Karaelmas Science and Engineering Journal
Journal home page: https://dergipark.org.tr/tr/pub/karaelmasfen
DOI: 10.7212/karaelmasfen.1297963

Research Article
Received / Geliş tarihi : 16.05.2023
Accepted / Kabul tarihi : 18.09.2023

Characterization, Antioxidant and Antimicrobial Activities of Iron Nanoparticles Synthesized Using Firethorn Fruit (*Pyracantha coccinea* Roemer) Extracts

Ateş Dikeni Meyvesi (Pyracantha coccinea Roemer) Ekstraktları Kullanılarak Sentezlenen Demir Nanopartiküllerinin Karakterizasyonu, Antioksidan ve Antimikrobiyal Aktiviteleri

Volkan Karakullukçu[1], Bülent Akar[2*], Cemalettin Baltacı[2], Azer Özad Düzgün[3], Ömer Karpuz[4]

[1]Gümüşhane University, Graduate Education Institute, Department of Biotechnology, Gümüşhane, Türkiye

[2]Gümüşhane University, Faculty of Engineering and Natural Sciences, Department of Food Engineering, Gümüşhane, Türkiye

[3]Gümüşhane University, Faculty of Health Sciences, Department of Occupational Health and Safety, Gümüşhane, Türkiye

[4]Gümüşhane University, Faculty of Engineering and Natural Sciences, Department of Genetic and Bioengineering, Gümüşhane, Türkiye

Abstract

The main focus of this study was to explore the biological activity of metal nanoparticles that were green-synthesized from firethorn fruits. Specifically, iron nanoparticles (Fe-NP) were synthesized from extracts obtained from fruits and seeds of the firethorn plant (*Pyracantha coccinea)* grown on Gumushane University's campus for landscaping purposes. The antioxidant and antimicrobial properties of both extracts and synthesized iron nanoparticles were evaluated.

To characterize the Fe-NPs, FT-IR spectroscopy, scanning electron microscopy (SEM), and X-Ray Diffraction (XRD) analyses were performed. The antioxidant activity of the fruit extracts and Fe-NPs was assessed using various antioxidant activity tests, including ABTS radical scavenging activity, DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant content (TAC). The results of all antioxidant activity tests indicated that the iron nanoparticles exhibited higher antioxidant activity than the fruit extracts.

Moreover, the minimum inhibitory concentrations (MIC) of the fruit extracts and nanoparticles against Gram-negative bacteria (*Yersinia pseudotuberculosis* ATCC 911, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 13315, and *Pseudomonas aeruginosa* ATCC 43288) and Gram-positive bacteria (*Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus* ATCC 25923, and *Bacillus subtilis* ATCC 6633) were determined the using the medium microdilution procedure. Similar to the antioxidant activity findings, the Fe-NPs demonstrated stronger antimicrobial activity compared to the fruit extracts.

Keywords: Antimicrobial, antioxidant, firethorn, iron nanoparticle, pyracantha coccinea

Öz

Bu çalışmanın amacı, ateş dikeni meyvelerinden yeşil sentez yolu ile elde edilmiş metal nanopartiküllerin biyolojik aktivitesini belirlemektir. Gümüşhane Üniversitesi kampüsünde peyzaj amacıyla yetiştirilen ateş dikeni bitkisinin (*Pyracantha coccinea*) meyve ve tohumlarından elde edilen ekstraktlardan demir nanopartikülleri (Fe-NP) sentezlenmiştir. Hem ekstraktların hem de sentezlenen demir nanopartiküllerinin antioksidan ve antimikrobiyal özellikleri değerlendirilmiştir.

*Corresponding author: akarblnt@gmail.com

Volkan Karakullukçu orcid.org/0000-0002-0893-6423
Bülent Akar orcid.org/0000-0002-1421-374X
Cemalettin Baltacı orcid.org/0000-0002-4336-4002
Azer Özad Düzgün orcid.org/0000-0002-6301-611X
Ömer Karpuz orcid.org/0000-0002-0546-9831

Fe-NP'leri karakterize etmek için FT-IR spektroskopisi, taramalı elektron mikroskobu (SEM) ve X-Işını Kırınımı (XRD) analizleri gerçekleştirilmiştir. Meyve özlerinin ve Fe-NP'lerin antioksidan aktivite etkinliği, ABTS radikal süpürme aktivitesi, DPPH radikal süpürme aktivitesi, demir indirgeyici antioksidan güç (FRAP), toplam fenolik içerik (TPC), toplam flavonoid (TFC) ve toplam antioksidan içeriği (TAC) gibi antioksidan aktivite belirleme testleri kullanılarak tespit edilmiştir. Tüm antioksidan aktivite testlerinin sonuçları, demir nanopartiküllerinin meyve ekstraktlarından daha yüksek antioksidan aktivite sergilediğini göstermiştir.

Ayrıca Gram-negatif bakterilere (*Yersinia pseudotuberculosis* ATCC 911, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 13315 ve *Pseudomonas aeruginosa* ATCC 43288) ve Gram-pozitif bakterilere (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615) karşı meyve ekstraktlarının ve nanopartiküllerin minimum inhibe edici konsantrasyonu (MIK), mikrodilüsyon besiyeri yöntemi kullanılarak belirlenmiştir. Antioksidan aktivite bulgularına benzer şekilde, Fe-NP'ler meyve özlerine kıyasla daha güçlü antimikrobiyal aktivite göstermiştir.

