The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(1): 470-476, 2025 www.thebioscan.com

BIOLOGICAL SYNTHESIS OF Fe_2O_3 NANOPARTICLES: STRUCTURAL ANALYSIS AND ANTIMICROBIAL POTENTIAL

A.R. Bhosale[a], R.B.Chavan[a], A.A. Surwase[b], P.V. Chavan[a*]

[*a] Department of Chemistry, Yashavantrao Chavan Institute of Science, Satara Karmaveer Bhaurao Patil University, Satara-415001, Maharashtra, India

[b] Department of Microbiology, Yashavantrao Chavan Institute of Science, Satara Karmaveer Bhaurao Patil University, Satara-415001, Maharashtra, India

Corresponding Author: - pvchavanchem@gmail.com

DOI: https://doi.org/10.63001/tbs.2025.v20.i01.pp470-476

KEYWORDS
Biological synthesis, saussurea obvallata, Fe2O3: nanoparticles, co-precipitation method, antimicrobial activity.

Received on:

04-01-2025

Accepted on:

04-02-2025

Published on:

08-03-2025

ABSTRACT

This research examines the biological synthesis of Fe_2O_3 nanoparticles (NPs). The synthesis of Fe_2O_3 nanoparticles was carried out using *saussurea obvallata* pant extract, employing biological approach while following the conventional co-precipitation method. The synthesized Fe_2O_3 nanoparticles' structural and morphological characteristics were examined utilizing Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX), and Transmission electron microscopy (TEM),Ultraviolet spectroscopy (UV).The antibacterial activity of the biogenically synthesized Fe_2O_3 NPs was also evaluated, demonstrating excellent antimicrobial efficiency in response to both Gram-positive and Gram-negative bacteria. The synthesized nanomaterials was exhibited excellent antimicrobial activity against bacterial strains Staphylococcus aureus (NCIM-2654), Bacillus cereus (NCIM-2703), Pseudomonas aeruginosa (NCIM-5032) and Proteus vulgaris (NCIM-2813). This study highlights the potential of plant-mediated green synthesis as an eco-friendly and sustainable approach for fabricating Fe_2O_3 NPs with promising biomedical applications.

INTRODUCTION

The increasing global issue of antimicrobial resistance impacts a range of pathogens, including bacteria, viruses, and fungi. It poses serious threats to both individual health and the worldwide economy.[1] Recent statistics suggest that bacterial antimicrobial resistance (AMR) is responsible for nearly 4.95 million fatalities annually. [2] Nanotechnology offers a promising solution to the challenges of antimicrobial resistance by introducing innovative methods for synthesizing nanoparticles (NPs) with antimicrobial properties.[3] Nanoparticles have attracted considerable gained focus in recent years because of their distinctive characteristics, various fabrication techniques, and extensive range of applications.[4] On the other hand, green synthesis approaches utilize biological sources such as bacteria, fungi and plant offering more environmentally friendly as well as sustainable option. These methods rely on biological molecules to facilitate the decreases of metal ions as well as the stabilization related to nanoparticles, improving their biocompatibility, stability, and biological effectiveness [5,6] The use of medicinal plants, which contain substances that affect living things, has been one of the oldest human achievements in treating most diseases. Throughout the development of human civilizations, there has always been a close relationship between man and plants [7]. Bioactive nanoparticles gain great attention due to their wide range of applications, antibacterial activity and medical application [8].
Nanotechnology has recently gained significant attention in biomedical fields, including areas like biosensors [9], cytotoxicity [10], magnetic resonance imaging [11] and drug delivery [12]. It is also explored for uses in catalyst support [13], and various biomedical applications such as magnetic carriers for bio separation [14], and the immobilization of enzymes and proteins [15].
In last decade the use of leaf extract for the biosynthesis of nanomaterials has gained significant attention due to its eco-friendly and cost-effective nature [16]. Bacteria have the ability to produce nanoparticles (NPs) through two primary mechanisms: by accumulating metal ions inside their cells or by utilizing their metabolic byproducts to convert these ions into NPs.[17] This method serve as a sustainable alternative to traditional chemical reduction and physical synthesis techniques. Leaf extract is reach in phytochemicals that act as reducing stabilising agents facilating the formation of nanoparticles without the need of for toxic chemicals additionally, these green synthesis approach aligns with

the principles of green chemistry promoting sustainability in nanomaterial production. The production of metal nanoparticles utilizing leaf extracts, fruit extracts, tree-derived gums, and fungi is a sustainable and eco-friendly method. The bioactive and phytochemical mixture present in these essences function as both minimizing and stabilizing agents, facilitating the development and synthesis of designed metal nanoparticles [18,19]

