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Antioxidant, Antimicrobial and Cytotoxic Activities of Endemic *Astragalus argaeus* Boiss. from Turkey

Türkiye'den Endemik *Astragalus argaeus* Boiss.'in Antioksidan, Antimikrobiyal ve Sitotoksik Aktivitesi

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ABSTRACT

In this study, phenolic compound, the total phenolic and flavonoid contents, in vitro antioxidant, antiradical, hydrogen peroxide scavenging, metal chelating, total reducing power, iron ion reducing antioxidant potential (FRAP), copper (II) ion reducing antioxidant capacity (CUPRAC) activities of the extracts obtained from underground and aerial parts of *Astragalus argaeus*, which is endemic to Turkish flora were investigated. The ferulic acid has been identified as main compound in the both extracts. Both extracts showed weak antioxidant activities. The extracts showed weak antibacterial activity against only *Pseudomonas aeruginosa* among the 15 microorganisms by agar well diffusion assay. The cytotoxic effects of extracts on MCF-7 (human breast cancer cell lines) and fibroblast cells during 24 and 48 hours were determined by MTT method. The extracts showed weak cytotoxic activity on MCF-7 after 24h.

Keywords

Astragalus argaeus, antimicrobial activity, antioxidant activity, cytotoxic.

ÖZ

Bu çalışmada, Türkiye florası için endemik olan *Astragalus argaeus*'un toprak üstü ve altı kısımlarından elde edilen ekstrelerin toplam fenolik ve flavonoid madde miktarları, in vitro antioksidan, antiradikal, hidrojen peroksit giderme, metal şelatlama, toplam indirgeyici güç, demir indirgeyici toplam antioksidan aktivite (FRAP), bakır (II) iyon indirgeyici antioksidan aktiviteleri araştırılmıştır. Her iki ekstrede de ferulik asit başlıca bileşen olarak tespit edilmiştir. Her iki ekstrede zayıf antioksidan aktivite göstermiştir. Ekstreler agar difüzyon yönteminde 15 mikroorganizmadan sadece *Pseudomonas aeruginosa*'ya karşı düşük antibakteriyel aktivite göstermişlerdir. Ekstrelerin MCF-7 (insan meme kanser hücre hattı) ve fibroblast hücrelerine karşı sitotoksik etkileri 24 ve 48 saat uygulama sonrası MTT yöntemi ile tespit edilmiştir. Ekstreler 24 saat sonrasında MCF-7 hücrelerine zayıf sitotoksik aktivite göstermişlerdir.

Anahtar Kelimeler

Astragalus argaeus, antimikrobiyal aktivite, antioksidan aktivite, sitotoksik.

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INTRODUCTION

Since immemorial time, medicinal plants have been used to treat and prevent various human ailments and they are considered a reservoir of bioactive compounds [1]. Plants produce a wide variety of secondary metabolites, among which phenolic compounds, whose interest is important for humans in pharmacological and food areas [2]. Phenolic compounds are considered to be the most abundant source of natural antioxidants and are usually found in sources such as different plant organs (leaves, roots, etc), fruits and vegetables. Their ingestion either through natural sources or through dietary supplements can be associated with a reduced risk of cardiovascular diseases, stroke and certain forms of cancer [3].

Microbial contamination and side effects of synthetic antioxidants are two important major concerns of food and pharmaceutical industries. Increasing propensity for replacing synthetic antioxidant by natural one on one side and development of microbial resistance to existing antibiotics from the other has encouraged researchers toward appraising medicinal plants for dual antioxidant and antimicrobial properties [1]. The research on phenolic extracts has been growing because of the increasing worldwide demand for phenolic compounds and their increasing application in the food industry such as anti-microbial and antioxidant agents and food stabilizers [4]. Until now, enormous efforts have been performed to discover novel, safe and non-toxic plant sources or plant-derived natural compounds as alternative or complementary medicines for the treatment of various chronic diseases [5].

Astragalus L. is the largest genus in the family Leguminosae with over 3000 species and is represented in Turkey by 445 species, of which 224 are endemic [6]. *Astragalus* is widely distributed in temperate regions of the Northern Hemisphere. The greatest numbers of species are found in the arid, continental regions of western North America and central Asia. Some *Astragalus* species are used as forage plants, to control erosion, as ornamentals or as medicinal plants [7]. The dried roots and leaves of some *Astragalus* species are very old and well known in traditional medicine for treatment of nephritis, leukemia, hypertension, diabetes, cirrhosis and uterine cancer [8-11]. Also, they are utilized against stomach ulcer, cough, chronic bronchitis, gynecological disorders and venomous bites of scorpion [12]. Several *Astragalus* species have many biological properties such as hepatoprotective, immunostimulant, antiviral [12-14], anti-aging, antitumor, antioxidant [15], antimicrobial, antimalarial, antileishmanial [16], antiperspirant, anti-inflammatory, diuretic and tonic [8,9]. In East Anatolia, while gums of *Astragalus* ssp. have been used to cure stomach aches, cancer, and sore throat, the roots have been used for treatment of jaundice and diabetes as a decoction [6]. Many phytochemical studies on *Astragalus* genus were showed the presence of saponin, phenolic, alkaloids, nitro compounds, mucilages, sterols etc. [7,12,14].