Anahtar Kelimeler: Antimikrobiyal, antioksidan, ateş dikeni, demir nanopartikül, pyracantha coccinea

1. Introduction

Organisms employ various strategies to cope with adverse biotic and abiotic environmental factors in order to survive. Animals rely on their mobility, enabling them to flee from predators, avoid extreme temperatures, and orient themselves toward light sources. In contrast, plants, lacking the ability to move, have evolved different mechanisms to combat these stressors. When faced with environmental changes, plants detect stress conditions and respond at the cellular level to ensure their survival (Gull et al., 2019). This cellular response leads to the production of secondary metabolites, which help minimize the harmful effects of stress on the plant. Secondary metabolites can be significantly induced in response to stress (Pagare, 2015; Isah, 2019). These compounds play a vital role in the adaptation of plants to environmental changes and also hold economic value as they are utilized in pharmaceuticals, food flavorings, cosmetics, and insecticidal chemicals (Thirumurugan, 2018). The primary types of secondary metabolites include terpenoids, alkaloids, phenolics, glycosides, tannins, and saponins (Paiva et al., 2010; Kabera, 2014). These secondary metabolites possess a wide array of properties, such as anti-inflammatory, antimalarial, anthelmintic, analgesic, antimicrobial, antioxidant, antiarthritic, antidiabetic, antihypertensive, antifungal, anticancer, cardioprotective, antispasmodic, and antihistamine properties (Zehra et al., 2019).

As plants serve as the primary source of these essential compounds, their importance to human health is substantial. To determine the biological activities of these compounds in plants, researchers commonly conduct studies on antioxidant and antimicrobial activity. New methods are continuously being developed to enhance the biological activity of extracts containing these secondary metabolites from plants. One such method is the production of nanoparticles through green synthesis using plants in nanotechnology. Nanotechnology involves manipulating matter using chemical and physical techniques to create materials with specific properties suitable for various applications (Herlekar et al., 2014). While chemical and physical methods can yield well-defined nanoparticles, they are often costly and can contribute to environmental pollution (Pattanayak and Nayak, 2013; Gottimukkala et al., 2017). The alternative approach of synthesizing nanoparticles from biological sources, such as microorganisms and plant biomass, is more economical and environmentally friendly (Pattanayak and Nayak, 2013). Additionally, nanoparticles derived from biological materials have unique optical, chemical, photoelectrochemical, and electronic properties, making them particularly interesting for research. This has led to a growing focus on "green synthesis" procedures using various organisms, ranging from bacteria and fungi to plants (Mohanpuria et al., 2008). During metallic nanoparticle green synthesis, a redox reaction occurs through the reduction capacity of cellular or extracellular compounds, such as carbohydrates, proteins, phenols, organic acids, and other metabolites. These compounds, whether single metabolites or metal groups, donate electrons to metal cations, converting them into metallic nanoparticles at the nanometric scale with zero-charge (Vitta et al., 2020). This reaction typically occurs spontaneously at room temperature without requiring significant changes in pressure and is completed within minutes. Ultimately, the phytochemicals present in plant extracts convert iron ions into iron nanoparticles (Ebrahiminezhad, 2018).

The firethorn plant (*Pyracantha coccinea* Roemer), belonging to the *Rosaceae* family, is the subject of this study. This plant naturally grows in Southern and Southeastern Europe, Italy, the Balkans, the Crimea, the Caucasus, and Turkey in the form of shrubs and is also cultivated as an ornamental plant (Tunç et al., 2020). The fruits of the firethorn plant have been traditionally used in medicine for their diuretic and heart-tonic properties (Tunç et al., 2020).

The aim of this study is to synthesize iron nanoparticles from the fruits of the firethorn plant (FF) (*Pyracantha coccinea*) grown as an ornamental plant for landscaping purposes on the Gumushane University-Main Campus area. The researchers aim to determine the antimicrobial and antioxidant activities of these nanoparticles and FF extracts and observe how the activity values change with nanoparticle formation.

2. Materials and Methods

2.1. Sampling

The FFs were collected in November 2022 from plants grown for landscaping purposes within the campus area of Gümüşhane University. The collected fruits are left to dry at room temperature and without light.

2.2. Extraction

First, the dried fruit samples were ground into a powder with a blender. Then, 500 g of the powdered sample was mixed with 7.5 L of water. The mixture was boiled in a closed container for 2.5 hours and cooled. Then, filtration of aqueous extract was performed through sieves with pore aperture of 2.00 mm, 1.00 mm, and 0.25 mm, respectively. Then, the total soluble solids of the FF extract were determined as 4.00%±0.25% by analysis with a digital refractometer (Hanna HI96801, Hanna Instruments, Milan, Italy). The total soluble solids of the FF extract were then increased to 10% under a pressure of 150 mbar at 60 °C using a vacuum evaporator (Gedikli 2022).

