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RESEARCH ARTICLE

# Biosynthesis of Zinc Ferrite Nanoparticles Using Polyphenol-rich extract of Citrus aurantium flowers

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**ABSTRACT**

This study was conducted to examine the synthesis of $ZnFe_2O_4$ nanoparticles, using the hydroethanolic extract of *Citrus aurantium* flowers, through the application of Iron chlorides, (II) and (III), and Zinc Acetate. The as-synthesized $ZnFe_2O_4$ nanoparticles was adequately dispersed and stabilized in an aqueous solution through biological ligands extracted from *C. aurantium* flowers, a widely distributed plant used in the folk medicine. The active groups existing in the crude extract particularly the polyphenol compounds act like reducers and stabilizers to synthesis of nanoparticles. The synthesized magnetic nanoparticles have been identified using various practical techniques, including UV–Vis spectroscopy, TEM, DLS, XRD, FT-IR, XPS analyses, etc. The EDS and FT-IR methods were capable of detecting the presence of the Zinc element inside the nanocomposite structure. However, magnetic properties of the Zn ferrite materials are reduced significantly due to the agglomeration, particularly in aqueous solution. In this research, it was attempted to synthesize polyphenol coated Zn ferrite through the green synthesis method in order to prevent agglomeration among the nanoparticles. The FT-IR and TEM techniques confirmed the presence of polyphenols on the surface of $ZnFe_2O_4$ ferrite. Meanwhile, the XRD and TEM results indicated that both degree of crystallinity and particle size of the materials increased with an increase in the precipitation temperature.

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## INTRODUCTION

Nowadays, green synthesis of nanoparticles (NPs) by use of bio-reducers is economical and eco-friendly way, without needing high pressure, high temperature, and toxic regular chemicals. Synthesis of nanoparticles using biomaterials especially plant extracts is an attractive feature because of their biocompatibilities (Kouhbanani et al., 2019c, Talaiekhozani and Amani, 2019, Iravani, 2011). Due to natural origin of nanoparticles, the considerable toxicity was not observed when they were exposed to either body or bodily fluids. Iron-oxide magnetic NPs are the most promising materials in the field of biomedicine. Low levels of toxicity, bioavailability, substantial magnetic attitudes (superparamagnetism), and versatile surface functionalization allow different

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biomedical applications. These include diagnostic functionalities as contrast agents in magnetic resonance imaging (Shahraki et al., 2012, Bock et al., 2009), biosensors (Hasanzadeh et al., 2015), cellular labeling (Tefft et al., 2015), and therapeutic functions including magnetic hyperthermia (Laurent et al., 2011), controlled drug release (Zhang et al., 2017), tissue reproduction (Gonçalves et al., 2017), as well as gene therapy (Cheong et al., 2009). Recently, in the area of nanodrugs, a combination of diagnosis and therapeutic functions led to coming out of intelligent nanomaterials revealing a provision of synergistic effects of nanomedicines known as "theragnostics" (Mousavi et al., 2019, Hajba and Guttman, 2016).

Ferrites are important nanomagnetic materials in the chemical industry due to their unique characteristics and potential uses, including magnetic resonance imaging, treatment of cancer, and biomedical drug delivery (Beheshtkhoo et al., 2018, Kouhbanani et al., 2018, Meidanchi et al., 2015, Issa et al., 2013). Meanwhile, the relatively high magnetic properties of zinc modified ferrite (Zn ferrite) nanoparticles have intensively attracted the researchers' attention in all areas of biomedicine and bioengineering, such as contrast-enhanced magnetic resonance imaging, cell separation, hyperthermia, detoxification of biological fluids, drug delivery, and tissue regeneration (Iacovita et al., 2019, Lohrasbi et al., 2019, Sawant et al., 2016, Issa et al., 2013, Bárcena et al., 2008). These MNPs enjoy high magnetization values and sizes smaller than 100 nanometers and thus have similar chemical and physical properties. It is also necessary that the surfaces of particles have been coated with non-toxic materials and also be biocompatible (Vatta et al., 2006, Kodama, 1999). Furthermore, metallic nanoparticles made of iron, zinc, nickel or cobalt are ignored and are easily oxidized in the presence of water and oxygen mostly due to their chemical instability for biological applications (Mousavi et al., 2018a). Therefore, coating agents, such as humic acid, gold, and silica, are normally used to protect MNPs because their core-shell structure will cause sustainable metal nanoparticles (Liu et al., 2008, Yi et al., 2005, Lin et al., 2001). Different types of capping agents, such as polyphenols were assessed as anchors for easy attachment of polyphenol coatings on magnetic nanoparticles (Hou et al., 2016, Jiang et al., 2014).

