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RESEARCH PAPER

Thermal, Magnetic Properties and Antimicrobial Effects of Magnetic Iron Oxide Nanoparticles Treated with *Polygonum cognatum*

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Abstract
This study aimed to synthesize magnetic Fe_3O_4 nanoparticles treated with *Polygonum cognatum* and then evaluate its thermal, magnetic, and antimicrobial activity. The surface morphology of MNP was characterized by SEM and image mapping. The XRD measurements were carried out to calculate the crystallite size of the sample, and the cubic structure of the iron oxide nanoparticles was identified in the XRD pattern. FT-IR spectroscopy confirmed that the bioactive molecules in the plant structure are attached to the MNP surface. Thermal analysis showed that the plant extract reduced the thermal stability of pure MNP. From the magnetization curves obtained by VSM, it was seen that MNP treated with the plant has superparamagnetic properties and its saturation magnetization value is 26.073 emu/g. Besides, the antimicrobial effects of MNP, plant extract, and MNP treated with plant extract against *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Bacillus megaterium*, and *Candida albicans* were examined.

Keywords Fe_3O_4 nanoparticles · *Polygonum cognatum* · Antibacterial · SEM · Thermal

1 Introduction

Nanomaterials have many application areas due to their large surface area and pores and the ability to be arranged according to the intended purpose (Salman et al. 2020; Tian et al. 2013). Magnetic nanoparticles, which have a very large surface area, have become interesting due to their wide application areas, such as magnetic resonance imaging (MRI) (Seo et al. 2006), drug release (Chouly et al. 1996; Zhang et al. 2002), tissue repair, heavy metal adsorption (due to the ability to quickly and easily separate from the aquatic environment) (Yılmaz et al. 2019), cell separation, and data storage (Inbaraj et al. 2012; Tamer et al. 2010). At the same time, studies are underway on the use of nanoparticles in the treatment of cancerous brain tumor cells and breast cancer cells (Subramani et al. 2009). It is also believed that a drug system based on the use of an external magnetic field will be developed to intervene in the areas where emergency treatment is required, by using the magnetic properties of magnetic nanoparticles (Pareta et al. 2008; Tran et al. 2010). To use these nanoparticles in the field of biochemistry and bioengineering, they must have a narrow particle size distribution and high magnetization to maintain their physical and chemical properties (Sun et al. 2002).

Different types of nanoparticles such as gold, silver, and cobalt have been synthesized and modified, then their antibacterial properties have been thoroughly investigated. Generally, polymers are modified with MNPs due to their properties such as low volume/surface area ratio, high adsorption capacity, and selective adsorption of the target molecule (Bilici et al. 2018). The antibacterial effects of superparamagnetic nanoparticles modified with chitosan on the surface (Inbaraj et al. 2012) and electromagnetic exposure of magnetic nanoparticles on some bacteria (Antoniea et al. 2012) can be shown as an example of studies on magnetic nanoparticles. Arokiyaraj et al. investigated the antibacterial properties of magnetic nanoparticles treated with *Argemone mexicana* leaf extract

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(Arokiyaraj et al. 2013). Additionally, studies such as gold nanoparticles used as antimicrobial agents (Chen et al. 2010) and antibacterial effects of silver nanoparticles on some bacteria (Shahverdi et al. 2007) have been performed using different types of nanoparticles.

The abundance of herbal diversity in the world, and even though scientific data on the biological effects and mechanisms of action of many plants are still insufficient, the interest in this issue is increasing day by day (Pekdemir et al. 2020), so much so that most developing countries continue to use herbal remedies, also known as "folk medicine". Another factor influencing this view that synthetic drugs harm people and nature has led to a shift to herbal medicines (Chariandy et al. 1999).

Polygonum cognatum, commonly known as "Madımak", is an edible plant species belonging to the Polygonaceae family with small pink flowers 15–30 cm long (Baytop 1994). This plant has a wide range of habitats such as roadsides, pastures, cliffs, agricultural areas, and grows especially at an altitude of 700–3000 m above sea level (Yıldırım et al. 2003). *Polygonum cognatum* is used pharmacologically as well as for food, depending on the region in which it grows. The roots of the plant are effective in diabetes, stomach indigestion, and also in kidney stones (Üçer 2010; Ulubelen et al. 1992). The plant appears to be an important antioxidant due to its 86.21 mg ascorbic acid (Vitamin C) content, moreover, to being rich in oil, protein, calcium, phosphorus, and sodium (Aker 1989; DEMİR).

