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The Medicinal Effects of Different Solvent Extracts of *Pyracantha Coccinea* Roem. Fruits: Heavy Metal Content, Antioxidant, and Antimicrobial Properties

Pyracantha Coccinea Roem. Meyvelerinin Farklı Çözücü Ekstraktlarının Tıbbi Etkileri: Ağır Metal İçeriği, Antioksidan ve Antimikrobiyal Özellikler

Neslihan Tekin KARACAER

Aksaray University Faculty of Science and Letters, Department of Molecular Biology and Genetics, Aksaray, Turkey

ABSTRACT

Objective: The current investigation has been conducted to assess the total flavonoid content, total phenolic content, heavy metal composition, and antioxidant and antimicrobial activities of *Pyracantha coccinea* Roem. fruit extracts prepared with different solvents.

Methods: Ethanol, diethyl ether, and hot water extraction were used as solvents to prepare the extract of *Pyracantha coccinea* Roem. fruit. Total phenolic ingredient was assessed by Folin-Ciocalteu assay, and the total ingredient of flavonoids was measured spectrophotometrically via $AlCl_3$ assay. The antioxidant activities of the extracts were investigated via free radical scavenging assays, DPPH, and ABTS. The fruits were analyzed by Inductively Coupled Plasma/Mass Spectrometer to determine heavy metal content. The antimicrobial activities of the extracts were investigated using agar well diffusion method against *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus,* and *Candida albicans*.

Results: It was determined that the total flavonoid ingredient, total phenolic ingredient, ABTS and DPPH activities of the hot water extract were significantly higher than the other fractions. These parameters were found to be significantly higher in ethanol extract compared to ether extract. All extracts exhibited antimicrobial activity against *Bacillus cereus* and *Pseudomonas aeruginosa* while the hot water fraction exhibited the highest antibacterial effect against *Pseudomonas aeruginosa*. It was determined that Cr, Co, Ni, and Cu contents exceeded the toxicity thresholds that might be found in plants.

ÖZ

Amaç: Mevcut araştırma, farklı çözücülerle hazırlanan *Pyracantha coccinea* Roem. meyve ekstraktlarının toplam flavonoid içeriği, toplam fenolik içeriği, ağır metal bileşimi ve antioksidan ve antimikrobiyal aktivitelerini değerlendirmek için yapılmıştır.

Yöntemler: *Pyracantha coccine*a Roem. meyvesinin ekstraktını hazırlamak için çözücü olarak etanol, dietil eter ve sıcak su ekstraksiyonu kullanıldı. Toplam fenolik bileşen Folin-Ciocalteu yöntemi ile toplam flavonoidlerin içerik $AlCl_3$ yöntemi ile spektrofotometrik olarak ölçüldü. Ekstraktların antioksidan aktiviteleri, serbest radikal süpürme deneyleri, DPPH ve ABTS ile araştırıldı. Meyveler, ağır metal içeriğini belirlemek için İndüktif Eşleşmiş Plazma-Kütle Spektrometresi ile analiz edildi. Ekstraktların antimikrobiyal aktiviteleri, *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus* ve *Candida albicans*'a karşı agar kuyusu difüzyon yöntemi kullanılarak araştırıldı.

Bulgular: Sıcak su ekstraktının toplam flavonoid bileşeninin, toplam fenolik bileşeninin, ABTS ve DPPH aktivitelerinin diğer fraksiyonlara göre anlamlı derecede yüksek olduğu belirlendi. Bu parametrelerin etanol ekstraktında eter ekstraktından önemli ölçüde daha yüksek olduğu bulundu. Tüm ekstreler *Bacillus cereus* ve *Pseudomonas aeruginosa*'ya karşı antimikrobiyal aktivite sergilerken, sıcak su fraksiyonu *Pseudomonas aeruginosa*'ya karşı en yüksek antibakteriyel etkiyi sergiledi. Cr, Co, Ni ve Cu içeriklerinin bitkilerde bulunabilecek toksisite eşiklerini aştığı belirlendi.

Address for Correspondence: Neslihan TEKİN KARACAER, Aksaray University Faculty of Science and Letters, Department of Molecular Biology and Genetics, Aksaray, Turkey
E-mail: neslihan_tekin@hotmail.com **ORCID ID:** orcid.org/0000-0002-0091-6428

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Conclusion: These results suggest that *Pyracantha coccinea* Roem. fruit may be considered as a natural source of antioxidants and antimicrobial agents.

Keywords: ABTS, antimicrobial activity, antioxidant activity, DPPH, *Pyracantha coccinea* Roem.

