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Green synthesis and characterization of ferrous nanoparticles using Psidium guajava (Linn.) leaf extract

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Abstract
Ferrous nanoparticles were produced using the aqueous leaf extracts of Psidium guajava as a dual reducing and stabilizing agent. A color shift from pale yellow/brown to dark brown/black was observed immediately after reducing ferric chloride ($FeCl_3$) solution (1 mM) to FeNPs by mixing with plant extract (300 mL). The synthesized FeNPs were characterized using UV-Visible spectroscopy and FTIR. Ferrous nanoparticles (FeNPs) were successfully synthesized using aqueous leaf extracts of Psidium guajava as both reducing and stabilizing agents. A rapid color change from pale yellow/brown to dark brown/black indicated the reduction of $FeCl_3$ (1 mM) upon mixing with 300 mL of plant extract. UV-Visible spectroscopy confirmed nanoparticle formation via a surface plasmon resonance (SPR) peak at 360 nm, indicating Fe^{3+} reduction to Fe^{2+}. FTIR analysis revealed key functional groups involved in nanoparticle stabilization and synthesis: C-O stretching (1154–1034 cm^{-1}; alcohols/ethers), alkane C-H (2923 cm^{-1}), alkene C=C (1648 cm^{-1}; flavonoids), and O-H stretching (3417 cm^{-1}; polyphenols/carboxylic acids). These phytochemicals primarily polyphenols, terpenoids, and flavonoids facilitated eco-friendly nanoparticle synthesis, offering a sustainable and nontoxic alternative for environmental and biomedical applications.

Keywords: Eco-friendly nanotechnology, Ferrous nanoparticles (FeNPs), Green synthesis**,** Phytochemical reduction**,** *Psidium guajava,* Surface plasmon resonance (SPR)

1. Introduction
Nanotechnology offers novel opportunities across diverse sectors by focusing on materials at the nanoscale (1–100 nm) (Arowora et al.., 2023). In biomedical research, it plays a pivotal role in the development of therapeutic nanoparticles. Among these, ferrous nanoparticles (FeNPs) stand out due to their high surface area, magnetic properties, and biocompatibility, making them promising candidates for medical applications. Green synthesis using plant extracts to reduce metal ions into nanoparticles has gained popularity for being cost-effective and environmentally sustainable (Saeidienik et al., 2018). While various plant-mediated syntheses of FeNPs have been reported, limited studies have explored the specific phytochemical mechanisms and functional group interactions involved in *Psidium guajava*-mediated synthesis. Moreover, few investigations have provided detailed spectroscopic evidence (for example, UV-Vis and FTIR) to confirm the role of guava leaf constituents in nanoparticle stabilization and reduction. This study addresses these gaps by characterizing FeNPs synthesized via *P. guajava* extract, identifying key functional groups

responsible for reduction and stabilization, and demonstrating the potential of this green method for biomedical and environmental applications.

2. Literature review

Guava, or *Psidium guajava*, is known to have anti-diabetic, anti-diarrheal, anti-mutagenic, and anti-microbial qualities. Its safety has also been shown by toxicity studies. Nanoparticles, favored for dye removal, exhibit unique characteristics such as high surface area, chemical reactivity, and mechanical strength. *Psidium guajava* is utilized in synthesizing iron nanoparticles (FeNPs), valued for their enhanced stability and positive cellular interactions (Allu et al., 2022).

Nanotechnology primarily focuses on the synthesis of nanoparticles with diverse sizes, shapes, and chemical compositions, enabling their application in various fields (Shahwan *et al.*, 2011). This innovative approach demonstrates significant potential for addressing numerous challenges across multiple disciplines, owing to its adaptability and effectiveness. The biosynthesis method uses various parts like roots, leaves, seeds, flowers, fruits, peels, petals, the entire plant, and seed husk, which are abundant in various biomolecules including carbohydrates, amino acids, flavonoids, proteins, saponins, terpenoids, and nitrogenous compounds that act as reducers, stabilizers, redox mediators, and capping agents in the formation of nanoparticles (Mittal et al., 2013; Royand Das, 2015; Rajeshkumar and Bharath et al., 2017; Mirza et al., 2018; Nadeem et al., 2018; Vasantharaj et al., 2019; Mondal et al., 2020). In general, plants are considered easily accessible, manageable, benign, and inexpensive resources for the production of various types of nanoparticles (Noruzi, 2015).

