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In vitro Antimicrobial Activity, Antioxidant Activity and Synergistic/Antagonistic Effect of Arum maculatum L. in Mersin Province, Turkey

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ABSTRACT

Arum maculatum is used for the treatment of colitis and internal bleeding haemorrhoids among the local people in Turkey. This study was designed to evaluate the antioxidant and antimicrobial activity and protective effects of the leaf and root methanol extract of Arum maculatum L. The phenolic compound content of the root and leaf extract was determined as 207.135±0.07 µg GAE g-1, and 386.054±0.7 µg GAE g extract-1, respectively. The total flavonoid amount was determined as 53.386±0.220 µg QE g-1 and 347.704±0.352 µg QE g-1 extract, respectively. DPPH free radical scavenging of leaf and root extracts was determined as 76.69% and 67.33%, respectively.. The metal chelating effect was determined as 46.585±0.025 mg EDTA/g extract and 35.610±0.087 mg EDTA g extract-1 for roots and leaves, respectively. All extracts were effective against all tested bacteria, and Staphylococcus mutans ATTC 10449 was the most sensitive bacterium with the lowest MIC value (0.20 mg mL-1, 0.81 mg mL-1) for leaf and root extracts, respectively. The biggest diameter of growth inhibition zone (8±2.44 mm, 9±3.26) compared to Chloramphenicol. Arum leaf extracts showed a synergistic effect with OFX against Escherichia coli and Staphylococcus mutans, and Arum root extracts have a synergistic effect with OFX against Staphylococcus aureus and Staphylococcus mutans. These results displayed that A. maculatum has notable natural bioactive compounds with antioxidant and antibacterial properties.

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Keywords
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Arum maculatum L.'nin in vitro Antimikrobiyal Aktivitesi, Antioksidan Aktivitesi ve Sinerjistik/Antagonistik Etkisi

ÖZET

Arum maculatum kolit ve iç kanamalı hemoroid tedavisinde kullanılmaktadır. Bu çalışmada, *Arum maculatum* L.'nin yaprak ve kök metanol ekstraktının in vitro antimikrobiyal aktivitesi, antioksidan aktivitesi ve sinerjistik/antagonistik etkisinin belirlenmesi amaçlanmatadır. Kök ve yaprak ekstraktının fenolik bileşik içeriği sırasıyla 207.135±0.07 µg GAE g^{-1}, 386.054±0.7 µg GAE g^{-1} ekstrakt olarak belirlenmiştir. Toplam flavonoid miktarı sırasıyla 53.386±0.220 µg QE g^{-1} ve 347.704±0.352 µg QE g^{-1} ekstrakt olarak belirlenmiştir. Yaprak ve kök ekstraktlarının DPPH serbest radikal süpürmesi sırasıyla %76.69 ve %67.33 olarak belirlendi. Metal şelatlama etkisi yapraklar ve kök için sırasıyla 35.610±0.087 mg EDTA g^{-1} ve ekstrakt 46.585±0.025 mg EDTA/g ekstrakt olarak tespit edilmiştir. Tüm ekstraktlar, test edilen tüm bakterilere karşı etkili olmuş ve *Staphylococcus mutans* ATTC 10449, yaprak ve kök ekstraktları için sırasıyla en düşük MİK değeri (0,20 mg mL^{-1} 0,81 mg mL^{-1}) ile en duyarlı bakteri olmuştur. Kloramfenikol ile karşılaştırıldığında en büyük zon inhibisyon bölgesi çapı (8±2,44 mm, 9±3,26) bulunmuştur. Arum yaprak ekstraktı *Escherichia coli* ve *Staphylococcus mutans*'a karşı OFX ile sinerjistik bir etki göstermiştir ve Arum kök ekstraktı *Staphylococcus aureus* ve *Staphylococcus mutans*'a karşı OFX ile sinerjistik bir etkiye sahiptir. Bu

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sonuçlar, *A. maculatum*'un antioksidan ve antibakteriyel özelliklere sahip kayda değer doğal biyoaktif bileşiklere sahip olduğunu göstermiştir.

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INTRODUCTION

Despite their toxic properties, Arum plants have been used for nutritional and medicinal purposes for hundreds of years (Everest & Ozturk 2005; Azab 2017). *Arum maculatum L.*, a locust plant belonging to the Araceae family, is popularly called "yeast root" (Uzun et al., 2004). *A. maculatum,* a natural wild plant, produces bright red fruits in late summer (Christie 2003). Its leaves are cooked and consumed as a soup (Erbil et al., 2018). *A. maculatum*, a perennial herb whose roots form tubercles, has bright green leaves that look like arrowheads at the end of a long stem. Uzun et al. (2004) determined the wild-growing plant species used as a folk medicine in Sakarya province after a 2-month field study. It has been reported that the roots of *A. maculatum* have an antifungal effect and the fresh roots are crushed and mixed with flour and used by preparing tablets. In ethnopharmacological records from western Turkey, *A. maculatum* is a cure for haemorrhoid and, at the same time, applied for colitis (Tuttolomondo, 2014; Everest & Ozturk, 2005).

It is a rich source of important chemical compounds in its fresh leaves and tubers. When the phytochemical properties of arum plants are examined, it has been determined that these plants contain alkaloids, polyphenols, glycosides (flavonoids, saponin and cyanogenic groups), proanthocyanidins, 2-heptanone, p-cresol, (E)-caryophyllene, monoterpenes, two sesquiterpenes and lectins (Safari et al., 2014).