Although a large number of studies about *Astragalus* genus and its chemical constituents have been performed worldwide because of their diverse activities, there is no data about *A. argaeus.* This present study was conducted to evaluate *in vitro* antioxidant, antimicrobial and cytotoxic activities of the methanol extracts obtained from underground and aerial parts of *A. argaeus* Boiss. naturally growing in Turkey.

MATERIALS and METHODS

Plant Material

Underground and aerial parts of *Astragalus argaeus* Boiss., which are locally endemic to Turkey were collected from Erciyes Mountain, Kayseri, Turkey ($38^0$32′041″ N-$35^0$27′860″ E, 3143 m, 15.08.2012, Voucher No: Aksoy 2463). The plant was identified by Dr. Ahmet Aksoy. The voucher specimens were deposited at the Herbarium of the Department of Biology in Erciyes University, Kayseri, Turkey.

Extraction

Grinded underground and aerial parts of the plant were separately extracted using methanol by a Soxhlet-type extractor. The obtained extracts were filtered (Whatman No. 1) and evaporated with a rotary evaporator (T< 40 ˚C). The extracts yield were calculated and stored at 4°C.

LC-MS Analysis of Phenolic Compounds in the Extracts

A liquid chromatography was equipped with electrospray ion sources mass spectrometer working with Mass Hunter software package. Zorbax SB-C18 (150x2.1 mm, 1.8 µm) column was used. The flow rate was 0.25 ml/min and the injection volume 10 µl. Positive and negative ion mode was performed. The nitrogen temperature was 350°C at a flow rate of 8 L/min. The mobile phase composition was: (5/95:h/h) methanol: water (eluent A) and methanol (eluent B) both containing 0.01% formic

acid and 5 mM ammonium formate. The gradient program was as follows: 5% B (0-1 min), 30% B (1-3 min), 60% B (3-4 min), 60% B (4-5 min), 70% B (5-6 min), 80% B (6-8 min), 5% B (8.01 min), 5% B (8.01-10 min).

Gallic acid, cinnamic acid, caffeic acid, ferulic acid, chlorogenic acid, protocatechuic acid, ellagic acid, catechin hydrate, epicatechin, epigallocatechin gallate, rosmarinic acid, syringic acid, quercetin, quercetin-3-β-D-glucoside, myricetin, phloridzin hydrate, rutin, p-coumaric acid, o-coumaric acid, kaempferol, bergapten, and psoralen were used as standard.

Determination of Total Phenolic

Folin-Ciocalteau assay was performed to detect of the total phenolic amount in the extract [17]. Forty µl aliquot of the extract was mixed with 200 µl of Folin-Ciocalteu reagent and 600 µl (20% Na_2CO_3) of sodium carbonate. The mixture was vortexed and incubated at room temperature for 2h. Absorbance was then read at 765 nm by the spectrophotometer. The standard curve was formed with gallic acid. Total phenolic was expressed in terms of milligrams of gallic acid equivalents (mg GAE)/ gram extract.

Total Flavonoid

Total flavonoid was assessed using Aluminum chloride colorimetric assay according to Pourmorad et al. [18]. The extract was mixed with methanol (1.5 ml), aluminum chloride (0.1 ml), 1 M potassium acetate (0.1 ml) and distilled water (2.8 ml). The absorbance of the mixture was read at 415 nm. The result is expressed as mg of quercetin equivalents (QE)/g extract.

Antioxidant Activity

Total Antioxidant Activity

Phosphomolybdenum method was used [19]. 4 ml of reagent solution (28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate) was added to the extract solution. The tubes were stored at 95 ˚C for 90 min. The absorbance was read at 695 nm. The antioxidant activity was expressed as mg of ascorbic acid equivalents (AAE)/g extract.

DPPH Method

The scavenging ability to 2,2-diphenyl-1-picrylhydrazyl (DPPH) was evaluated according to Lee et al. [20]. 0.1 - 2 mg/ml concentrations of the extract was mixed with DPPH solution (0.1 mM). The absorbance was read at 517 nm after 30 min incubation period. IC_{50} (concentration required to scavenge 50% DPPH) value was determined. BHT (Butylated hydroxytoluene) was used as control. Percent Inhibition (%) was calculated in the following way:

$$\text{Inhibition}\,\% = (A_{control} - A_{sample} / A_{control}) \times 100$$

β-Carotene Bleaching Assay

Lipid peroxidation inhibitory effect in β-carotene bleaching system was assessed [21]. An aliquot of β-carotene in chloroform was mixed with Tween 40 and linoleic acid. The chloroform was evaporated. Then, distilled water saturated with oxygen was added. The solution was added to the extract solution. After the tubes were stored at 50˚C for 2h, the absorbance was read at 470 nm. The same procedure for BHT was carried out.