In order to increase the possibility of using nanoparticles, an attempt has been made to determine the biocompatibility of nanoparticles in biological systems (Akçinar et al. 2020). In this study, magnetic Fe_3O_4 nanoparticles were synthesized by the co-precipitation method, then it was characterized by FT-IR, SEM, and XRD. We investigated the magnetic and thermal properties of MNP treated with ethanol extract. At the same time, antibacterial properties of MNP treated with *P. cognatum* were studied by disk diffusion method against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus megaterium*, and *Candida albicans* microorganisms.

2 Materials and Methods

2.1 Preparation of the *Polygonum cognatum* Ethanolic Extract

Polygonum cognatum plant was collected from Turkey (Elazığ) and dried in the season, then powdered with an electric blender. 20 g of the powdered plant was dissolved in 200 mL of ethanol and mixed in an ultrasonic homogenizer for 10 min. The homogenate was shaken at 25–30 °C for 24 h in a shaker incubator at 100 rpm. The solution was filtered with Whatman filter paper 1, and the solvent was evaporated at 37 °C with a vacuum evaporator (Pekdemir et al. 2020). The obtained plant extracts were kept at – 20 °C.

2.2 Synthesis of Magnetic Fe_3O_4 Nanoparticles (MNP)

Fe_3O_4 nanoparticles were synthesized by the co-precipitation method. Fe (III) and Fe (II) ions were used for this purpose in a ratio of 2:1 (Pekdemir et al. 2012). 50 mL solution was prepared with 0.1 M $FeCl_2.4H_2O$ and 0.2 M $FeCl_3{\cdot}6H_2O$ in deionized water. Then, 250 mL of 0.3 M NaOH solution was added dropwise into the obtained solution and stirred in parallel for 40 min. The precipitated black magnetite was collected with a magnet and washed with distilled water. The iron salts were oxidized, washed with 0.03 M HCl to convert to Fe_3O_4, and kept in HCl solution for 1 night until it turned brown. Positively charged magnetic particles were centrifuged at 13,000 rpm for 15 min to remove excessive amounts of acid. The magnetic nanoparticles synthesized under room conditions were washed 3 times with deionized water and dried in a vacuum oven at 40 °C. The chemical reaction that occurs during the synthesis of magnetic nanoparticles can be described as follows (Gupta et al. 2005).

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O.$$

2.3 Microorganisms

In this study, *Staphylococcus aureus* ATCC25923, *Klebsiella pneumoniae* ATCC700603, *Escherichia coli* ATCC25322, *Bacillus megaterium* DSM32, and *Candida albicans* FMC17 microorganisms were used. Microorganism cultures were obtained from Firat University, Faculty of Science, Department of Biology, Microbiology Laboratory culture collection.

2.4 Treatment of MNP with *Polygonum cognatum*

The extracted *Polygonum cognatum* plant and synthesized MNP were mixed in a ratio of 1:1 and dispersed in 5 mL ethanol. The prepared solution was incubated in a shaking incubator at 40 °C for 5 h. The incubated solution was prepared for thermal and antimicrobial measurements.

2.5 Antimicrobial Assay of MNP Treated with Ethanolic Extract of *Polygonum cognatum*

The antimicrobial activity of the ethanol extracts of *P. cognatum* was determined according to the disk diffusion method (Collins et al. 1987). Bacterial strains, including *Staphylococcus aureus* ATCC25923, *Klebsiella pneumoniae* ATCC700603, *Escherichia coli* ATCC25322, and *Bacillus megaterium* DSM32, were inoculated in Nutrient Buyyon (Difco) and incubated at 35 ± 1 °C for 24 h. Yeast strains (*Candida albicans* FMC17) were inoculated in Malt Extract Buyyon (Difco) and incubated at 25 ± 1 °C for 48 h. Cultures of the prepared bacteria and yeast were inoculated into Müeller Hinton Agar and Sabouraud Dextrose Agar at a rate of 1%, respectively (10^6 bacteria/mL, 10^4 yeast/mL). After shaking thoroughly, 25 ml was poured in sterile Petri dishes with a diameter of 9 cm, and homogeneously of the medium was dispersed. The discs (6 mm diameter), each impregnated in 100 µl of different extracts, were added to the appropriate agar media inoculated with microorganisms. Then, Petri dishes were stored at 40 °C for 2 h. The inoculated Petri dishes were incubated in bacterial strains at 37 ± 0.10 °C at 24 h for and also in yeasts at 25 ± 0.10 °C for 72 h. As a control, different standard discs were used for bacteria (Ceftrioxane 30 µg/disk) and yeasts (Nystatin 30 µg/disk). Dimethyl sulfoxide (DMSO) was used for negative control. Inhibition zones formed on the medium at the end of the period were evaluated in mm.