The antimicrobial, proinflammatory, and antioxidant activity of Arum species was investigated (Uzun et al., 2004; Alencar et al., 2005; Dayısoylu, 2010). Additionally, its insecticidal activity was reported by Majumder and Mondal (2005). Substances such as glycosides and saponins in the plant content have made the plant toxic (Kızılarslan, 2008; Kocabaş & Gedik, 2016; Gül & Topcu, 2017). In addition to this feature, saponins provide the plant with a strong antifungal activity (Çolak et al., 2009). For this reason, saponins, one of the phytochemicals considered an alternative to antibiotics, is a herbal ingredient that is concentrated. Although this plant varies from region to region, it is called names such as (Şimşek Yurt et al., 2019), Tirşik, Andırın doktoru, Pancar (Demirci ve Özatay, 2012), Yılan Dili ve Yılan Bıçağı (Turkish Herbology Association 2020). Turkey is located in the geography where this plant grows naturally and is among the plants exported from country. However, it is forbidden to collect the onions or tubers of Arum species from nature and export them.

In this study, phytochemical properties, antimicrobial activity, and protective effect of root and leaf methanol extracts of *A. maculatum* obtained from Mersin province were investigated.

MATERIAL and METHOD

Chemicals

All analytical reagents used in the study were of analytical grade and were purchased from Sigma. Nutrient agar (NB) (Merck 105443) was used for bacterial culture and Mueller–Hinton broth and agar (MHA) (Merck, 105437) was used for microbiological assays.

Collection and processing of plant samples

Healthy disease-free leaves and roots of *A. maculatum L.* were collected from 293 m in the orange garden of Buluklu village of Mersin in April 2021. The description of the plant was made by Mustafa Kargıoğlu from the Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, and herbarium number was determined as AKU9962.

Extract preparation

Twenty-five grams of powdered leaf was continuously extracted with 300 mL methanol for six hours using Soxhlet extraction. Methanolic root and leaf extracts of *A.maculatum L.* were concentrated under reduced pressure with a rotary evaporator. Thereafter, the collected crude methanolic extract was carefully stored in an airtight container until further use (Korcan et al., 2013).

Determination of Total Phenolic Content and Total Flavonoids by the Colorimetric Method

The total phenolic content was determined by the Folin–Ciocalteau method (Dipankar & Murugan, 2012; Öztürk et al., 2018). Gallic acid was used as a standard for the determination of total phenolic content (mg mL^{-1}) using a calibration curve (A = 0,0037c + 0,1666, r^2 = 0.99, n = 3) and content was expressed as Gallic acid equivalents. The total flavonoid content of Arum

extracts was estimated using the colorimetric method as described by Baba and Malik (2015). As a standard, Quercetin (QE) was used for the determination of total flavonoids contents using a calibration curve (A = 0,0088x c + 0,0552, r^2 = 0.99, n = 3) and content was expressed as Gallic acid (GA) equivalents (mg GA g dryweight $^{-1}$).

Fig.1 *Arum maculatum L.*
Resim 1 *Arum maculatum L.*

In vitro antioxidant assays

DPPH radical scavenging assay

When the DPPH solution is mixed with a substance that can donate hydrogen atoms (antioxidant), the reduced form is formed with the loss of the dark violet color (Lee et al., 1998). Briefly, 100 µL (1.56-100µg mL^{-1}) of each extract was mixed with 1 mL of DPPH solution, vigorously shaken, and incubated for 20 min at room temperature in the dark.

The CUPRAC method

This method is based on the reduction of Cu(II) to Cu(I) by the antioxidants (reductants) present in the sample (Apak et al., 2004). The chromogenic reagent bathocuproin (2,9-dimethyl-4,7-diphenyl-1,10phenanthroline) forms a complex with Cu (I) in a ratio of 2:1. This complex has a maximum absorbance at 490 nm. The cupric reducing antioxidant capacity (CUPRAC) was calculated based on the Trolox calibration curve and expressed as mM trolox/mg extract.

Metal chelating activity

The chelating activities of Arum extracts and standard (EDTA) were estimated using the method of Dinis et al.(1994). The method is based on the competition of ferrozine, which has high iron chelating power, and metalbin ding compounds in the extracts. If the iron binding power of the compounds in the extracts is high, the formation of the red colored. Ferrozine+Fe^{+2} complex will be prevented. 370 µl of distilled water and 10 µl of 2 mM $FeCl_2$ solution were added to the test tubes containing the different concentrations (62,5-100 µg mL^{-1}) of 100 µl of extract and left for incubation at room temperature for 5 min. The reaction was started by adding the ferrozine (5 mM; 20 µl). After shaking forcefully, the mixture was incubated at room temperature for 10 min. The absorbance was recorded at 562 nm with UV–vis spectrophotometer. EDTA (1–100 µg mL^{-1}) was used as a standard for the preparation of a calibration curve. The metal chelating ability of antioxidant was expressed as mg EDTA g^{-1}.

Determination of Antimicrobial Activities of Root and Leaf Extract of Arum maculatum

Test Microorganisms

Escherichia coli ATCC 35213, *Staphylococcus aureus* ATCC 12600, *Pseudomonas aeroginosa* ATCC 11778, and *Staphylococcus mutans* ATTC 10449 were obtained from the culture collections of the Uşak University, Department of Medical Laboratory Techniques.