2.3. Preparation of Iron Nanoparticles

In order to prepare iron nanoparticles from FF samples, a 125 mL solution that contains 2 M Fe^{+3} and 1 M Fe^{+2} was prepared from $FeCl_3.6.H_2O$ and $FeSO_4$ chemicals. The pH of 250 mL of FF extract with total solids of 10% was set to 11 with 1.0 M NaOH using a pH meter (OHAUS Starter 3000, Ohaus Corporation, Parsippany, NJ, USA) The solution was then mixed in a magnetic mixer (500 rpm) while Fe^{+2}/Fe^{+3} solution was added to the extract mixture at a speed of 1 second/1 drop. Then the beaker was covered for 60 minutes and mixed at 500 rpm.

Color change (Complete conversion to black) was observed in the solution due to the formation of Fe_3O_4. The resulting FF based iron nanoparticle (FF-FeNP) was centrifuged (NÜVE NF 800R) at 12000 rpm. The residue was centrifuged with the addition of pure water for washing purposes, and this step was repeated four times. Finally, drying was carried out in the oven at 60 °C (Yusefi et al. 2020, Gedikli 2022).

2.3.1. Characterization of FF-FeNPs

At the East Anadolu High Technology Application and Research Center of Atatürk University, analyses of FF-FeNPs using XRD, FTIR, and SEM were conducted. Using the Opus program on the Bruker VERTEX 70v (Bruker Optik GmbH, Rosenheim, Germany) device, FT-IR spectrometer readings were done as absorbents at 4 cm^{-1} accuracy 32 times in the 400–4000 range. SEM images and elemental analyses of nanoparticles were measured through the in-lens detector in the scanning electron microscope (SEM-Zeiss Sigma 300, Carl Zeiss AG, Oberkochen, Germany). The crystal size was examined via the Panalytical Empyrean XRD (Empyrean, Almelo, The Netherlands) at 45 kV, 40 mA, and 0.05 degrees per second scanning speed.

2.4. Antioxidant Analysis

2.4.1. ABTS radical scavenging activity

150 µL of the sample and 10 mg of Fe-NP was taken, and 2.85 mL of ABTS solution was added. After vortexing the solution, the mixture was kept in the dark for 2 hours. The absorbances were read via spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 734 nm. The same procedure was repeated by taking 150 µL of standard ascorbic acid (Ahmed et al. 2015).

2.4.2. DPPH radical scavenging activity

100 µL of the sample and 10 mg of Fe-NP was taken, and 3 mL of DPPH was added to the working solution. The mixture was vortexed and held for 30 minutes. A 517 nm spectrophotometer reading followed this. The same procedure was performed for the standard ascorbic acid. The DPPH radical scavenging activity amounts are calculated as indicated below in Equation 1 (Ahmed et al. 2015).

$$\% \text{ inhibition capacity} = ((Ac-As)/Ac)) \times 100 \quad (1)$$

Ac: Blind Sample Absorbance

As: Sample Absorbance

2.4.3. Ferric reducing antioxidant power (FRAP)

A 250 µL sample and 10 mg of Fe-NP was taken, and 2750 µL FRAP solution was added. The mixture was vortexed and held for 30 minutes. 250 µL were taken from the standards, and the same procedure was carried out. The quantities of FRAP substances were determined using the linear equation of the calibration curve obtained with $FeSO_4$ (10,

25, 50, 100, and 200 µl/ml) solution. FRAP was determined as mg $FeSO_4$ equivalent/g (Ahmed et al. 2015).

2.4.4. Total antioxidant capacity (TAC)

500 *µL* of a sample and 10 mg of Fe-NP was added to 2.5 mL of deionized water. Then, 1 mL of molybdate reactive was added to the mixture. The mixture was vortexed and incubated at 95°C in a water bath for 90 minutes. The samples are kept for 30 minutes to reach room temperature after incubation. The absorbances of the mixtures were read on a spectroscopic photometer at 695 nm. The same procedure was followed for the standards. The TAC amount of the samples was determined based on the linear equation of the calibration curve generated using the solution of the ascorbic acid standard (25, 50, 100, 150, 400, and 800 *µg*/mL) as the TAC mg AA equivalent/g sample (Prieto et al. 1999).

2.4.5. Total phenolic content (TPC)

The total phenolic substance content of the FF was analyzed using the Folin–Ciocalteu's reactive (Slinkard and Singleton 1977). In the first phase, a 0.3 mL sample and 10 mg of Fe-NP was taken, 3.4 mL of pure water was added, 0.5 mL of methanol was added to the mixture, and 200 *µL* of the Folin-Ciocalteu's reactive was added. Then, the mixture was vortexed and held for 10 minutes in room conditions, and 0.6 mL of 10% liquid sodium carbonate solution was added. After the final mixture was vortexed again, it was incubated at room temperature in the dark for 2 hours. At the end of this period, the absorbance value of the mixture was recorded by reading on a spectrophotometer at 760 nm. In addition, a mixture of 500 *µL* methanol, 100 *µL* folin–ciocalteu's reactive, 600 *µL* sodium carbonate, and 3.7 mL water has been used as a blind.

The amounts of TPC in the samples are shown as mg GA Equivalent/L samples based on the linear equation of the calibration curve generated using gallic acid (160, 120, 80, 60, 40, and 20 *µg*/mL) solution.