Considering these observations and aligning with our ongoing commitment to advancing green eco-friendly synthetic methodologies.[20,21] In this study we synthesize Fe_2O_3 by utilizing co-precipitation method and *saussurea obvallata* leaf extract functioning as a reductant. The development related to nanoscale iron oxide nanoparticles with outstanding characteristics, including shape- and size-dependent properties, is crucial for applications in medicine, biosensors.[22] its conventional applications, spiritual importance in Hindu mythology, and contribution to the formulation of Ayurvedic remedies.[23]

2.Methodology procedure :

2.1 Formulation of *saussurea obvallat*a leaf extract:

Seven grams of *Saussurea obvallata* leaves were gathered and placed inside a clean beaker. The leaves were rinsed twice with deionized water and dried by exposure to air at room temperature for 48 hours in the experiment laboratory. Once dried, they were cut into small pieces using a small bag. These pieces were then boiled in 250 mL of deionised water at 100°C to ensure complete extraction of the essence, following the Soxhlet method for five cycles. The resulting pale greenish solution was filtered using Whatman filter paper. The extract was then left to chill for two hours in a beaker before being preserved in a storage vessel for future use.

2.2 Synthesis belonging to Fe_2O_3 nanoparticles:

Entire chemicals employed were of analytical grade and were used lacking further purification. Iron oxide nanoparticles were prepared using a straightforward co-precipitation method. Initially, 10gm of $feso_4.7H_2O$ was dissolved in 150 ml of pure water while continuously stirring at room temperature. Next 6 ml of NH_4OH solution was slowly introduced dropwise into the stirring the mixture at room temperature at a rate of 1 ml per minute. The pH level was regulated at 11 throughout the synthesis process. The resulting black dispersion was stirred continuously for 1 hour at room temperature, followed by heating at 80^0C for 2 hours facillate evaporation, ultimately yielding a brown powder. The obtained product it was left to reach room temperature and then calcinated within 500^0C for 4 hours all samples were analysed without undergoing any washing or purification.

The size, composition and surface characteristics of the as-synthesized and annealed Fe_2O_3 nanoparticles where characterized. An X-ray diffractometer (XRD) was utilized for determine the crystalline phase as well as estimate the crystal size.

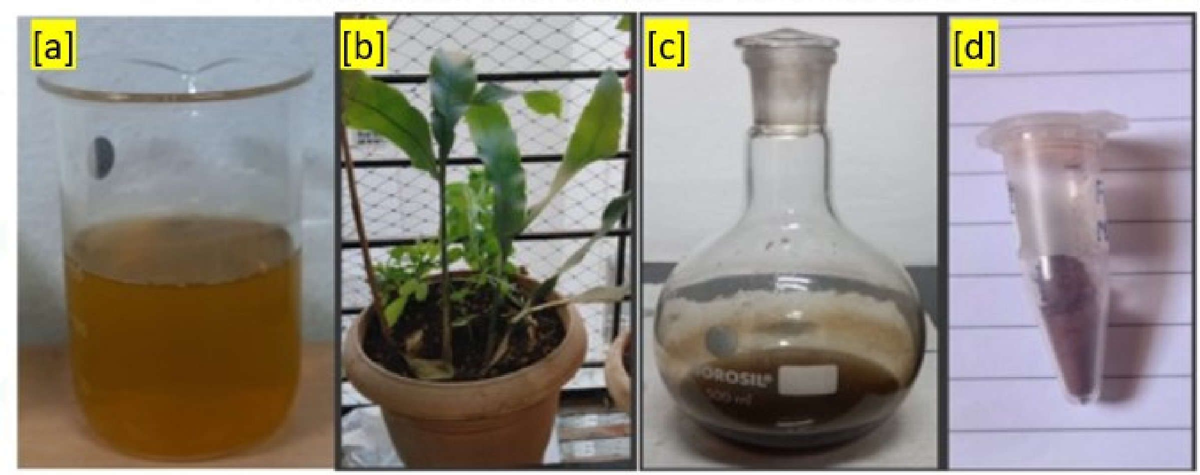

Fig no.1 (a) Ferrous sulphate heptahydrate solution, (b) *saussurea obavallata* leaf, (c) prepared leaf extract, (d) Fe_2O_3 nanoparticles

3.Characterization :

