Biogenic synthesis of magnetic iron oxide nanoparticles using inedible borassus flabellifer seed coat: characterization, antimicrobial, antioxidant activity and in vitro cytotoxicity analysis

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Abstract

An environment friendly green synthesis of iron oxide nanoparticles using the seed coat extract of B. flabellifer was investigated. The nanoparticles were characterized using XRD, UV–vis spectroscopy, FTIR, TGA, SEM and EDS. The x-ray diffraction spectrum showed the formation of crystalline inverse spinel magnetite nanoparticles with crystallite size of 35 nm and the UV–vis absorption recorded characteristic peak at 352 nm for iron oxide nanoparticles. The surface functionalization of the nanoparticles was confirmed from the various functional group peaks present in the FTIR spectrum and the thermal decomposition of the synthesized nanoparticles from TGA. The morphological study using SEM showed the formation of hexagonal shaped, well dispersed nanoparticles. The cytocompatibility of the iron oxide nanoparticles was studied using MTT assay and haemolytic analysis. The antimicrobial activity of the nanoparticles against E. coli, S. aureus, B. subtilis, Shigella, A. niger and Candida albicans were measured and the nanoparticles showed significant activity against all the microorganisms which increased with increase in the nanoparticle concentration. The free radical scavenging activity of the nanoparticles against DPPH, Hydrogen peroxide and hydroxyl radical was performed which showed efficient antioxidant activity.

1. Introduction

Environmentally benign solutions for the synthesis of nanoparticles are being widely sought after and are attracting recent research. There exist numerous chemical routes to synthesize these nanoparticles [1, 2]. However these chemical methods are usually toxic, expensive and time consuming [3, 4]. Hence it emphasizes the need for an alternative eco-friendly, cost effective and rapid synthesis method. Green synthesis approach provides a non-toxic, eco-friendly route for the synthesis of multifunctional nanoparticles. Recent years have witnessed green chemistry become a major focus in the synthesis of metal nanoparticles due to their cost effectiveness, ease in nanoparticle synthesis, biocompatibility and eco-friendly process. This technique involves metal nanoparticle synthesis using various biological sources like plants [5], bacteria [6], fungi [7], yeast [8], algae and diatoms [9, 10].

However, among the various biological systems for nanoparticle synthesis, plant mediated synthesis has captured major attention due to its role in rapid synthesis of nanoparticles, low cost, easy availability, safe handling and presence of broad range of biomolecules like tannins, flavonoids, alkaloids, phenols and hence this gives it the edge as the chassis of choice for an environment friendly and sustained application [11].

Nanoparticles have been synthesized using many different plant sources, to mention a few, silver nanoparticles using Cordia dichotoma fruit [12], iron oxide nanoparticles using Cynometra ramiflora leaf [13], copper nanoparticles using Solanum lycopersicum leaf [14], zinc oxide nanoparticles using Phyllanthus emblica leaf [15], gold nanoparticles using Artocarpus Lakoocha fruit, cerium oxide nanoparticles using Sida acuta leaf [16], titanium nanoparticles using Vigna Radiata seeds [17].
Among metal nanoparticles, magnetic iron oxide nanoparticles always hold greater significance for their wide range of biomedical, bioremediation [18, 19], information storage [20], catalytic [13] and biosensor [21] applications due to their superparamagnetic property apart from other unique size and shape dependent physicochemical and biological properties [18, 19]. These magnetic nanoparticles when synthesized using plant sources become non-toxic, biocompatible and eco-friendly [22]. Plant extracts apart from acting as a reducing agent, also help in stabilizing the nanoparticles by acting as a capping agent [23]. The biomedical and pharmacological application of iron oxide nanoparticles require that they possess no toxicity and are compatible to the biological system. The therapeutically vital bioentities from the extract get coated onto the nanoparticles thereby enhancing their medicinal value and biocompatibility further and making it a more potent candidate for biomedical and bioremediation applications.

