



Determining total phenolic content and antioxidant activity in fruits and flowers of naturally grown *Arbutus andrachne* L. in Artvin

Artvin'de doğal yetişen Arbutus andrachne L.'nin meyve ve çiçeklerinin toplam fenolik madde içeriği ve antioksidan aktivitesinin belirlenmesi

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Abstract

Arbutus andrachne L. is widespread as Mediterranean enclave in the Black Sea Region, while it is found naturally in the Mediterranean and Aegean Region of Turkey. In this study, antioxidant activity, total phenolic and total flavonoid contents were determined in the fruits and flowers of *A. andrachne* samples obtained from Artvin. The antioxidant activity was analyzed using the DPPH radical scavenging activity and FRAP methods. In addition, while the total phenolic content was determined using the Folin-Ciocalteu assay, the aluminum chloride assay was used to measure the total flavonoid content. According to the results, the flowers of *A. andrachne* have higher contents in respect to antioxidant activity ($104.81 \pm 4.75 \mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$ dry sample), phenolic ($43.57 \pm 2.85 \text{ mg GA/g}$ dry sample) and flavonoid ($114.28 \pm 1.49 \text{ mg Que/g}$ dry sample) than its fruits. It was concluded that the flowers and fruits of *A. andrachne* are good source of phenolic and antioxidant.

Özet

Arbutus andrachne L. Akdeniz ve Ege bölgesinde doğal olarak bulunurken, Karadeniz bölgesinde Akdeniz enklav'ı olarak yayılır. Bu çalışmada Artvin'den alınan *A. andrachne* örneklerinin meyve ve çiçeklerinin antioksidan aktivite, toplam fenolik ve flavonoid madde içerikleri belirlendi. Antioksidan aktivite, DPPH radikal temizleme aktivite ve FRAP yöntemleri kullanılarak belirlenmiştir. Toplam fenolik madde miktarı Folin-Ciocalteu yöntemi, toplam flavonoid miktarı alüminyum klorür yöntemi kullanılarak tespit edilmiştir. Sonuçlara göre, *A. andrachne*'nin çiçekleri meyvelerinden daha yüksek antioksidan aktiviteye ($104.81 \pm 4.75 \mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$ kuru madde) fenolik ($43.57 \pm 2.85 \text{ mg GA/g}$ kuru madde) ve flavonoid ($114.28 \pm 1.49 \text{ mg Que/g}$ kuru madde) içeriğe sahiptir. *A. andrachne*'nin çiçek ve meyvelerinin iyi bir fenol ve antioksidan kaynağı olduğu sonucuna varılmıştır.

INTRODUCTION

The genus *Arbutus* belonging to Ericaceae family is represented by two species; *Arbutus andrachne* L. and *Arbutus unedo* L. These two species are different from each other in respect to flowering time, inflorescence and bark characteristics. *A. andrachne* is an evergreen shrub or a small tree with edible fruits (Stevens 1978). The fruits of *A. andrachne* are sweet and can be eaten as fresh or dried (Hedrick 1972; Facciola 1990). It has rich mineral contents such as K, Ca and P, and sugar in the form of fructose and glucose (Serçe et al. 2010).

A. andrachne is widespread as enclaves in the Black Sea Region, while it is found naturally in the Mediterranean and Aegean Region of Turkey. Moreover, it is also found

in some regions of Artvin showing Mediterranean climate characteristics along with other Mediterranean flora elements including *Cistus creticus* L., *C. salviifolius* L., *Pinus pinea* L., *Olea europaea* L. var. *syvestris* (Mill.) Lehr, *Punica granatum* L. and *Jasminum fruticans* L., especially at altitudes of 200-750 m (Eminağaoğlu and Anşin 2003, Eminağaoğlu 2015).

A. andrachne is used traditionally as astringent and urinary antiseptic (Said et al. 2002; Sakar et al. 1991). In addition, it is the most effective plant to expel gallstones (Dingil 1990). Medically, these plants have a strong antioxidant activity. Phytochemicals are called biomolecules which are synthesized by plants. It has properties such as signaling in plants (messenger),

antioxidant, antimicrobial, stress-resistant agents, protective against insecticides and herbicide (Treutter 2006; Yang et al. 2011; Costa et al. 2013). Phytochemicals can be found especially in vegetables and fruit phenolics, flavonoids and carotenoids. These compounds show a high correlation with antioxidant activity (Einbond et al. 2004). Many studies have shown that high concentrations of phenolics and flavonoids reduce the risk of diseases such as cardiovascular disease (Neil 2008), cancer (Wen et al. 2014), cirrhosis (Saral et al. 2016).

