

Prunus divaricata var (Yonuz erik) Metanol Ekstraktının Toplam Fenolik Bileşik ve Toplam Antioksidan/Oksidan Kapasitesinin Araştırılması

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Total fenolik bileşik

Total antioksidan/oksidan kapasite

ÖZ

Doğal olarak yetişen bitkilerin antioksidan kapasitesini belirleyen biyoaktif bileşiklerin tespiti, oksidatif strese bağlı hastalıkların tedavisinde kullanımlarını sağlayabilir. *Prunus divaricata var* (yerel olarak yonuz eriği, yabani erik, yunus eriği ve ekşi erik olarak da bilinir) gibi yüksek rakımlarda yetiştirilen bitkiler, besin değeri yüksek, hastalıkları azaltıcı ve tedavi edici potansiyele sahiptir. Bu çalışmada; metanol ekstraktının toplam antioksidan kapasitesi (TAK), toplam oksidan kapasitesi (TOK), oksidatif stres indeksi (OSI) ve toplam polifenolik bileşik (TFB) içerikleri belirlenmiş ve terapötik potansiyelleri değerlendirilmiştir. Meyveler Eylül ayında toplanmış, oda sıcaklığında ve rutubetsiz ortamda ortalama 20 gün kurutulmuştur. Kurutulmuş meyveler (10 g) metanol (500 mL) içinde eritilmiş ve toplam antioksidan/oksidan kapasiteleri ekstraksiyon ve buharlaştırma ile belirlenmiştir. TFB, Folin-Ciocalteu reaktif yöntemi kullanılarak spektrofotometrik olarak ölçülmüştür. Sonuç olarak; yabani erik meyve ekstraktının TAK seviyesi 3.595 ± 0.05 mmol Trolox eşdeğeri/L, TOK seviyesi 15.853 ± 0.09 $\mu\text{mol H}_2\text{O}_2$ Eşdeğeri/L ve OSI 0.441 ± 0.006 (AU) ve toplam TFB içeriği 41.52 ± 0.17 mg GAE g^{-1} olarak tespit edilmiştir. *P. divaricata* fenolik bileşikler açısından zengin olup güçlü bir antioksidan ve oksidan aktiviteye sahiptir. Birçok antioksidan bileşiğin birbirleriyle etkileşimleri sonucunda prooksidan etkiye sahip olabileceğine inanıyoruz. *P. divaricata*'nın güçlü oksidan aktivitesi, beslenme ve tedavi amaçlı kullanım için dikkatli bir şekilde değerlendirilmelidir.

Investigation of the Total Phenolic Compound and Total Antioxidant/Oxidant Capacity of Methanol Extract of *Prunus divaricata var* (Yonuz plum)

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ABSTRACT

Detection of bioactive compounds that determine the antioxidant capacity of naturally grown plants may provide their use in the treatment of oxidative stress-related diseases. Plants grown at high altitudes, such as *Prunus divaricata var* (locally known as yonuz plum, wild plum, dolphin plum, and sour plum) have high nutritional value, disease-reducing and therapeutic potential. In this study, total antioxidant capacity (TAC), total oxidant capacity (TOC), oxidative stress index (OSI) and total polyphenolic compound (TPC) contents of methanol extract were determined and their therapeutic potential was evaluated. The fruits were collected in September and dried at room temperature and in a humidity-

free environment for an average of 20 days. Dried fruits (10 g) were dissolved in methanol (500 mL), and their total antioxidant/oxidant capacity was determined by extraction and evaporation. TPC was measured spectrophotometrically using the Folin-Ciocalteu reagent method. In conclusion, the TAC level of the wild plum fruit extract was 3.595 ± 0.05 mmol Trolox equiv./L, the TOC level was 15.853 ± 0.09 $\mu\text{mol H}_2\text{O}_2$ Equiv./L and OSI 0.441 ± 0.006 (AU), and the total TPC content was 41.52 ± 0.17 mg GAE g^{-1} . *P. divaricata* is rich in phenolic compounds and has a strong antioxidant and oxidant activity. We believe that many antioxidant compounds can have a pro-oxidant effect as a result of their interaction with each other. The strong oxidant activity of *P. divaricata* should be carefully evaluated for nutritional and therapeutic use.