Sonuç: Bu sonuçlar, *Pyracantha coccinea* Roem. meyvesinin doğal bir antioksidan ve antimikrobiyal ajan kaynağı olarak kabul edilebileceğini göstermektedir.

Anahtar Kelimeler: ABTS, antimikrobiyal aktivite, antioksidan aktivite, DPPH, *Pyracantha coccinea* Roem.

Introduction

Reactive oxygen species (ROS) are produced in oxidative metabolism, which is required to generate the energy needed by most living organisms and to fuel other biological processes. On the other hand, overproduced ROS harm cells by affecting proteins and causing damage to DNA. Therefore, ROS have significant effects on the pathogenesis of numerous diseases such as cancer, neurodegenerative disorders, cardiovascular diseases, atherosclerosis, inflammation, and cataracts (1). Natural antioxidants have extensive variety of biological activities such as alteration of intracellular redox potential, prevention of ROS formation, and direct or indirect scavenging of free radicals. Antioxidants are essential for the survival of living organisms against injuries provoked by uncontrolled manufacturing of ROS and accompanying protein damage, lipid peroxidation, and DNA chain breakage. In addition, antioxidants contribute to the inhibition of degenerative disorders by preventing the oxidation of other molecules. However, it has been reported that the intake of natural antioxidants such as polyphenol-rich nutrients, vegetables, and fresh fruits can resist oxidative deterioration of free radicals (2).

Phytochemical antioxidants are important in therapeutic applications against animal and human pathogens including fungi, bacteria, and viruses. These secondary metabolites which are produced by plants are highly structurally diverse organic chemicals which are used in the nutraceutical industry that play various functions including bactericidal, bacteriostatic, antimicrobial, and chemotherapeutic functions (3). Plants (herbs, vegetables, and fruits) possess extensive diversity of free radical scavenger molecules such as nitrogen compounds, vitamins, phenolic compounds, terpenoids, and other endogenous metabolites which are abundant in antioxidant activities (2). Flavonoids contain tannins and phenolic acids. All these active ingredients can act as antioxidants through lipid oxidation prevention, radical scavenging, or reduction power. Flavonoids have antiproliferative, antitumor, antifungal, anti-inflammatory, antiviral, and antibacterial features (4). In this regard, screening plants for their antimicrobial properties pose significant potential to discover novel compounds for medicinal usage (5). On the other hand, it should be known that when medicinal plants are used in the treatment of certain diseases, they may be toxic besides their pharmacological effect if their heavy metal content increases. Although the efficacy of medicinal plants is mainly associated with their components such as secondary metabolites and essential oils, heavy metals such as Ni, Pb, Zn, Cd, and other impurities are thought to cause health problems if they are above threshold concentrations (6).

There are recent studies on many herbs which produce health boosting effects such as antimicrobial properties and antioxidant properties, yet the potential of many herbs as sources for novel medicine still remain uninvestigated (5). Pyracantha (firethorn) is a genus belonging to Rosaceae family (Amygdaloideae subfamily, tribe Maleae) with various species and hybrids scattered throughout Eurasia. *Pyracantha coccinea* Roem. (Red pyracantha) is a thorny shrub that grows up to 3 m in height at different altitudes in China, Himalayas (4), Italy, and Turkey. It has small bright red, fruits which may be cooked for marmalades, jams, jellies, and sauces. In addition, its fruits are used in conventional medicine for their cardiac, tonic and diuretic features (7).

Pyracantha coccinea Roem. fruit extracts have rarely been explored for their phytochemical properties and biological effects when prepared with different solvents. Therefore, the purposes of this research are: 1) to investigate the total phenol, total flavonoid, and heavy metal contents of *Pyracantha coccinea* Roem. fruits extracted with different solvents (ethanol, diethyl ether, and hot water); and 2) to assess the antimicrobial and antioxidant activities of *Pyracantha coccinea* Roem. fruits.

Methods

Plant samples: *Pyracantha coccinea* Roem. fruit samples were collected and identified from Aksaray in September 2020. The Flora of Turkey and The East Aegean Islands (8) and the Checklist of the Flora of Turkey - Vascular Plants (9) were used for identification of the plant specimens. The plant species was identified at Anadolu University Plant Medicine and Scientific Research Center. They were preserved in Aksaray University Herbarium with the code of M. Tekşen 2982 (Aksu). The fruit parts of the collected plants were separated and washed with and then rinsed in distilled water. Afterwards, the fruits were dried in shade at room temperature and powdered.