3.1 UV-visible spectrophotometer:

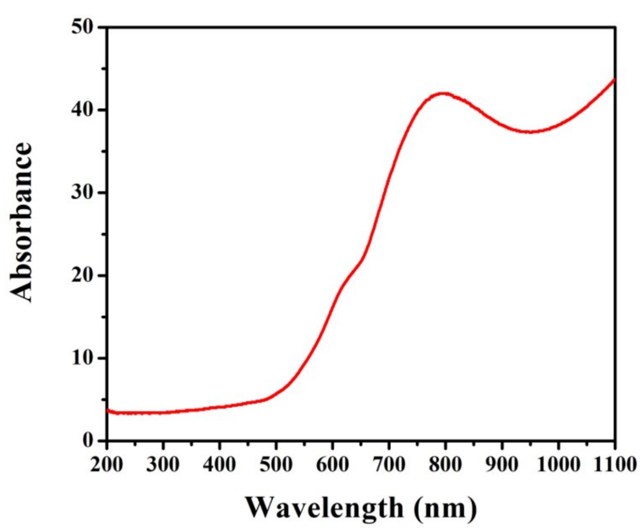

Fig no.2 UV-Spectrophotometer of Fe_2O_3

The UV-visible spectrophotometer was used to examine the optical characteristics of the synthesized nanoparticles (UV-2600i Shimadzu Japan) within the wavelength range of 800 nm

3.2 Powder x-ray diffraction evaluation:

X-ray diffraction (XRD) evaluation was conducted using a Perkin Elmer X-ray diffractometer within the 2θ range of 20° to 80° to investigate the well-ordered structure of Fe_2O_3 nanoparticles synthesized utilizing *Saussurea obvallata* leaf extract. The obtained spectrum displayed prominent diffraction peaks associated with specific (hkl) values (2 2 0), (3 1 1),(4 4 0), (4 2 2), (5 5 1), (4 0 0).

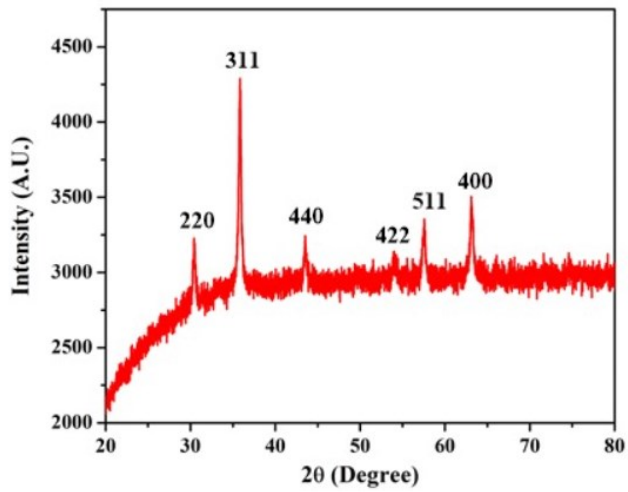

Fig no. 3 EDX synthesized Fe_2O_3

3.3 FTIR spectral analysis:

The Fourier transform infrared analysis of Fe_2O_3 nanoparticles synthesized using *Saussurea obvallata* leaf extract was conducted, as shown in Figure 4. The chemical constituents in the leaf extract influenced the bonding characteristics of the functional groups.

Fig no.4 The FTIR synthesized Fe_2O_3

The broadband observed at 553cm-1, 568cm-1,580 cm-1 can be attributed to Fe-O vibrational mode functional group of and 1114 cm-1 assigned to functional group of C–O stretching. The presence of Fe-O nanoparticles was verified by the prominent absorption peak in the spectrum 553 cm^{-1} for Fe_2O_3.[24]

3.4 SEM, EDX, TEM analysis:

The surface build of iron oxide nanoparticles synthesized using *Saussurea obvallata* leaf extract was examined through SEM analysis. The particle distribution appeared irregular, with tiny round shaped structures dispersed across the recorded spectrum. The interaction between phytochemical compounds from the leaf extract and the starting material influenced the nanoparticle shape, resulting in the formation of accumulated particles observed in the sample. [25]

