

Astragalus diphtherites FENZL var. *diphtherites* ve *Astragalus gymnaopecias* RECH. FIL'in Gövde ve Kök Kısımlarından Farklı Çözücüler ile Elde Edilen Özütlerin İn vitro Antioksidan ve Antimikrobiyal Özelliklerinin Belirlenmesi

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ÖZET

Bu çalışmanın amacı farkı polariteye sahip çözücü serilerinden geçiren *Astragalus diphtherites* var. *diphtherites* (*A. diphtherites*) ve *Astragalus gymnaopecias* (*A. gymnaopecias*) türlerinin gövde ve kök kısımlarının antioksidan ve antimikrobiyal özelliklerinin araştırılmasıdır. Antioksidan özellikleri belirlemek için özütlerin total fenolik, total flavonoid, DPPH, metal şelatlama, indirgeme gücü ve hidroksi radikalini söndürme aktiviteleri test edildi. En yüksek total fenolik bileşen miktarı *A. diphtherites*' de gövdede metanol, kökte ise aseton özütünden elde edilirken *A. gymnaopecias*' ta gövdede metanol kökte ise etil asetat özütünde tespit edildi. *A. diphtherites* ve *A. gymnaopecias*' ta en yüksek total flavonoid miktarları ve indirgeme gücü aktiviteleri gövdede aseton kökte ise etil asetat özütlerinden elde edildi. *A. diphtherites* için en yüksek DPPH radikalini söndürme aktivitesi, gövdede metanol kökte ise aseton özütünde, *A. gymnaopecias*' ta gövdede metanol kökte ise etil asetat özütünde tespit edildi. *A. diphtherites*' te gövde kısmından elde edilen metanol ve kök kısmından elde edilen hekzan özütleri, *A. gymnaopecias*' ta ise gövde ve kök kısımlarının metanol özütleri en yüksek metal şelatlama aktivitesi gösterdi. Her iki türde de gövde ve kök etil asetat özütleri en yüksek hidroksil radikali söndürme aktivitesi gösterdi. *A. gymnaopecias*'ın gövde aseton ve metanol özütlerinin *Streptococcus pyogenes*'in büyümesi üzerinde inhibisyon etkisine sahip olduğu tespit edildi.

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Anahtar Kelimeler

Astragalus diphtherites FENZL var. *diphtherites*,
Astragalus gymnaopecias RECH. FIL.,
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Biyolojik aktivite

Araştırma Makalesi

Determination of in vitro antioxidant and antimicrobial properties of shoot and root extracts of *Astragalus diphtherites* FENZL var. *diphtherites* and *Astragalus gymnaopecias* RECH. FIL. obtained by different solvents.

ABSTRACT

Objective of this study was to investigate the antioxidant and antimicrobial properties of shoot and root parts of *Astragalus diphtherites* var. *diphtherites* (*A. diphtherites*) and *Astragalus gymnaopecias* (*A. gymnaopecias*) species which were subjected to solvent series with different polarity. Total phenolic, total flavonoid, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), metal chelating, reducing power and hydroxy radicals scavenging activities of the extracts were tested to determine antioxidant properties. The highest total phenolic content of *A. diphtherites*, was obtained from the methanol extract of shoots and the acetone extract of root parts. On the other hand, the highest total phenolic content in *A. gymnaopecias* were achieved from methanol extract of shoot parts and the ethyl acetate extract of the root part. The highest amount of total flavonoids and reducing power activities of *A. diphtherites* and *A. gymnaopecias* were obtained from the acetone extracts in the shoot part and from

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the ethyl acetate extracts in the root part. While the highest DPPH radical scavenging activity was determined in the methanol extract in the shoot part and the acetone extract in the root part of *A. diptherites*. The highest DPPH radical scavenging activity for *A. gymnaepecias* was determined in the methanol extract of the shoot part and the ethyl acetate extract of the root part. The highest metal chelating activity was seen in the methanol extracts from shoot parts and in the hexane extracts from the root part of *A. diptherites*. The ethyl acetate extracts of the shoot and root part in both species showed the highest hydroxyl radical scavenging activity. It was determined that acetone and methanol extracts of the shoot part of *A. gymnaepecias* have inhibition effect on the growth of *Streptococcus pyogenes*.