2.4.6. Total flavonoid contents (TFC)

A 150 µL and 10 mg of Fe-NP sample was taken in the first stage, and 3.2 mL methanol (30% v/v) was added. 150 *µL* of 0.5 M sodium nitrite solution and 150 *µL* of 0.3 M aluminum chloride solution were added to the mixture. Then 5 minutes waited, and 1 mL of 1 M sodium hydroxide solution was added. Subsequently, the mixture was vortexed again, and after a 10-minute wait, the absorbance was read at 506 nm on the spectrophotometer. The same procedure was followed for the standards. The TFC in the samples were determined by using the linear equation of the calibration curve generated using the solution of quercetin (25, 50, 100, 200, and 400 *µg*/mL) as the total flavonoid mg catechin equivalent/g sample (Kasangana et al. 2015).

2.5. Minimum Inhibition Concentrations (MIC)

MIC of the FF extracts and FF-FeNPs against Gram-negative (*Yersinia pseudotuberculosis* ATCC 911, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 13315, and *Pseudomonas aeruginosa* ATCC 43288) and Gram-positive (*Streptococcus pyogenes* ATSC 19615, *Staphylococcus aureus* ATCD 25923, and *Bacillus subtilis* ATCB 6633) bacteria was determined by fluid microdilution method. The concentration of the FF extract was set at 90-0.17 mg/ml, and the nanoparticle concentration was 60-0.05 mg/mL. The experiments were carried out using 96 well-plate three times. 50 *µl* of Luria Broth (LB) feed was added to each well (except for the 12th well), and 100 *µl* LB was added to the 12th well. In order to evaluate sterility control, the 12th well was used. Furthermore, the eleventh well growth (50 *µl* LB and 50 *µl* bacteria) was assessed as the control. 50 *µL* of the FF extract or the first concentration of the nanoparticles were taken from the first well to the 10th well. The first concentration of MIC in which Fe-NPs inhibit the strains, incubated at 37°C, was determined, and ampicillin was used as a control (Üçüncü et al. 2020).

3. Results and Discussion

3.1. Characterization of FF-FeNPs

Researchers have used Fourier transform infrared spectroscopy (FTIR) to identify phenolic compounds, which are biomolecules that exhibit capping and reducing properties in the formation of iron nanoparticles synthesized from biological sources.

Figure 1 displays the FTIR spectra of iron nanoparticles and FF extract. Various vibration bands observed in the range of 650-4000 cm^{-1} indicate that functional groups are responsible for the reduction of iron (Figure 1). O–H stretching vibrations of the C-OH and H_2O groups were observed around 3419 cm^{-1} and 3373 cm^{-1}. The peak around 1622 cm^{-1} shows C=C stretching. C-O-C absorption peaks of carboxylated acid groups in the structure are seen around 1030 cm^{1}.

Peaks around 1430 and 1388 cm^{-1} indicate polyphenolic O-H bending and aromatic structure. The peak at 1068 cm^{-1} shows the presence of polyphenols due to the C-N stretching vibration, which represents aliphatic amines. The groups

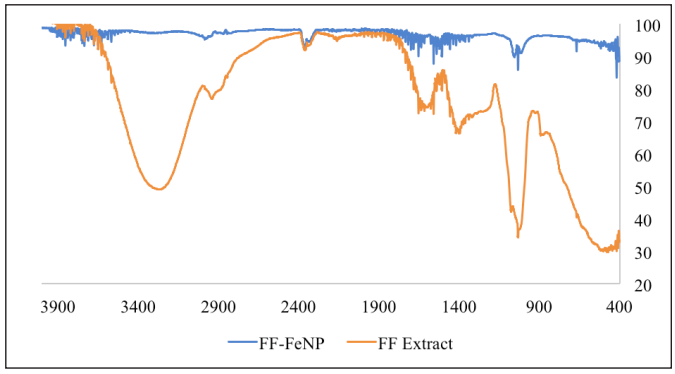

Figure 1. FTIR spectra of firethorn fruit iron nanoparticles (FF-FeNP) and firethorn fruit extract (FF Extract).

of methyl and methylene belong to the plant extract lipids caused stretching vibration at the band of 2950 cm^{-1}.

The O-H (1250–1310 cm^{-1}, 675–815 cm^{-1}), C=O (1690–1740 cm^{-1}), C=C (1520–1590 cm^{-1}), N-H (1415–1490 cm^{-1}), C-H (2750–2860 cm^{-1}), O-H (3310–3390 cm^{-1}), C–O–C (1025–1195 cm^{-1}), C-N (2310–2350 cm^{-1}) bands reported by Khan et al. (2020) seen in FTIR give traces of aromatic components in the structures examined. The bands in the spectrum, at 870 cm^{-1} and 668 cm^{-1}, are attributed to CH bending. As described above, it is typical of the heterocyclic compounds of flavonoids and alkaloids found in plant extract (Alsammarraie et al. 2018). Therefore, the FTIR spectra of FF-FeNP produced by green synthesis and FF extracts exhibit some similar characteristics. The results indicate that the Fe_3O_4 structure is capped by FF organic structures. Fico et al. (2000) detected about 30 metabolites in flowers and fruits of firethorn plant. Both flowers and fruits of firethorn have been reported to have high flavonoid content (Fico et al. 2000).

