Medbiotech Journal

MBTJ, 5(2): 15-22, 2021

Research Paper

Green synthesis of hematite nanoparticles using aqueous extract of *Teucrium Polium* after microwave-assisted extraction: synthesis, characterization and evaluation of some biological activities

Zahra Emamifard [a] , Hamid Reza Rajabi [*b]

[a] Chemistry Department, Yasouj University, Yasouj, 75918-74831, Iran
[b] Department of Animal Sciences, Faculty of Agriculture, Yasouj University, Yasouj, Iran

Received: 30 March 2021 Accepted: 16 May 2021 Published: 03 June 2021

Abstract

This work presented a green approach for the synthesis of hematite (α-Fe2O3) magnetic nanoparticles (MNPs) using the aqueous extract of Teucrium Polium plant. The aqueous extracts were prepared by the maceration and microwave-assisted extraction (MAE) techniques and performed as the natural precursor for one-step green synthesis of α-Fe2O3 MNPs, at room temperature. The as-prepared α-Fe2O3 MNPs were characterized by different techniques including UV-Vis absorption spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray(EDX), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and vibrating sampling magnetometer (VSM). The XRD results confirmed the high crystalline structure of magnetic α-Fe2O3 MNPs. The average particle size of MNPs was obtained around 60 nm. In addition, the antibacterial properties of the as-synthesized α-Fe2O3 MNPs against various pathogenic bacteria were investigated by well-diffusion test. Based on the observations, α-Fe2O3 MNPs were more sensitive against Gram-negative bacteria than the Gram-positive bacteria. Moreover, the DNA cleavage potential of the samples was studied, too.

Keywords: α-Fe2o3 magnetic Nanoparticles; Aqueous extract; Microwave- assisted extraction; Antimicrobial activity.

How to cite the article:
Z. Emamifard, HR. Rajabi, Green synthesis of hematite nanoparticles using aqueous extract of Teucrium Polium after microwave-assisted extraction: synthesis, characterization and evaluation of some biological activities, Medbiotech J. 2021; 5(2): 15-22. https://dorl.net/dor/20.1001.1.22092528.2021.05.02.2.8

1. Introduction

Magnetic nanoparticles (MNPs) were gaining significant importance in the fields of advanced biological and medical. The important applications of MNPs in various fields such as separation science, cancer hyperthermia, fluorescence imaging, water treatment, drug delivery, and magnetic resonance imaging make them attractive nanoparticles [1]. In green chemistry, MNPs can be covered with some biological molecules to generate the samples with less toxicity and different optical, magnetic, biological activities, and surface characteristics. Besides, due to the magnetic properties of these nanoparticles, it can easily separate MNPs from the media by an external magnetic field [2]. Among MNPs, iron oxides (IO) NPs have been gaining extreme importance due to their magnetic characteristic and wide applications such as wastewater treatment, coating process, catalytic materials, sensor design, ion exchange process , bio-separation, and magnetic recording devices. The most common forms of IO magnetic nanostructures are hematite (α-Fe_2O_3), magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) are among them.

* Corresponding author E-mail: h.rajabi@mail.yu.ac.ir, hr.rajabi@gmail.com (H.R. Rajabi)

Among them, α-Fe_2O_3 is one of the most interesting metal oxides n-type semiconductors with antiferromagnetic properties [3].

In the past years, many efforts have been done to use green and eco-friendly methods for the synthesis of nanoparticles. One of these methods is employing the extract of different parts of the plants such as root, leaf, vegetable juice, stem, and seed. The organic compounds in the extracts were used as reducing, capping, and stabilization agents in the green synthesis of NPs. *Teucrium polium* or *Polygermander* plant is a kind of mint from *Lamiaceae* family with Persian names of "*kalpoure*" or "*halpeh* [4].

In this study, α-Fe_2O_3 MNPs are prepared by using the aqueous extract of *T. polium* plant after microwave-assisted extraction (MAE). The ideal extraction method must possess the shortest processing time, minimum production cost, particularly the production of the effective constituents, and the use of the lowest volume of organic solvent [5]. The low solvent consumption, short extraction time, less risk of oxidization, and decomposition of pleasant compounds make this technique a favorable and interesting technique.

