

Original Research Article

Facile Green Synthesis of Iron Oxide Nanoparticles
Using *Phoenix Dactylifera L.* Seed Extract and Their
Antibacterial Applications

UNDER PEER REVIEW

ABSTRACT

Aim: The synthesis methods of iron oxide nanoparticles (IONPs) have got great attention in recent years, due to their variety in physicochemical properties and applications. This study aimed for the green synthesis of the IONPs using an aqueous extract of *P. Dactylifera L.* seeds for its antibacterial applications.

Methodology: IONPs were prepared in an aqueous seed extract of *P. Dactylifera L.* The physicochemical characterisations were performed with transmission electron microscopy (TEM) to study the shape of obtained nanoparticle, energy-dispersive spectroscopy (EDS) for elemental confirmation of iron and oxygen, dynamic light scattering (DLS) for particles size measurement, vibrating sample magnetometer (VSM) for saturation magnetisation, Infrared spectroscopy (FTIR) for chemical confirmation of function groups and X-ray diffraction (XRD) for crystalline nature. The polyphenols contents were also determined; we suggest that the presence of phenolic compounds is the key element involved in the formation and the stabilisation of IONPs. Antibacterial activity was determined using disc diffusion assay and minimum inhibitory concentration (MIC) of the IONPs were determined by broth dilution assay.

Results: The biosynthesised IONPs showed crystalline magnetite spherical morphologies with average diameter 30 nm. Discs with all the concentrations of IONPs tested in this study (10 - 100 $\mu\text{g.mL}^{-1}$) showed antibacterial activity against bacterial strains tested (*Staphylococcus epidermidis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). MIC values were found to be 30 $\mu\text{g.mL}^{-1}$, 50 $\mu\text{g.mL}^{-1}$ and 60 $\mu\text{g.mL}^{-1}$ for *S. epidermidis*, *K. pneumoniae* and *P. aeruginosa* respectively.

Conclusion: The biosynthesis of IONPs has provided, reliable, safe, simple and eco-friendly method. Hence, this study has focused on biological method of synthesis of IONPs. The IONPs synthesized in this study can be used as the therapeutic agents against bacteria.

Keywords: Green nanoparticles, superparamagnetic iron oxide nanoparticles, antimicrobial efficacy, antibacterial activity,

1. INTRODUCTION

Multifunctional metal oxides have emerged as promising materials in wide range of applications, such as biomedicines, contrast imaging, heavy metal removal, separation, cosmetics, diagnosis, catalysis and bioremediation [1]. In recent years, the interface of green chemistry and nanotechnology has provided an alternate approach to physicochemical methods of synthesis of the iron oxide nanoparticles (IONPs) [2]. The biogenic methods are more eco-friendly, safer and more cost-effective for metal oxide nanoparticles productions. Plant extracts and microorganism are amongst the best promising approach for “green” synthesis. However, aqueous plant extracts are preferred since their ease of availability and simplicity to use and the scalability, compared to biohazards and complex step of cell culture maintenances [3]. Aqueous plant extracts comprise a compelling array of antioxidants, such as polyphenols, tannins, alkaloids and flavonoids, amino acids, glycosides and nitrogenous bases that can reduce the metal ions in metal salt environment [4]. The reduction of metal ions leads in creation of the nucleation point, avoiding the further deposition. The incorporation of adjoining nucleation points subsequently, leads to the formation of nanoscale materials. Phytochemicals of the plant extract associate with such nanoscale materials and as a result provide a better stability. Such phytochemicals are hydrophilic, non-toxic and biodegradable. In addition, these are the key elements for reduction and capping of the resulting nanoscale materials. Moreover, such nanoparticles have demonstrated lesser toxicity compared to nanoparticles synthesised using traditional physicochemical approaches [4].