3 Results and Discussion

3.1 Characterization of MNP

The FT-IR spectrum of the pure MNP, the extracted *P. cognatum*, and the MNP treated with the *P. cognatum* are demonstrated in Fig. 1. MNP shows characteristic bands at 3445 cm^{-1} (O–H stretching vibration, 1631 cm^{-1} (O–H deformed vibration caused by water adsorbed on the surface), and 630 cm^{-1} (Fe–O stretching vibration) (Pekdemir et al. 2020; Shen et al. 2004). In FT-IR spectra of plant extract and MNP treated with plant extract, 2920–2850 cm^{-1} signals belong to aliphatic C-H stretching vibration (Arokiyaraj et al. 2013). These stretching vibrations were not observed in the spectrum of pure MNP. The peaks between 1800 and 1000 cm^{-1} could be attributed to the functional groups of bio compounds such as flavonoid, phenolic, and fatty acid components in the extracted *P. cognatum* (Pekdemir et al. 2020). Moreover, in the FT-IR spectrum of MNP treated with the plant, the signal is seen

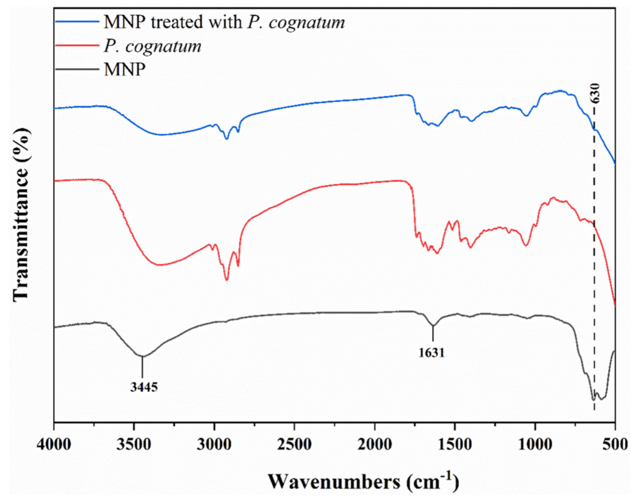

Fig. 1 FT-IR spectra of MNP, *P. cognatum* and MNP treated with *P. cognatum*

at 630 cm^{-1} proves the presence of the Fe_3O_4 nanoparticle in the structure.

The XRD measurement of the magnetic Fe_3O_4 nanoparticles, shown in Fig. 2, was performed using a D8 Advance model diffractometer device. The X-ray source for this measurement was CuKα radiation with a wavelength of 0.15406 nm. The characteristic peaks (2θ angles) of the MNP were 30.5°, 35.5°, 43.1°, 53.4°, 57.6°, and 62.8°. The diffraction peaks of these angles correspond to (220), (311), (400), (422), (511), and (440) (Woo et al. 2004). XRD results show that the synthesized magnetic nanoparticles have a cubic crystal structure. Also, the mean crystallite size of the nanoparticles was calculated from the XRD results using the Debye–Scherrer equation (Mahdavian et al. 2010) and was found to be 17.83 nm.

