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Plant Mediated Co-NPs & Fe-NPs by using Borage officinalis; Characterization and Biological Potential

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ABSTRACT

Green synthesis of metal nanoparticles using plant extracts is a promising route towards the synthesis of biologically active nanomaterials. Cobalt (Co) and iron (Fe) nanoparticles were produced in the current study using the *Borago officinalis* leaf extract which acted as a reducing and stabilizing agent. The UV-Visible spectroscopy revealed absorption peaks at about 520-540nm for Co-NPs and 280-320nm for Fe-NPs. Fourier-transform infrared spectroscopy (FTIR) was used to determine different functional groups like hydroxyl, carbonyl, and amine which can be attributed to phytochemicals of plants in the reduction and capping of the nanoparticles. The morphology of the nanoparticles in scanning electron microscopy (SEM) images revealed that they were almost spherical in shape with minimal agglomeration and average diameters ranging between 60-100nm. The obtained nanoparticle exhibited significant, concentration dependent antibacterial properties against Gram-positive and Gram-negative bacterial strains; cobalt nanoparticles formed larger zones of inhibition, an average of 18 mm, than iron nanoparticles, an average of 15 mm, but still lower than the conventional antibiotic. By the DPPH scavenging system, antioxidant activity reached maximum inhibition of about 78 and 67% in Co-NPs and Fe-NPs respectively at 1250 ug/mL, which equals IC50 of near 550 and 750 ug/mL respectively. All these findings highlight the potential of cobalt and iron nanoparticles based on *Borago officinalis* as safe antibacterial and antioxidant agents.

Introduction

The field of nanotechnology has become one of the most critical areas of modern studies due to the ability to produce materials that possess better physicochemical and biological properties (Yusuf et al., 2023). The nanoparticles of metals have demonstrated a wide range of uses in antimicrobial therapy, catalysis and environmental applications (Sánchez-López et al., 2020). In this framework, cobalt and iron nanoparticles are receiving growing interest in the field of their magnetism, biocompatibility, and strong antimicrobial activity (Arias et al., 2018) (Ameen & Majrashi, 2023).

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The traditional production of metal nanoparticles often requires toxic substances and other processes that are harmful to the environment (Dikshit et al., 2021). Green synthesis has been embraced as alternative which is sustainable and make use of natural biological systems to reduce metal ions and stabilize nanoparticles (Radulescu et al., 2023). The plant extracts represent a particularly promising opportunity, as they can be found, are cost-efficient, and contain a large amount of bioactive phytochemicals (Ghabru et al., 2025).

The presence of phytochemicals (phenolics, flavonoids, terpenoids and proteins) in plant extracts, which also work as reducing and capping agents in the generation of nanoparticles (Pirsaheb et al., 2024). They decrease microbial size, shape and stability, and at the same time, increase the antimicrobial activity of the compounds (Mauriello et al., 2021). *Borage officinalis* is a well-known medicinal species known as an antioxidant, anti-inflammatory, and antimicrobial agent, and its success is due to the high concentration of polyphenols (Gakhar, 2021).

Cobalt and iron nanoparticles were also characterised by the strong antimicrobial and catalytic properties and the widely studied application to the environment and biomedicine (Hosseinzadeh, 2025). The application of green synthesis of these nanoparticles using the leaf extract of *B. officinalis* could have a synergistic effect, thus enhancing the antimicrobial efficacies (Askary & Malih, 2021). Characterization of the synthesized nanoparticles is an important step in the process of validating the formation of these nanoparticles, as well as understanding their properties. Ultraviolet- visible spectroscopy was used to validate nanoparticle synthesis, Fourier- transform infrared spectroscopy was used to identify functional groups used in reduction and stabilization and scanning electron microscopy provided detailed data on surface morphology and particle dimensions.

Due to the urgent demand to find alternative antimicrobial agents, the current research intends to prepare the *Borage officinalis* leaf extract to synthesize cobalt (CoNPs@BO) and iron (FeNPs@BO) nanoparticles using green synthesis methods, characterization of the nanoparticles using UV-vis spectroscopy, FTIR, and SEM, and determination of their antimicrobial activity on some of the unwanted pathogenic microorganisms.

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Experimental Section

Preparation of *Borage officinalis* (BO) leaf aqueous extract:

5g BO leaves were pulverized to get fine powder after they were weighed, cleaned and dried under shade. The powder was taken in a beaker, and using de-ionised water, volume was made to 100ml. Then heated it on a hot plate and brought it up to boil. Boiled for twenty minutes, cooled, then filtered using Whatman filter paper. The filtrate obtained was centrifuged and filtered again to remove suspended particles and further used for the synthesis of nanoparticles.

