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Investigation of Antimicrobial and Antioxidant Activities of *Paliurus spina-christi* Mill. in Kahramanmaras, Turkey

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ABSTRACT

In this study, the antioxidant and antimicrobial activities of the water and ethanol extracts obtained from the fruit and leaves of *Paliurus spina-christi* Mill, from Kahramanmaras city were investigated. The antioxidant activities of the extracts were determined using the cupric ion (Cu^{+2}) reducing antioxidant capacity (CUPRAC), 1,1-diphenyl-2-picryl-hydrazyl-hydrate (DPPH), and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods. The results were evaluated by comparing them with those of butylated hydroxytoluene (BHT) and Trolox, as standard substances. On the other hand, the antimicrobial activities of the plant extracts were identified using the agar well diffusion method. According to the CUPRAC method, the antioxidant activities of the plant extracts at a concentration of 800 µg/mL were found to be lower than those of the standard antioxidant substances. At the same concentration, the DPPH radical scavenging activity of the ethanolic extract of the leaves of the plant was found to be higher than that of Trolox. In the ABTS method, the radical scavenging activity of the fruit of the plant was found to be high and close to those of the standard antioxidants. According to the data obtained from antimicrobial studies, ethanol extracts obtained from fruit and leaf parts on all microorganisms except *Pasteurella multicoda* and *Bacillus cereus* bacteria have the highest zone diameter. The ethanol extract of fruit of the plant was found to be more effective in *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*.

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Kahramanmaraş İlindeki *Paliurus spina-christi* Mill. Bitkisinin Antimikrobiyal ve Antioksidan Aktivitelerinin Araştırılması

ÖZET

Çalışmada, Kahramanmaraş ilinde bulunan *Paliurus spina–christi* Mill. (Karaçalı) bitkisinin meyve ve yaprak kısımlarından elde edilen su ve etanol ekstrelerinin antioksidan ve antimikrobiyal aktiviteleri incelenmiştir. Ekstrelerin antioksidan aktiviteleri, kuprik iyonu (Cu^{+2}) indirgeme (CUPRAC), 1,1-Difenil 2-pikril hidrazil (DPPH) ve 2,2-azinobis (3-etilbenzo-tiyazolin-6-sülfonik asit) (ABTS) yöntemleri ile belirlenmiştir. Sonuçlar, BHT (Bütil hidroksitoluen) ve Troloks standart maddeleriyle karşılaştırılarak değerlendirilmiştir. Bitki ekstrelerinin antimikrobiyal aktiviteleri ise agar kuyu difüzyon yöntemi kullanılarak belirlenmiştir. CUPRAC yöntemine göre, bitki ekstrelerinin 800 µg/ml konsantrasyondaki antioksidan aktivitelerinin, standart antioksidan maddelere göre daha düşük olduğu tespit edilmiştir. Aynı konsantrasyonda, bitkinin yaprak kısmının etanol ekstresinin DPPH radikali giderme aktivitesinin Trolokstan daha yüksek olduğu tespit edilmiştir. ABTS yönteminde ise, bitkinin meyve kısmının radikal giderme aktivitesinin yüksek ve standart antioksidanlara yakın olduğu tespit edilmiştir. Antimikrobiyal çalışmalardan elde edilen verilere göre *Pasteurella multicoda* ve *Bacillus cereus* bakterileri dışında tüm

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mikroorganizmalar üzerinde meyve ve yaprak kısmından elde edilen etanol ekstrelerinin en yüksek zon çapına sahip olduğu, bitkinin meyve kısmının etanol ekstresi ise *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* ve *Candida albicans*'da daha etkili olduğu tespit edilmiştir.

