



Structural Characterization, Antimicrobial and Antioxidant Assay of Biogenically Synthesized Maghemite ($\gamma\text{-Fe}_2\text{O}_3$) Nanoparticles

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study focused on the optical and morphological characterization, antioxidant and antimicrobial activities of the biogenically synthesized iron oxide nanoparticles.

Methods: The preliminary phytochemical screening was done for the leaf extract of *Annona reticulata* L. The leaf extract and Ferrous Sulphate heptahydrate were used to synthesize the iron oxide nanoparticles under room temperature. The determination of antioxidant activity was done using DPPH free radical scavenging assay and the determination of antimicrobial activity using disc diffusion method.

Results: The UV-visible spectra showed the sharp absorption peak at 278 nm. The Fourier-transform infrared spectroscopy studies revealed the role of phytochemical constituents in the leaf extract for the iron oxide nanoparticles formation. X-ray diffraction pattern showed the presence of γ phase of Fe_2O_3 nanoparticles. Scanning electron microscope analysis showed the moderately spherical morphology of $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles and Energy-dispersive X-ray peaks showed the presence of iron and oxygen in the synthesized nanoparticles. Particle size analysis showed that the synthesized $\gamma\text{-Fe}_2\text{O}_3$ possessed an average size of 115.9 nm.

Conclusion: The synthesized $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles have potential antioxidant and antimicrobial activity.

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Keywords: γ -Fe₂O₃ nanoparticles; biogenic synthesis; XRD; SEM; antioxidant; antimicrobial activity.

1. INTRODUCTION

Nanoparticles are broadly used in nature and are as materials for the study of fields in science like Chemistry, Biology, Physics and Geology. Even though it is transition from bulk molecules or atoms, they exhibit some phenomena which do not exist in the bulk molecules or atoms. The ultrafine nature is observed in nanoparticles whose diameter is between 1nm to 100nm. Due to the unique properties of nanoparticles, they have more applications [1-5]. But if they are mishandled, they can be harmful and extremely destructive. Some of them cause health problems and impacts in the environment by the chemical methods of synthesis of nanoparticles using organic solvents, non-biodegradable agents and toxic chemicals cause health problems and impacts in the environment. Therefore, nanoparticles are synthesized via green synthetic method or biogenic synthesis. The field of green nanotechnology carried out by using the naturally available waste materials like plant's parts extract, microorganism's metabolites and natural humic materials as capping and reducing agents [6-8]. Green synthetic method of nanoparticles uses the natural reducing agents such as microorganisms, polysaccharide, plant extract and enzyme. From the biological ideas, plants are considered as nano manufacturing factories, which produces the best pathway to accumulation of food chain biologically and environmentally. It is a safe and bio-benefit method. Due to the largest plant diversity, syntheses of nanoparticles from plant is known to be a more interesting subject. In recent

years, the major focus of researchers is to synthesize the metal oxide nanoparticles using efficient methods of green chemistry [9], this method provides a faster production of nanoparticles which is ecofriendly [10]. Nowadays, iron oxide nanoparticles taken into much consideration and attractions due to their unique properties like surface-to-volume ratio, superparamagnetic, greater surface area and their easier separation method [11]. Thus, in this study, a simple green method is used for the iron oxide nanoparticles synthesis from *Annona reticulata L.* leaf extract.

This plant is used traditionally for epilepsy treatment, infestations of worm and parasites, dysentery, heart problem, fever, infections due to bacteria, ulcer, constipation, insecticide [12,13]. Its bark is used as a tonic and leaves used to treat helminthiasis [14]. Phytoconstituents present in stem are compounds of phenolic, tannins, alkaloid; in leaves are carbohydrates, proteins, flavonoids, glycosides; in roots are acetogenin, tannins, carbohydrates. This plant is rich in minerals like Na, Mg, Zn, Se, Cu, Ca, Cr, Ni, Mn, S, Cl, P, Co, K [15,16].