CUPRAC Assay

The cupric ion reducing antioxidant activity of the extract was detected [22]. The extract, Neocuproine (7.5 mM), $CuCl_2$ (10 mM) and NH_4Ac buffer (1 M) solutions were mixed into a test tube. Then, the total volume was completed to 4.1 ml and incubated for 30 min. The absorbance of the mixture was read at 450 nm. Trolox was used as a positive control.

FRAP

Ferric ion reducing the capacity of the extract was examined [23]. FRAP is based on the reduction of ferric 2,4,6-tris(2-pyridyl)-1,3,5-triazine [Fe(III)-TPTZ] to the ferrous complex at low pH. FRAP reagent was made by mixing of $FeCl_3$. $6H_2O$ (20 mM), acetate buffer (300 mM), and TPTZ (10 mM). An aliquot of each plant extract was added to diluted FRAP reagent and stored during 30 min at 37°C, and the absorbance was read at 595 nm. The results were expressed as mmol/l of Fe^{2+}.

H_2O_2 Scavenging Activity

The extract (25-500 µg/ml) was mixed with 43 mM H_2O_2 solution. After 10 min, the absorbance of the solution was read at 230 nm [24]. Gallic acid, BHA (Butylated hydroxyanisole) and BHT were used as control. The percentages of scavenged hydrogen peroxide of extract and standards were calculated using the following equation:

$$\text{Scavenged}\,H_2O_2\,\% = (A_{control} - A_{sample} / A_{control}) \times 100$$

IC_{50} values were determined graphically.

Reducing Power

The reducing power of the methanolic extract was detected [25]. The extracts (0.5-10 mg/ml) were added to 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] and was stored for 20 min at 50°C. Then, the solution was centrifuged at 3,000 rpm for 10 min after trichloroacetic acid (2.5 ml) was added. The upper layer solution was added to $FeCl_3$ (0.5 ml). The absorbance was read at 700 nm. BHT was used as control. The high absorbance value reflects the high reducing power.

Chelating Activity on Ferrous Ion

Ferrous ions (Fe^{2+}) chelating activity of the extract was examined [24]. Aliquots of the extract (5 mg/ml) were added to $FeCl_2$ (2 mM, 0.1 ml). After incubation, ferrozine (5 mM and 0.2 ml) was added to the reaction solution. The absorbance was read at 562 nm. The chelating ability was compared with that of ethylenediaminetetraacetic acid (EDTA). Chelating activity was calculated by the following formula:

Chelating activity % = [1-(Absorbance of sample /Absorbance of control)] x 100

Determination of Antimicrobial Activity

The following microorganisms were tested:
Aeromonas hydrophila ATCC 7965, *Bacillus cereus* RSKK 863, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Escherichia coli* O157:H7, *Klebsiella pneumoniae* ATCC 27736, *Listeria monocytogenes* 1/2B, *Mycobacterium smegmatis* RUT, *Morganella morganii, Proteus mirabilis* BC 3624, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* NRRLE 4463, *Staphylococcus aureus* ATCC 29213, *Yersinia enterocolitica* ATCC 1501 and *Candida albicans* ATCC1223.

Agar-well diffusion assay was performed to detect of antimicrobial effect [26]. Suspensions of each microorganism were regulated to 10^6-10^7 colony-forming units (cfu)/ml and added to sterile growth medium. The mix was poured into Petri plates (9 cm). The wells (5 mm in diameter) were cut from the agar. 50 µl of extract (10%, 5%, 2.5% and 1% concentrations) was added to the wells. The methanol was used as a control. The growth inhibition zones were measured in millimeters. Inhibition zones of standard antibiotics namely Tetracycline TE-30 (10 mg/ml) and Oxacillin OX-5 (10 mg/ml) were compared.

MTT Assay

MCF-7 (Human breast cancer) cell line was incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with heat-inactivated 100 ml/l fetal bovine serum (Gibco), 100 U/ml penicillin, 100 mg/ml streptomycin. Fibroblast cell line was incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM glutamine. The cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C, and the medium was changed every other day.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was used to examine effects of extracts on the cell growth and survival [15]. 1 x 10^4 cells were dispersed to a 96 well plate. After 24h incubation, the cells were treated with extracts (10, 25, 50, 100, 200 and 400 µg/ml) and incubated for 24, 48 and 72h. Then 10 ml of MTT in PBS (5mg/ml) was added into each well and the cells were further incubated for 4h. The MTT formazan crystals were solubilized by adding 200 ml of DMSO to each well. Absorbance was measured at 570 nm with Microplate Reader. The absorbance of the control cells was taken as 100% viability. The results are expressed as percentages of viable cells versus the respective controls.