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INTRODUCTION

Today, the use of some herbs for therapeutic purposes, intended for maintaining a healthy life, and the subsequent consumption of natural antioxidants is increasing with each passing day, as are the number of studies on this subject. Many antioxidant compounds found naturally in vegetal sources have been identified as free radicals or active oxygen scavengers. Reactive oxygen derivatives, known as free radicals, induce the oxidation reactions in foods, causing spoilage and the loss of color and aroma, as well as many diseases in humans (Valko et al., 2006). Antioxidant substances are used to lessen the damage of free radicals and some kinds of reactive oxygen, as well as extend the shelf life of foods. These compounds are abundant in vegetables, shelled and unshelled fruits, seeds, leaves, flowers, roots, and shells (Pratt and Hudson, 1990; Damien Dorman et al., 1995; Tomaino et al., 2005). Natural antioxidants delay the progression of many chronic diseases by protecting the human body from free radicals, and also delay oxidative spoilage in foods (Gülçin et al., 2003). Synthetic antioxidants are too often used in the food industry to extend the shelf life of foods. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) are the most commonly used synthetic antioxidants in this field (Fkı et al., 2005). However, restrictions have been imposed on the use of synthetic antioxidants due to their side effects; hence, today, the natural antioxidants found in foods have gained more importance.

Due to the complex structures of the plant extracts, the evaluations were conducted using multiple methods instead of a single method in the identification of the antioxidant characteristics (Chu et al., 2000). The methods used studies, such as CUPRAC, DPPH, and $ABTS^{\cdot+}$, have been commonly used in the evaluation of plant extracts. Essential oils and plant extracts obtained from various vegetal sources are known to have antimicrobial activities on some microorganisms. Antimicrobial agents that also have aromatic properties are used for protecting raw and processed foods from spoilage, in the treatment of infectious diseases in medicine, in the field of alternative medicine, and in natural treatment processes (Hammer et al., 1999, Prince and Prabakaran, 2011).

Paliurus spina-christi Mill.(*Rhamnaceae)*, colloquially known by different local names, such as blackthorn, Sincan thorn, bush thorn, and Christ's thorn, is spread across the Balkans, the Caucasus, Southern Europe, and particularly, in Turkey (Deligöz et al., 2007). *P. spina-christi*, which is used with various medical methods by local people, is also important in terms of the substances it contains. The plant is known to have significant biological activities, since it contains alkaloids, flavonoids, glycosides, polyphenols, tannins, methyl esters of natural fatty acids, sterols, and free fatty acids (Medić-Šarić, M et al., 1996). Studies have also reported that the plant is used as an antidiarrheal, diuretic and antirheumatic medication, and that it has different extracts that comprise antimicrobial, hypolipidemic, antioxidant, antidiabetic, and antigenotoxic activities (Brantner and Males, 1990, 1999; Schirarend and Olabi, 1994; Brantner et al., 1996; El Rabey et al., 2014; Zor et al., 2017; Takım, 2021).

This study was intended to determine the antioxidant and antimicrobial activities of *P. spina-christi*, located in the city of Kahramanmaras, which is used for medical purposes by the local people. For this reason, the antioxidant and antimicrobial properties of the water and ethanol extracts obtained from the fruit and leaves of *P. spina-christi* were investigated.

MATERIALS and METHODS

Chemicals

2,9-dimethyl-1,10-phenanthroline [Neocuproine], 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) [ABTS] , butylated hydroxytoluene [BHT], 1,1-diphenyl-2-picryl-hydrazyl [DPPH] and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [Trolox] were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were analytical grade and obtained from either Sigma-Aldrich or Merck.

Plant materials

The *P. spina-christi* sample used within the scope of the study was collected from the Süleymanlı town of Onikisubat in Kahramanmaras Province, between May and June in 2018. The plant sample was identified and confirmed by Asst. Prof. Dr. Seyran PALABAŞ UZUN. A voucher specimen was deposited in the Kahramanmaras Sütçü Imam University Forest Faculty Herbarium (KASOF 1324). The fruits and leaves of the plant were left to dry completely in the shade at room temperature. The completely dried

fruits and leaves of the plant were ground into a fine powder using a blender (Waring Commercial). The ground samples were kept at 4 °C until performing the experiments.