Green Synthesis of Nanoparticles

Cobalt Nanoparticles (CoNP's@BO)

1mM aqueous solution of Cobalt nitrate hexahydrate was prepared by dissolving 0.029103g Co $(NO_3)_2.6H_2O$ to make 100ml solution. 40 ml salt solution was mixed with 10ml BO leaf extract. This solution was stirred at 60°C for 2 hours until a color change from light brown to dark brown was observed. This solution was kept for 24 hours, centrifuged and solid particles were dried at 60°C in drying oven to obtained solid dried nanoparticles (Hafeez et al., 2020).

Iron Nanoparticles (FeNP's@BO)

1mM ferrous chloride tetrahydrate aqueous solution was prepared by dissolving 0.019881g of $FeCl_2.4H_2O$ to prepare 100ml solution. 50 ml of salt solution was taken and mixed with 10 ml BO leaf extract. Stirring at 80°C for 2 hours was done until the light-yellow color to dark brown color change was visible. The solution was left overnight for 24 hours, centrifuged and solid particles were dried at 80°C using drying oven to get solid dried nanoparticles (Kumar et al., 2023).

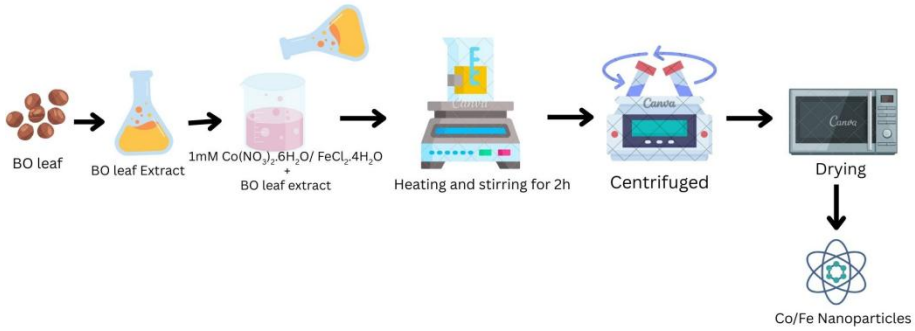


Figure 1: Green Synthesis of Co/Fe Nanoparticles

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Characterization Techniques

Functional group and chemical structure of the samples prepared were characterized by using an FTIR-Vertex 70 spectrophotometer (Bruker, Karlsruhe, Germany) within the spectral range of 400–4,000 cm^{-1}. Field Emission SEM (FE-SEM, Zeiss, Model-Sigma 300) instrument was employed to find the morphology and particle size of NPs.

Biological Potential

Nanoparticles prepared through green synthesis are considered to have the capability of having anti-bacterial, antifungal and indeed antioxidant characteristics. A microbiological assay substrate to be used in the microbiological assays was chosen to be Mueller-Hinton agar medium (MHA, Merck, Germany). To determine antibacterial efficacy, gram-negative bacterial strain was used, namely, *Escherichia coli*, and gram-positive one, namely, *Bacillus subtilis (Buarki et al., 2022).* To complement the bacterial test, fungal strains of *Aspergillus niger* and *Fusarium oxysporum* were also added to determine the antifungal action of the nanoparticles. The agar plates and the height of the zone of inhibition were measured and used to quantify the inhibitory activity of the nanoparticles by determining the diameter (in millimetres) (Bukhari et al., 2021).

Antioxidant Assay

Radical Scavenging Assay of DPPH Radicals

To assess the antioxidant capacity of the synthesized nanoparticles, 2,2-diphenyl-1-picrylhydrazyl (DPPH), one of the most stable radicals at room temperature was utilized. The assay was performed by incubating 50µl of cobalt and iron nanoparticles solutions with a concentration of 250, 500, 750, 1000, and 1250µg/ml of DPPH reagent and 0.1 ml of Tris -HCl buffer (pH 7.4). After incubating the solution in the dark (60 minutes), the absorbance was measured at 517nm. The quantitative measure of antioxidant activity was the IC_{50} which was determined as the concentration needed to reduce the DPPH signal by half (Govindasamy et al., 2022). The following formula was used to compute percentage inhibition of the nanoparticles:

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$$\%\ scavenging\ activity\ (DPPH) = \frac{\boldsymbol{A}\ control - \boldsymbol{A}\ sample}{\boldsymbol{A}control} \times 100$$