RESULTS and DISCUSSION

The yields of methanol extracts obtained from underground and aerial parts of ***A. argaeus*** on a dry weight basis were 19.31 and 15.60%, respectively (Table 1). Identification of the phenolic compositions of the extracts was carried out using LC-MS/MS. The amounts of phenolic compounds in the extracts were calculated as ppm (Table 2).

The total amounts of phenolic in the extracts evaluated by the Folin-Ciocalteu assay were indicated as gallic acid equivalent (GAE) and the results are given in Table 1. The total phenolic content of the extract obtained from aerial parts (10.39 mg GAE/g extract) was higher than that of the extract obtained from underground parts (4.59 mg GAE/g extract).

The amount of total flavonoid in the extracts measured by the aluminum chloride colorimetric assay was expressed as the quercetin equivalent (mg QE/g extract) and the results are given in Table 1. The total flavonoid content of the extract obtained from aerial parts (5.88 mg QE/g extract) was found to be higher than that of the extract obtained from underground parts (1.63 mg QE/g extract).

The phenolic compositions of the extracts from underground and aerial parts of *A. argaeus* were examined by LC-MS (Table 2). The amount of each compound is

Table 1. The yields, total phenolic contents, total flavonoid contents and antioxidant activities of *A. argaeus*.

A. argaeus	Underground Part	Aerial Part
Yield (%)	19.31	15.60
Total Phenolic (mg GAE/g)	4.59±0.2	10.39±0.3
Total Flavonoid (mg QE/g)	1.63±0.1	5.88±0.1
Antioxidant activity (mg AAE/g)	108.40±0.2	121.56±1.8
β -Carotene % Inhibition	37.31±0.0	47.26±1.72
DPPH IC_{50}(µg/ml)	194.28	140.96
H_2O_2 IC_{50} (µg/ml)	187.93	212.65
FRAP mM/L	0.49	0.68

*Values expressed are mean±standard deviation of three experiments. Total phenolic content expressed as gallic acid equivalent (GAE), total flavonoid content expressed as quercetin equivalent (QE), total antioxidant activity expressed as ascorbic acid equivalent (AAE).

Table 2. The quantity of some phenolic compounds (ppm) determined in *A. argaeus* extract by LC-MS.

Compounds	Underground Part	Aerial Part
Rosmarinic acid	-	-
Rutin	-	-
Quercetin-3-β-D-glucoside	-	-
Quercetin	274.00	402.08
Kaempferol	-	202.72
Protocatechuic acid	-	-
Chlorogenic acid	-	-
Caffeic acid	38.62	224.84
Syringic acid	317.90	313.00
p-coumaric acid	-	-
Ferulic acid	359.20	481.40
Total	989.72	1624.04

-: not detected

demonstrated as ppm. Quercetin, caffeic acid, syringic acid and ferulic acid were identified in the extract obtained from underground and aerial parts of *A. argaeus.* Also, kaempferol was identified in the extract obtained from aerial parts of *A. argaeus*. Ferulic acid was determined as main compound in the both extract (359.20 and 481.40 ppm in the extracts obtained from underground and aerial parts, respectively).

The total antioxidant activity of the extracts measured by the phosphomolybdenum method is indicated by the ascorbic acid equivalent (mg AAE/g extract) and the results are shown in Table 1. The total antioxidant activities of *A. argaeus* extracts obtained from underground and aerial parts were found to be 108.40 and 121.56 mg AAE/g extract, respectively.

The percent inhibition values of the extracts caused on the oxidation of the β-carotene-linoleic acid emulsion are given in Table 1. As shown in Table 1, *A. argaeus* extracts obtained from underground and aerial parts showed 37.31 and 47.26% inhibition at 2 mg/ml, respectively. % inhibition values of the extracts were lower than the inhibition values of BHA (107.96±1.72%) and BHT (105.47±2.28%).

The antiradical activities were tested by the DPPH assay, and the results were expressed as % inhibition. The results are shown in Figure 1. The obtained values were compared with the standard antioxidant BHT. The extract obtained from aerial parts of the plant showed stronger antiradical activity than the extract obtained from underground part of the plant in all of the concentrations tested. At the concentration of 2 mg/ml, the scavenging rates of extracts obtained from underground and aerial parts were 16.32 and 38.25%, respectively. At this concentration, the percent inhibition rate of BHT (as standard antioxidant) was found to be 92.15%. The concentration causing the 50% inhibition (IC_{50}) was detected by plotting the graph including percent inhibition values versus concentrations for each extract (Table 1). The