Energy-dispersive X-ray (EDX) spectroscopy in Scanning Electron Microscopy (SEM) can provide elemental maps showing properties at the atomic level. The EDX spectra of Fe-NPs synthesized using FF extracts are shown in Figure 2 and their numerical values are shown in Table 1. The elements evaluated in EDX analyses are iron, carbon and oxygen. The percentage-to-weight ratios whose elements are detected in FF-FeNPs are 47.33 for oxygen, 38.46 for carbon, and 14.21 for iron. The percentages of carbon, oxygen and iron atoms were determined as 49.92, 46.11 and 3.97, respectively. The presence of the Fe and O phase in the EDX spectra in the samples and their high quantity confirms that the FF-FeNPs are synthesized at high purity. A high percentage of oxygen indicates that nanoparticles are composed of iron oxide (Demirezen et al. 2019). The presence of carbon and oxygen can be due to the organic compounds' existence. Gedikli (2022) obtained separate iron nanoparticles from black tea and green tea. While iron, carbon and oxy-

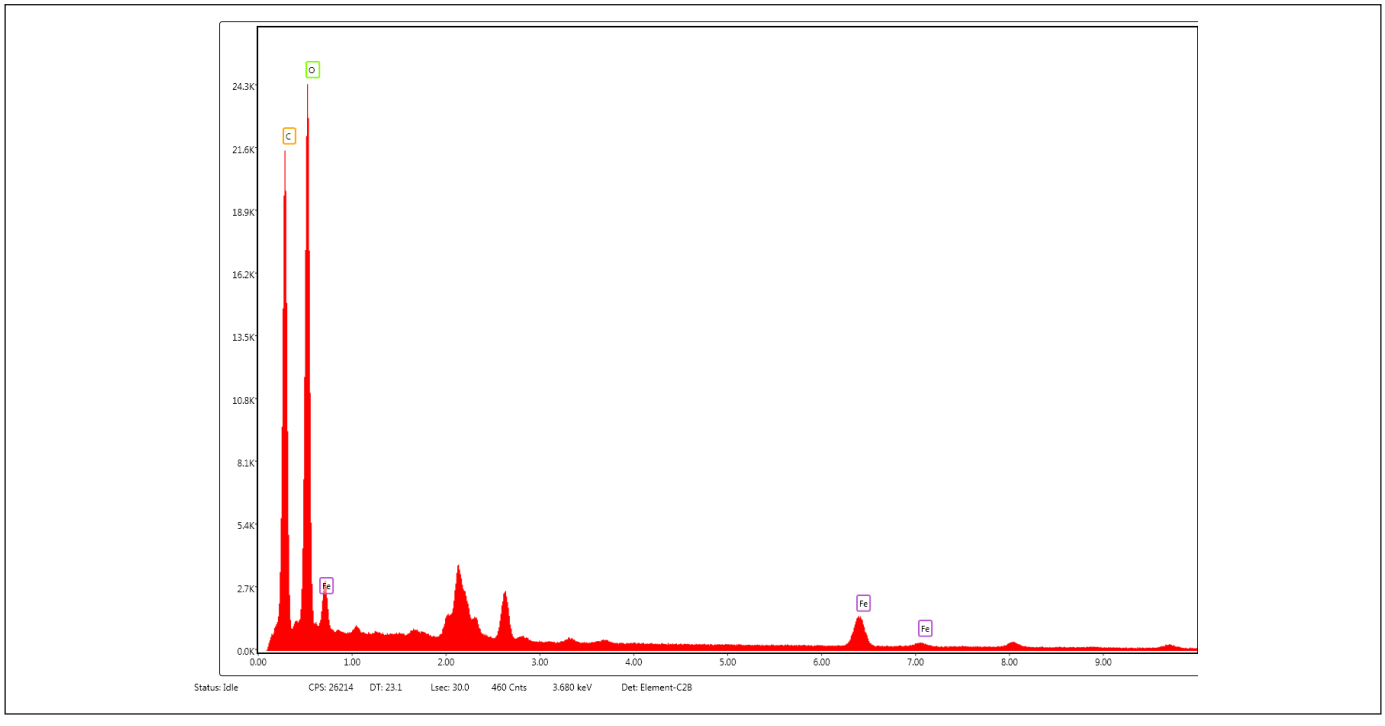

Figure 2. EDX spectrum of synthesized firethorn fruit iron nanoparticles (FF-FeNP).

gen in these iron nanoparticles obtained from black tea were 8.62, 40.15 and 46.85 %, respectively, in green tea they were 7.41, 41.97 and 49.31 %. Chatterjee et al. (2021) concluded that the strong presence of iron and oxygen elements in the EDX analysis of the iron nanoparticle that were synthesized from rice seeds is a sign of the synthesis of Fe-NPs.

Table 1. EDX element values of Fe-NPs obtained from the fruits of firethorn plant.

Element	% Weight	% Atom
C	38.46	49.92
O	47.33	46.11
Fe	14.21	3.97

Scanning Electron Microscopy images of FF-FeNPs at 80.00 KX magnifications are given in Figure 3. It has been observed that FF-FeNPs are nano-sized and spherical. In Figure 3, the dimensions of the nanoparticles marked in the SEM images at 80.00 KX were measured as 11.25 and 13.75.