Determination of Antibacterial Activity

The paper disc diffusion method was used to detect the antibacterial activity (Avaz et al. 2013). 200 mL mg^{-1} stock solutions were prepared by dissolving the extracts in 5 mL methanol. Sterilized filter paper discs (5 mm) were soaked with 50 µl of a stock solution. The soaked discs were put inoculated with McFarland 0.5 tested bacterium on MHA. The plates were incubated at 37°C for 24 h. The diameter (mm) of inhibition zones was measured. Chloramphenicol (C30) was used as a positive control, and methanol was used as a negative control. All tests were done in triplicate.

Determination of minimum inhibitory concentration (MIC) by the microdilution method

The microdilution method was used to investigate the extracts obtained from plants and their antimicrobial effects. Sterile microdilution plates (Nunc® 96 DeepWell™) with 96 U-bottom wells were used for antimicrobial tests. The turbidity of the bacterial suspension was adjusted to 0.5 McFarlanda and then diluted to 10^6 cfu mL^{-1}. Different concentration (from 200 to 0.20 mg mL^{-1}) of the extracts was prepared. MHB, which is used as a medium, was prepared and sterilized at 121°C for 15 minutes. Then, 95 µl of Mueller-Hınton Broth (Merck 110293) (MHB), 5 µl of bacteria, and 100 µl of antibacterial extract were added with an automatic pipette. The 96-well plates were covered with a sterile label and incubated for 24 hours in an incubator at 37 °C. As a result of incubation, 100 microliter samples were taken from each well and planted on Mueller-Hınton Agar (Merck 1.05437) (MHA). Chloramphenicol (C30) was used as a positive control, and methanol was used as a negative control. The inoculated petri dishes were incubated for 24 hours at 37°C, and the Minimum Inhibition Concentrations (MIC) were determined according to the lowest final concentration with bacterial growth as a result of the incubation (Jennifer 2001).

Time-killing assay

For each extract, 900 µl of active ingredient was taken from 5 mL of stock solution and the first 10^{-1} dilution was prepared with 100 µl of nutrient broth medium. Serial dilution was prepared by adding 100 µl of nutrient broth to all eppendorf, starting from the first dilution and transferring up to 10^{-6}, and 100 µl of microorganism was added to each eppendorf. This process was prepared in two series for Arum root and Arum leaf: the first was prepared for *Pseudomonas aeruginosa,* and the second was prepared for *Escherichia coli.* From the prepared stocks, three replications were made in the form of spot sowing on nutrient agar at 0 min. It is incubated for 24 hours at 37 °C. Additionally, a negative control containing microorganisms is cultivated in 1 mL of nutrient broth medium without an active substance, and as a positive control, 1 mL of nutrient broth medium + microorganism + OFX (oflaxin) antibiotic disc stock is cultivated. The same procedures are repeated from each stock solution at the 4th, 8th, and 24th hours. The same procedures are repeated for the positive and negative controls.

Antibiotic Combination Tests

As in the Kirby-Bauer method, the bacterial suspension at 0.5 McFarland turbidity was spread on the petri dish, the combination effect of the extract was investigated, and Ofloxacin(OFX5), Vancomycin(VA30), and Clindamycin (DA2) antibiotic discs were placed side by side. In the evaluation after incubation, expansion or bridge formation on the side of the two-zone diameters facing each other is defined as synergy (Köksal, 2010).

RESULT AND DISCUSSION

Determination of Total Phenolic and Flavonoid Content

The total amount of phenolic compounds of *A. maculatum L.* extracts was determined as gallic acid equivalent using Folin-Ciocalteu Reagent (FCR) (Table 1). Solutions of gallic acid were used as standard and plant extracts were prepared in methanol. For the gallic acid calibration curve (Fig 2), six different concentrations of methanol solutions of gallic acid were prepared.

Phenolic compounds and flavonoids are important types of phytochemicals with antioxidant activity. Phenolic contents are one of the important plant components with free radical scavenging effect (Hatano et al., 1980).

Accordingly, the phenolic compound content of the root extract was determined as 207.135±0.07 µg GAE/g extract and the leaf extract as 386.054±0.7 µg GAE g $extract^{-1}$. In the results in Table 1, it was observed that *A. maculatum L.* leaf extract contained a higher amount of phenolic substance compared to the root extract. Erbil et al., (2018) found the amount of total phenolic compounds in the berries as 960.6 mg GAE 100 mg^{-1} whereas the total phenolic compounds were detected as 134.9±2.3 mg GAE 100 mg^{-1} in the extracts obtained from leaves by these researchers. Jaradat and Abualhasan (2016) found the total phenolic content in the methanolic extract of *A.elongatum* to be 27.49 mgGAE/g.

In addition to phenolic compounds, plants' most common antioxidant components are flavonoids (Nagy & Attaway, 1992). Flavonoids having rich hydroxyl groups are emphasized with antioxidant activity, metal ion chelation activity, radical scavenging capacity, and enzyme activators (Packer et al., 1999).

