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Original Research Article

Preparation and Comparative Antioxidant Activity of Iron nanoparticles from Extracts of the Leaf and Root of *Bridelia ferruginea*

Olubunmi S. Oguntoye*, Oluwatobi O. Amusan, Kayode Adeniran

Department of Chemistry, University of Ilorin, Kwara State, Nigeria

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ABSTRACT

Nanomaterial plays an important role in the development of biologically active agents. Several methods used for synthesizing these nanomaterials have been associated with various challenges such as high toxicity and environmental pollution. This has led to the consideration and adoption of greener means of synthesis. The preparation of iron nanoparticle using crude plant extracts in this study was aimed at a non-toxic and environment friendly means of synthesis with determination of their antioxidant activity. Iron nanoparticles were prepared from ferrous sulphate using the crude extracts from the leaf and root of *Bridelia ferruginea* as stabilizing and capping agents. The antioxidant activity of the synthesized iron nanoparticles was investigated using DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethybenzothiazoline-6-sulphonic acid) radical scavenging activity under different dosages of the iron nanoparticles. The prepared iron nanoparticles were characterized using different analytical techniques. The absorption at 400 and 390 nm by leaf- and root-mediated iron nanoparticles, respectively confirmed the formation of iron nanoparticles which was further analyzed by X-ray diffraction showing peaks at 2θ to confirm their crystal structures. Scanning Electron Microscopic (SEM) images showed a rod-like and spherical morphology while the Energy Dispersive X-ray Spectroscopy (EDX) revealed the elemental compositions of the synthesized iron nanoparticles. It was confirmed that the root-mediated iron nanoparticles exhibited a higher antioxidant activity at the various dosages that were investigated by DPPH and ABTS decolourization assay than the leaf-mediated iron nanoparticles.

Keywords: Iron nanoparticles, DPPH, ABTS, Scavenging Activity.

Introduction

Nanotechnology is a field that is gaining wide recognition and attraction among scientists recently. The existence of materials in the smallest possible size with possible modifications to their physical properties, thereby increasing the surface area while maintaining high level of activity, makes nanotechnology an interesting field of study having applications in fields such as medicine, environmental remediation, electronics, energy, catalysis, cosmetics and several other fields for human application.[1,2]

Various physical and chemical methods have been used for the synthesis of nanoparticles due to its novel applications in several fields because of its catalytic ability and less toxicity. The activities of the synthetic approaches have been monitored and found to pose harmful effect on the environment, hence the less toxic and environmentally friendly green synthetic approach.[3-5]

The presence of secondary metabolites in medicinal plants makes them useful in the synthesis of nanoparticles. The metal ions are reduced to their metallic nanoparticles by the presence of alkaloids, phenolics, terpenoids, saponins and other metabolites in plants.[6-8]

The reduction of metal salts to their metallic nanoparticles is a promising method due to the abundance of plants in nature and the less toxicity associated with their synthesis. Plant materials acts as both reducing agents as well as capping agents in the synthesis of iron nanoparticles from the corresponding salt precursor.

Iron nanoparticles as one of the synthesized nanomaterials have been reported to exhibit anticancer, antimicrobial, antioxidant, and anti-inflammatory activities. These biological activities of Iron nanoparticles have generated their interest in medicine and drug delivery.[9-11]The synthesis of iron nanoparticles using plant extracts through a green synthetic approach is a novel technique to overcome the limitations of other conventional methods by the use of non-toxic and environmental friendly synthesis. In this method, different parts of plants such as root, leaf and bark are being used for nanoparticle synthesis by extracting the biomolecules such as polyphenolic compounds, alkaloids, proteins, polysaccharides and terpenoids to be used as reducing and capping agents in the reduction of the salt precursor to synthesize iron nanoparticle.[12] Iron nanoparticles have been synthesized using various plant extracts.[13-15]

This study aimed to prepare environmentally friendly Iron nanoparticles with comparative investigation of their antioxidant activity from extracts of the root and leaf of *Bridelia ferruginea*.

Materials and Methods

Plant Material and Chemicals

The leaves and roots of *Bridelia ferruginea* were obtained on 22nd January, 2018 at University of Ilorin Botanical Garden and the plants were identified and deposited at the herbarium of Department of Plant Biology, University of Ilorin, Ilorin, Kwara State with voucher

*Corresponding author. E mail: stevorol@unilorin.edu.ng
Tel: +2348066490198

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number UILH/002/988. The salt precursor ($FeSO_4.7H_2O$), ABTS (2,2'-azino-bis-3-ethybenzothiazoline-6-sulphonic acid), DPPH (1,1-diphenyl-2-picryl hydrazyl hydrate) were obtained from Sigma-Aldrich Company, Germany.