Borassus flabellifer also called Palmyra palm belongs to the family Arecaceae and is an easily cultivable tree, abundantly found in Indian subcontinent and south east asian countries. In India it is also called Indian toddy palm. The tree bears fruits with soft edible jelly seeds inside the kernels. The jelly is enclosed in a thin yellowish inedible brown coat inside the socket which occur in 2–4 inside the fruit. The male flowers possess analgesic, antipyretic and antidiabetogenic properties [24, 25]. The sap collected from the shoot called as toddy is edible and known for its cooling property and nutritive value with the presence of elements like Calcium, Iron, Zinc, Copper and vitamin A. The fermented toddy is rich in niacin, riboflavin and thiamine [26, 27].

The edible seed in the fruit is consumed whereas the seed coat is discarded and goes waste. This seed coat extract contains phytochemicals like tannins, flavanoids, saponins, glycosides and terpenoids and is proved to have good antimicrobial and free radical scavenging activity [28, 29]. The present investigation reports the utilization of this less explored inedible seed coat of Borassus flabellifer L. in the facile synthesis of magnetic iron oxide nanoparticles for the first time to the best of our knowledge and evaluating its antimicrobial activity and cytocompatibility in NIH-3T3 fibroblast cell lines. The research work finds its significance in the synthesis of biogenic magnetic iron oxide nanoparticles with antimicrobial property against both bacteria and fungi. The synthesized nanoparticles possess good antioxidant activity and are found to be highly biocompatible.

2. Materials and methods

2.1. Plant material

Borassus flabellifer tender seeds were collected from local market in the summer season near Anna University, Chennai, Tamilnadu (figure 1(a)). The seeds were peeled and the seed coat obtained were washed with double distilled deionized water and dried under shade (figure 1(b)). The dried peels as shown in figure 1(c) were stored in air tight bags at room temperature for further use.

2.2. Chemicals

Ferric chloride and Ferrous sulphate used as precursors were purchased from Sigma Aldrich. Double distilled deionised (DI) water was used as solvent and Nitric acid used as pH regulator was purchased from Alfa Aesar. All the chemicals used in this experiment were of analytical grade and used without any further purification.

2.3. Preparation of plant extract

The extraction is done using a soxhlet apparatus. The dried seed coat was crushed using mortar and pestle and 30 g of this crushed seed coat is loaded in the thimble placed inside the Soxhlet extractor. 250 ml of ethanol is used as the solvent in a round bottom flask. The extract is collected after 30 completed cycles and allowed to cool down and then refrigerated for further use.
2.4. Synthesis of iron oxide nanoparticles

Precursor solution was prepared by adding Ferric chloride (0.2 M) and Ferrous sulphate (0.1 M) in 2:1 ratio. 25 ml of this solution was added to 25 ml of the extract under constant stirring in a magnetic stirrer at room temperature. The pH of the above solution was adjusted between 10–11 using 0.1 M NaOH. An intense black coloured precipitate immediately formed. This change in the colour of precursor solution from yellow to black indicated the formation of iron oxide nanoparticles as shown in the figure 2. The black precipitate thus obtained was washed thrice with double distilled water and twice with ethanol and dried at room temperature to obtain magnetic iron oxide nanoparticles.

2.5. Analysis of iron oxide nanoparticles

The as synthesized iron oxide nanoparticles were characterized for their crystallinity and phase purity using Bruker’s D2 Phaser x-ray diffractometer (XRD) with CuKα radiation λ = 0.1540 nm, step size of 0.02° in the range of 5° to 80°. The optical property was analysed using UV–vis absorption spectrometer (UV-8000 Shimadzu). Different functional groups present in the nanoparticles were determined using Bruker ALPHA Fourier Transform Infra red spectrometer (FTIR) and the spectra of the nanoparticles was recorded in transmission mode (4000 to 500 cm⁻¹). The morphological characteristics and the particle size were analysed using ZEISS Field Emission Scanning Electron Microscope (FESEM). The presence of extract incorporated bioentities on the nanoparticles was confirmed by analyzing the percentage weight loss of Iron oxide nanoparticles with a heating rate of 10 °C min⁻¹ in the temperature range of 30 °C–800 °C using Thermo Gravimetric Analyser (TGA, Mettler Toledo, Switzerland).