Using zinc ferrite NPs in pharmaceutics necessitate a comprehensive study of the plausible toxicity. In the previous studies, some useful applications have been reported such as induction of chromosomal aberration in the sunflower root meristematic cells (Foca-nici et al., 2010), the production of geno- and cytotoxicity in human amnion epithelial cell lines (WISH) (Saquib et al., 2013), oxidative stress in different human cells (Alhadlaq et al., 2015), and cytotoxicity and antioxidant activity (Kanagesan et al., 2016). The experimental inconsistencies observed in methods, materials, and used cell lines, however, make it difficult to achieve acceptable conclusions. In addition, nanotoxicological assessments should be considered as a physicochemically comprehensive characterization of the materials. Hence, the need for development of standardized nanotoxicity methods in order to utilize the promising biomedical applications is underlined. These purposes are well addressed by spinel ferrites. Zinc ferrite ($ZnFe_2O_4$) NPs and/or zinc-doped magnetite ($Zn_XFe_{3-X}O_4$ ($0 < x < 1$)) NPs specifically stand out toward other ferrites because of their appropriate thermal and chemical stability (Shahraki et al., 2012) along with their lower toxicity (Wan et al., 2012). Interestingly, Iron and Zinc elements are involved in a wide range of biological processes. They are expected to be safely incorporated in the easily altered pools of both elements (Thambiayya et al., 2012, Kruszewski and Iwanenko, 2003). The system has also specific magnetic properties due to the non-magnetic nature of the zinc atom, which makes them change and improve simply through adjusting the chemical position on the NPs (Gholami et al., 2019, Shahraki et al., 2012, Naseri et al., 2011). It is essential to study the toxicity possibly induced by NP-administration relations for the potential use of zinc ferrite NPs in pharmaceutics. These biocompatible green-synthesized nanoparticles might be applied in many areas of medicine, particularly in diagnosis and treatment of cancers, drug delivery, gene delivery, and biosensors.

A simple and single-step synthesis method was used in this study to produce safe nanoparticles. The prepared NPs were then characterized using the following techniques: TEM; transmission electron microscopy, EDS; energy-dispersive X-ray spectroscopy, XRD; X-ray diffraction, VSM; vibrating-sample magnetometer (VSM), and FT-IR; Fourier transform infrared spectroscopy.

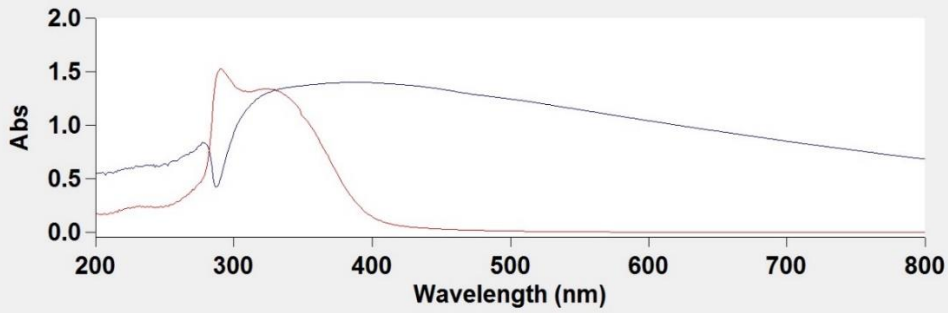


Fig. 1. The optical properties of $ZnFe_2O_4$ nanoparticles.

## EXPERIMENTAL PROCEDURE

### *Materials*

The analytical grade chemicals applied in this study were purchased from Merck Chemicals (Darmstadt, Hessen, Germany) and used without any further purification. These chemicals included Zinc acetate ($Zn(CH_3CO_2)_2.2H_2O$), iron chloride (II) tetrahydrate ($FeCl_2.4H_2O$), iron chloride (III) hexahydrate ($FeCl_3.6H_2O$), acetic acid ($CH_3COOH$), sodium hydroxide (NaOH), and ammonium hydroxide ($NH_4OH$, 25 wt %).