Preparation of plant extracts: Powdered fruit particles were used to obtain *Pyracantha coccinea* Roem. fruit extracts. Three solvents were preferred for the extraction: ethanol, diethyl ether, and hot water (100 °C). 300 mL of solvent was added on 50 g of fruit particles in all three extractions. The extracts were kept in an ultrasonic sonicator (Bandelin Sonorex) at 37 °C for 30 minutes. Next, they were kept in the shaker for a total extraction duration of 24 hours. Then the plant particles were removed from the solvent with the help of a paper coarse filter. After the ether and ethanol were removed from the extracts prepared with ether and ethanol, they were re-dissolved in ethanol (50 mL). The other extract was centrifuged at 8,000 g for 10 minutes. The acquired extracts were maintained at +4 °C to be used in measurements.

Total phenolic content measurement: Total phenolic matter analysis was assayed based on the Folin-Ciocalteu method with minor modifications (10) by adding 900 µL of distilled water and 5 mL of 0.2 M Folin-Ciocalteu reagent onto 100 µL of the extract. After adding 5mL (7.5%) of Na_2CO_3 and incubation for 2 hours at room temperature, the absorbance was determined at a wavelength of about 760 *nm* in a spectrophotometer (Biochrom S70 Dual). Gallic acid was used to prepare a standard curve. The data are stated as mg gallic acid equivalent per g dry plant weight (DW).

Total flavonoid content measurement: Total flavonoid substance was determined based on in the assay developed by Dewanto et al. (11) with minor modifications using $AlCl_3$ and $NaNO_2$ as reagents. 4 mL of pure water and 0.3 mL of $NaNO_2$ (5%) were added onto 0.4 mL of extract and incubated for 5 minutes. Then 0.5 mL of 10% $AlCl_3$ was added to the mixture and kept for 6 more minutes. Next, the absorbance of the mixture, into which 2 mL of 1M NaOH and 3 mL of distilled water were added, was read at 510 nm in a spectrophotometer (Biochrom S70 Dual). Catechin concentrations 0.01-0.25 mg/mL were used to generate a calibration curve, and the results were stated as mg catechin equivalent per g DW.

Assay of DPPH scavenging activity: Free radical scavenging activity of *Pyracantha coccinea* Roem. fruit extracts was measured based on DPPH assay previously carried out by Brand-Williams et al. (12) with minor modifications (12). A 25 mg/L DPPH solution was prepared with methanol. After adding 0.1 mL of extract to 3.9 mL of DPPH solution, it was kept in a shaker in a dark environment at room temperature for 30 minutes. Absorbance measurements were conducted at 517 nm wavelength in a spectrophotometer (Biochrom S70 Dual). The calculations were performed using the formula below.

DPPH radical scavenging %=$[(A_C-A_E)/A_C \text{ x}100]$

The A_C is the absorbance of DPPH solution, and A_E is the absorbance of the sample.

Assay of ABTS scavenging activity: The antioxidant capacities of the samples were determined based on the spectrophotometric measurement method developed by Re et al. (13) with slight changes. Briefly, a 2,2′-Azinobis-(3-Ethylbenzthiazolin-6-Sulfonic Acid) ($ABTS^+$) cation radical solution was produced by reacting ABTS (2 mM) in H_2O and $K_2S_2O_8$ solution (final concentration: 2.45 mM) for 12 hours in dark at room temperature. Before $ABTS^+$ radical solution was used, the absorbance of the control solution at 734 nm was adjusted to a value between 0.750 nm-0.800 nm with phosphate buffer with 0.1 M and pH 7.4. 1 mL of ABTS solution was added on 80 µL of extract samples in different concentrations, and the total volume was completed to 4 mL with phosphate buffer. These samples were incubated for 30 minutes by vortexing, and their absorbance was evaluated at 734 nm via a spectrophotometer (Biochrom S70 Dual). ABTS radical scavenging activity inhibition percentages were calculated with the formula given below:

% ABTS Inhibition=$[(A_C-A_E)/A_C \text{ x}100]$

The A_C is the absorbance of ABTS solution, and A_E is the absorbance of the sample.

Profiling of mineral elements in *Pyracantha coccinea* Roem. Fruit: The analysis of heavy metals (Cr, Cd, Mn, Pb, Fe, Ni, Co, Zn, As, and Cu) in *Pyracantha coccinea* Roem. fruits were performed by an Inductively Coupled Plasma/Mass Spectrometer (ICP-MS) based on the method by Hajar et al. (14) with minor changes. Approximately 0.2 g of plant sample was put into the microwave tube, and 10 mL of nitric acid was added on it. The solution mixture was mixed and kept for 10 minutes for preburning. Later, the tubes were covered and exposed to heat in a microwave system. Initially, the microwave was heated up to 190 °C for 20 minutes. Then it was kept at 190 °C for 15 minutes. The pressure was set at 800 psi and the power at 900-1800 watts. Next, it was cooled from 190 °C down to room temperature in 15 minutes. The samples that were taken from the tubes after burning were completed up to 50 mL with ultrapure water. A CEM MARS6 model microwave oven was used for microwave-assisted digestion of the plant material. The heavy metal contents in the examined plant material were analyzed using Bruker Aurora M90 ICP-MS. Firstly, a standard and a blank were read for each heavy metal content using ICP-MS instrument. The calibration solutions were prepared by the suitable dilution of the single element certified reference materials. Then the calibration charts were plotted. Later, the samples and blind of the samples were analyzed by reading. The operating conditions of ICP-MS instrument are given in Table 1.

