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Eco-Friendly Production and *In-Vitro* Assessment of Antibacterial Iron Nanoparticles Synthesized from *Achyranthes aspera* Leaf Extract: A Comprehensive Characterization Study

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Abstract

The remarkable antibiotic properties of *Achyranthes aspera* leaf extracts have been harnessed in this study to produce iron nanoparticles (FeNPs) with potent antibacterial activity against various pathogens. Serving as both a reducing and capping agent, the phytochemical and protein-rich composition of *A. aspera* leaves facilitated the eco-friendly synthesis of FeNPs. This study delves into an innovative, environmentally conscious strategy for the production of iron nanoparticles (FeNPs) by utilizing *A. aspera* leaf extract as a dual-function reducing and capping agent. The resulting FeNPs, endowed with powerful antibacterial attributes, are crafted from the rich phytochemical and protein composition of *A. aspera* leaves. The comprehensive characterization of these nanoparticles, employing techniques such as energy dispersive X-ray (EDX) analysis, scanning electron microscopy (SEM), Fourier transmission infrared spectroscopy (FTIR), X-ray diffraction (XRD), and UV-visible spectrophotometry, revealed diverse morphologies and agglomeration. The latter, confirmed by EDX analysis, was potentially stabilized by plant proteins. These agglomerated FeNPs are subjected to rigorous *in-vitro* antibacterial assessments against prevalent human pathogens, namely *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. The minimum inhibitory concentration (MIC) values are discerned, with the MIC established at 500 µg/ml for *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, while *S. aureus* exhibits a MIC of 1000 µg/ml. This study underscores the ecologically sustainable production of FeNPs through a plant-mediated approach and emphasizes their potential for diverse applications in nanotechnology and biomedicine, presenting *Achyranthes aspera* as a valuable resource in the quest for sustainable and potent therapeutic solutions.

Keywords: *Achyranthes aspera*, Eco-friendly synthesis, Iron nanoparticles (FeNPs), Minimum inhibitory concentration (MIC).

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1. Introduction

In the dynamic landscape of nanomaterials and their diverse applications, the investigation into green-synthesized FeNPs stands as a burgeoning area of interest. This inquiry aligns with the contemporary shift towards eco-friendly products, driven by a growing awareness of the adverse effects associated with synthetic drugs. The envisioned end-product of commercialized applications not only aspires to deliver optimal results but also prioritizes environmental sustainability. The pursuit of such products on a mass scale holds the promise of fostering a sustainable ecosystem. Consequently, there is an escalating societal demand for the adoption of eco-friendly drugs. Against the backdrop of rising concerns surrounding antibiotic resistance and the quest for innovative approaches to combat microbial diseases, the potential of these nanoparticles as potent antibacterial agents has captured significant attention [1]. Iron oxide nanoparticles (FeNPs) have become a well-known family of nanoscale materials in the quickly developing field of nanomaterials. These FeNPs, composed of iron atoms, have unique properties due to their minute size. Iron oxide nanoparticles come in a variety of forms, including magnetite (Fe_3O_4) and maghemite (-Fe_2O_3), which are both known for their catalytic and magnetic properties [2]. Till date use of physically synthesized and chemically prepared [3-7].

FeNPs recorded with many limitations with as they required high temperature, high energy, governs discharge of toxic byproducts and many times onsite increased pressure for synthesis. To overcome these problems, make use of green synthesis approach for FeNPs is advocated and noted with many advantages such as better biodegradability, more stability and its low cost of production [8-10]. The antibacterial activity of nanoparticles formed by plant extract has mainly been linked with polyphenols and those generate a complex with formed nanoparticles to give an antibacterial feature [11]. In detailed, analysis of chemical constituents of *Achyranthus aspera (*AA) carried out and reported number of bioactive compounds once tested & purified from AA extracts such as 1- hexadecanol, 3- Eicosyme, Squalene, Phytol, 9- octadecenamide, and others mainly been linked with antibacterial activity [10,12]. It was evident from the literature that polyphenols of AA are good in antibacterial activity [10,13-14]. Literature clearly states that polyphenols linked with AA leaves are quantified in greater details but not linked with many pharmacological points of view. As estimated by high performance liquid chromatography (HPLC) presence of polyphenols in AA leaves are on higher side which may be responsible for reducing nanoparticles [15]. As previously also noted by Devatha *et al.*, (2018) that presence of polyphenols is accountable to act as a capping agent but its fate as antimicrobial agent especially of AA leaves not been put forward till date and additionally these polyphenols remain safe and biodegradable to produce bio-compound based nanoparticles if used in treatment [11]. Nanoparticles, as is usually believed, interact with biodegradable compounds, altering the properties of biomaterials. The nano-scale characteristics of the nanoparticles, such as their size, surface chemistry, and surface roughness, are what essentially control these changes [16]. These inorganic compounds especially are of metal oxides governs high affinity that gives antimicrobial properties. The exact mechanism of iron oxide nanoparticles-bacteria interaction are put forward with reference to positive antimicrobial activity by Arakha *et al.*, (2015) [17]. Iron nanoparticles possess positive surface potential that gives better surface for bacterial attachment so that it can shoot up interaction at the interface which in turn increases the reactive oxygen species (ROS) in media [11]. Till date numbers of studies were available for antibacterial application of FeNPs. Recently, Batool *et al.*, (2021) reported antibacterial activity of FeNPs from *Phoenix dactylifera* prepared from different plant organs [15]. Similar report by Chandrasekar *et al.*, (2013) reported potent antibacterial activity of iron- oxide, iron- cobalt core shell and zero valent ion nanoparticles prepared by various methods [18]. Literature discloses that FeNPs synthesized from green technology gaining more importance in last few years as they guarantee the benefits of eco-friendly production by cost – effective approach. Consequently, an attempt is made to prepare FeNPs by green technology by using *A. aspera* leaf extract to prepare new edge FeNPs by green synthesis method. Here it has also been put forward that exact mechanism of action of nanoparticle is tough or less known till date in terms of toxicity and safety in response many studied recommended using plant reduced nanoparticles for medicinal usage [19]. Many nanoparticles like zinc oxide, titanium dioxide and magnesium dioxide nanoparticles are proving to be useful [20]. The ability to control water borne diseases by bacteria was also found to be possible by using nanoparticles once demonstrated in industrial applications [21-22]. The design of this study was based on three primary goals. The first step was to start an ecologically friendly process for making iron oxide nanoparticles (FeNPs) using the leaves of *A. aspera* (AA). The second step was to fully characterize the FeNPs using a variety of analytical methods, such as spectroscopy, FTIR (Fourier-transform infrared spectroscopy), SEM (scanning electron microscopy), EDX (energy-dispersive X-ray spectroscopy), and XRD (X-ray diffraction). Finally, to evaluate the antibacterial efficacy of the FeNPs against a panel of human pathogens, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, using the Minimum Inhibitory Concentration (MIC) spectroscopic approach. *A. aspera* leaves were specifically chosen as the raw material because they are widely distributed in India and contain high levels of polyphenols, making them a prime option to produce green nanoparticles. Thus, the goal of this work was to investigate the potential of these greenly produced FeNPs for a range of uses, notably in the context of nanotechnology and antimicrobial agents.