### *Methods*

#### *Synthesis of magnetic $ZnFe_2O_4$ NPs*

Zinc-doped magnetite NPs, $ZnFe_2O_4$, with the average diameter of 15.7 nm were prepared once a co-precipitation method using hydroethanolic extract of *C. aurantium* flowers was applied to turn the $ZnFe_2O_4$ nanoparticles into synthesis of sorts. To do so, 5.4 g of $FeCl_3.6H_2O$ plus 1.99 g of $FeCl_2.4H_2O$ and 0.8 g of $Zn(CH_3CO_2)_2.2H_2O$ were solved in 50 ml of deionized water. This process was followed by shaking the solution strongly in order to increase the pH up to 10. Therefore, 25% of the weight of $NH_4OH$ was also brought into the process. The ensuing reaction lasted two hours while the ambient atmosphere gas was $N_2$, and the surrounding temperature was kept at 80 °C (Gholami et al., 2019). Having chilled the synthesized nanoparticles, they were then washed with deionized water until reaching to a neutral pH.

#### *Characterization techniques*

The UV–Visible absorption spectra were analyzed using a UV–visible spectrophotometer, (Varian, model; Carry 100) in the wavelength range of 200–800 nm. The visual appearance and the morphology of the $ZnFe_2O_4$ nanoparticles were examined through different methods, including transmission electron microscopy (TEM), using a ZEISS 10A conventional TEM model Carl Zeiss-EM10C-100 KV (Germany). The FT-IR analyses were performed, employing a Fourier transform infrared spectrophotometer (Nicolet IS10, Thermo Scientific, USA). The wavenumbers were scanned in the range of 400–4000 $cm^{-1}$, using KBr pellets. A PANalytical X'Pert Pro (UK) diffractometer was used to characterize the crystalline structure characterized through Powder X-ray diffraction. A vibrating-sample (VSM, LBKFB, Meghnatis Daghigh. Kavir Co) magnetometer was used to measure magnetic properties and obtain magnetization curves.

## RESULTS AND DISCUSSIONS

### *UV–Visible spectroscopy*

Fig. 1 displays the optical properties of $ZnFe_2O_4$ nanoparticles synthesized via ethanolic extract of *C. aurantium* flowers. According to the UV–Vis absorption spectra in the wavelength range from 200 to 800 nm, the pink spectrum is related to the polyphenols, which peaked at the ranges of 290 nm to 360 nm representing the phenolic moieties in the structures. While, the Grey graph is belonging to the synthesized Zinc ferrite NPs, the broad absorbance peak at 500 nm which could be because of electrons' excitation from O-2p to Fe-3d level in spinel type compounds (Kouhbanani et al., 2019a, Sriramulu et al., 2018, Meidanchi et al., 2015).

### *Transmission electron microscopy (TEM)*

The TEM image of *Citrus aurantium* synthesized zinc ferrite nanoparticles makes it quite clear that the polyphenol-coated nanoparticles are very well covered. Fig. 2 illustrates the image of the synthesized ferrite magnetite nanoparticles taken by the electron microscope. Most of the

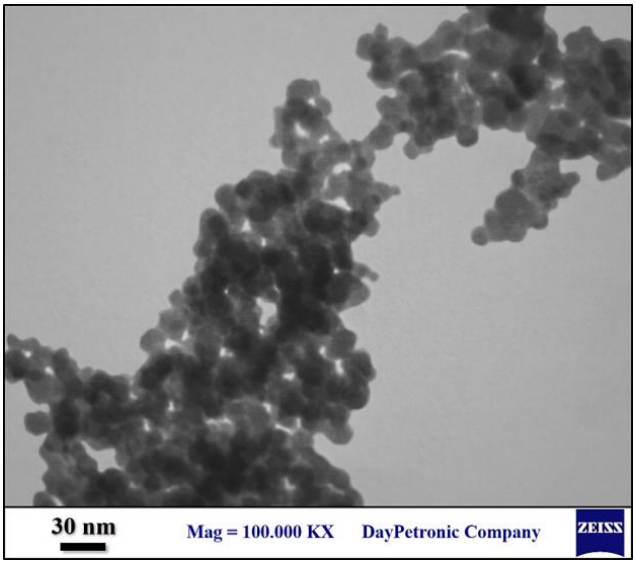


Fig. 2. TEM image of $ZnFe_2O_4$ nanoparticles.