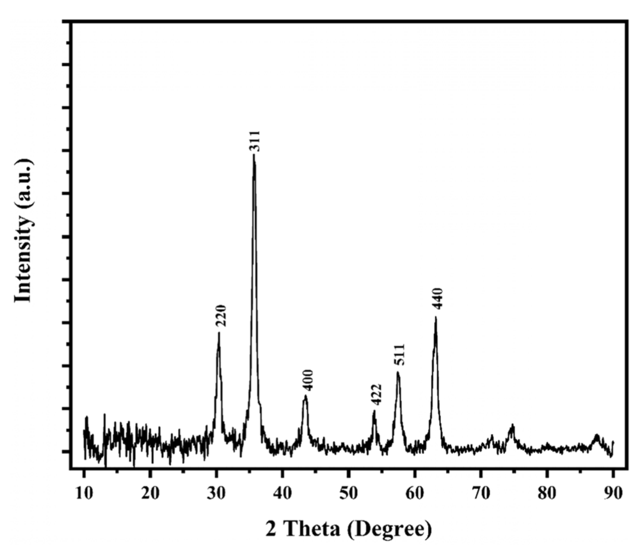

Fig. 2 XRD spectra of MNP

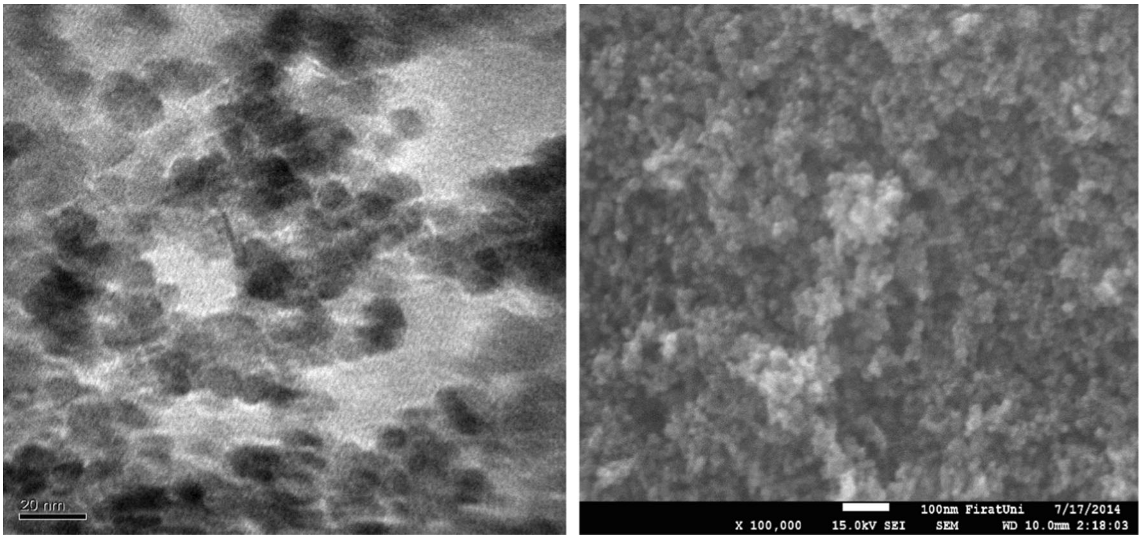

Fig. 3 **a** TEM and **b** SEM images of MNP

Figure 3 depicts the SEM and TEM images of densely packed spherical Fe_3O_4 nanoparticles. Although agglomeration was rarely seen in the SEM images due to the high energy and large surface area of the nanoparticles, they were generally observed to have a uniform distribution without agglomeration. Obviously, determining the particle size of the SEM image is not very easy due to the very fine particles and the presence of agglomeration of particles. The TEM image shows that the mean size of the nanoparticles is less than 20 nm and very close to the value determined by XRD analysis (17.83 nm).

Figure 4 reveals Fe and O image mapping of the magnetic nanoparticles. It confirms the homogeneous distribution of Fe and O atoms on the MNP.

3.2 Thermal Investigation

TGA curves of the pure magnetic Fe_3O_4 nanoparticle and treated MNP with the extracted *P. cognatum* are shown in Fig. 5. For MNP, 2.8% mass loss between 100 and 500 °C is probably related to water loss and dehydroxylation. MNP treated with *P. cognatum* showed a thermal decomposition with two stages. The initial and second decomposition temperatures are 120 and 320 °C, respectively. Besides, a 40% mass loss between 100 and 500 °C is probably related to biomolecules in the plant. TGA results indicate that the pure nanoparticle has reduced thermal stability after treatment with plant extract.

