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# DETERMINATION OF ANTIRADICAL CAPACITY OF EXTRACTS *SCUTELLARIA ORIENTALIS* L. SUBSP. *BICOLOR* (HOCHST.) EDMONDSON

**ABSTRACT**

In this study, the antiradikal activity of water and ethanol extracts of *Scutellaria orientalis* L. subsp. *bicolor* (Hochst.) Edmondson was evaluated by various antiradical assay, ABTS• (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging capacity and DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity. Those various antiradical activities were compared to standard antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol. The extracts of *Scutellaria orientalis* showed strong antioxidant activity. The results obtained in the present study indicated that *Scutellaria orientalis* is a potential source of natural antioxidant.

**Keywords:** *Scutellaria orientalis*, Antiradical, Extract, Water, Ethanol

# *SCUTELLARİA ORİENTALİS* L. SUBSP. *BİCOLOR* (HOCHST.) EDMONDSON EKSTRAKTLARININ ANTİRADİKAL KAPASİTELERİNİN BELİRLENMESİ

**ÖZET**

Bu çalışmada, *Scutellaria orientalis* L. subsp. *bicolor* (Hochst.) Edmondson' in su ve etanol ekstrelerinin antiradikal aktivitesi ABTS• (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)) radikal temizleme kapasitesi and DPPH• (2,2-diphenyl-1-picrylhydrazyl) radikal temizleme kapasitesi gibi çeşitli antiradikal yöntemler tarafından değerlendirildi. Bu çeşitli antiradikal aktiviteler bütillendirilmiş hidroksianisol (BHA), bütillendirilmiş hidroksitoluen (BHT) ve α-tokoferol gibi standart antioksidanlarla karşılaştırıldı. *Scutellaria orientalis*' in ekstreleri kuvvetli antioksidan aktivite gösterdi. Mevcut çalışmada alınan sonuçlar *Scutellaria orientalis*' in doğal antioksidanların potansiyel bir kaynağı olduğunu gösterdi.

**Anahtar Kelimeler:** *Scutellaria orientalis*, Antiradikal, Ekstrakt, Su, Etanol

## 1. INTRODUCTION (GİRİŞ)

The human body has several antioxidant defense systems to protect healthy cell membranes from active oxygen species and free radicals [1 and 2]. The innate defense systems may be supported by antioxidative compounds taken as foods, cosmetics and medicine. Therefore, the antioxidative compounds provided by the diet may enrich the antioxidative status of living cells and thus reduce the damage, particularly in the elderly [3]. The most widely used antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been restricted recently because of serious concerns about their carcinogenic potential [4]. Therefore, there is great interest in finding new and safe antioxidants from natural sources [5]. Recently, natural plants have received much attention as sources of biologically active substances including antioxidants, antimutagens and anticarcinogens [6]. Numerous studies have been carried out on some plants such as rosemary, sage and oregano, which resulted in the development of natural antioxidant formulations for food, cosmetic and other applications. However, scientific information on antioxidant properties of various plants, particularly those that are less widely used in culinary and medicine is still scarce. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals [7].

Plants contain a wide variety of free radical scavenging molecules, such as flavonoids, anthocyanins, cartenoids, dietary glutathionine, vitamins and endogenous metabolites and such natural products are rich in antioxidant activities [8]. Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), singlet oxygen ($^1O_2$) and non-freeradical species such as hydrogen peroxide ($H_2O_2$) are various forms of activated oxygen and often generated by oxidation product of biological reactions or exogenous factors [9]. Electron acceptors, such as molecular oxygen, react easily with free radicals to become radicals themselves, also referred to as reactive oxygen species (ROS). ROS have aroused significant interest among scientists in the past decade. Their broad range of effects in biological and medicinal systems has drawn on the attention of many experimental works [10].

