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Biogenic Synthesis of Iron Oxide Nanoparticles using *Bergenia ligulata* Rhizome Extract and their Biological Activities

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Over the last few decades, nanoparticles were synthesized using various methods. Currently, studies are more focussed towards the biogenic synthesis of nanoparticles which has proven to simple, environmental-friendly, convenient and non-toxic as compared to other conventional methods. This work describes the biosynthesis of iron oxide nanoparticles (FeO NPs) using *Bergenia ligulata* rhizome extract. The synthesis of FeO NPs was validated by visual inspections when the colour of solution changed from dark brown to blackish brown. Other characterization including UV-visible spectroscopy, FTIR, XRD, DLS and stability tests were also performed. UV-visible spectrum of FeO NPs revealed high signal at 290 nm. The FTIR spectroscopy analysis revealed various functional groups at different bands. XRD results confirms the crystalline structure of FeO NPs. The size and stability of the synthesized nanoparticles was studied by DLS analysis and stability test. Plant extracts and NPs produced were used for a quantitative study that revealed phenolic and flavonoid compounds in high concentration. ABTS and DPPH tests were carried out to assess the *in vitro* radical scavenging activities. Further, the antifungal activity was performed against *Rosellinia necatrix*, *Fusarium* spp, *Sclerotinia sclerotiorum*. This study confirms that the synthesized FeO NPs are a novel natural preservative with possible uses in agricultural applications.

Keywords: *Bergenia ligulata*, Iron oxide nanoparticles, Antifungal activity, Antioxidant activity.

INTRODUCTION

Nanotechnology is a fascinating and rapidly expanding area of study that has made significant advances in the contemporary era of technology [1]. Nanoparticles are incredibly small particles (1 to 100 nm in diameter) that have higher catalytic activity, non-linear visual performance, high stability and thermal conductivity because of its superior surface area to volume ratio [2]. Amongst some of the different nanoparticles, metallic nanoparticles are generally considered acceptable for both human beings and nature. Metal oxide nanoparticles have sparked interest due to their potential in a variety of sectors such as materials science, medical, agricultural, information systems, environment, energy and so on [3,4]. Recently, many researchers have become interested in using nanoparticles made of different metals in biotechnological and pharmaceutical uses. Common examples of these nanoparticles include zinc oxide, copper, silver, gold, platinum, *etc*. [5].

Of the various metallic oxides nanoparticles, iron oxide (FeO) is the most intriguing inorganic oxide and it is broadly applied due to its biocompatibility, excellent magnetic properties, simple surface flexibility, varied oxidation states, crystal structures, low cost and renewability allow for a huge variety of adsorption sites [6]. Several researchers are interested in FeO nanoparticles for its application in electronics, energy, veterinary biotechnology, bioremediation, biomedical and agriculture [7]. They offer antimicrobial properties against harmful bacteria and fungus [8]. Iron oxide (FeO) nanoparticles can be fabricated using traditional chemical and mechanical techniques. The mechanical technique demands the utilization of expensive tool, extreme pressure and heat and a significant space for machinery [9]. The chemical approach requires the utilization of harmful chemicals which produces toxic waste as a byproduct and cause detrimental effect to both the environment and the person using them [10].

Due to the drawbacks of traditional synthesis procedures, the researchers are currently focusing on developing non-toxic, sustainable, biocompatible and environmental friendly methods to generate iron oxide nanoparticles and noted that this can only be done using biological sources. It generally employs various intracellular and extracellular biological extracts from plants and microorganisms having biomedical activities [11,12]. Currently, substantial research is being conducted on the fabrication of FeO nanoparticles using different plant extracts. The nanoparticles are integrated with plant biological components, which improves the shape and maintain stability [13]. The biological agents lead to the production of nanoparticles which are non-toxic and environmentally benign. According to literature, several hundreds plant extracts are employed for the synthesis nanoparticles of various sizes [13-17]. Therefore, green synthesis of FeO NPs are considered to be an ideal method and mostly chosen over other conventional techniques because of their stability.