Extraction of Crude Plants

The fresh leaves and roots of *Bridelia ferruginea* were washed with distilled water to remove impurities. 7.00 g of leaves and roots were weighed and chopped into small sizes and soaked in 100 mL distilled water which was heated at 70°C for 15 minutes to extract the secondary metabolites in the plant materials without denaturation. The solution was allowed to cool, centrifuged at 10,000 rpm, before using whatmann No 1 filter paper to filter the extract and stored in a refrigerator at -4°C for further use.

Synthesis of Iron Nanoparticles

To 100 mL of 0.1 M $FeSO_4.7H_2O$ under continuous stirring at 500 rpm and 55°C using a magnetic stirrer with temperature sensor, 100 mL of *Bridelia ferruginea* extract was added slowly under continuous stirring for 30 minutes. The colour of the mixture changed from light brown with the formation of black precipitate observed. The black precipitate was centrifuged at 10,000 rpm washed with distilled water, filtered and washed repeatedly using ethanol to obtain a pure black powder. The black powder was oven-dried at 50°C for 10 minutes to obtain a dry powder, labelled and stored in air-tight bottles for further use.

Characterization of Iron Nanoparticles

The prepared iron nanoparticles were characterized using Ultraviolet-Visible and Fourier Transform Infrared (FTIR) Spectroscopic techniques to confirm its formation by obtaining the absorption peak and detection of specific functional group in the plant extracts that might be responsible for the reduction of the metal salt, respectively. X-ray Diffraction (XRD) Analysis was done to investigate the structure of the iron nanoparticles formed; Energy Dispersive X-ray Spectroscopy (EDX) was also used to determine the elemental composition.However, Scanning Electron Microscopy (SEM) was used to determine the morphology of the prepared Iron nanoparticles.

Evaluation of Antioxidant Activity

The radical scavenging activity of the prepared Iron nanoparticles were measured using the stable free radical scavenger ABTS (2,2'-azino-bis-3-ethybenzothiazoline-6-sulphonic acid) and DPPH (1,1-diphenyl-2-picrylhydrazyl hydrate) decolourization assays.[16,17] In this method, iron nanoparticles were diluted into different concentrations. Ascorbic acid was used as standard. DPPH and ABTS were dissolved in methanol (10^{-4} M, in 95% methanol) and the absorbance taken. Different concentrations of synthesized Iron nanoparticles were added to each differently, shaken thoroughly and incubated for 30 minutes. The absorbance of the solution was measured at 517 nm against a blank. All experiments were carried out in triplicates and percentage inhibition was calculated using the equation:

$$\% \text{ Inhibition} = (A_s - A_i/A_s) \times 100\ \%$$

Where, A_s = the absorbance of standard.
A_i = the absorbance of the iron nanoparticles.

Results and Discussion

Fourier Transformed Infra-Red Spectroscopy (FTIR)

FTIR analyses of crude extracts from the leaf and root of *Bridelia ferruginea* are shown in Figures 1 and 2, respectively. Also shown are the FTIR spectra of the synthesized Iron nanoparticles in order to detect the functional groups of biomolecules that were responsible for the reduction of Iron metal as well as capping of the Iron nanoparticles from the metal salt precursor.The FTIR of extract as shown in Figure 1a showed vibrations stretching at 1637 $cm^{-1}$for C=C and 3461 $cm^{-1}$for O-H stretch which are responsible for the reduction of the salt precursor. Comparison of the FTIR of leaf extract to that of the FTIR of the product (Iron nanoparticles) showed broad O-H group stretch at 3385 cm^{-1}, C=C at 1628 cm^{-1}, C-O at 1197 cm^{-1} and Fe-O at 612 cm^{-1}(Figure 1b).

The FTIR of root extract showed vibrations stretching at 1628 $cm^{-1}$for C=C and 3461 $cm^{-1}$for O-H stretch which were responsible for the reduction of the salt precursor (Figure 2a). Comparison of the FTIR of root extract to that of the FTIR of the product (Iron nanoparticles) showed broad O-H group stretch at 3385 cm^{-1}, C=C at 1619 cm^{-1}, C-O at 1128 cm^{-1} and Fe-O at 634 and 602 cm^{-1}(Figure 2b).