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INTRODUCTION

Plants have been used extensively for therapeutic purposes since ancient times (Ortega-Ramirez et al., 2014). Herbal medicines, which have been begun to be ignored with the development of the pharmaceutical industry, have come to the fore again due to adverse side effects caused by synthetic medicines. Studies in this area have focused on revealing the therapeutic properties of plants that are found in nature (Pu et al., 2015). According to a report prepared by World Health Organization (WHO), the numbers of medicinal plants that are used for treatment are around 20.000 (WHO, 2002). Herbs produce secondary metabolites in order to defense themselves against to herbivores and microorganisms, and maintain their generations (Mulabagal and Tsay, 2004). The therapeutic properties of plants are due to the secondary metabolites in which they contain. It is estimated that there are about 100.000 kinds of secondary compounds in plants. Antimicrobial and antioxidant properties of secondary metabolites that are produced by plants have been extensively investigated in recent years (Kashani et al., 2012). One of the most important of the herbal secondary metabolite groups is undoubtedly antioxidants. Oxygen, which acts in many metabolic processes, can gain radical properties during the use phase of metabolic pathways, and in consequence of that a variety of reactive oxygen species are occurred. Reactive oxygen can cause DNA mutation and disrupting in the membrane structures of cells and tissues resulting from lipid peroxidation. Radical scavenger antioxidant substances are needed to remove the effects of reactive oxygen (Aktepe et al., 2015; Keskin, 2015, Keskin et al., 20017). Plants can defend against destructive effects of free radicals by virtue of flavonoids, anthocyanins, carotenoids, glutathione, vitamins, endogenous metabolites that are found in their structures and the many natural products with strong antioxidant activity like these. It

is also known that antioxidant effects of plants having medical value are derived from phenolic compounds and flavonoid content in particular (Roidoung et al., 2016; Eghbaliferiz and Iranshahi, 2016; Lia et al., 2017a; George et al., 2017). Flavonoids are known as biological response modifiers of nature (Nakagami et al., 1995). The reason is that they have the ability to regulate the body's response against to other compounds such as allergens, viruses, carcinogens by means of their proven anti-inflammatory, antiallergic, antiviral and anticarcinogenic properties. Nonetheless, they are potent antioxidants that provide significant protection against oxidative stress and free radical damage. They are one of the classes of the most common and most found secondary plant phenolics by these properties (Formica and Regelson, 1995; Guardia et al., 2001; Kim et al., 2004; Song et al., 2005).

There are approximately 2500 species of the type of *Astragalus* in the world (Akan and Civelek, 2001). It was reported that *Astragalus* species contain bioflavonoids, choline and astragalins B and amino acids, triterpene glycosides, flavonoids, isoflavonoids and saponins (Cui et al., 2003; Shao et al., 2004; Huang et al., 2009). Since they also contain important polysaccharides playing a role in the regulation and strengthening of the immune system they are seen as an alternative for the treatment of cancer and virus diseases. Moreover *Astragalus* is known with its feature that protects the liver against to fibrosis. (Gui et al., 2006; Wang et al., 2016; Lai et al., 2017b).

In this study, the antioxidant potential of the extracts which obtained from shoot and root parts of *A. diptherites* and *A. gymnaepecias* taxa by using solvents with different polarity, the amount of total phenolic and total flavonoid, the activity of DPPH radical scavenging, the metal chelating, the reducing power and the activities of hydroxyl radical scavenging were determined by testing in order to contribute to understanding of the importance of medicinal plants.

Antimicrobial properties of plant extracts were investigated using disk diffusion method.

MATERIAL and METHOD

Material

A. diptherites and *A. gymnaopecias* (Endemic samples collected from the vicinity of Mardin (Bakakri/37°19'15"N, 40°45'23"E) and Diyarbakır (Devegeçidi dam lake road 3. km/38°05'25"N, 40°00'48"E) respectively. Taxonomic identification of the plant samples were confirmed by Dr. A. Selçuk Ertekin from Dicle University. Plant samples were stored in the Herbarium (voucher no. DUF 9711, 9344 respectively) of same institutions.

Tested Microorganisms

Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615 *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 11774 standard bacterial strains and *Candida albicans* ATCC 10231 yeast were used for antimicrobial evaluation of collected plant extracts. Tested microorganisms were obtained from the Dicle University, Faculty of Medicine, and Department of Microbiology.

Reagents

Commercially obtained butylated hydroxy anisole (BHA), gallic acid (3,4,5-trihydroxy benzoic acid), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt), ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide (H₂O₂), butylated hydroxytoluene (BHT), sodium carbonate (NaCO₃), deoxyribose (Sigma-Aldrich), potassium phoshate monobasic (KH₂PO₄), quercetin dihydrate, aluminium nitrate [Al(NO₃)₃], potassium acetate (CH₃COOK), folin&ciocalteu's phenol reagent, potassium ferricyanide [K₃Fe(CN)₆], 2-thiobarbituric acid (TBA), ascorbic acid (Fluka), ferrous chloride 4-hydrous, trichloro acetic acid (TCA), iron (III) chloride (FeCl₃), dimethyl sulfoxide (DMSO), hexane, ethyl acetate, methanol and acetone (Merck) were used for antioxidant assays. The polarity index of hexane, ethyl acetate, acetone and methanol are 0, 4.3, 5.4 and 6.6 respectively (Snyder, 1974).

Standard antibiotic discs containing Erythromycin (E, 15 µg), Amoxicillin (20 µg)/ Clavulanic acid (10 µg) (AMC, 30 µg), Ofloxacin (OFX, 5 µg), Netilmisin (NET, 30 µg) (Oxoid) and Amphotericin B (30 µg) (Bristol-Myers Squibb) were purchased commercially.

Method

Preparation of crude plant extracts

The root and shoot parts of the plant, dried at room temperature, were made ready to analysis by grinded.