Bergenia ligulata belongs to the Saxifragaceae family is an evergreen perennial herb and distributed in the temperate region of Himalayas. *B. ligulate* also provides certain biological activities such as antimicrobial, anti-inflammatory, antiulcer, antioxidant, antitussive, insecticidal, diuretic, anti-bradykinin, antidiabetic, antiviral, anticancer, anti-obesity, antimalarial and so [18]. In addition, *B. ligulata* contains phytochemicals such as bergenin, gallic acid, tannic acid and terpenes and is considered to be an efficient capping and reducing agent [19]. In present work, the biogenic synthesis of FeO NPs was accomplished in this study utilizing the rhizome extract of *Bergenia ligulata* and characterized with different techniques. The synthesized FeO nanoparticles were evaluated for their antioxidant and antifungal activity against fungi species *viz. Fusarium* spp, *Rosellinia necatrix* and *Sclerotinia sclerotiorum.*

EXPERIMENTAL

Collection of samples: *Bergenia ligulata* was utilized as a reducing agent for the biogenic synthesis of iron oxide (FeO) nanoparticles. The rhizome of *B. ligulata* were collected in the month of April and May 2022 from the Herbarium located at the School of Biological and Environmental Sciences, Shoolini University, Solan, India [20].

Drying and grinding of samples: The rhizomes were first cleaned in running water to remove soil and other extraneous materials prior to dry in a shady area. The dehydrated substance was then crushed into a powder form using a grinder, kept at ambient surrounding in an airtight jar. The extract was then utilized for further investigations.

Preparation of rhizome extract: Rhizome powder (10 g) was heated with 100 mL water for 30 min at 60 ºC in 250 mL flask and then cooled to 25 ºC. A 0.2 µm Whatman filter paper was used to filter the solution and the aqueous layer was stored at 4 ºC for further steps [21].

Green synthesis of FeO NPs: Biosynthesis of FeO nanoparticles was achieved by the modified method [22]. Ferric chloride and ferrous sulphate were prepared and then mixed in 2:1 ratio (40 mL ferric chloride + 20 mL ferrous sulphate). Then the above solution (40 mL) was mixed with 40 mL rhizome extract) and covered with foil. The mixture was then stirred continuously for about 30 min at 50 ºC on a magnetic stirrer and the pH was adjusted to 10-11. It was stirred for another 2 h at 80 ºC. After 10 min, the reaction began and a colour shift from colourless to clear yellow at different intervals followed by a dark brown when stored in the shady place at ambient condition for 24-48 h. The centrifugation of the mixture was done at 10,000 rpm for 10 min for eradication of any undesirable biological molecules. The pellet obtained after centrifugation was re-dispersed in water. For achieving efficient separation of unbound entity from FeO nanoparticles, the methods of re-dispersion and separation in sterile water was performed thrice. Finally, the suspended pellet was purified with absolute ethanol. The purified pellets were then placed in petri-dishes and dried in a 60 ºC oven for 24 h.

Characterization The ultra-violet absorption spectra of the samples were evaluated by employing Shimadzu UV-Vis V-530A spectrophotometer with a range of 200 to 800 nm. The grain size and purity of the nanoparticles were determined using X-ray diffraction at 40 kV and 30 mA. Dynamic light scattering (DLS) was achieved out using the DynaPro Plate Reader (Wyatt Technology).

Assessment of phenolic and flavonoid content

Phenolic content: The Folin-Ciocalteu test was used to quantify the phenol content. Rhizome extract of 10 g was taken and diluted with 1 mL of sterile water. The tube filled with 200 mL of folate concentration and 1 mL of water and the solution was set aside to settle for 5 min at ambient temperature. It was again treated with 2 mL of Na_2CO_3 and 2 mL of gallic acid and left to sit for 0.5 h at room condition. The optical density (OD) was obtained between 650 and 730 nm [23].

Flavonoid content: Flavonoid was estimated *via* $AlCl_3$ technique. To make the extract, 10 mg of rhizome was diluted in 1 mL of water and added to a test tube sample (200 mL) containing 75 µL of 5% $NaNO_2$ and allowed to settle for 5 min followed by the addition of 150 µL of 10% $AlCl_3$ and then stand for another 5 min at room temperature. Finally, 1 M NaOH was allowed to mix at room temperature for 30 min and the optical density (OD) was taken at 515 nm [24].