There are increasing suggestions by considerable evidence that the free radicals induce oxidative damage to biomolecules (lipids, proteins and nucleic acids), the damage which eventually causes atherosclerosis, ageing, cancer, diabetes mellitus, inflammation, AIDS and several degenerative diseases in humans [1;11]. Several methods have been developed to measure the free radical scavenging capacity (RSC), regardless of the individual compounds which contribute towards the total capacity of a plant product in scavenging free radicals. The methods are typically based on the inhibition of the accumulation of oxidized products, since the generation of free radical species is inhibited by the addition of antioxidants and this gives rise to a reduction of the end point by scavenging free radicals. The reliable method to determine RSC involves the measurement of the disappearance of free radicals, such as 2,2-azino-bis (3-ethylbenzenthiazoline-6-sulphonic) acid radical ($ABTS^{\bullet +}$), the 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet +}$) or other colored radicals, with a spectrophotometer [12 and 13]. Owing to the increasing demand for information about the total RSC of all types of plant extracts, an easy, rapid and reliable method for the determination

of RSC of various samples might be useful. The method should not be time-consuming, but sensitive enough to screen differences between plants parts used for herbal medicine, which include the flower, top, aerial and roots [14].

## 2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

The ethyl acetate and methanol extracts of the 33 *Scutellaria* species were assayed for their antioxidant activity at 250, 500, and 1000 µg/ml concentrations. In DPPH radical quenching activity test, the ethyl acetate extracts exerted a low activity, whereas occurrence of very high quenching effect against DPPH radical was observed in most of the methanol extracts. At 250 µg/ml, the lowest concentration tested, many subspecies of *S. orientalis* possessed the most potent activity, being *S. orientalis* subsp. *pinnatifida* (87.62%) the best, followed by *S. orientalis* subsp. *macrostegia* (65.29%), *S. orientalis* subsp. *bornmulleri* (62.37%), *S. orientalis* subsp. *alpina* var. *alpina* (61.55%), *S. orientalis* subsp. *bicolor* (60.17%). Most of the species displayed a high radical scavenging effect in this test at 500 and 1000 µg/ml, which is quite comparable to that of gallic acid, the reference compound. In FRAP assay, the ethyl acetate extracts had insignificant effect, where only some of the methanol extracts belonging to several subspecies of *S. orientalis* exerted moderate reducing activity on ferric ion. On the other hand, the ethyl acetate extracts were found to be inactive in ferrous ion-chelating test except only those of *S. brevibracteata* subsp. *brevibracteata* and *S. hastifolia*. Additionally, the methanol extracts also showed insignificant activity in this test having chelating capacity below 50%. Total phenol contents of the methanol extracts were calculated according to the equation ($y = 0.0009x - 0.0145$, $r^2 = 0.9956$) as gallic acid equivalent (GAE, mg/g extract), while their total flavonoid contents were determined in accordance with the equation ($y = 2.1257x - 0.02902$, $r^2 = 0.9858$) obtained by calibration curves as quercetin equivalent (QUE, mg/g extract). The richest extract considering total phenol content belonged to *S. orientalis* subsp. *pichleri* (393.34 ± 1.89 mg/g extract GAE), followed by *S. orientalis* subsp. *carica* (295.56 ± 1.44 mg/g extract GAE). *S. brevibracteata* subsp. *pannosula* was found to have most abundant total flavonoid content (125.27 ± 1.65) as QUE. However, no correlation was observed between total phenol and flavonoid contents and antioxidant activity of the extracts [15].

Furthermore, the antioxidant activity and radical scavenging capacity of *A. schischkinii* and *A. teretifolia* has not previously been published. In this study, in vitro antioxidant, radical scavenging and antimicrobial properties of the methanol, water and chloroform extracts of two *Achillea* species growing in the eastern part of Turkey were investigated.