Bacterial and fungal strains and culture conditions: The pathogenic microorganisms which were studied in the investigation were produced from the microbial culture collection at Aksaray University Scientific and Technological Application and Research Center Microbiology Laboratory. *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (ATCC 10231), and *Staphylococcus aureus* (ATCC 25923) were grown using Brain Heart Infusion Broth (BHI) (Merck) medium. *Candida albicans* (ATCC 10231) strain was investigated for antifungal activity. The microorganisms which were to be tested were grown in Mueller Hinton broth overnight in a rotary shaker at 37 °C. Every strain in the present study was used after adjusting it at

Table 1. Operating conditions of ICP-MS instrument

Parameters	
RF power (kW)	1.40
Plasma Gas Flow Rate (L min^{-1})	15
Auxiliary Gas Flow Rate (L min^{-1})	1.5
Sheath Gas Flow Rate (L min^{-1})	0.11
Nebulizer Gas Flow Rate (L min^{-1})	0.95
Read delay (sn)	40
Gas	Argon
Purge gas	Hydrogen
Repeat/sample reading	5
Scan replicate	5
Scan mode	Peak hopping
Hydrogen Gas Flow Rate (mL min^{-1})	80

ICP-MS: Inductively Coupled Plasma/Mass Spectrometer

a concentration of 10^8 cells/mL with a 0.5 McFarland standard (15). *Candida albicans* was prepared from a 48-hours culture of fungal isolates in potato dextrose broth (16). The fungal spore density at a final concentration of 10^6 spores/mL was acquired using a spectrophotometer (595 nm). Each microorganism was kept by subculturing regularly on BHI medium and storing at +4 °C before being used in the tests.

Analysis of Antimicrobial Activities of *Pyracantha Coccinea* Roem. Fruits: The antimicrobial effects of *Pyracantha coccinea* Roem. fruit extracts on various pathogenic bacteria and fungi were investigated by agar well diffusion method. The agar well diffusion assay was used to monitor the antifungal and antibacterial activities of various solvent extracts (17). A 100 µL of fresh bacterial or fungal culture was inoculated in the middle of a sterile petri dish with a Mutueller hinton agar medium, and smear was performed. Wells were made into microorganism seeded media using a sterile cork borer (5 mm diameter). Next, 20 µL of each extract (20% w/v concentration if any residual) was added to the respective wells. The prepared plates were kept in the refrigerator for 30 minutes to ensure that the extracts penetrated the agar thoroughly. Then the petri dishes were incubated at 37 °C for 24 hours. The antimicrobial activity was determined by assessing the zone of inhibition (containing well diameter) that appeared after the incubation period. Gentamicin (10 UI) was used as a positive control, while Dimethyl sulfoxide (DMSO at 10% concentration) was used as a negative control. All tests were done three times.

Statistical Analysis

Data analyses were performed with Statistical Package for Social Sciences (SPSS) version 18.0 statistical software package (SPSS Inc, Chicago, Illinois). Statistical significance was verified by One-Way analysis of variance (ANOVA) with Tukey's post-hoc test. The outcomes were presented as means ± standard deviation (n=3 per each test sample). The differences between the applied doses with $p<0.05$ was recognized as statistically significant.

Results

Figure 1 exhibits the total phenolic ingredient and total flavonoid ingredient of *Pyracantha coccinea* Roem. fruit extracted with various solvents. Among *Pyracantha coccinea* Roem. fruit extracts prepared with the solvents, the highest content of phenolic compounds was found in hot water extracts in comparison to the ethanol and diethyl ether extracts ($p<0.001$, $p<0.001$). However, the total phenolic content in the ethanol extract was found to be higher than the diethyl ether extract ($p<0.001$). The extraction with hot water showed the highest total flavonoid content compared to the ethanol and diethyl ether extracts ($p<0.001$, $p<0.001$, respectively). However, the total flavonoid content was found to be higher in the ethanol extract compared to the diethyl ether extract ($p<0.001$).