Beside the numerous studies on the synthesis of silver, zinc and gold nanoparticles using various plant extracts such as sorghum [5], Aloe vera [6], black tea [7], coffee [8] and fruit extracts [9], however efficient synthesis of the IONPs using the plant extracts is much difficult. Moreover, IONPs have higher susceptibility to form agglomerates. This is because the large surface area to volume ratio of the IONPs that increases the energy associated to surface area (a characteristic phenomenon may be aggravated with low surface charge). As a result, chemical and magnetic activities of the IONPs cause the rapid clearance in biological system [10]. IONPs stability can be improved with electrostatic repulsion through the surface coating or surface augmented ions [10]. IONPs have been preferred over other nanoparticles due to their ability to absorb light [11]. The heat or “hyperthermia” induces the cell apoptosis or sometime kills the cells. This promising feature of IONPs can be exploited in tumour therapy. Temperature 38 °C to 45 °C has demonstrated the synergic efficacy of chemotherapy and radiotherapy [12]. IONPs produces heat over the exposure of magnetic field from the associated energy loss with Néel rotation and Brownian relaxation of magnetic moment. Magnetic field induced heat generation can control the release of payloads (therapeutics) at desired temperature range 38 °C – 45 °C.

Previous studies demonstrated that IONPs synthesised with improved stability using tea, coffee and sorghum extracts [5]. IONPs of different shapes and sizes were formed instantaneously in aqueous extract of tea. The resulting size and shapes of IONPs were dependent on the concentration of tea extracts, particularly these were hexagonal metallic iron, magnetite, maghemite and amorphous iron [13]. Furthermore, 40 – 50 nm IONPs were produced in sorghum bran extract. Bran extracts are well-reported to contain higher amount of freely available polyphenols [5]. The biogenic IONPs were evaluated to be non-toxic in comparison with IONPs produced using traditional NaBH_4 and NH_4OH reduction methods [14]. Moreover, such biogenic IONPs were reported for their model applications such as drug delivery and organic pollutant degradation [15]. Despite the synthesis of IONPs in numerous plant extracts have been studied, this green synthesis approach needs an immediate improvement for optimisation of novel cheaper extracts to achieve stable, uniform size and shape. This would be conducive to obtain large scale synthesis of IONPs for biomedical, environmental remediation, hazardous waste treatment and hyperthermia applications [15].

In this study, we characterised the synthesised IONPs using iron chloride salt as sources in *P. dactylifera L.* seed extract. Furthermore, we evaluated the antimicrobial efficacy of biosynthesised IONPs.

2. MATERIALS AND METHODS

2.1 Plant extracts preparation and characterisation

P. Dactylifera L. (member of palm family *Areaceae* plant) fruit seeds were used. Seed were ground into fine powder then water was added to 100 g.L^{-1} , which was mixed and heated for 30 min. The extract was filtered through muslin, the filtrate was centrifuged for 10 min at 1000 G. The supernatant was further filtered through $0.45 \mu\text{m}$ filters (Millipore) [16].

2.2 IONPs synthesis using date dectlefera seed extracts

IONPs were synthesised according to method [17] with small modifications. Iron source (mixture of FeCl_2 and FeCl_3 molar ratio 1:2) was used for this study. 0.1 M solution of mixture of FeCl_2 and FeCl_3 were also prepared with seed extracts. The solutions were mixed continuously at $80 \text{ }^\circ\text{C}$ for 2 h. The resulting suspension was vacuum filtered and black precipitates were collected. Afterwards, black precipitates were washed three times with distilled water and dried under vacuum. The synthesised IONPs were characterised using transmission electron microscope (TEM), energy dispersive spectroscopy (EDS), X-ray diffraction (XRD) spectroscopy, vibrating sample magnetometer (VSM), Fourier transform infrared (FTIR) spectroscopy and dynamic light scattering (DLS) techniques.

TA LEO912 AB OMEGA TEM operating at 100 kV was used for image captur and EDS spectra data acquisition. The diluted (1:100) nanoparticles were dropped to

Formvar coated copper grids and left to dry at room temperature. EDS spectra were analysed simultaneously in TEM to achieve elemental composition of synthesised IONPs.