2. Materials and Methods

2.1. Collection of Plant

The foliage of Apamarga, known as "*Achyranthes aspera*" in scientific circles, was meticulously gathered from the verdant landscapes of Chhindawara district, nestled within the heart of Madhya Pradesh. Each leaf was meticulously identified and authenticated by the esteemed expertise of the herbarium. Subsequently, the leaves underwent a meticulous drying process within a specialized drier, as illustrated in Figure 1, prior to further processing.

2.2. Preparation of plant extract and synthesis of FeNPs

Iron nanoparticles of plant origin were synthesized utilizing the rich reserves of polyphenols found in *Achyranthes aspera* leaves. The process commenced by introducing 1% $FeCl_3.H_2O$ into a solution containing plant extract at a ratio of 1:9 (Plant: $FeCl_3.H_2O$ 1%), followed by an incubation period of 24 hours in darkness. The emergence of a distinct color change after this incubation period signified the formation of iron oxide nanoparticles, a transformation that was meticulously tracked for a total duration of 96 hours. Subsequently, the solution underwent centrifugation at a formidable speed of 15,000 RPM for 30 minutes, yielding a discernible pellet which was then carefully separated from the supernatant. To eliminate any residual moisture, the pellet underwent treatment with ethanol and was subsequently subjected to a 24-hour drying period in an oven set at 60°C. The desiccated material was then meticulously stored in plastic centrifuge tubes, shielded from light, in anticipation of further analytical endeavors.

2.3. Characterization of iron nanoparticles

2.3.1. X-ray diffraction (XRD)

The meticulous characterization of iron nanoparticles (Fe NPs) entailed a comprehensive array of analytical techniques. Initially, X-ray diffraction (XRD) was

conducted using the state-of-the-art Bruker Eco D8 advance instrument, featuring CuKα radiation (λ=1.5405A°) with nickel filtration. The determination of the average crystallographic size (t) was facilitated through Scherer's relation (t=0.9 λ/ß cos Θ), establishing a correlation between t, the X-ray wavelength (λ), and the full width (ß) at half maximum (FWHM) of the XRD peaks. This meticulous XRD analysis furnished crucial insights into the structural characteristics underpinning the Fe NPs, offering a foundational understanding essential for further investigation.

2.3.2. Spectrophotometric analysis

Conducting a sophisticated spectrophotometric analysis spanning a duration of 72 hours, researchers employed a cutting-edge single-beam spectrophotometer (Labtronics LT-291) to delve into the phenomenon of plasmon resonance within the nanoparticles. This meticulous examination allowed for the in-depth exploration of the optical properties of the nanoparticles across the 300–800 nm spectral range, providing valuable insights into their stability dynamics over the designated time frame.

2.3.3. FTIR (Fourier-transform infrared)

Employing FTIR (Fourier-transform infrared) spectroscopy, the nanoparticles were meticulously encapsulated within KBr pellets to facilitate analysis. This sophisticated procedure was conducted utilizing the state-of-the-art IR-tracer-100 facility housed at the esteemed Kalasalingam Academy of Research and Education. By scrutinizing the FTIR spectra, researchers gained invaluable insights into the intricate network of chemical bonds and functional groups present within the samples, affording a comprehensive understanding of their chemical composition and properties.

2.3.4. Scanning electron microscopy (SEM) and Energy Dispersive X-ray (EDX)

Following this, SEM (Scanning Electron Microscopy) was employed to scrutinize the FeNPs across varying resolutions spanning from 1 µm to 10 µm. These SEM images offer intricate insights into the size and morphology of the nanoparticles, providing a detailed understanding of their structural characteristics. Furthermore, concurrent Energy Dispersive X-ray (EDX) analysis was conducted to elucidate the elemental composition of the nanoparticles. This synergistic approach significantly enhances our comprehension of both the morphological features and elemental makeup of the FeNPs, thereby enriching our overall knowledge of their properties.

2.4. Analysis of antimicrobial activity of FeNPs

The bacteriostatic efficacy of FeNPs derived from *A. aspera* leaves was meticulously evaluated using the Minimum Inhibitory Concentration (MIC) assay. *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, representing clinically relevant pathogens, were selected as test microorganisms for this investigation. To establish a comprehensive dosage-response profile, FeNPs were prepared at four distinct concentrations (62µg/ml, 125µg/ml, 500µg/ml, and 1000µg/ml) in a nutrient-rich broth medium. Standardizing the bacterial cultures to an optical density of 0.5 using the MacFarland standard ensured uniformity across experimental conditions. Subsequently, bacterial cultures were treated with varying concentrations of FeNPs solutions, with a control group maintained for comparison. Following an incubation period, the optical density at 560 nm was measured using sophisticated spectroscopic techniques. By analyzing the resultant data, the MIC values for each pathogen were meticulously determined, providing crucial insights into the extent of growth inhibition across a spectrum of FeNPs concentrations.