Here, we report on the preparation of crystalline and pure mesoporous hematite MNPs by a simple, rapid, eco-friendly, and one-step hydrothermal method using the extract of *T. Polium* plant. Due to the above benefits of MAE, this technique was applied for extraction of the secondary metabolite of *T. Polium*. The hematite MNPs were synthesized by using the obtained extracts and characterized by different techniques. Finally, the antibacterial activity of the as-prepared hematite was investigated.

2. Experimental

2.1. Chemicals and apparatus

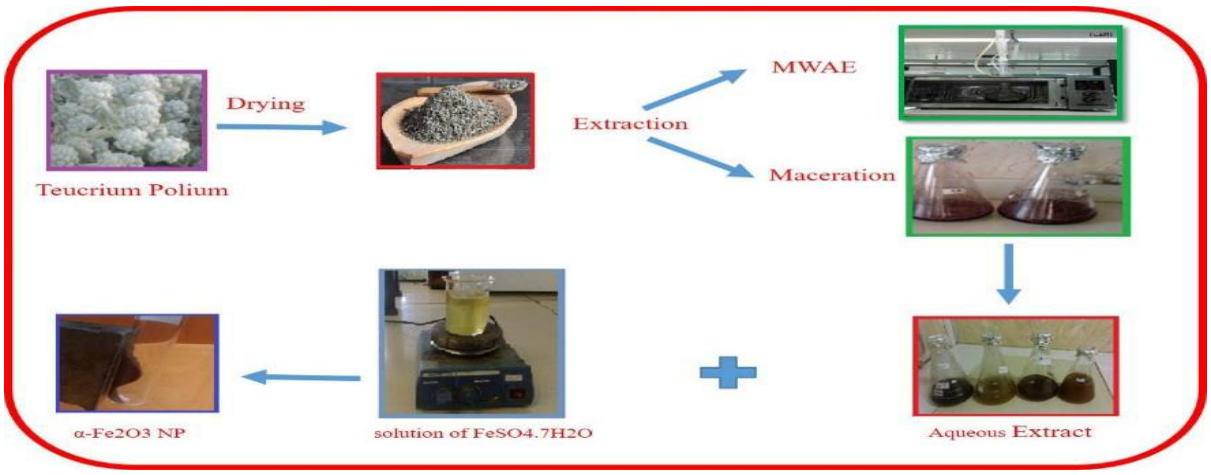
$FeSO_4.7H_2O$ was purchased from Merck and Aldrich companies without purification. All solutions were prepared in double-distilled water. UV–Vis absorption spectra were recorded by using a Lambda 25 (make, Perkin-Elmer) spectrophotometer. The FT-IR patterns of hematite nanoparticles were analyzed by using a FT-IR spectrophotometer (model JASCO-680), using the KBr pellet method. In the MAE technique, a Daewoo microwave device (model KOC-9N8T, Daewoo Company, Korea) was used for aqueous extract preparation. The morphology of the as-synthesized hematite NPs was examined by scanning electron microscopy (SEM) model Philips XL30. The crystal structure properties of the samples were investigated by X-ray diffraction (XRD) with Cu Kα radiation (λ= 0.154 nm) model of X'PertPro. The magnetism identification of hematite NPs were carried out by a vibrating sample magnetism (VSM) device made by Magnetic Kavir Kashan Company (Kashan University, Kashan, Iran).

2.2. Microorganisms

Four bacterial strains were chosen for antibacterial assay including two Gram-negative bacteria (*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027)) and two Gram-positive (*Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633)). For the preparation of solid culture medium bacteria, the Mueller-Hinton agar powder (Merck, Germany) was applied, while Mueller Hinton Broth (Scharlab) was used as the liquid culture media.