A Philips X'Pert Pro instrument was used for the X-ray diffraction analysis. The x-ray source consisted of Cu K α radiation ($\lambda = 1.54 \text{ \AA}$). Samples were scanned was performed at range of $20 - 70^\circ$ within 2Θ . The functional groups of IONPs were analyzed using FTIR (JASCO FTIR 4700). Zetasizer Nano ZS (Malvern Instruments, UK) was used for the determination of size and surface charge of synthesised IONPs using seed extract into 1 cm cell. The measurements were achieved using H-Ne laser (633 nm). Data were analysed using Dispersion Technology Software (DTS) version 5.10.

2.3 Antibacterial Activities

Antibacterial activity was determined using disc diffusion assay [18] against three bacterial strains (*Staphylococcus epidermidis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Optical density of fresh cultures was standardised to $1 \times 10^8 \text{ CFU.mL}^{-1}$. 100 μL of standardised culture was transferred on Mueller Hinton Agar (Oxoid) and uniform microbial lawn were prepared using sterilised glass spreader. Discs with 10 μL of IONPs (impregnated with 10 $\mu\text{g.mL}^{-1}$ to 100 $\mu\text{g.mL}^{-1}$) were placed on each bacterial lawn. A positive control was prepared using gentamycin (10 $\mu\text{g.mL}^{-1}$). Plates were incubated at 37°C for 24h. After incubation, zones of the inhibitions were measured in mm. In addition, the minimum inhibitory concentration (MIC) of IONPs was also determined by broth dilution assay [19].

2.4 Total Phenolic Content

Total phenolic content of the seed extract was investigated using Folin-Ciocalteu's phenol reagent [18]. The absorbance was monitored at 725 nm (Jenway 6715, Spectrophotometer, UK). The total phenolic content of the seeds extract was depicted in gallic acid equivalents (GAE) per gram.

3. RESULTS AND DISCUSSION

A rapid, simple, cost-effective, and green method for the biosynthesis of IONPs has been established successfully, using *P. dactylifera* L seed extract for the first time. Bioinspired synthesis methods are attractive strategies in health science applications compared to other physical and chemical methods. *P. dactylifera* L seeds are already in use for folk nutrition and medicines. Phenolics, such as gallic acid, caffeic acid, sinapic acid, synergic acid and tocopherols have been studied from *P. dactylifera* L seeds [20].

3.1 Physicochemical Characterisation

TEM images were recorded to obtain the morphology of bioinspired IONPs. Recorded TEM images of IONPs depicted the spherical shape (Fig. 1 a). However, images also revealed that the most of nanoparticles were aggregated, this might be due to presence of hydroxyl groups from extract or thickening of extract. In addition, aggregation on IONPs (as Fe_3O_4) is not surprising due to their ultrasmall size and magnetic properties [21]. DLS of synthesised IONPs showed average size of nanoparticles was 20 nm (± 2.5 nm). The crystallite size from XRD pattern was 16.5 nm, which is in good agreement with TEM results.

FTIR spectroscopy revealed the absorption bands at 3445, 2925, 1675, 1235, 1065, 930, 845, and 705 cm^{-1} (Fig. 1 b), comparably, absorption bands of biosynthesized IONPs were observed at 3441, 2920, 1634, 1233, 1075, 958, 842, and 558 cm^{-1} . The peak at 3445 cm^{-1} in seed extract depicted vibration of O-H stretching. The band at 2925 cm^{-1} revealed the stretching vibration of CH_2 (C-H). In IONPs, a new peak appeared that contributed to Fe-O. This metal oxide band corresponded to octahedral-metal stretching of Fe-O. The IONPs (Fe_3O_4) are confirmed with peak at region between 400 to 600 cm^{-1} for metal oxide bonds [22].