A primary reason for this is because bacteria and fungus necessitate extended incubation periods, but the phytochemicals present in plants can reduce metal ions more rapidly (Singh et al., 2018). Recent research indicates that plant extracts from *Solanum trilobatum*, *Ziziphora tenuior*, *Persea americana*, *Abutilon indicum*, *Azadirachta indica*, *Camellia sinensis*, and green tea leaves have been used for the synthesis of IONPs across various size ranges (Khalil et al., 2017; Arularasu et al., 2018). Current findings also suggest that nanoparticles synthesized with plant extracts possess greater stability compared to those produced through conventional methods (Bibi et al., 2019) and typically exhibit distinct shapes such as spherical, cubical, cylindrical, needle-like, stem-like, prism-like, and dendritic forms (Abdullah et al., 2020).

The properties of synthesized nanoparticles are largely determined by specific factors, including the type of plant extract utilized, the volume ratios of the extract and metallic salt solutions, and the reaction conditions (such as pH, temperature, and incubation time) (Abdullah *et al.*, 2020; Singh *et al.*, 2018). Because of this, researchers have used a variety of methods to synthesize iron oxide nanoparticles based on these important parameters. These methods vary slightly in terms of the starting materials chosen, the plant extracts prepared, the iron salts chosen, the inclusion or exclusion of NaOH, the reaction conditions, and the methods used to collect the synthesized nanoparticles. Matter is altered chemically and/or physically in nanotechnology to create materials with specific qualities suitable for a variety of applications (Silva, 2004). A microscopic particle with at least one dimension smaller than 100 nm in diameter is referred to as a nanoparticle (NP) (Webster et al., 2011; Roy & Ghosh, 2017). Because of their unique optical, thermal, electrical, chemical, and physical characteristics (Majeedkhan et al., 2011; Panigrahi et al., 2004), nanoparticles (NPs) have a wide range of uses in heavy industry, consumer goods, chemistry, medicine, environmental science, energy, agriculture, and information and communication technology (Castillo-Henríquez et al., 2020; Thiyagarajan et al., 2018; Tyagi, 2016).

Conventional techniques for creating nanoparticles, such as pyrolysis and attrition, have drawbacks such producing faulty surfaces, limited production efficiency, and expensive, energy-intensive production (Charitidis et al., 2014). Hazardous byproducts and contamination from precursor materials can result from the use of poisonous compounds in chemical synthesis techniques such as chemical reduction and the sol-gel technique (Şengül et al., 2008; Tyagi et al., 2016). Consequently, there is a growing need to develop safe, non-toxic, and environmentally friendly technologies for synthesizing nanoparticles.

When compared to traditional physical and chemical methods, biological synthesis techniques have a number of obvious advantages: (i) they don't use harmful chemicals, which makes them a clean and environmentally sustainable method (Uzair et al., 2020; Usman et al., 2018; Tyagi et al., 2012; Senapati et al., 2005); (ii) biological agents, such as enzymes, act as capping and reducing agents, lowering the overall cost of the synthesis process (Senapati *et al.,* 2005); (iii) even in large-scale production, small nanoparticles can be produced (Klaus *et al.*, 1999); and (iv) no external conditions, such as high energy inputs or high pressure, are required, which results in significant energy savings (Bansal et al., 2004).

Because of its affordability and non-toxicity, the green production of iron nanoparticles has garnered interest (Saif et al., 2016). Since no dangerous chemicals are used in this process, it is environmentally benign. Iron nanoparticles are highly effective and have a lot of promise for solving a number of environmental problems.