Antioxidant activity

DPPH analysis: The scavenging capacity of rhizome extract was assessed *via* procedure outlined by Barros *et al.* [25]. To make the DPPH solution, 20 mg of DPPH was combined with 100 mL of methanol (stock solution). The absorbance of this solution at 515 nm (the control solution) was measured in 3 mL and fixed to 0.75. The stock solution of DPPH was covered in aluminium foil and left in a shady area for 1 day to shield against the oxidative stress. Rhizome extract of 5 g was dispersed in 5 mL of methanol to generate stock solutions. From stock solutions, various dilutions (25, 50, 75 and 100 g/mL) were prepared *via* serial dilution. A 2 mL solution of DPPH was combined with 2 mL of each dilution then stored in a shady place for 15 min. All of the experiments conducted for the comparative research used vitamin C as a standard agent. The proportion of DPPH radical inhibition by rhizome extract was estimated using the following equation:

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c: OD of the control, A_s: OD of the extract/standard.

ABTS analysis: The antioxidant effectiveness of extracts was tested using the ABTS free radical scavenging test [26]. By combining ABTS in water with potassium persulphate (1:1) for 12 to 16 h in a shady area at ambient condition, an ABTS cation radical was formed. Then, the ABTS solution was mixed with alcohol until it reached an intensity of 0.70 at 734 nm. The intensity was measured after 30 min after combining 3.9 mL of diluted ABTS solution and 5 L of plant extract. In each test, an acceptable solvent blank was used and readings were taken three times.

Antifungal activity: The fungicidal activity of synthesized FeO NPs was determined by examining the growth responses of three different fungal strains (*Rosellinia necatrix*, *Fusarium* spp., *Sclerotinia sclerotiorum*). For fungal growth, the potato dextrose agar was utilized. Phull's method [27] was somewhat modified for assessing the fungicidal capabilities. The extract (50 mg/mL) was applied to sterilize PDA and a 6 mm diameter actively developing ring of pathogenic microorganism's culture from 6-7 days old was kept in the middle of plate. The negative control was plates without extract, whereas the positive control was hygromycin at 5 mg/mL. The rate of growth was evaluated and compared to a negative control. *Fusarium* spp., *Sclerotinia sclerotiorum* and *Rosellinia necatrix* were cultured at 25 ºC for 6 days. After 6 days, the circular proliferation of mycelium was measured. The zone of inhibition around the discs were measured as follows:

$$I = \frac{C - T}{C} \times 100$$

where C is colony diameter in control (cm); I is percentage of inhibition and; T is diameter of colony in treatment (cm).

RESULTS AND DISCUSSION

The iron oxide (FeO) nanoparticles were obtained by employing the rhizome extract of *B. ligulata via* environmental friendly synthetic approach. The plant polyphenols contribute as both an encapsulating and a reducing agent, eventually results in green nanoscale zero-valent iron particles which is stable with different properties [28]. The colour transformation in the solution serves as preliminary screening for the generation of nanoparticles. During the 24-48 h reaction, the colour of mixture changed from dark to blackish brown, confirming presence of FeO nanoparticles. Due to surface vibrations, FeO nanoparticles solution exhibits a dark brown to blackish brown colour, according to literature studies [29,30].

UV-visible studies: A UV-visible spectroscopy study was carried out to demonstrate the biogenic synthesis of FeO nanoparticles. The UV-visible spectrum was found from 200 to 800 nm. The presence of FeO nanoparticles (Fig. 1) in the mixture was confirmed by the high signal at 290 nm [21].

XRD studies: The nanoparticles were highly crystalline in nature as indicated by the high-intensity XRD peaks (Fig. 2) [31]. An XRD analysis revealed the formation of the crystalline