Iron oxide nanoparticles are familiar for its super paramagnetic properties, used as a catalyst in catalysis, used as labeling agent in MRI scan for imaging the highly sensitive biomolecular tissues, used in nano therapy of cancer by magnetic spin properties in the free radicals, helpful to load with drugs for treating tumor and also injected to the cancer cells for destroying them at various temperature [17].



Fig. 1. Annona reticulata L. plant

2. MATERIALS AND METHODS

2.1 Preparation of Leaf Extract

The leaves of *Annona reticulata* L. collected from somarasampettai, Trichy district. The leaves are cleaned, dried in shade and powdered. From the dry powder of the leaves, the constituents were extracted by the addition of 10g of the leaf powder to the 100mL of the double distilled water, boiled for 30 minutes, cooled, filtered, collected in amber bottle and stored in refrigerator for further use.

Preliminary phytochemical (qualitative) analysis was carried out to find the phytoconstituents like carbohydrates, reducing sugars, non-reducing sugars, proteins, steroids, glycosides, flavonoids, tannins, alkaloids, saponin, emodols and betacyanins using the standard procedure [18].

2.2 Synthesis of Iron Oxide Nanoparticles

1mM solution of Ferrous Sulphate heptahydrate is weighed and made up in a 100mL standard flask using double distilled water. Different concentrations of the leaf extract in μL were added to the solution in order to determine the correct ratio of the leaf extract to the metal solution. Upon addition of 50 μL of the leaf extract, the colorless ferrous sulphate solution changes to greenish black. These mixtures kept for stirring for 30 minutes. The synthesized iron oxide nanoparticles collected by cooling centrifuge, washed with double distilled water thrice and dried in hot air oven for 6 hours at 80°C.

2.3 Characterization Techniques

The iron oxide nanoparticles were confirmed by Ultraviolet-Visible HITACHI U-2910 spectrophotometer, IR Affinity-1S FTIR spectrophotometer (Bruker Tensor - 27), X-ray diffraction analysis (PANalytical X Pert3 Powder), Scanning Electron microscope using Tescan instruments, Energy-dispersive X-ray analysis using Oxford instruments and Particles Size analyzer (Micromeritics, Model: Nano Plus).

2.4 Determination of Antioxidant Activity using DPPH Free Radical Scavenging Assay

Freshly prepared DPPH solution kept at 4°C in dark by 2.7mL of 96% ethanolic addition. Thus,

0.05 mM ethanolic solution of DPPH was prepared. 40 μL of aqueous iron oxide nanoparticles with different concentrations was added to the solution, shaken vigorously and left for 5minutes. The absorbance at 517nm was measured by spectrophotometrically. The Ascorbic acid used as standard. The same procedure carried out in the blank solution. All these were done in triplicate.

Percentage of inhibition = $[(A-B)/A] \times 100$. A= absorbance of blank, B= absorbance of iron oxide nanoparticles [19]. The plot between the percentage of inhibition and concentration of aqueous iron oxide nanoparticles were expressed the results.

2.5 Determination of Antimicrobial Activity Using Disc Diffusion Method

Sabouraud dextrose broth used as culture media. *Candida albicans* and *Aspergillus flavus* are the fungal strain collected from NCL, Pune, India. These fungal strains in separate broth were inoculated for 6 hours and checking of suspensions approximately provided at 10⁵ CFU/mL.

3. RESULTS AND DISCUSSION

From ancient times, plants are still in use to prevent or treat illness. Researchers were attracted by the plant *Annona reticulata* L. because of its novel phytochemical constituents with more bioactivities. For their aqueous leaf extract, the phytochemical qualitative analysis were carried out.

3.1 Qualitative Analysis for the Aqueous Leaf Extract of *Annona reticulata* L.

From ancient times plants are still in use to prevent or treat illness. Researchers were attracted by the plant *Annona reticulata* L. because of its novel phytochemical constituents with more bioactivities. Hence, in our present study phytochemical qualitative analysis were carried out for the aqueous leaf extract of the plant *Annona reticulata* L.

