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Facile one-pot sustainable microwave-assisted green synthesis of Fe_2O_3 nanoparticles with *Coleus scutellarioides* leaf extract and their antibacterial and photocatalytic activity

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Abstract

The green method of synthesis of nanoparticles has become an extremely promising technique because of its environment-friendly performance and non-toxic nature. In the present study, iron oxide nanoparticles were prepared through a microwave-assisted approach by using leaf extract of *Coleus scutellarioides* with the addition of $FeCl_3.6H_2O$ and $FeSO_4$ under atmospheric conditions. The confirmation of these iron oxide nanoparticles was done by using various characterization techniques like UV-Visible spectroscopy, X-ray diffraction (XRD), Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). UV-Visible spectroscopy has shown absorption spectra in the range of 240-800 nm. XRD has shown the crystalline nature of these iron oxide nanoparticles. SEM results have described the average particle size in the range of 50-60 nm. FTIR detected different functional groups like -OH, -NH, C=H, -NO, that were present on the surface of nanoparticles. The synthesized iron oxide nanoparticles were successful in degrading the toxicity of dyes like rhodamine and bromocresol green by decolorizing them which shows that these nanoparticles can be used to reduce toxicity and harmful compounds in the wastewater and make the water useful for other purposes. The antibacterial activity of iron oxide nanoparticles has also been checked against *Pseudomonas, Staphylococcus aureus, and Bacillus anthracis* bacterial strains. These nanoparticles have shown moderate to good activity in resisting these bacteria from growth.

Keywords: Nanoparticles, Fe_2O_3 green synthesis, *C. scutellarioides,* Photocatalytic, Antibacterial

1. Introduction

Nanotechnology is a department of science that deals with the manufacture, manipulation, and use of materials ranging in nanometers. Suggestion of nanotechnology was proposed by Richard Feynman and the term was used in scientific fields in 1974. The arrival of nanotechnology has brought large-scale research in recent years by intersecting with different other branches of science and making an impact on all forms of life [1] It is believed that nanotechnology is capable of massively increasing manufacturing production at significantly lower prices [2]. Nanoparticle synthesis involves the methods for generating nanoparticles. Currently, two principal applications are being applied for synthesizing nanoparticles, known as the top-down approach and the bottom-up approach [3]. Concisely, if we discuss the top-down method, nanoparticles are made as a result of the size reduction of bulk material into small particles with the help of lithographical processes and mechanical processes [4]. These processes are simple and dependent on eliminating or break-up of bulk material to form the intended structure with relevant properties. Whereas, if we talk about the bottom-up method, compact building materials cluster by cluster are brought together forming a huge structure [5]. Synthesis of nanoparticles can be carried out by different methods including physical methods, chemical methods, and biological methods [6]. There are two ways physical and chemical methods are generally used to create regular-sized nanoparticles having long-lasting stability [7-9]. These two methods have some disadvantages as these methods are costly and harmful to the environment. The chemical approach for the fabrication of nanoparticles required different types of chemicals due

to the use of harmful chemicals the application of these nanoparticles becomes limited. To improve the quality and field of application it is important to reduce the use of these harmful chemicals in the fabrication of nanoparticles [10]. During the last few decades, a tremendous amount of research has been carried out to develop a novel green synthesis approach for the fabrication of nanoparticles. In these green synthesis approaches different type of plant leaves, fruit extract algae, and different kind of extract is used to reduce the use of harmful and toxic chemicals. This greener approach for the fabrication of different kinds of ceramic and semiconductor nanoparticles received much more attention during the last decades [11].

Purification of the wastewater contaminated with effluents from different industries like textile, pharmaceutical, and paint industries generated critical environmental threats. Different physical, chemical, and biological methods are utilized to remove the contaminants from the wastewater. But most of the available methods are costly and not safer for the environment [12].