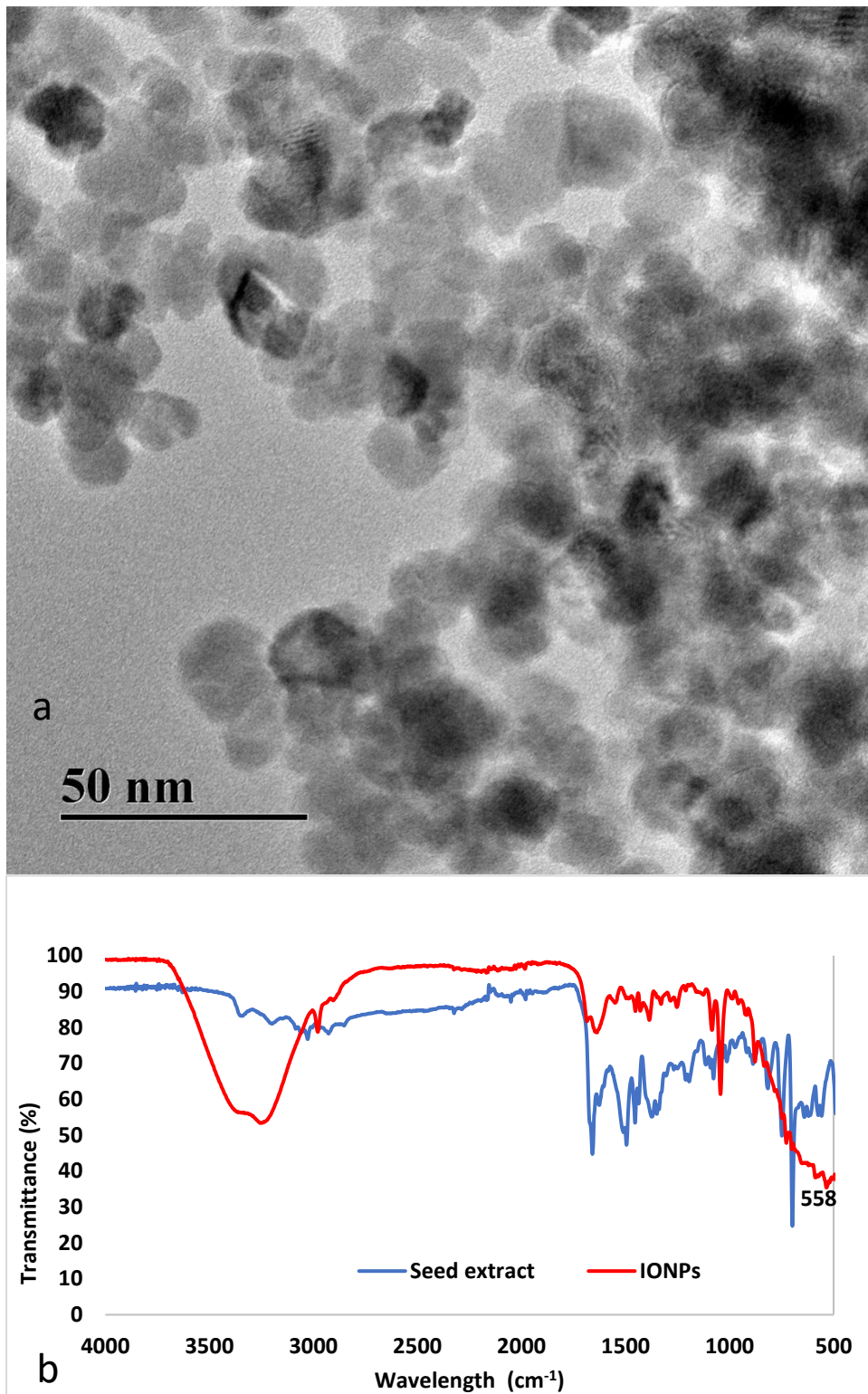


Fig. 1 (a)TEM image of biosynthesised IONPs (b) FTIR spectra of seed extract and bioinspired IONPs

XRD pattern of biosynthesised IONPs using seed extract is shown in Fig. 2 a. Pattern depicted diffraction peaks at 0.4° , 35.8° , 43.5° , 54.1° and 57.4° 2θ , that contribute to crystalline plane of (220), (311), (400), (511) and (440) for Fe_3O_4 . The

diffraction pattern is in good agreement with XRD standard of Fe_3O_4 in spinal phase structure [23].