Preparation of ethanol and water extracts

For the water extracts, 20 g of dried fruit and leaf samples were weighed separately (at a ratio of 1:20), and 400 mL of pure water was added to each, and then they were mixed on a magnetic stirrer for 30 min (Gülçin, 2005). The extracts were filtered through Whatman No. 1 filter paper. The collected extracts were frozen at –20 °C, and once frozen, the extracts were lyophilized for a period of 18 h.

For the ethanol extracts, 20 g of the samples were weighed separately, and 400 mL of pure ethanol was added to each, and then they were mixed on a magnetic stirrer at 3000 rpm for a period of 6 h. The ethanol extracts were filtered through Whatman No. 1 filter paper, and the ethanol was removed from the collected extracts using an evaporator at 40 °C. Samples at concentrations of 12.5, 25, 50, 100, 200, 400, and 800 µg/mL were prepared using the extracts.

Cupric ions reducing antioxidant capacity assay

The cupric ion reduction activities of the water and ethanol extracts obtained from the fruit and leaves of *P. spina-christi* were identified CUPRAC method of Apak et al. (2004). First, 1×10^{-2} M of $CuCl_2$ solution was added into the test tubes, and then 7.5×10^{-3} M of neocuproin solution and 1 M of ammonium acetate buffer were added. After mixing these solutions, the water and ethanol extracts obtained from the fruit and leaves of the plant, prepared at different concentrations (12.5–800 µg/mL), were added to the mixture, and absorbance was recorded at 450 nm. BHT and Trolox were used as the standard.

1,1-Diphenyl-2-picryl-hydrazyl-hydrate free radical scavenging assay

The free radical scavenging activities of the water and ethanol extracts obtained from the fruit and leaves of *P. spina-christi* were identified using DPPH, according to the method of Blois (1958).

First, 1 mL of 10^{-3} M DPPH radical solution was added to the 12.5–800 µg/mL concentrations of the water and ethanol extracts of the fruit and leaves of the plant. After keeping the mixtures at room temperature for 30 min, the absorbance at 517 nm was recorded against the blank, using the control solution (consisting of ethanol and DPPH). BHT and Trolox were used as the standard. A decrease in the absorbance value indicated the presence of an antioxidant substance in the environment. All of the antioxidant activity experiments were done with three repetitions. The values were calculated using the formula below.

$$\% \text{ Inhibition} = \frac{A\ control - A\ sample}{A\ control} x100$$

$A_{control}$ is the absorbance value of the control sample containing the DPPH radical solution alone, A_{sample} is the measured absorbance value of the sample prepared by adding a solution containing the DDPH radicals to the extracts.

2,2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity assay

The ABTS radical scavenging activities of the water and ethanol extracts obtained from the fruit and leaves of *P. spina-christi* were identified according to the method described by Re et al. (1999). For the implementation of the method, the 2 mM ABTS solution was mixed with potassium persulfate solution at a ratio of 1/0.5, and then the mixture was kept in a magnetic stirrer in the dark for 1 night to obtain the $ABTS^{\cdot+}$ radicals. Before using the resultant radical solution, its absorbance at 734 nm was adjusted to 0.700 ± 0.025 nm with a phosphate buffer of 0.1 M and pH = 7. The required amount was taken from the water and ethanol extracts obtained from the fruit and leaves of the plant, prepared at different concentrations (12.5–800 µg/mL), and then 1 mL of $ABTS^{\cdot+}$ solution was added, and the resultant mixture was allowed to stand for 30 min. The absorbance at 734 nm was recorded against a blank consisting of the buffer solution. A decrease in the absorbance value indicated the amount of $ABTS^{\cdot+}$ radicals eliminated from the environment. The implementations were performed in triplicate, and the % inhibitions of the extracts and standards (BHT and Trolox) were calculated using the following equation.

$$\% \text{ Inhibition} = \frac{A\ control - A\ sample}{A\ control} x100$$

$A_{control}$ is the absorbance value of the control sample containing the ABTS radical solution alone, and A_{sample} is the measured absorbance value of the sample prepared by adding a solution containing the ABTS radicals to the extracts.