During the last few decades, the photocatalytic degradation of organic pollutants is receiving much attention due to its low cost and eco-friendly approach. Photocatalytic degradation is an advanced oxidation procedure in which the nanoparticles as a photocatalyst play a crucial role. Metal (Ag, Au) and metal oxide (ZnO, TiO_2) nanoparticles are very effective nanocatalysts due to their large surface area and unique physical and chemical properties. Among the various available metal and metal oxide nanoparticles iron oxide nanoparticles received much attention due to their excellent photocatalytic properties. One of the most important reasons behind the popularity of iron oxide nanoparticles as one the most suitable candidate for the photocatalysis of dye food and agricultural waste and one of the most important advantages in the utilization of the iron oxide nanoparticles in separation is the ease of the separation of nanoparticles from the test solution. As the separation of the nanoparticles is a very costly and tedious process [13]. In the photocatalytic treatment of the waste water mainly advanced oxidation process is involved. In this process when the developed nano Photocatalyst irradiated with the light having a band gap higher than the band gap of the nanocatalyst then the electron-hole pairs are generated on the surface of the catalyst which ultimately generated the reactive oxygen species. These reactive oxygen species are considered to be the key factor in the degradation of organic pollutants. [14]

The biological way for the development of nanoparticles is considered to be one of the most eco-friendly low-cost approaches. The biological way of the development of nanoparticles utilize different kinds of plant material having significant amount of alkaloids terpenes and phenol play a key role in the development of nanoparticles [15]. Green synthesized iron oxide nanoparticles are less toxic as compared to the nanoparticles synthesized by using different chemical procedures. Microwave-assisted green synthesis is slightly different from conventional green synthesis and chemical synthesis. However, microwave-assisted synthesis has certain advantages, In this synthesis the materials are heated uniformly hence the particle size is uniformly distributed and the rate of the reaction is very fast as compared to other methods. With this background in the present investigation, we are trying to synthesize iron oxide nanoparticles by utilizing the leaf extract of *Coleus scutellarioides* plant. The synthesis nanoparticles follow the sol-gel technique. The photocatalytic potential of the developed nanoparticles was investigated against the rhodamine and bromocresol green dye. The antibacterial activities of the developed nanoparticles were also investigated against the selected bacterial strain.

2. Materials and methods

2.1 Chemicals

$FeCl_3.6H_2O$, $FeSO_4$, Ethanol (CH_3CH_2OH), Distilled water, Rhodium dye, Bromocresol green dye, Nutrient agar solution all these chemicals were High Purity Laboratory Chemicals Pvt. Ltd, India. All the chemicals used in this study were of analytical grade and used without any further purifications. Bacteria: *Pseudomonas, Staphylococcus aureus and Bacillus anthracis* were received from the microbiology department of our university.

2.2 Collection of plant

The plant *C. scutellarioides* (Figure 1) leaves were collected from different regions of Dehradun, Uttarakhand, India. The plant is then washed with clean water 3 to 4 times to ensure that there is no dust or impurities left behind on the leaves. A shade drying method has been carried out for drying these leaves. For this, leaves were stored in a place away from sunlight for 9-10 days (about 1 and a half weeks). Leaves should be dried enough to be crushed and transformed into powered form to continue the further process of making the extract.

C. scutellarioides belongs to the species of flowering plant under the Lamiaceae family. It is an aromatic, evergreen plant. It is mostly used for decorative purposes because of its leaves having different colored zones appearance. It is found in different regions, with leaves showing a variety of colors varying in shades (pale yellow, wine red, dark purple, mostly outlined with green color) and shapes.

Figure 1 *C. scutellarioides.*

2.3 Preparation of plant extract

Dried leaves were crushed and transformed into powdered form. After this Soxhlet extraction technique was performed for preparing the extract. For this, an instrument named Soxhlet was used which is cylindrical having two parts upper part and a lower part. In the upper part, it contains a condenser system through which water runs in it and in the lower part crushed leaves were placed. This system runs on electricity and needs a constant water supply for work. At the lower end, a solvent flask was connected which contained ethanol (200 mL) as a solvent. After 4-5 h the extract that has been collected in the solvent flask is taken into a beaker and placed on a hot plate for around one hour for distillation maintaining the temperature around 50-60°C. The plant extract has been prepared (Figure 3) [16].

Figure 2 Prepared plant extract.