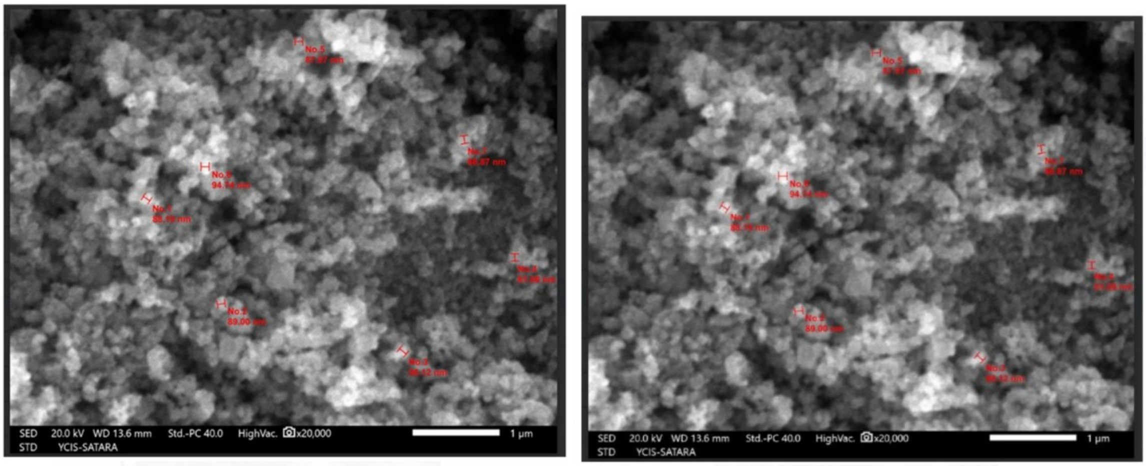

Fig no.5 SEM image synthesized Fe_2O_3

The elemental composition of the synthesized nanoparticles was analyzed using the EDX spectrum. Distinct peaks corresponding to iron (Fe) and oxygen (O) were identified, showing variations in intensity. The successful synthesis of iron oxide nanoparticles utilizing *Saussurea obvallata* leaf extract was confirmed. The weight percentage of iron and oxygen in the samples was determined, maintaining a specific ratio, with no detectable impurities in the EDX spectrum. The SEM image and EDX spectrum of Fe_2O_3 nanoparticles synthesized from *Saussurea obvallata* leaf extract were examined.

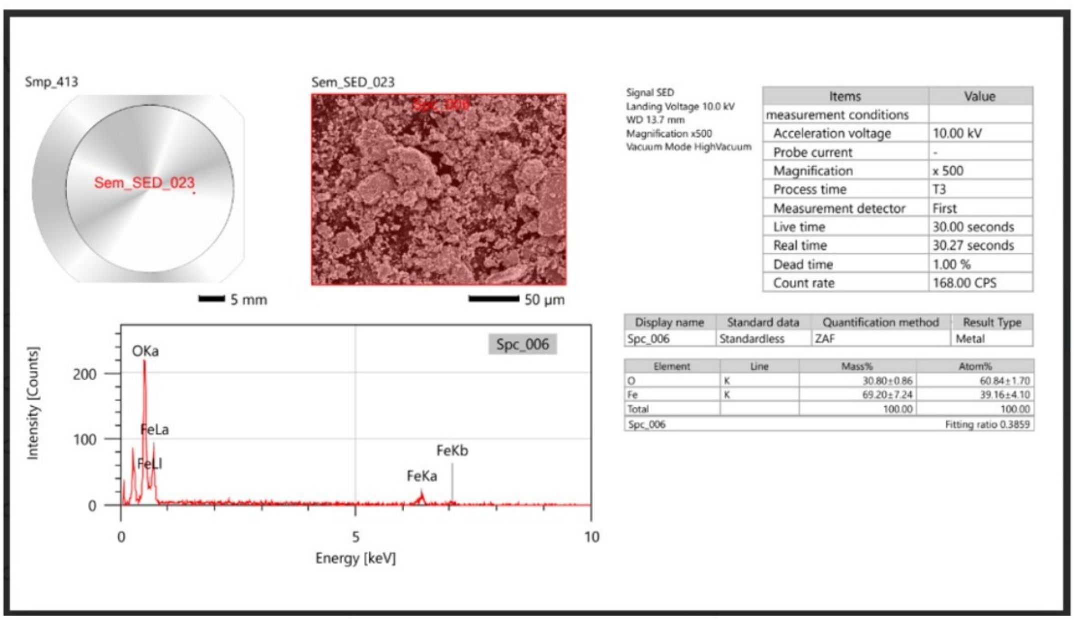

Fig no.6 EDX Spectrum Fe_2O_3

The HR-TEM results are shown in inset Fig.7 TEM images revel that the particles are arranged in a specific pattern, likely due to magnetic properties of biological synthesizes iron oxide nanoparticles ($IONP_S$) additionally, HR-TEM images confirm the structure of iron oxide nanoparticles ($IONP_S$) calcinate at 500^0C. [26]