2.5.1. Antimicrobial activity

Antimicrobial activity of the biogenic iron oxide nanoparticles was determined using agar well diffusion method against *Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella*, *E. coli*, *Aspergillus Niger* and *Candida albicans*. The test microbial cultures were adjusted to 0.5 McFarland turbidity standard equivalent to a microbial suspension of 1.5*10⁸ colony forming units (CFU/ml). For the antibacterial studies, Muller Hinton Agar (MHA) plates were poured into sterile petri plates and 100 μl of bacterial cultures were spread on the agar plates using sterile L-rod. The seeded agar plates were punctured into 5 mm sized well using a sterile cork borer. The biogenic iron oxide nanoparticles were loaded into the wells at three different concentrations of 50 μg ml⁻¹, 100 μg ml⁻¹ and 500 μg ml⁻¹. The plates were incubated overnight at 37 °C. Millipore water was used as the negative control. The inhibition zones against the test organisms were measured in millimetre. Similarly for the antifungal testing, Potato dextrose Agar (PDA) plates were inoculated with the cultures of *A.Niger* and *Candida Albicans* and the test samples loaded for determining the zone of inhibition. The experiments were performed in triplicate.

2.5.2. MTT assay

MTT assay was employed to determine the cell viability in NIH 3T3 cells using phytomediated iron oxide nanoparticles according to the method described earlier with slight modifications [30]. The cells were plated in a 96 well plate with 20,000 cells per well and incubated in a 5% CO₂ atmosphere. Plating was done in quadruplicates for every cell number. After 24 h, the media was removed as the cells would have attached to the bottom of the well and the nanoparticles were added to the wells (2000 cells/well) at different concentrations of 50 μg ml⁻¹, 150 μg ml⁻¹, 250 μg ml⁻¹, 350 μg ml⁻¹, 500 μg ml⁻¹ and incubated for 24 h and 48 h. After the
incubation period, 5 mg ml\(^{-1}\) (12 mM) of MTT stock solution was prepared in filtered PBS solution and 100 \(\mu l\) of this MTT solution was added to each well and further incubated for 4 h at 37 \(^\circ\)C in dark. After incubation, formazan crystals formed in viable cells were dissolved with 100 ml of 100% DMSO after removal of MTT solution carefully with micropipette. The absorbance value corresponding to the viable cells was read at 490 nm using a microplate reader.

### 2.5.3. Antioxidant analysis

#### 2.5.3.1. Free radical scavenging activity on DPPH

The antioxidant property of biogenic iron oxide nanoparticles was determined using DPPH free radical scavenging assay with slight modifications \([31]\). Briefly, a methanolic solution of 0.2 mM of DPPH was prepared. Ascorbic acid was used as reference standard. 2 ml of different concentrations (20, 40, 60, 80, 100 \(\mu g\) ml\(^{-1}\)) of aliquots of iron oxide nanoparticles in water was mixed with 2 ml of the DPPH methanolic solution. The nanoparticles were stirred vigorously and kept in dark at 35 \(^\circ\)C for 30 min. The absorbance of DPPH radicals reducing action was measured spectrophotometrically at 517 nm using UV–vis absorption spectrometer (UV-8000 Shimadzu). The free radical scavenging activity was measured using the following formula,

\[
\text{Radical scavenging effect} (\text{% inhibition}) = \left[\frac{(A_0 - A)}{A_0}\right] \times 100
\]

Where, \(A_0\) is the absorbance value of control and \(A\) is the absorbance value of the sample. The results were expressed as plot between % DPPH free radical scavenging activity and the concentration of iron oxide nanoparticles and the control (\(\mu g\)/ml).

#### 2.5.3.2. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging ability of the phyto mediated iron oxide nanoparticles was measured according to the method followed by Ruch \textit{et al} \([32]\). 40 mM of hydrogen peroxide solution was prepared in phosphate buffer solution. Ascorbic acid was used as the positive control. 100 \(\mu g\) ml\(^{-1}\) of iron oxide nanoparticles in water was dissolved in 0.6 ml of 40 mM hydrogen peroxide solution. The absorbance values were measured at 230 nm after 10 min and the percentage of inhibition activity was calculated using the following formula,

\[
\text{Percentage inhibition} = \left[\frac{(\text{control OD} - \text{sample OD})}{\text{Control OD}}\right] \times 100
\]