3. Result and discussion

The biological synthesis approach, often referred to as "green synthesis," stands as a superior alternative to numerous physico-chemical methods, heralding from its utilization of organisms such as plants, fungi, bacteria, algae, and others, obviating the necessity for costly, hazardous chemicals, or high energy consumption [23]. In our investigation, this methodology unfolded through the dynamic interaction between precursor salts and reducing agents under continuous agitation, yielding the coveted Fe NPs. Remarkably, our study harnessed the potent reducing properties of *A. aspera*, a botanical marvel abundant in the Chhindwara region of Madhya Pradesh, to effectuate the transformation of $FeCl_3.H_2O$ into FeNPs. A pivotal highlight of this endeavor lies in the utilization of *A. aspera* plant extract for FeNPs synthesis, a practice that not only circumvents the hazards associated with conventional chemical methods but also mitigates the overall environmental footprint of nanomaterial production, embodying the ethos of environmentally benign "green" technology. Notably, precedent research has also underscored the efficacy of *A. aspera* in the successful synthesis of silver nanoparticles, further affirming its versatility and potential for sustainable nanomaterial production [24].

3.1. Spectroscopic analysis

Central to our investigation was a meticulous examination of the absorbance variations induced by the reduction of ferric chloride hexahydrate via *A. aspera* plant extract, culminating in the synthesis of iron oxide nanoparticles (FeNPs). Initially, the absorption spectra of the *A. aspera* extract exhibited a prominent peak at 320 nm, yet underwent a profound shift upon its interaction with ferric chloride hexahydrate, indicative of FeNPs formation. The emergence of a plasmon resonance peak at 425–430 nm, characteristic of successful FeNPs synthesis through the reduction process, served as a tangible marker for this transformative event. Furthermore, the observed stability of the plasmon resonance over a duration of 96 hours underscores the efficient encapsulation of these FeNPs by plant biomolecules, thereby preserving their stability (Figure 2). Notably, the resultant FeNPs exist in a stable state as a complex with plant biomolecules, encapsulating them and imparting stability, encapsulating them and imparting stability, encapsulating them and imparting stability. This phenomenon aligns with prior findings where apple peel-derived FeNPs similarly exhibited absorption maxima between 200-450 nm, corroborating our observations [25]. Similarly, absorption maxima for FeNPs derived from various sources, including corn, leaves, waste, and fruits,

have been documented within the range of 325 to 365 nm, 256 to 325 nm, and 335 to 375 nm, respectively [26-28]. Furthermore, prior studies have reported absorption at 430 nm in conjunction with iron nanoparticles, further reinforcing the consistency of our findings [29].

3.2. XRD analysis of iron nanoparticles

The presence of a dominating peak at 2θ = 46.920°, corresponding to the (110) plane of a face-centered cubic crystal structure, in the XRD pattern analysis indicates the strong crystallinity and stability of the FeNPs (Figure 3). The zerovalent state (Fe°) is significant because it improves the nanoparticles' ability to catalyze reactions. This crystallinity and structural stability are positives for catalytic applications, where stable and homogenous nanoparticles are greatly desired [30]. As per the result formed iron nanoparticles noted predominant crystalline nature and zerovalent sate (Fe°). When compared to Rengasamy *et al.*, (2016) study, same data was obtained, which is consistent with the results shown by once created FeNPs from castor oil [31]. In a similar report, synthesis of zerovalent iron nanoparticles using $FeCl_3.6H_20$ was made realistic using chemical method [32].

3.3. FTIR analysis of iron nanoparticles

Further elucidating the intricate biomolecular interplay underpinning FeNPs synthesis, a comprehensive Fourier-transform infrared (FTIR) study was conducted. Examination of the *A. aspera* leaf extract revealed a distinctive array of bands at various wavenumbers, including 3426 cm^{-1} (associated with hydroxyl group stretching vibrations denoted as -OH), indicative of polyphenols, and 1637 cm^{-1} (attributed to the amide-1 group of proteins).

Amide molecules emerge as pivotal constituents involved in capping the iron nanoparticles (FeNPs), as evidenced by the discernible peaks in the FTIR spectra at 1633 cm^{-1} (nitrile group, -CN) and 2310 cm^{-1} (carboxyl group, -C=O). Additionally, the peak at 1346 cm^{-1} may be assigned to -N= O bonding, while the absorption at 1063 cm^{-1} corresponds to -C-N stretching vibration of aliphatic amines. Notably, the robust absorption peak at 532 cm^{-1} is indicative of Fe-O stretching within the iron nanoparticles (Figure 4). This aligns with previous findings reported by Rengasamy *et al.* (2016) and Yuvakkumar *et al.* (2011) [31,33].

The collective appearance of distinctive peaks at 3423, 2310, 1633, 1346, and 1020 cm^{-1} suggests that the synthesized FeNPs are enveloped by a milieu of polyphenols and proteins. These findings underscore the pivotal role of biomolecules, such as polyphenols and proteins present in *A. aspera* leaves, in facilitating the reduction of Fe^{3+} ions and stabilization of iron nanoparticles, as corroborated by previous studies [18,31,34].

The functional groups present on the surface of FeNPs, such as polyphenols, amide groups, and carboxyl groups, are of paramount significance for their potential applications in biomedicine. These groups offer exciting prospects for the development of targeted drug delivery systems, as they enable the facile attachment of bioactive molecules or medications, enhancing therapeutic efficacy [35]. Moreover, the inherent stability exhibited by these nanoparticles underscores their suitability as a dependable platform for sustained medication delivery, ensuring prolonged therapeutic benefits over extended durations.

3.4. SEM and EDX analysis of iron nanoparticles

The SEM images presented in Figure 5 offer a revealing glimpse into the morphology of iron nanoparticles synthesized using *A. aspera* leaves. These images depict a diverse array of shapes and sizes among the formed nanoparticles, suggestive of agglomeration phenomena. Indeed, previous studies have linked such agglomeration to the presence of attached biomolecules [36]. Our SEM analysis further delineated the variability in the forms and dimensions of *A. aspera* FeNPs, with at least one dimension measuring 100 nm and comprising approximately 64% iron content, alongside fractions of oxygen, aluminum, phosphorus, and sulfur, aligning closely with prior findings [37].