Phenolic compounds in *A. unedo* fruits (Ayaz et al. 2000; Özcan and Haciseferoğulları 2007; Pallauf et al. 2008; İşbilir et al. 2012; Mosele et al. 2016), leaves (Kıvçak et al. 2001; Malheiro et al. 2011; Nenadis et al. 2015; Moualek et al. 2016), flowers (İşbilir et al. 2012) and roots (Djabou et al. 2013) are well documented. However, number of such studies in *A. andrachne* is very limited. Total phenolic content and antioxidant activity were studied in various parts of *A. andrachne* plants (Tawaha et al. 2007; Kirca and Arslan 2008; Özgen 2009; Serçe et al. 2010; Ergun et al. 2014). In these studies the flowers were not included except Ergun et al. (2014), where antioxidant activity was studied using DPPH. To our knowledge there is no study conducted on flavonoid content in the fruits, or flavonoid and phenolic contents, and antioxidant activity using FRAP analysis in the flowers of *A. andrachne*. In addition, all previous studies employed samples were from the Mediterranean and the Aegean region. Enclave *A. andrachne* population in Artvin has never been studied for total phenolic content and antioxidant activity. Therefore, the goal of this study is to determine phenolic content, flavonoid content and antioxidant activity in *A. andrachne* fruits and flowers from the plants naturally found in northeastern Turkey.

MATERIALS and METHODS

Preparation Samples

The flowers and fruits of *A. andrachne* were collected near Fıstıklı Village (Artvin) in May and December of 2014, respectively. Fruits were collected at red stage. Herbarium samples were deposited at the Artvin Coruh University Herbarium (ARTH). Taxonomical identification of the plant was made according to Stevens (1978).

The fruits and flowers were dried in the oven at 40°C before analysis. Dry samples were ground with a blender. About 10 g of dried samples were used to prepare methanolic extracts. Then, methanolic extracts were filtered with filter paper and taken filtrates which were used to determine antioxidant activities.

Total Phenolic Assay

The total phenolic content of fruits and flowers were determined by using the Folin-Ciocalteu assay (Slinkard and Singleton 1977). 20 µl of various concentrations of gallic acid (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/ml) and 20 µl methanolic samples (1 mg.ml⁻¹), 400 µl of 0.5 N Folin-Ciocalteu reagents and 680 µl of distilled water were mixed. Then the mixture was vortexed. After following 3-minute incubation, 400 µl of Na₂CO₃ (10%) solution was added and vortexed, then the mixture was incubated for 2 hours. The absorbance of the mixtures was measured at 760 nm. The concentrations of total phenolic compounds were calculated as mg of gallic acid (GA) equivalents per g of the dry weight of samples.

Total Flavonoid Assay

The total flavonoid content was measured by using the aluminum chloride assay (Chang et al. 2002). 0.5 ml of Quercetin (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/ml) and 0.5 ml samples, 4.3 ml methanol 0.1 ml 10% Al(NO₃)₃ and 0.1 ml 1 M NH₄CH₃COO were added and mixed. The mixtures were incubated for 40 minutes. After incubation, the absorbance was measured at 415 nm. The total flavonoid contents of fruits and flowers were expressed as mg quercetin (Que) equivalents per g of dry weight sample.

Determination of Antioxidant Activity

The antioxidant activities of the samples were determined by using FRAP and DPPH methods. The FRAP method is based on the measurement of the iron reducing the capacities of the samples (Benzie and Szeto 1999). The 3 ml of FRAP reagent (containing TPTZ, FeCl₃, and acetate buffer) and 100 µl of sample were added to the test tube and mixed. After incubated for 4 min at 25°C, the absorbance was measured at 593 nm. The absorbance was compared to the standard curve (100-1000 µmol/l). The data were expressed as µmol

FeSO₄·7H₂O equivalents per gram of dry matter. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is based on the color change of the DPPH solution from purple to yellow as the radical is deactivated by the antioxidants (Pokorny et al. 2001). Briefly, different concentrations 0.75 ml of parts of sample extracts were mixed with 0.75 ml of a 0.1 mM of DPPH in methanol. The absorbance measured at 517 nm at the end of the incubation period of 50 min. BHT (Butylated hydroxytoluene) is used as standards and the values are expressed as IC₅₀ (mg sample per ml), the concentration of the samples that causes 50% scavenging

of DPPH radical. IC₅₀ values are calculated by linear regression analysis. The lower the IC₅₀ value of DPPH scavenging is so high.