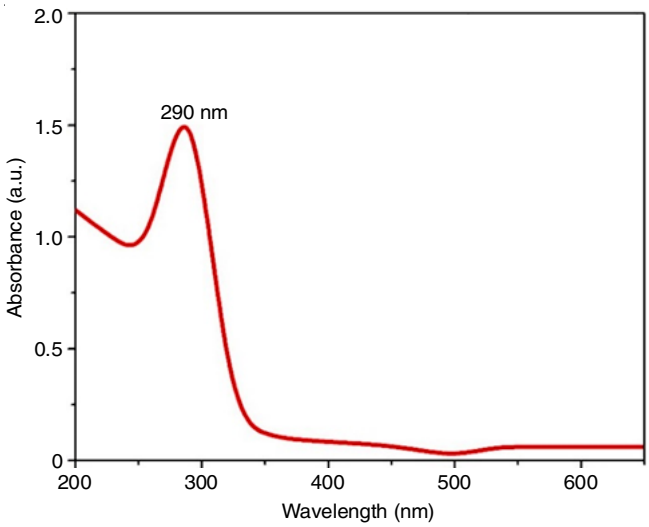

Fig. 1. UV-visible spectroscopic analysis of FeO nanoparticles

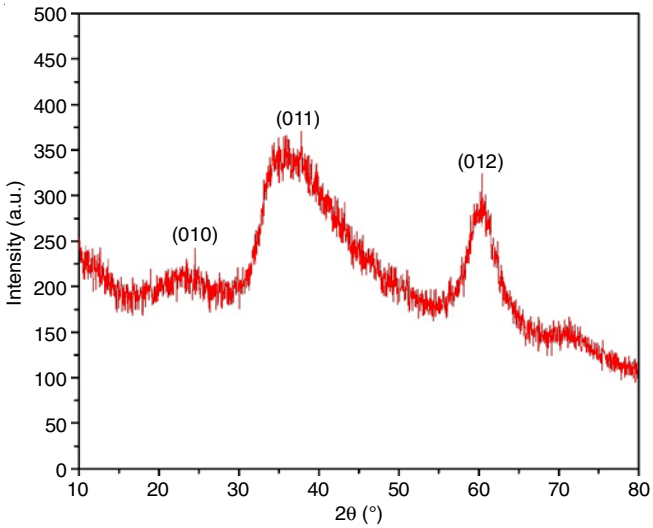

Fig. 2. XRD analysis of FeO nanoparticles

structure of FeO nanoparticles. The resulting X-ray diffraction analyses were compared to the standard FeO spectra. The Debye-Scherrer's equation was used to compute the crystalline size of the nanoparticles. Assuming there are no non-uniform stresses, we may calculate their Debye-Scherrer's equation as follows:

$$D = \frac{0.9\lambda}{\beta \cos\theta}$$

where D is the size of the particle, λ is the wavelength of the X-ray, β is the full width at half maximum (FWHM) of the diffraction peak and θ is the angle of the diffraction.

Dynamic light scattering (DLS) studies: The size of the synthesized FeO nanoparticles in the solution was calculated using the fluctuation in light shift. The hydrodynamic diameter of the produced nanoparticles was also assessed using DLS. DLS studies revealed that the typical particle size was 80-100 nm (Fig. 3).

Stability studies: The stability of the biosynthesized FeO nanoparticles was assessed using UV-visible spectra at different time intervals. The stability of the synthesized nanoparticles

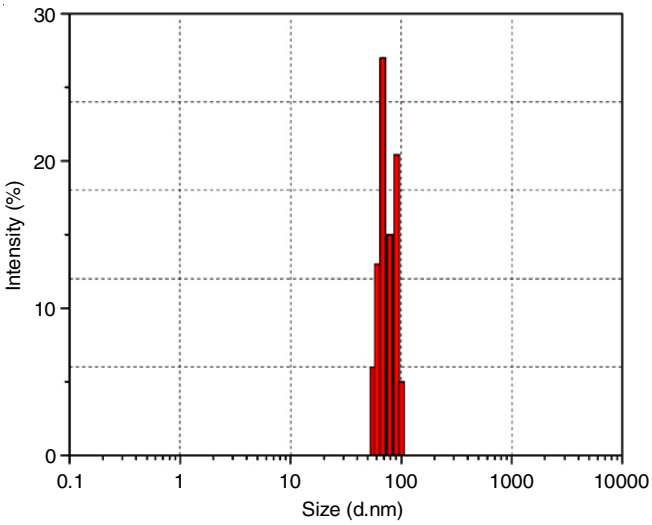

Fig. 3. DLS analysis of FeO nanoparticles

was examined for one month using SPR monitoring at 0, 7, 14, 21 and 28 days intervals. The peak's absorbance strength was greatest at 290 nm (Fig. 4).