The totally powdered plant samples in 100 g, were taken in a glass flask and extracted in a magnetic stirrer with organic solvents such as hexane, ethyl acetate, acetone and methanol which differ in terms of polarity in constant stirring rate at 250 rpm. The obtained extracts were undergone to evaporation process after having been undergone to filtrating (Watman No. 1 filter) process and the crude extracts were obtained. This process was repeated three times for each solvent. Fractionated hexane, ethylacetate, acetone and methanol extracts were stored at -20 C° in deep freezer for later use.

In order to determine the total phenolic contents in hexane, ethyl acetate, acetone and methanol extracts obtained from the shoot and root parts of *A. diptherites* and *A. gymnaopecias* species and to determine their DPPH, metal chelating, reducing power and the hydroxyl radical scavenging activities their concentrations in ethyl alcohol were prepared in a way of 1 mg/ml in all extracts, and in order to determine the total amount of flavonoid compounds the stock solutions were prepared in a way of 2 mg/ml.

In vitro activity measurement methods (Antioxidant assay)

Total phenolic content

The total amount of phenolic compounds in the obtained extracts was determined according to the Folin-Ciocalteu colorimetric method (Slinkard and Singleton, 1997). Gallic acid (50–500 µg/ml concentration) was used as standard to construct a linear calibration curve ($\lambda=765$ nm). The equation was as $y = 0.0021 \times [\text{GAE}] - 0.036$, $R^2 = 0.997$.

Total flavonoid content

Total flavonoid content of plant extracts were determined based on the formation of flavonoid-aluminium complex by using quercetin (50–500 µg/ml concentration) as the standard flavonoid described by Moreno et al., 2000. The equation that was used is $y = 0.0152 \times [\text{QE}] - 0.0052$, $R^2 = 0.999$ ($\lambda=415$ nm). Using this equation, the total amount of flavonoid content in the test samples was calculated as the quercetin equivalent (QE) for shoot and root parts of *A. diptherites* and *A. gymnaopecias*.

DPPH radical scavenging activity

The free radical scavenging activities of plant extracts were quantitatively tested using a DPPH according to Shimada et al., (1992) method. UV-VIS measurements were performed at 515 nm and results were calculated from the equation given in literature (Dorman and Hiltunen, 2004). The IC₅₀ values were calculated in µg/ml by plotting the percentage inhibition versus the increased extract concentration. IC₅₀ (µg/ml) values of plant extracts were calculated using the linear

regression analysis test in the Prism Version 2.0 program.

Metal chelating activity

The metal chelating activity of root and shoot extracts of fractionated *A. diphtherites* and *A. gymnaopecias* species was measured by modified method (Dinis et al., 1994). EDTA, with strong chelating activity, was used as a positive control. In the Fenton type reactions, while Fe⁺² form the hydroxyl radical by reacting with H₂O₂ itself also oxidize to Fe⁺³. The formation of OH radical leads to lipid peroxidation, protein modification and DNA damage. In the presence of *Astragalus* extracts, the conversion to Fe⁺³/Fe⁺² was investigated by Oyaizu method ($\lambda=700$ nm) (Oyaizu, 1988).

OH radical scavenging activity

The hydroxyl scavenging activity of plant extracts were measured with deoxyribose method (Halliwell et al., 1987). This method is based on some equations. The hydroxyl radical attacks the deoxyribose and at the end of the series reaction are formed to malondialdehyde (MDA). MDA reacts with TBA, and pink-colored MDA-TBA complex is formed ($\lambda=532$ nm). DMSO was used as positive control. Inhibition values of hydroxyl radical scavenging activity of *A. diphtherites* and *A. gymnaopecias* shoot and root parts were calculated according to the below formula.

$$\% I = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Antimicrobial activity assay

Preparation of the stock plant solution

8 mg/mL stock solutions of hexane, ethyl acetate, acetone and methanol extracts obtained from the shoot and root parts of *A. diphtherites* and *A. gymnaopecias* were prepared in methanol. Prepared stock solutions were filtered by using sterile 0.45 μm -por membrane filters in order to separate from insoluble fragments.

Preparation of microbial cultures and application of disc diffusion assay

Antimicrobial activity was determined with disk diffusion test by taking N.C.C.L.S (National Committee for Clinical Laboratory Standards) rules into account (Clark and Jacobs, 1998). Disc diffusion test was performed by using Mueller Hinton Agar (MHA, oxoid), Nutrient Agar (NA, oxoid) and Sabouraud Dextrose Agar (SDA, merck) medium. The bacterial strains that were used by inoculated in 20 ml Mueller Hinton Broth medium, and the yeast strain that were used by inoculated in 20 ml Sabouraud Dextrose Broth medium, were incubated at 37 °C for overnight in a water bath at 120 rpm. According to the growth needs of the tested microorganisms MHA (*B. subtilis*, *S. pyogenes*), NA (*P. aeruginosa*, *S. aureus*, *E. coli*) and SDA (*C. albicans*) that were sterilized and