Subsequently, EDX analysis was conducted to ascertain the elemental composition of the synthesized iron nanoparticles (Figure 4C). The resulting spectrum unveiled the presence of oxygen, aluminum, phosphorus, sulfur, and iron, with respective weight percentages of 32.3%, 1.3%, 0.7%, 0.8%, and 64.9%. Notably, the presence of oxygen, phosphorus, sulfur, and aluminum likely originates from biomolecules within *A. aspera* leaf extracts, acting as capping agents; a premise supported by earlier studies [9]. These findings harmonize with previously reported XRD and FTIR results, collectively confirming the successful synthesis of FeNPs through spectroscopic, SEM, and EDX analyses. The absorption peak observed at 430 nm in the spectrophotometry data unequivocally corresponds to iron nanoparticles. Additionally, the diverse forms and sizes of the FeNPs observed via SEM underscore the phenomenon of agglomeration, while the elemental composition revealed by EDX analysis sheds light on the biomolecular involvement in nanoparticle capping. Notably, the documented green synthesis of zerovalent iron nanoparticles from *Azadirachta indica* plants further bolsters the robustness of our findings [38].

3.5. Antibacterial activity

Iron oxide nanoparticles (FeNPs) have been evaluated for their antibacterial efficacy utilizing spectroscopic Minimum Inhibitory Concentration (MIC) analysis on a panel of human pathogens. FeNPs were evaluated at concentrations of 62 µg/ml, 125 µg/ml, 500 µg/ml, and 1000 µg/ml. The results showed that none of the tested pathogens, including *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), and Pseudomonas aeruginosa (P. aeruginosa),* were significantly affected by FeNPs at the lowest dose of 62g/ml. Apart from *Staphylococcus aureus (S. aureus)* at the 500 g/ml concentration, FeNPs showed considerable growth inhibition against all of the pathogens tested at higher doses of 500 µg/ml and µ1000 g/ml (Table 1).

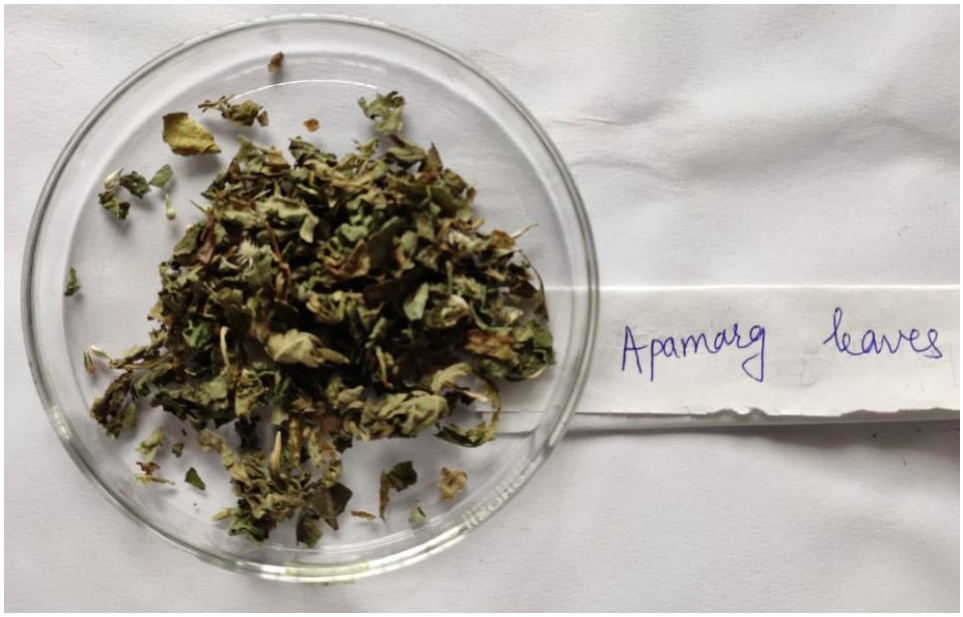


Figure 1: Dried leaves of *Achyranthus aspera*.

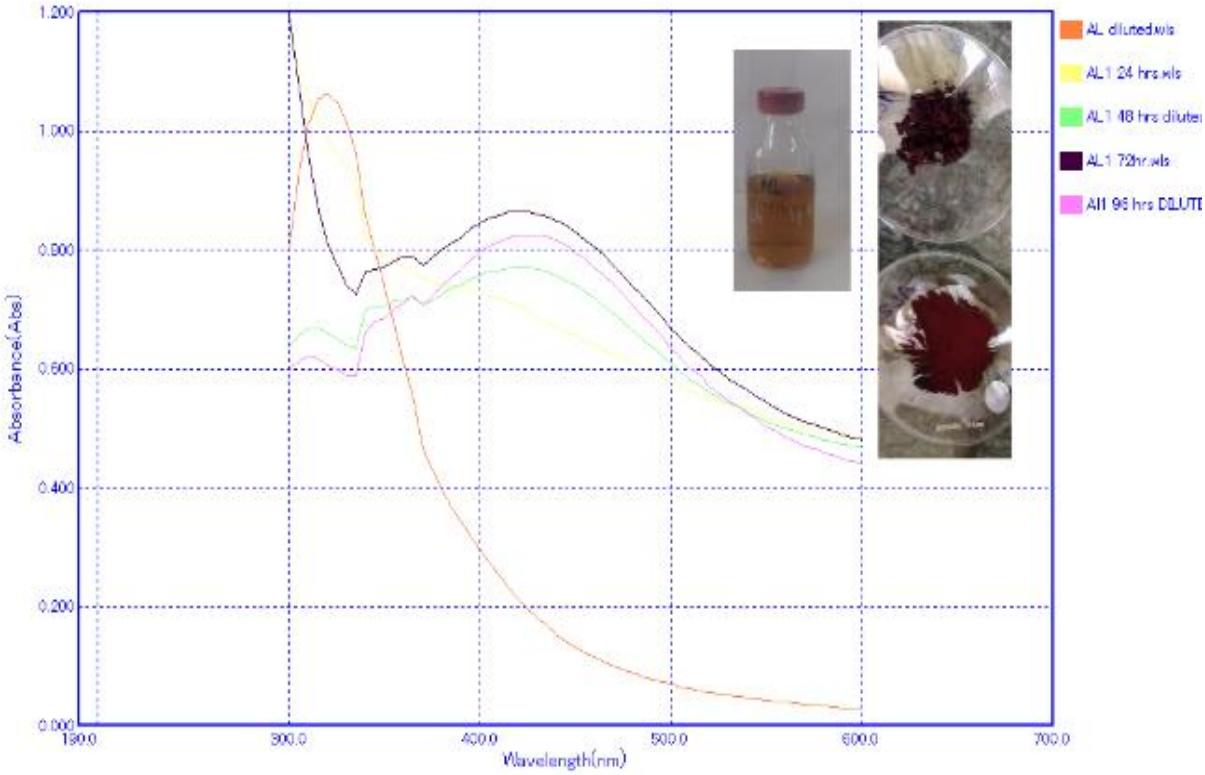


Figure 2: Absorption spectra of *A. aspera* leaf extract (AL diluted) compared with Formed iron nanoparticles after 24 hours (AL1 24), 48 hours (AL1 48), 72 hours (AL72) and 96 hours of formations indicated the stable plasmon resonance till 96 hours indicated that plant biomolecules capped the nanoparticles and stabilized them. In inset *A. aspera* leave extract (bottle) added with $Fecl_3.6H_2O$ recovered for FeONPs as seen in cavity plate.