Ultraviolet-Visible (UV-Visible) Spectroscopy

UV-Visible spectrum was run between 300-700 nm for the biosynthesized iron nanoparticles. *Bridelia ferruginea* Root-mediated Iron nanoparticles (FeNP-BFR) showed absorption peak at 390 nm as shown in Figure 3a. These values confirmed the formation of iron nanoparticles. Similar result was obtained using *Eichhornia crassipe*.[17] *Bridelia ferruginea* Leaf-mediated Iron nanoparticles (FeNP-BFL) showed absorption peak at 400 nm as shown in Figure 3b which was in agreement with the result obtained by Mahdavi *et al.* (2013) using seaweed extract.[18]

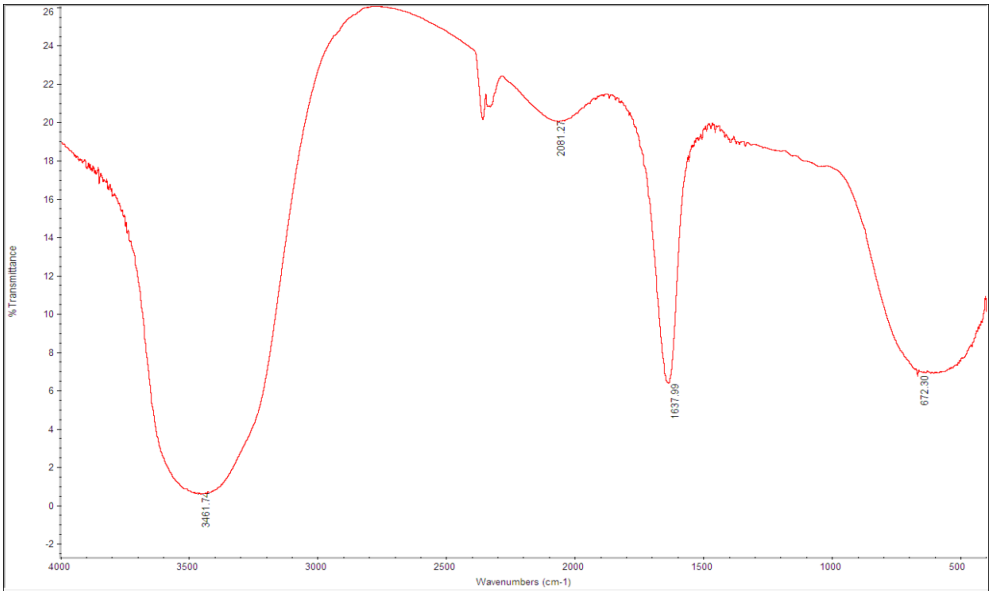

Figure 1a: FTIR Spectrum of the Leaf Extract of *Bridelia ferruginea*

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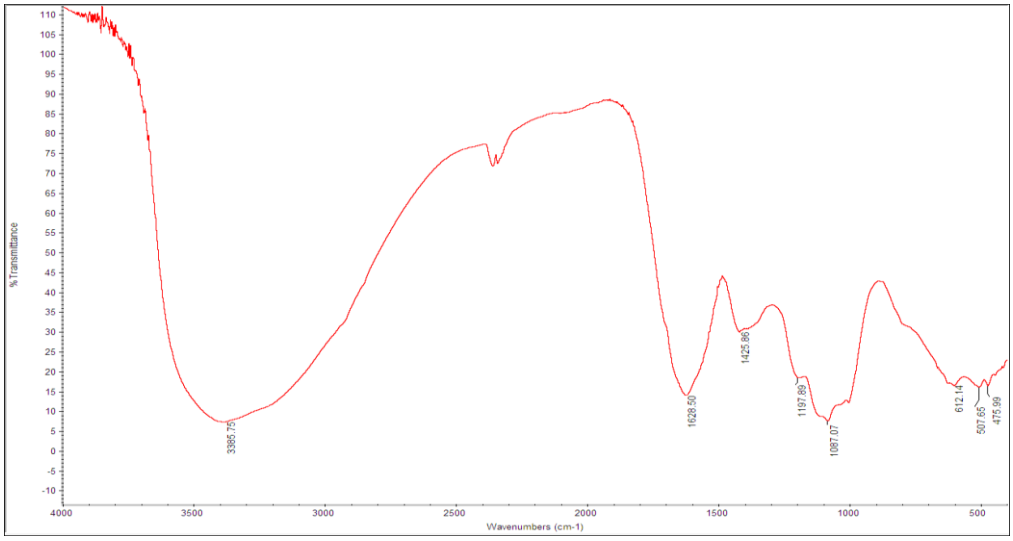

