1}) and gooseberry leaves (38.50 μg mL^{-1}). Again, IC_{50} values of lyophilized water and alcohol extracts of flaxseed were found as: flaxseed for water (53.30 μg mL^{-1}), flaxseed for alcohol (49.50 μg mL^{-1}) (Han 2012; Han et al. 2018). When WESS and EESS IC_{50} values were compared with standard antioxidants, it was determined that BHA, α-tocopherol and trolox had a high effect, but BHT, WESS and EESS had a lower interaction. In addition, EESS showed a slightly higher effect than WESS. Unlike the ABTS procedure, the $DMPD^{\cdot+}$ method guarantees a stable endpoint. This is an important point, especially when large-scale scanning is required. The main disadvantage of the $DMPD^{\cdot+}$ method is that its sensitivity and reproducibility are significantly reduced when hydrophobic antioxidants such as α-tocopherol or BHT are used (Gulcin, 2020). Therefore, these two standard antioxidants, BHT and α-tocopherol, were not used in this radical scavenging experiment.

When the $DMPD^{\cdot+}$ scavenging activity is evaluated; For WESS and EESS, the IC_{50} values for $DMPD^{\cdot+}$ radical scavenging activity were compared with standard antioxidants, with trolox showing the highest efficacy. WESS and EESS showed close values, BHA showed lower antioxidant effect than Trolox, and WESS showed higher antioxidant effect than EESS. A low IC_{50} (μg mL^{-1}) value indicates high antioxidant activity. Accordingly, it is as follows; Trolox (9.90) > BHA (24.75) > EESS (46.20) > WESS (49.14).

Considering the data on the ABTS radical scavenging capacity of EESS and WESS within the scope of the study, when the IC_{50} (μg mL^{-1}) values were evaluated from decreasing to increasing,

standard antioxidants gave very close results. In this case, it indicated high antioxidant activity. It can be said that the highland rowan extracts used show low antioxidant activity compared to the standards. Accordingly, it is as follows; BHA (2.74) > Trolox (3.14) > BHT (3.61) > α-Tocopherol (4.95) >EESS (33.00) > WESS (36.47).

Tablo 2. Comparison of the IC_{50} (μg mL^{-1}) Values of the Radical Scavenging Activities

Antioxidants	$ABTS^{\cdot+}$ scavenging	$DMPD^{\cdot+}$ scavenging	DPPH· scavenging
BHT	3.61	*	43.31
BHA	2.74	24.75	7.70
Trolox	3.14	9.90	4.98
α-Tocopherol	4.95	*	10.83
WESS	36.47	49.14	173.25
EESS	33.00	46.20	138.60

*It does not show activity in the $DMPD^{\cdot+}$ removal method (Mutlu et al., 2023).

Lipid peroxidation is a common occurrence during food harvesting, storage, and processing, leading to chemical deterioration and resulting in rancidity, diminished nutritional value, altered aroma, compromised safety, and changes in texture in various food products such as milk, dairy, meat, fruits, vegetables, and pharmaceuticals. To combat this, food manufacturers utilize antioxidants to stabilize food lipids, which is considered the most effective method to control lipid oxidation (Kiziltas, Goren, Alwasel, & Gülçin, 2022). Antioxidants play a crucial role in inhibiting lipid peroxidation and protecting against cellular damage caused by free radicals (Öztürk Sarıkaya, 2015).

Undesirable properties like unpleasant taste and rancidity in food are often linked to non-enzymatic peroxidation or lipid peroxidation triggered by lipoxygenase enzymes in plants. Consequently, antioxidants are commonly regarded by food scientists as inhibitors of lipid peroxidation and subsequent food spoilage (M. Topal, 2018). For instance, a study revealed that the consumption of black currant and apple juice by human volunteers decreased lipid peroxidation but increased oxidative protein damage (Young et al., 1999).

The initiation of lipid peroxidation involves the attack on the side chain of a fatty acid by a radical, leading to the removal of a hydrogen atom from a methylene carbon. Fatty acids with more double bonds are more susceptible to radical attack, making monounsaturated and saturated fatty acids more resilient to radicals compared to polyunsaturated fatty acids (Apak et al., 2022). Consequently, the removal of lipid peroxides has proven to be significantly effective. These values are given in Table 3.

Tablo 3. Peroxidation Inhibition Percentage Amounts of Linoleic Acid Emulsion

Antioxidants	Lipid Peroxidation (%) Inhibitions
BHT	99.00
BHA	98.67
Trolox	98.00
α-Tocopherol	91.26
WESS	70.93
EESS	82.63

As a result, it was found that the percentage of inhibition of lipid peroxidation of the extracts was lower than that of all the standard antioxidants.

Considering the amount of phenolic compounds, there is no significant difference between WESS (43.5 μg GAE mg^{-1} extract) and EESS (43.0 μg GAE mg^{-1} extract) of highland rowan. On the contrary, almost the same amount of phenolic compounds was detected with each other (Table 4).

Table 4. Total Phenolic and Flavonoids in WESS and EESS

Extract	Total Phenolic (µg GAE/mg extract)	Total Flavonoid (µg QE/mg extract)
WESS	5.64	43.50
EESS	10.69	43.00

Flavonoids, a prominent subgroup of plant phenolics, are abundant in various plant-based foods (Shahidi, Janitha, & Wanasundara, 1992). They constitute a diverse group of polyphenolic compounds renowned for their efficacy in combating chronic diseases (Baxter, Puri, Harborne, Hall, & Moss, 1998; Gulcin, 2020). Despite their prevalence, flavonoids are typically poorly absorbed from dietary sources (Formica & Regelson, 1995).

These compounds serve as potent antioxidants, playing a pivotal role in shielding against cardiovascular diseases by mitigating the oxidation of low-density lipoproteins. Fruits, vegetables, and herbal teas are rich sources of flavonoids, making them integral components of our diet. On average, daily flavonoid intake reaches several hundred milligrams, with over 4000 naturally occurring flavonoids identified to date (Ghosh et al., 2015). The flavonoid content of this fruit is also quite high, as seen in Table 4. Accordingly, the total flavonoid content of the plant extracts was calculated as 43.50 µg QE/mg extract for WESS and 43 µg QE/mg extract for EESS. This amount is quite high for antioxidant plants. It is thought that its effectiveness in other antioxidant results is due to its high flavonoid content.

CONCLUSION

In recent years, the antioxidant properties of many fruits, plants and purified substances have been investigated by different experiments. In this study, which was carried out to support antioxidant foods, it was determined that the highland mountain ash showed an average antioxidant property. Although there are plants and fruits with much better antioxidant properties in the current situation, it was considered appropriate to be used in the food industry if preferred. Its slightly sour taste and red color make it attractive for use in red fruit juices such as sour cherry. However, like all antioxidants, it needs to be tested, although it may have synergistic or antisynergistic properties in food. Although there is not much information about the antioxidant capacity of the *Sorbus subfusca*, it is important to investigate its vitamin, mineral and other properties.

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Conflict of Interest

The authors declare no conflict of interest.

Author's Contributions

The authors declare that they have contributed equally to the article

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