cooled to 45-50 °C, were ensured to be solidify properly by poured into sterile petri dishes of 25 ml and 9 cm in diameter. The mediums were kept in oven at 37 °C for overnight. The cultures of bacterial (10⁸ cell/ml) and yeast strains (10⁷ cell/ml) that had been left in the water bath were taken and distributed to sterile petri dishes using sterile cotton swab by taken 150 μL from them. It was ensured them to be distributed in the medium as a homogenous. The 10 μL (80 $\mu\text{g}/\text{disc}$), and 20 μL (160 $\mu\text{g}/\text{disc}$) plant extracts were impregnated to sterile blank paper discs (6 mm in diameter). Negative controls were prepared using the same solvents (methanol) utilized to dissolve the tested extracts. The discs, whose plant solutions were impregnated, were placed on the solidified agar by being pressed lightly on them. Petri dishes that were prepared in this way were incubated at 37 °C for 24 hours. At the end of this period, the inhibition zone diameters formed on the medium, were measured in mm using a ruler. In the same conditions, this test was repeated 3 times. Commercially purchased standard antibiotic discs were used as positive control.

RESULT and DISCUSSION

The total phenolic and flavonoid compound contents of the plants constitute one of the important parameters in determining antioxidant properties. In this study, the antioxidant and antimicrobial activities of extracts obtained with solvents that vary from non-polar to polar were investigated.

The highest amount of total phenolic compound in *A. diphtherites* was obtained from the methanol (76.1 μg GAE/mg extract) in shoot and the acetone (48.2 μg GAE/mg extract) extract in root. The highest amount of total flavonoids in the same plant was obtained from the acetone (42.2 μg QE/mg extract) extracts in the shoot and from the ethylacetate (4.23 μg QE/mg extract) extracts in the root. The highest amount of total phenolic compound was detected in the methanol extract (54.66 μg GAE/mg extract) obtained from the shoot part of the *A. gymnaopecias* and the ethylacetate extract (35.83 μg GAE/mg extract) obtained from the root part, and the highest amount of total flavonoid content was detected in the acetone extract (80.15 μg QE/mg extract) obtained from the shoot part of the plant and the ethylacetate extract (14.01 μg QE/mg extract) obtained from the root part (Table 1,2).

Flavonoid-rich foods consumed by humans can show substantial anti inflammatory, anti allergic and anticancer activities (de Castro and Anderson, 2015). According to these results, it is seen that the shoot parts of both plants contain higher polar phenolic and flavonoid compounds than the root parts of them. The radical scavenging capacities of different phenolic antioxidants such as flavonoid, tannin, kumarin and xanthone vary depending on the amount of compound.

Plant extracts with more polar compounds exhibit better radical scavenging properties although there are very small differences (Yeşilada et al., 2000; Kang et al., 2003; Zlotec et al., 2016). It was found in studies on different *Astragalus* species that antioxidant capacities of species with higher polar phenolic compounds are higher (Cai et al., 2004; Wong et al., 2006; Tahawa et al., 2007; Borneo et al., 2009). Free radical scavenging activities of the extracts that were obtained from both plants were determined by activity of scavenging the DPPH radical. The results were compared with the BHT and BHA values that were used as positive control in the tested concentrations. The results obtained show that the polar extracts obtained from root and shoot parts of both plants have

close or higher radical scavenging activity than BHA and BHT, which were used as positive control in certain concentrations (Table 3).

It is known that the antioxidant activities of the compounds are derived from polar compounds including aromatic hydroxyl group (Yeşilada et al., 2000). In a study in which methanol and hexane/dichloromethane extracts obtained from aboveground parts of some *Astragalus* species were examined in terms of DPPH activity, it was reported that IC₅₀ (µg/ml) values of methanol extracts that were obtained from plants were in the range of 68-400 and their non-polar extracts did not show DPPH activity (Adıgüzel et al., 2009).

Table 1. Total phenolic contents of hexane, ethyl acetate, acetone and methanol extracts of shoot and root parts of *A. diphtherites* and *A. gymnaepecias*.

Plant	Total phenolic content* (µg GAE/mg extract)							
	Shoot				Root			
	Heg	EtOAc	Ace	MeOH	Heg	EtOAc	Ace	MeOH
<i>A. diphtherites</i>	19.4±1.4*	23.5±0.9	32.4±0.8	76.1±0.9	32.5±1.4	27.2±0.9	48.2±1.5	30.7±1.5
<i>A. gymnaepecias</i>	32.33±1.8	40.83±1.3	42.33±2.8	54.66±2.3	32.33±1.5	35.83±1.8	19.66±1.8	17.66±1.3

*Data are presented as mean values; ±standard deviation (SD) of triplicate values.

Table 2. Total flavonoid contents of hexane, ethyl acetate, acetone and methanol extracts of shoot and root parts of *A. diphtherites* and *A. gymnaepecias*.