Table 1 Total phenolics, tannin, and flavonoid contents of *A. maculatum* extract
Çizelge 1 *A. maculatum ekstraktının toplam fenolikler, tanen ve flavonoid içerikleri*

Samples	Total phenolics (µg GAE g $extract^{-1}$)	Flavonoids (µg QE g $extract^{-1}$)
Root (M)	207.135±0.07	53.386±0.220
Leaf (M)	386.054±0.7	347.704±0.352

Values are means of three independent analyses ± standard deviation (n = 3)

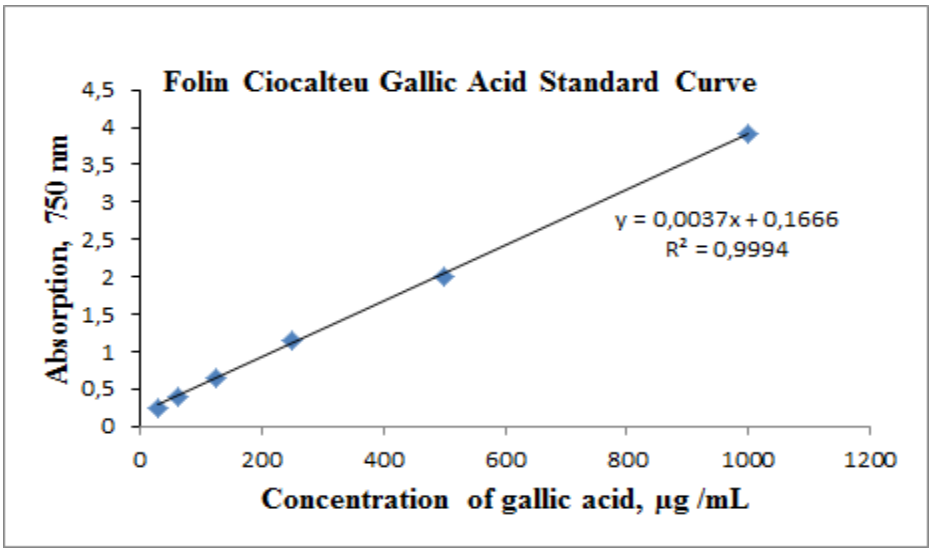

Fig. 2 Gallic acid calibration curve graph.
Şekil 2 *Gallik asit kalibrasyon eğrisi grafiği*

The total flavonoid content of the extracts was determined by determining the equivalent amount of quercetin, a flavonoid substance (Table 1). The concentration of total flavonoid content was determined using an equation derived from the quercetin calibration curve (Fig. 3) (r^2=0.9983, n=4).

In this study, as a result of the calculations made by obtaining the quercetin calibration curve, the total flavonoid amount was determined as 53.386±0.220 µg QE /g extract of the root extract and 347.704±0.352 µg QE g $extract^{-1}$ of the leaf extract. Farahmandfar et al., (2019) reported the phenolic content of *A. maculatum* as 51.09 mg GA g^{-1} and the flavonoid value as 4.42 mg CE g^{-1} in the extracts prepared in ethanol: water (50:50) by the ultrasonic extraction method.

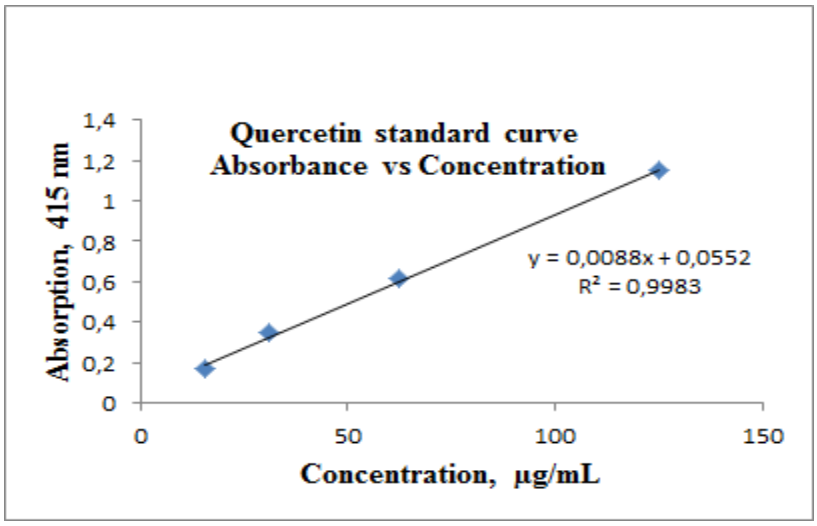

Fig 3. Quercetin calibration curve graph
Şekil 3. *Quercetin kalibrasyon eğrisi grafiği*

DPPH radical scavenging assay

Polar (methanol) extracts were obtained from *A. maculatum L.,* and antioxidant and antimicrobial activities of these extracts were determined. Polar extracts obtained from the roots and leaves of *A. maculatum L.* were subjected to various test systems for the determination of antioxidant activity. The DPPH free radical scavenging capacity of the extract is shown in Figure 4. It was observed that the absorbance values increased depending on the dose in the measurements performed based on 0.032, 0.0625 and 0.125, 0.25, 0.5, and 0.1 $mg.mL^{-1}$ concentration levels. While DPPH free radical scavenging was determined at the rate of 76.69% in methanolic arum leaf extract at 0.1 mg mL^{-1} concentration, it was determined as 67.33 % in methanolic arum root extract. These values are also relatively close to the synthetic antioxidant BHA (83.86% ± 0.01). Ibraheem and Mohammed, (2015) reported that 0.500 mg mL^{-1}

concentration of *A. maculatum* displayed a DPPH radical cleaning activity as 93.33±0.58% inhibition. Mahomoodally et al. (2023) found the highest antioxidant activity in the MeOH/water extract of *Arum elongatum* plant, equivalent to 38.90 mg Trolox per gram, against DPPH* radical and Ethylacetate extract showed the lowest antioxidant activity against DPPH (1.17mgTE g^{-1}). In a study of Alan (2018) A. *elongatum Stevens* extracts, ethanol and purified water extracts were found to have low DPPH activity. Antioxidant activity has been attributed to the presence of defined phytochemicals such as chlorogenic acids (Manuja et al., 2013), which donate hydrogen atoms to reduce free radicals and cause oxidation of phenoxy radicals (Liang and Kitts, 2015). As a result, in this study, it was observed that the leaf and root extracts showed DPPH radical scavenging activity against different concentrations, and this was more in the leaf extract.