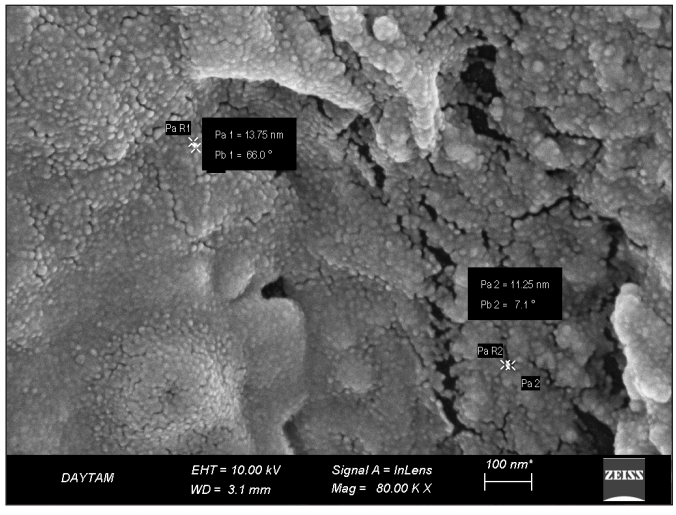

Figure 3. Scanning electron microscopy (SEM) images of the firethorn fruit iron nanoparticles (FF-FeNP).

X-ray diffraction analysis (Figure 4) showed no significant peak for green synthesized Fe_3O_4 NPs. This lack of peaks indicates an amorphous structure of the nanoparticles produced (Machado et al. 2014, Wang et al. 2014a, Machado et al. 2015, Ebrahiminezhad et al. 2016). The presence of amorphous iron nanoparticle structure has been detected in previous studies on waste or leaf extracts such as eucalyptus and sorghum bran (Njagi et al. 2011, Wang et al., 2014a,

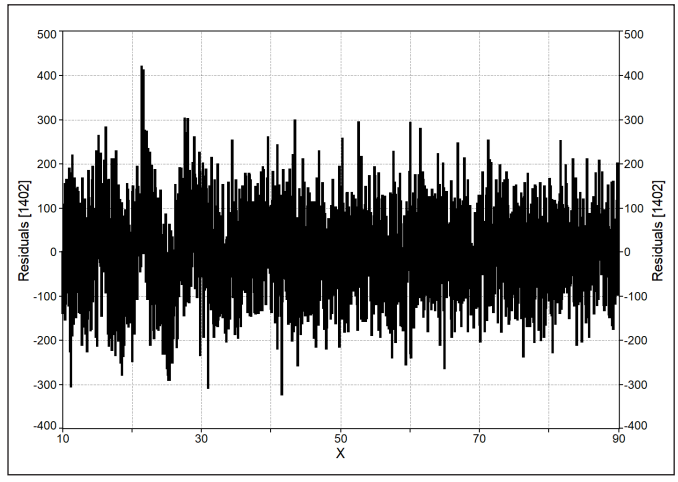

Figure 4. X-ray diffraction (XRD) analysis of synthesized firethorn fruit iron nanoparticles (FF-FeNP).

Wang et al. 2014b). The wide shoulder range of 2 Theta values between 10° and 20° degrees has been suggested to be due to the existence of organic materials in leaf extracts as stabilizing agents for NPs (Wang 2014a, Ebrahiminezhad et al. 2016).

3.2. Antioxidant Activity

The antioxidant activities of FF extracts and FF-FeNPs obtained by green synthesis were evaluated through six different mechanisms (Table 2).

ABTS, a radical scavenging assay, is widely used to determine the antioxidant activity of compounds. In our study, the ABTS values of the FF extract and produced Fe-NP were 7.92 mg/g and TEAC equivalents of 15834.57 mg/g. It was determined that the capacity of Fe-NPs was much higher than the FF extract. A study on the extracts of *P. coccinea* (Keser 2014) showed that ethanol, methanol, and acetone extracts exhibit higher ABTS and DPPH radical scavenging activity than BHT. The presence of high phenolic compounds in FF was therefore concluded to be an excellent source of natural antioxidants (Keser 2014). In another study on the fresh fruits of firethorn, ABTS ethanol extract was found to be 4.00, and water extract was 0.71 mg TEAC/g (Sarikurkcu and Tepe 2015).

The scavenging activity of the DPPH radical forms the basis of the DPPH antioxidant test (Sharma and Bahat 2009). In the present study, DPPH values of the FFs and FF-FeNP were determined as 11.47±0.26 and 15400±400 mg AA/g, and DPPH % inhibition values were determined as 91.44±0.44 and 94.97±0.11, respectively. As in ABTS method, the activity values of Fe-NP in the DPPH method

Table 2. Antioxidant activities of FF extract and FF-FeNPs.

Analyses	Samples	Concentration mg/g	
ABTS$^{\bullet+}$ mg TEAC	FF extract	7.92	±0.1
	FF-FeNPs	15834.57	±197.53
DPPH mg AA	FF extract	11.47	±0.26
	FF-FeNPs	15400	±400
DPPH % Inhibition	FF extract	91.44	±0.44
	FF-FeNPs	94.97	±0.11
FRAP mg $FeSO_4$	FF extract	30.55	±0.62
	FF-FeNPs	20368.89	±411.11
TAC mg AA	FF extract	14.83	±6.52
	FF-FeNPs	7332.81	±945.32
TPC mg GA	FF extract	11.46	±0.22
	FF-FeNPs	5741.67	±771.24
TFC mg QUE	FF extract	46.55	±4.75
	FF-FeNPs	8633.33	±642.91

FF extract: Firethorn fruit extract, **FF-FeNPs:** Firethorn fruit iron nanoparticles, n:3.

were much higher than in the FF extract. Sarikurkcu and Tepe (2015) measured DPPH values of ethanol and water extracts of FF as 6.12 ± 0.292 and 2.05 ± 0.101 mg TEs/g, respectively. Keser et al. (2014) determined DPPH % inhibition values of the same plant fruit as ethanol (78.73±0.27), water (27.62±0.09), methanol (93.43±0.81), acetone (81.18±0.21) and diethylether (7.807.80±0.190.19). Other studies have also been carried out to determine the activity value of FFs using the DPPH method (Kerasioti et al. 2019, Tunç et al. 2020, Turu et al. 2020, Sharifi-Rad, 2021).