Figure 1b: FTIR Spectrum of Fe-NP from the Leaf Extract of *Bridelia ferruginea*

Figure 2a: FTIR Spectrum of the Root Extract of *Bridelia ferrugunia*

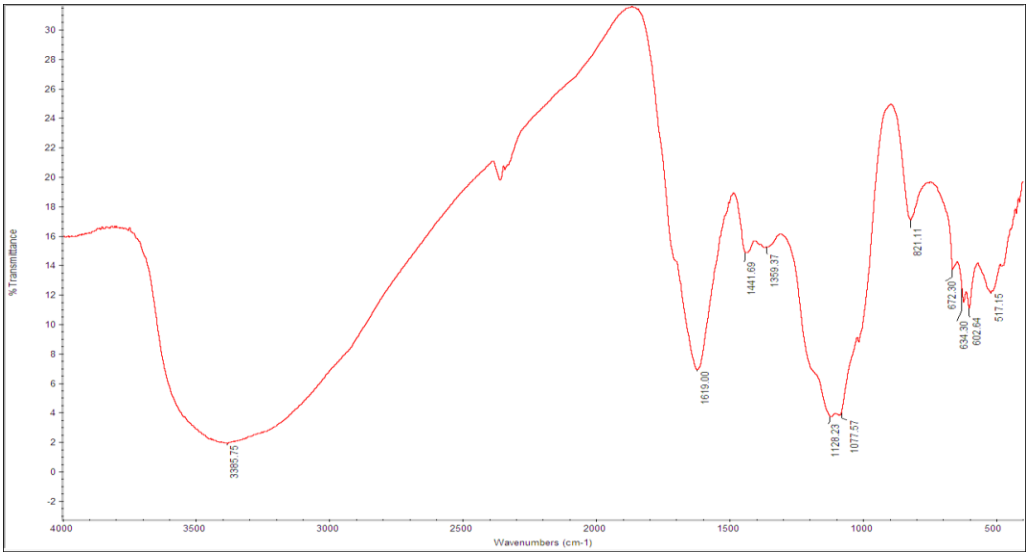

Figure 2b: FTIR Spectrum of Fe-NP from Root Extract of *Bridelia ferruginea.*

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X-Ray Diffraction Analysis (XRD)

X-Ray Diffraction Analysis was done to prove the phase of *Briedelia ferruginea*-mediated Iron nanoparticles. In Figure 4a, the XRD peaks at 2θ values match to the crystal planes of 28° = 111 of *Bridelia ferruginea* leaf extract-mediated Iron nanoparticles. The analyzed diffraction peaks matched well with the standard magnetite XRD patterns using JCPDS (card number 87-1164). These results closely agreed with the XRD results obtained for *Abelmoschus esculentus* extract which showed good crystallinity.[19]

The XRD peaks at 2θ values as shown in Figure 4b matched to the crystal planes of 18° = 220 and 24° = 200, of *Bridelia ferruginea* root extract-mediated Iron nanoparticles. The analyzed diffraction peaks matched well with the standard magnetite XRD patterns using JCPDS (card number 19–0629). These results closely agreed with the XRD results obtained for *Eichhornia crassipes* leaf extract which was used to synthesize pure Fe_3O_4 with a spinal structure.[18]

Scanning Electron Microscopy (SEM)

The morphological studies and the structure of the synthesized iron nanoparticles were analyzed as shown in Figures 5a and 5b. SEM images of FeNP-BFL were well separated with slight aggregation. The SEM results were consistent with reported results of synthesized FeNPs using aqueous sorghum bran extracts as the reducing agent.[20] SEM images of FeNP-BFR showed a clear rod-like spherical morphology while that of FeNP-BFR looks spherical. The difference in their morphology might be due to the presence of different secondary metabolites in the Leaf and roots extracts of the plant.