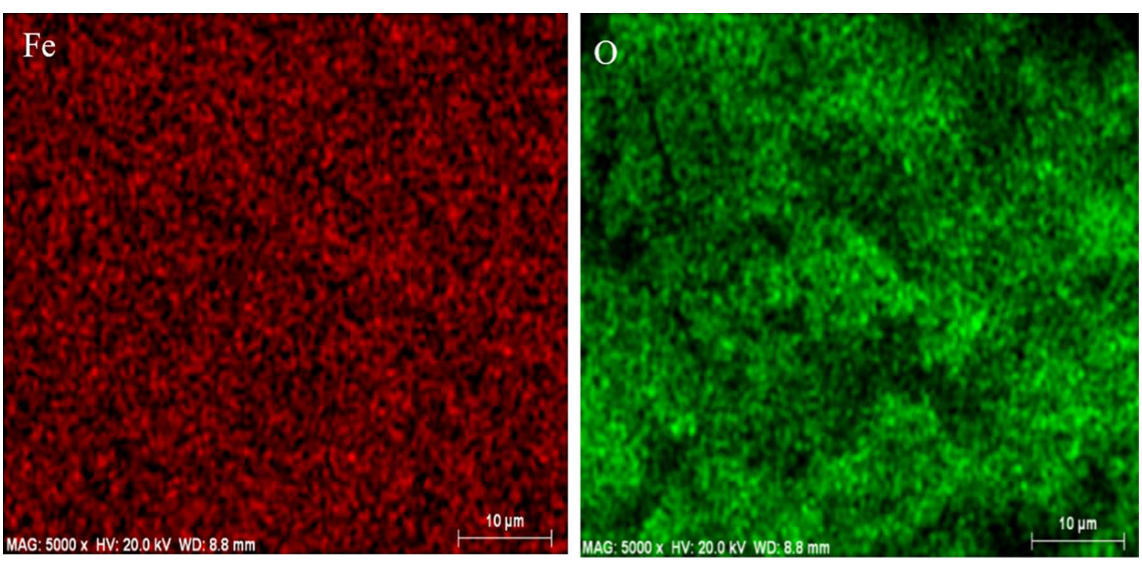

Fig. 4 SEM/EDX image mapping of MNP

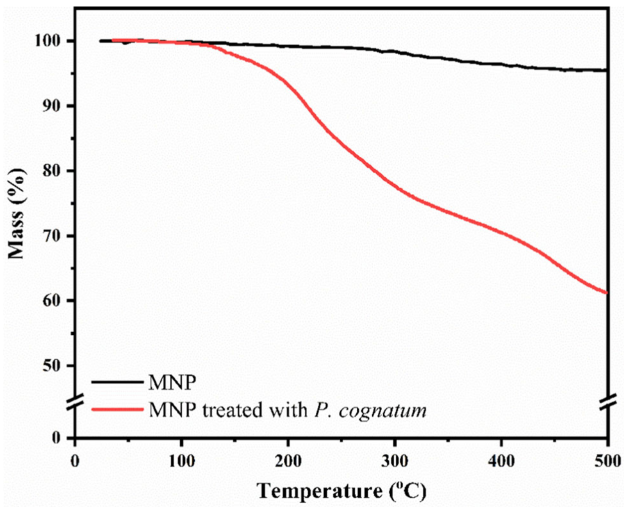

Fig. 5 TGA curves of **a** MNP and **b** MNP treated with *P. cognatum*

3.3 Magnetic Properties

The magnetic properties of the pure MNP and MNP treated with *P. cognatum* were characterized by a vibrating sample magnetometer (VSM) at 300 K. Curves obtained from magnetic field versus moment are shown in Fig. 6. In the previous study, we reported that the saturation magnetization (M_s) value of a pure MNP is 53.275 emu/g (Pekdemir and Coşkun 2020). While in this study, we found that the M_s value of MNP treated with *P. cognatum* is 26.073 emu/g. Thus, it can be stated that the saturation magnetization of pure MNP decreased significantly after treatment with the *P. cognatum* plant extract, which can be due to the biochemical components in the structure of the plant. Figure 6 shows that the magnetization curve of both samples has no hysteresis loop (H_c = 0 Oe), indicating the superparamagnetic nature of the MNP treated with *P. cognatum* (Alimirzalu et al. 2014).