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1. Introduction

Turkey is one of the important countries in terms of its geographical location, soil structure, ecology, climate, and plant diversity. Approximately one-third of thousands of plant species grown in our country are endemic, and it is known that Turkey is the gene source of many wild fruits (Kendir and Güvenc 2010; Ketenoglu, 2011; Cömlekcioglu et al., 2020).

Plants are also known as 'super antioxidants' because they contain bioactive compounds that effectively treat many diseases, especially those associated with oxidative stress (Deveci et al., 2016; Mattioli et al., 2018). In particular, anthocyanin flavonoids and polyphenolic compounds that give fruits and vegetables their dark (red, blue, purple) color contain bioactive compounds with high antioxidant content (Blackhall et al., 2018 Cömlekcioglu et al., 2020). These bioactive compounds show a protective effect against cancer, diabetes, cardiovascular diseases, neurological diseases (Alzheimer's, Multiple sclerosis, Parkinson's), and other chronic diseases as well as diseases related to oxidative stress (Deveci et al., 2016; Adefegha, 2018, Mattioli et al., 2018; Deveci et al., 2022). Obtaining antioxidants from natural herbal sources such as vegetables and fruits, instead of using them as medicine, facilitates their absorption and elimination. If these antioxidants are taken in a balanced way through various vegetables and fruits, they do not reach toxic levels in the body. Thus, other functional components help to increase the functional effect with a synergistic effect (Liu, 2003; Prior, 2003; Coskun, 2005; Ozkaya, 2021).

Plant sources are important in maintaining general health status as they are a source of minerals, vitamins, and fiber. Especially wild fruits have many positive effects on nutrition and healthy life with their polyphenolic compounds. In addition, these wild fruits are used as natural therapeutics to treat various diseases (Baytop, 1999; Ebrahimzadeh et al., 2008; Ebrahimzadeh et al., 2009; Septembre-Malaterre et al., 2018). In recent years, after the positive effects on health have been revealed, fruits are consumed more, and studies on fruits are increasing day by day (Ebrahimzadeh et al., 2008; Ebrahimzadeh et al., 2009; Nabavi SF et al., 2013; Septembre- Malaterre et al., 2018; Cömlekcioglu et al. 2020).

Many wild fruit species are grown in our country, and most of them are used by the public as a source of food and therapy. Edible wild fruits are more intense in taste, odor, and minerals than the fruits grown through agriculture. *P. divaricata* var, belonging to the genus *Prunus* L., is one of the wild fruits grown in high-altitude regions. This type of plum, which has a yellow-red, stretched outer skin, has an intense aroma and is not suitable for fresh consumption with its bitter-sour taste; it is consumed in the form of marmalade, jam, and fruit pulp (Wöhrmann et al., 2011; Demirci and Ozhatay., 2012; Baskaya-Sezer et al., 2016; Cömlekcioglu et al., 2020). In the light of all this information, the TAC, TOC, OSI, and TPC content of *P. divaricata* methanol extract was determined, and its therapeutic potential was evaluated.

2. Material and Method

Collection of P. divaricata Fruit

In this study, fruit samples of *P. divaricata* var plant were collected in September 2020 from Hanagzi Village Karaagac locality of Islahiye district in Gaziantep province, 37° 04' 13" N and 36° 36' 06" E coordinates.

Preparation of P. divaricate fruit extracts

The fruits of *P. divaricata* were brought to the laboratory on the day of collection and washed with distilled water, and the fruits were spread in such a way that they did not touch each other. Approximately 250 g of fruit were pitted and left to dry for approximately 20 days at room temperature (24-30 °C) in natural airflow until the moisture content dropped to $\geq 90\%$. Then, the dried fruits were crushed in a porcelain mortar and ground into powder. Powdered fruit samples were stored in dark glass bottles, protected from light and moisture until extraction, polyphenol and oxidative stress parameters analysis. 10 g of the *P. divaricata* sample stored in powder bottles was weighed, and 500 ml of methanol (CH₃OH) (106009, Merck) was added to it, and extraction was achieved with a Soxhlet system (Wise Therm, Ildam) at 60°C for approximately 18 hours. Methanol was removed from the obtained extract by evaporation method in a rotary evaporator (Heidolp, Laborota 4000) at 48°C. The prepared extract was stored at -18 °C until analysis (Heleno et al., 2016).