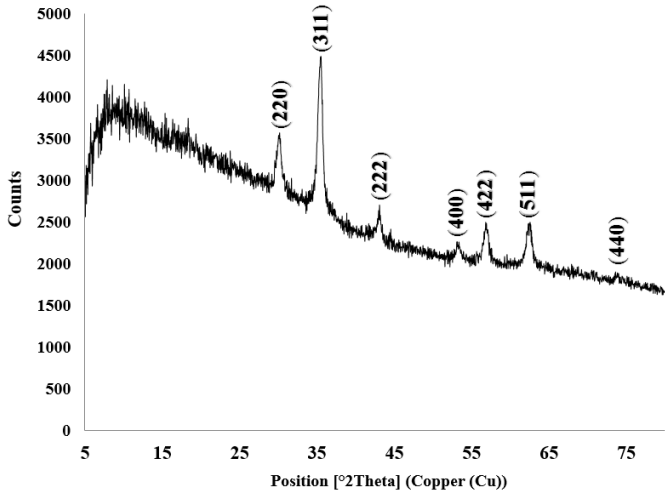


Fig. 3. X-ray diffraction patterns of $ZnFe_2O_4$ nanoparticles

synthesized nanoparticles were spherical and ranges from 9 to 20 nm in size. In terms of shape, the nanoparticles deposit significantly and show permeation interactions, which constitute one of the properties of iron nanoparticles against other metal nanoparticles and not affected by the orange extract coated on the surface of the nanoparticles. Previous studies on synthesis of Zn-doped magnetite nanoparticles have also reported similar results about shape and size (Shahraki et al., 2012, Naseri et al., 2011).

*X-ray diffraction (XRD) measurements*

The X-ray powder diffraction analysis was carried out to assess the crystalline phase of the *Citrus aurantium* hydroethanolic extract-mediated $ZnFe_2O_4$ nanoparticles. As shown in Fig. 3, there are seven peaks 220, 311, 222, 400, 422, 511, and 440 corresponding the inverse magnetite crystal structure. The peaks are shorter than the magnetospheres reported in previous studies, implying the zinc potential to crystallize the nanoparticles in the magnet structure, based on the other studies (Tehranian et al., 2019, Kombaiah et al., 2016, Moghaddam et al., 2012). Furthermore, the absence of ZnO peak in the crystalline structure has led to the argument that all the $Zn^{2+}$ ions involved in the reaction constitute the crystalline structure of magnetite and have no surface oxidation.

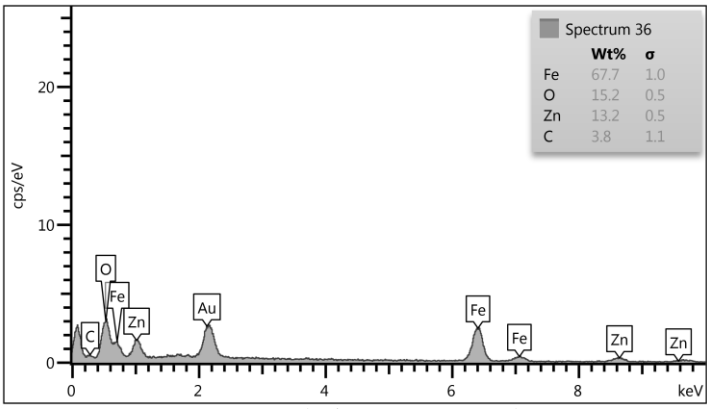


Fig. 4. EDS graph of $ZnFe_2O_4$ nanoparticles

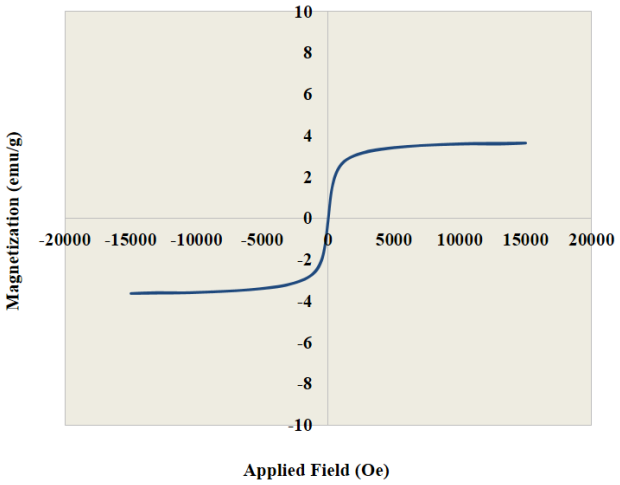


Fig. 5. VSM graph of the $ZnFe_2O_4$ nanoparticles.