#### 2.5.3.3. Hydroxyl radical (OH\(^{−}\)) scavenging activity

The capacity hydroxyl radical scavenging was determined by modified method described previously by Halliwell \textit{et al} \([33]\). Following stock solutions were prepared in de-ionized water, 1 mM EDTA, 10 mM \(H_2O_2\), 10 mM \(FeCl_3\), 10 mM deoxyribose and 1 mM of ascorbic acid. A solution of 0.1 mM EDTA, 0.01 mM \(FeCl_3\), 0.1 mM \(H_2O_2\), 0.36 ml deoxyribose, 1 ml of different concentrations (10, 20, 30, 40, 50 \(\mu g\) ml\(^{-1}\)) of the nanoparticles, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid added in sequence was prepared and incubated at 37 \(^\circ\)C for an hour. 1 ml of this incubated mixture was mixed with solution containing 1 ml of 10% TCA and 1 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) and the pink chromogen thus developed was measured at 532 nm. The reaction between iron-EDTA complex and \(H_2O_2\) in the presence of ascorbic acid produces hydroxyl radicals which interacts with deoxyribose and yields a pink chromogen on heating with thiobarbituric acid at low pH. In the presence of another hydroxy radical scavenger, there is mutual competition for the hydroxyl groups produced and hence this diminishes the formation of chromogen. Hence the radical scavenging activity was measured in terms of percentage of inhibition of deoxyribose degradation and calculated using the following formula,

\[
\text{Radical scavenging activity} (\%) = (1 - A\text{ sample} / A\text{ control}) \times 100
\]

Where, A sample and A control are the absorbance values of the sample and the control respectively.

#### 2.5.4. Haemolytic activity

The biocompatibility of the synthesized iron oxide nanoparticles with the red blood cells was determined by the haemolytic activity as per the method followed by \([34]\) with few modifications. Blood from a healthy volunteer was collected in a sterile glass tube containing EDTA and centrifuged at 1500 rpm for 10 min to collect the RBCs. The supernatant containing the plasma and platelets was discarded and the pellet containing the RBCs was washed with phosphate buffer saline (PBS). Different concentrations (25, 50, 75, 100 \(\mu g\) ml\(^{-1}\)) of the nanoparticles in phosphate buffer saline was made upto 1 ml volume in PBS containing RBCs (5% v/v). The above solution was vortexed and incubated at 37 \(^\circ\)C for 4 h. The supernatant was then used for measuring the absorbance value of haemoglobin at 575 nm using UV–vis absorption spectrometer (UV-8000 Shimadzu). RBCs in phosphate buffer saline solution and in 1% triton x-100 was used as negative and positive control.
respectively. The percentage haemolytic activity was measured as the ratio of difference in absorbance values (A) of sample and negative control to the difference in absorbance values of positive and negative control as given below.

\[
\% \text{ Haemolysis} = \frac{(A \text{ sample} - A \text{ negative control})}{(A \text{ positive control} - A \text{ negative control})} \times 100
\] (4)

2.5.5. Statistical analysis

All the experiments were replicated thrice and the obtained results were expressed as the mean value differences of the three independent results ± standard deviation (SD). The significance in mean value difference of the results was processed by one way analysis of variance (ANOVA) followed by duncan’s Multiple range test at significance level of \( P < 0.05 \) using Statistical Analysis Solution (SAS) (Version: SAS 9.4, SAS Institute Inc., Cary, NC).

3. Results and discussion

3.1. Synthesis and characterization of magnetic iron oxide nanoparticles

The presence of phytochemicals like flavanoids, tannins, saponins in the B. flabelifer seed coat extract act as a potential metal reducing agent in the synthesis of magnetic iron oxide nanoparticles. Similar results of phytoconstituents as a reducing agent in nanoparticle synthesis have been reported [35, 36]. Hence the addition of B. flabelifer seed coat extract to the precursor solution immediately turns the solution colour from yellow to black, leading to the formation of precipitate. This substantiates the synthesis of iron oxide nanoparticles [37, 38]. The synthesized nanoparticles were strongly attracted by an external magnet as shown in the figure 3.