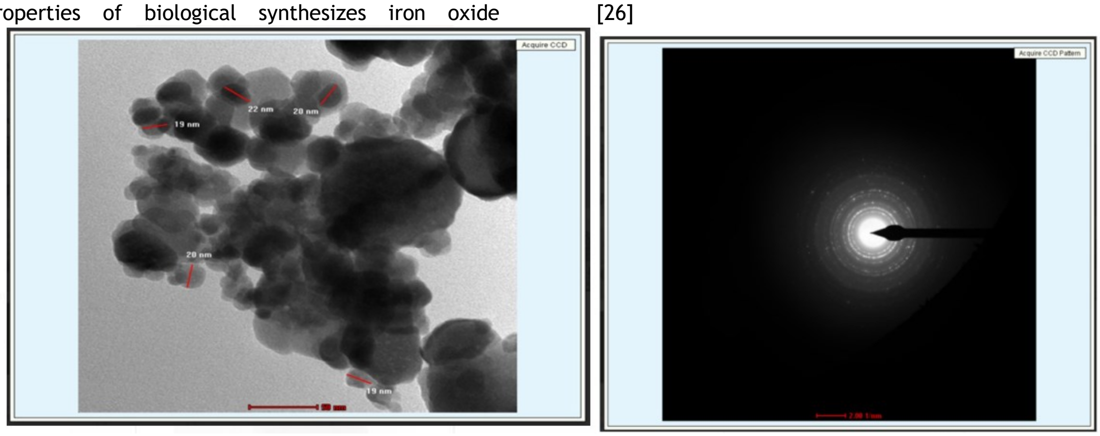
Fig no.7 TEM image of Fe_2O_3 20 nm size and SAED image

4.Experimental details:

4.1 Antimicrobial activity:

The microbial inoculums were prepared using sterile saline water. Nutrient agar plates served as the growth medium for bacterial cultures. Strains of S. aureus, B. cereus, P. aeruginosa, and P.

vulgaris were evenly spread on sterile nutrient agar plates. Wells, each measuring 7 mm in diameter, were created using a sterile cork borer. A green-synthesized nanomaterial (100 µg/mL) and a standard antibiotic were dissolved in sterile dimethyl sulfoxide (DMSO) and dispensed using a micropipette. The plates were then incubated at 37°C for 24 hours to assess antibacterial activity

4.2 Result and Discussion:

a) Antimicrobial activity :

The antimicrobial activity of chemically synthesized Fe_2O_3 nanoparticles (Fe_2O_3 Nps) was evaluated using the agar well diffusion method. Dimethyl sulfoxide (DMSO) was used as the negative control. The antimicrobial potential of the Fe_2O_3 Nps was tested against both gram-positive bacterial strains *Staphylococcus aureus* (S. aureus) and *Bacillus cereus* (B. cereus), as well as gram-negative strains *Pseudomonas aeruginosa* (P. aeruginosa) and *Proteus vulgaris* (P. vulgaris). Streptomycin was used as a positive control in these experiments.

The results showed that the synthesized Fe_2O_3 nanoparticles present in the agar wells inhibited the growth of all four bacterial strains (*S. aureus*, *B. cereus*, *P. aeruginosa*, and *P. vulgaris*). However, the antimicrobial activity was more pronounced against the gram-positive bacteria (*S. aureus* and *B. cereus*) compared to the gram-negative strains (*P. aeruginosa* and *P. vulgaris*). The data are presented as the mean ± standard error of the mean (SEM) from three replicate experiments. [27]

Chart Caption:1

Table: 1 Zone of inhibition of 1) Fe_2O_3 NPs, 2) Antibiotic and 3) Control Antibacterial effects against human disease-causing microorganisms

Entry	Antibacterial activity			
	Gram + Ve		Gram -Ve	
	Staphylococcus aureus (NCIM-2654)	Bacillus cerus (NCIM - 2703)	Pseudomonas aeruginosa (NCIM 5032)	Proteus vulgaris (NCIM - 2813)
1-Fe_2O_3 NPs	17.33 ± 0.57	16.33 ± 0.57	11.00 ± 1.00	12.33 ± 0.57
2-Antibiotic	23.66 ± 0.57	21.00 ± 1.00	17.66 ± 0.57	21.66 ± 0.57

1 The Staphylococcus aureus NCIM (2654) is gram positive nature.
2 The Bacillus cereus NCIM (2703) is gram positive nature.
3 The Pseudomonas aeruginosa NCIM (5032) is gram negative nature.
4 The Proteus vulgaris NCIM (2813) is gram negative nature.