Fig. 3b shows the saturation magnetisation measurements. The saturation magnetisation was 66.1 emu.g^{-1} . Coercivity (H_c) was negligible of hysteresis loop (5.6 kOe) and no remanence (1.3 emu.g^{-1}), which revealed the superparamagnetic behaviour of biosynthesised IONPs. Superparamagnetic property could be due to ultrasmall size on nanoparticle (20 nm) and could have only single domain.

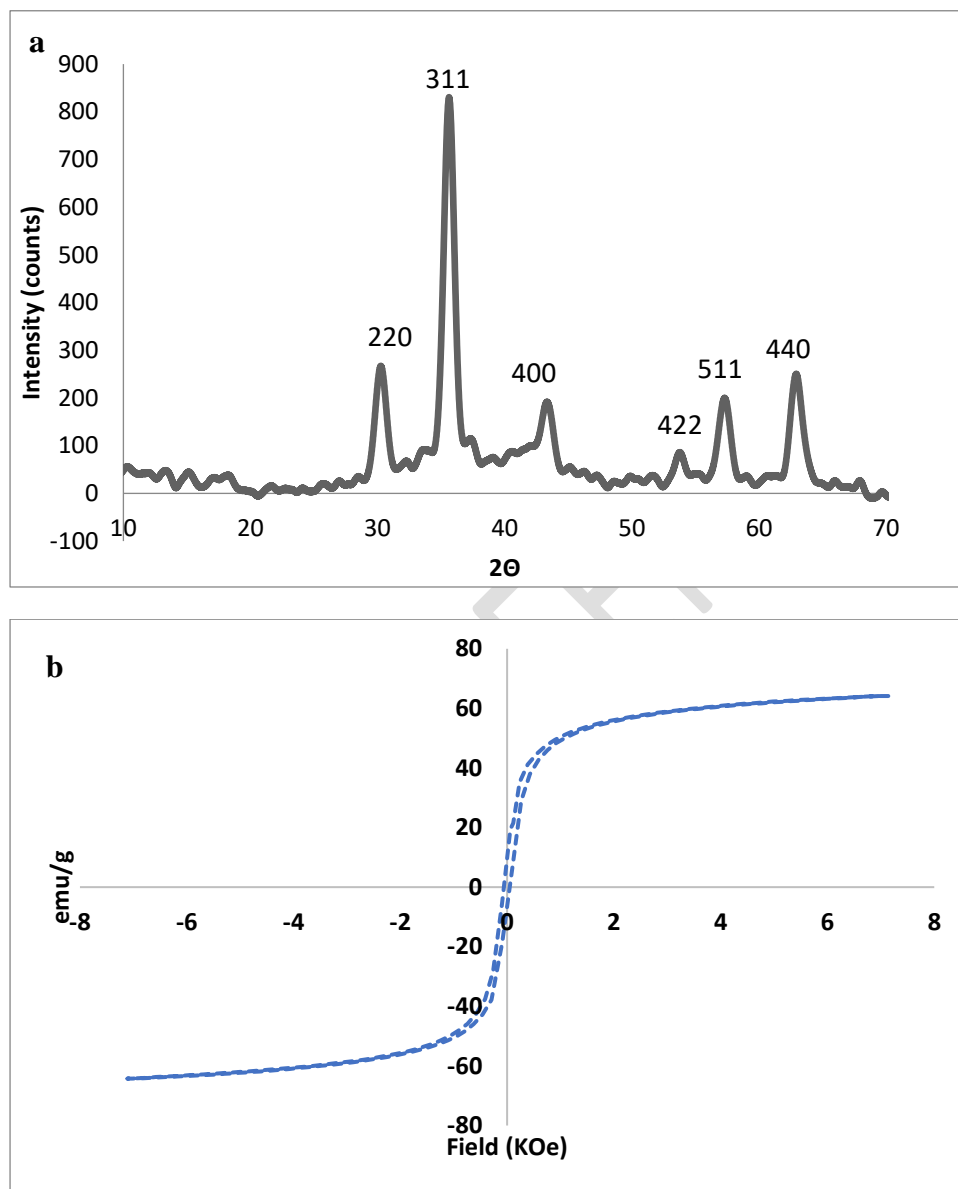


Fig.2. (a) X-ray diffraction pattern of bioinspired IONPs (b) Magnetic hysteresis curves of IONPs

3.2 Antibacterial Activities

Antibacterial activities were determined for green IONPs against three strains (*S. epidermidis*, *K. pneumoniae* and *P. aeruginosa*) using different concentration (10 - 100 $\mu\text{g.mL}^{-1}$) of discs and results are shown in Fig. 3. All strains were susceptible to bioinspired IONPs, where *S. epidermidis* was found least susceptible with MICs (30 $\mu\text{g.mL}^{-1}$), whereas for *K. pneumoniae* and *P. aeruginosa* MICs were 50 and 60 $\mu\text{g.mL}^{-1}$, respectively. It is pertinent to note that according to previous studies IONPs synthesised using chemical method showed higher MICs (50 mg). Similarly, IONPs synthesised with *Balanitesaegyptiaca* oil showed moderate antibacterial activity [24]. *P. dactylifera* L seed have already been reported for antibacterial potential, now used for IONPs biosynthesis. This study revealed significant antibacterial activities against such pathogenic strains.