3.2 UV-Visible Spectroscopy Analysis

The aqueous leaf extract of *Annona reticulata* was investigated and reported to consist of protein, flavonoids, alkaloids, emodols and betacyanins. These phytoconstituents plays a

vital role in the bio reduction of Ferrous Sulphate heptahydrate solution. To the colorless $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution, after the addition of the leaf extract, the solution becomes greenish black immediately, which indicates the iron oxide nanoparticles formation. The analysis of UV-visible spectroscopy for the color change was carried out and a sharp peak of absorption at 278nm was observed for the synthesized iron oxide nanoparticles and a similar result was reported by (Clarina., 2018) [20,21].

3.3 FTIR Spectroscopy Analysis

This analysis is used to determine the role of functional groups in the leaf extract as a bio

reductant in the synthesis of iron oxide nanoparticles. The band at 655, 603.72 cm^{-1} indicates the Fe-O-Fe stretching of $\gamma\text{-Fe}_2\text{O}_3$ (Maghemite) nanoparticles. The other bands are due to the phytoconstituents of *Annona reticulata* leaf extract which are responsible for the reduction of ferrous sulphate heptahydrate solution to iron oxide nanoparticles. The band at 3331.07 cm^{-1} due to hydroxyl group, the weak band at 2113.98 cm^{-1} due to alkyne stretching, 1637.56 cm^{-1} due to H_2O vibrational bending and 1251.80 cm^{-1} indicates the stretching of C-N in aromatic amine. The similar results were reported by [22,23].

Table 1. Qualitative analysis of the leaf extract

S. No.	Tests	Phytoconstituents in aqueous Leaf Extract
1	Carbohydrates	-
2	Reducing sugars	-
3	Non-reducing sugars	-
4	Protein	+
5	Steroid	-
6	Glycosides	-
7	Flavonoids	+
8	Tannins	-
9	Alkaloids	+
10	Saponin	-
11	Emodols	+
12	Betacyanins	+

(+) indicates the presence; (-) indicates the absence

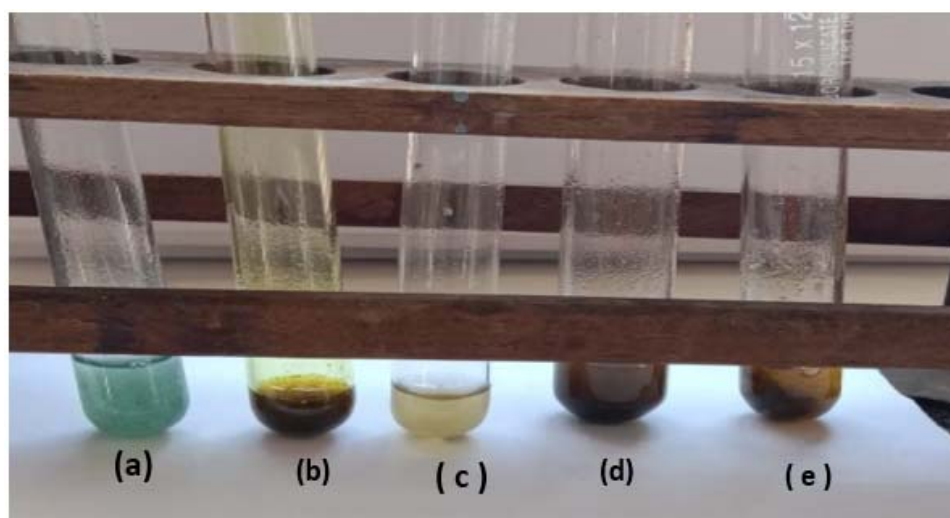


Fig. 2. a) Test for Protein; b) Test for flavonoids; c) Test for Alkaloids; d) Test for Emodols; e) Test for Betacyanins

3.4 XRD Analysis

This analysis is used to find the crystalline nature of the iron oxide nanoparticles. The peaks (2θ) at 14.91, 23.94, 49.51 were indicates the iron oxyhydroxide particles due to partial oxidation.

The 2θ at 28.55 denotes the $\gamma\text{-Fe}_2\text{O}_3$ particles; the peak at 18 due to the polyphenols in leaf extract. 2θ with miller indices 18.24(202), 23.94(132), 28.55(322), 38.59(111) were also revealed in XRD pattern. The similar results were obtained by (Silvia Groiss, 2019) [24,25].