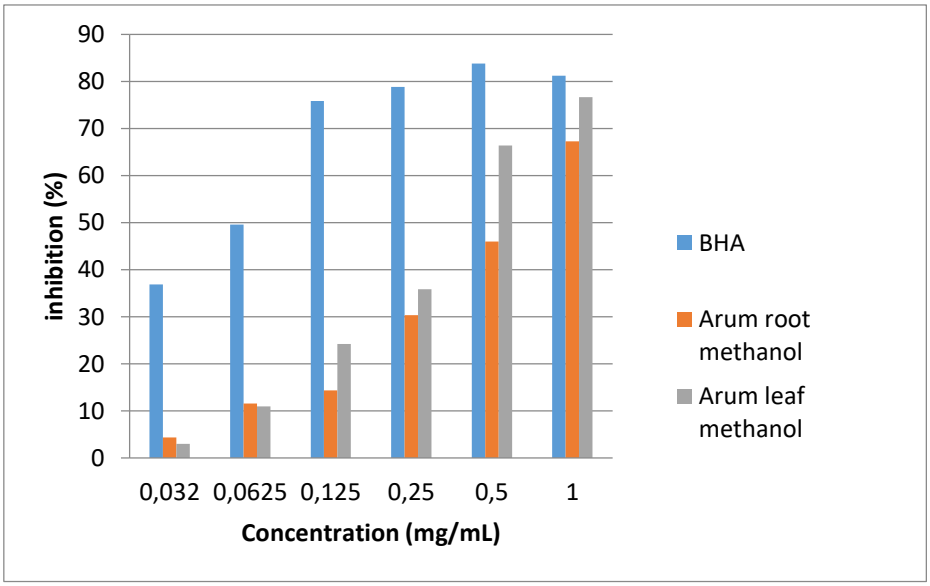

Fig. 4 Antioxidant activity (DPPH free radical scavenging assay) of ARM, ALM, and BHA.
Şekil 4 *ARM, ALM ve BHA'nın antioksidan aktivitesi (DPPH serbest radikal süprücü etkisi).*

CUPRAC assay

The extracts prepared from the leaves and roots of *A.maculatum L.* plant and the standard antioxidant compounds convert the Cu (II) neocuproin complex to Cu (I) neocuproin at 450 nm by the antioxidant compounds in the medium, and the Antioxidant Capacity (TEAC) of this complex equivalent to µg Trolox and ascorbic acid is shown (Table 2). Trolox and ascorbic acid were used as standard antioxidants. The graphs obtained for the CUPRAC assay are shown in Figure 5.

According to the CUPRAC antioxidant test results, it was determined that the leaf extract was equivalent to 36.018±0.03 Eq µg mL^{-1}Trolox and 157.086±0.044 Eq μg^{-1} Ascorbic acid mL^{-1} at 1mg mL^{-1} concentration while the root extract was 10.222±0.025 Eq µg mL^{-1} Troloks' at 1mg mL^{-1} concentrations and 41.962±0.037 Eq µg Ascorbic acid mL^{-1} was found to be equivalent. Additionally, a linear decrease is observed in equivalent amounts of Trolox and Ascorbic acid depending on the decrease in concentration. According to Mahomoodally et al. (2023) found CUPRAC values in *Arum elongatum* extracts were the highest (102.22 mgTE g^{-1}) in methanol/water extract and the lowest (50.44 mgTE g^{-1}) in ethyl acetate extract. Alan (2018) reported that 100 µL concentration of ethanol and purified water extracts and standards for A. elongatum species changed as BHT > BHA > A. *elongatum* (ethanol) > *A. elongatum* (pure water) in terms of reducing the power of Cu^{2+} ions according to the CUPRAC method. Uguzlar et al. (2011) determined the CUPRAC values equivalent to Trolox for antioxidant studies of *Arum dioscoridis* as 1.55±0.23 mg mL^{-1} in methanolic extract and 1.36±0.18 mg mL^{-1} in acetone extract. According to the results given in Table 2, since the *A. maculatum L.* leaf extract contains a higher amount of phenolic substances than the root extract, it was observed that the amount of TEAC was higher in the leaf than in the root by reducing more cupric ions to the cuprose.

Metal chelating activity

As can be seen from Table 3, the root extract has a metal chelating effect of 46.585±0.025 mg EDTA g^{-1} extract and the leaf extract 35.610±0.087 mg EDTA g $extract^{-1}$.

