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Eco-Friendly Synthesis of Magnetic Iron Oxide Nanoparticles Using Achillea Nobilis Extract and Evaluation of Their Antioxidant and Antibacterial properties

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ABSTRACT: Magnetite (Fe_3O_4) is a magnetic Iron Oxide encountered in many technological applications. The particle size and shape of magnetite nanoparticles allow tuning their properties to different applications such as targeted drug delivery, cancer diagnostic, magnetic resonance imaging, catalysts, pharmaceuticals, biomedicine, and agriculture. During the last two decades, the biosynthesis of nanoparticles has received considerable attention due to the growing need to develop environmentally sociable technologies in nanoparticle synthesis. Therefore, there is a need for the development of an eco-friendly process to synthesize nanoparticles through green chemistry using plants and microorganisms. The research work involves the development of a simple and reliable method for the bio-fabrication of magnetic Iron Oxide nanoparticles (IO-NPS) through the green method using Achillea Nobilis extract. The crystalline structure and morphology of IO-NPS were studied using various characterization techniques i.e. Fourier Transform Infrared Analysis (FTIR), Ultraviolet spectroscopy studies (UV–vis), X-ray diffraction, and FESEM. The antibacterial and antioxidant activity of the iron oxide nanoparticles was determined. Iron Oxide nanoparticles exhibited potent antibacterial activity against gram-positive and gram-negative bacterial strains tested. From the results, this method can be applied to different medical and industrial applications.

Keywords: *Antioxidant Activity, Antimicrobial Activity, Biosynthesis, Iron Oxide, Nanoparticle.*

Introduction

Nanoparticles have various properties such as small size and higher surface area. They have high also thermal and electrical conductivity and photocatalytic activity. Iron oxides have good biocompatibility. For this reason, they are used in medicine as magnetic resonance imaging contrast agents and in pharmacology as drug delivery agents (Bishnoi, KumaAr, and Selvaraj, 2018). In recent years, scientists have focused on the synthesis of magnetic iron oxide nanoparticles (Wu *et al.*, 2015). Controlling their size, shape and morphology are the most important research aims. Numerous methods have been reported for the synthesis of nanoparticles, including hydrothermal methods, solvothermal methods, co-precipitation, and sonochemical methods (Zhu *et al.*, 2018). Lately, green synthesis of

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several nanoparticles from different plants such as green tea, Azadirachta, Camellia Sinensis, Solanum nigrum, Saraca Indica, Clitoria ternatea, Mangiferaindica, and Ocimum Basilicum has been reported (Sorescu *et al.*, 2016; Rajendran and Sengodan, 2017). Iron oxide nanoparticles have received great consideration due to their applications in the fields of medicine such as biosensors. Last studies have also shown that magnetic nanoparticles can even positively affect glucose metabolism and neuronal interactions (Karimzadeh *et al.*, 2017; Tong *et al.*, 2017). Nanomaterials could be used in the future as antioxidants in industry and therapeutic applications. In recent years, researchers have investigated the antioxidant activity of different metal nanocomposites, like iron, nickel oxide, and gold for usage as nano antioxidants (Khalil *et al.*, 2020; Sundaram Sanjay and Shukla, 2021). Among these, magnetic iron oxide nanoparticles have become particularly important due to their antioxidant activity against pathogenic oxidants (Shah *et al.*, 2017).

19 herbaceous aromatic species of Achilles have been identified in Iran. One of the native species of this genus is Achillea Nobilis. Several parts of Achillea species are used in traditional medicine because of their antiseptic, anti-inflammatory, antihistamine, and antioxidant properties (Azimi, Sefidkon, and Monfared, 2016).

In this study, we adopted a green chemistry approach for the synthesis of Fe_3O_4-NPS using the extract plant of the Achillea Nobilis as a reducing agent. Furthermore, the antibacterial properties of nanoparticles were studied against gram-negative and gram-positive bacteria. The antioxidant activity of the synthesized nanoparticles has also been measured based on the free radical scavenging activity by the DPPH (1, 1-diphenyl 2-picrylhydrazyl) method.