3.4 Antimicrobial Activity

Antimicrobial activity results against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus megaterium*, and *Candida albicans* of the ethanol extracts of *P. cognatum,* MNP, and MNP treated with *P. cognatum* are given in Table 1 and Fig. 7. Phenolics, terpenoids, essential oils, alkaloids, lectins, polypeptides, and polyacetylenes, grouped according to their chemical structures in plants, act as antimicrobial agents (Cowan 1999). According to Table 1, the highest inhibition zone of *Polygonum cognatum* extract was observed against *Klebsiella pneumoniae.* Although *P. cognatum* ethanol extract is known to contain a high amount of phenolic compounds (Pekdemir et al. 2020), it was determined that it showed moderate activity against *S. aureus, E. coli*, *B. megaterium* bacterium, and *C. albicans* yeast.

There are some reasons why magnetic Fe_3O_4 nanoparticles are bactericidal. It is thought to be due to ROS, which includes superoxide radical, hydrogen peroxide, and hydroxyl radical (Kohanski et al. 2007; Sies 1997). Since MNP causes ROS formation, it inhibits the growth of bacteria (Tran et al. 2010).

We observed that pure Fe_3O_4 nanoparticles have a low and the same level of antimicrobial effect against all microorganisms (inhibition zone:7 mm). However, it was determined that MNP treated with *P. cognatum* ethanolic extract did not show antimicrobial effect against all microorganisms. It can be thought that MNP interacts with the phytochemical components in the plant and completely

Fig. 6 Magnetization curves of MNP and MNP treated with *P. cognatum*

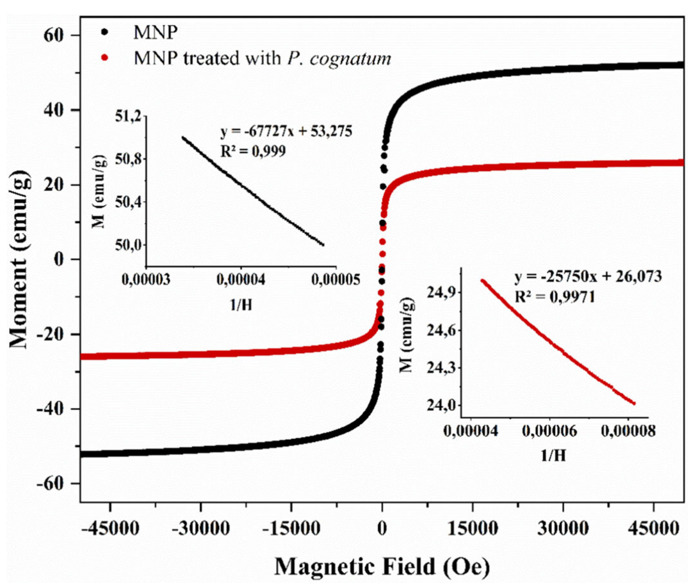

Table 1 Antimicrobial effect of *P. cognatum,* MNP and MNP treated with *P. cognatum*

Microorganism	*P. cognatum*	MNP	MNP + plant extract	Ceftriaxone	Nystatin
Staphylococcus aureus	8	7	–	10	–
Klebsiella pneumoniae	10	7	–	10	–
Escherichia coli	8	7	–	10	–
Bacillus megaterium	8	7	–	11	–
Candida albicans	8	7	–	–	11