Energy Dispersive X-Ray (EDX) Spectroscopy

The EDS was exploited to reveal the elemental composition of the prepared nanoparticlesas as shown in Figures 6a and 6b. These EDS values were used in reflecting the nuclear content on the surface regions of the synthesized Iron nanoparticles. The Fe signal is that of the synthesized iron nanoparticles. Carbon signals are credited mainly to the polyphenol groupsand other carbon containing molecules in the plant extracts. Other elements such as oxygen and sulphur signals must be originating from $FeSO_4.7H_2O$ used as the salt precursor.

Antioxidant Activity

A) DPPH Assay

The radical scavenging activity of the prepared iron nanoparticle from the plants extracts at different concentrations (0.01, 0.02, and 0.05 µg/mL) were measured using the stable free radical scavenger, DPPH (1, 1-diphenyl-2-picrylhydrazyl hydrate) decolourization assay. From figure 7a, all the synthesized iron nanoparticles showed inhibition that is comparable to the standard used at different concentrations that were investigated. For the concentration of 0.01 and 0.02 mg/mL, the difference in the inhibition may be statistically insignificant as the FeNP-BFL gave an inhibition of 95 and 89% while that of FeNP-BFR gave an inhibition of about 98 and 90%, respectively. At concentration of 0.05 mg/mL, the difference in the inhibition might be statistically significant as the FeNP-BFL gave an inhibition of 88% while that of FeNP-BFR gave an inhibition of 70%. Thus, it can be deduced from the overall inhibition that the root-mediated Iron nanoparticle showed a moderate activity than the leaf-mediated Iron nanoparticles.