The formation of magnetic iron oxide nanoparticles were confirmed using XRD analysis. The powder x-ray diffraction pattern of the iron oxide nanoparticles shows majorly the presence of magnetite phase and traces of hematite phase as shown in the figure 4. The intense diffraction peaks of synthesized magnetic iron oxide nanoparticles appear at 29°, 35.5°, 36.8°, 43°, 55°, 57°, 62.8°, 73° correspond to (220), (311), (222), (400), (422), (511), (440), (622) crystallographic planes of inverse spinel magnetite phase iron oxide nanoparticles. The diffraction at \( 2\theta = 35.5° \) can be accounted to Fe₂O₃ which is in accordance with the JCPDS card No.-19-0629 [39]. Traces of hematite phase at diffraction angles at 28°, 33°, 40° corresponding with planes (012), (104), (113) were also found which match with JCPDS card number 33-0664. Similar results for magnetic nanoparticles have been obtained [40, 41]. The high crystallinity of the synthesized nanoparticles is indicated by the narrow and sharp diffraction peaks obtained. The average crystalline size was found to be 35 nm using the Scherrer equation,
\[ d = \frac{k \lambda}{\beta \cos \theta} \]

where \( k \) is the scherrer constant (0.54), \( \lambda \) is the x-ray wavelength (1.54 Å), \( \beta \) is the half width of the peak and \( \theta \) is the Bragg’s angle.

The UV–visible spectra of the synthesized nanoparticles showed broad absorption spectrum at 352 nm as shown in figure 5. Magnetic nanoparticles show UV absorption band in the range 330 nm–450 nm due to the absorption and scattering of light by the nanoparticles. Similar results were obtained in previous literatures [42, 43]. Furthermore, the change in colour of the precursor solution from yellow to black colour during the synthesis process can be attributed to the phenomenon of excitation of Surface Plasmon Resonance [44]. The band gap energy was calculated using \( E = \frac{h^* c}{\lambda} \) (h-Plank’s constant c-velocity of light \( \lambda \) is the wavelength) and was found to be 3.52 ev.

The extract reduced iron oxide nanoparticles are surface functionalized by the functional moieties present in the extract. The FTIR spectra of the extract and the synthesized nanoparticles are shown in figure 6. The spectrum of the extract shows peaks at 3267 cm\(^{-1}\) attributed to OH stretching vibration of the phenolic compounds present in it and peaks at 2923 cm\(^{-1}\), 2850 cm\(^{-1}\) arises due to C–H stretching of alkanes. The band at 1744 cm\(^{-1}\) corresponds to C=O stretching vibration. Peaks at 1635 cm\(^{-1}\) and 1545 cm\(^{-1}\) occur due to N–H bend of primary amines and N–O asymmetric stretching vibration in nitro compounds respectively. Bands at \(~1391\) cm\(^{-1}\), \(1237\) cm\(^{-1}\) are contributed by C–C stretch of aromatic constituents and C–N stretch of amines respectively. The peak at \(1019\) cm\(^{-1}\) appears due to the C–O stretch vibration of alcohol, carboxylic functional groups. The synthesized iron oxide nanoparticles showed peaks at \(3358\) cm\(^{-1}\), \(1617\) cm\(^{-1}\), \(1454\) cm\(^{-1}\), \(1246\) cm\(^{-1}\), \(1102\) cm\(^{-1}\), \(1069\) cm\(^{-1}\), \(835\) cm\(^{-1}\) and \(796\) cm\(^{-1}\). On comparing both the IR spectra, a relative shift in the peaks of extract could be seen at \(3267\) cm\(^{-1}\), \(1635\) cm\(^{-1}\), \(1545\) cm\(^{-1}\), \(1237\) cm\(^{-1}\) and \(1019\) cm\(^{-1}\).
corresponding to polyphenols (O–H, 3358 cm$^{-1}$), amines (N–H bend, 1617 cm$^{-1}$), nitro group (N–O, 1454 cm$^{-1}$), aliphatic amines (C–N, 1246 cm$^{-1}$) functional groups. The bands between 650 cm$^{-1}$–1000 cm$^{-1}$ in the spectrum of nanoparticles are attributed to C–H bend of alkenes. The shift in the bands can be attributed to the involvement of the functional groups of the extract in the synthesis of nanoparticles [12, 15].