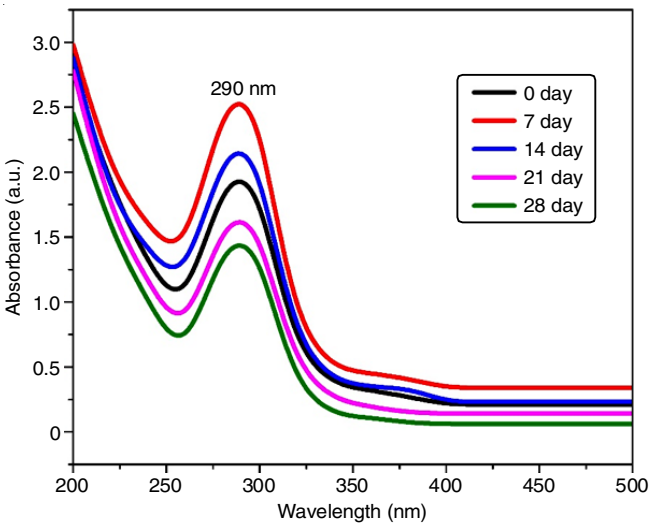

Fig. 4. Stability study analysis of FeO nanoparticles

Total flavonoid content (TFC) and total phenolic content (TPC): The total flavonoid content was determined with the help of rutin standard curve and the equation $y = 0.0465x + 0.123$ contents. The high flavonoid content of extract and FeO nanoparticles was determined to be 48.411.38 to 52.429.87 mg QE/g (Fig. 5). TFC was also shown to be greater in FeO nanoparticles. Flavonoids inhibit oxidative stress through direct induction of reduction reactions or indirect chelation of iron as a result of their ant oxidative function by scavenging the reactive oxygen species [32]. It seems that determining the contributing level of iron blend in leaf extract play a vital role in increasing or decreasing the flavonoid contents in plants. Ghorbanpour [33] reported an increase in flavonoid level from the use of iron chelates in high quantity. The total amount of phenol was calculated using the following equation:

$$y = 0.0344x + 0.1775$$

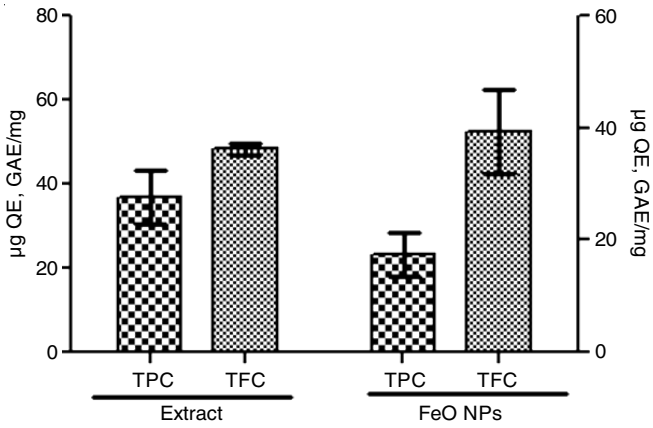

Fig. 5. Total flavonoid and phenolic content of *B. ligulata* rhizome extracts and FeO nanoparticles

The phenolic content of rhizome extract of *B. ligulata* and FeO nanoparticles was determined to be 36.976.34 to 23.235.10 mg GAE/g. The FeO nanoparticles showed a lower TPC value than the extract alone as observed, which could be as a result of contribution of phenolic compounds in the reduction reaction of FeO nanoparticles biosynthesis. It is affirmed that both phenols and their quinones can stabilize the metal nanoparticles [34,35].

Antioxidant studies: The anti-oxidative characteristics of green production of FeO NPs were investigated by applying the ABTS and DPPH techniques for measuring compound reduction power.

DPPH activity: The potency of the inhibitory action of the synthesized FeO nanoparticles was greater than that of the plant extract at levels extending from 25 µg/mL to 100 µg/mL. The IC_{50} value of *B. ligulata* DPPH free radical scavenging activity was 402.35 µg/mL, while the value for FeO nanoparticles was 52.24 µg/mL (Fig. 6). As a control, vitamin C was used and its IC_{50} was 16.55 µg/mL. It was found that FeO nanoparticles have a stronger scavenging action than *B. ligulata* rhizome extract. Similar results were observed from the antioxidant potential of both aqueous extract of *E. robusta* leaf and FeO nanoparticles through the entrapment of the DPPH radical [36].