The outcomes for ABTS free radical scavenging activity of *Pyracantha coccinea* Roem. fruit extracts are provided in Figure 2A. The hot water extracts exhibited higher ABTS values with higher radical scavenging activity compared to the ethanol and diethyl ether extracts ($p<0.001$, $p<0.001$). ABTS values were found to be lower in the diethyl ether extract compared to the ethanol extract ($p<0.001$). As shown in Figure 2B, the highest DPPH scavenging ability among *Pyracantha coccinea* Roem. fruit extracts were obtained in hot water compared to the ethanol and diethyl ether extract ($p<0.001$ and $p<0.001$, respectively). DPPH scavenging level was found to be lower in the diethyl ether extract compared to the ethanol extract ($p<0.001$).

The outcomes of antimicrobial activity against the tested microorganisms are summarized in Table 2. The extracts of *Pyracantha coccinea* Roem. fruit in different solvents exhibited antimicrobial activities against *Bacillus cereus* and *Pseudomonas aeruginosa*. In *Bacillus cereus*, there were no significant differences in the levels of antimicrobial activity in different solvent extract applications with *Pyracantha coccinea* Roem. fruit ($p>0.05$). On the other hand, *Pyracantha coccinea* Roem. fruit hot water extract displayed *antimicrobial activity higher* than ethanol and diethyl ether extracts ($p<0.01$, $p<0.01$).

The present study also determined the contents of heavy metal elements Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Cd, and Pb in *Pyracantha coccinea* Roem. fruit (Table 3). The presences of Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, and Pb were confirmed, and

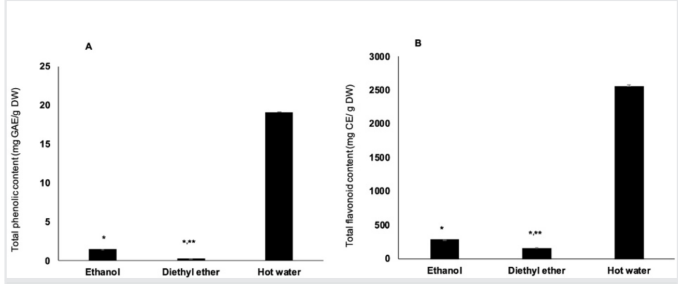

Figure 1. Total phenol (A) and total flavonoid (B) contents of *Pyracantha coccinea* Roem. fruit extracts prepared with various solvents. The results are expressed as means ± standard deviation from three independent experiments. *Indicates $p<0.001$ versus hot water extract, and **Indicates $p<0.001$ versus ethanol extract

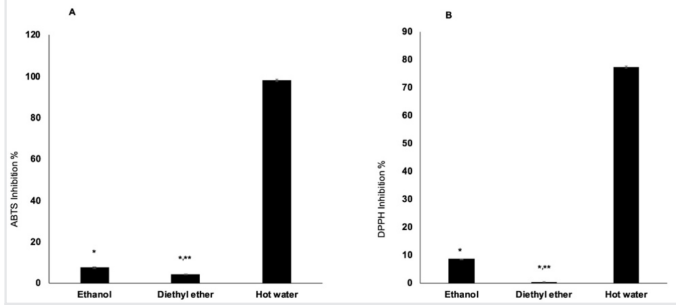

Figure 2. Antioxidant properties of *Pyracantha coccinea* Roem. fruit extracts prepared with various solvents: ABTS radical scavenging activity (A) and DPPH radical scavenging activity (B). The results are expressed as means ± standard deviation from three independent experiments. *Indicates $p<0.001$ versus hot water extract, and **Indicates $p<0.001$ versus ethanol extract

their concentrations were defined. However, Cd was not detected. The heavy metal contents of *Pyracantha coccinea* Roem. fruits were evaluated with reference to the normal and toxic heavy metal concentrations found in previously studied medicinal plants (6). Consequently, it was determined that Mn, Fe, Zn, As, Mo, Cd, and Pb concentrations in *Pyracantha coccinea* Roem. fruit were within the safety limits while Cr, Co, Ni and Cu concentrations were above the safety limits.

Discussion

There is a lot of existing evidence on the effects of free radicals on the formation of a number of disorders such as neurodegeneration, some inflammatory diseases, and cancer (18). Although there is an antioxidant defense system in human body, it is often insufficient to neutralize multiple attacks that increasingly affect the body. In order to maintain a balance among antioxidants and oxidants in the body, substances that are effective against ROS are used as nutritional support. On the other hand, the consumption of some artificial antioxidants has been recently suggested to be limited due to their carcinogenic and toxic effects (19). Because of toxicological apprehensions related with synthetic preservatives and antioxidants, the antioxidant and antimicrobial effects of numerous curative herbs are being studied worldwide (18). Plants include some phytochemicals such as alkaloids, flavonoids, terpenoids, and vitamins, which have antioxidant and antimicrobial properties (7). Antioxidant substances and phytochemicals in plant samples are often influenced by diverse conditions such as time, temperature, solvent concentration, and solvent polarity during extraction and purification. Therefore, the diverse phytochemicals are extracted in solvents of varied polarity because one single solvent may not be sufficient to extract all phytochemicals (20). Accordingly, the present study aims to define the heavy metal composition, total flavonoid and total phenolic ingredients and to investigate the antimicrobial and antioxidant activities of *Pyracantha coccinea* Roem. fruits obtained with different solvents.