2.3. Synthesis of hematite nanoparticles

For the synthesis of hematite MNPs, firstly, 30 ml of the aqueous extract with the initial pH of 4 was transferred to a volumetric flask. The temperature was stabilized at 80 C. Then, an aqueous solution of $FeSO_4.7H_2O$ (30 mL; 0.1 M) was added drop by drop to the extract solution, under vigorous stirring. At the beginning of the reaction, the color change from brown to black was observed, clearly. Afterward, the formed black hematite precipitates were separated by a magnet and kept in the oven at 90 ° C for one day. The resulted powder was washed twice with methanol and double-distilled water, respectively, and centrifuged at 3000 rpm for 5 min. The obtained hematite NPs were collected and used for further experiments (Scheme 1).



(Scheme 1)

2.4. Well diffusion method

In this method, initially, 15 mL of Mueller-Hinton agar media was decanted into each Petri plate and allowed to be solidified at a refrigerator for 10 min. Then, 100 µl of the bacteria picked up. The bacteria had previously cultivated in the Muller Hinton broth by a micropipette and poured onto the solid medium and spread with the Pasteur and the wells were created by a Pasteur pipette. Subsequently, 25 mL of the MNPs suspension with different concentrations (25, 12.5, 6.25 $mg.ml^{-1}$) was poured into the wells and incubated at 37 °C for 24 h. The inhibition zone was measured with a caliper.

2.6. DNA Cleavage

For cleavage tests, in the individual microtubes, 4 µL of each sample (25 mg mL^{-1} in DMSO) was mixed with 4µL of plasmid DNA and incubated at 37 °C for 2 h. For monitoring the possible DNA cleavages, chromosomal DNA was treated with 30% H_2O_2 (X) and chromosomal DNA alone were used as negative and positive controls, respectively.

3. Results and discussion

3.1. Characterization

The phase identification and crystalline structures of the nanoparticles were characterized by XRD. The XRD patterns obtained for the α-Fe_2O_3 synthesized using *T. polium* extract is shown in Fig.1 (A,B). Some strong diffraction peaks with 2θ values of 24.8, 32.8, 35.9, 40.6, 49.8, 54.6, 57.4, 62.2, 64.7, 72.1, 75.4, 77.1 and 80.1 corresponding to the crystal planes of (012), (104), (110), (113), (024), (116), (018), (214), (300), (1010), (220), (036) and (128) were detected for crystalline α-Fe_2O_3, respectively. The results confirm the rhombohedral phase structure of the as-synthesized hematite MNPs [6]. The results were in good agreement with the standard XRD (JCPDS card no. 00-024-0072). However, the presence of some additional peaks in XRD patterns of the nanoparticles synthesized by aqueous extracts is previously related to the presence of some unknown compounds such as organic matters or amorphous impurities, which may be remained at the surface of the nanoparticle [7]. Meanwhile, the average particle size of α-Fe_2O_3 MNPs synthesized by MAE was calculated using the Deby-Sherrer equation ($D=k\lambda/\beta\cos\theta$) [8]. The average crystalline sizes of the hematite MNPs are found to be ~16 nm.

Moreover, the EDS analysis was carried out to clarify the elemental composition and stoichiometry of the synthesized hematite nanoparticles (Fig.1). As it can be seen, oxygen and iron signals have been detected. The other present signals may be coming from the bioactive molecules in *T. Polium* extract, which presented at surface of the as-prepared α-Fe_2O_3 MNPs.