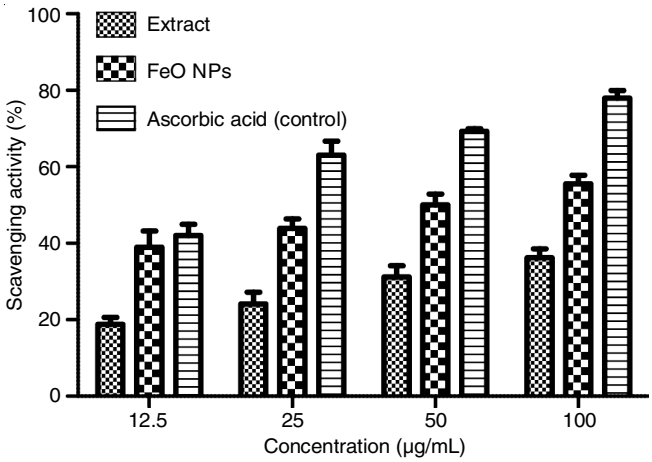

Fig. 6. DPPH Assay showing enhanced antioxidant potential of rhizome extract and FeO NPs

ABTS activity: The scavenging strength of biogenic generated FeO nanoparticles using an ABTS scavenging assay with vitamin C as a positive control was evaluated. The ABTS assay results demonstrated that both plant extracts and biosynthesized nanoparticles inhibited the free radicals effectively. The IC_{50} value of *B. ligulata* ABTS free radical scavenging activity was 383.5 µg/mL, while for FeO nanoparticles was 47.64 µg/mL (Fig. 7). Similarly, vitamin C was employed as a control and its IC_{50} value was 22.14 µg/mL. The proportion of inhibition in FeO nanoparticles was found to be higher than in other materials. The percentage of inhibition in FeO nanoparticles was found to be higher than in *B. ligulata* rhizome extract. Previous study found that the concentration of FeO nanoparticles increased with increasing concentration, with an IC_{50} value of 63.8 µg/mL [24].

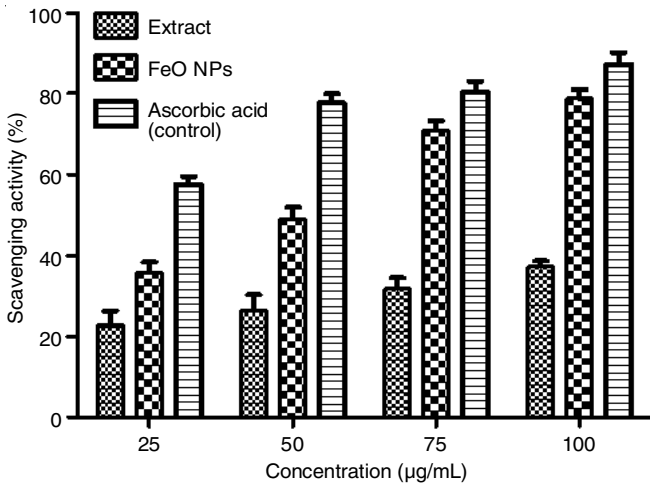

Fig. 7. ABTS Assay showing enhanced antioxidant potential of rhizome extract and FeO NPs

Antifungal activity: The efficiency of rhizome extracts and synthesized iron oxide nanoparticles as antifungal agents towards certain fungi strains was investigated in the study. In previous studies, determination of amount of fungal growth by El-Mohamedy & Abadallah [37] demonstrates that *Moringa oleifera* seed extract has fungicidal action towards all pathogens tested and these observations support the experiment outcomes (Table-1). Thus, iron oxide nanoparticles from rhizome extract of *B. ligulata* have the prospective being used as antimicrobials, resolving the issues in fungal infection management due to rapid development of resistance to standard fungicides.

TABLE-1
ANTIFUNGAL ANALYSIS (ZONE OF INHIBITION)

Fungus	Negative control (cm)	NPs (cm)	Positive control	Zone of inhibition (%)
Rosellinia necatrix	3.7	1.5	ND	45.9
Fusarium spp.	4.0	2.4	ND	37.5
Sclerotinia sclerotiorum	3.5	1.9	ND	55.5

Conclusion

In this study, green synthesis was used to synthesize iron oxide (FeO) nanoparticles using *Bergenia ligulata* rhizome extract in a cost-effective and environmentally friendly manner. The FTIR, DLS, XRD, UV-vis studies and stability analysis were employed for the identification of the biosynthesized FeO nanoparticles. Furthermore, iron oxide nanoparticles generated showed antifungal action against three fungal species. *In vitro* antioxidant studies using various approaches revealed that FeO nanoparticles synthesized biologically had significant action. This study paves the way for further research into the synthesis of cost-effective FeO nanoparticles from the medicinal plants that are environmentally favourable.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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