Antioxidants are very crucial because of their capability of neutralizing free radicals (18). Polyphenols with antioxidant functions can preserve cells from oxidative injury and hence decrease the risk of numerous degenerative illnesses related with oxidative stress caused by free radicals (19). Phenolic compounds have hydroxyl groups in their structures and are important plant components due to their free radical neutralizing capacity through these groups. Therefore, the total phenolic content can be utilized as a basis for rapid determination of antioxidant capacity (18). The phenolic compounds of plants are divided into several categories, and the leading compounds are flavonoids with strong antioxidant activities. It is known that the flavonoids which are found naturally in plants have significant positive effects on human health. Investigations on flavonoid derivatives have revealed an extensive variety of antiviral, antibacterial, anticancer, anti-inflammatory, and anti-allergic activities. Flavonoids have been shown to be very effective scavengers for most oxidizing molecules and to contain various free radicals and singlet oxygen which play a beneficial role against various diseases (21). The total flavonoid and phenolic ingredients

Table 2. Antimicrobial effect of *Pyracantha coccinea* Roem. fruit extracts prepared with different solvents against *Bacillus cereus* (ATCC 10231), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 10231) demonstrated by Agar well diffusion method

Microorganisms	Ethanol	Diethyl ether	Hot water
Bacillus cereus	7.00±0.001^{ns}	5.67±0.58^{ns}	7.3333±1.16^{ns}
Staphylococcus aureus	-	-	-
Pseudomonas aeruginosa	5.33±0.58*	5.00±0.00*	7.33±0.58
Candida albicans	-	-	-

The results are expressed as means ± SD from three independent experiments. *Indicates $p<0.01$ versus hot water extract. $^{ns}p>0.05$ indicates statistically insignificant

Table 3. Total content of heavy metals (mg kg-1) of *Pyracantha coccine*a Roem. fruit and reference values for trace elements as normal and toxic concentrations in plants

Element	*Pyracantha coccinea* Roem. fruit heavy metals content (mg kg-1)	Normal concentrations in plants[10] (mg kg-1)	Toxic concentrations in plants[10] (mg kg-1)
Cr	116.38±0.03	<0.1-1	2
Mn	31.32±0.06	15-100	400
Fe	58.48±0.41	50-250	(>500)
Co	27.08±0.31	0.05-0.5	30-40
Ni	669.72±1.75	0.1-5	30
Cu	531.08±3.89	3-15	20
Zn	26.28±0.7	15-150	200
As	22.12±0.45	10-60	<2
Cd	Not detected	<0.1-1	10
Pb	1.8±0.45	1-5	20

in the extracts are also determined to predict the relationship between the free radical scavenging activity and the polyphenolic ingredient (22). Thus, the total phenolic and flavonoid contents in *Pyracantha coccinea* Roem. fruit extracts were explored in the present study. The amount of ingredients was observed to differ in the extracts prepared with the different solvents used. The data obtained from the analyzes showed that there was a statistical difference in total flavonoid and phenolic contents of the extracts prepared with various solvents. In the current study, hot water extract displayed the highest total phenolic content and had the best antioxidant capacity compared to the other two extracts. The amount of total phenolic substance was found to be higher in ethanol extract than diethyl ether extract. In the study conducted by Keser (23), the highest total phenolic content among the firefly extracts prepared with various solvents was detected in the extract prepared with ethanol, followed by water and ether extracts. Sarıkurkcu and Tepe (7) stated that the total flavonoid content in Pyracantha in the ethanol extract was higher than the water extract. In the current study, the highest amount of total flavonoid content was detected in hot water extract, followed by the ethanol and ether extract. However, total flavonoid content was found to be higher in the ethanol extract than the diethyl ether extract. The differences in the flavonoid and phenolic contents of the different solvent extracts may be due to the polarity of the solvents used and the chemical structure of the endogenous extractable compounds (24). The polarity of a solvent is determined to be crucial for the total phenolic and flavonoid contents: a solvent with a higher polarity yields higher phenolic and flavonoid contents. Water is a highly polar solvent which can extract a higher diversity of compounds (25). The result in the present study may be related, partially, to the fact that water aids in the diffusion of extractable compounds through plant tissues (26). On the other hand, the high temperatures in the extraction procedure may have enhanced the transition of these substances to the solvent. It is commonly confirmed that numerous biological activities and curative utilities of plants can be recognized based on the antioxidant activity of the phenolic compounds and flavonoids they include (27). The total phenol and flavonoid contents which were determined in the recent study suggested that *Pyracantha coccinea* Roem. fruit had a number of potential health-associated biological properties thanks to its antimicrobial and antioxidant effects.