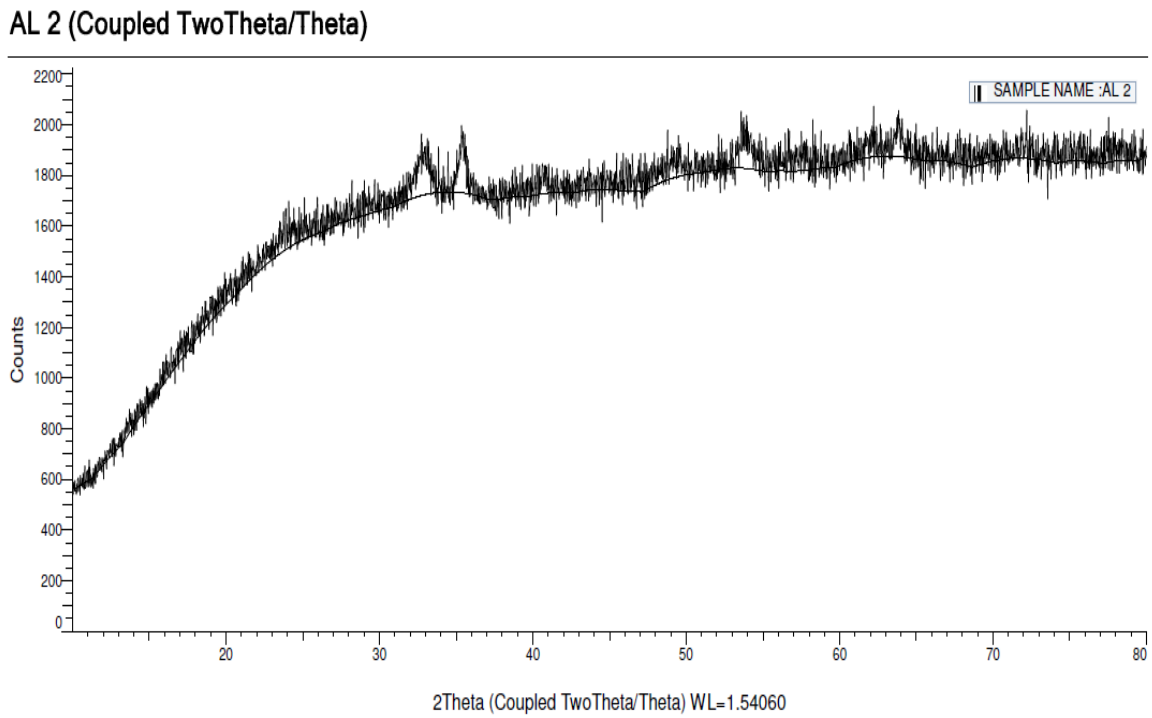


Figure 3: XRD pattern of iron nanoparticles.