The TGA curve shows continuous weight loss between 0 °C–700 °C as shown in figure 7. The initial weight loss (15%) observed up to 100 °C in the TGA curve is attributed to the evaporation of trapped or adsorbed moisture in the synthesized nanoparticles. Similar results for weight loss due to trapped moisture on coated Fe$_2$O$_3$ nanocomplex were reported by Sangeetha et al [45]. A major weight loss of 52.32% is seen between 100 °C–500 °C. This could be attributed to the decomposition of the bioentities from the extract capped on the synthesized iron oxide nanoparticles. This thermal decomposition can be yet another confirmation for the presence of therapeutically important phytoconstituents that are functionalized on the nanoparticles. Similar results of thermal decomposition of phytochemicals coated on the nanoparticles have been reported [46].

3.1.1. Morphological and elemental analysis

The morphology of the synthesized nanoparticles is analysed using scanning electron microscopy (SEM). The SEM images show the formation of iron oxide nanoparticles at different magnification levels in figures 8, (a)–(c). The images indicate the formation of hexagonal shaped iron oxide nanoparticles. The nanoparticles are well dispersed with slight aggregation. Higher magnifications show the average size of nanoparticles ranging between 30–200 nm. The morphology reveals the presence of majority of individual nanocrystals with a smooth surface and well defined hexagonal facets. The images also show the formation of minor irregular shaped

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**Figure 6.** FTIR spectra of (A) B. flabellifer mediated iron oxide nanoparticles, (B) Extract.

**Figure 7.** TGA graph of synthesized iron oxide nanoparticles.
nanostructures. The edax result in the figure 9 indicates the composition of the synthesized iron oxide nanoparticles. The B. flabellifer mediated iron oxide nanoparticles are composed of 52.81% of Iron and 29.7% of Oxygen and 17.5% of other elements like Sodium, Potassium and Sulphur. The presence of sulphur can be attributed to the organosulphur compounds and potassium and sodium elements that might be present in the extract acting as capping agents during the synthesis of iron oxide nanoparticles. Similar results for the presence of elemental composition from the extract have already been reported [47]. The phytochemical constituents in the seed coat extract have been screened for the presence of tannins, flavanoids, saponins, terpenoids, glycosides [28], whereas further investigation needs to be done in order to determine the elemental composition of the seed coat extract.

Figure 8. (a)–(c). SEM images of the synthesized iron oxide nanoparticles at different magnifications.

Figure 9. EDAX spectra of iron oxide nanoparticles.
3.1.2. Antimicrobial activity using Agar Well diffusion

Antimicrobial activity of iron oxide nanoparticles against *E. coli*, *S. aureus*, *Shigella*, *B. subtilis*, *A. niger* and *C. albicans* was investigated using Agar well diffusion method at three different concentrations of $50 \mu g/ml$, $100 \mu g/ml$ and $500 \mu g/ml$ shown in figure 10. The seed coat extract mediated iron oxide nanoparticles showed significant activity against the microorganisms with an increase in the activity as the concentration of the nanoparticles increased. Table 1 represents the zone of inhibition values (in mm) for all the microorganisms at three different concentrations. The zone of inhibition was the highest at a concentration of $500 \mu g/ml$ measured to be 23 mm for *E. coli*, 20 mm for *S. aureus*, 22 mm for *Shigella*, 26 mm for *B. subtilis*, 15 mm for *A. niger* and 13 mm for *C. albicans*. *B. subtilis* showed the maximum zone of inhibition of 18 mm, 24 mm and 26 mm at all the concentrations of $50 \mu g/ml$, $100 \mu g/ml$ and $500 \mu g/ml$ respectively. This is in agreement to the previous literature where the extract alone showed highest activity against *B. subtilis* [28]. The larger zone of inhibition can be accredited to the synergistic activity of the iron oxide nanoparticles and the phytocomponents.