Antioxidants work by chelating metals, scavenging a number of free radical species produced in oxidative reactions, and inhibiting free radical formation through reduction of precursors (5). DPPH is a free radical compound that is widely used to assess the free radical scavenging ability of diverse samples because of its easy use, stability (in radical form) and reproducibility. DPPH test is utilized to assess the capacity of antioxidants to scavenge free radicals, which are recognized to be an important factor in biological injury triggered by oxidative stress (3). Similarly, $ABTS^{\bullet+}$ scavenging test is a great assay for assessing the antioxidant activity of chain breaking and hydrogen donating antioxidants (28). The number of studies which have been conducted to assess the antioxidant and general properties of *Pyracantha coccinea* Roem. fruit is quite limited. In a study on the radical scavenging potential of *Pyracantha coccinea* extracts prepared with water, ethanol, acetone, methanol, and diethyl ether, Keser (23) determined that methanol, ethanol, and acetone extracts displayed DPPH and ABTS radical scavenging activity. He suggests that *Pyracantha coccinea* can be an important source of natural antioxidants because of the existence of phenolic compounds (23). In another study, Yoshimura stated that DPPH radical scavenging activity of *Pyracantha coccinea* might arise from the therapeutically effective flavonoid glycosides and other polyphenols it contained (29). In the current investigation, the antioxidant capacities of the extracts which were acquired through various solvents were determined by ABTS and DPPH methods, which expressed scavenging of free radicals (30). DPPH radical scavenging effect was the highest in hot water extract and subsequently in ethanol, while the lowest effect was found in the ether extract. On the other hand, the highest ABTS radical scavenging effect was found in the hot water extract followed by the ethanol extract, while the lowest effect was found in the ether extract. However, DPPH and ABTS activities were found to be higher in the ethanol extract than the diethyl ether extract. Considering the data, hot water and then ethanol solvents appeared to have a good capacity to extract antioxidant molecules because *Pyracantha coccinea* Roem. fruit extracts exhibited varying degrees of antioxidant activity with the potential to act as free radical scavengers. On the other hand, it meaned that the phenolic and flavonoid components which were found to be high in the extraction with hot water exhibited a distinguishable effect on the free radical scavenging.

Most minerals contribute significantly to normal growth, even at threshold levels, and play an important role in biochemical functions, particularly essential enzyme systems, but they can be toxic and pose a health risk at high levels (31). Although the effectiveness of medicinal plants is mainly associated with their components, it has been stated that they may cause health problems due to the heavy metals they may contain if taken for a long-term (32). Therefore, it is very essential to define the levels of these compounds in popular, common, and extensively used herbs (31). To date, there are no reports on the mineral content of *Pyracantha coccinea* Roem. fruit. In the present study, Cr, Co, Ni, and Cu amounts were determined above normal limits, while other metal concentrations were within safety limits. On the other hand, Cd was below detection limits. Co is an important element that is essential for the production of vitamin cobalamin. Although it effects several functions in the human body such as the formation of amino acids and neurotransmitters, excessive Co accumulation in the body induces asthma, fibrosis in the lungs, and high erythrocyte production. Similarly, while Cu is a vital element for the proper functioning of organs and systems in the body, it can be toxic even in slightly high levels. On the other hand, the biochemical function of Ni in animals and humans is still not fully known (33). As one of the trace metal nutrients necessary for humans and animals, the main role of Cr is to help maintain normal glucose tolerance in the body. On the other hand, the maximum intake limit has not been determined as no toxic effect of Cr is known (31). Cd is an element which is not required by humans or plants and can easily cause toxic effects in

humans at low amounts (6). Therefore, the fact that no Cd that was detected in *Pyracantha coccinea* Roem. fruit in the present study was a highly desirable result. However, heavy metals are among the most important pollutants in the environment. Phytoremediation is recently utilized as an environmentally friendly and potentially cost-effective technology used to clean contamination from soil, sediment, and water (34). *Pyracantha coccinea* Roem. fruits used in the present study were collected from a region in the immediate vicinity of a highway. Therefore, some heavy metals (Co, Cr, Cu, and Ni) might be found to be high. Since some of the heavy metals in this study had high levels, it could be suggested that *Pyracantha coccinea* Roem. fruit could be used to remove heavy metals from the soil using its ability to absorb metals necessary for plant growth.