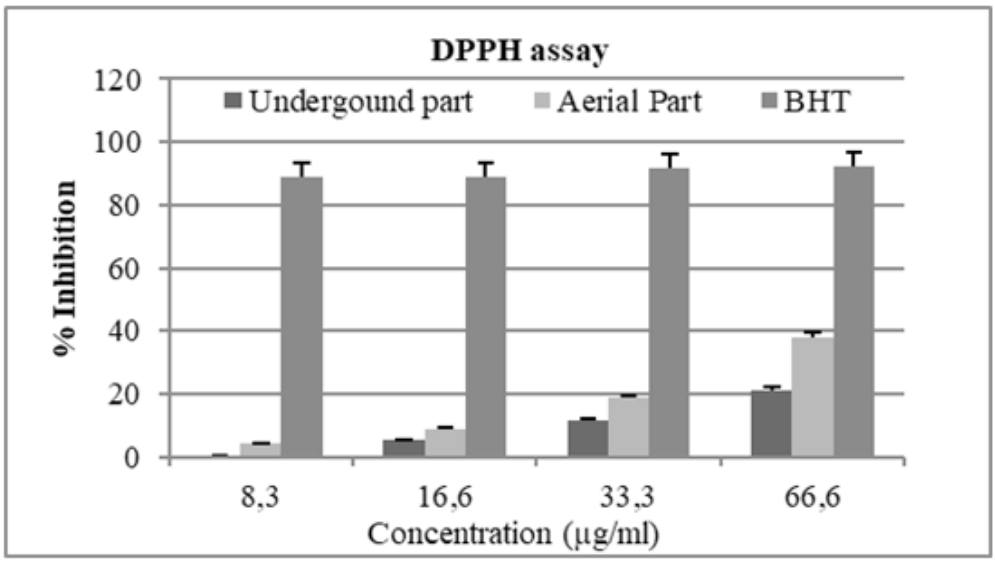

Figure 1. %Inhibition values of *A. aragaeus* extract by DPPH assay.

half inhibition concentrations (IC_{50}) of *A. argaeus* extracts obtained from underground and aerial parts were 194.28 and 140.96 µg/ml, respectively (Table 1).

The reduction activity of the copper (II) -neopurin complex of the extracts to the copper (I) -neocuproine complex was measured by the CUPRAC method [22]. The resulting complex gives maximum absorbance at 450 nm. The results are shown in Figure 2 as absorbance values at 450 nm and compared with the values of Trolox. As shown in Figure 2, the absorbance values of *A. argaeus* extracts obtained from underground and aerial parts were 0.50 and 0.68 at 1 mg/ml. Depending on the concentration, the absorbance and activity were increased. The absorbance values of the both extracts were much lower than that of the trolox (2.85) used as control.

FRAP is commonly used to measure of reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants [27]. The results are expressed as mM/l (Table 1). The obtained values were compared with the value of a standard L-ascorbic acid. The activity of the extract (0.68 mM/l) obtained from aerial parts of plant was higher than that of underground parts (0.49 mM/l). The iron ion-reducing powers of extracts were very lower than that of L-ascorbic acid (4.25 mM/l).

The hydrogen peroxide scavenging activity of the extracts was tested; the results are expressed as % inhibition and compared with the% inhibition values of BHT,

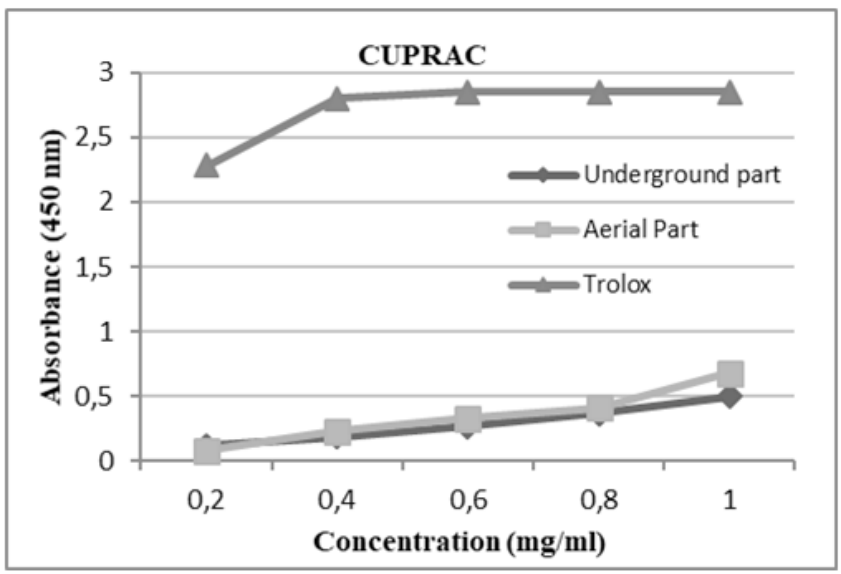

Figure 2. Antioxidant capacity of *A. aragaeus* extract by CUPRAC method.