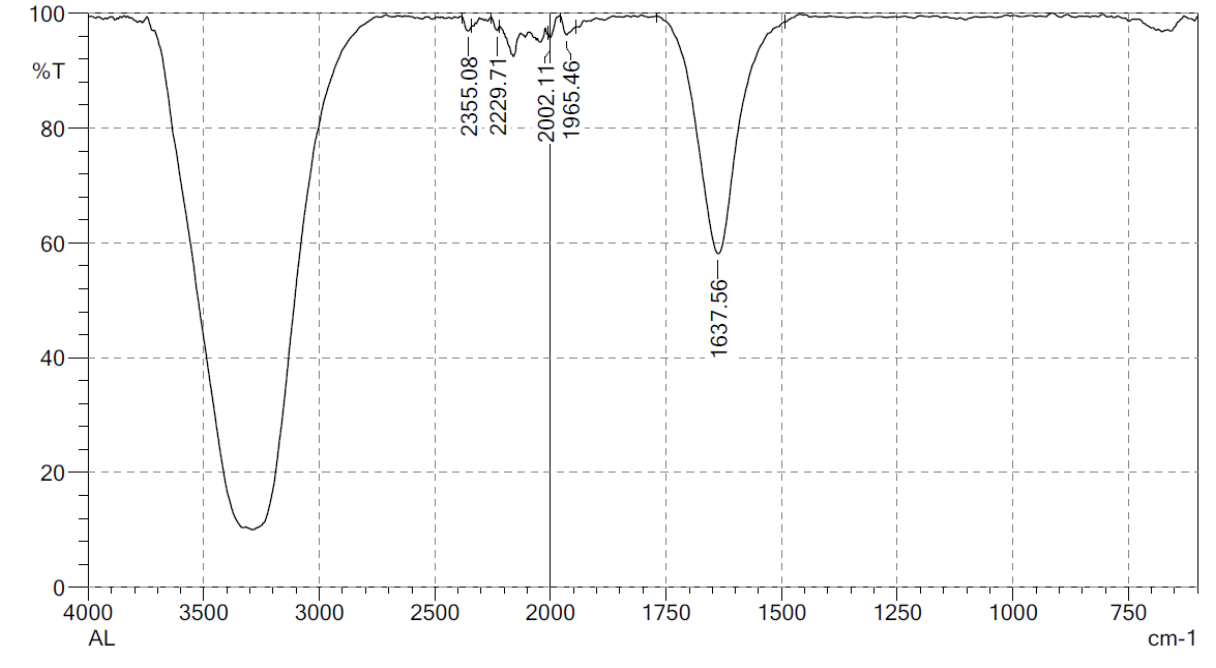
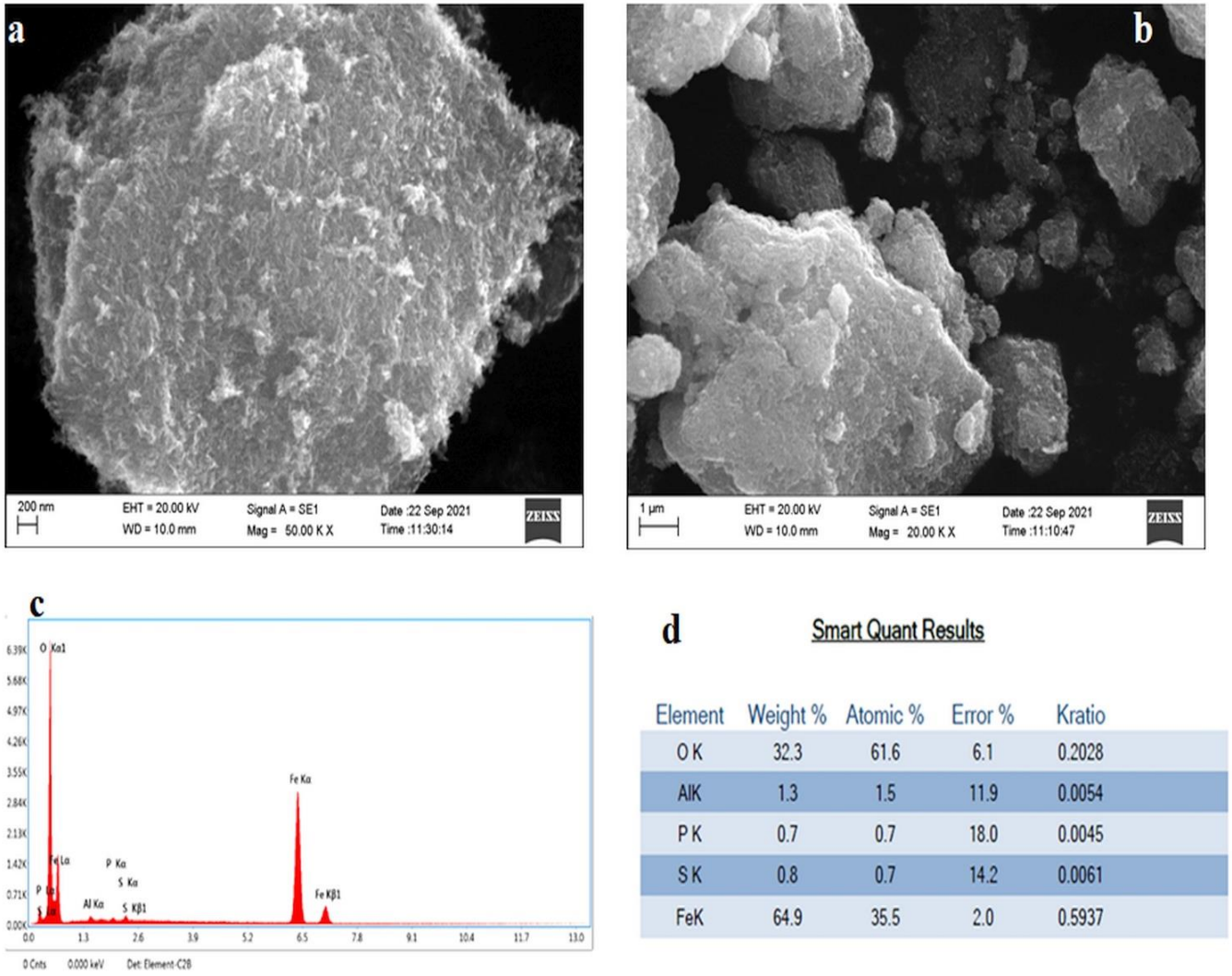


Figure 4: FTIR spectra of Plant extract.



Element	Weight %	Atomic %	Error %	Kratio
O K	32.3	61.6	6.1	0.2028
Al K	1.3	1.5	11.9	0.0054
P K	0.7	0.7	18.0	0.0045
S K	0.8	0.7	14.2	0.0061
Fe K	64.9	35.5	2.0	0.5937

Figure 5*:* (a and b) SEM images of the *A. aspera* reduced iron nanoparticles at different magnification; (c) EDX analysis and (d) Smart Quant Results.

Table 1: Antibacterial potency of FeNPs against pathogenic bacteria in humans evaluated via spectroscopic MIC determination.

Isolates	**Concentrations per ml**			
	62 µg	125 µg	500 µg	1000 µg
E. coli	NI	NI	I	I
K. pneumoniae	NI	NI	I	I
S. aureus	NI	NI	NI	I
P. aeruginosa	NI	NI	I	I
NI- No Growth Inhibition; I- Growth Inhibition				

Numerous studies have documented the remarkable efficacy of iron oxide nanoparticles in combatting a wide array of bacteria and fungi, many of which hold significant epidemiological relevance [39]. Recent investigations have elucidated that these nanoparticles exhibit a remarkable inhibitory effect on a spectrum of pathogens, including *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus*, achieving a minimum inhibitory concentration (MIC) as low as 65 ± 1.5 µg/ml. Moreover, findings akin to our current study reveal that at higher concentrations, ranging from 800 to 1000 µg/ml, iron oxide nanoparticles display enhanced efficacy against a broader range of bacteria, such as *E. coli*, *K.*

pneumoniae, and *S. aureus* [40]. Noteworthy results from another study underscore the positive impact of D-8-fe-0 nano composites on bacteria like *S. aureus* and *B. subtilis* at concentrations of 500 and 250 mg/ml [41]. These collective findings underscore the concentration-dependent antibacterial prowess of FeNPs, particularly against notorious pathogens like *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, while exhibiting slightly diminished effectiveness against S. aureus. This underscores the potential of FeNPs as potent antibacterial agents, offering a promising avenue for combating bacterial infections. Moreover, their controlled release mechanisms mitigate collateral damage to surrounding tissues, making them suitable candidates for the development of antimicrobial coatings or wound dressings.