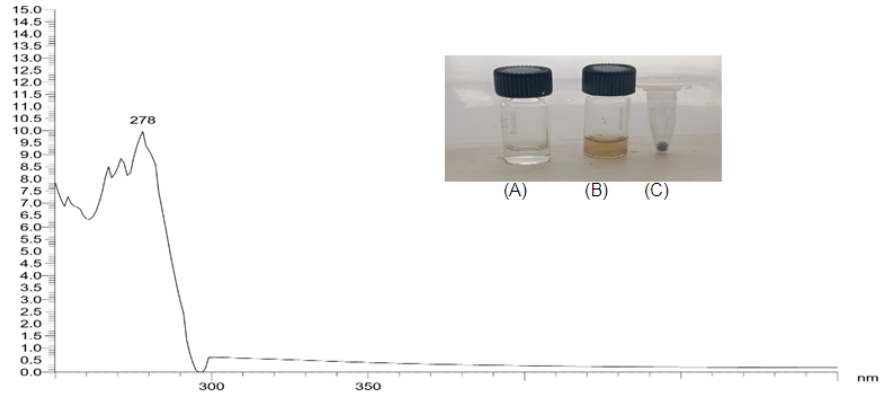


Fig. 3. Visual Observation: (A) Ferrous Sulphate heptahydrate solution; (B) Ferrous sulphate solution with extract; (C) Iron oxide nanoparticles in dried state and UV-Visible spectra of synthesized iron oxide nanoparticles

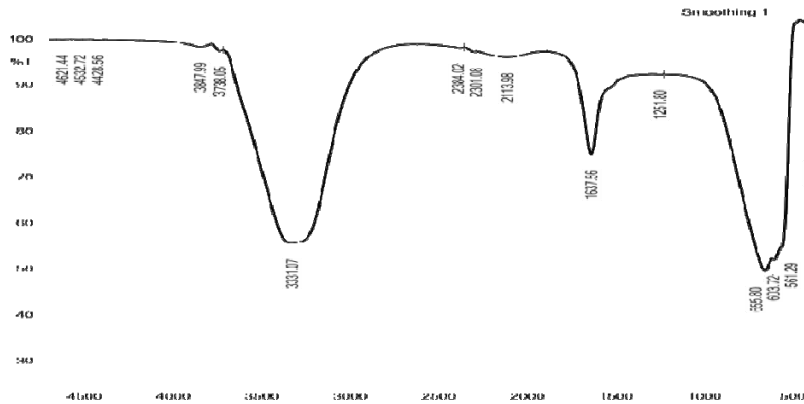


Fig. 4. FTIR spectra for the synthesized iron oxide nanoparticles

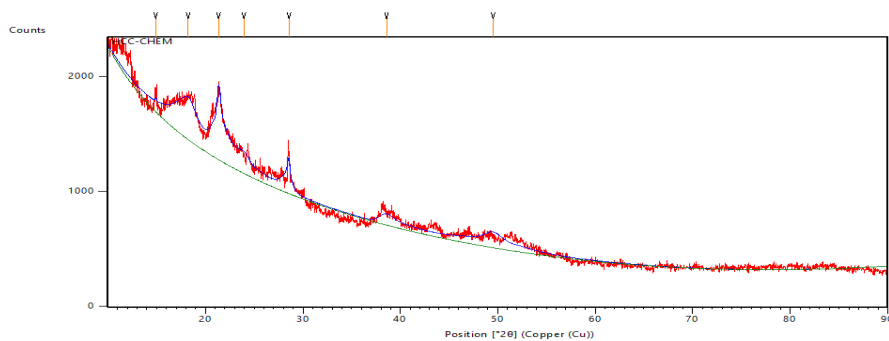


Fig. 5. XRD pattern for the synthesized iron oxide nanoparticles

3.5 SEM Analysis

The morphology of the iron oxide nanoparticles was investigated by SEM analysis. The SEM images revealed that the particles were moderately spherical in shape and agglomerated and the similar results were reported by (Huang.L2014) [26-28].