Another method for measuring antioxidant activity is the iron-reducing antioxidant power (FRAP) method developed by Benzie and Strain (1996). FRAP activity values of FF extracts and FF-FeNPs were measured as 30.55±0.62 mg $FeSO_4$/g for FF extracts and 20368.89±411.11 mg FeSO4/g for FF-FeNP. Similar to the previous methods above, FF-FeNPs had FRAP values considerably higher than FF extracts. In other studies on firethorn, the FRAP values of the FF extract were 3.18 ± 0.050 in ethanol extract, 0.73± 0.082 mg TEs/g fresh fruit in water extract (Sarıkurkcu and Tepe 2015), 6.75±0.52 AAE/g (Sharifi-Rad 2021). The FRAP value of the methanol extract of Himalayan firethorn (*Pyracantha crenulata* (D.Don) M.Roem.) fruits, which is classified in the genus *Pyracantha*, to which the firethorn plant belongs as well, was 5.40±0.05 mm AAE/100g (Saklani et al. 2011), and FRAP value of *Pyracantha fortuneana* (Maxim.) Li species water extracts was 18.1 ± 0.14 (Fu et al. 2010). Many factors, such as genetic diversity, environmental factors, and fruit ripening times, may impact FRAP activity results in the studies mentioned.

In TAC analysis, which is one of the six antioxidant activity determination methods used, the activity values of FF-FeNPs produced in our study were measured quite higher than those of FF extracts. TAC values were 14.83±6.52 mg AA/g in firethorn fruit extracts, and 7332.81±945.32 mg AA/g in Fe-NPs. Sarıkurkcu and Tepe (2015) reported the total antioxidant capacity values of ethanol and water extracts of FFs as 6.69 ± 0.744 and 1.96 ± 0.381, respectively, and Sharifi-Rad et al. (2021) reported 3.97 ± 0.28 mg AAE/g. These values are lower than our study results. The TAC values of the methanol extracts of the *Pyracantha crenulata* species in the *Pyracantha* genus at different concentrations were measured in the range of 18.05 -66.68 μM Trolox equivalents (Pal et al. 2013).

TPC, also defined as secondary metabolites and mostly found in plant tissues such as vegetables and fruits, show antioxidant activity as well as antimicrobial (Akar et al. 2020, Baltacı et al. 2022), antifungal (Simonetti 2020, Possamai Rossatto et al. 2021). There are many studies showing

that it has anticancer and antiangiogenic (Abbaszadeh et al. 2019), anthelmintic (Kaska et al. 2018), and anti-inflammatory (Demir et al. 2019) activities. TPC analysis showed that TPC values of FF-FeNP were considerably higher than FF extracts. TPC values of FF extracts and FF-FeNP were determined as 11.46±0.22 mg AA/g and 5741.67±771.24. In a study on fresh FF, TPC in methanol and water extracts were reported as 1.31±0.198 and 0.38±0.022 mg GAEs/g, respectively (Sarıkurkcu and Tepe 2015). Sharifi-Rad et al. (2021) found the TPC values of methanol extracts of FF to be 14.48±1.09 higher than the results in this study. However, there was no significant difference between the two activity values.

According to the TFC, which is one of the most used antioxidant analyses, the activity value of FF-FeNPs (8633.33±642.91) was found to be considerably higher than the activity value of FF extract (46.55±4.75), in accordance with previous analyses. Sarikurkcu and Tepe (2015) reported the ethanol and water extract TFC values of firethorn fresh fruit as 0.22 ± 0.014 and 0.03 ± 0.002 mg REs/g, respectively. Sharifi-Rad et al. (2021) determined 0.13 ± 0.01 mg QE/g in methanol extracts of firethorn fruits.

Although scientific literature has revealed that FF has high antioxidant activity, there are differences in activity values. Factors affecting the chemical composition of plants, such as different solvents used in plant extraction, variations between different plant parts, individual genetic diversity, different developmental stages of plants, production process conditions and environmental changes (soil, air, water, pH, temperature, light, pollutants), may be the reason for these differences (Figueiredo, 2008, Akar 2021).