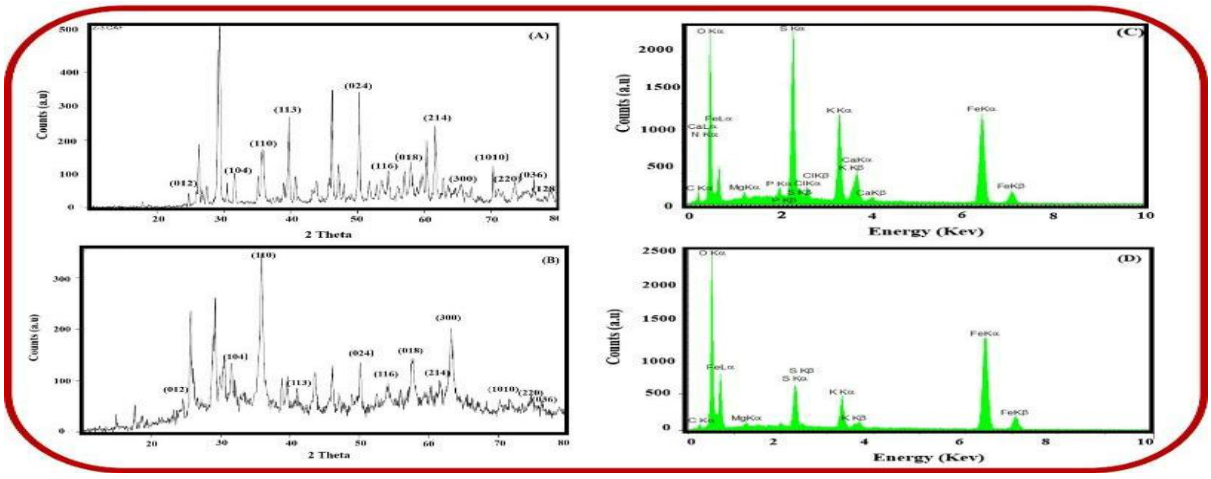


Fig 1: The XRD and EDX patterns of α-Fe_2O_3 MNPs synthesized using the aqueous extracts obtained by (A,C) MAE and (B,D) maceration techniques.

The optical properties of the as-synthesized hematite MNPs were investigated by UV-Vis absorption spectroscopy. Fig. 2 shows the UV-Vis absorption spectra of as-prepared hematite synthesized using the aqueous extract obtained by maceration (a) and MAE (b) methods. As observed, after mixing of $FeSO_4$. $7H_2O$ with *T. polium* aqueous extract an obvious color change of the solution from brown to black due to the formation of hematite nanoparticles was happened (Scheme 1). The FT-IR spectra of the α-Fe_2O_3 MNPs synthesized using *T. polium* aerial parts extract are showed in Fig. 2, too. The peaks at 3390 cm^{-1} are assigned to the OH stretching of alcohol and phenol. The peak at 1122 cm^{-1} indicates the C-C groups from aromatic rings that are present in *T. polium* extract [9]. The peak at 2358 cm^{-1} indicates the presence of -CH stretching of aliphaticcompounds and the peak at 1627 cm^{-1} is assigned to the C=O band of aldehyde, keton, and carboxylate. The formation of α-Fe_2O_3 was also confirmed by an obvious band below 700 cm^{-1}, which corresponds to the Fe-O band. Therefore, the FT-IR analysis confirmed that the bio-reduction of Fe^{2+} ions in hematite nanoparticles is due to some capping materials present in the aqueous extract of *T. polium* aerial parts.

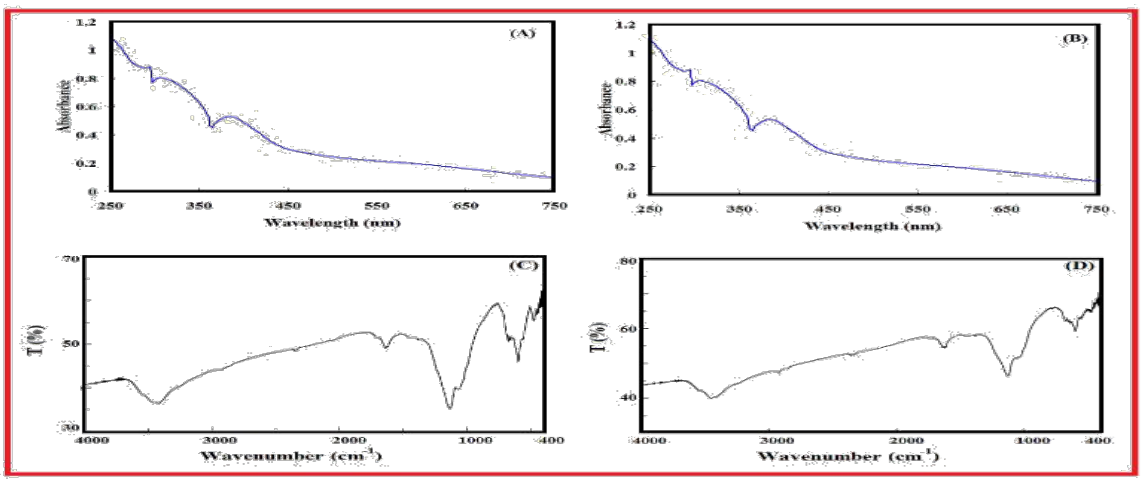


Fig 2: UV-Vis absorption and FT-IR spectra the as- prepared α-Fe_2O_3 MNPs by using maceration (A,C) and MAE (B,D) techniques.