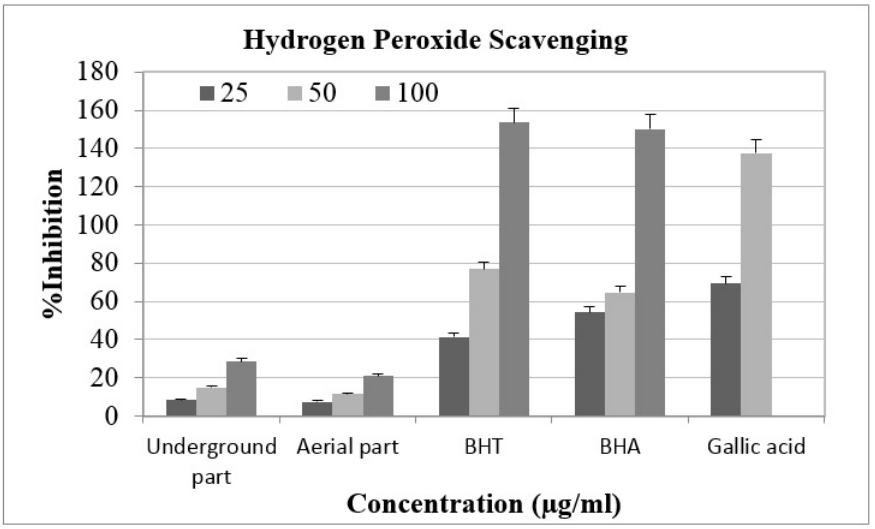

Figure 3. %Inhibition values of the *A. aragaeus* extract and standards by H_2O_2 assay.

BHA and Gallic acid. The% inhibition values of the extracts are shown in Figure 3. It was observed that hydrogen peroxide scavenging activity increased with increasing concentration. The concentrations (IC_{50}) causing 50% inhibition were detected by plotting the graph including percent inhibition values versus concentrations for each extract and values were given in Table 1. The hydrogen peroxide scavenging activity of the extract (IC_{50}= 212.65 µg/ml) obtained from aerial parts of plant was lower than that of underground parts (IC_{50}= 187.93 µg/ml). It has been observed that the scavenging activities of the both extracts are much lower than that of the standards (IC_{50}= 31.09 for BHT, IC_{50}= 23.16 for BHA, and IC_{50}= 17.62 for gallic acid).

The reducing powers of both extracts have been measured. In this bioanalytical method used in antioxidant studies, the color of the test solution transforms into green color due to the reducing activities of the antioxidant substances in the medium [28]. The results are shown in Figure 4 as absorbance values at 700 nm. The obtained values were compared with the value of BHT. The absorbance of the extract of obtained from underground parts of *A. argaeus* (1.70) was higher than that of aerial parts (1.55) at 10 mg/ml. Increased absorbance value indicates increased antioxidant activity. The reducing powers of both extracts were lower than that of BHT (2.25) at same concentration.

The metal chelating activity of the both extracts was

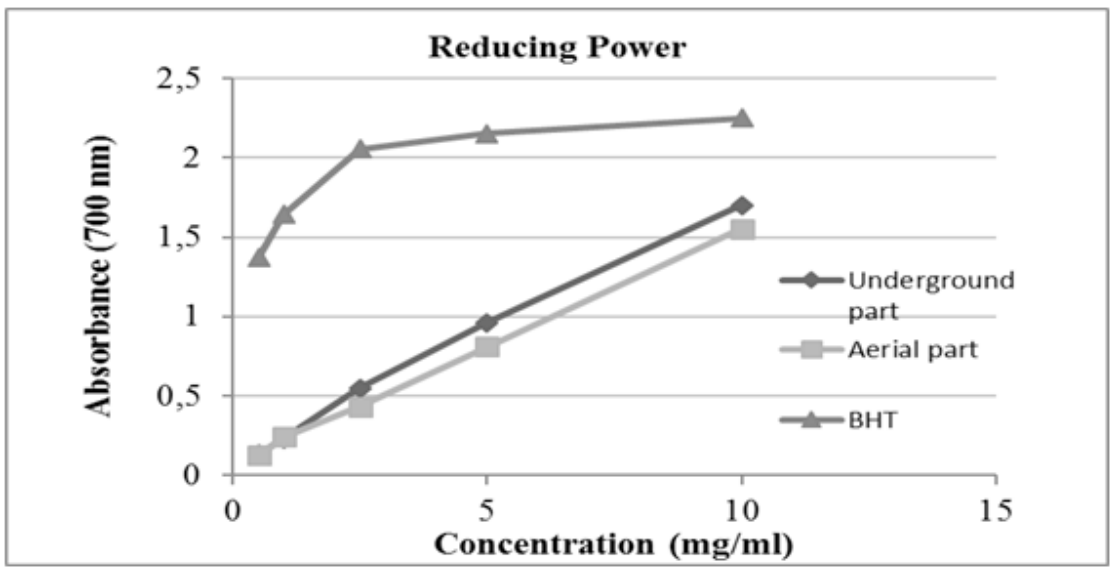

Figure 4. Reducing power of methanolic extracts from *A. aragaeus.*

measured as of 5 mg/ml and the results are indicated as% inhibition. The metal chelating activities of the extracts obtained underground and aerial parts of *A. argaeus* and EDTA were found to be 62.79%, 55.19% and 99.45%, respectively.