Results and Discussion

UV–Visible Spectral Analysis

Since UV-Visible spectroscopy is a viable and effective method used to confirm the synthesis of iron and cobalt nanoparticles produced through a green route with the use of *Borage officinalis* leaf extract, it was used to initially confirm the formation of these nanoparticles. In Figure 2, the UV spectrum of FeNPs has a specific absorption band peaked at 280-290nm, as is typical of surface plasmon resonance (SPR) or electronic transitions involving iron nanoparticles and iron oxides formed in the synthesis involving phytochemicals. This spectral range has been widely reported to show parallel absorption bands of plant-mediated iron nanoparticles and is believed to be due to the presence of Fe–O related charge-transfer transitions and formation of nanoscale particles.

Figure 2, UV- visible spectrum of CoNPs, shows a strong absorption peak at 290-300 nm, which proves the reduction of cobalt ions to cobalt nanoparticles was successful. This observation of the absorption band is consistent with previous studies of green-synthesized cobalt nanoparticles where broad absorption in the UV is often observed because of collective oscillations of conduction electrons and metal-ligand interactions induced by phytochemical capping agents.

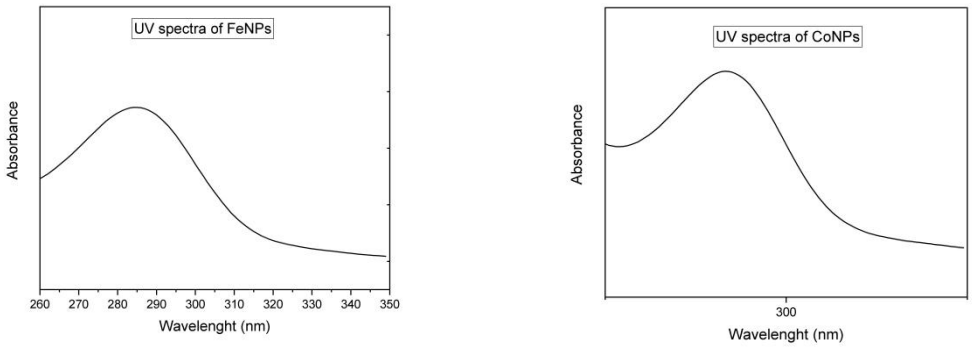
The broad nature of the absorption bands in both spectra is a characteristic of a polydisperse system of nanoparticles with varying particle sizes and morphologies, typical of green synthesis procedures. The difference in the maximum of the absorption between FeNPs and CoNPs can be attributed to changes in the composition of metals, size distribution, and interactions of the nanoparticles with plant-derived biomolecules that worked as reducing and stabilizing agents.

Together, the UV-visible data is in support of the successful production of iron and cobalt nanoparticles using a green method and supports the FTIR and SEM characterizations that follow. The absorption behaviour was like the reported UV spectra of plant-mediated metal nanoparticles in the past and thus validated the effectiveness of *Borage officinalis* leaf extract in nanoparticle synthesis (Roy et al., 2022).

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http://amresearchreview.com/index.php/Journal/about



(a) (b)

Figure 2: UV-Vis Spectra of Cobalt and Iron Nanoparticles

FTIR Spectroscopy

The FTIR spectrum of the iron nanoparticles produced with the help of plant extract (FeNP's@BO) shows the presence of plant-derived phytochemicals in the nanoparticle's preparation and stabilization. It is expected that the broad absorption at 3300-3400cm^{-1} is due to the O -H stretching vibrations of phenolic and alcoholic groups, which could explain their role of reducing agents, which is in line with previously reported green synthesis of iron nanoparticles using plant extracts. The weak bands at 2920-2850cm^{-1} are C-H stretching of aliphatic groups, which points to the presence of organic moieties on the surface of a nanoparticle.

The presence of the absorption band 1630-1650cm^{-1} C=O (amide I) or C=C is attributed to the presence of proteins and flavonoids, which are potential capping agents, as was previously reported in experiments of plant-mediated iron nanoparticle synthesis. It is explained by the presence of bands in the 1050-1150cm^{-1} area related to C -O stretching vibrations of alcohols and polysaccharides, which are involved in the stabilization of nanoparticles. Notably, the band with high absorption at around 500-580cm^{-1} is related to the Fe -O stretching vibrations, which proves the successfulness of forming iron nanoparticles, as it is commonly reported with green-synthesized iron nanoparticles and iron oxide nanoparticles (Roy et al., 2022).