Materials and Methods

- *Chemicals*

Achillea Nobilis was collected from Quchan and was identified by the Research Center for Natural plant sciences, Ferdowsi University of Mashhad (Iran) under identification number 38311(FUMH). High-purity chemical reagents were purchased from Merck Chemical Company Germany. $FeCl_2.4H_2O$, $FeCl_3.6H_2O$, NaOH, NH_4OH, and deionized water were used in experimental works (Table 1).

Table 1. The characteristics of the used material

compound	CAS number	purity	supplier
$FeCl_2.4H_2O$	13478-10-9	99	Sigma-Aldrich
$FeCl_3.6H_2O$	10025-77-1	99	Sigma-Aldrich
NaOH	1310-73-2	98	Sigma-Aldrich
NH_4OH	1336-21-6	> 98	Sigma-Aldrich

- *Instruments*

UV–vis spectral analysis was performed on the Cary-8454 spectrophotometer. The morphology of the prepared Metal Oxide(MO) NPS was observed by FE-SEM (S-4160, Hitachi), Zeta Potential (CAD, Zeta Compact, France), particle size analyzer (Nano-Sizer, Vasco3, Cordouan, France), EDX (MIRA III, Tescan), and TEM (CM30, Philips) analysis. MO NPS structure and composition were analyzed by XRD (PW1730, Philips). Further characterization was done using FTIR (Spectrum100, Perkin Elmer).

- *Preparation of aqueous Achillea Nobilis Extract*

The fine powder was obtained from flowers and stems using a kitchen blender. Then, 5 g powders of the plant with 100 mL of deionized water under vigorous mechanical stirring (1000 rpm) were boiled for 20 min at 60 °C. Then the extract was cooled at room temperature, filtered through Whatman No.1 filter paper, and stored at 4 °C for further experimental analysis (Ahmed,

Chaudhry, and Ikram, 2017) (Figure 1). The methanolic extract of the plant was also prepared at room temperature. The methanolic extract is used to determine the antioxidant properties.

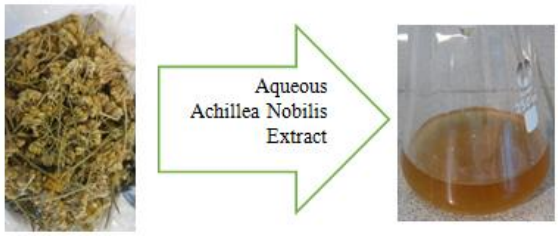

Fig. 1. Preparation of aqueous Achillea Nobilis Extract

- Preparation of Nanoparticles via chemical and green co-precipitation method

- Synthesis of Fe_3O_4 nanoparticles via chemical co-precipitation method

The magnetite nanoparticles were prepared via the chemical co-precipitation method by the following; 1.625 g (8 mmol) $FeCl_2.4H_2O$ and 4.430g (16 mmol) $FeCl_3.6H_2O$, with the molar ratio of ferric ion to ferrous ion in the solution of (2:1 molar ratios), were dissolved in 200 ml of deionized water under vigorous mechanical stirring (1000 rpm) for 2 h at 25 °C 10 ml of 25 wt% NH_4OH (excess base concentration) was added to the solution, then the solution color changed from orange to black rapidly (Figure 2). The black product was centrifuged at 5000 rpm for 20 min and washed three times with deionized water to remove excess ions, and finally dried in a vacuum at 80°C for 12h (Gurenko, Tolstoy and Gulina, 2017).

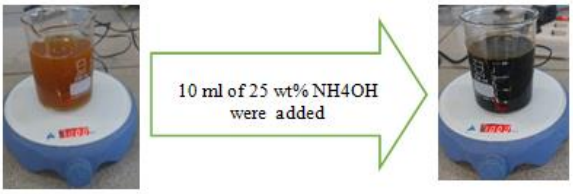

Fig. 2. Preparation of Fe_3O_4 nanoparticles

- Biosynthesis of Fe_3O_4 nanoparticles

1.1 g $FeCl_3.6H_2O$ and 0.5 g $FeCl_2.4H_2O$ (1:2 molar ratios) were dissolved in 100 ml of deionized water. Then heated at 80 °C with mild stirring under atmospheric pressure. After 5 minutes, 5 ml of the aqueous Achillea Nobilis extract was added to the mixture and the light orange color of the Achillea Nobilis extract was converted to black color. After 30 minutes, 20 ml aqueous solution of sodium hydroxide (1M) was added to the mixtures for uniform preparation of Iron Oxide precipitations. The mixture was cooled at room temperature and the Iron Oxide nanoparticles were obtained. The nanoparticles were washed 3 times with deionized water and finally dried in a vacuum at 80◦C for 12h (Asoufi, Al-Antary, and Awwad, 2018).