The morphology and size of the as-synthesized α-Fe_2O_3 were determined by SEM analysis. Fig.3 demonstrates the SEM image of α-Fe_2O_3 synthesized by MAE and maceration method. The results demonstrated the formation of regular, uniform, and nano-sized particles. The average particle size of the formed α-Fe_2O_3 was estimated below 60 nm. However, SEM results of the hematite synthesized by the maceration method show the irregular particles with wide size

distribution and various shapes. Therefore, compared to maceration, the MAE technique can provide an efficient and rapid approach for the preparation of α-Fe_2O_3 nanoparticles with more uniformity.

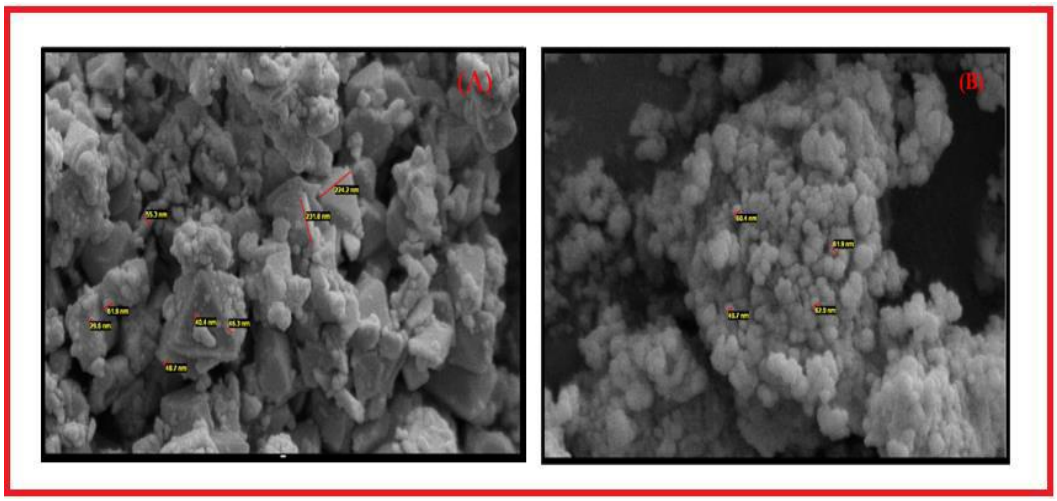


Fig 3: SEM images of α-Fe_2O_3 MNPs synthesized by (A) maceration and (B) MAE techniques.

In order to study the magnetic behavior of α-Fe_2O_3, magnetization measurements were performed with the VSM technique. As can be observed in Fig.4, the saturation magnetization value was observed 1.35 and 13 emu/g for α-Fe_2O_3 synthesized by MAE and maceration techniques, respectively. The results demonstrated that the saturated magnetization value decreases continuously with decreasing particle size of α-Fe_2O_3 MNPs. The decrease of saturation magnetization for hematite NPs prepared by MAE compared to maceration is may be due to the efficient capping of α-Fe_2O_3 by the formation of a diamagnetic organic layer around the α-Fe_2O_3 surface [10]. However, the resulted hematite MNPs can be easily separated by a common magnet from the solution.