## 3. EXPERIMENTAL METHOD-PROCESS (DENEYSEL ÇALISMA)

### 3.1. Chemicals (Kimyasallar)

Ammonium thiocyanate was purchased from E. Merck. α-tocopherol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), butylatedhydroxyanisole (BHA) and butylated hydroxytoluene (BHT), were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were of analytical grade and were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

### 3.2. Plant Material and Extraction (Bitki Materyali ve Ekstraksiyon)

The herbal parts of *S. orientalis* was collected from Kuşakçı Mountain Elazığ-Turkey when flowering (June 2009). The aerial parts of the plant material were dried in shade at room temperature and then ground to a fine powder in a mechanic grinder. Then the powdered plant materials (10 g) were extracted with 100 ml of ethanol and water in a Soxhlet extractor. After the filtration of the solvent, the organic phases were independently concentrated under vacuum by evaporating to dryness. The residues were dissolved in the same solvent and stored at -20 °C until studied.

### 3.3. ABTS• Radical Scavenging Capacity (ABTS• Radikali Temizleme Kapasitesi)

ABTS also forms a relatively stable free radical, which decolorizes in its non-radical form Shirwaikar *et al.* [16]. The spectrophotometric analysis of $ABTS^{\bullet+}$ radical scavenging capacity was determined according to the method of Re *et al.* [17]. $ABTS^{\bullet+}$ was produced by reacting 2mM ABTS in $H_2O$ with 2.45mM potassium persulfate ($K_2S_2O_8$), stored in the dark at room temperature for 12h. The $ABTS^{\bullet+}$ solution was diluted to give an absorbance of 0.750±0.025 at 734 nm in 0.1M sodium phosphate buffer (pH 7.4). Then, 1 mL of $ABTS^{\bullet+}$ solution was added to 3mL of *S. orientalis* extracts in ethanol at 100 µg/mL concentrations. The absorbance was recorded 30 min after mixing and the percentage of radical scavenging was calculated for each concentration relative to a blank containing no scavenger. The extent of decolorization is calculated as percentage reduction of absorbance.

The scavenging capability of test compounds was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_o - A_1)/A_o] \times 100$$

where $A_o$ is the absorbance of the control, and $A_1$ is the absorbance in the presence of the sample of *S. orientalis* extracts or standards.

### 3.4. DPPH• Radical Scavenging Capacity (Dpph• Radikali Temizleme Kapasitesi)

The free radical scavenging capacity of *S. orientalis* extracts was measured by 2,2-diphenyl-1-picryl-hydrazil (DPPH•) using the method of Shimada *et al.* [18]. Briefly, 0,1mM solut ion of DPPH• in ethanol was prepared and 1 mL of this solution was added 3 mL of *S. orientalis* extracts solution in water at different concentrations (50, 100 and 250 µg/mL). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The capability to scavenge the DPPH• radical was calculated using the following equation:

$$\text{DPPH}^{\bullet} \text{ Scavenging Effect } \% = [(A_o - A_1)/A_o] \times 100$$

where $A_o$ is the absorbance of the control reaction and $A_1$ is the absorbance in the presence of the sample of *S. orientalis* extracts.

## 4. FINDINGS AND DISCUSSIONS (BULGULAR VE TARTISMALAR)

### 4.1. ABTS Radical-Scavenging Capacity (ABTS• Radikali Temizleme Kapasitesi)

All the tested compounds exhibited effective radical cation scavenging activity. The scavenging effect of *S. orientalis* (100 µg/mL concentrations) and standards on $ABTS^{\bullet+}$ decreased in the order (fig 1.): BHA> BHT> α-tocopherol> ethanol extract of *S. orientalis*> water extract of *S. orientalis* (99.8%, 97.3%, 96.9%, 90.8% and 90.7%, respectively) at the concentration of 100 µg/mL (Tablo 1). No significant differences in $ABTS^{\bullet+}$ scavenging potential were found among of water and ethanol extract of *S. orientalis*, BHA, BHT and α-tocopherol.