3.6 EDAX Analysis

The presence of main elemental and their composition in the synthesized iron oxide

nanoparticles was found out using the energy dispersive X-ray analysis. The EDAX data revealed the presence of iron and oxygen as main element in the synthesized nanoparticles.

3.7 Particle Size Analysis

Particle size analysis was studied using Dynamic light scattering for determining the hydrodynamic size of the synthesized iron oxide nanoparticles. The average size of the particles found to be 115.9 nm.

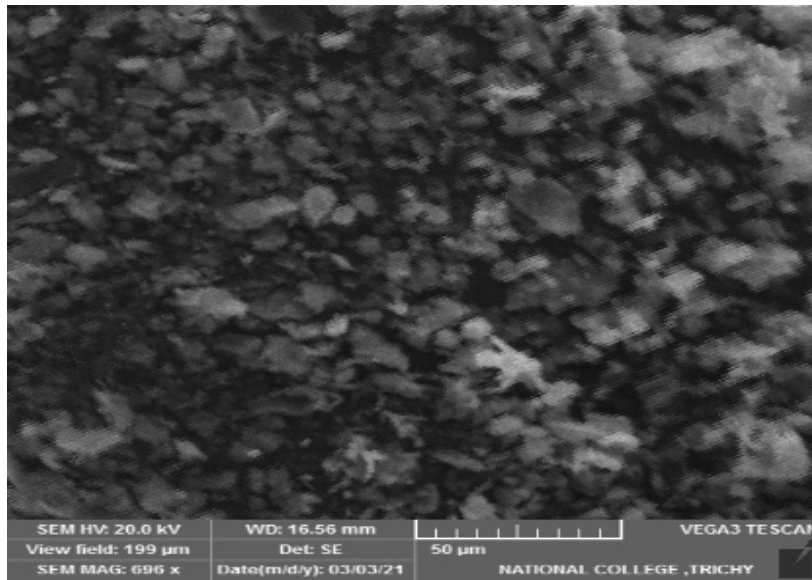


Fig. 6. SEM image of the synthesized iron oxide nanoparticles

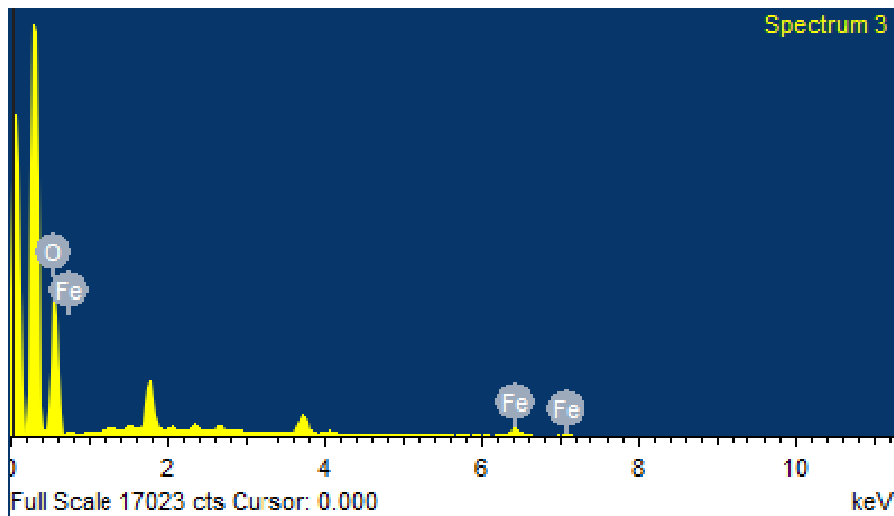


Fig. 7. EDAX of the iron oxide nanoparticles

Table 2. Elemental composition of iron oxide nanoparticles