Plant	Total flavonoid content* (µg QE/mg extract)							
	Shoot				Root			
	Heg	EtOAc	Ace	MeOH	Heg	EtOAc	Ace	MeOH
<i>A. diphtherites</i>	8.58±0.3*	5.44±0.2	42.20±0.5	39.31±0.2	2.31±0.3	4.23±0.3	4.1±0.2	2.31±0.2
<i>A. gymnaepecias</i>	11.20±0.3	77.67±2.4	80.15±0.3	36.81±0.3	4.21±0.2	14.01±0.1	10.43±0.2	11.20±0.1

*Data are presented as mean values; ±standard deviation (SD) of triplicate values.

Table 3. Effect of different solvent extracts of *A. diphtherites* and *A. gymnaepecias* shoot and root extracts on the inhibition of DPPH free radical (n=3, mean±standard deviation)*.

	% IC ₅₀ µg/mL*						
	5µg/mL	25µg/mL	50µg/mL	100µg/mL	150µg/mL	250µg/mL	350µg/mL
A1S Heg	7.20±0.9	10.57±0.5	22.37±0.6	27.10±0.4	22.56±0.2	27.12±0.5	27.07±0.9
A1S EtOAc	12.98±0.2	20.87±0.7	33.38±1.1	41.39±1.0	43.23±0.7	50.15±0.9	57.34±1.1
A1S Ace	14.53±0.7	22.76±0.7	32.72±0.4	46.78±0.9	52.95±0.7	64.34±0.3	70.48±0.5
A1S MeOH	9.85±0.6	19.73±0.8	30.58±0.6	58.16±0.8	62.92±0.9	68.06±0.4	79.01±0.7
A1R Heg	7.20±0.9	17.97±0.5	16.27±0.6	27.32±0.7	24.63±0.5	29.96±0.5	35.92±0.6
A1R EtOAc	16.70±0.6	35.71±0.3	55.47±0.6	58.99±0.5	85.06±0.8	90.91±0.1	90.26±0.4
A1R Ace	20.39±0.7	40.01±0.7	44.46±0.4	71.13±0.9	87.64±0.8	90.38±0.6	91.55±0.2
A1R MeOH	10.72±0.5	13.19±0.2	28.66±0.1	36.53±0.2	42.52±0.2	50.74±0.8	58.41±0.9
A2S Heg	11.74±0.2	13.98±0.7	14.22±0.2	10.31±0.2	13.90±0.2	14.29±0.7	18.31±0.9
A2S EtOAc	14.02±0.4	15.74±0.8	18.84±0.7	21.77±0.4	24.78±0.9	29.20±0.9	31.68±0.9
A2S Ace	11.86±0.9	21.85±0.9	25.97±0.3	33.60±0.5	41.82±0.4	52.02±0.3	55.61±0.8
A2S MeOH	14.59±0.9	22.66±0.3	36.36±0.9	59.62±0.6	78.02±0.6	85.13±0.9	86.83±0.2
A2R Heg	4.20±0.2	5.71±0.2	6.59±0.4	7.65±0.2	10.06±0.2	13.34±0.5	16.62±0.2
A2R EtOAc	4.27±0.8	16.35±0.7	33.98±0.9	43.71±0.5	58.69±0.2	76.84±0.7	84.10±0.9
A2R Ace	5.58±0.2	10.11±0.7	16.23±0.1	21.03±0.9	25.57±0.9	45.91±0.6	56.07±0.6
A2R MeOH	4.44±0.4	5.73±0.9	8.22±0.8	15.24±0.3	30.10±0.5	32.65±0.9	39.62±0.9
BHA	37.17±0.4	43.19±0.2	88.98±0.4	89.96±0.5	90.49±0.6	90.92±0.5	95.46±0.6
BHT	22.99±0.2	35.59±0.3	52.88±0.4	72.63±0.2	81.67±0.5	88.00±0.3	88.68±0.1

A1: *A. diphtherites*, A2: *A. gymnaepecias*, S: Shoot, R: Root, Heg: Hegzane, EtOAc: Ethylacetate, Ace: Acetone, MeOH: Methanol.

*Data are presented as mean values±standard deviation (SD) of triplicate values.

In another study Jaradat et al (2017) investigated the DPPH radical scavenging activities of various solvent extracts

(aqueous, acetone, methanol and dichloromethane) of *A. allepicus*, *A. angustifolius*, *A. annularis* and *A. boeticus* and

they reported that highest IC₅₀ values were observed in methanol extracts. In a study that was made to be determined the antioxidant properties of some plants, IC₅₀ values were reported to be found 156.98%, 13.19%, 19.62%, 52.31% and 145.31% µg/ml for *Astragalus glycyphyllos*, *Trifolium pannonicum*, *Lathyrus binatus*, *Onobrychis scardia* and *Coronilla emerus* species, respectively (Godevac et al., 2008). In our study, the IC₅₀ values obtained for DPPH activity showed similarity with the studies above (Table 3).