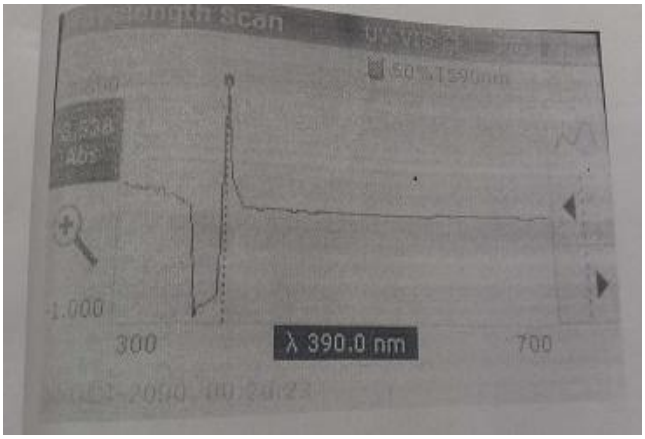

Figure 3a: UV Spectrum of FeNP-BFR

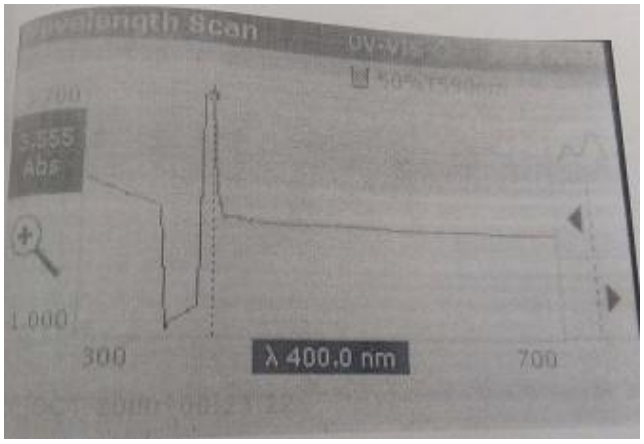

Figure 3b: UV Spectrum of FeNP-BFL

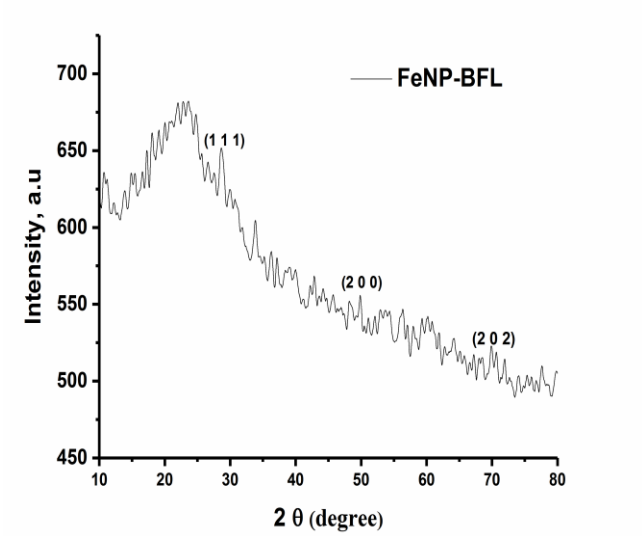

Figure 4a: XRD Pattern for Fe-NP from the Leaf Extract of *Bridelia ferruginea.*

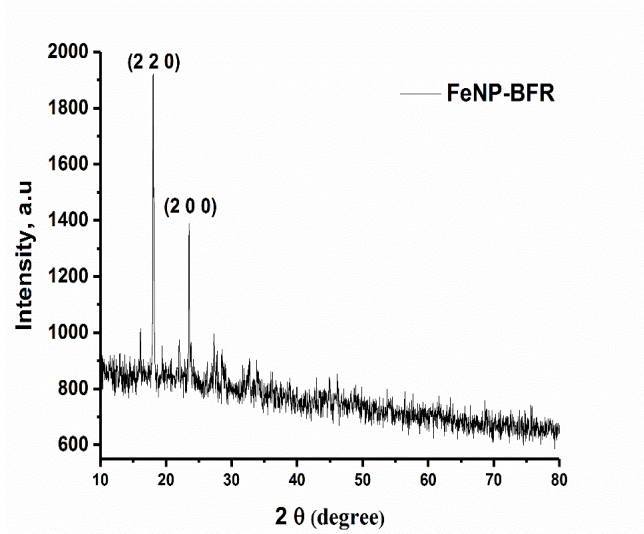

Figure 4b: XRD Pattern for Fe-NP from the Root Extract of *Bridelia ferruginea.*

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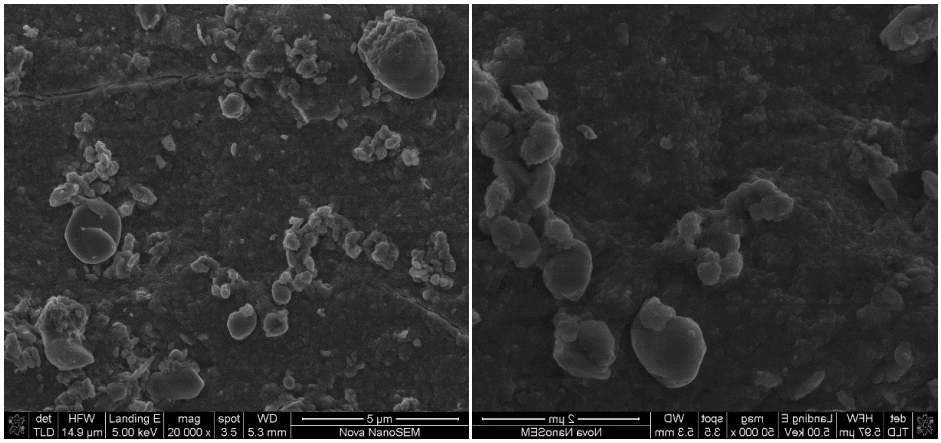