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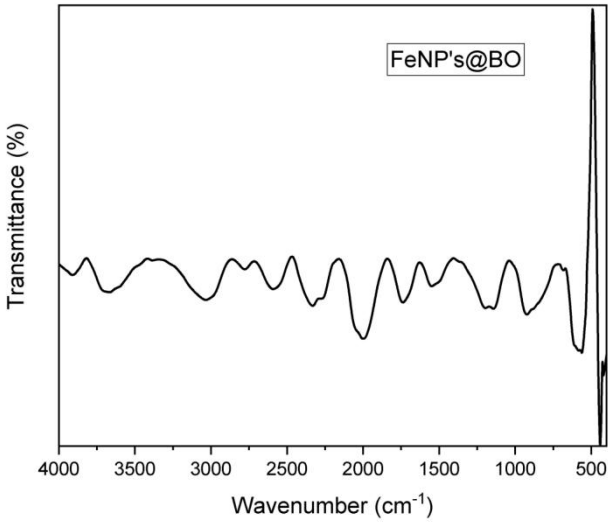


Figure 3: FTIR Spectra of Iron Nanoparticles

SEM Analysis

SEM micrographs of cobalt nanoparticles (CoNPs) and iron nanoparticles (FeNPs) showed distinctive morphological characteristics depending on the type of the metal and the route through plant mediate synthesis (Figures 4). The SEM image of CoNPs exhibits irregular and spherical particles with strong agglomeration to compose thick cluster with a coarse surface texture. This aggregation has always been observed with cobalt nanoparticles, which is explained by their magnetic interactions and large surface energy, even in the presence of phytochemical capping agent. According to the SEM observation, the mean distance between the particles of CoNPs is estimated at a range of about 40-80nm, which is in line with other reported, green-synthesized cobalt nanoparticles using plant extracts.

Conversely, the SEM image of FeNPs represents a relatively smaller and unseparated morphology, in which particles will be represented as aggregated but finer nanoscale domains and integrated into an organic matrix. It seems that the surface is smoother in comparison with CoNPs, which implies that iron nanoparticles interact better with the biomolecules of the plant, which are valid stabilizing and capping agents (Ahmed et al., 2021). The size of the FeNPs is estimated to be between 20-50nm in diameter which implies that the particles are smaller than CoNPs. Green-synthesized iron nanoparticles with similar

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http://amresearchreview.com/index.php/Journal/about

morphologies and size distributions have been reported, in which phytochemicals highly limit the growth of particles (Nahari et al., 2022).

In general, the comparative SEM analysis suggests that FeNPs have a smaller particle size and comparatively with CoNPs have a better dispersion ability, and this can be explained by the differences in the reduction kinetics and metal phytochemical interactions. The nanoscale dimensions of CoNPs as well as FeNPs and surface roughness of the particles play to their benefit in responding to antimicrobial purposes since a greater surface area increases the interaction between the nanoparticles and microbes, which is consistent with literature reports on green-synthesized metal nanoparticles.