- Antioxidant activity (DPPH free radical scavenging assay)

The antioxidant activity of IO-NPS was characterized based on the scavenging effect on the DPPH free radical activity. 3 ml of the ethanolic solution of DPPH (0.1 MilliMolar) was added to 600 µl of IO-NPS solution with different concentrations (1000, 500,100 and, 20 ppm). DPPH solution was freshly prepared and stored in the dark at 4 °C. The mixture was kept for 30 minutes and then the absorbance was measured at 517 nm. A blank sample containing an equivalent amount of ethanol and DPPH was also prepared. The DPPH free radical scavenging activity was determined from the different bio-metal oxide NPS at different concentrations (1000, 500, 100 and, 20 ppm). This assay showed that an increase in the concentration of IO-NPS led to a rise in the radical scavenging activity (Park *et al.*, 2019). For the determination of the radical scavenging activities, the percentage of inhibition was calculated. In this formula, AA is the absorbance value of the test, and AB, absorbance value of blank samples. For these calculations, the curve of percent

inhibition versus concentration was plotted (Eq. 1).

Percent (%) inhibition of DPPH activity = [(AB – AA) / AB] * 100 (1)

The antioxidant properties of the methanolic extract of nanoparticles and methanolic extract of the plant were compared.

- Antibacterial assay

In order to evaluate the antibacterial effects, inhibition zone diameter of two multidrug-resistant pathogens, Escherichia coli PTTC 1399 (gram negative bacteria) and Staphylococcus aureus 1112 PTTC (gram positive bacteria) bacteria using the agar well diffusion method was measured. The agar well diffusion method consists of making wells of 6 mm in diameter by punching an agar plate with a glass tube under sterilized conditions. Aliquots of 50 µL of each IL were placed on the wells, using physiological water for the control wells without. The agar plates were incubated at 37 °C for 18-24 h and then, the radius of the inhibition zone around the wells was determined (Missoun *et al.*, 2020). First, for the bacterial strain, the cell suspension with 0.5 McFarland turbidity (1.5×10^8 CFU/ml) was prepared, and each well of the ELISA plate was filled with 200 µL of Muller-Hinton broth (MHB), and then 100 µL of IO-NPS was poured into the first cast well; Afterward, 100 µL of this mixture was removed and transferred to the next well. This process continued to the last well. Finally, the concentrations of IO-NPS (200, 100, 75, 50, and 25 µg/mL) were obtained (Khatami *et al.*, 2017).

Results and Discussion

- UV-Spectrophotometer analysis

The synthesized nanoparticles via chemical co-precipitation and green method were characterized through UV–Vis absorption, and the results are shown in Figure 3(a,b). Wavelengths between 200 and 700 nm are generally used for characterizing metallic nanoparticles, and the results of the UV-Vis absorption spectra of Iron oxide nanoparticles at 220 nm(Rajendran and Sengodan, 2017) are preseuted accordingly.

- FT-IR analysis

FTIR spectroscopic measurements were carried out to identify the possible biomolecules in extracts to the efficient stabilization of metal oxide nanoparticles (Sorbiun *et al.*, 2018). Extract FTIR showed a band at 3400 and 2890 cm^{-1}, which have been due to stretching vibrations of the primary and secondary amines, O−H stretching of alcohols, and C−H stretching of alkanes. Purified samples of IO-NPS were dried and mixed well with KBr powder for pellet preparation for FTIR measurements. FTIR analysis was carried for the reduction of iron ions with a spectral range of 400-4000 cm^{-1}.