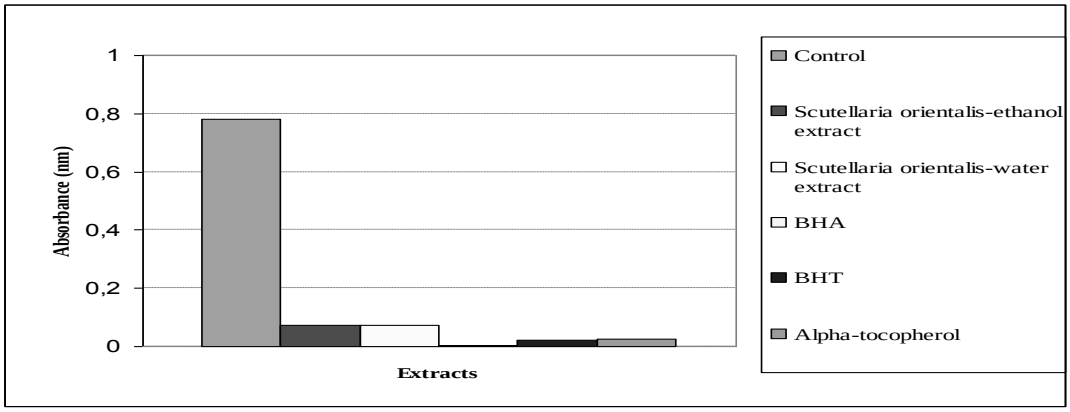


Figure. 1. $ABTS^{\bullet+}$ radical-scavenging capacity of water and ethanol extracts of *S. orientalis*, BHA, BHT and α -tocopherol.
(Şekil 1. *S. orientalis*' in su ve etanol ekstraktları, BHA, BHT ve α-tokoferolün ABTS• radikali temizleme kapasitesi)

Table 1. (%) ABTS• radical-scavenging capacity of water and ethanol extracts of *S. orientalis*, BHA, BHT and α-tocopherol.
(Tablo 1. *S. orientalis*' in su ve etanol ekstraktları, BHA, BHT ve α-tokoferolün % ABTS• radikali temizleme kapasitesi)

| Extracts (100 µg/ml) | ABTS assay (%) |
|---|---|
| Scutellaria orientalis ethanol extract | 90,8 |
| Scutellaria orientalis water extract | 90,7 |
| BHA | 99,9 |
| BHT | 97,3 |
| α-tocopherol | 96,9 |

### 4.2. DPPH• Radical Scavenging Capacity (DPPH• Radikali Temizleme Kapasitesi)

DPPH• is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [20]. The reduction capability of DPPH• ·radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, DPPH• is often used as a substrate to evaluate antioxidative activity of antioxidants [21]. Fig. 2 illustrates decrease in the concentration of DPPH• radical due to the scavenging ability of the extracts of S. orientalis. We used α-tocopherol as standards. The scavenging effect of water and ethanol extracts of S. orientalis and standards on the DPPH• radical decreased in that order: α-tocopherol> ethanol extract of

S.orientalis > water extract of S. orientalis (Fig. 3). 100 µg of water and ethanol extracts of S. orientalis exhibited 68%, 69.2%, 59.2%, 75.9%, 69.6% and 60.1% DPPH˙ scavenging capacity, respectively. In the other hand, at the same dose, α-tocopherol exhibited 95% DPPH˙ scavenging capacity. These results indicates that S. orientalis extracts have a noticeable effect on scavenging free radical.