Total Antioxidant/Oxidant Capacity Analysis

TAC of the fruit extract prepared using methanol as a solvent was analyzed spectrophotometrically (Epoch, Biotek, USA) with the Total Antioxidant Status kit (Rel Assay Kit Diagnostics, Turkey) developed by Erel (Erel, 2004). TAC value was calculated as mmol Trolox equivalent/L. The TOC of the fruit extract was analyzed spectrophotometrically (Epoch, Biotek, USA) with the Total Oxidant Status kit (Rel Assay Kit Diagnostics, Turkey) developed by Erel (Erel, 2005). TOC value was calculated as $\mu\text{mol H}_2\text{O}_2$ equivalent/L. TAC and TOC analyzes were repeated three times, and the results were averaged. The OSI value, expressed as a percentage of the ratio of the obtained TOC

value to the TAC value, was calculated according to the TAC and TOC results. While calculating OSI values, the mmol value in the TAC result unit was converted to μmol unit as in the TOC result. OSI value was calculated according to the formula below (Eren et al., 2015).

$$\text{OSI} = [(\text{TOC} / \text{TAC}) \times 100]$$

Total Polyphenolic Compound Analysis

TPC analysis was performed by spectrophotometer, modified from the Folin-Ciocalteu reagent method (Slinkard and Singleton 1977). This method is based on the fact that phenolic compounds dissolved in water or organic solvents form a purple-violet color with the Folin-Ciocalteu reagent (47641, Sigma) in an alkaline environment. Since this colored compound gave maximum absorbance at 760 nm, it was analyzed spectrophotometrically (Epoch, Biotek, USA) at 760 nm. Gallic acid (G7384, Sigma) was used as a standard and the total amount of phenolic compound was calculated as mg gallic acid equivalent (GAE) g^{-1} . TPC analysis was repeated three times and the results were averaged.

Statistical Analysis

The results are the average of three independent measurements (mean \pm SD).

3. Findings and Discussion

In the current study, the highest TAC (3.664), TOC (15.914), OSI (0.447), and TPC (41.348) values, and the lowest TAC (3.560), TOC (15.748), OSI (0.429) and TPC (41.695) values were determined. The results of oxidative metabolism and polyphenolic content are shown in Table 1.

Table 1. Oxidative stress parameters of dried *P. divaricata* var methanol extract and TPC analysis results.

TAC (mmol Trolox equiv./L)	TOC ($\mu\text{mol H}_2\text{O}_2$ Equiv./L)	OSI (Arbitrary unit)	TPC (mg GAE g^{-1})
3.595 \pm 0.05	15.853 \pm 0.09	0.441 \pm 0.006	41.52 \pm 0.17

Data are presented as mean \pm SD. TAC; Total antioxidant capacity, TOC; Total oxidant capacity, OSI; Oxidative stress index, TPC; Total polyphenolic compound.

The obtained TAC values are similar to those known and show antioxidant nutrients and higher capacity than some. Compared to the dry forms of these products obtained from large geographical regions such as Africa, Asia, and South Africa, where broad and medicinal plants are concentrated, this capacity was obtained from 100 g, while the data in our study were obtained from 10 g of dry extract (Carlsen et al., 2010). To protect and maintain human health and to protect ourselves from many diseases, we need to be protected from oxidant substances formed in our bodies. At this point, atoms, molecules or ions, and free radicals containing unpaired electrons are already harmful as oxidant whitewash, and consuming foods with this content is an invitation for diseases triggered by

oxidative stress. Our data show that the TOC level is higher than the TAC level. Therefore, besides the consumption of the fruit, the excess may cause the oxidant quality to come to the fore. Phenolic substances are now considered non-essential and non-nutritive compounds. However, it is also known that it has effects on human health. Flavonoids, also called bioflavonoids, have an antioxidative effect. The antioxidant effects of herbal products are due to their phenolic compounds. These products prevent the formation of many diseases such as cancer, diabetes, Alzheimer's, and heart diseases by preventing the reactions caused by free radicals. It is known that plant-derived phenolic compounds have pro-oxidant properties as well as antioxidant properties. Our data were obtained as high in dry content in terms of polyphenolic content compared to common foods (Caliskan, 2015).