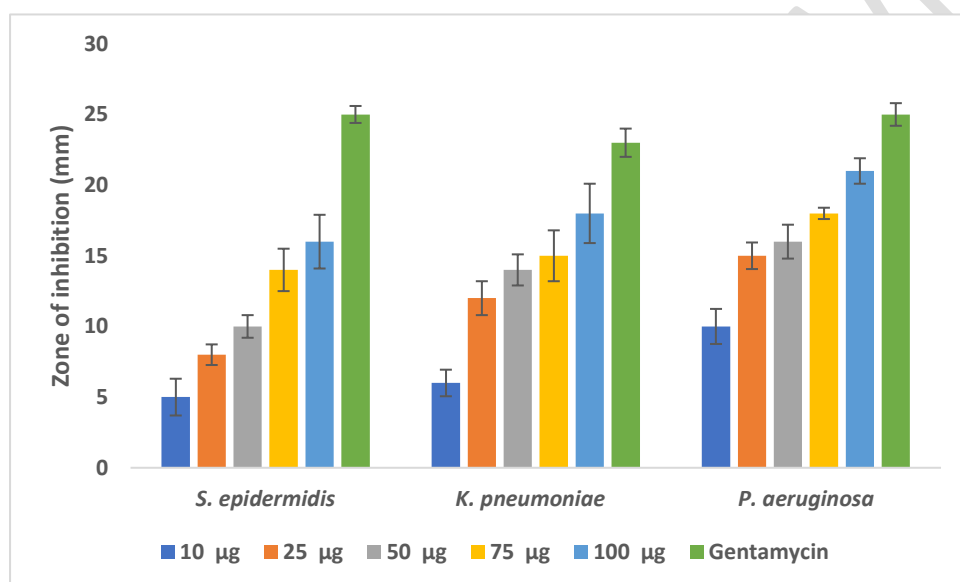


Fig. 3. Antibacterial activities of bioinspired IONPs

Numerous studies have explained the antimicrobial effect of IONPs synthesised using different approaches [25]. Most studies are focused on considering IONPs as generation of reactive oxygen species that lead to oxidative damage to cells as mode of action for their antimicrobial activity. Other researchers also considered non-oxidant factor *via* sorption of nanoparticles at membrane interface leading to perturbation of membrane bilayers [26]. Moreover, defects in surface of nanoparticles morphology could cause the membrane disruption or disorientation. This could increase the antimicrobial efficacy of nanoparticle up to ten-folds higher. We also explore the role of phenolics adhered from the **aqueous *P. Dactylifera* L. seed extract have significant contribution to antibacterial potential.**

4. CONCLUSION

Increasing attention for green chemistry and biological approaches has led to develop eco-friendly methods of synthesis of metal oxide nanoparticles. In contrast physicochemical process involves hazardous chemicals such as use of sodium hydroxide, ammonium hydroxide or borohydrates. A green chemistry approach for the biosynthesis of crystalline IONPs was demonstrated using eco-friendly, less expensive, rapid and simple method. The use of plants and waste products of agriculture as sustainable resources are advantageous to produce industry grade nanoparticles over other methods, such as use of microbes which are more costly due to their culture maintenance and purification cost. Detailed physicochemical characterisation and antimicrobial potency was performed. Our findings indicated the impressive potential of biosynthesised IONPs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nikolova MP, Chavali MS: **Metal oxide nanoparticles as biomedical materials.** *Biomimetics* 2020, **5**(2):27.
2. Vaid P, Raizada P, Saini AK, Saini RV: **Biogenic silver, gold and copper nanoparticles- A sustainable green chemistry approach for cancer therapy.** *Sustainable Chemistry and Pharmacy* 2020, **16**:100247.
3. Jasrotia T, Chaudhary S, Kaushik A, Kumar R, Chaudhary G: **Green chemistry-assisted synthesis of biocompatible Ag, Cu, and Fe₂O₃ nanoparticles.** *Materials Today Chemistry* 2020, **15**:100214.