*Energy-dispersive X-ray spectroscopy (EDS)*

Fig. 4 indicates the weight percentage of the elements making up the *Citrus aurantium* ethanolic extract-mediated $ZnFe_2O_4$ nanoparticles, applying EDS analysis. The obtained data revealed that zinc constituted 13.2% of the nanoparticles, which raises doubts on whether zinc is well doped in the magnet structure. This considerable amount of zinc increases the magnetic properties of magnetite nanoparticles, as in other studies where it has shown that if the magnetite structure consists of a large amount of zinc, it results in a photo and decreases the magnetic property (Kombaiah et al., 2016). The presence of carbon and oxygen elements among the elements also denotes that the polyphenol-rich extract of *C. aurantium* flowers has synthesized the nanoparticles.

*Magnetic measurements*

The magnetic attitude of *Citrus aurantium* hydroethanolic extract-mediated $ZnFe_2O_4$ nanoparticles was investigated using VSM technique at room temperature (Fig. 5). In terms of magnetization curve of the synthesized $ZnFe_2O_4$ nanoparticles, the hysteresis loop with a measurable remanence and coercivity values was observed. Likewise, the level of magnetic saturation was found to be 30 emu.g$^{-1}$, which is significant against the magnetic saturation ($M_s$) of the nanoparticles synthesized in green found in previous studies (Sriramulu et al., 2018, Yadav et al., 2017, Kombaiah et al., 2016).

*Determination of thermal properties of $ZnFe_2O_4$ nanoparticles*

The thermal stability of the green synthesized nanoparticles was evaluated through the thermal gravimetric analysis (TGA), and the nanoparticles were exposed to high temperatures of 50 °C to

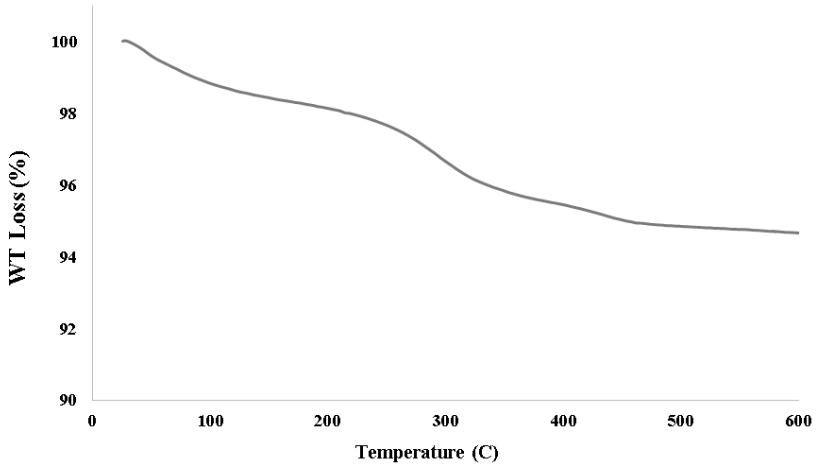


Fig. 6. TGA diagram of $ZnFe_2O_4$ nanoparticles.

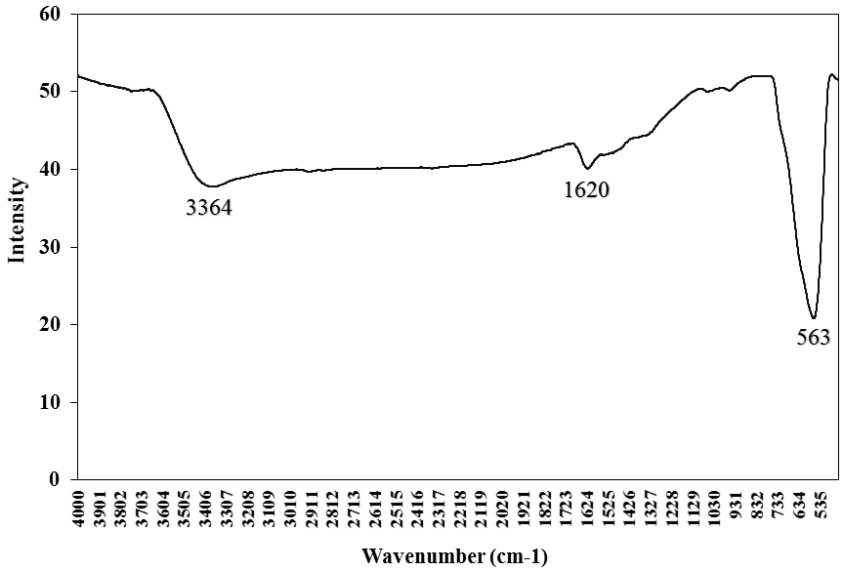


Fig. 7. FT-IR spectra of calcinated $ZnFe_2O_4$ nanoparticles.