It has been reported that *Arum italicum* collected around Ordu/Fatsa, it has a metal chelating capacity of 82% at a concentration of 0.1 g L^{-1} (Yılmaz, 2008). Uguzlar et al. (2011) reported that chelating ability of

the standard (92.1±1.1%), rutin, was approximately similar to that of the methanolic extract from *Arum dioscoridis* (85.4±2.1%) whereas chelating ability of the methanolic extract was more effective than that of the acetone extract (42.3±1.2%) at an extract concentration of 0.5 mg mL^{-1}

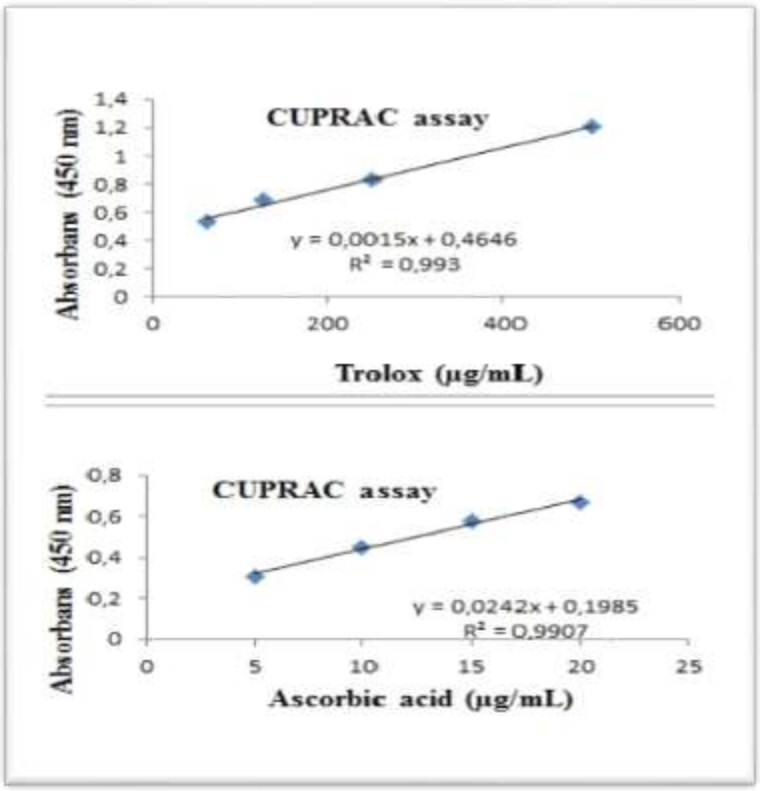

Fig.5 Standard curve of Trolox and Ascorbic acid
Şekil 5 *Trolox ve Askorbik asidin standart eğrisi*

Table 2. Metal chelating activities of methanol extract of *A. maculatum L.*
Çizelge 2. *Arum maculatum L. metanol ekstraktının metal şelatlama aktiviteleri.*

Conc. (µg mL^{-1})	Metal chelating activity (mg EDTA g $extract^{-1}$) A = -11,335c + 0,3012 (r = 0,9933)	
	Extract	
	Root	Leaf
1000	35.610±0.087	46.585±0.025
500	20.488±0.005	23.659±0.045
250	15.366±0.089	17.073±0.067
125	7.073±0.075	9.756±0.01
62.5	2.195±0.036	4.390±0.004

Values are means of three independent analyses ± standard deviation (n = 3).

It was observed that the reducing capacity of *A. maculatum L.* extracts increased with increasing concentration as well as antioxidant activity. The reducing power can be an important factor in the antioxidant activity of a compound (Meir et al., 1995). However, the antioxidant properties of any pure substance can go on different mechanisms. In summary, antioxidant compounds can exhibit their antioxidative properties by different mechanisms such as binding transition metals, breaking down peroxides, and removing radicals (Diplock, 1997).

Antimicrobial Activities of Root and Leaf Extract of A. maculatum L.

The antimicrobial activity of leaf and root methanol extracts obtained from *A. maculatum L.* was determined by the disk diffusion method and MIC tests, and the results are given in Table 4.

In a study investigating the antimicrobial activity of *A. maculatum* extract on Gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), Although the plant extract was found to be effective against the tested bacteria, it was determined that the most sensitive bacteria was *L. monocytogenes* (Farahmandfar et al., 2019). Çolak et al., (2009) reported that extracts of *A. maculatum* had good activity against *Bacillus cereus, Micrococcus luteus, Pseudomonas phaseolicola, Yersinia enterocolitica, Enterobacter aerogenes,* and *Aspergillus niger*.

Table 3. CUPRAC assay of metanol extract of *A. maculatum L.*
Çizelge 3. *A. maculatum* L'nin metanol ekstraktının CUPRAC testi.

Conc. (µg mL $^{-1}$)	CUPRAC assay Trolox A = 0.108c + 0.11 (r = 0.9977) (Eq µg mL $^{-1}$ Troloks)		CUPRAC assay Ascorbic acid A = 0,0242c + 0,1985 (r = 0.9907) (Eq µg Ascorbic acid ml^{-1})	
	Extract		Extract	
	Leaf	Root	Leaf	Root
1000	36.018±0.03	10.222±0.025	157.086±0.044	41.962±0.037
500	22.611±0.034	5.833±0.014	97.252±0.012	22.376±0.025
250	11.851±0.028	3.944±0.065	49.235±0.009	13.946±0.093
125	7.287±0.045	2.379±0.087	28.863±0.023	6.962±0.058
62.5	4±0.001	1.537±0.047	14.194±0.019	3.202±0.004

Values are means of three independent analyses ± standard deviation (n = 3).