4. Selim YA, Azb MA, Ragab I, Abd El-Azim MH: **Green synthesis of zinc oxide nanoparticles using aqueous extract of *Deverra tortuosa* and their cytotoxic activities.** *Scientific reports* 2020, **10**(1):1-9.
5. Ziotti ABS, Ottoni CA, Correa CN, de Almeida OJG, de Souza AO, Neto MCL: **Differential physiological responses of a biogenic silver nanoparticle and its production matrix silver nitrate in *Sorghum bicolor*.** *Environmental Science and Pollution Research* 2021:1-14.
6. Anju T, Parvathy S, Veetil MV, Rosemary J, Ansalna T, Shahzabanu M, Devika S: **Green synthesis of silver nanoparticles from Aloe vera leaf extract and its antimicrobial activity.** *Materials Today: Proceedings* 2021.
7. Xiao C, Li H, Zhao Y, Zhang X, Wang X: **Green synthesis of iron nanoparticle by tea extract (polyphenols) and its selective removal of cationic dyes.** *Journal of Environmental Management* 2020, **275**:111262.
8. Desai NM, Gilbert Stanley J, Murthy PS: **Green coffee nanoparticles: optimisation, in vitro bioactivity and bio-release property.** *Journal of microencapsulation* 2020, **37**(1):52-64.
9. Renuka R, Devi KR, Sivakami M, Thilagavathi T, Uthrakumar R, Kaviyarasu K: **Biosynthesis of silver nanoparticles using *Phyllanthus emblica* fruit extract for antimicrobial application.** *Biocatalysis and Agricultural Biotechnology* 2020, **24**:101567.
10. Kim C, Lee J, Schmucker D, Fortner JD: **Highly stable superparamagnetic iron oxide nanoparticles as functional draw solutes for osmotically driven water transport.** *NPJ Clean Water* 2020, **3**(1):1-6.
11. Soetaert F, Korangath P, Serantes D, Fiering S, Ivkov R: **Cancer therapy with iron oxide nanoparticles: Agents of thermal and immune therapies.** *Advanced Drug Delivery Reviews* 2020.
12. Hedayatnasab Z, Dabbagh A, Abnisa F, Daud WMAW: **Polycaprolactone-coated superparamagnetic iron oxide nanoparticles for in vitro magnetic hyperthermia therapy of cancer.** *European Polymer Journal* 2020, **133**:109789.
13. Gul S, Khan SB, Rehman IU, Khan MA, Khan M: **A comprehensive review of magnetic nanomaterials modern day theranostics.** *Frontiers in Materials* 2019, **6**:179.
14. Wu C-C, Chen D-H: **Spontaneous synthesis of gold nanoparticles on gum arabic-modified iron oxide nanoparticles as a magnetically recoverable nanocatalyst.** *Nanoscale research letters* 2012, **7**(1):1-7.
15. Wu W, He Q, Jiang C: **Magnetic iron oxide nanoparticles: synthesis and surface functionalization strategies.** *Nanoscale research letters* 2008, **3**(11):397-415.