The activity of the reducing power was found to be higher in the shoot and root acetone extracts of *A. diphtherites* having the highest total flavonoid and total phenolic contents. It was seen that the extracts obtained from both root and shoot parts of *A. gymnaopecias* exhibited similar reducing power activity depending on the increase in concentration. Although both the root and shoot acetone extracts of *A. diphtherites* had the

highest reducing power activity, it was found that they had moderate activity in comparison with BHT and BHA, which were used as positive controls. However, it was determined that the extracts obtained from *A. gymnaopecias* showed increase in activity depending

on the concentration ratio, but they had very low activity values when compared with positive controls (Table 4). Luo and Fan (2011) reported that ethyl acetate extract of *Astragalus* demonstrated highest reducing power and DPPH radical scavenging activity when compared to other extracts (butanol, petroleum ether and water). The highest metal chelating activity in the studied concentration ranges (25-350 µg/ml) were showed the shoot and root acetone extract of *A. diphtherites* and the ethyl acetate extract (shoot and root) of *A. gymnaopecias*.

Table 4. Reducing potential of different solvent extract of *A. diphtherites* and *A. gymnaopecias* shoot and root parts and BHA, BHT as standards (n=3, mean±standard deviation)*.

	Fe ³⁺ /Fe ²⁺ reducing power (A _{700nm})* (µg/mL extract)					
	25µg/mL	50µg/mL	100µg/mL	150µg/mL	250µg/mL	350µg/mL
A1S Heg	0.04±0.01	0.05±0.01	0.07±0.01	0.07±0.01	0.11±0.03	0.15±0.01
A1S EtOAc	0.04±0.00	0.04±0.02	0.05±0.00	0.05±0.01	0.06±0.01	0.07±0.01
A1S Ace	0.05±0.00	0.07±0.00	0.09±0.01	0.12±0.00	0.19±0.01	0.23±0.03
A1S MeOH	0.06±0.00	0.07±0.01	0.09±0.02	0.12±0.00	0.15±0.00	0.18±0.03
A1R Heg	0.05±0.00	0.07±0.00	0.09±0.00	0.09±0.02	0.11±0.0	0.18±0.01
A1R EtOAc	0.05±0.00	0.05±0.01	0.06±0.00	0.07±0.00	0.08±0.02	0.10±0.01
A1R Ace	0.7±0.01	0.08±0.00	0.13±0.02	0.14±0.01	0.23±0.00	0.27±0.00
A1R MeOH	0.05±0.00	0.06±0.00	0.07±0.01	0.08±0.00	0.09±0.03	0.11±0.02
A2S Heg	0.07±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.10±0.03	0.13±0.01
A2S EtOAc	0.09±0.00	0.09±0.01	0.10±0.00	0.11±0.00	0.13±0.00	0.17±0.00
A2S Ace	0.08±0.00	0.09±0.00	0.10±0.01	0.11±0.00	0.13±0.01	0.16±0.03
A2S MeOH	0.08±0.00	0.09±0.01	0.09±0.02	0.10±0.00	0.12±0.00	0.14±0.03
A2R Heg	0.09±0.00	0.11±0.00	0.11±0.00	0.13±0.01	0.14±0.0	0.15±0.01
A2R EtOAc	0.09±0.00	0.09±0.01	0.11±0.00	0.12±0.00	0.13±0.02	0.16±0.00
A2R Ace	0.09±0.00	0.09±0.00	0.10±0.02	0.10±0.01	0.12±0.00	0.13±0.00
A2R MeOH	0.09±0.00	0.09±0.01	0.09±0.00	0.10±0.00	0.10±0.03	0.11±0.00
BHA	0.19±0.00	0.32±0.02	0.44±0.01	0.47±0.02	0.54±0.00	0.65±0.03
BHT	0.09±0.00	0.14±0.01	0.24±0.00	0.35±0.02	0.45±0.00	0.53±0.03

It was determined that the metal chelating activity of extracts obtained from the shoot part of *A. diphtherites* increased with the increasing polarity of the solvents used in the extraction process and the plant showed an rising in proportional to the amount of phenolic compound. It was determined that the metal chelating activities of the hexane and methanol extracts obtained from the root part of the plant had higher and closer activity values than the ethylacetate and acetone extracts. The increase in the metal chelating activity in the extracts obtained from the shoot part of *A. gymnaopecias*, showed variability depending on the polarity as in the *A. diphtherites*.

When the extracts that were obtained from shoot part of the plant was examined, it was determined that activities of hexane, ethyl acetate and acetone extracts were close to each other whereas activities of methanol extracts were very high. It was determined that metal chelating activity was less in low concentrations when *A. diphtherites* was compared to standard EDTA, while at high concentrations, root hexane and methanol extracts showed moderate activity, but the acetone and

methanol extracts of the shoot showed the same activity with positive control. In the *A. gymnaopecias*, it was determined that methanol extracts obtained from the stem part, showed metal chelating activity that is very close to positive control at concentrations of 250 and 350 µg/mL. To sum up, it was found that the high metal chelating activity was generally obtained from methanol extracts of high polarity (Table 5).

The groups which make transition with polar solvents are known to be more polyphenolic. Polyphenols have the ability to chelate divalent cations by virtue of the hydroxyl groups in which they contain (Hatano et al., 1989; de las Heras et al., 1998; Lopes et al., 1999; Kang et al., 2003). The activities of different concentrations of plant extracts in scavenging of the hydroxyl radical, in the Fe²⁺/Ascorbat/EDTA/H₂O₂ system, was examined by deoxyribose method. When compared with the DMSO (used as a positive control), it was found that their capacity of the obtained extracts in scavenging of OH radicals increased depending on increasing in concentration and that the antioxidant capacities of them increased (Table 6).