Microorganisms

The standard strains, which comprised *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 11774), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 4352), *Pasteurella multocida* (ATCC 12945), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 6358), *Yersinia enterocolitica* (ATCC 27729), and *Candida albicans* (ATCC 10231), were used to determine the antimicrobial activity of the *P. spina-christi* fruit and leaf extracts.

The strains used in the study were obtained from the Microbiology Laboratory of Atatürk Vocational School of Health Services, Kafkas University, Kars, Turkey. Mueller-Hinton broth and agar (Merck, Darmstadt,

Germany) were used to revitalize the bacteria, and Sabouraud dextrose broth and agar media (Merck) were used to resuscitate the yeast.

Determination of the antimicrobial activity and the agar well diffusion technique

First, 50 g of the fruit and leaves of *P. spina-christi* were weighed separately, and then ground. The extracts obtained from a Soxhlet device were evaporated at 50 °C. The fruit and leaf extracts taken from the evaporator were kept at 4 °C until analysis. The agar well diffusion method was used to identify the antimicrobial activity.

The bacterial strains taken from the stock cultures were separately incubated in 5 mL of liquid medium for 2 to 5 h at 35 °C and 30 °C, respectively. Next, 100 µL of the cultures that grew at the end of the incubation period was planted in petri dishes in such a way as to ensure that 10^8 cfu/mL was available for the cultural bacteria and 10^6 cfu/mL was available for the yeast. They were inoculated by frequently combing them in a petri dish, with a sterile swab being rubbed in 3 different directions. After this process, all of the petri plates were left to dry at room temperature for a period of 5 to 15 min. After completion of the drying process, 50 µL of the plant extracts were placed into small wells with a diameter of 5 mm, opened on the agar, and then the bacteria were incubated at 35 °C for 24 h, while the yeast was incubated at 30 °C for 48 h. At the end of the incubation period, the diameters (mm) of the resultant inhibition zones around the wells were measured. The antimicrobial activity experiments against all test microorganisms were done with three repetitions.

Statistical analyses

The analysis results were created using Microsoft Office Excel 2010 and one-way ANOVA. The statistical analyses performed using IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). The experimental results were performed in triplicate.

RESULTS and DISCUSSION

CUPRAC assay results

The results of the Cu^{+2} reducing power, CUPRAC assay, are shown in Figure 1. The Cu^{+2} reduction power of the fruit ethanol (EEF) and water (WEF) extracts and leaf ethanol (EEL) and water (WEL) extracts increased in direct proportion to the increasing concentration. When the absorbance of the WEF, WEL, EEF, and EEL of the plant were compared with standard antioxidants, at a concentration of 800 µg/mL, the result was as follows: Trolox > BHT > WEF > EEL> WEL > EEF, respectively.

When the EEF and EEL of the plant were compared, EEL of the plant were found to have the highest Cu^{+2}