RESULTS and DISCUSSION

The total phenolic content, total flavonoid content and antioxidant activity (FRAP and DPPH assays) of *A. andrachne* fruits and flowers are presented in Table 1 and Figure 1.

Table 1. The results of total phenolic and total flavonoid contents, and FRAP assay of *A. andrachne* fruit and flower growing in Artvin

Sample	Total Phenolic (mg GA/g dry weight)	Total Flavonoid (mg Que/g dry weight)	FRAP ($\mu\text{mol FeSO}_4\cdot 7\text{H}_2\text{O/g dry weight}$)
Flower	43.57±2.85	114.28±1.49	104.81±4.75
Fruit	7.29±1.02	6.17±0.02	3.41±0.25

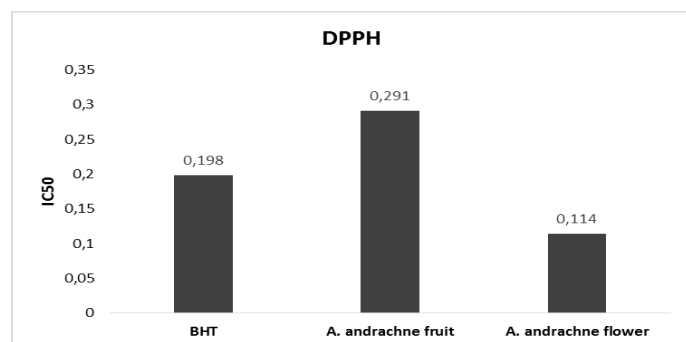


Figure 1. The results of DPPH for *A. andrachne* fruit and flower.

It is important to note that this study is the first in determining the total flavonoid contents both in flowers and fruits of *A. andrachne*. In addition, the total phenolic content and antioxidant activity using the flowers of *A. andrachne* were also investigated for the first time in this present research. The results indicated that the flowers of *A. andrachne* growing in Artvin have higher total phenolic content, total flavonoid content and ferric reducing power than its fruits (Table 1).

In earlier studies, total phenolic content and antioxidant activity of various plant parts (excluding flower) were investigated. In a study by Tawaha et al. (2007), out of 51 plant species investigated *A. andrachne* had the highest antioxidant activity (720±2.7 $\mu\text{mol TE/g dry weight}$) and total phenolic content (57.6±20.8 mg GAE/g dry weight). The total phenolic content and antioxidant activity (FRAP) in fruit samples of *A. andrachne* were found 2422-4102 $\mu\text{g GA/g fw}$ and 16.8-29.5 mmol/TE g fw, respectively

(Serçe et al 2010). In another study, *A. andrachne* fruits were collected in different maturation stages (green, green-orange, orange-red and red) and compared in respect their antioxidant activity. The results of this study showed that the total phenolic content (3.904 mg GA/kg fw) and the FRAP value (21.8 $\mu\text{mol TE/g fw}$) were the highest at the red stage (Özgen et al. 2009). While *A. andrachne* fruits (red stages) collected from Artvin appear to have higher phenolic content (7.29 mg GA/g dry weight) than those reported earlier (Serçe et al. 2010, Özgen et al. 2009), it should be noted that dried fruit samples were used to determine phenolic content in this study.

Flowers and fruits of *A. andrachne* from Artvin were subjected to DPPH analysis to determine their IC₅₀ values (Fig.1). DPPH radical scavenging activity of the flowers (0.114 mg/ml) was higher than that of the fruits (0.291 mg/ml). Similarly, Okmen (2015) reported higher radical scavenging activity (using ABTS method) in *A. andrachne* flowers than in the leaves. Furthermore, Kirca & Arslan (2008) and Ergün et al. (2014) also reported high DPPH radical scavenging activity in *A. andrachne* flowers and leaves.

In a similar study, İşbilir et al. (2012) used the other naturally-grown species of strawberry tree, *A. unedo*, and reported that its flowers showed higher total phenolic contents and DPPH radical scavenging activity than its

fruits, supporting the results of this study that the flowers of *A. andrachne* had also more total phenolic content and DPPH radical scavenging activity than its fruits.

Overall, when the results of previous studies along with the current research are evaluated, it can be concluded that such findings may be related to the differences in geographical locations, climatic features and extraction procedures.

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