Table 5. Metal chelating activity of different solvent extract of *A. diphtherites* and *A. gymnaopecias* shoot and root parts at different concentrations (n=3, mean ± standard deviation)*.

	Metal chelating activity (A_{562nm})* (Fe ²⁺ -Ferrozine- μ g/mL extract)							
	5 μ g/mL	10 μ g/mL	25 μ g/mL	50 μ g/mL	100 μ g/mL	150 μ g/mL	250 μ g/mL	350 μ g/mL
A1S Heg	0.0±0.0	0.00±0.0	7.4±0.1	15.6±0.1	19.0±0.1	24.1±0.2	31.5±0.2	37.6±0.3
A1S EtOAc	0.0±0.0	0.00±0.0	12.5±0.1	14.1±0.1	20.5±0.1	22.0±0.2	39.4±0.2	40.5±0.2
A1S Ace	0.0±0.0	0.00±0.0	13.1±0.3	16.3±0.4	32.7±0.3	51.0±0.2	65.1±0.3	68.9±0.3
A1S MeOH	0.0±0.0	0.00±0.0	4.1±0.1	14.3±0.1	28.6±0.1	33.4±0.2	62.1±0.2	74.4±0.2
A1R Heg	0.0±0.0	0.00±0.0	12.2±0.1	22.2±0.1	36.2±0.2	38.7±0.2	49.3±0.1	62.4±0.2
A1R EtOAc	0.0±0.0	0.00±0.0	11.±0.1	16.6±0.1	20.8±0.1	25.1±0.1	31.5±0.1	32.7±0.2
A1R Ace	0.0±0.0	0.00±0.0	4.1±0.1	4.5±0.1	5.2±0.1	13.3±0.1	14.2±0.1	16.0±0.2
A1R MeOH	0.0±0.0	0.00±0.0	13.1±0.1	16.0±0.1	17.6±0.1	26.2±0.1	34.6±0.2	51.1±0.2
A2S Heg	0.0±0.0	0.00±0.0	17.9±0.1	19.1±0.1	20.5±0.1	27.8±0.1	41.4±0.1	46.7±0.1
A2S EtOAc	0.0±0.0	0.00±0.0	19.1±0.4	25.3±0.1	28.0±0.1	30.6±0.2	56.5±0.2	75.0±0.2
A2S Ace	0.0±0.0	0.00±0.0	11.8±0.1	13.6±0.1	15.8±0.1	31.2±0.2	53.7±0.2	73.0±0.2
A2S MeOH	0.0±0.0	0.00±0.0	22.0±0.1	30.4±0.2	46.5±0.1	67.4±0.2	86.9±0.1	89.6±0.1
A2R Heg	0.0±0.0	0.00±0.0	13.7±0.1	14.5±0.1	15.8±0.2	24.6±0.1	36.0±0.2	46.2±0.2
A2R EtOAc	0.0±0.0	0.00±0.0	10.3±0.1	12.7±0.1	15.3±0.2	21.7±0.2	38.0±0.2	43.6±0.2
A2R Ace	0.0±0.0	0.00±0.0	8.3±0.1	8.7±0.1	12.8±0.1	17.1±0.2	25.2±0.2	37.1±0.2
A2R MeOH	0.0±0.0	0.00±0.0	11.6±0.1	14.9±0.2	27.5±0.1	38.0±0.2	58.8±0.2	76.4±0.3
EDTA	33.1±0.3	60.3±0.2	99.8±0.1	99.8±0.1	99.8±0.1	99.9±0.1	99.9±0.1	99.9±0.1

Table 6. Hydroxyl (OH) radical scavenging activities of different solvent extracts of *A. diphtherites* and *A. gymnaopecias* according to TBA method (n=3, mean ± standard deviation)*.