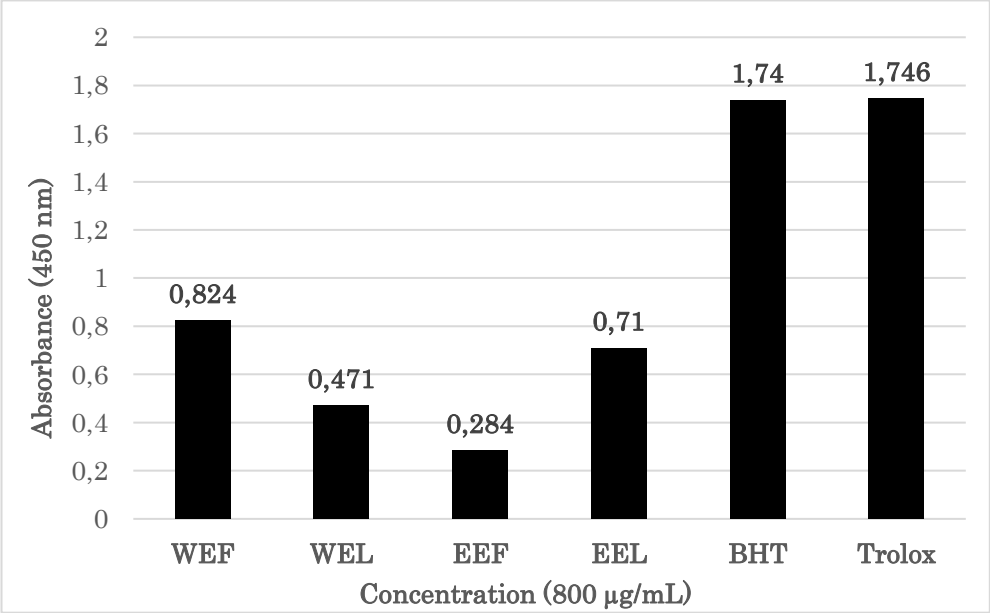

Figure 1. Comparison between the absorbance values of the standard antioxidants (BHT ve trolox) at a concentration of 800 µg/mL, and those of the water and ethanol extracts obtained from the fruit and leaves of *P. spina-christi* , using the CUPRAC method.

(BHT: butylated hydroxytoluene, WEF: water extract of the fruits, WEL: water extract of the leaves, EEF: ethanol extract of the fruits, EEL: ethanol extract of the leaves)

Şekil 1. P. spina-christi bitkisinin meyve ve yaprak kısımlarından elde edilen etanol ve su ekstrelerinin (800 µg/ml) konsantrasyonundaki absorbansların CUPRAC yöntemine göre standart antioksidanlar (BHT ve troloks) ile karşılaştırılması

reducing activity. In the water-phase extracts, the highest Cu^{+2} reduction activity was found in the WEF of the plant. Likewise, an increase was also found in the absorbance values obtained with an increased concentration of the water and ethanol phase extracts of the plant. The higher absorbance values of the standard antioxidants (BHT and Trolox) were thought to possibly have been due to the fact that the extracts of the fruit and leaves had lower antioxidant compound contents or exhibited less antioxidant effect when compared to the standard antioxidants used (BHT and Trolox).

DPPH assay results

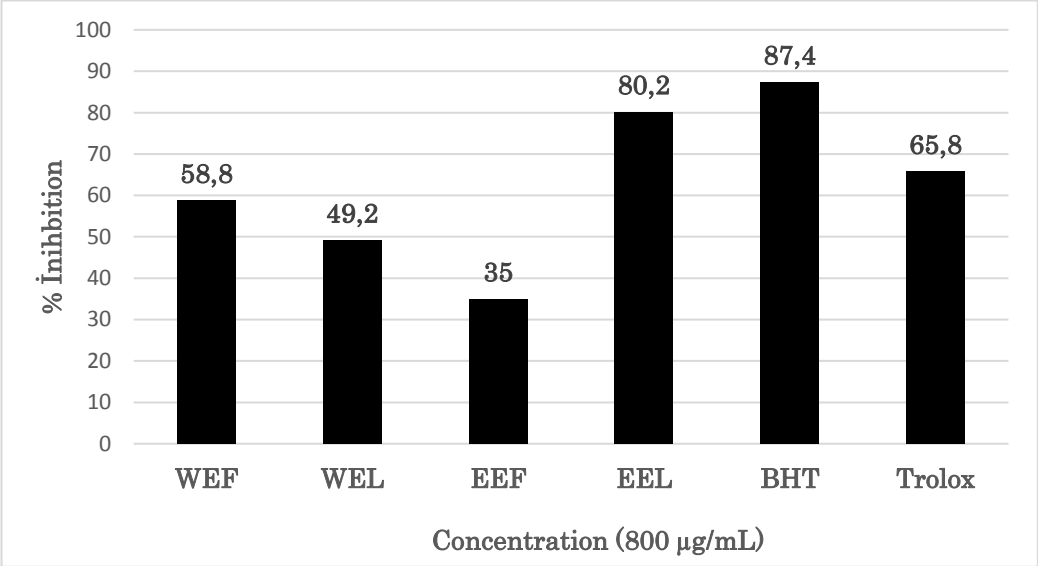

Figure 2. Comparison between the % inhibition values of the standard antioxidants (BHT and Trolox) at a concentration of 800 µg/mL, and those of the water and ethanol extracts obtained from the fruit and leaves of *P. spina-christi*, using the DPPH method.