Table 4. Antimicrobial activities of *A. maculatum L.* against test microorganisms
Çizelge 4. *A. maculatum L.'nin test mikroorganizmalarına karşı antimikrobiyal aktiviteleri*

Microorganisms	Inhibition zone (mm)(SD) (200mg mL^{-1})			MIC (mg mL $^{-1}$)	
	Leaf	Root	C30	Leaf	Root
Escherichia coli ATCC 35213	14±1.63	9±0.81	34±0.83	**0,41**	3.25
Staphylococcus aureus ATCC 12600	13±1.63	14±1.64	21±0.84	1,625	3.25
Pseudomonas aeroginosa ATCC 11778	8±2.44	8±2.44	32±1.64	13	1.625
Staphylococcus mutans ATTC 10449	8±2.44	9±3.26	8±1.67	**0.20**	**0.81**

Chloramphenicol: C30 All values are presented as "means ± SD"(n=3)
MIC : Minimum Inhibitor Concentration (mg/mL).

The antimicrobial effect of *A. maculatum* was investigated by Kianinia and Farjam (2016) with extracts prepared with petroleum ether, methanol and ethyl acetate. Minimum Inhibiting Concentration (MIC) of 17 microorganisms including 10 bacteria, 6 molds, and 1 yeast were investigated. The strong antimicrobial effects of the plant were found on *Staphylococcus aureus* (MIC= 32 mg mL^{-1}), *Staphylococcus epidermidis* (MIC=4 mg mL $^{-1}$), and *Escherichia coli* (MIC=4 mg mL^{-1}) (Kianinia & Farjam, 2016).

A. maculatum exerted inhibition feature on these microorganisms. Erbil et al. (2018) determined that *Arum maculatum* leaf extract showed more effective antibacterial properties than fruit extract. TA 98 and TA 100 strains of *Salmonella typhimurium* were used to test the antimutagenic activities of the extracts. In total, 10 and 80 µl plaque^{-1} doses of the leaves were effective against mutagenesis of *Salmonella typhimurium* TA 98 strain. Moreover, *A. maculatum L.* essential oil demonstrated higher MIC against *S. aureus* (32 mg mL^{-1}) and *E. coli* (4 mg mL^{-1}) than the outcomes revealed in this study (Kianinia & Farjam, 2018). Uzun et al. (2004) reported that petroleum ether extract of *A. maculatum* displayed antibacterial effect against *Staphylo-coccus* epidermidis with MIC value of 39.1 µg mL^{-1} .

In this study, inhibition against Escherichia *coli* ATCC 35213 microorganism in leaf extract was measured as 14±1.63 mm, and it was found to be more effective than root extract. It was determined that root extract (9±3.26 mm) showed greater diameters of zone inhibition against *Staphylococcus aureus* ATCC 12600 microorganism and even showed a higher antimicrobial effect than Chloramphenicol (8±1.67 mm), which was used as a positive control. Chloramphenicol standard antibiotic disc was used as a positive control in antimicrobial activity tests (30 µg/disc). The result of microdilution assay showed that the leaf and root extracts had the lowest MIC values and at the same time showed high inhibition against *Staphylococcus mutans* ATTC 10449 (Çizelge 4). Among the Arum species, *A. maculatum* is the most promising one for further bioactive research (ComLekcioglu et al., 2021) .Mansur et al., (2015) showed that methanol extracts were the best producing-antimicrobial activity. Therefore, in this study, methanol extracts were used to determine the antimicrobial activity. Nitiema et al. (2012) reported that phenolic compounds may have antimicrobial activity. The antimicrobial effect of phenolic ingredients may be due to their ability to alter the membrane permeability of microbial cells (Shahidi & Naczk, 1995).

Time Killing

Gram-negative bacteria, which have additional protection from the cell wall, are more resistant to antimicrobial agents than gram-positive bacteria. So far, the necessity of this rare combination of properties

has prevented the accumulation of many antimicrobials in Gram-negative pathogens (Kristen et al., 2021). For this reason, in our study, a time-killing assay was performed to observe the time-dependent antimicrobial effect of flow pumps in gram-negative bacteria.

Dilutions of 10^{-1} to 10^{-4} of *A.maculatum* root extract *Pseudomona aeroginosa* showed a cidal effect in the first 4 hours while dilutions of 10^{-5} and 10^{-6} did not show a cidal effect after 24 hours. For *Escherichia coli*, only 10^{-1} and 10^{-2} dilutions showed a cidal effect after 4 hours, 10^{-3} dilutions at 8 hours, and 10^{-4} dilutions after 24 hours (Table 5). In the samples taken from *A.maculatum* leaf extracts after 24 hours, a weak growth was detected in all dilutions of both bacteria. It was observed that Arum maculatum root extract was more effective than leaf extract in 24 hours, reducing growth but not killing bacteria.