2.4 Microwave accelerated green approach for the development of Iron oxide (Fe_2O_3) nanoparticles

For the synthesis of nanoparticles, 10 mL $FeCl_3.6H_2O$ and 10 mL of $FeSO_4$ solution was taken in a beaker and 5 mL of prepared *C. scutellarioides* leaf extract was added into that beaker, then the solution was placed in a magnetic stirrer, ethanol was added to this mixture drop by drop at the interval of 15 min. The mixture was checked constantly and after some time the mixture would become darker in color and tiny particles can be seen deposited on the beaker. Now placed the beaker in microwave (800 W) for irradiation (1 min). The color of the test solution becomes almost black, and the color change initially indicates the formation nanoparticles. Finally, the developed nanoparticles were isolated by the centrifugation (5000 rpm at 30 min) of the test solution. Isolate nanoparticles were washed thoroughly with distilled water to remove the excess phytochemicals. The developed nanoparticles were dried in the oven at 60°C for 5 h (Figure 3) [17].

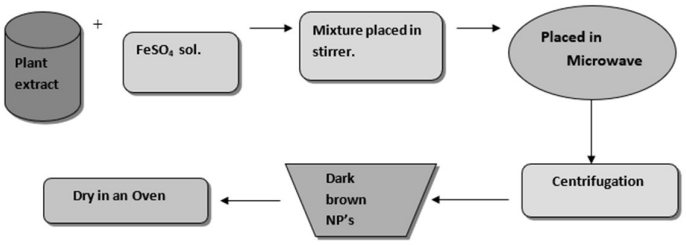

Figure 3 Diagram representing the process of green synthesis of iron oxide NP's.

2.5 Characterization

Synthesized nanoparticles were subjected to various characterization analyses. Fourier transform infrared spectroscopy (FTIR) characterization analysis has been carried out using a Thermo scientific FTIR spectrophotometer for the sample of obtained nanoparticles, functional groups that are present in the desired nanoparticles have been characterized by performing this analysis, maintaining wavenumber region around 1400-2000 cm^{-1}. Scanning electron microscopy (SEM) has been performed on a Zeiss-evo-18 scanning electron microscope for knowing the particle size and morphological structure of the nanoparticles to study the features these nanoparticles possess. The purity of these synthesized nanoparticles and their crystallographic nature were recorded through XRD (X-ray diffraction) analysis that has been performed using a Regaku diffractometer. The property of light absorption of these prepared nanoparticles was demonstrated with the help of UV-Visible spectroscopy techniques (on Systonics UV- spectrometer) that were found to be in the range of 200-800 nm.

2.6 Photocatalytic degradation activity

Photocatalytic degradation activity has been performed using these nanoparticles for studying and knowing their ability to which extent these nanoparticles can degrade the solution of different dyes. For this study, two different samples of dyes named Rhodamine and Bromocresol green were used. A solution of dyes was prepared. For this, a 50 L solution of both dyes was prepared by mixing 1 L of distilled water with 4 mg of both dyes separately in two beakers. Now in the prepared dye solution an appropriate amount of the developed nanoparticles (0.1 g/L) was added to the 20 mL of both dye solutions. Now the suspension of the dye and nanocatalyst was subjected to solar light irradiation. After the regular interval (10 min) withdraw the sample solution from the test solution and centrifuged to remove the solid suspended particles then the absorption spectra of the test solution were recorded [18].

2.7 Antibacterial activity

These prepared nanoparticles were also tested against antibacterial action using various microorganisms. For this entire process, firstly we made the solution of nutrient agar by dissolving 5.6 grams of dry nutrient agar in 200 mL of distilled water in a beaker. Then three Petri dishes with covers have been taken, and sterilized properly, then the thick layer of nutrient agar solution was poured into all three Petri dishes then properly folded the Petri dishes into a piece of paper, and carefully placed in an oven for one and a half hours to solidify the agar layer properly. In between this time, nanoparticles solution of different concentrations (20 %, 30%, and 40%) has been prepared in ethanol by weighing the iron oxide nanoparticles accordingly. All three solutions of 20 %, 30 %, and 40 % concentrations have been prepared separately and placed in a sonicator for 30 min. The Petri dishes were removed from the oven and placed in a laminar for further processing. In each of the three Petri dishes three different bacteria name-*Pseudomonas, Staphylococcus aureus, Bacillus anthracis*, have been spread equally with the help of sterilized swabs, this part of the process should be done very carefully because of the infectious pathogenic bacteria we are using, now when all the three bacteria have been applied properly, it is left to dry out. Now four holes have been made using a micropipette in each of the three layers in different Petri dishes. The antibacterial was placed in each of the one hole in three Petri dishes and in the other three holes the nanoparticles solution of different concentrations has been filled, then the Petri dishes were covered properly and placed in an incubator for 22-24 h (full night). In this period of incubation, the solution of the sample has been converted into gel form and growth of microorganisms has been inhibited. The next day petri dishes were taken out from the incubator and the results were observed carefully and the zone of inhibition that was developed in the plate is measured [19].