Plants are the most important source of bioactive compounds with strong biological activities and are widely used for traditional treatment both in the world and in our country (Erdemoglu et al., 2003; Lin et al., 2014; Demir et al., 2017; Cömlekcioglu et al., 2020). Plant extracts obtained from parts of plants such as roots, leaves, fruits and flowers constitute the raw material source of many medicinal drugs (Ebrahimzadeh et al., 2008; Mosihuzzaman, 2012; Ozkol et al., 2017; Ozyurt et al., 2021). Phenolic compounds found in plants are secondary metabolites that protect plants from pathogens and ultraviolet rays. The number of phytochemicals in the phenolic compounds detected in natural products such as cereals, vegetables, and fruits is more than 8000 (Hussain et al., 2016, Demir et al., 2017, Ozkaya, 2021). It is reported that dietary phenolic compounds reduce the risk of cancer, cardiovascular and neurodegenerative diseases as well as their antioxidant and anti-inflammatory properties (Commenges et al., 2006; Morris et al., 2006; Xu et al., 2017; Demir et al., 2017).

Motamed and Naghibi (2010) determined the total phenolic content of the methanol extract of the fruit as 11.35 ± 0.48 mg GAE g^{-1} in their study with various plants, including *P. divaricata*. Baskaya-Sezer et al. (2016) found the total phenolic content of fresh plum marmalade to be 45.38 ± 0.09 , and the total phenolic content of wild plum marmalade to be 45.67 ± 1.4 mg GAE g^{-1} . In a study conducted by Cömlekcioglu and colleagues (2020), they compared the phenolic content of the extracts obtained by two different extraction methods (Soxhlet and ultrasonic water bath) of *P. divaricata* collected from two different locations. Accordingly, they stated that the phenolic content of the fruit extracts obtained by the soxhlet extraction method varied between 27.40-34.90 mg GAE g^{-1} , and the phenolic content of the fruit extracts obtained by the ultrasonic water bath extraction method ranged between 37.40-62.50. In our study, the total phenolic compound content of *P. divaricata* methanol extract was found to be 41.52 ± 0.17 mg GAE g^{-1} . Accordingly, we can say that this study's total phenolic compound content is higher than other studies conducted using the same extraction method.

Today, plants have become indispensable elements of natural nutrition because people prefer more natural foods in their diets. The fact that bioactive compounds in plants are a natural source of antioxidants and that these natural antioxidants play an active role in maintaining a healthy life attracts people's attention to this direction. In many studies, it has been reported that bioactive compounds in plants prevent cell death by preventing oxidative stress, which leads to the onset of many diseases

(Ebrahimzadeh et al., 2009; Nabavi SF et al., 2013; Raafat et al., 2014, Kurutas, 2015; Deveci et al., 2016, Septembre- Malaterre et al., 2018; Ozyurt et al., 2021). Although it was stated in a limited number of previous studies that the antioxidant activity of *P. divaricata* fruit extracts was high, no study was found on total oxidant capacity and oxidative stress index. Therefore, in this study, which is an original study, the total oxidant capacity and oxidative stress index of *P. divaricata* fruit extract were studied for the first time. In previous studies with different antioxidant measurement methods, it has been reported that the antioxidant activity of *P. divaricata* fruit extracts is high (Motamed and Naghibi, 2010; Baskaya-Sezer et al., 2016; Cömlekcioglu et al., 2020).

According to the results obtained, it was determined that both antioxidant capacity and oxidant capacity of *P. divaricata* fruit were high. However, when OSI, which is expressed as a percentage of the ratio of TOC value to TAC, is evaluated, it is seen that *P. divaricata* fruit has strong antioxidant activity. The reason why the total oxidant capacity of the *P. divaricata* fruit is higher than expected may be that this fruit has a large number of antioxidant compounds and these antioxidant compounds have a pro-oxidant effect.

4. Conclusion

According to the literature review, there are a limited number of studies on the determination of the total antioxidant capacity and phenolic compound content of *P. divaricata*, therefore we believe that this study will contribute to the literature. In this study, it was determined that *P. divaricata*, one of the wild fruits frequently consumed by the public both as a food and as a traditional treatment method, is rich in phenolic compounds and has strong antioxidant activity. However, it should not be forgotten that many antioxidant compounds found in such wild fruits may have a pro-oxidant effect as a result of their interaction and may cause health problems in excessive consumption. However, it is necessary to consider these properties when consuming plants with strong antioxidant activity such as *P. divaricata* and when using them therapeutically.

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Declaration of Conflict of Interest

The author(s) declares that there is no conflict of interest regarding the study.

Researchers' Contribution Rate Statement

The authors declare that they have contributed equally to the study.

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