Şekil 2. P. spina-christi bitkisinin meyve ve yaprak kısımlarından elde edilen etanol ve su ekstrelerinin (800 µg/ml) konsantrasyonundaki %inhibisyon değerlerinin DPPH yöntemine göre standart antioksidanlar (BHT ve troloks) ile karşılaştırılması

When the activities of the WEF, WEL, EEF, and EEL of the plant were compared with standard antioxidants, at a concentration of 800 µg/mL, the free radical scavenging activity were as follows: BHT > EEL > Trolox > WEF > WEL > EEF, and the % inhibitions were as follows: 87.4 > 80.2 > 65.8 > 58.8 > 49.2 > 35, respectively (Figure 2).

The % inhibition values of both the water and ethanol extracts obtained from the fruit and leaves of *P. spina-christi* were found to increase with an increase in the concentration. The inhibition value (80.2%) of the EEL was higher than that of Trolox, the standard antioxidant substance (65.8%). Therefore, the antioxidant activity of the EEL was considerably high.

The maximum inhibition value of the WEF was 58.8%, while the maximum inhibition value of the EEF was 35%. This difference was thought to have been due to the fact that the fruit was hydrophilic, i.e. it had the ability to bond better with water.

DPPH and $ABTS^{\bullet+}$ radical scavenging activity were used to measure the radical scavenging activities of plant extracts, mixtures, and pure substances. This method is based on the fact that when a solution containing DPPH is mixed with an antioxidant that tends to give hydrogen atoms, they react with each other, and consequently, the DPPH radical in the environment is reduced and the solution loses its original purple color. The transfer of hydrogen atom from the antioxidant to the stable DPPH radical reduces the absorbance by reducing the DPPH radical in the environment (Miller 1996; Gülçin et al., 2007).

ABTS assay results

The findings obtained with the method, which was based on the fact that adding an antioxidant-containing sample to the $ABTS^{\bullet+}$ radical solution produced through the oxidation of ABTS reduced the radical (Re et al., 1999), are shown in Figure 3. When the activities of the WEF, WEL, EEF, and EEL were compared with standart antioxidants, at a concentration of 800 µg/mL in the ABTS radical scavenging activity method, the result was as follows: Trolox > BHT > EEF = WEF > EEL > WEL, and the % inhibitions were: 97.8 > 97.2 > 96.2 = 96.2 > 95.9 > 95.5, respectively. When the EEF and EEL were compared, the fruit of the plant was found to have the highest ABTS radical scavenging activity.

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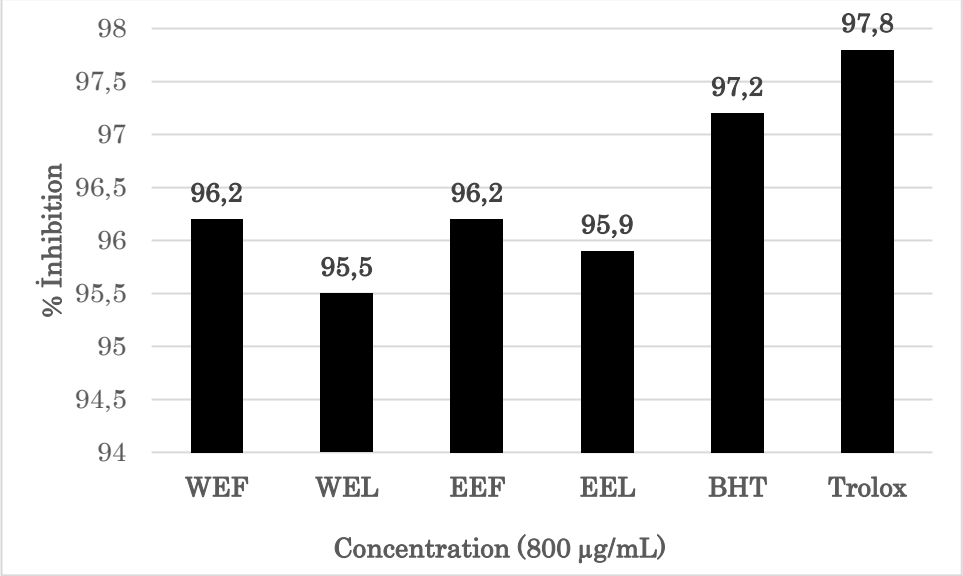