3. Results and discussion

In the proposed research work we prepare iron oxide nanoparticles by using leaf extract of *C. scutellarioides.* The leaf extract acts as a key ingredient in the development of nanoparticles. The leaf extract of the selected medicinal plant contains various phytochemicals like alkaloids, flavonoids, and polyphenols. These chemicals play a key role in the development of nanoparticles. Phytochemicals act as reducing and stabilizing agents in the fabrication of nanoparticles. We can prepare metal oxide nanoparticles by using the sol-gel technique. The fabricated nanoparticles were structurally characterized by using various analytical techniques.

3.1 Characterization

3.1.1 UV-Vis analysis

One of the most important properties includes optical property that is related to the shape of the particles, the size of the particles, their color, and their composition [20]. All these things are of immense importance for knowing the ability of nanoparticles for absorbing light at room temperature. UV-Visible absorbance spectra of prepared nanoparticles have been shown in figure-4. The absorption spectra of the developed nanoparticles were scanned in the range of 200-800 nm. A characteristic absorption peak that appears at 410 nm initially indicates the formation of Fe_2O_3 NPs [21, 22]. The nanoparticles have been observed to be dark brown in color.

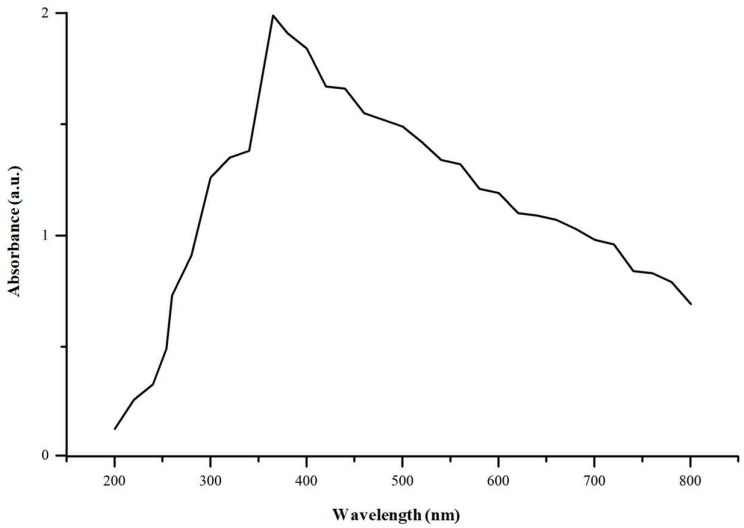

Figure 4 Ultraviolet visible spectra of Fe_2O_3 nanoparticles.

3.1.2 SEM analysis

SEM (Scanning electron microscopy) technique was used to analyze the surface morphology of the developed nanoparticles. Figure 5 showing an image obtained as a result of SEM describes the morphological structure ofFe2O3NPs. that is found to be crystalline needle-like in shape with cluster, because of these cluster formations it was not easy to describe the clear structural shape of these nanoparticles and that is why the particles are seen in variable shapes and of varied sizes [23]. The average particle size of the developed nanoparticles was discovered in the range of 50-60 nm. Few of them appear to be bigger due to cluster development; this might be because the surface of the particles may contain some kind of biological components present on them [24].