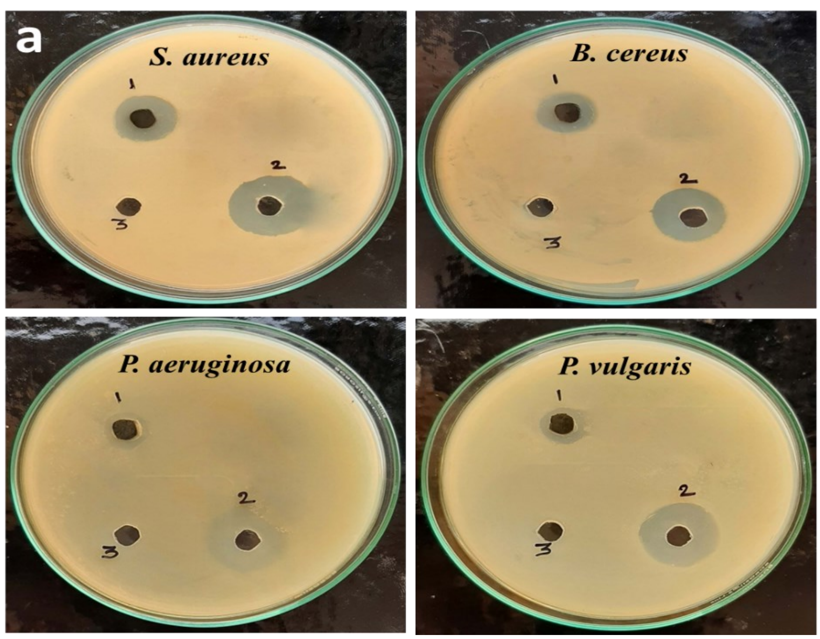

Fig.no.8(a) Antimicrobial activity on the *Staphylococcus aureus* (NCIM -2654), *Bacillus cerus* (NCIM 2703), *Pseudomonas aeruginosa* (NCIM 5032) *and Proteus vulgaris* (NCIM - 2813) zone of inhibition for 1- Fe_2O_3 NPs, 2-Antibiotic and 3-Control

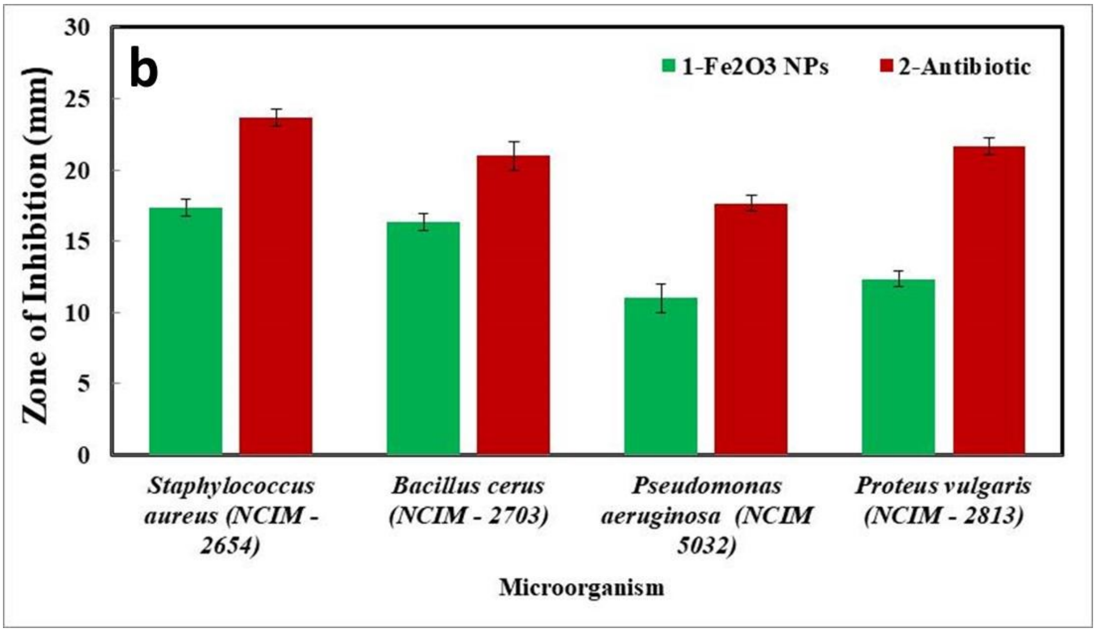

Fig.no. 8(b) The statistical analysis of Fe_2O_3 NPs and standard antibiotic against bacterial human pathogens