Guava leaf extract is taken into consideration in this study for the synthesis of ferrous nanoparticles. The guava, or *Psidium guajava* L., is a tropical American plant that is a member of the *Myrtaceae* family. It has been grown in tropical regions over time and has spread to other areas with suitable conditions. In traditional medicine, guava is highly prized. Different portions of the plant are used to cure a variety of conditions, such as cholera, wounds, ulcers, and intestinal problems. This illustrates its importance in indigenous healing traditions and therapeutic potential (Begum et al., 2002). *Psidium guajava*'s bark, fruit, and leaves have been shown in pharmacological investigations to possess antibacterial, hypoglycemic, anti-inflammatory, antipyretic, spasmolytic, and central nervous system depressive qualities (Begum et al., 2002). It is admirable to promote the use of plant extracts rather than artificial and chemical methods since it promotes green technologies. High pressure or high temperatures are not necessary with this method. The goal of this study is to create ferrous nanoparticles in an environmentally friendly manner using guava leaves. UV-visible and Fourier transform infrared spectroscopy will be used to characterize the particles.

3. Research methodology

3.1. Materials

3.1.1. Glasswares and Facilities

Mortar and pestle, digital analytical weighing balance (ohaus: pa-1000): beakers, whatman number 1 filter paper, conical flask (pyrex), spatula, measuring cylinder (pyrex), aluminum foil, cotton wool, separating funnel, plastic funnels, thermostatic water cabinet (Model:HH-W420): spectrophotometer(UV-Visible double beam light), shimadzu-FTIR-8400s-spectrometer.

3.1.2. Reagents and Chemicals

All reagents used were of analytical grades. They are ferric chloride ($FeCl_3$) (LOBA Chemie laboratory, reagents and fine chemicals. India).

3.1.3. Collection and Preparation of Plant Materials

The fresh leaf of *Psidium guajava* were collected from Government reservation Area (G.R.A) Wukari, Taraba State. To get rid of contaminants, the guava leaves were rinsed with distilled water after being repeatedly cleaned with water. After being cleaned, the leaves were allowed to air dry for one week until they were crispy, and then they were ground into a powder using a dry, clean mortar and pestle.

3.2. Methods

3.2.1. Preparation of the Extract

A slightly modification of the Saranyaadevi et al. (2014) approach was used for the extraction process. In particular, 500mL of distilled water and 50g of *Psidium guajava* were added to a 250mL beaker after being carefully weighed. After that, the mixture was heated to 60ºC for 30 minutes while being constantly stirred. To ensure ideal extraction conditions, the beaker was heated, covered with cotton wool and aluminum foil to avoid contamination, and then left undisturbed for a whole day. The leaf extract was meticulously gathered and filtered via Whatman No. 1 filter paper after a 24-hour period.

3.2.2. Ferric Chloride ($FeCl_3$) Solution Preparation

Specifically, 0.1622g of ferric chloride ($FeCl_3$) was dissolved in a 1000mL volumetric flask to create a 1mM solution, and then distilled water was added to reach the desired level.

3.2.3. Ferrous Nanoparticle Synthesis

Then, using a hot plate magnetic stirrer, 300mL of *Psidium guajava* filtrate was slowly added to the produced $FeCl_3$ solution while being continuously stirred for 30 minutes at 60°C. Iron nanoparticle production is shown by the color changing from brown/yellowish brown to black (Saranyaadevi *et al.*, 2014). The flasks were sealed with foil paper and cotton wool to keep out light, and they were then placed in a water bath for three hours to help reduce the Fe^{2+} ions. For later usage, the finished solution was kept at 4^{O}C.

3.2.4. Characterization of Ferrous Nanoparticles Using UV- Visible Spectroscopy

Synthesized ferrous nanoparticles (FeNPs) were subjected to UV-visible spectra recording at different time intervals using a spectrophotometer equipped with a 1.0 nm slit width and spectral bandwidth. Optical absorbance scans were performed between 200 and 500 nm at 20 nm intervals to characterize FeNPs made from *Psidium guajava* extract. A UV-visible spectral measurement was performed after comparing a 1 mL aliquot with 1 mL of distilled water as a blank in order to evaluate the bio-reduction of iron ions in an aqueous solution. A Shimadzu UV-2500PC dual beam spectrophotometer, which operates with a 20 nm range spanning the 200-500 nm wavelength range, was used to perform the spectral analysis. Only one replicate was used for this analysis.

3.2.5. Characterization of FeNPs Using FTIR

The sample is placed in a holder in the path of the infrared source. A detector reads the analog signal and converts the signal to a spectrum. A computer is used to analyse the signals and identify the peaks. An IR beam goes through a partially silvered mirror, which splits the beam into two beams of equal intensity. FTIR analysis was also conducted using a single sample replicate.