The FT-IR spectrum of Chem - Fe_3O_4 NPS and Biosynthesized using Achillea Nobilis extract in figure 4 has the characteristic absorbance bands of nano-sized magnetite, in the range of 400-700 cm^{-1}, the vibrations relating to the Fe—O bond show peaks at 621, 589 cm^{-1}, which are characteristic absorbance bands of nano-sized magnetite. The absorption bands at 3434, 1628, 1723, 1108, and 621 cm^{-1} in iron oxide nanoparticles synthesized using chemical method wound be shifted into 3405, 1621, 1062, and 589 cm^{-1} of synthesized Bio- Fe_3O_4 NPS (Karimzadeh *et al.*, 2017; Ramesh *et al.*, 2017).

- FESEM

The surface morphology of as-synthesized Iron oxide nanoparticles prepared from Achillea Nobilis Aqueous Extract was primarily characterized by field emission scanning electron microscopic (FESEM) analysis. The biosynthesized

Fe_3O_4 NPS were observed using FESEM and the resultant images at different magnification levels are shown in Figure 5(a,b). It can be observed that most of the Fe_3O_4 nanoparticles are on the nanometer scale and are mostly cubic (Figure 5a, 5b). EDX spectrum is shown in Figure 5c.

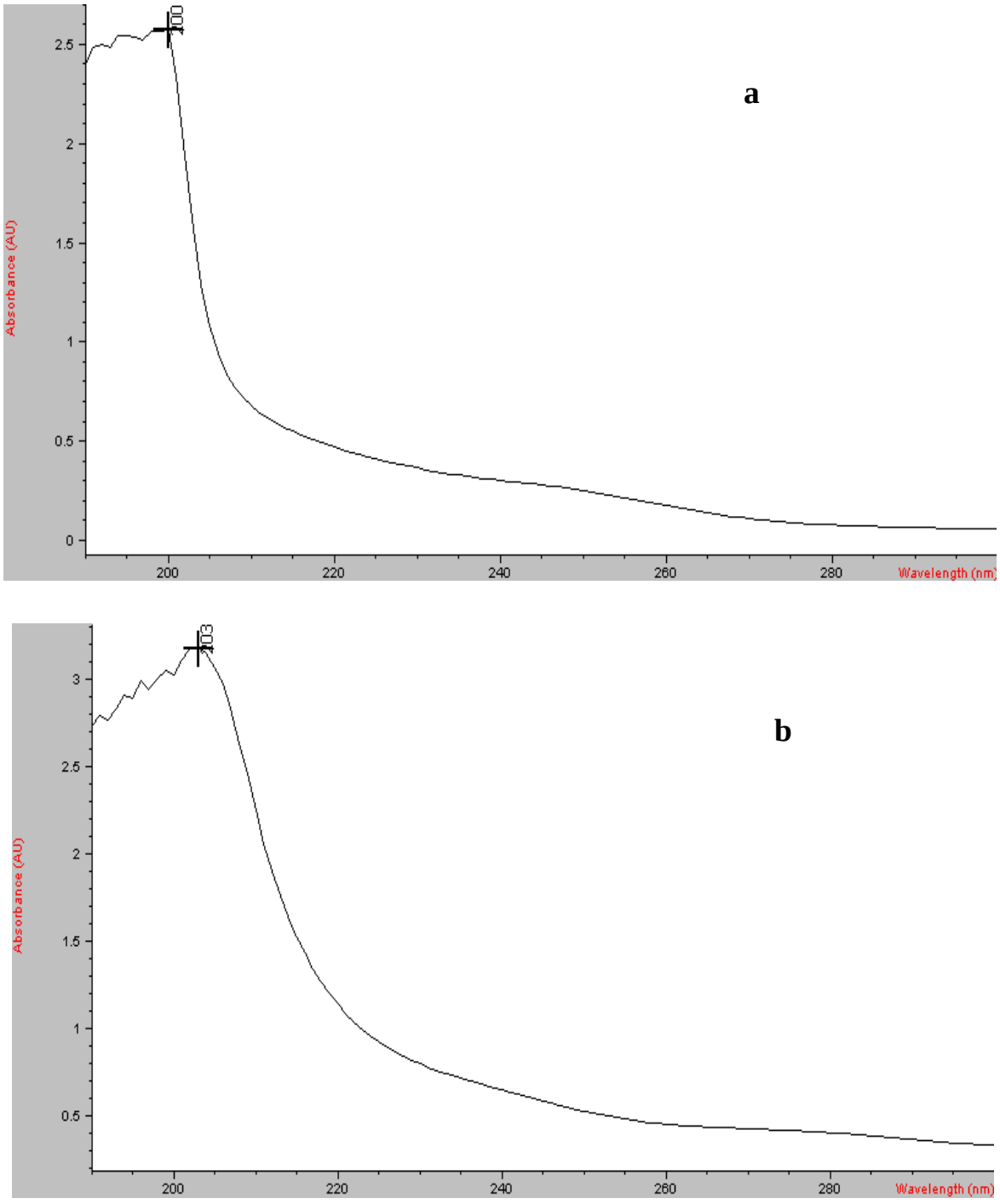

Fig. 3. UV-Vis absorption spectra of Iron Oxide nanoparticles synthesized using a) chemical co-precipitation method, b) green method.