Figure 5a: SEM images of FeNP-BFL at Different Magnification

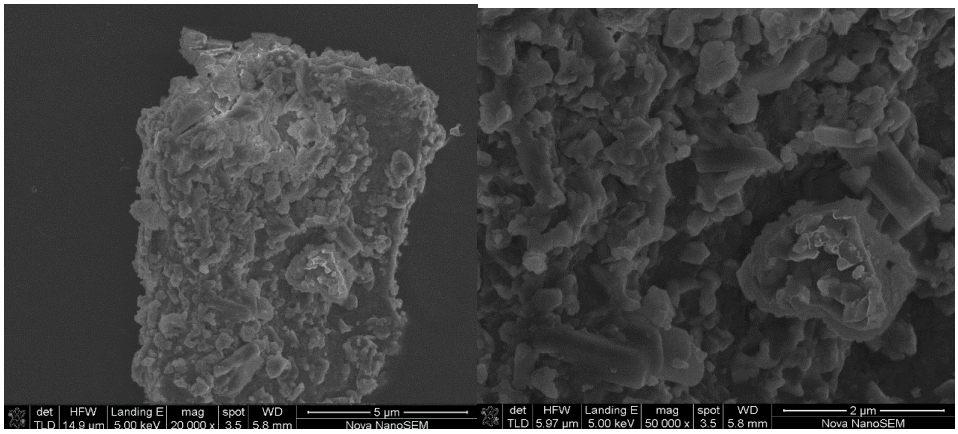

Figure 5b: SEM images of FeNP-BFR at Different Magnification

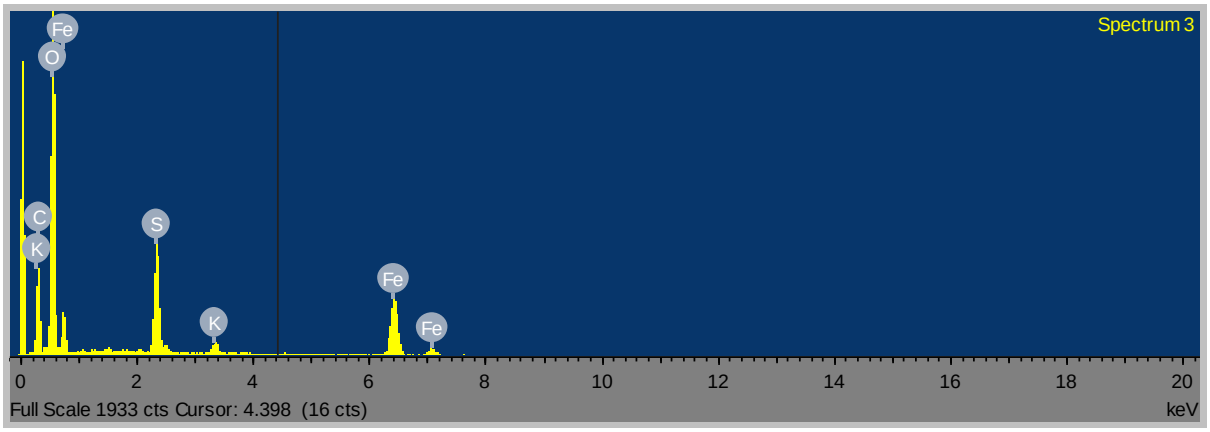

Figure 6a: EDX images of FeNP-BFL

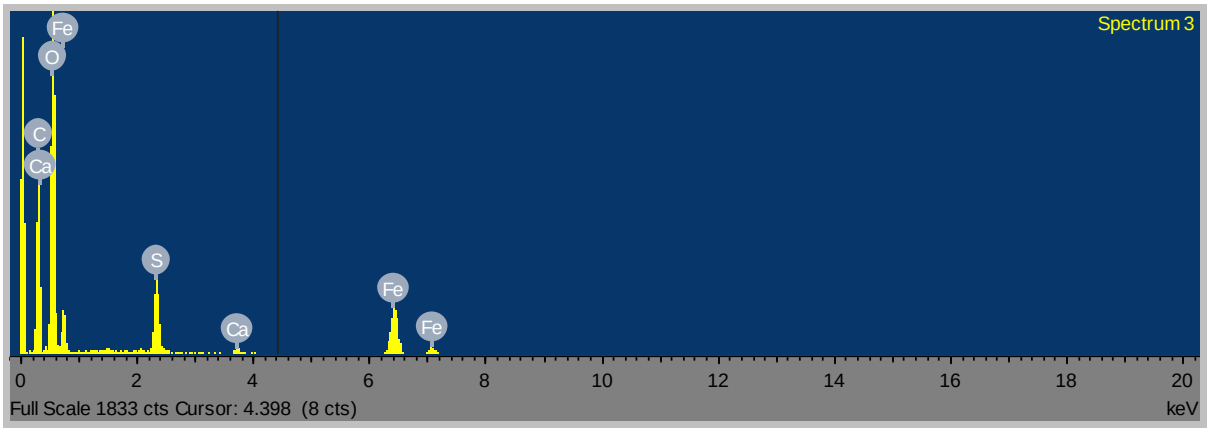

Figure 6b: EDX images of FeNP-BFR

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B) ABTS Assay

The radical scavenging activity of the prepared Iron nanoparticles from the plants extracts at different concentrations (1, 10, 15 mg/mL) were measured using the stable free radical scavenger ABTS (2,2'-azino-bis-3-ethybenzothiazoline-6-sulphonic acid) decolourization assay as shown in figure 7b with ascorbic acid used as standard. The concentration at 1 and 10 mg/mL, showed the difference in the inhibition which might be statistically significant as the FeNP-BFL gave an inhibition of 15 and 40% while that of FeNP-BFR gave an inhibition of 70 and 75%, respectively. At concentration of 15 mg/mL, the difference in the inhibition might be statistically insignificant as the FeNP-BFL gave an inhibition of 70% while that of FeNP-BFR gave an inhibition of 75%. Therefore, it can be found from the overall inhibition that *Bridelia ferruginea* Root-mediated Iron nanoparticles showed higher inhibition than Leaf-mediated one at all concentrations that were investigated.