	Hydroxyl radical scavenging activity (%)*					
	5 μ g/mL	10 μ g/mL	20 μ g/mL	50 μ g/mL	75 μ g/mL	100 μ g/mL
A1S Heg	56.4±4.0	58.1±4.1	60.2±3.3	61.1±3.3	61.9±3.1	62.7±3.4
A1S EtOAc	73.7±2.2	75.2±2.2	76.3±2.1	77.7±1.4	79.5±2.1	82.6±1.3
A1S Ace	62.8±3.7	63.8±3.5	64.9±3.5	66.6±3.4	67.6±3.5	69.1±3.1
A1S MeOH	73.0±2.6	74.7±2.3	75.8±2.0	77.2±1.4	79.0±1.9	81.3±1.6
A1R Heg	60.5±3.1	62.1±3.4	62.1±3.4	64.7±2.9	66.5±3.1	67.5±2.8
A1R EtOAc	83.7±1.2	84.7±1.0	85.6±1.1	87.1±0.9	88.4±0.7	88.8±0.4
A1R Ace	81.5±0.6	82.6±0.5	83.9±0.8	85.1±0.3	85.8±0.3	86.4±0.6
A1R MeOH	72.4±4.7	73.9±4.4	75.2±4.6	76.6±4.4	77.7±3.9	78.5±3.6
A2S Heg	45.0±4.2	52.4±2.6	59.1±2.1	60.1±2.4	63.3±3.6	72.9±1.5
A2S EtOAc	75.0±1.8	79.4±0.9	83.9±0.8	85.9±1.0	88.4±0.8	90.9±0.9
A2S Ace	72.3±1.7	76.7±3.1	79.6±3.9	83.5±2.7	87.3±1.4	90.2±1.3
A2S MeOH	73.5±1.9	75.9±1.6	80.0±1.2	81.8±1.4	86.4±0.6	90.0±0.7
A2R Heg	74.0±0.7	79.6±0.3	82.5±1.8	84.2±0.9	85.7±0.6	87.3±0.6
A2R EtOAc	69.3±1.1	74.3±1.4	78.6±4.9	82.9±3.4	87.0±2.0	89.3±0.5
A2R Ace	71.1±0.7	77.0±4.5	80.5±3.5	84.2±1.6	85.6±0.5	89.0±1.1
A2R MeOH	70.7±0.5	77.2±0.7	80.6±1.5	81.5±1.0	82.6±0.8	84.5±0.8

The inhibition effects of extracts of *A. diphtherites* and *A. gymnaopecias* species obtained from shoot and root parts by using different solvents on the *Candida albicans* yeast and Gram (+) and Gram (-) bacteria strains, were compared with the standard antibiotics. It was found that the acetone and methanol extracts (160 μ g/paper disc) of *A. gymnaopecias* shoot formed inhibition on the growth of *S. pyogenes*, but the other extracts showed no inhibition effect on the tested microorganisms (Fig 1).

Digrak et al (1998) investigate the antimicrobial activity of *A. schizophtherus* chloroform extract (500 μ g/paper disc) and reported low antimicrobial activity (7-10 mm inhibition zone) against tested microorganisms (*E. coli*, *E. aerogenes*, *M. luteus* and *P. fluorecens*). Likewise, Adıgüzel et al., (2009), Shan et al., (2007) and Pistelli et al., (2002) investigate the antimicrobial properties of some *Astragalus* species and observed so weak or no activity on the tested microorganisms.

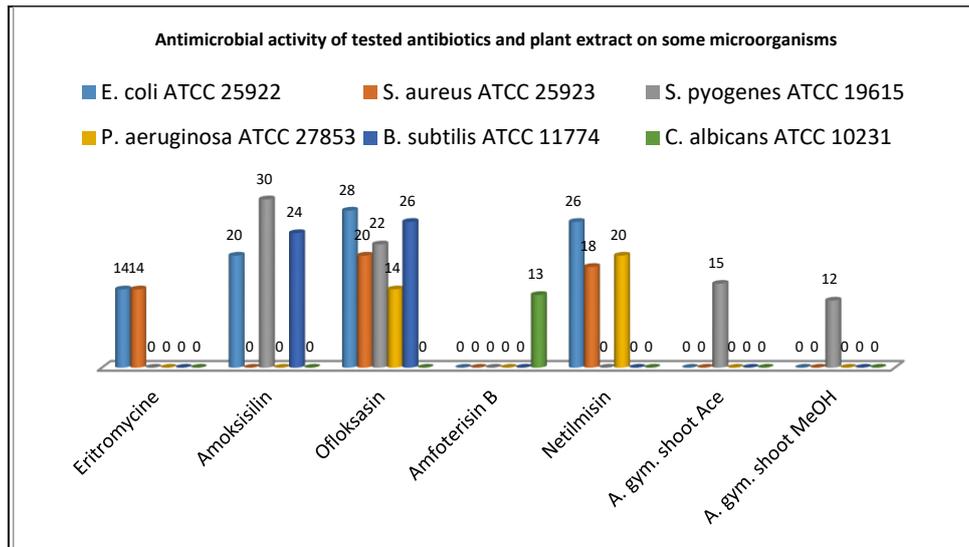


Figure 1. Antimicrobial activity against tested microorganisms; Eritromycine (15 µg/paper disc), Amoksisilin (20 µg/paper disc), Ofloksasin (5 µg/paper disc), Netilmisin (30 µg/paper disc), *A. gym. shoot Ace* (160 µg/paper disc)

CONCLUSION

It was found that the extracts obtained with the solutions having different polarities from the root and shoot parts of the taxa *A. diphtherites* and *A. gym. shoot Ace* showed different activities from each other. This situation may be associated with the transition of biologically active compounds depending on the polarity of the solvents that was used. That crude extracts obtained by using methanol, ethyl acetate and acetone with different polarity compared to positive controls show high antioxidant activity is very important for the possible use of *Astragalus* species in medical sciences especially in cancer studies, cosmetics and food industry. In the light of the results that were obtained, it is possible to obtain more meaningful results for the purpose of the research with detailed fractionation and pure compound obtaining studies.

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