The antimicrobial activities of the extracts were studied by agar diffusion assay against 15 microorganisms at 1, 2.5, 5 and 10% concentrations. The both extracts showed low antibacterial activity against only *P. aeruginosa* among the tested bacteria. The inhibition zones of *P. aeruginosa* for the extract obtained from underground parts were found as 8.0, 8.0 and 7.0 mm at 10, 5.0 and 2.5% concentration, respectively. No inhibition was observed at 1% concentration. The extract obtained from aerial parts was exerted 7.0 and 7.0 mm inhibition zones at 10 and 5.0 % against *P. aeruginosa,* respectively. The inhibition zones of standart antibiotics Tetracycline and Oxacillin were 18.0 and 12.0 mm at 10 mg/ml against *P. aeruginosa,* respectively. The both extracts had no anticandidal activity against *C. albicans.*

Cytotoxic effects of the both extracts on MCF-7 (Human Mammary Metastatic Carcinoma Cells) and Fibroblast cells were assessed by MTT assay. The extracts were applied to MCF-7 and Fibroblast cells for 24 and 48h at 10, 25, 50, 100, 200 and 400 µg /ml. % Viability values were calculated. The results are graphically showed in Figure 5. When MCF-7 cells were treated for 24h at 25 µg/ml, percentages of cell viability values were 77.06 and 67.19% for the extracts obtained from underground and aerial parts of *A. argaeus*, respectively. Whereas, there was no inhibition on the growth of MCF-7 cells when incubated with the extract obtained from underground parts for 48h at all tested concentrations. The percentage of cell viability value was 87.34% when incubated with the extract obtained from aerial parts for 48h at 25 µg/ml.

When fibroblast cell lines, used as positive control, were incubated with the extract obtained from underground parts for 24h at 25, 50 and 100 µg/ml concentrations, the percentage of cell viability values were found to be 86.36, 85.12 and 86.59%, respectively. At same conditions, these values for the extract obtained from aerial parts were found to be 79.54, 76.99 and 84.27%, respectively. For 48h incubation, the extract obtained from underground parts exhibited minor inhibition on the growth of fibroblast cell lines only at 200 and 400 µg/ml. There was no inhibition on the growth of fibroblast cell lines, used as positive control, when incubated with all concentration of the extract obtained from aerial parts for 48h. To best of our knowledge, this study demonstrated for the first time the cytotoxic potential on MCF-7 cell lines of *A. argaeus* extract.