4. Conclusions

The ecologically conscious synthesis of iron oxide nanoparticles using *A. aspera* plant extract represents a significant stride with expansive potential. The synthesis process outlined in this study not only delineates an economical, rapid, and efficient route but also underscores the dual functionality of *A. aspera* leaves as both a reducing and stabilizing agent in the green synthesis of FeNPs. The successful synthesis and inherent biological compatibility of these FeNPs find robust support in their plasmon resonance, manifested through shifts in absorbance maxima, crystalline nature, and nanoparticle stability. Their efficacy in addressing bacterial infections is underscored by compelling antibacterial activities, as evidenced by the minimum inhibitory concentration (MIC) experiment. Anticipating diverse applications in biology, catalysis, materials science, and nanotechnology, these FeNPs signify a promising avenue for future exploration. However, to fully unlock their potential while ensuring responsible and sustainable utilization, comprehensive studies are imperative. This includes an in-depth exploration of their safety profile, scalability in manufacturing, and a nuanced investigation into their optical and magnetic characteristics. Such endeavors will not only enhance our understanding but also pave the way for the conscientious application of these nanoparticles in a spectrum of fields.

Declaration of competing interest

The authors have no financial or non-financial interests to disclose.

References

[1] G. Waktole, B. Chala. (2023). The Role of Biosynthesized Metallic and Metal Oxide Nanoparticles in Combating Anti-Microbial Drug Resilient pathogens. Journal of Biomaterials and Nanobiotechnology. 14 (1): 1-22.

[2] M. Yazdanian, P. Rostamzadeh, M. Rahbar, M. Alam, K. Abbasi, E. Tahmasebi, H. Tebyaniyan, R. Ranjbar, A. Seifalian, A. Yazdanian. (2022). The potential application of green-synthesized metal nanoparticles in dentistry: a comprehensive review. Bioinorganic Chemistry and Applications, 2022.

[3] S. Baskoutas, P. Giabouranis, S.N. Yannopoulos, V. Dracopoulos, L. Toth, A. Chrissanthopoulos, N. Bouropoulos. (2007). Preparation of ZnO nanoparticles by thermal decomposition of zinc alginate. Thin solid films. 515 (24): 8461-8464.

[4] G. A. Beaulah, A. M. Sadiq, R. J. Santhi. (2011). Antioxidant and antibacterial activity of *Achyranthes aspera*: An in vitro study. Annals of biological research. 2 (5): 662-667.

[5] T. Tosco, M. P. Papini, C. C. Viggi, R. Sethi. (2014). Nanoscale zerovalent iron particles for groundwater remediation: a review. Journal of cleaner production. 77: 10-21.

[6] W. X. Zhang. (2003). Nanoscale iron particles for environmental remediation: an overview. Journal of nanoparticle Research. 5: 323-332.

[7] M. Z. Kassaee, E. Motamedi, A. Mikhak, R. Rahnemaie. (2011). Nitrate removal from water using iron nanoparticles produced by arc discharge vs. reduction. Chemical Engineering Journal. 166 (2): 490-495.

[8] S. Machado, S. L. Pinto, J. P. Grosso, H. P. A. Nouws, J. T. Albergaria, C. Delerue-Matos. (2013). Green production of zero-valent iron nanoparticles using tree leaf extracts. Science of the Total Environment. 445: 1-8.

[9] L. Huang, X. Weng, Z. Chen, M. Megharaj, R. Naidu. (2014). Synthesis of iron-based nanoparticles using oolong tea extract for the degradation of malachite green. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 117: 801-804.

[10] C. L. Priya, G. Kumar, L. Karthik, K. V. B. Rao. (2010). Antioxidant activity of *Achyranthes aspera* Linn stem extracts. Pharmacologyonline. 2 (2): 228-237.

[11] C. P. Devatha, K. Jagadeesh, M. Patil. (2018). Effect of Green synthesized iron nanoparticles by Azardirachta Indica in different proportions on antibacterial activity. Environmental Nanotechnology, Monitoring & Management. 9: 85-94.

[12] P. A. Mary, R. S. Giri. (2017). GC-MS Analysis of Bioactive Compounds of Acryranthes aspera. World Journal of Pharmaceutical Research. 7 (1): 1015-1056.

[13] H. Borzouei, S. M. Mirdamadi, J. Hosseini. (2011). Affective factors in commercialization of nanotechnology in Iran's agricultural sector. Annals of Biological Research. 2 (6): 56-61.

[14] A. R. Ndhlala, H. Ghebrehiwot, B. Ncube, A. O. Aremu, J. Gruz, M. Šubrtová, K. Doležal, C. P. du Plooy, H. A. Abdelgadir, J. VanStaden. (2015). Antimicrobial, anthelmintic activities and characterization of functional phenolic acids of *Achyranthes aspera* Linn.: a medicinal plant used for the treatment of wounds and ringworm in east Africa. Frontiers in Pharmacology. 6: 274.

[15] F. Batool, M. S. Iqbal, S. U. D. Khan, J. Khan, B. Ahmed, M. I. Qadir. (2021). Biologically synthesized iron nanoparticles (FeNPs) from Phoenix dactylifera have anti-bacterial activities. Scientific Reports. 11 (1): 22132.

[16] R. P. Singh, V. K. Shukla, R. S. Yadav, P. K. Sharma, P. K. Singh, A. C. Pandey. (2011). Biological approach of zinc oxide nanoparticles formation and its characterization. Advanced Materials Letters. 2 (4): 313-317.

[17] M. Arakha, S. Pal, D. Samantarrai, T. K. Panigrahi, B. C. Mallick, K. Pramanik, B. Mallick, S. Jha. (2015). Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. Scientific reports. 5 (1): 14813.

[18] N. Chandrasekar, K. M. Mohan Kumar, K. S. Balasubramnian, K. Karunamurthy, and R. Varadharajan. (2013). Facile synthesis of iron oxide, iron-cobalt and zero valent iron nanoparticles and evaluation of their anti- microbial activity, free radicle Scavenginging activity and antioxidant assay. Digest Journal of Nanomaterials & Biostructures (DJNB). 8 (2).

[19] K. Kumar, R. K. Singh, P. K. Tyagi, D. Gore. (2020). Assessment of Toxicity and Safety Profiles of Nanoparticles. Letters in Applied NanoBioScience. 10: 1877-1888.

[20] A. Shrivastava, R. Singh, P. Tyagi, D. Gore. (2021). Synthesis of zinc oxide, titanium dioxide and magnesium dioxide nanoparticles and their prospective in pharmaceutical and biotechnological applications. Journal of Biomedical Research & Environmental Sciences. 2 (1): 011-020.