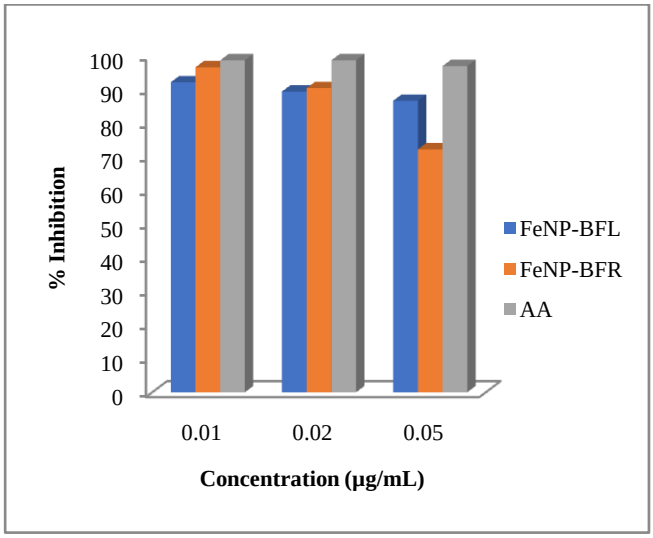

Figure 7a: Comparative DPPH Scavenging Activity of Iron Nanoparticles with Standard.
FeNP-BFL - *Brideliaferruginea*leaf-mediated Iron nanoparticles; FeNP-BFR –*Brideliaferruginea*Root-mediated Iron nanoparticles; AA-Ascorbic acid.

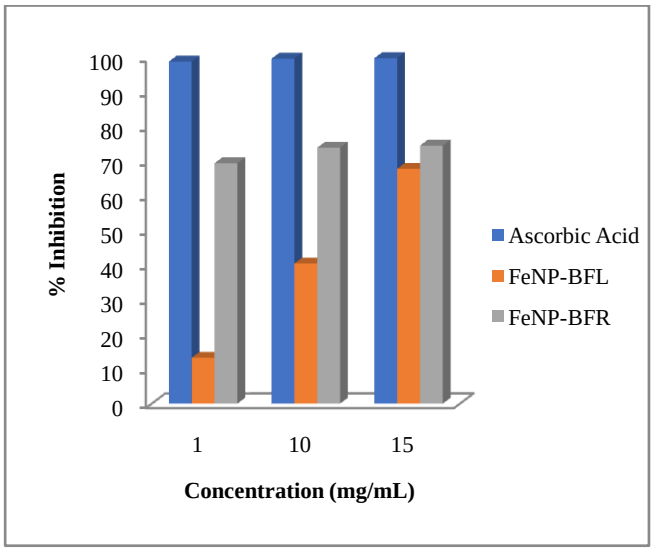

Figure 7b: Comparative ABTS Scavenging Activity of Iron Nanoparticles with Standard.
FeNP-BFL –*Brideliaferruginea*Leaf mediated Iron nanoparticles
FeNP-BFR –*Brideliaferruginea*Root mediated Iron nanoparticles

Conclusion

Nanotechnology is currently an area of intense research for scientists. There have been many reported physical and chemical synthetic methods. However, green synthesis of nanoparticles involving the use of crude extracts from *Bridelia ferruginea* as reducing as well as capping agents for the reduction of $FeSO_4.7H_2O$ to iron nanoparticles provides an environmentally friendly method for preparing nanoparticles due to less toxicity associated, short reaction time and reduced by-products. The preparation of iron nanoparticle using crude extracts from plants presents a non-toxic and environment friendly means of synthesis. This study concluded that even though iron nanoparticles is a good antioxidant, root-mediated iron nanoparticles is a better antioxidant than the leaf-mediated iron nanoparticles. Current research is on the isolation of specific compound(s) in the root extract for the preparation of Iron nanoparticles.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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