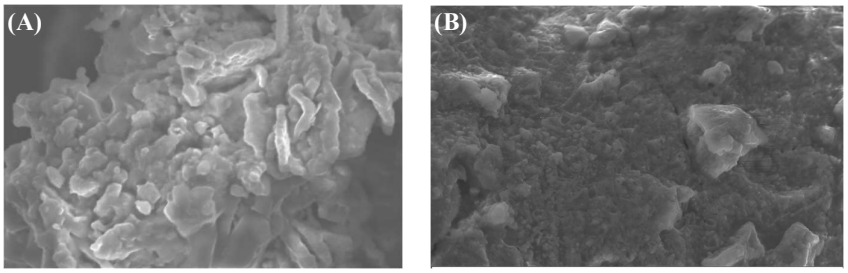

Figure 5 Image obtained as a result of SEM analysis: (A) 2 µm (B) 4µm.

3.1.3 XRD analysis

XRD analysis is basically done for knowing the properties that are related to structure and orientation of the particles. X-ray diffraction of prepared nanoparticles has been carried out to know about the nature of nanoparticles [25]. Figure 6 represents the peaks and crystal planes of the nanoparticles. The responses from the

XRD show the peaks at 25.09^0, 35.68^0, 36.54^0, 40.54^0, 48.45^0, $57.34^0$and 59.42^0 correspond to the lattice plane at (012), (104), (110), (113), (024), (116) and (018) respectively.

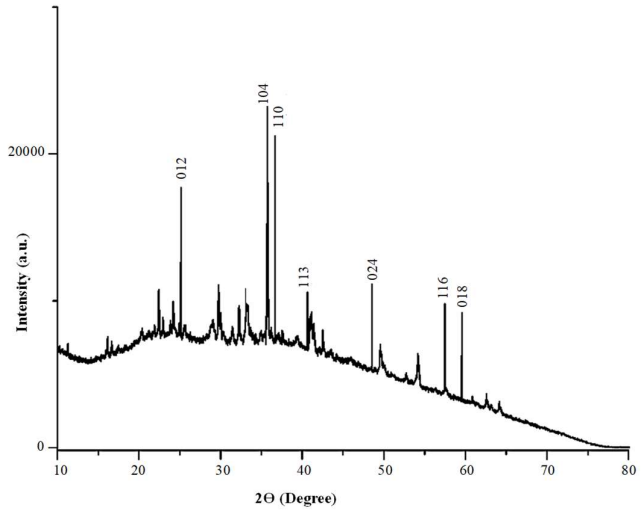

Figure 6 Showing the results obtained from XRD technique.

This XRD pattern indicates the crystalline nature of the fabricated iron oxide nanoparticles [26, 27].The results that were obtained were in good agreement with other reported observations (JCPDS fle 019–0629)[28].

3.1.4 FTIR analysis

FTIR (Fourier transform infrared spectroscopy) characterization method was carried out for the synthesized iron oxide nanoparticles. The analysis detected various functional groups that are present in the nanoparticles and phytochemicals that were present on the surface of prepared nanoparticles [29]. The absorption spectra of prepared iron oxide (Fe_2O_3) can be seen in (Figure 7). The peak 622 cm^{-1} at the right side of the graph depicts the OH-stretching vibrational bands that are related to polyphenol compounds [30]. The peak 1404 cm^{-1} displays the presence of nitro group (N-O) bands. Bands that are visualized under 544 cm^{-1} – 449 cm^{-1} at the right side of the graph show the groups containing Fe-O bond in the sample.

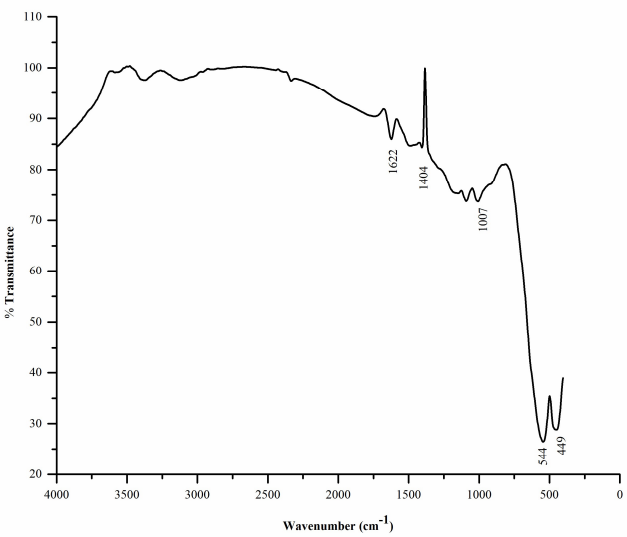

Figure 7 Vibrational Properties of the green synthesized Fe_2O_3 Nanoparticles.