Plants commit a natural source of antimicrobial substances. It has been stated that the antimicrobial activity of plants is associated with the defense mechanism against microorganisms (19). However, the increased resistance of bacteria to common antimicrobial agents and the unwanted side effects of synthetic antimicrobial agents have led medical researchers to subject their attention to the possible antimicrobial properties of herbs. In addition, the antibacterial effects of several medicinal herbs are being investigated because of toxicological concerns related with synthetic preservatives and antioxidants (35). As a result of the literature review, there were only two studies which investigated the antimicrobial effects of *Pyracantha coccinea* Roem. In the study conducted by Turker et al. (36), antimicrobial activity could not be determined on *Staphylococcus aureus* in the extracts prepared by *Pyracantha coccinea* Roem. with cold water, and hot water and cold ethanol. They determined an inhibitory effect of the extract prepared with hot ethanol on *Staphylococcus aureus* (36). It was determined by Turu et al. (37) that *Pyracantha coccinea* Roem. extract which was prepared with ethanol and applied in different doses had antimicrobial effects against *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Candida albicans.* In their study, antimicrobial activity was determined for *Pyracantha coccinea* extracts against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus,* and *Candida albicans.* According to the results of the present study, *Pyracantha coccinea* Roem. exhibited antimicrobial activity only against *Bacillus cereus* and *Pseudomonas aeruginosa*. The current study has differences (37) as well as consistency with other studies (36,37). Contrary to the present study, Turu et al. (37) found that *Pyracantha coccinea* Roem. fruit extracts displayed antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*. This difference may have stemmed from the extract preparation method and the applied extract dosage. On the other hand, the application of *Pyracantha coccinea* Roem. fruit extracts which were prepared with different solvents against *Bacillus cereus* did not make a difference in antimicrobial activity based on the statistical data. However, it was determined that the antimicrobial effect of the extract prepared with water in *Pseudomonas aeruginosa* was higher than the other two extracts. The detected antimicrobial activity suggests that these extracts contain compounds that can inhibit the growth of microorganisms. Secondary compounds found in plants may play a role in the defense of plants through cytotoxicity against pathogenic microorganisms, and it may prove their usefulness as antimicrobial drugs for humans (38). In addition, flavonoids (low mass polyphenolic compounds) have antibacterial, antiviral, antifungal, antitumor, antiproliferative, and anti-inflammatory properties (4). It can be said that the antimicrobial effects reported in the present study may have arisen from the contribution of phenolic and flavonoid compounds. However, the fact that *Pyracantha coccinea* Roem. fruit extracts do not show inhibition on *Staphylococcus aureus* and *Candida albicans* suggests that they can be used as a narrow spectrum antimicrobial. However, the cell membrane, cytoplasm, metabolism, and structural functions of the cell of microorganisms are negatively effected by some meatal ions (39). The analyses in the present study show that some heavy metals have high amounts and may have caused antimicrobial effects against *Bacillus cereus* and *Pseudomonas aeruginosa.*

Study Limitations

The main limitation of the present study was that there was no analysis to detect which flavonoid and phenolic compounds in the extracts.

Conclusion

People have recently turned their attention to using natural alternatives such as plant extracts to solve health and environmental problems (19). The current investigation displays the extraction of *Pyracantha coccinea* Roem. fruit via different solvents. Among the studied solvents, water was proved to be the finest for extracting bioactive compounds from *Pyracantha coccinea* Roem. fruit since it resulted in the highest total content of phenolic and flavonoid. Compared with other extracts, the water extract of *Pyracantha coccinea* Roem. fruit showed the highest antimicrobial and antioxidant activity. These findings propose that water is the finest solvent for the extraction of bioactive compounds from *Pyracantha coccinea* Roem. fruit and that the water extract is a promising antimicrobial and antioxidant agent for further drug development. Therefore, the extracts of *Pyracantha coccinea* Roem. fruit could be novel resources to improve new plant based cures for the management of illnesses. *Pyracantha coccinea* Roem. fruit may be a good candidate for further investigations on its usefulness in disorders caused by oxidative stress due to its phenolic and flavonoid contents and antioxidant activity. Additional discoveries are required to break down and purify the extract to discover molecules liable for the detected antioxidant and antimicrobial activity.

Ethics

Ethics Committee Approval: Was not obtained as no animals or humans were used in the study.

Peer-review: Externally peer reviewed.

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