Elements	Weight (%)	Atomic (%)
O	87.81	96.18
Fe	12.19	3.82
Totals	100.00	100.00

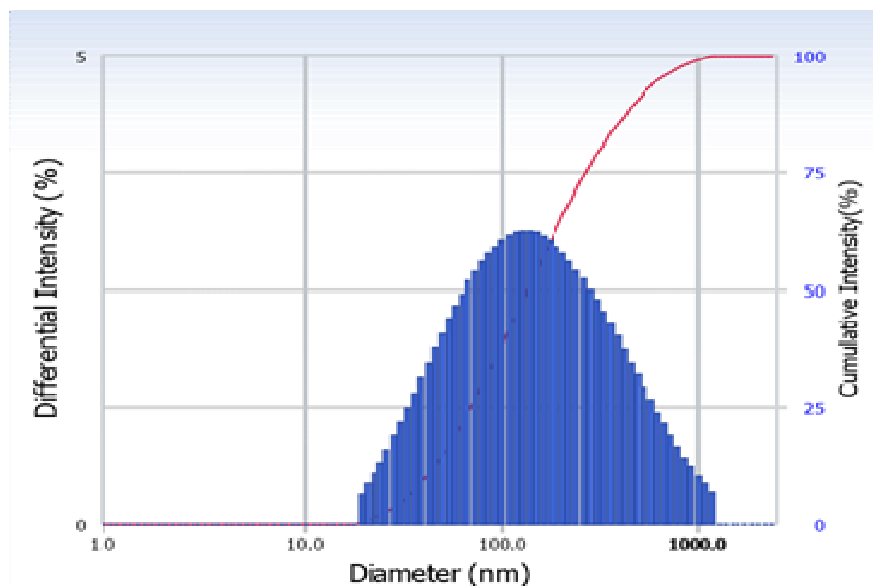


Fig. 8. Particle size analysis for iron oxide nanoparticles

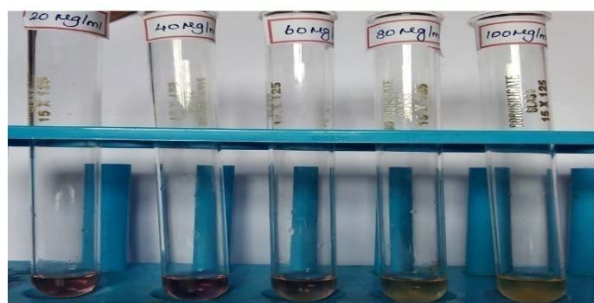


Fig. 9. Antioxidant activity of ascorbic Acid by DPPH method

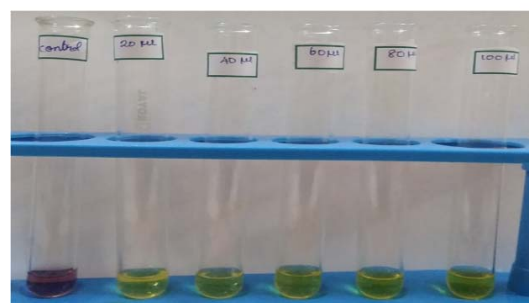


Fig. 10. Antioxidant activity of iron oxide Nanoparticles by DPPH method

Table 3. Antioxidant activity for the iron oxide nanoparticles and ascorbic acid by dpph method

S.NO.	Concentrations	Scavenging % in γ - Fe_2O_3 nanoparticles	Scavenging % in Ascorbic acid nanoparticles
1	20 $\mu\text{g/mL}$	43.41	72.09
2	40 $\mu\text{g/mL}$	48.83	76.74
3	60 $\mu\text{g/mL}$	55.03	79.06
4	80 $\mu\text{g/mL}$	58.13	81.39
5	100 $\mu\text{g/mL}$	62.01	83.72