The peak at 1007 cm^{-1} shows the N-H vibrational bands that depict the presence of amine group in the nanoparticles [31]. Weak stretching bands are describing the extent of unsaturation in the synthesized Fe_2O_3 nanoparticles.

3.1.5 Photocatalytic degradation activity

The photocatalytic degradation activity of these iron oxide nanoparticles was checked by degrading the reactive rhodamine and bromocresol green dye. It has been observed that the developed Fe2O3 NPs were able to degrade the dye to a quite great extent by converting the harmful components into harmless ones or decreasing the ability of these harmful components that were present in the solution of dyes. These Fe2O3 NPs have been proven effective in degrading the solution of both of these dyes and the results can be shown in the figures below (Figures 8 A, B).

When the test solution was irradiated with solar light the suspended nanoparticles absorb the radiation and produce hydroxyl free radicals which covered the toxic dye material into the nontoxic substances. In general, when the nanoparticles were irradiated with solar light having energy greater than the band gap between the valence band and conduction band then the transfer of electrons takes place from the valence band to the conduction band. The excitation of electrons generated a positive hole in the valence band and a free electron in the conduction band. The positive hole (h+) interacts with the water molecules and generates a hydroxyl free radical which is the key player in the oxidative photodegradation of the dye and converted it into nontoxic substances [32] (Figure 9).

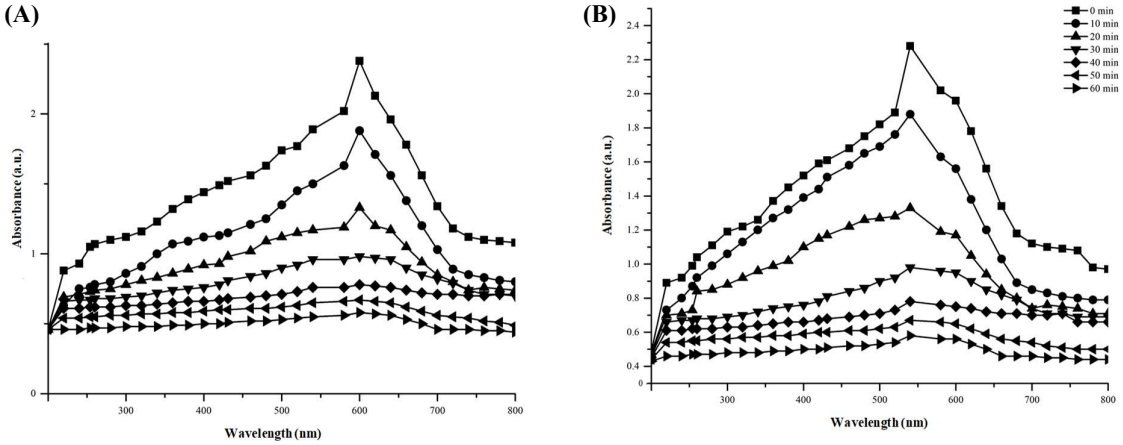

Figure 8 Photocatalytic degradation (A) bromocresol green dye by synthesized Fe_2O_3 NPs, and (B) rhodamine dye by using green synthesized Fe_2O_3 NP_S. Bromocresol green=4 ppm, pH=2, catalyst=0.1g/L

It can be depicted from Figure 8 (A) and (B), that after every 10 min the peak that is seen at first reading has been decreased constantly and after 40 min the peak has been disappeared which is showing that the nanoparticles were effective in degrading the sample of both the dyes.