16. Ardekani MRS, Khanavi M, Hajimahmoodi M, Jahangiri M, Hadjiakhoondi A: **Comparison of antioxidant activity and total phenol contents of some date seed varieties from Iran.** *Iranian journal of pharmaceutical research: IJPR* 2010, **9**(2):141.
17. Gupta AK, Gupta M: **Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications.** *biomaterials* 2005, **26**(18):3995-4021.
18. Carson C, Riley T: **Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*.** *Journal of applied bacteriology* 1995, **78**(3):264-269.
19. Chakraborty M, Mahmud NU, Gupta DR, Tareq FS, Shin HJ, Islam T: **Inhibitory effects of linear lipopeptides from a marine *Bacillus subtilis* on the wheat blast fungus *Magnaporthe oryzae* Triticum.** *Frontiers in Microbiology* 2020, **11**:665.
20. Padhi A, Nanjundaswamy K, Masquelier C, Okada S, Goodenough J: **Effect of structure on the Fe³⁺/Fe²⁺ redox couple in iron phosphates.** *Journal of the Electrochemical Society* 1997, **144**(5):1609.
21. Ruia A, Gupta RK, Bandyopadhyay G: **Implication of Malnutrition on Human Capital: Bridging the Inequality through Robust Economic Policies.** *Indian Journal of Public Health Research & Development* 2018, **9**(1).
22. Jasinski R, Iob A: **FTIR measurements of iron oxides on low alloy steel.** *Journal of the Electrochemical Society* 1988, **135**(3):551.
23. Shishkovsky I, Scherbakov V, Morozov Y: **Layerwise laser-assisted sintering and some properties of iron oxide core/PEEK shell magnetic nanocomposites.** *Microelectronic Engineering* 2015, **146**:85-91.
24. Khalil AT, Ovais M, Ullah I, Ali M, Shinwari ZK, Maaza M: **Biosynthesis of iron oxide (Fe₂O₃) nanoparticles via aqueous extracts of *Sageretia thea* (Osbeck.) and their pharmacognostic properties.** *Green Chemistry Letters and Reviews* 2017, **10**(4):186-201.
25. Saqib S, Munis MFH, Zaman W, Ullah F, Shah SN, Ayaz A, Farooq M, Bahadur S: **Synthesis, characterization and use of iron oxide nano particles for antibacterial activity.** *Microscopy research and technique* 2019, **82**(4):415-420.
26. Chen YC, Hsiao JK, Liu HM, Lai I-Y, Yao M, Hsu SC, Ko BS, Chen YC, Yang CS, Huang DM: **The inhibitory effect of superparamagnetic iron oxide nanoparticle (Ferucarbotran) on osteogenic differentiation and its signaling mechanism in human mesenchymal stem cells.** *Toxicology and applied pharmacology* 2010, **245**(2):272-279.