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A Study on the Antioxidant Activity of Methanolic Extracts from Leaf, Stem and Flower Parts of Astragalus ovatus DC.

Astragalus ovatus DC Yaprak, Sap ve Çiçek Kısımlarının Metanolik Ekstraktlarının Antioksidan Aktivitesi Üzerine Bir Çalışma

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Abstract

The present study investigated the antioxidant activity of flower, stem and leaf extracts of *Astragalus ovatus* DC. plant. Total phenolic and flavonoid contents were determined in methanolic extracts of *Astragalus ovatus* DC. flower, stem and leaves. Leaf of *Astragalus ovatus* DC. Exhibited the highest activity of those parts tested. Total phenolic content and total flavonoid content for this part of plant were determined as 6.88 mg GAE/g and 2.80 mg QUE/g, respectively. FRAP value was found as 30.50 µmol $FeSO_4.7H_2O$/g. This is the first study to report the antioxidant activity of flower, stem and leaf extracts of *Astragalus ovatus* DC.

Keywords: *Astragalus ovatus* DC., Antioxidant Activity, Total Phenolic, Total Flavonoid

Özet

Bu çalışmada *Astragalus ovatus* DC bitkisinin çiçek, sap ve yaprak ekstraktlarının antioksidan aktivitesi araştırılmıştır. *Astragalus ovatus* DC çiçek, sap ve yapraklarının metanolik ekstraktlarının toplam fenolik ve flavonoid içerikleri belirlenmiştir. Bitkinin analiz edilen kısımlarından en yüksek antioksidan aktiviteyi *Astragalus ovatus* yaprakları göstermiştir. Yaprakların toplam fenolik ve flavonoid içeriği sırasıyla 6.88 mg GAE/g ve 2.80 mg QUE/g olarak tespit edilmiştir. FRAP değeri ise 30.50 µmol $FeSO_4.7H_2O$/g olarak bulunmuştur. Bu çalışma *Astragalus ovatus* DC'nin çiçek, sap ve yaprak ekstraktlarının antioksidan aktivitesini ortaya koyan ilk çalışmadır.

Anahtar kelimeler: *Astragalus ovatus* DC, Antioksidan Aktivite, Toplam Fenolik, Toplam Flavonoid

Abbreviations: GAE, gallic acid equivalent; QUE, quercetin equivalent

1. INTRODUCTION

Astragalus L., which is used for medical purposes and consists of approximately 3000 taxa, is the largest genus of the Fabaceae family (Hardion et al., 2010). There are 466 taxa belonging to Astragalus in Turkey and it is stated that 218 of them are endemic (Uzun, Palabaş Uzun & Durmaz, 2019). Studies have shown that Astragalus species have biological properties such as antioxidant, antimicrobial and anti-inflammatory (Albayrak& Kaya, 2018; Allam et al., 2013; Chen et al., 2012; Li et al., 2011).

Since ancient times, humanity has used plants to treat various diseases, meet their nutritional needs, and cosmetics (Albayrak & Kaya, 2019). Plants produce secondary metabolites such as phenolics, alkaloids and terpenoids. These metabolites are responsible for the biological activity of plants (Kabera, Semana, Mussa & He, 2014). Researchers have also begun to intensify their work on natural medicinal plants due to the increasing interest of humans in natural compounds recently (Albayrak & Kaya, 2019).

While there are a limited studies in the literature on the biological activity of different *Astragalus* species used for medical purposes, there is no study on thissubject on *Astragalus ovatus.* Therefore, in our study, we aimed to determine the antioxidant activity of the leaf, flower and stem (peduncle) part of the *Astragalus ovatus.*

2. MATERIALS AND METHODS

2.1. Plant Material

Leaf, flower and stem (including peduncle) parts of *Astragalus ovatus* were used as a research material. Those plant parts were collected from Macka district of Trabzon province (Uzun and Terzioğlu, 2020). (Figure 1, Table 1). Representative plant samples were placed at KATO Herbarium (Karadeniz Technical University, Faculty of Forestry).

Figure 1. *Astragalus ovatus* DC. (photo A.Uzun).

Astragalus ovatus can be observed locally in stony and grassy pseudomacchie areas at low altitude (Uzun & Terzioğlu, 2020). The population is localised but abundant. This species was re-collected after than Sintenis in 1894 (Davis, 1965-85). The threat category of this species was evaluated as"DD" (Data deficient) in Red Data Book of Turkish Plants (Ekim et al., 2000), but later it was proposed as "EN" (Endangered) by Ekici (2010).

Table 1. Location of plant.

Taxon	Location	Herbarium of KTU, Faculty of Forestry
Astragalus ovatus	Çeşmeler, grassy place, 583-910 m, 01.06.2008, A.Uzun 1061	KATO: 17880
	ibid., 910 m, 18.06.2008, A.Uzun 1097	KATO: 17881
	ibid., stony hill, 583 m, 07.07.2008, A.Uzun 1155	KATO: 17882

2.2. Preparation of Plant Extracts

Plants were divided into three parts as flowers, stems and leaves.The plant parts were dried at room temperature and ground to a fine powder with a grinder. Approximately 5 g of each sample were weighed and 30 mL of methanol was added. Then these parts were mixed for 24 h at room temperature and filtered with filter papers. Samples were stored at +4°C until analyzes were performed.