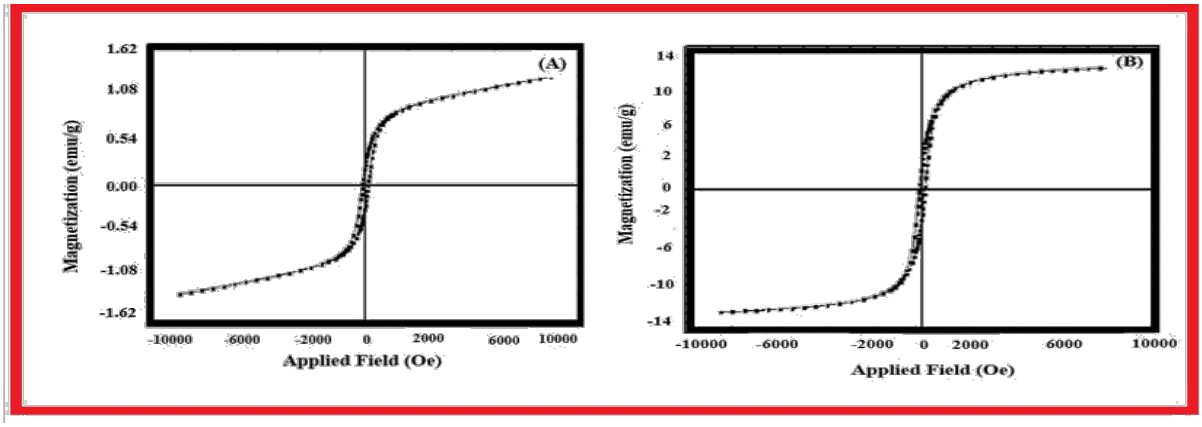


Fig 4: VSM patterns of α-Fe_2O_3 MNPs by using MAE (A) and maceration (B) techniques

3.3. Antibacterial properties and DNA cleavage study

The antibacterial activity of α-Fe_2O_3 MNPs synthesized by MAE and maceration methods was investigated against various pathogenic bacteria, at three concentrations (6.25, 12.5, and 25 mg/ml). The diameter of the inhibition zone corresponding to the antibacterial activities of α-Fe_2O_3 MNPs against some microorganisms was measured (Fig.S3). Fig. 5 illustrated the effect of different concentrations of α-Fe_2O_3 against different microorganisms. As seen, the as-synthesized α-Fe_2O_3 MNPs showed significant antibacterial activities against gram-positive as well as gram-negative bacteria. According to the results, the MNPs concentration was the main factor of their antibacterial activity, so that the antibacterial activity at high concentrations against four microorganisms is larger than in low concentration.

Besides, it is worth noting that α-Fe_2O_3 MNPs do not negatively efficacy all cells. It can be explained

that with a suitable external magnetic field, these nanoparticles may be directed to destroy bacteria as required all over the body. The maximum zone of inhibition was observed toward *E. coli* (11 mm), followed by *B. subtilis* (10.7 mm) and *P. aeruginosa* (10.5 mm). In all three methods, *B. subtilis* was more damaged than *P. aeruginosa*. Also, in microwave methods, Gram-positive bacteria were more damaged than Gram-negative but in the maceration method, Gram-negative bacteria were more damaged than Gram -positive.

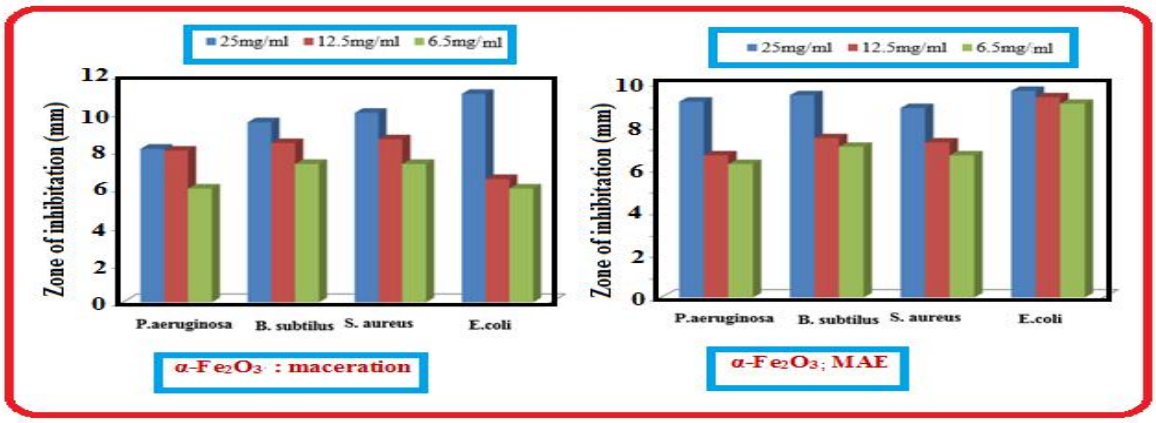


Fig 5: Antibacterial results of α-Fe_2O_3 nanoparticles synthesized by using MAE and maceration method against various microorganisms.