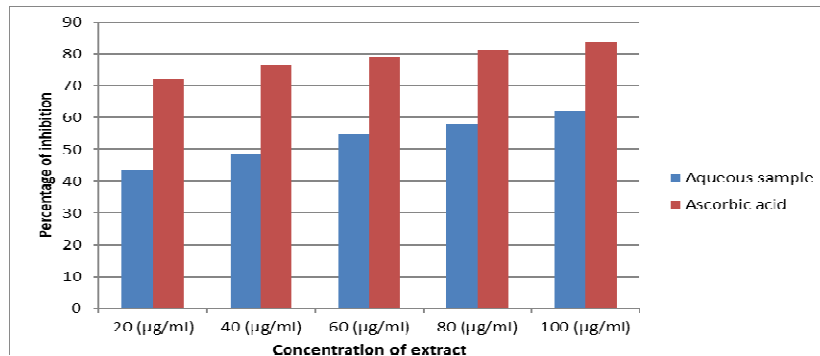


Fig. 11. Antioxidant activity of Ascorbic acid and iron oxide nanoparticles by DPPH method

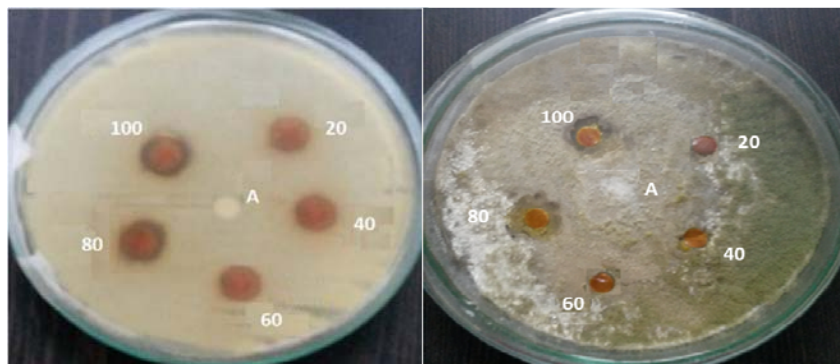


Fig. 12. Antifungal activity of iron oxide nanoparticles towards *Candida albicans* and *Aspergillus flavus* respectively

3.8 Determination of Antioxidant Activity

The green synthesized iron oxide nanoparticles showed the potential of scavenging activity in DPPH free radicals [29-32]. The Ascorbic acid was utilized for positive control and taken the range of same concentration as the concentration of aqueous solution of iron oxide nanoparticles. The DPPH with different concentration of $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles was taken and the percentage of scavenging effect is found to be between 43.41 to 62.01%. The IC50 value for the ascorbic acid is 80.22% and IC50 for the synthesized iron oxide nanoparticles is 66.58%.

3.9 Determination of Antifungal Activity of Iron Oxide Nanoparticles

The Sabouraud's dextrose agar was prepared in petridishes of 60mm in diameter and test organisms were inoculated. With use of sterile forceps, the sterile disc of 6mm in width with 10µL of crude extract were impregnated at different range of concentrations from 20µg/mL

to 100µg/mL. It showed that the iron oxide nanoparticles were more potentially active and effective against *Aspergillus flavus* at 100µg/mL and zone of inhibition at 6mm. The iron oxide nanoparticles exhibit 5mm zone of inhibition in *Aspergillus flavus* and 4mm zone of inhibition in *Candida albicans* at 80µg/mL of concentration.

4. CONCLUSION

In conclusion, iron oxide nanoparticles were synthesized using the leaf extract of *Annona reticulata L.* which reduced the Ferrous Sulphate heptahydrate solution to iron oxide nanoparticles. The synthesized iron oxide nanoparticles were characterized using UV-visible spectroscopy and the sharp absorption peak found at 278nm. FTIR analysis revealed that band at 655 and 603.72 cm^{-1} were due to $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles. XRD studies showed the 2θ peak at 28 which indicates the presence of $\gamma\text{-Fe}_2\text{O}_3$. SEM analysis showed the result that the synthesized nanoparticles was moderately spherical in shape and agglomerated. EDAX studies confirmed the presence of Fe and O in

the synthesized iron oxide nanoparticles. Particles size analysis outcomes with the average size of the particles were 115.9 nm. Antioxidant activity was studied by DPPH method which showed greater potential of iron oxide nanoparticles towards free radical scavenging activity. Antifungal activity was also carried out in *Candida albicans* and *Aspergillus flavus*.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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