Table 5. Time-kill effect of the bactericidal effect of *A. maculatum* L. Root and Leaf extract
Çizelge 5. *A. maculatum L. Kök ve Yaprak ekstraktının bakterisidal etkisinin zaman öldürücü etkisi*

Arum Root extract												
	Pseudomonaaeroginosa						*Escherichia coli*					
Time	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
0. min.	++	++	+++	+++	+++	+++	++	++	+++	+++	+++	+++
4.hour	-	-	-	-	++	+++	-	-	++	++	++	++
8.hour	-	-	-	-	++	++	-	-	-	++	++	++
24.hour	-	-	-	-	+	+	-	-	-	-	+	+
Arum Leaf extract												
	Pseudomona aeroginosa						*Escherichia coli*					
Time	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
0. min.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
4.hour	+++	+++	+++	+++	+++	+++	++	++	+++	+++	+++	+++
8.hour	+	+	+	++	++	++	+	++	++	++	+++	+++
24.hour	+	+	+	+	+	+	+	+	+	++	++	++

(+++=heavy growing,++=moderate growing,+=low growing,- =no growing)

Table 6. Antibiotic Combinations and Synergistic Interactions (Kirby Bauer disc diffusion method)
Çizelge 6. *Antibiyotik Kombinasyonları ve Sinerjik Etkileşimler (Kirby Bauer disk difüzyon yöntemi)*

Microorganisms	Arum extract	Antibiotic Combinations and Synergistic Interactions		
		Vancomycın (VA30)	Clındamycın (DA2)	Ofloxacın (OFX5)
Pseudomonas aeruginosa	L	Ad	Ad	Ad
	R	Ad	Ad	Ad
Escherichia coli	L	Ad	Ad	Sy
	R	Ad	Ad	Ad
Staphylococcus aureus	L	An	Sy	An
	R	An	Sy	Sy
Staphylococcus mutans	L	An	Ad	Sy
	R	Ad	Ad	Sy

Ad: Additive, Antagonism: An, Synergism: Sy, L: Leaf, R: Root

Three different interactions occur between antibiotics in combination antibiotic therapy. These are synergy, additive effect, and antagonism (Bal, 1999, Koksal, 2010). If the effect of the tested antibiotics together is significantly higher than the effect of using each antibiotic alone, it is called synergistic effect. There is a positive interaction here. If the effect of the tested antibiotics together is significantly lower than the effect of using each drug alone, it is called antagonist effect. There is a negative interaction here. If the effect of antibiotics used in combination is the sum of their separate effects, this is called additive effect also known as partial synergy. With the emergence of multidrug-resistant organisms, there are few or no treatment options for infections with certain microorganisms, as bacteria are resistant to 21 different antibiotics, and each isolate is resistant, on average, to 7-8 antibiotics (D'Costa et al., 2006). Several studies have shown various interactions between plant extracts and antibiotics (Braga et al., 2005; Kumar et al.,2009; Aiyegoro et al., 2011). In this study, the combination of Arum root and leaf extracts with different antibiotics was investigated against potential pathogens in vitro to identify synergistic or antagonistic antibacterial combinations that could provide empirical use and treatment of poly-microbial infections where combination therapy is required. From our previous studies, Vancomycin(VA30)

Clindamycin (DA2) Ofloxacin (OFX5) antibiotics were found to be effective, and these antibiotics were preferred (Table 6). Arum leaf extracts showed a synergistic effect with OFX against *E. coli* and *S. mutans*. It has been determined that Arum root extracts have a synergistic effect with OFX against *S. aureus* and *S. mutans.* Arum leaf and root extracts have a synergistic effect with DA against *S. aureus* (Fig.6).

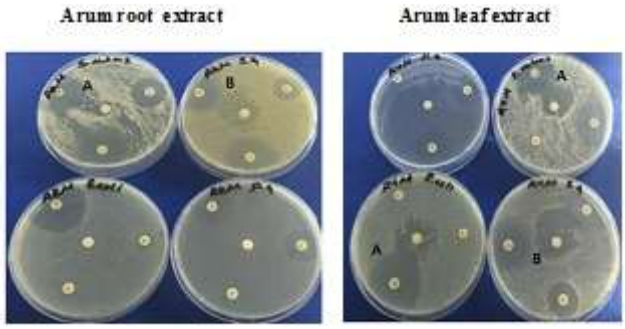

Fig 6. Antibiotic Combinations and Synergistic Interactions, A=Sinerjizim, B= Antogonizm

Şekil 6. *Antibiyotik Kombinasyonları ve Sinerjik Etkileşimler, A=Sinerjizim, B= Antogonizm*

CONCLUSION

The total phenolic substance and flavonoid content of A. maculatum L. leaf extracts was higher than the root extract. Additionally, it was observed that the DPPH radical scavenging and cupric ion-reducing properties of the leaf extract were stronger. This is because phenolic compounds and flavonoids can easily donate hydrogen in their hydroxyl groups. The bond between oxygen and hydrogen in phenolic compounds can be easily broken down homologously due to the stability of the phenol radical to be formed, and it can give hydrogen containing one electron. Thus, while scavenging the radical itself, it transforms into a more stable radical. As a result of our study, the presence of both glucosides and saponins in the leaf extracts of A. maculatum is the main reason for the antimicrobial activity demonstrated by the leaf extracts. Since the development of new classes of antibacterial agents is of great importance, methanolic extracts of A. maculatum have been found to show potential synergy when combined with some antibiotics against clinically important resistant bacteria. This study showed that A. maculatum methanol extract is both a strong source of antioxidants and a high level of antimicrobial activity. It has been shown that this plant can be a source of new chemotherapeutic agents in the production of synthetically developed therapeutic agents that can be combined with antibiotics. Further studies are underway, including the isolation of bioactive phytochemical compounds, investigation of their mechanism of action, and in vivo cancer studies to confirm the plant's potential.

Conflict of Interest

The authors declare no conflict of interest.

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