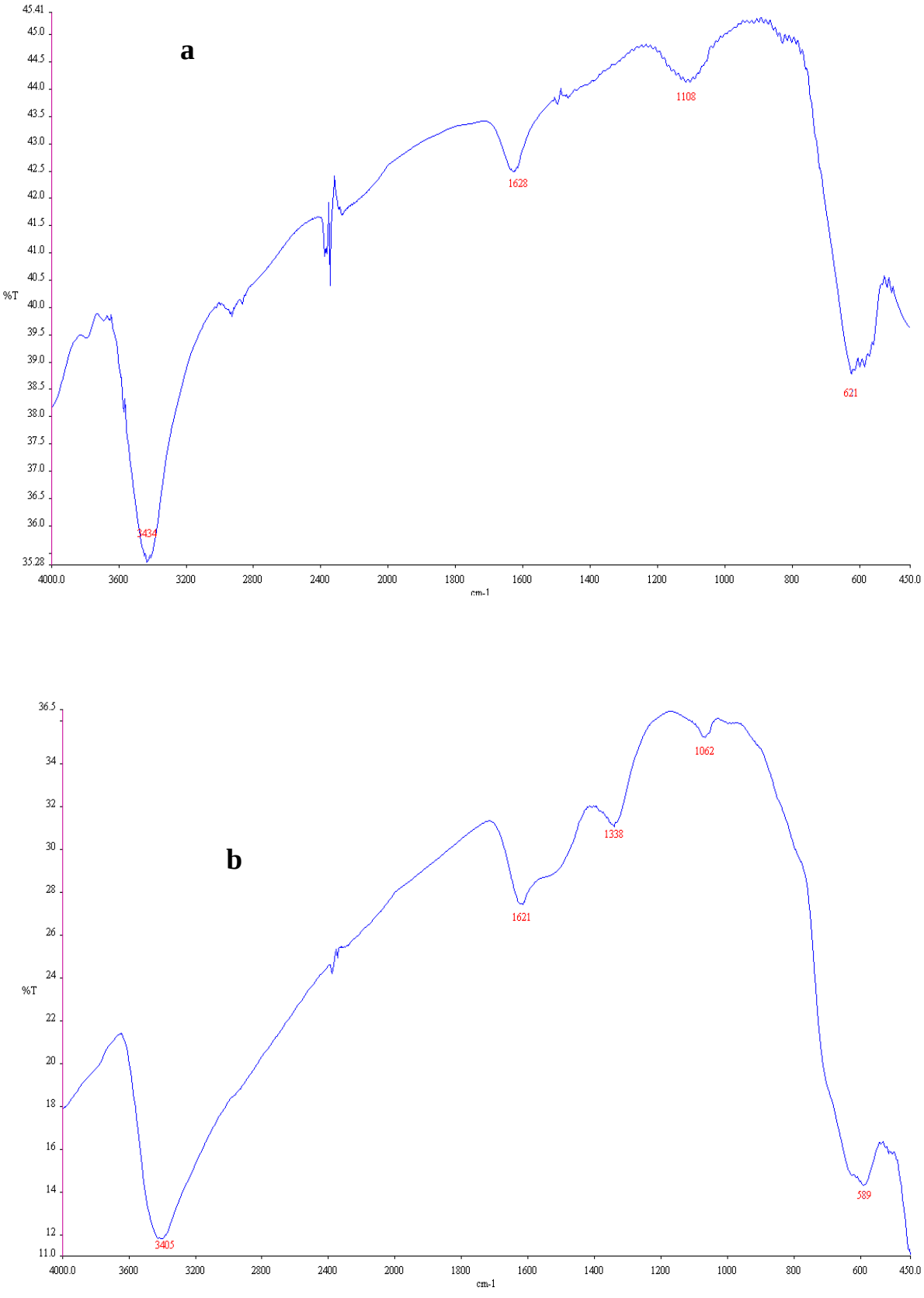

Fig. 4. FT-IR spectra of Iron Oxide nanoparticles using a) chemical co-precipitation method, b) green method.

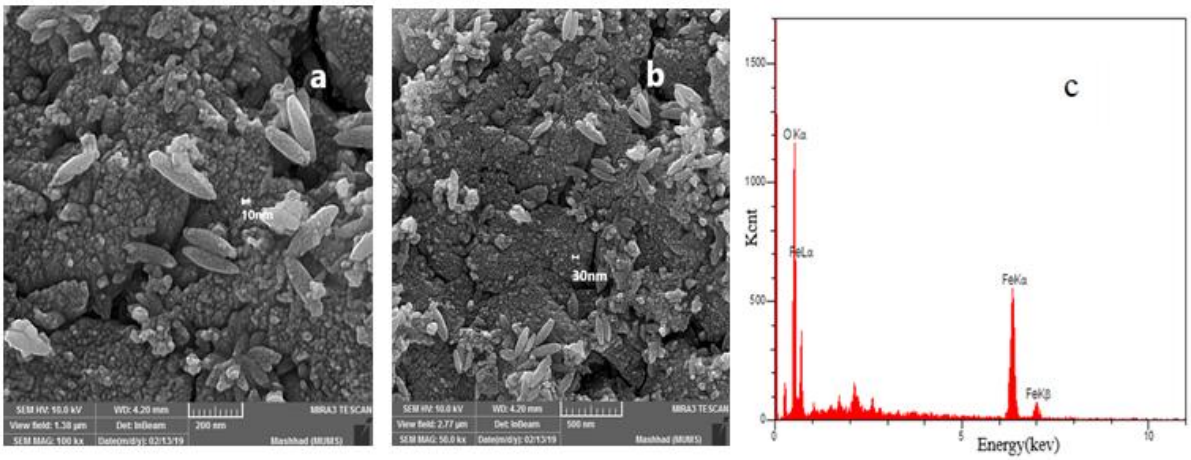

Fig. 5. FESEM images of Fe_3O_4 NPS(a, b), (c) EDX spectrum.

- X-Ray Diffraction

The X-ray diffraction patterns (XRD) are addressed as an important analytical method. Generally, XRD is used for the recognization and determination of various crystalline forms which be in powder and solid samples (El-Naggar *et al.*, 2017). The structure of metal oxide nanoparticles was specified by X-Ray diffraction analysis (PW1730, Philips) at room temperature. The XRD pattern of Bio-synthesized Fe_3O_4 nanoparticles from an aqueous extract of Achillea Nobilis is shown in Figure 6. The XRD pattern of bio-synthesized Fe_3O_4 nanoparticles from an aqueous extract of Achillea Nobilis is shown in figure 6. The XRD of the synthesized Fe_3O_4 nanoparticles shows broad peaks at 2θ values of 30.03, 35.33, 62.47, 71.03, and 74.02 were assigned to (220), (311), (440), (620) and (622) planes respectively. The peak positions of samples exhibit the cubic structures of Fe_3O_4, which are comparable with ICCD Ref No. 04-009-8439. The positions and relative intensities of the reflection peak of Fe_3O_4 NPS agree with the XRD diffraction peaks of standard Fe_3O_4 samples (Karimzadeh *et al.*, 2017; Ramesh *et al.*, 2017; Asoufi, Al-Antary and Awwad, 2018). The average crystalline size of synthesized metal oxide nanoparticles (D) could be calculated using the Debye Scherrer formula.