The antioxidant activities of plant extracts based on Fe-NPs are considerably higher than those of plant extracts. This situation occurs due to the intense bonding of organic compounds such as alkaloids, flavonoids, tannins, phenols, and saponins in plant extracts around Fe_3O_4 (Mahendiran et al. 2017, Rehana et al. 2017). Vitta et al. (2020) determined the antioxidant activities of *Eucalyptus robusta* Sm plant extracts and Fe-NP obtained from these extracts using DPPH, TPC, and TFC antioxidant activity determination methods. They reported DPPH activity IC50 values as 423.14 µg/mL, 81.63 µg/mL, and 7.19 µg/mL for *E. robusta* plant extract, plant extract-derived Fe-NP, and standard quercetin, respectively. As seen from the standard in this test, a lower value indicates higher activity. In the same study, the values obtained for TPC (158.47 mg GAE /g in plant extract, 98.21 mg GAE /g in Fe-NP) and TFC (131.12 mg QE /g in plant extract; 40.54 mg QE /g in Fe-NP) were higher in plant leaf extract than in Fe-NP. Vitta et al. (2020) stated that the low TPC and TFC in nanoparticles might be due to the reduction reaction include these compounds in Fe-NP biosynthesis. In addition, regarding the existences of phenolic compounds in FeNPs, they stated that some of these compounds were not reduced and were related to the fact that they could interplay in the stabilization of nanoparticles.

3.3. Antimicrobial Activity

The antibacterial effects of FF extract and FF-FeNP were investigated using seven isolates. MIC values in the FF extract were 20 mg/mL against *S. pyogenes* and *P. aeruginosa* strains, 40 mg/mL against *S. aureus* and *P. vulgaris*, 10 mg/mL for *E. coli*, 1.25 mg/mL for *Y. pseudotuberculosis* and 5 mg/mL for *B. subtilis* (Table 3). The MIC value of FF-FeNPs obtained using the plant extract was 0.75 mg/mL for *S. pyogenes*, *B. subtilis*, *P. vulgaris, E. coli*, *Y. pseudotuberculosis*, and *S. aureus*. The MIC value of Fe-NPs against *P. aeruginosa* was 0.375 mg/ml (Table 3).

It was observed that FF-FeNPs from plant extract had 53 times more activity on *P. aeruginosa*, *S. aureus*, and *P. vulgaris*, 27 times more on *S. pyogenes*, and 13 times more on *E. coli* than plant extract. It was determined that the plant extract and FF-FeNPs had 1.7 and 7 times more antimicrobial activity against *Y. pseudotuberculosis* and *B. subtilis*, respectively. The plant extract showed the highest activity against *Y. pseudotuberculosis*. Fe-NP was found to have the highest activity against *P. aeruginosa* strain. The antimicrobial activity differences were influenced by both the sensitivity of bacteria and the iron oxide content of the nanoparticles, as reported by Ezealigo et al. (2021). Particularly noteworthy is the higher antimicrobial activity exhibited by

Table 3. Antimicrobial activity of FF extract and FF-FeNPs.

Bacteria	FF Extract MIC (mg/mL)	FF-FeNP MIC (mg/mL)
B. subtilis	5	0.75
S. aureus	40	0.75
S. pyogenes	20	0.75
E. coli	10	0.75
P. aeruginosa	20	0.375
P. vulgaris	40	0.75
Y. pseudotuberculosis	1.25	0.75

green-synthesized Fe-NPs compared to FF extracts against all bacteria. This enhancement in activity can be attributed to the presence of plant-specific functional groups on the surface of the NPs, which significantly contribute to their increased antimicrobial efficacy. Moreover, the smaller size of the NPs facilitates their penetration into the bacterial cell wall, ultimately leading to cell death. Consequently, both the nanoparticle size and the structure of the bacterial cell wall play crucial roles in determining the antimicrobial activity of the nanoparticles, as emphasized by Kanagasubbulakshmi and Kadirvelu (2017).

Studies in the scientific literature have reported that the antimicrobial activities of Fe-NPs obtained by using different plant extracts are high. Antimicrobial activities of aqueous extracts from the leaves of *Eucalyptus robusta* Sm species and Fe-NPs based on these extracts were determined by Vitta et al. (2020), and they concluded that Fe-NP showed higher antimicrobial activity than the plant extract against *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis* species. Likewise, in a study by Irshad et al. (2017), where they compared the antimicrobial effects of *Punica granatum* L. bark extracts and iron oxide nanoparticles synthesized with these extracts, it was conclusively demonstrated that the bacterial cell (*Pseudomonas aeruginosa*) experienced denaturation and shrinkage in response to the treatment with FeNPs. On the other hand, the treatment with *Punica granatum* bark extract alone resulted in relatively less denaturation.

4. Conclusion

Green synthesis was used to produce FF-FeNPs from firethorn fruit extracts. The size and shape of the nanoparticles were examined using SEM images. Analysis of the EDX spectra revealed that the Fe-NPs had high purity, with significant amounts of Fe and O phases. XRD spectra analysis indicated that the nanoparticles had an amorphous structure. The FT-IR analysis results showed the involvement of polyphenols in the synthesis of FF-FeNPs.

In terms of antioxidant activity, FF-FeNPs demonstrated higher levels compared to the fruit extracts, as assessed by various methods, including ABTS, DPPH, FRAP, TAC, TPC, and TFC. The antibacterial properties of both the FF extract and FF-FeNPs were evaluated against seven different isolates, and it was found that Fe-NPs exhibited stronger antimicrobial activity than the fruit extracts.

These findings suggest that FF-FeNPs, synthesized using firethorn fruit extracts, possess notable antioxidant and antibacterial activity, making them promising candidates for non-toxic and environmentally friendly nano or micro-scale materials in various applications.

Conflict of Interest

The authors declared that there is no conflict of interest.

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