![Figure 10. Antimicrobial activity of iron oxide nanoparticles against (a) E. coli, (b) S. aureus, (c) Shigella, (d) B. subtilis, (e) A. niger, (f) C. albicans.](image)

| Concentration ($\mu g/ml$) | E. coli | S. aureus | Shigella | B. subtilis | A. niger | C. albicans |
|---------------------------|---------|-----------|----------|-------------|----------|-------------|
| 50                        | 14      | 11        | 17       | 18          | 9        | 9           |
| 100                       | 20      | 18        | 19       | 24          | 11       | 10          |
| 500                       | 23      | 20        | 22       | 26          | 15       | 13          |

3.1.2. Antimicrobial activity using Agar Well diffusion

Table 1. Zone of inhibition of the iron oxide nanoparticles.

The larger zone of inhibition can be accredited to the synergistic activity of the iron oxide nanoparticles and the phytocomponents.
coated on the nanoparticles [48]. The seed coat extract has already been proven to exhibit good antimicrobial activity and free radical scavenging activity and the presence of phytochemical increases the antimicrobial activity significantly [29].

3.1.3. Cytocompatibility using MTT analysis and Haemolytic activity

The cytocompatibility of the phytosynthesized iron oxide nanoparticles at concentrations of 50 μg ml⁻¹, 150 μg ml⁻¹, 250 μg ml⁻¹, 350 μg ml⁻¹, 500 μg ml⁻¹ on the viability of NIH 3T3 cells at incubation periods of 24 h and 48 h were recorded. The optical density of the cells on treatment with the nanoparticle were measured at different concentrations at 24 h and 48 h incubation time as shown in table 2 and figure 11 represents their relative cell viability percentage that is graphically plotted to assess the cell proliferation efficiency. 10% DMEM is used as positive control (control for maximum cell viability) and 40% DMSO is used as the negative control (control for minimum cell viability). The optical density values of the treated cells decreased from 0.786 to 0.689 as the concentration increases from 50 μg ml⁻¹ to 500 μg ml⁻¹. The cells displayed percentage viability between 94%–82% until the maximum concentration. At 48 h incubation, the optical density value decreased from 0.754 to 0.689 and the percentage viability measured ranged between 95%–86%. The viability percentage recorded was above 80% in all cases which shows the high cytocompatibility of the nanoparticles towards the fibroblast cells. Similar results for non toxicity of iron oxide nanoparticles synthesized against NIH 3T3 fibroblast cell line were reported [49]. The biocompatibility of the synthesized iron oxide nanoparticles on erythrocyte cells was investigated. The assay involved different concentrations of the nanoparticles (25, 50, 75 and 100 μg ml⁻¹) on the erythrocytes. Erythrocytes in PBS and in 1% Triton x-100 were used as the negative and positive control.

| Concentration (μg/ml) | 24 h | 48 h |
|-----------------------|------|------|
| PC                    | 0.832| 0.792|
| 50                    | 0.786| 0.754|
| 150                   | 0.776| 0.738|
| 250                   | 0.752| 0.722|
| 350                   | 0.732| 0.701|
| 500                   | 0.689| 0.689|
| NC                    | 0.04 | 0.02 |

Figure 11. Percentage cell viability after NIH 3T3 fibroblast cells treatment with the nanoparticles.

Table 2. Cell viability absorbance values after treating NIH 3T3 fibroblast cells with the iron oxide nanoparticles.
respectively. The nanoparticles were found to exhibit no haemolytic activity at the different concentrations. The absorbance values of the erythrocytes is mentioned in table 3. Any biological material should possess haemolysis percentage below 5\% [34]. The measured haemolysis percentage was well within the admissible level. Hence the biogenic iron oxide nanoparticle prove to be a potential cytocompatible material for biological applications.

### 3.1.4. Antioxidant activity

The antioxidant activities of the iron oxide nanoparticles are depicted in figures 12(a)–(c). The nanoparticles show potential free radical scavenging activity against all the three, DPPH, Hydrogen peroxide and hydroxyl radicals. Ascorbic acid was used as a positive control in the same concentration range. The DPPH scavenging activity at five different concentrations of the nanoparticles, 20 \( \mu \text{g ml}^{-1} \), 40 \( \mu \text{g ml}^{-1} \), 60 \( \mu \text{g ml}^{-1} \), 80 \( \mu \text{g ml}^{-1} \), 100 \( \mu \text{g ml}^{-1} \) was in the range from 43.04\%–85.53\% respectively with a minimum inhibitory concentration value, IC\textsubscript{50} of 61.06 \( \mu \text{g ml}^{-1} \) and that of Hydrogen peroxide varied from 44.12\%–91.79 with IC\textsubscript{50} value of