$$D = 0.89\,\lambda / \beta\,(\cos\theta) \qquad (2)$$

In this equation, β is the full-width at half maximum (FWHM) in radians of the Fe_3O_4 (311) lines and θ the scattering angle in degree. The average crystallite size D was calculated using equation (2), which was found to be 9 nm for Fe_3O_4, respectively**.**

- Zeta potential particle size analysis

The particle size and zeta potential of green synthesized Fe_3O_4 NPS were shown in an aqueous solution using a particle size analyzer (Nano-Sizer, Vasco3, Cordouan, France). The zeta potential of Fe_3O_4-NPS was measured at pH = 6.62 and 24.15 °C. Zeta potential value was determined at 11.78 meV that proves the synthesized NPS is highly stable due to the strong negative surface charge (Kanagasubbulakshmi and Kadirvelu, 2017). The average particle size of nanoparticles was obtained using a particle size analyzer. It was observed that the size of Fe_3O_4-NPS was within a range of 5-24 nm.

-Antioxidant activity

The results confirmed that Fe_3O_4-NPS

has antioxidant activity. Figure 7 demonstrates a significant reduction in the concentration of DPPH that is due to the scavenging ability of methanolic extract of all the nanoparticles. Methanolic Extract of Achillea Nobilis has greater antioxidant activity as compared to IO-NPS (Majumdar and Parihar, 2012).

- *Antibacterial activity*

In agar, the good diffusion method IO-NPS showed significant antibacterial activity on all the two bacterial strains (Naika *et al.*, 2015). The results of the bactericidal effect of the metal oxide NPS on the pathogenic bacteria are shown in Table 2. The bacterial strains tested, Chem-Fe_3O_4 nanoparticles showed a low inhibitory effect on the growth of Gram-negative bacteria Escherichia coli (4.8 mm), at a concentration of 25 µg/ml and strongly inhibited the growth of Gram-positive bacteria Staphylococcus aureus (8.25mm) and at a concentration of 200 µg/ml. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the Iron oxide NPS for the antibacterial activities were presented in Table 3. Based on the obtained data; these results are statistically significant (P-value< 0.05).

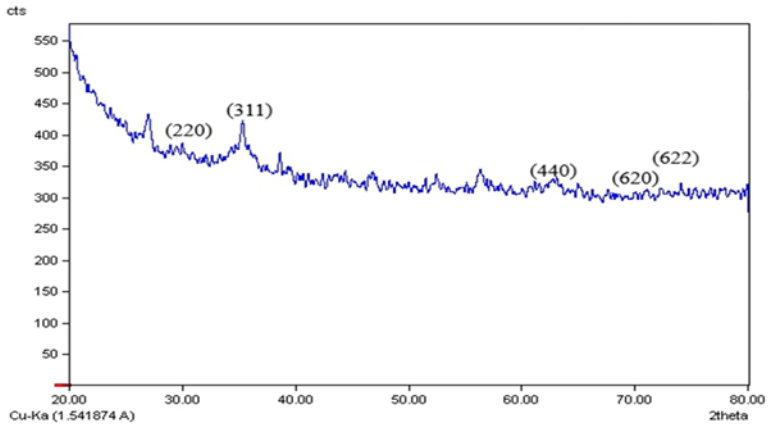

Fig. 6. XRD patterns of Fe_3O_4 NPS.