2.3. Total Phenolic and Total Flavonoid Content

Total phenolic contents were determined by the Folin-Ciocalteau procedure (Slinkard & Singleton, 1977) and using gallic acid as standard. Firstly, 20 µL of various concentrations of gallic acid and 20 µL extracts (1 mg/mL), 400 µl of 0.5 N Folin-Ciocalteu regents and 680 µL of distilled water were added and mixture was vortexed. Following 3-minute incubation, 400 µL of Na_2CO_3 (10%) solution was added and after vortexing. After 2 h incubation period at the room temperature, absorbances of the mixtures were measured at 760 nm. The concentrations of total phenolic compounds were calculated as mg of gallic acid equivalents (GAE) per g of dry sample.

Total flavonoid content was measured by using thealuminum chloride assay (Chang, Yang, Wen &Chern, 2002). Quercetin was used as a standard. 0.5 mL various concentrations of Quercetin, 4.3 ml methanol 0.1 mL 10% $Al(NO_3)_3$ and 0.1 mL 1 M NH_4CH_3COO were added and mixed. After 40 minutes incubation, absorbance was measured at 415 nm. The total flavonoid contents of samples were expressed as mg quercetin equivalents (QUE) per g of dry sample.

2.4. Ferric Reducing Antioxidant Power (FRAP)

FRAP method was used for the determination of total antioxidant capacity, based on the reduction of yellow Fe^{3+} -TPTZ complex to the blue Fe^{2+} -TPTZ complex by electron donating substance under acidic condition (Benzie& Szeto, 1999). The 3 mL of FRAP reagent (containing TPTZ, $FeCl_3$, and acetate buffer) and 100 µL of sample were added and mixed. Absorbance values at 593 nm were recorded after 4 min at 25°C. FRAP value was expressed as µmol $FeSO_4.7H_2O$ equivalents per gram of dry sample.

3. RESULTS AND DISCUSSION

The total amounts of phenolic compounds in samples evaluated by the Folin-Ciocalteu assay were given as gallic acid equivalent (GAE) and the results are given in Table 2.

Table 2. Total phenolic contents of three different part of *A. ovatus*

Samples	TPC (mg GAE/g dry sample)
Flower	1.81±0.32[a]
Leaf	6.88±0.44[b]
Stem	1.74±0.25[a]

*Values with different superscript letters are significantly different ($p < 0.05$).

As shown in Table 2, there is no statistical variation in TPC content for flower and stem parts of plant examined. The highest total phenolic content belongs to leaf of *A. ovatus*. TPC were calculated as 1.81, 6.88, and 1.74 mg gallic acid equivalent/g sample for flower, leaf and stem of *A. ovatus*, respectively; while TFC were found as 1.10, 2.80, and 0.32 mg quercetin equivalent/g sample for flower, leaf and stem of *A. ovatus*, respectively (Table 3).

Table 3. Total flavonoid contents of three different part of *A. ovatus*

Samples	TFC (mg QUE/g dry sample)
Flower	1.10±0.03[b]
Leaf	2.80±0.11[c]
Stem	0.32±0.02[a]

*Values with different superscript letters are significantly different ($p < 0.05$).

A review of *Astragalus* genus stated that more than 100 species of this plant has been investigated until 2016 (Bratkov, Shkondrov, Zdraveva&Krasteva, 2016). This plant has been investigated mainly for three main groups of biologically active compounds polysaccharides, flavonoids, and saponins (Verotta& El-Sebakhy, 2001). Flavonids represent the largest group of polyphenolic compounds occurring in *Astragalus* species (Bratkov et al., 2016).

Leaves had the highest flavonoid contents among studied parts of plant. Biochemical characteristics of *A. gummifer*, *A. microcephalus*, *A. talasseus* and *A. acmophyllus* from the flora of Turkey have been studied by Albayrak and Kaya (2018). They found total phenolic content between 5.49 mg GAE/g and 13.49 mg GAE/g for those species. In our study, flower and stem parts of *A. ovatus* had lower phenolic contents than *A. gummifer*, *A. microcephalus*, *A. talasseus* and *A. acmophyllus*.

Ferric reducing/antioxidant power values of three different parts of *A. ovatus* are given in Table 4. The results of assay show that leaf of *A. ovatus*was exhibited the highest antioxidant activity. FRAP assay results was confirmed by total phenolic and flavonoid contents i.e. the richest part in terms of flavonoids and phenolics is leaf of *A. ovatus.*

Table 4. Ferric reducing antioxidant power values of three different parts of *A. ovatus*

Samples	Ferric reducing antioxidant power (µmol $FeSO_4.7H_2O$/gdry sample)
Flower	24.88±0.59[b]
Leaf	30.50±1.04[c]
Stem	13.96±1.66[a]

*Values with different superscript letters are significantly different ($p < 0.05$).

4. CONCLUSION

This manuscript deals with antioxidant properties of flower, stem and leaf extracts of *Astragalus ovatus* DC. plant. Significant differences were found among analyzed plant parts in terms of total phenolic and flavonoid contents ($p<0.05$). It

is thought that this study has the feature of being a preliminary research. Further studies are needed to illuminate bioactive characteristics and health benefits of this plant.

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