CONCLUSION

The study successfully synthesized Fe_2O_3 nanoparticles through biological methods and examined their structural and antimicrobial properties. Structural analysis confirmed the formation of nanosized Fe_2O_3 with distinct morphological characteristics influenced by the synthesis approach. The antimicrobial assessment revealed notable effectiveness against various pathogenic microorganisms, with biologically synthesized nanoparticles demonstrating superior activity, likely due to the presence of bioactive compounds. These findings emphasize the potential of Fe_2O_3 nanoparticles as efficient antimicrobial agents and support biological synthesis as an environmentally friendly alternative.

Furthermore, the study confirmed that biologically synthesized Fe_2O_3 nanoparticles exhibited significant antimicrobial activity, particularly against Gram-positive bacteria (S. aureus and B. cereus), compared to Gram-negative bacteria (P. aeruginosa and P. vulgaris). The results, obtained through the agar well diffusion method and validated with three replicates, reinforce the potential of Fe_2O_3 nanoparticles as effective antibacterial agents.

Acknowledgement:

We sincerely our gratitude to department of chemistry, YCIS Satara for providing the necessary facilities and resources for this work. We extended our heartfelt thanks to the Principal, Y.C.I.S. Satara and our guide/mentor, Dr. P. V. Chavan for their invaluable guidance, constant encouragement and insightful suggestions.

REFERENCES

- Larsson, D. J., & Flach, C. F. (2022). Antibiotic resistance in the environment. Nature Reviews Microbiology, 20(5), 257-269.
- Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., ... & Tasak, N. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The lancet*, *399*(10325), 629-655.
- Gupta, N., Rai, D. B., Jangid, A. K., & Kulhari, H. (2019). Use of nanotechnology in antimicrobial therapy. In Methods in microbiology (Vol. 46, pp. 143-172). Academic Press.
- Thorley, A. J., & Tetley, T. D. (2013). New perspectives in nanomedicine. Pharmacology & therapeutics, 140(2), 176-185.
- Ghasemi, S., Harighi, B., & Ashengroph, M. (2023). Biosynthesis of silver nanoparticles using Pseudomonas canadensis, and its antivirulence effects against Pseudomonas tolaasii, mushroom brown blotch agent. Scientific Reports, 13(1), 3668.
- Khan, F., Jeong, G. J., Singh, P., Tabassum, N., Mijakovic, I., & Kim, Y. M. (2022). Retrospective analysis of the key molecules involved in the green synthesis of nanoparticles. Nanoscale, 14(40), 14824-14857.
- Jafari-Sales, A., Rasi-Bonab, F., & Sayyahi, J. (2019). The survey on antimicrobial effects of methanolic extract of Carum copticum L. on Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa in laboratory conditions. Paramedical Sciences and Military Health, 13(4), 19-25.
- Saif, S., Tahir, A., & Chen, Y. (2016). Green synthesis of iron nanoparticles and their environmental applications and implications. Nanomaterials, 6(11), 209.
- Morais, P. C., Garg, V. K., Oliveira, A. C., Silveira, L. B., Santos, J. G., Rodrigues, M. M. A., & Tedesco, A. C. (2009). Nanoscaled biocompatible magnetic drug-delivery system: preparation and characterization. In ISIAME 2008: Proceedings of the International Symposium on the Industrial Applications of the Mössbauer Effect (ISIAME 2008) held in Budapest, Hungary, 17–22 August 2008 (pp. 269-275). Springer Berlin Heidelberg.
- Villanueva, A., Canete, M., Roca, A. G., Calero, M., Veintemillas-Verdaguer, S., Serna, C. J., ... & Miranda, R. (2009). The influence of surface functionalization on the enhanced internalization of magneticnanoparticles in cancer cells. Nanotechnology, 20(11), 115103
- Farahmandjou, M., & Soflaee, F. (2015). Synthesis and characterization of α-Fe_2O_3 nanoparticles by simple co-precipitation method. Physical Chemistry Research, 3(3), 191-196.
- Tomitaka, A., Hirukawa, A., Yamada, T., Morishita, S., & Takemura, Y. (2009). Biocompatibility of various ferrite nanoparticles evaluated by in vitro cytotoxicity assays using HeLa cells. Journal of Magnetism and Magnetic Materials, 321(10), 1482-1484.
- Perez, J. M., Josephson, L., O'Loughlin, T., Högemann, D., & Weissleder, R. (2002). Magnetic relaxation switches capable of sensing molecular interactions. Nature biotechnology, 20(8), 816-820
- Yoon, T. J., Lee, W., Oh, Y. S., & Lee, J. K. (2003). Magnetic nanoparticles as a catalyst vehicle for simple and easy recycling. New Journal of Chemistry, 27(2), 227-229.
- Doyle, P. S., Bibette, J., Bancaud, A., & Viovy, J. L. (2002). Self-assembled magnetic matrices for DNA separation chips. Science, 295(5563), 2237-2237