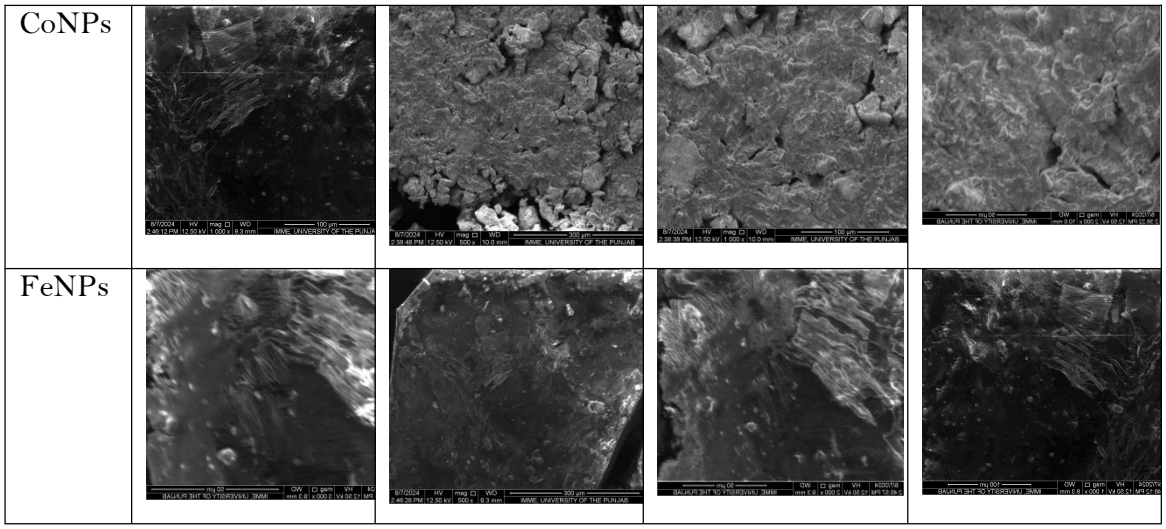


Figure 4: SEM Images of Co and Fe Nanoparticles

Biological Potential

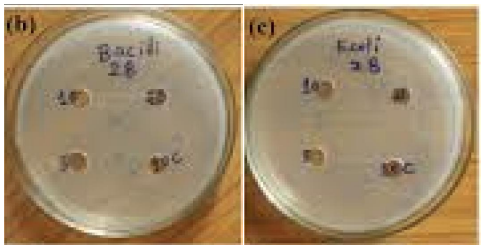


Figure 5: Antibacterial Activity of CoNPs against B. subtilis and E. coli

Antibacterial efficacy of cobalt (Co -NPs) and iron nanoparticles (Fe -NPs) using *borago officinalis* as the mediator was evaluated through an agar diffusion test in relation to representative Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli* bacteria. The longest zone of inhibition was found with the standard

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http://amresearchreview.com/index.php/Journal/about

antibiotic which ranged between about 22 and 25 mm in length making the assay reliable. Conversely, Co -NPs produced with green synthesis showed strong concentration-dependent activity, whereby the zones of inhibition were approximately 10 -12 mm, 16 -18 mm at the lowest and highest concentration respectively. Antibacterial effects were also observed in Fe-NPs although with relatively smaller areas with the distance appearing around 8-10 mm at low concentration and 13-15 mm at high concentration. The increased area of inhibition of Co -NPs indicates the better antibacterial activity compared to the Fe -NPs, which could be explained by the increased reactivity of the surface and more effective release of metal ions.



Figure 6: Antibacterial Activity of FeNPs against B. subtilis and E. coli

A sharp difference occurred between Gram-positive and Gram-negative bacteria. Gram-positive strains gave larger inhibition zones of the two types of nanoparticles, and it may be attributed to the lack of the outer lipopolysaccharide membrane, which provided a favorable environment of nanoparticle penetration and attachment to the peptidoglycan shell. On the other hand, Gram-negative bacteria were less susceptible, in accordance with their further outer membrane that serves as a barrier to the metal ions and nanoparticles permeability. The suggested antibacterial action implicates the production of the reactive oxygen species (ROS), impairment of the membrane integrity, and intracellular damage through the liberation of Co^{2+} and Fe^{2+}/Fe^{3+} ions, which is promoted by phytochemical capping agents of *B. officinalis*. In general, even though the antibacterial activity of the nanoparticles was lower in comparison with that of the conventional antibiotic, the concentration-dependent and the broad-spectrum effect, especially of the Co-NPs, indicates their potential application as environmentally safe antibacterial agents especially against Gram-positives.

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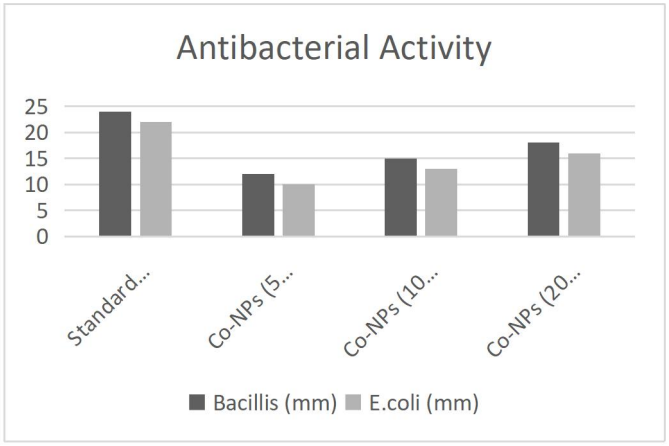


Figure 7: Graphical representation of Antibacterial Activity of CoNPs against B. subtilis and E. coli