The presence of the inhibition zone demonstrates a biocidal activity of the MNPs that may involve the disrupting of bacterial membrane [11]. The expanse of bacterial inhibition depends on the initial bacterial concentration, as well as, MNP concentration. The smaller particle size leads to increased membrane penetrance and cell disruption [12]. The smaller particles with bigger surface interplay area will indicate more antibacterial effect than the bigger particles. The reaction of reactive oxygen species (ROS) produced from different nanoparticles with microorganisms is due to antibacterial activity [13]. This free radical generation causes intracellular pressures, depolymerized polysaccharides, DNA string breakage, inactivates enzymes, and initiates lipid peroxidation leading to cellular death [14]. The concentration of the MNPs is effective in the area of bacterial inhibition [15]. Meanwhile, good dispersion of α-Fe_2O_3 particles is a requisite for sufficient contact and interaction between α-Fe_2O_3 and microbial species. Fig 5 showed the inhibition zone of α-Fe_2O_3 nanoparticles synthesized by using MAE and maceration methods against various microorganisms.

Based on our observations, the α-Fe_2O_3 nanoparticles are more sensitive against Gram-negative bacteria than the Gram-positive bacteria. The morphological differences between Gram-positive and Gram-negative bacteria, due to different polarities of their cell membrane are the reason for the different sensitivities of these microorganisms. S. aureus was demonstrated to have a less negatively charged and softer surface than that of E. coli [16]. This would accept a higher level of permeation of negatively charged free radicals and cause destroy, S. aureus cell wall at lower concentrations than those required to damage E. coli. The Gram-positive stains contain only an outer peptidoglycan layer. Nano zero valence (NZV) could then react with intracellular oxygen, causing an oxidative pressure and, finally, interrupting the cell membrane [12]. From the DNA cleavage experiments, α-Fe_2O_3-NPs synthesized by MAE (Z_4) show a stronger effect than that of α-Fe_2O_3-NPs synthesized by maceration (Z_3) (Fig. 6). Overall above-mentioned studies bring us to the conclusion that α-Fe_2O_3 MNPs can be used as a good antibacterial nanomaterial. The investigation of the gel after electrophoresis clearly revealed a significant influence of nanoparticles on DNA and as soon difference was observed in the bands (Lane Z3 and Z4) compared to the control DNA of *E. coli*. Meanwhile, chromosomal DNA control alone does not demonstrate any clear cleavage and mainly damages the presence of MNPs.

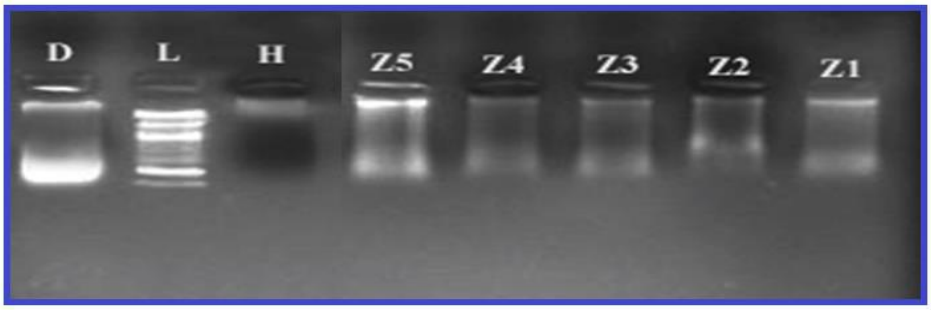


Fig 6: DNA cleavage results; D: DNA bacteria, L: Ladder, H: H_2O_2, Z_3: Maceration, Z_4: MAE.