To the best of our knowledge, this study is the first

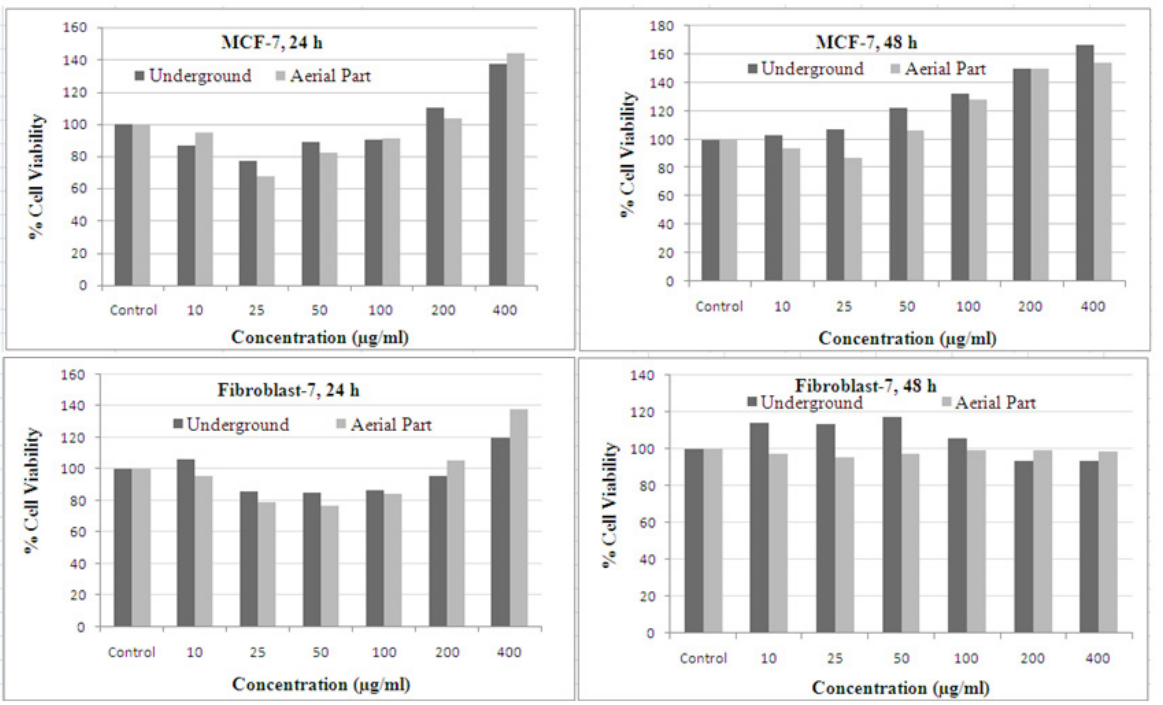

Figure 5. Effect of *A. aragaeus* extracts on proliferation of MCF-7 and Fibroblast cells for 24 and 48h.

report on the phenolic composition and biological activitiy of extracts of *A. argaeus* with respect to antioxidant, antimicrobial and cytotoxicity. The total phenolic and flavonoid content were found as 279.9 and 175.2 mg/g in the acetone extract of *A. sinicus* seed by Lim et al. [13]. In this research, the acetone extract exerted the high DPPH radical scavenging activity of 95.1% at 10.0 mg/ml and hydroxyl radical scavenging activity of 89.4% at 15 mg/ml [13]. Asgarpanah et al. [7] reported that the methanol extract obtained from flowering aerial parts of *A. squarrosus* has low phenolic (23.3 mg/g) and flavonoid content (26.0 mg/g) and also showed weak free radical scavenging activity by the DPPH method. Previous phytochemical investigations performed on some *Astragalus* species have resulted in the isolation of a series of cycloartane-type saponin [9, 29- 40]. The antimicrobial and antifungal properties of several crude extracts and pure saponins from the aerial parts of *A. verrucosus* were investigated by Pistelli et al. [41]. Abbas and Zayed [42] showed antibacterial and antifungal activities of seven saponins from the aerial parts of *A. suberi.* Methanolic and ethanolic extracts which were prepared using dried root of *A. membranaceus* were found to have alkaloids, saponins, terpenoids, flavonoids and cardiac glycosides. In the same study, both extract showed good inhibitory activity against *E. coli, Salmonella enteritidis, Shigella,* and *Campylobacter* [43]. In another study which conducted to evaluate the antimicrobial and antioxidant activities of the hexane and methanol extracts of the aboveground parts and roots of some *Astragalus* species grown in the eastern Anatolia, Turkey, the methanol extract, which was obtained from the aboveground parts of *Astragalus* species, exhibited mild free radical scavenging activity (IC_{50}= 68.8 - 400.4 µg/ml) [44]. The antimicrobial activities of methanol and acetone extracts of *A. flavescens* were determined against *E. coli, Enterecoccus fecalis, S. typhimurium, Bacillus subtilis, P. vulgaris, Micrococcus luteus, L. monocytogenes, P. aeruginosa, B. cereus* and *Yersinia* by Akyil et al. [45]. They reported that *A. flavescens* extracts were not vigorously efficient as antimicrobial agents with highest 13 mm inhibition zone. The antibacterial activities of aqueous, methanol and ethanol extracts of *A. brachypterus* were evaluated against *Streptococcus pyogenes, S. aureus, Staphylococcus epidermidis, E. coli, P. aeruginosa, S. typhimurium, Serratia marcescens, P. vulgaris, Enterobacter cloacea,* and *K. pneumoniae* by Turker and Yıldırım [46]. In this previous study, extracts of *A. brachypterus* were found to be only active against *S. pyogenes* with 15-17 mm inhibition zones.

The antibacterial and cytotoxic activities of extracts from the leaves of *A. gombiformis* were investigated by Teyeb et al. [47] and the strongest cytotoxic activity against the human A549 lung epithelial carcinoma cell line was detected for dichloromethane extracts at IC_{50} = 85±21.7 µg/ml for 48h and the methanol extract was found to be the most active against *P. aeruginosa* and *S. typhimurium* (14 mm and 13 mm inhibition zones at 50 mg/ml, respectively) [47].

In conclusion, from these results it is clear that the both extracts obtained from underground and aerial parts of *A. argaeus* showed weak antioxidant and cytotoxic activities. The both extracts demonstrated no antibacterial activity except *P. aureginosa.* The *in vitro* biological activities of *A. argaeus* have not been previously reported. This appears to be the first report concerning the total phenolic and flavonoid contents, and antioxidant, antimicrobial against a wide range of microorganisms, and cytotoxic activities of *A. argaeus* the methanol extracts.

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