[21] A. Kaushik, R. K. Singh, P. K. Tyagi. (2023). Green Synthesized Nanoparticle Based Drug Delivery: Recent Trends and Future Prospects. Precision Nanomedicine. 6 (4): 1109-1131.

[22] P. K. Tyagi, R. Singh, S. Vats, D. Kumar. (2012). Nanomaterials use in wastewater treatment. In Proceedings of the international conference on nanotechnology and chemical engineering (Vol. 21).

[23] A. Singh, N. B. Singh, I. Hussain, H. Singh. (2017). Effect of biologically synthesized copper oxide nanoparticles on metabolism and antioxidant activity to the crop plants Solanum lycopersicum and Brassica oleracea var. botrytis. Journal of biotechnology. 262: 11-27.

[24] T. P. Amaladhas, M. Usha, S. Naveen. (2013). Sunlight induced rapid synthesis and kinetics of silver nanoparticles using leaf extract of *Achyranthes aspera* L. and their antimicrobial applications. Advanced Materials Letters. 4 (10): 779-785.

[25] A. S. Y. Ting, J. E. Chin. (2020). Biogenic synthesis of iron nanoparticles from apple peel extracts for decolorization of malachite green dye. Water, Air, & Soil Pollution. 231 (6): 278.

[26] J. K. Patra, K. H. Baek. (2017). Green biosynthesis of magnetic iron oxide (Fe_3O_4) nanoparticles using the aqueous extracts of food processing wastes under photo-catalyzed condition and investigation of their antimicrobial and antioxidant activity. Journal of Photochemistry and Photobiology B: Biology. 173: 291-300.

[27] G. Sathishkumar, V. Logeshwaran, S. Sarathbabu, P. K. Jha, M. Jeyaraj, C. Rajkuberan, N. Senthilkumar, S. Sivaramakrishnan. (2018). Green synthesis of magnetic Fe_3O_4 nanoparticles using Couroupita guianensis Aubl. fruit extract for their antibacterial and cytotoxicity activities. Artificial cells, nanomedicine, and biotechnology. 46 (3): 589-598.

[28] J. K. Patra, M. S. Ali, I. G. Oh, K. H. Baek. (2017). Proteasome inhibitory, antioxidant, and synergistic antibacterial and anticandidal activity of green biosynthesized magnetic Fe3O4 nanoparticles using the aqueous extract of corn (Zea mays L.) ear leaves. Artificial cells, nanomedicine, and biotechnology. 45 (2): 349-356.

[29] I. M. Grabs, C. Bradtmöller, D. Menzel, G. Garnweitner. (2012). Formation mechanisms of iron oxide nanoparticles in different nonaqueous media. Crystal growth & design. 12 (3): 1469-1475.

[30] D. L. Huber. (2005). Synthesis, properties, and applications of iron nanoparticles. Small. 1 (5): 482-501.

[31] M. Rengasamy, K. Anbalagan, S. Kodhaiyolii, V. Pugalenthi. (2016). Castor leaf mediated synthesis of iron nanoparticles for evaluating catalytic effects in transesterification of castor oil. RSC advances. 6 (11): 9261-9269.

[32] R. Yuvakkumar, V. Elango, V. Rajendran, N. Kannan. (2011). Preparation and characterization of zero valent iron nanoparticles. Digest journal of nanomaterials and biostructures. 6 (4): 1771-1776.

[33] B. Bhushan, A. Basumallick, N. Y. Vasanthacharya, S. Kumar, D. Das. (2010). Sr induced modification of structural, optical and magnetic properties in Bi1− xSrxFeO3 (x= 0, 0.01, 0.03, 0.05 and 0.07) multiferroic nanoparticles. Solid State Sciences. 12 (7): 1063-1069.

[34] F. Luo, Z. Chen, M. Megharaj, R. Naidu. (2014). Biomolecules in grape leaf extract involved in one-step synthesis of iron-based nanoparticles. RSC Advances. 4 (96): 53467-53474.

[35] F. Fatima, S. Siddiqui, W. A. Khan. (2021). Nanoparticles as novel emerging therapeutic antibacterial agents in the anti-biotics resistant era. Biological Trace Element Research.199 (7): 2552-2564.

[36] Z. Markova, P. Novak, J. Kaslik, P. Plachtova,, M. Brazdova,, D. Jancula, K. M. Siskova, L. Machala, B. Marsalek, R. Zboril, R. Varma. (2014). Iron (II, III)–polyphenol complex nanoparticles derived from green tea with remarkable ecotoxicological impact. ACS Sustainable Chemistry & Engineering. 2 (7): 1674-1680.

[37] Y. Wang, X. He, K. Wang, X. Zhang, W. Tan. (2009). Barbated Skullcup herb extract-mediated biosynthesis of gold nanoparticles and its primary application in electrochemistry. Colloids and Surfaces B: Biointerfaces. 73 (1): 75-79.

[38] M. Pattanayak, P. L. Nayak. (2013). Green synthesis and characterization of zero valent iron nanoparticles from the leaf extract of Azadirachta indica (Neem). World Journal of Nano Science & Technology. 2 (1): 06-09.

[39] M. Kanoksil, A. Jatapai, S. J. Peacock, D. Limmathurotsakul. (2013). Epidemiology, microbiology and mortality associated with community-acquired bacteremia in northeast Thailand: a multicenter surveillance study. PloS one. 8 (1): e54714.

[40] T. Ahmed, Z. Wu, H. Jiang, J. Luo, M. Noman, M. Shahid, I. Manzoor, K. S. Allemailem, F. Alrumaihi, B. Li. (2021). Bioinspired green synthesis of zinc oxide nanoparticles from a native Bacillus cereus strain RNT6: characterization and antibacterial activity against rice panicle blight pathogens Burkholderia glumae and B. gladioli. Nanomaterials. 11 (4): 884.

[41] M. A. Mahdi, S. R. Yousefi, L. S. Jasim, M. Salavati-Niasari. (2022). Green synthesis of DyBa2Fe3O7. 988/$DyFeO_3$ nanocomposites using almond extract with dual eco-friendly applications: photocatalytic and antibacterial activities. International Journal of Hydrogen Energy. 47 (31): 14319-14330.