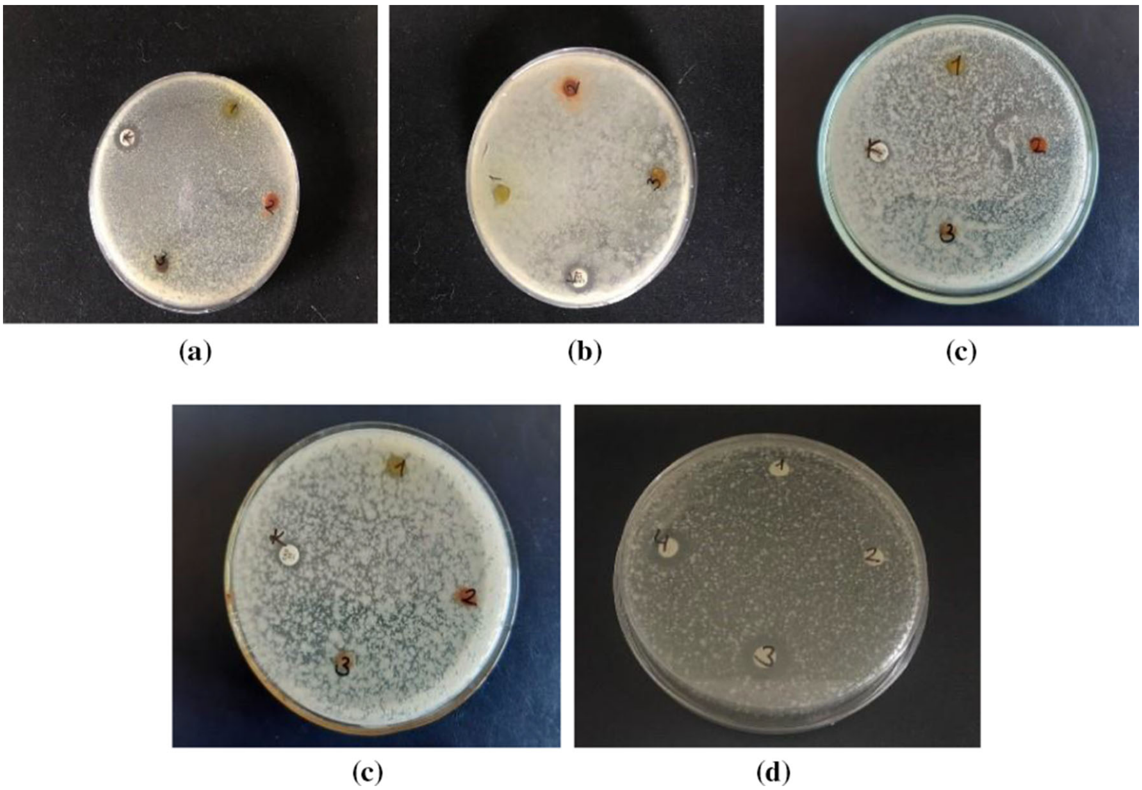

Fig. 7 Antimicrobial effect of *P. cognatum,* MNP and MNP treated with *P. cognatum* (1) ethanol extracts of *P. cognatum,* (2) ethanol extracts of NP, (3)ethanol extracts of MNP treated with *P. cognatum*, (4)Control; **a** *S. Aureus*, **b** *C. albicans*, **c** *E. coli*, **d** *B. Megaterium*, **f** *K. Pneumoniae*

eliminates the moderate antimicrobial effect of pure plant extract.

4 Conclusions

Magnetic Fe_3O_4 nanoparticles synthesized by the co-precipitation method were treated with ethanolic extract of the *P. cognatum.* In the FT-IR spectrum of the MNP treated with *P. cognatum*, the characteristic Fe–O stretching vibration at 630 cm^{-1} is proof of MNP in the structure. When TGA curves were examined, it was seen that the thermal stability of MNP treated with plant extract was lower than pure MNP. Although the saturation magnetization value of MNP treated with *P. cognatum* extract was lower than pure MNP, it showed superparamagnetic properties. Finally, *P. cognatum* ethanolic extract has a moderate antimicrobial effect against *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Bacillus megaterium*, and *Candida albicans,* while the synthesized MNP treated with *P. cognatum* did not show antimicrobial effect. In other words, Fe_3O_4 magnetic nanoparticle destroyed the existing antimicrobial effect of the pure plant extract.

Author's Contribution Mustafa Ersin Pekdemir synthesized magnetic nanoparticles and treated with plant extract. Sibel Pekdemir and Mehmet Çiftci collected the plant and prepared the plant extract. Şule İnci and Sevda Kırbağ performed antimicrobial analysis. Additionally, all authors contributed to writing and controlling the manuscript.

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Data Availability The data associated with a paper are available, and under what conditions the data can be accessed.

Declarations

Conflict of interest The authors state that there is no conflict of interest in the printing of this manuscript.

Animal Research No animals were used in our study.

Consent to Participate Our manuscript does not report on or involve the use of any human data or tissue.

Consent to Publish The Authors hereby consents to publication of the Work in "Iranian Journal of Science and Technology, Transaction A, Science (ISTT)".

Plant Reproducibility Polygonum cognatum plant has be found in nature of Elazig/Turkey.

Clinical Trials Registration This study does not involve human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes.

Gels and Blots/Image Manipulation Removal of lanes from gels and blots or cropping of images has nor been performed for the images used in this study. Also, the images presented in the manuscript remain representative of the original data.

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