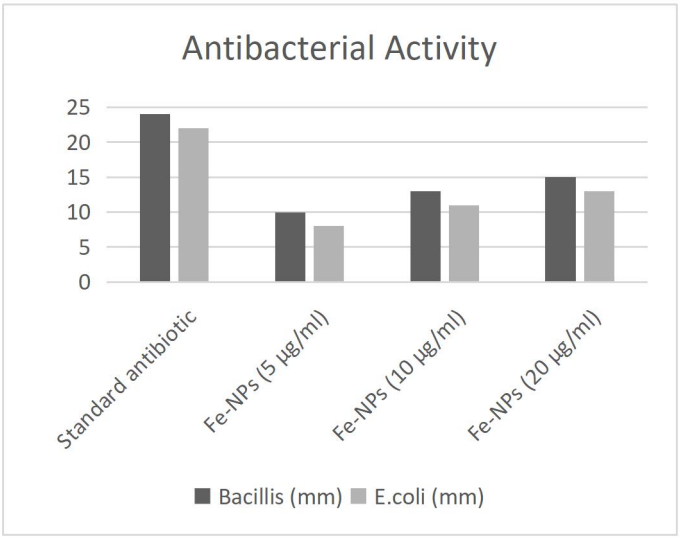


Figure 8: Graphical representation of Antibacterial Activity of FeNPs against B. subtilis and E. coli

Antioxidant Assay

Borago officinalis was used to prepare nanoparticles of iron (Fe) and cobalt (Co), the antioxidant potential of which was studied by utilizing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay at the concentration of 250-1250 µg/L. Findings showed that the DPPH radical scavenging capacity was concentration-dependent, as indicated by a gradual reduction in absorbance at 517nm.

Fe-NPs and Co-NPs had moderate scavenging rates (28-32% and 35-38%) at the lowest dose (250 mL^{-1}) respectively. Gradual increase in concentration produced

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Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

significant enhancement of antioxidant property. As an example, Fe-NPs showed an inhibition value of approximately 48-52% at 750 ug/mL while, Co-NPs showed higher scavenging potential in the range of 58-62%. FeNPs reached a percentage of approximately 65-68% at the highest level of concentration studied (1250 ug/mL^{-1}), whereas CoNPs exhibited greater efficacy with a percentage of approximately 75-80% inhibition of DPPH radicals.

The IC50 values were estimated to be in the ranges of 720-780 ugmL to be within the ranges of 520-580 ugmL, respectively, and were used as the concentration required to attain 50 percent DPPH radical scavenging with the Fe-NPs and Co-NPs. These results prove that Co-NPs have a better antioxidant potential as compared to Fe-NPs (Figure 9).

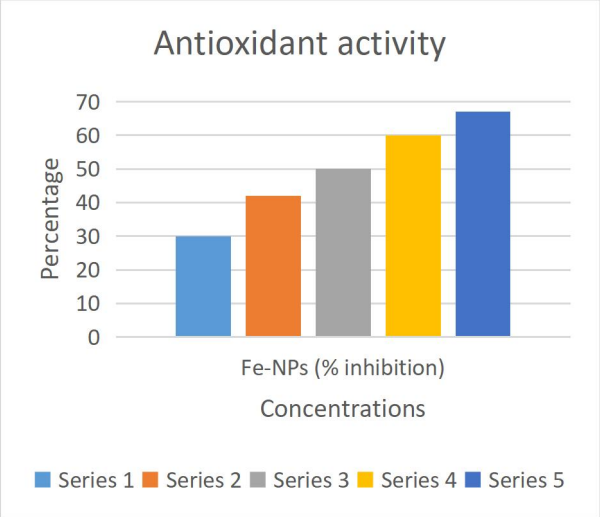


Figure 9: Graphical representation of Antioxidant Activity of FeNPs

Conclusion

In conclusion, both cobalt and iron nanoparticles were prepared using a green, eco-friendly, method using a reduced green leaf extract of *Borago officinalis* that was used as both a reducing and stabilising agent. The spectroscopic and microscopic studies (UV-Vis, FTIR, SEM) confirmed the presence of stable, nanosized, and mostly spherical particles, capped by phytochemicals of plant origin. The nanoparticles biosynthesised had concentration-dependent antibacterial properties against Gram-positive and Gram-negative and cobalt nanoparticles were more effective. Also, the

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two types of nanoparticles had a large antioxidant activity in the DPPH assay, with cobalt nanoparticles offering decreased IC^{50} and increased free-radical scavenging capacity compared to iron nanoparticles. The interactions of the metal nanoparticles with bioactive constituents of *B. officinalis* can explain the better bioactivity. Altogether, this paper highlights potential applications of the cobalt and iron nanoparticles synthesised through the mediation of the *B. officinalis* as sustainable and efficient antibacterial and antioxidant substances in the context of biomedical, pharmaceutical, and antimicrobial material development.

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