- Hassanisaadi, M., Bonjar, A. H. S., Rahdar, A., Varma, R. S., Ajalli, N., & Pandey, S. (2022). Eco-friendly biosynthesis of silver nanoparticles using Aloysia citrodora leaf extract and evaluations of their bioactivities. Materials Today Communications, 33, 104183.
- Nadeem, M., Khan, R., Shah, N., Bangash, I. R., Abbasi, B. H., Hano, C., ... & Celli, J. (2021). A review of microbial mediated iron nanoparticles (IONPs) and its biomedical applications. Nanomaterials, 12(1), 130
- Eluri, R., & Paul, B. (2012). Microwave assisted greener synthesis of nickel nanoparticles using sodium hypophosphite. Materials Letters, 76, 36-39.
- Oma, G., Di Braccio, M., Grossi, G., Mattioli, F., & Ghia, M. (2000). 1, 8-Naphthyridines IV. 9-Substituted N, N-dialkyl-5-(alkylamino or cycloalkylamino)[1, 2, 4] triazolo [4, 3-a][1, 8] naphthyridine-6-carboxamides, new compounds with anti-aggressive and potent anti-inflammatory activities. European journal of medicinal chemistry, 35(11), 1021-1035.
- Chavan, P. V., Pandit, K. S., Desai, U. V., Kulkarni, M. A., & Wadgaonkar, P. P. (2014). Cellulose supported cuprous iodide nanoparticles (Cell-CuI NPs): A new heterogeneous and recyclable catalyst for the one pot synthesis of 1, 4-disubstituted–1, 2, 3-triazoles in water. RSC advances, 4(79), 42137-42146.
- Chavan, P. V., Charate, S. P., Desai, U. V., Rode, C. V., & Wadgaonkar, P. P. (2019). Bentonite-Clay-Supported Cuprous Iodide Nanoparticles (BENT-CuI NPs): A New Heterogeneous Catalyst in Diversity-Oriented Synthesis of 1, 2, 3-Triazoles in Aqueous Medium. ChemistrySelect, 4(24), 7144-7150
- Anandan, K., & Rajendran, V. (2012). Structural, optical and magnetic properties of well-dispersed NiO nanoparticles synthesized by CTAB assisted solvothermal process. Nanosci. Nanotechnol. Int. J, 2(4), 24-29.
- Mukhopadhyay, N., Jain, D., Tripathi, A., & Bhaskar, P. (2024). A Comprehensive Insight into the Pharmaceutical Potential of Saussurea obvallata. Current Pharmacology Reports, 10(6), 349-359.
- Mert, B. D. Green Synthesis and Characterization of Fe_2O_3 Nanoparticles. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 28(3), 1059-1067.
- Chaudhuri, S. K., & Malodia, L. (2017). Biosynthesis of zinc oxide nanoparticles using leaf extract of Calotropis gigantea: characterization and its evaluation on tree seedling growth in nursery stage. Applied Nanoscience, 7(8), 501-512.
- Hassan, D., Khalil, A. T., Saleem, J., Diallo, A., Khamlich, S., Shinwari, Z. K., & Maaza, M. (2018). Biosynthesis of pure hematite phase magnetic iron oxide nanoparticles using floral extracts of Callistemon viminalis (bottlebrush): their physical properties and novel biological applications. Artificial cells, nanomedicine, and biotechnology, 46(sup1), 693-707.
- Survase, A. A., & Kanase, S. S. (2023). Green synthesis of TiO_2 nanospheres from isolated Aspergillus eucalypticola SLF1 and its multifunctionality in nanobioremediation of CI Reactive Blue 194 with antimicrobial and antioxidant activity. Ceramics International, 49(10), 14964-14980.