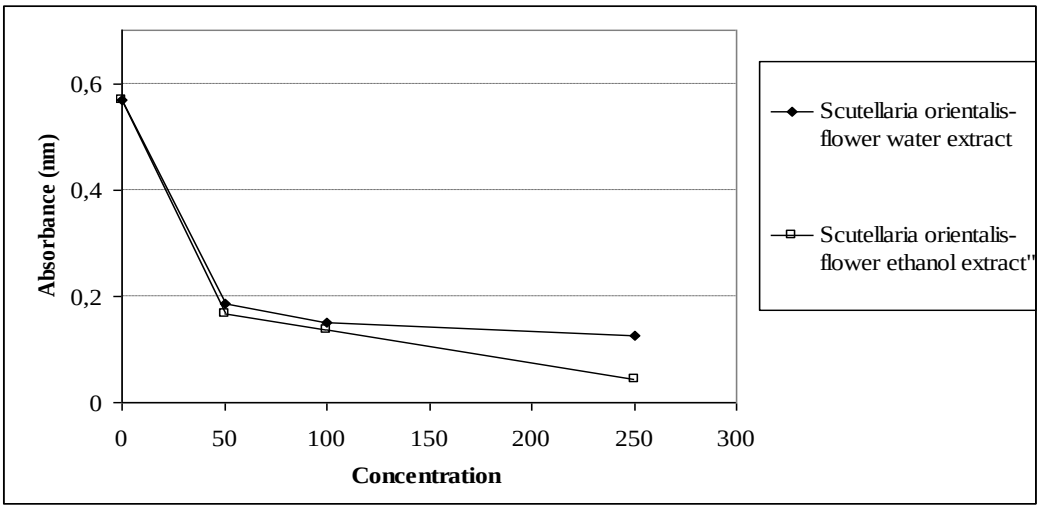


Figure 2. DPPH˙ radical scavenging capacity of water and ethanol extracts of *S. orientalis.*
(Şekil 2. *S. orientalis*' in su ve etanol ekstraktlarının DPPH˙ radikali temizleme kapasitesi)

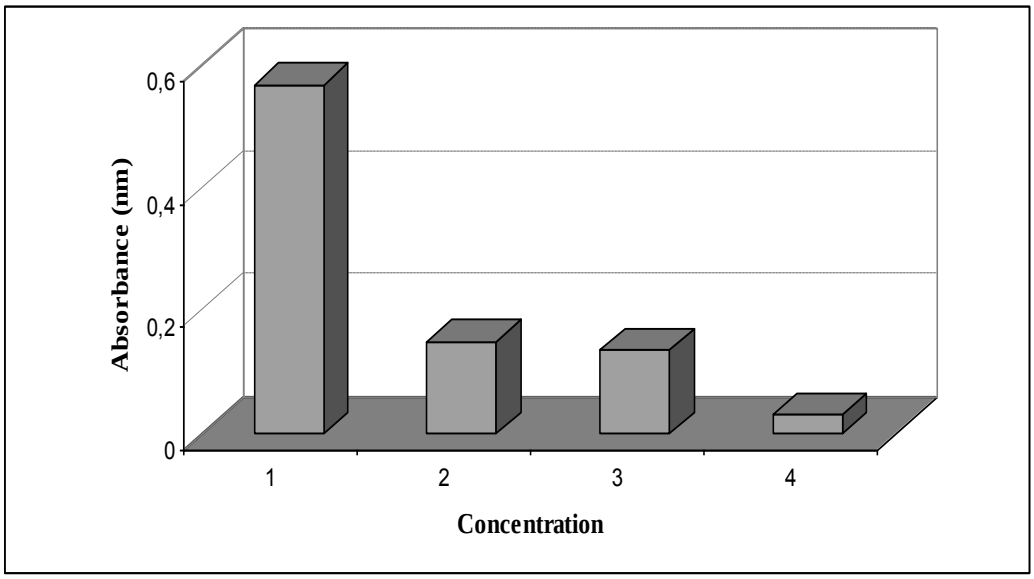


Figure 3. DPPH˙ radical scavenging capacity of water and ethanol extracts of *S. orientalis* (100 µg) and α-tocopherol. (1. Control 2. Water extract of *S. orientalis* 3. Ethanol extract of *S. orientalis* 4. α-tocopherol.)
(Şekil 3. *S. orientalis*' in su ve etanol ekstraktları (100 µg) ve α-tokoferolün DPPH˙ radikali temizleme kapasitesi)

## 5. CONCLUSION AND RECOMMENDATIONS (SONUÇ VE ÖNERİLER)

As a conclusion, the water and ethanol extracts of *S. orientalis* showed strong ABTS$^{\bullet}$ radical and DPPH$^{\bullet}$ radical when compared to standards such as BHA, BHT and α-tocopherol. The results of this study show that the water and ethanol extract of *S. orientalis* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. It can be used in stabilising food against oxidative deterioration.

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