![Figure 12. Antioxidant activity of the synthesized nanoparticles, (a) DPPH scavenging activity, (b) Hydrogen peroxide scavenging activity, (c) Hydroxyl radical scavenging activity.](image)

| Sample (n = 3) | Haemolysis % |
|----------------|--------------|
| PBS (control)  | 1.20 ± 0.11  |
| 1% triton X-100| 1.20 ± 0.18  |
| Iron oxide nanoparticle (25 \( \mu \text{g ml}^{-1} \)) | 1.23 ± 0.12  |
| Iron oxide nanoparticle (50 \( \mu \text{g ml}^{-1} \)) | 1.34 ± 0.17  |
| Iron oxide nanoparticle (75 \( \mu \text{g ml}^{-1} \)) | 1.39 ± 0.18  |
| Iron oxide nanoparticle (100 \( \mu \text{g ml}^{-1} \)) | 1.39 ± 0.18  |

Data represented as mean ± SD (n = 3).
62.95 μg ml$^{-1}$ and hydroxyl radical scavenging activity ranged from 54.35%–76%, with IC$_{50}$ value of 65.81 μg ml$^{-1}$.

The radical scavenging activities of the nanoparticles can be imputed to the presence of bioactive components in the extract that possess good antioxidant activity and hence the surface functionalized iron oxide nanoparticles exhibit appreciable scavenging activity against free radicals [28]. The phytochemicals like flavonoid, tannins, saponins, glycosides are known to be present in the extract and the free radical scavenging activity thus can be ascribed to the polyphenolic content present in the extract [28]. The green synthesized iron oxide nanoparticles hence can be a potential candidate in various biomedical applications due to their high cytocompatibility and antioxidant activity. The bioentity surface functionalization can be used to tailor the properties of nanoparticles in terms of their chemical functionality and applications [50]. The pharmacognostic properties of the phytochemicals enhance the therapeutic properties of the nanoparticles with improved biological activity [11]. The synthesized procedure being environment friendly, simple and rapid is an advantage over other conventional chemical routes of nanoparticle synthesis. Moreover the presented method is a cost effective route, wherein the left over waste of the seed is utilized in the synthesis.

### 4. Conclusion

In the present work, highly biocompatible magnetic iron oxide nanoparticles with antimicrobial activity against both bacteria and fungi have been green synthesized using the seed coat extract of B. flabellifer. The x-ray diffraction results confirm the formation of highly crystalline inverse spinel magnetite iron oxide nanoparticles with an average crystallite size of 35 nm. The UV Visible absorption spectrum exhibited characteristic iron oxide nanoparticle peak at 352 nm. Scanning electron microscopy images show well dispersed, hexagonal shaped iron oxide nanoparticles. The EDAX results indicate major composition consisting of iron and oxygen with trace amounts of other elemental composition like sulphur, sodium, potassium obtained from the extract during the reduction process. FTIR and TGA analysis confirm the surface functionalization of the synthesized nanoparticles with the biological molecules present in the extract. The nanoparticles exhibited efficient antimicrobial activity against E. coli, S. aureus, Shigella, B. subtilis, A. niger and C. albicans with the highest zone of inhibition of 26 mm at 500 μg ml$^{-1}$ for B. subtilis. Significant radical scavenging activity of the nanoparticles against DPPH, Hydrogen peroxide and hydroxyl radical was measured. This is attributed to the presence of polyphenols in the extract that enhance the antioxidant property of the nanoparticles. Haemolytic activity showed no cytotoxicity by the nanoparticles in the erythrocytes at different concentration ranging from 25 μg ml$^{-1}$–100 μg ml$^{-1}$, the maximum haemolytic percentage being less than 2%. MTT assay showed the viability percentage of NIH 3T3 cell lines at all the concentration from 50–500 μg ml$^{-1}$ being above 80%, thereby proving their cytocompatibility. Hence it could be concluded that this green synthesis route for iron oxide nanoparticle is an efficient method for the synthesis of highly biocompatible nanoparticle with enhanced therapeutic properties.

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