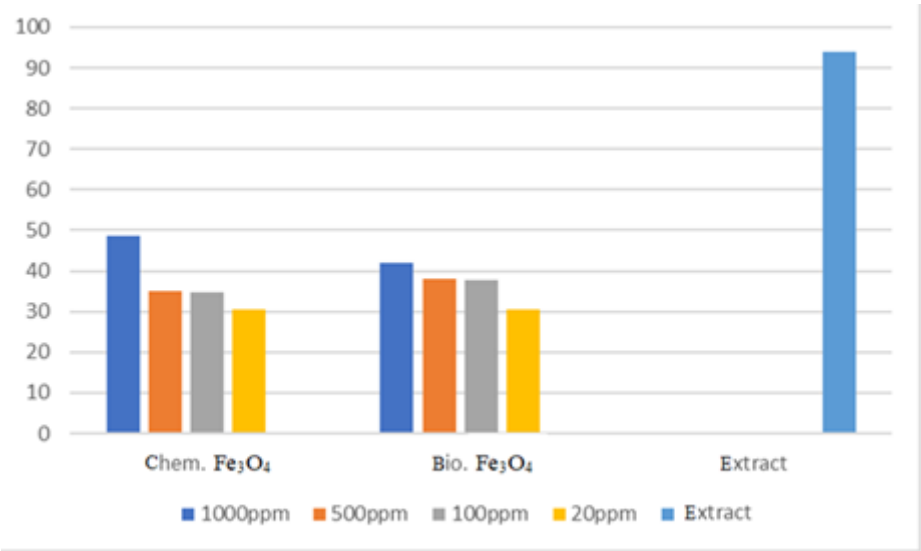

Fig. 7. Comparison of Antioxidant Activity of methanolic solution of Iron Oxide (Bio& Chem) NPS and methanolic extract of Achillea Nobilis.

Table 3 shows the results of the determination of MIC and MBC of Iron Oxide nanoparticles up to 200 µg/ml against the desired bacteria using the microdilution method. The lowest MIC value was noticed in Iron oxide nanoparticles against *Escherichia coli* (25 µg/ml), Additionally, Iron Oxide nanoparticles prepared from green and chemical methods were not capable of interfering with bacterial growth, but shown to be inhibitory.

Conclusion

Various methods have been reported for the synthesis of nanoparticles, including hydrothermal methods, co-precipitation, and sonochemical methods. Some of these reactions are performed in aqueous media in reactors or autoclaves where the pressure can be higher than 2000 psi and the temperature can be above 200 °C(Laurent *et al.*, 2008); Therefore these methods require a higher amount of energy and entail harmful chemicals. Therefore Magnetic Iron Oxide nanoparticles were synthesized via green and simple method using Achillea Nobilis extract. This method does not require high energy and the process does not use any harmful chemicals and thus is eco-friendly. The IO-NPS were confirmed by UV-Spectrophotometer analysis, FT-IR, Particle size, EDX, and FE-SEM. The antioxidant activity of iron Oxide nanoparticles was determined. Synthesized iron oxide nanoparticles in the size range of 5-24 nm showed good antioxidant properties. The antioxidant activities of the synthesized nanoparticles and the obtained plant extract were compared. The synthesis of nanoparticles via green and chemical methods for antibacterial and antioxidant properties were evaluated. The results indicated that nanoparticles were effective against both the Gram-positive and Gram-negative bacterial strains. Due to the results obtained from the antioxidant and antibacterial activities of synthesized nanoparticles, they have proper physical and chemical properties for biomedical and pharmaceutical applications.

Table 2. Zone of inhibition (mm in dia.) on the pathogen bacteria

Microorganism	Cont.	Chem-Fe_3O_4 nanoparticles	Bio-Fe_3O_4 Nano particles	Ampicillin	Gentamicin
Organism bacteria Staphylococcus aureus	25	5.3±0.32	5.8±0.24		
	50	5.77±0.3	6.25±0.25	18±0.85	17±0.31
	75	6.34±0.27	6.85±0.31		
	100	7.15±0.32	7.40±0.35		
	200	8.00±0.38	8.25±0.22		
Bacteria- Escherichia coli	25	4.8±0.24	5.23±0.30		
	50	5.2±0.15	5.5± 0.18	13±0.23	16±0.55
	75	5.65±0.15	5.95±0.22		
	100	6.38±0.31	6.45±0.25		

Table 3. MIC and MBC (µg/ml) for metal oxide nanoparticles against selected pathogens

Microorganism	MICs/MBCs		
	Organism type	Chem-Fe_3O_4 nanoparticles	Bio-Fe_3O_4 nanoparticles
Staphylococcus aureus	Gram Positive	12.5/__	12.5/__
Escherichia coli	Gram Negative	25/__	25/__

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