References

[1] W. Juddin, S. Arora, Superparamagnetic iron oxide nanoparticles: magnetic nanoplatforms as drug carriers, Int. J.Nanomed., 7 (2012) 3445- 3471.

[2] M. Mahmoodpour, M. Goharkhah, M. Ashjaee, Investigation on trajectories and capture of magnetic drug carrier after injection into a direct vessel, J. Magn. Magn. Mater. 497 (2020) 166065.

[3] A. Satoh, R. Cuadra, Experimental verification of negative magnetorheological characteristics in spindle-like hematite particle suspensions J. Mag. Mag. Mater. 469 (2019) 606-612.

[4] E. Darabpour, H. Motamedi, S. M. Seyyed Negad, Antimicrobial properties of Teucrium polium against some Clinical pathogens, Asian Pac. J. Trop. Med., 3 (2010) 124-127.

[5] Z. Moradi Alvand, H.R. Rajabi, A. Mirzaei, A. Masoumiasl, Ultrasinic and microwave assisted extraction as rapid and efficient techniques for plant mediated synthesis of quantum dots: Green synthesis, characterization of zinc telluride and comparison study of some biological activities, New J. Chem., 2019,43, 15126-15138.

[6] Z. Jing, S. Wu, Synthesis and characterization of monodisperse hematite nanoparticles modified by surfactants via hydrothermal approach Mater. Let, 58 (2004) 3637-3640.

[7] Y. Mo, Y. Tang, S. Wang, J. Ling, H. Zhang, D. Luo, Metal Oxide Nanoparticles as Bactericidal Agents, Mater. Lett., 138 (2015) 251-254.

[8] K. Bhattacharya, B. Gogoi, A. K. Buragohain, P. Deb, Fe2O3/C nanocomposites having distinctive antioxidant activity and hemolysis prevention efficiency, Mater. Sci. Eng. C, 42 (2014) 595-600.

[9] M. Mahdavi, F. Namvar, M. B. Ahmad, R. Mohamad, Green Biosynthesis and Characterization of Magnetic Iron Oxide (Fe3O4) Nanoparticles Using Seaweed (Sargassum muticum) Aqueous Extract, Molecules, 18 (2013) 5954-5964.

[10] S. Ankanna, N. Savithramma, Biological synthesis of silver nanoparticles by using stem of Shorea tumbuggaia Roxb and its antimicrobial efficacy, J. Pharm. Clin. Res., 4 (2011) 137-141.

[11] R. Y. Macías, A. M. Bonilla, M. A. De, J. Tellez, H. M. Textle, C. G. Sánchez, U. S. Schubert, R. G. Santos, Combinations of Antimicrobial Polymers with Nanomaterials and Bioactives to Improve Biocidal Therapies Polymers, 11 (2019).

[12] R. A. Ismail, G. M. Sulsiman, S.A. Abdulrahman, T. R. Marzoog, Antibacterial activity of magnetic iron oxide nanoparticles synthesized by laser ablation in liquid Materials. Sci. Eng. C., 53 (2015) 286-297.

[13] S. Phumying, S. Labuayia, C. Thomas, V. Amornkitbamrung, E. Swatsitang, S. Maensiri, Aloe vera plant-extracted solution hydrothermal synthesis and magnetic properties of magnetite (Fe_3O_4) nanoparticles Appl. Phys. A, 111 (2013) 1187-1193.

[14] F. A. Khan, M. A. Fisher, R. A. Khakoo, Association of hemochromatosis with infectious diseases: expanding spectrum, Int. J. Infect. Dis., 11 (2007) 482-487

[15] Y. T. Prabhu, K. Venkatesware, B. S. Kumari, V. S. Kumar, T. Pavani, Synthesis of Fe_3O_4 nanoparticles and its antibacterial application, Int. Nano. Lett., 5 (2015) 85-92.

[16] R. Sonohara, N. Muramatsu, H. Ohshima, T. Kondo, Difference in surface properties between Escherichia coZi and Staphylococcus aureus as revealed by electrophoretic mobility measurements Biophys. Chem., 55 (1995) 273-277.