Figure 3. Comparison between the inhibition values (%) of the standard antioxidants (BHT and Trolox) at a concentration of 800 µg/mL, and those of the water and ethanol extracts obtained from the fruit and leaves of *P. spina-christi*, using the ABTS radical scavenging method.

Şekil 3. P. spina-christi bitkisinin meyve ve yaprak kısımlarından elde edilen etanol ve su ekstrelerinin (800 µg/ml) konsantrasyonundaki % inhibisyon değerlerinin ABTS radikal giderme yöntemine göre standart antioksidanlar (BHT ve troloks) ile karşılaştırılması

According to the obtained data, regarding the ethanol and water extracts at a concentration of 800 µg/mL, the ABTS radical scavenging activities of the fruit was found to be higher than those of the leaves, and they were also found to be very close to those of the standard antioxidants (BHT and Trolox) used in the study.

The ethanol extracts were found to show higher antioxidant activity than the water extracts. In this study, conducted using the ABTS radical scavenging activity method, the % inhibition values were observed to increase in parallel with the increases in the concentrations of both the standard antioxidant substances and plant extracts.

The highest inhibition value (96.2%) was obtained with the ABTS method among the CUPRAC, ABTS and DPHH methods used for antioxidant activity. When the WEF, WEL, EEF, and EEL were evaluated, the antioxidant activities of the WEF were found to be higher than those of the WEL with all of the methods used.

When the literature studies were examined, it was seen that there was a limited number of studies on the antioxidant activities of *P. spina-christi.* Şen (2018) stated that, among the different solvent extracts obtained from the fruit, leaves, and branches of P. *spina-christi*, especially the ethyl acetate and ethanol extracts of the plant branches plant had strong antioxidant activity against DPPH and ABTS radicals. Ethyl acetate and ethanol extracts of branches of *P. spina-christi* showed the highest antioxidant activity in DPPH and ABTS assays with IC_{50} values of 15.54 and 22.06 µg/ml. In both ABTS and DPPH assays, all extracts showed low antioxidant activity when compared to standards. In this study, the ethanol extract of the leaves of *P. spina-christi* was found to have especially high antioxidant activity against the DPPH radicals when compared to Trolox, the standard antioxidant substance.

In study conducted by Kirca and Arslan (2008) to determine the antioxidant activity of the methanolic extract of the fruit of *P. spina-christi*, the inhibition value at the highest concentration was determined to be 70% using the ABTS radical scavenging activity method. In the present study, results higher than those in the literature were found using the ABTS radical scavenging activity method, with an inhibition value that was higher than 90%.

Takım and Isık (2020) examined the antioxidant activity of the fruit extracts of *P. spina-christi*, using the DPPH, ABTS, FRAP, and CUPRAC methods, and found that the plant had metal reduction and radical scavenging activity that was higher than those of the commonly used standard antioxidants. In addition, the radical scavenging rate of the fruit extract was found to be 77.41% using the ABTS method, while in the present study, the radical scavenging rate of the plant was found to be 96.2% using the same method.

Antimicrobial activity results

The agar well diffusion method is one of the methods

commonly used to determine antimicrobial activity. The inhibition zone diameters (mm) of different extracts of the fruit and leaves of *P. spina-christi* were determined using the agar well diffusion method (Table 1), where in, $p < 0.05$ was accepted as statistically significant.

Table 1. Zone diameters (mm) of the water and ethanol extracts obtained from the fruit and leaves of *P. spina-christi*, determined using the agar well diffusion method.