600 °C. Since the nanoparticles were well washed with both distilled water and acetone and became maximally free from the plant extract existing on their surface, the thermal stability of the nanoparticles was increased significantly. As illustrated by Fig. 6, the weight of nanoparticles has changed up to 2% at 200 °C and 4% at 600 °C.

*Fourier transform infrared (FT-IR) spectroscopy*

FT-IR spectrum of synthesized zinc ferrite is shown in Fig. 7, recorded in the range of 4000-400 $cm^{-1}$. An general overview on the Infrared spectrum reveals certain peaks at 3364 $cm^{-1}$, 1620 $cm^{-1}$, 1430 $cm^{-1}$, 1053 $cm^{-1}$, and 563 $cm^{-1}$ corresponding to the various functional groups in the *C. aurantium* extract that were involved in reduction and stabilization of the nanoparticles. The peaks at 3364 $cm^{-1}$, 1620 $cm^{-1}$, and 1053 $cm^{-1}$ were referred as hydroxyl (-OH), aromatic (-C=C-), etheric (C-O-C) groups, respectively due to the polyphenol compounds (Kouhbanani et al., 2019b). Meanwhile, the peaks under 1000 $cm^{-1}$ could be related to the metal-oxygen complex, thereby the tetrahedral $Zn^{2+}$ stretching and octahedral $Fe^{3+}$ vibration bands of synthesized zinc ferrite nanoparticles were appeared in 400-600 $cm^{-1}$. Thus, a peak in the 563 $cm^{-1}$ represents the Fe-O group and the presence of iron oxide

nanoparticles (Lohrasbi et al., 2019, Beheshtkhoo et al., 2018, Mousavi et al., 2018b).

## CONCLUSION

In the current study, a simple, cost-effective, eco-friendly method was introduced for production of zinc ferrite magnetite nanoparticles, using the hydroethanolic extract of *Citrus aurantium* flowers. The nanoparticles were produced without adding any chemicals. The biomolecules of the plant extract especially polyphenols in the process of nanoparticle production contribute to stabilization of the produced nanoparticles besides reducing the iron and zinc ions to ferrite magnetite nanoparticles. The produced nanoparticles were characterized using methods, including TEM, XRD, FT-IR, TGA, and VSM. The size distribution of the synthesized nanoparticles ranged from 9 - 20 nm, with the average size of 15.7 nm. The infrared spectroscopy studies revealed that the biomolecules containing phenol groups in the plant extract are most likely to play a key role in the production and stability of nanoparticles. The production of ferrite nanoparticles using plant extracts is very efficient and advantageous despite obvious limitations. The methods used for production of ferrite nanoparticles using plant extracts have been so far performed only *in vitro*. Extensive studies are required to optimize such methods and make them more effective in order to be able to supply the nanoparticles produced by plant extracts and the make them more competitive against those produced through common physical and chemical methods.

The main costs of producing metal nanoparticles through chemical techniques include metal precursor salts and reducing substances. However, the main costs of green synthesis of nanoparticles include only the metal precursor salt due to the possibility of using plant extracts and also food wastes as the reducing agents. Regarding the foregoing, industries, such as the food industry and the floral industry, can produce inexpensive metal nanoparticles, using plants and food wastes, besides their usual activity. Zinc ferrite nanoparticles might be used as antibacterial and also a material enhancing the antimicrobial of disinfectants and antibiotics, for treatment of cancer, hyperthermia, etc. in medicine besides the fact that the proposed synthesis of ferrite nanoparticles is easy and cost-effective, is produced out of renewable resources, and does not need complex laboratory conditions, devices, or expensive materials. Regarding the considerable benefits and above-mentioned limitations of the proposed method, further biological studies shall be performed in order to make use of the significant characteristics of the green synthesis of zinc ferrite nanoparticles appropriately.

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## CONFLICT OF INTEREST

The authors declare there is no any conflict of interest.

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