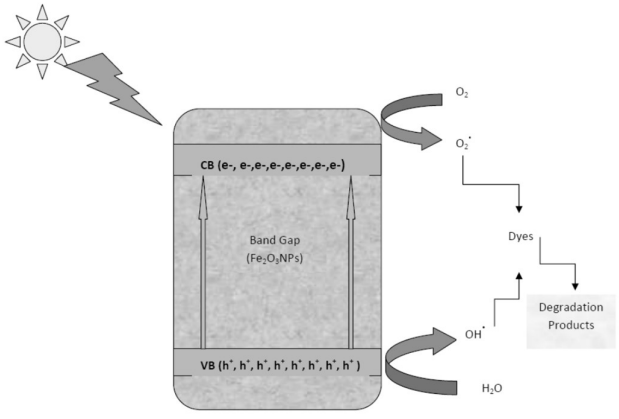

Figure 9 Photocatalytic Mechanism of synthesized Fe_2O_3NPs.

3.1.6 Antibacterial activity

Antibacterial properties of these prepared iron oxide nanoparticles have been tested by agar well diffusion method against bacteria strains that were *Pseudomonas, Staphylococcus aureus, and Bacillus anthracis.* All three

bacteria have shown a zone of inhibition at different concentrations of nanoparticle solution. Bacteria *Staphylococcus aureus* has shown a zone of inhibition of 10 mm at concentration 20, 13 mm at concentration 30, and 18 mm at concentration 40 and the control has shown the zone of inhibition to 27 mm. Bacteria *Bacillus anthracis* has shown a zone of inhibition of 11 mm at concentration 20, 15 mm at concentration 30, and 19 mm at concentration 40 and the control has shown the zone of inhibition to 26 mm. The bacteria *Pseudomonas*have shown a zone of inhibition of 12 mm at concentration 20, 17 mm at concentration 30, and 21 mm at concentration 40 and the control has shown the zone of inhibition of 27 mm. (Table 1) and images (Figure 11) obtained as a result of antibacterial activity has been represented below.

Table 1 Table showing the zone of inhibition at different concentration of NPs solution.

Bacteria	Control (mm)	20% (mm)	30% (mm)	40% (mm)
S. aureus	27	10	13	18
B. anthracis	26	11	15	19
Pseudomonas	27	12	17	21

By observing the images above, we can indicate that the nanoparticles at concentration at 40 have shown quite good antibacterial activity against all the three bacteria*, S. aureus, B. anthracis, Pseudomonas* and inhibit the growth of these bacteria to a good extent.

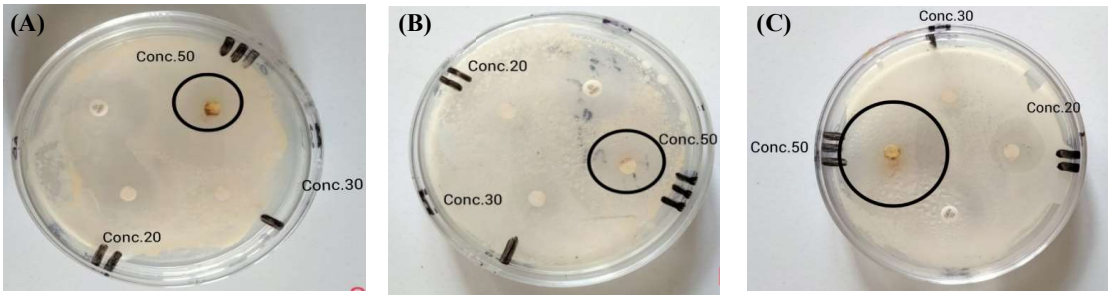

Figure 11 Antibacterial potential of synthesized nanoparticles against (A) *S.aureus* (B) *B. anthracis* and (C) *Pseudomonas.*

4. Conclusion

In summary, the present study demonstrates a very convenient low-cost approach for the development of Fe_2O_3 NPs by using leaf extract and microwave irradiation. The technique of synthesizing nanoparticles through the green method has been proven highly effective, biocompatible, sustainable, and environmentally friendly. The results that were obtained from different characterization techniques confirmed the development of Fe_2O_3 NPs by describing the properties that are exhibited by Fe2O3 NPs. The nanoparticles were able to degrade the bromocresol green and rhodamine dye by converting the harmful components into harmless ones. These nanoparticles have also shown significant antibacterial potential against selected microorganisms and proved that they are effective in inhibiting their growth.

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