Çizelge 1. P. spina-christi bitkisinin meyve ve yaprak kısımlarından elde edilen su ve etanol ekstrelerine ait agar kuyu difüzyon yöntemine göre elde edilen zon çapları (mm).

	Zone diameter / mm				
Test microorganisms	WEF	WEL	EEF	EEL	Penicillin 10 mg
B.cereus	R	10b	R	R	14a
B.subtilis	15b	10c	R	20a	10c
E.coli	10bc	12b	10bc	26a	26a
K.pneumoniae	R	10b	30a	R	12b
P.multocida	20a	12b	R	R	14b
P.aeruginosa	R	12c	16b	10c	20a
S.aureus	R	R	30a	30a	26b
Y.enterocolitica	R	R	R	R	16a
C.albicans	R	8c	24a	R	24a

R: Resistant

Values expressed with different letters in the same row are significant at $P < 0.05$.

It was observed that the water and ethanol extracts obtained from the fruit and leaves of *P. spina-christi* had no antibacterial effects against *B. cereus* and *Y. enterocolitica* bacteria and produced an inhibition almost equivalent to the penicillin antibiotic (Table 1). Regarding *E. coli* bacteria, the ethanol extract obtained from the leaves of the plant created an inhibition diameter equivalent to that of the penicillin antibiotic, while the other extracts had a comparatively lower antibacterial effect. The ethanol extract obtained from the fruit of the plant was observed to show the highest activity against *K. pneumoniae*, and this activity was higher than the activity of the penicillin antibiotics. It was seen that the water extract obtained from the fruit and leaves of the plant had antibacterial activity against *P. multicoda*, while the ethanol extracts did not form a zone diameter. It was seen that the ethanol extract obtained from the fruit of the plant had the highest activity against *P. aeruginosa*, while the water extract obtained from the fruit had no inhibitory effect. It was ascertained that the ethanol extracts obtained from the fruit and leaves of the plant formed the highest zone diameter (30 mm) against *S. aureus*, but the water extracts had no antibacterial activity against this bacterium. Regarding *C. albicans* yeast, it was seen that the ethanol extract obtained from the fruit of the plant created an inhibition diameter equivalent to that of the penicillin antibiotic, while the other extracts had either no or very low antifungal activity. The highest antimicrobial activity for the fruit and leaf extracts of the plant was found to have been created by the ethanol extracts.

When the literature studies were examined, it was seen that there were a very few studies on the antimicrobial activity of this plant. In a study conducted by Brantner et al. (1996), the highest zone diameter of the ethanolic root extracts of *P. spina-christi* was found to be greater than that of *S. aureus* (14 mm). In this study, it was observed that the highest zone diameter was greater than that of *S. aureus* (30 mm); the ethanol-phase leaf extract created a zone diameter equivalent to that of the penicillin antibiotic, especially against *E.coli*; and the obtained results were parallel to those in the literature. In another study, the antimicrobial activity of the hydrated extracts of the fruit of *P. spina-christi* was investigated by determining its antioxidant and minimal inhibition concentration using the DPPH activity method. Its antifungal activity against *P. eruginosa* and *S. aureus* was found to be very low (Orhan et al., 2012).

CONCLUSION

In this study, by using different antioxidant activity identification methods, it was found that *P. spina-christi* had antioxidant properties comparable to those of the standard antioxidant substances, and that the plant showed antioxidant properties close to the standard antioxidant substances used in the study, especially when considering the results obtained using the ABTS radical scavenging activity method. In antimicrobial studies, particularly the ethanol extracts of the fruit of the plant were found to have a high antimicrobial activity. Therefore, it seems promising in terms of its use in the food industry and various others, as an alternative to synthetic antioxidants and antimicrobial agents. In addition, it is believed that a new evaluation can be made by studying the antioxidant and antimicrobial effects of the growing conditions of the plant in different provinces using different standard substances, yeast, and bacteria, and that it can, consequently, provide a new perspective for

studies and the literature.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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