4. Results and discussions

In this study, ferrous nanoparticles were synthesized from *Psidium guajava* leaf. Exactly, five different peaks were observed which include: 200nm, 300nm, 320 nm, 340nm and 360nm respectively. The highest absorption characteristic peak of the ultraviolet-visible spectrum of the ferrous nanoparticles was observed at 360nm (Figure 1).

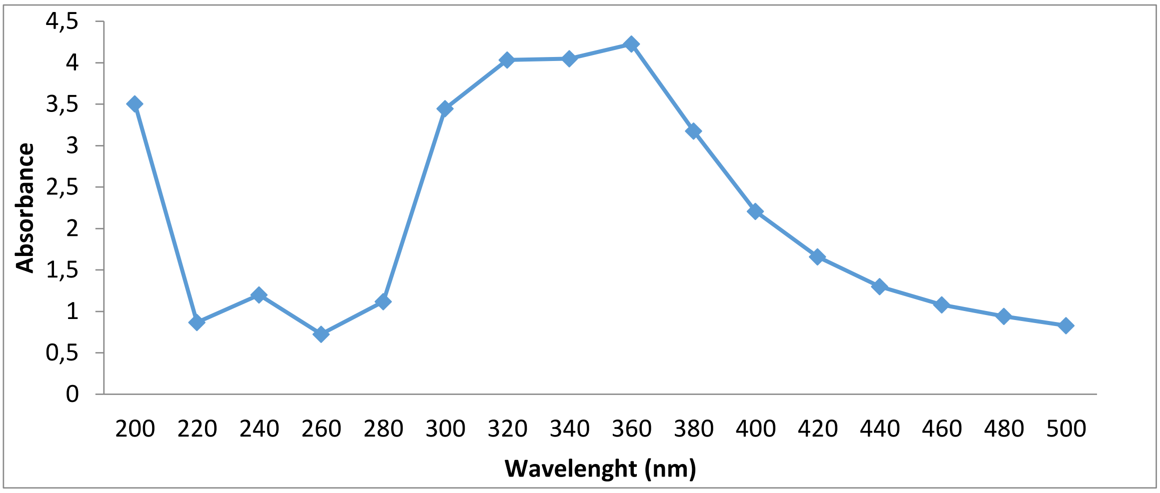

Figure 1: Characterization of Biosynthesized Ferrous Nanoparticles from *Psidium guajava* Leaf Using UV-Vis Spectroscopy

The crystal of green synthesized ferrous nanoparticles produced was subjected to FTIR spectroscopy for its characterization. The FTIR characterization different spectra and absorbance bands which have been observed in the region of 3417.01 cm−1, 2923.22 cm−1, 1648.23 cm−1, 1412.90 cm−1, 1154.43 cm−1, 1034.84 cm−1, 675.11 cm−1, 596.02 cm−1 and 419.53 cm−1 which indicate O-H group, alkane (C-H) group, alkene (C=C) group, alkane (C-H) group, Alcohols (C-O) group, Aromatic compounds (C-H out-of-plane bending), alkyl Bromide (C-Br) and alkyl iodide (C-I) respectively. The changes observed in the reaction converting $FeCl_3$ to Ferrous nanoparticles using FTIR analysis is represented below (Table 1).

Table 1: FTIR Spectroscopy Showing the Wavelength, Functional Groups and Inference

0S/N	WAVENUMBER (cm^{-1})	FUNCTIONAL GROUPS	INFERENCES
1	3417.01	O-H stretch	Hydroxyl group (Found alcohols and phenol
2	2923.22	C-H stretch	Alkanes
3	1648.23	C=C stretch	Alkenes
4	1412.90	C-H bending	Alkanes
5	1154.43	C-O stretch	Alcohols, ethers, Carboxylic acids and esters
6	1034.84	C-O stretch	Alcohols, ethers, Carboxylic acids and esters
7	675.11	C-H stretching out of plane bending	Aromatic compound
8	596.02	C-Br stretching	Alkyl bromides
9	419.53	C-I stretching	Alkyl iodides

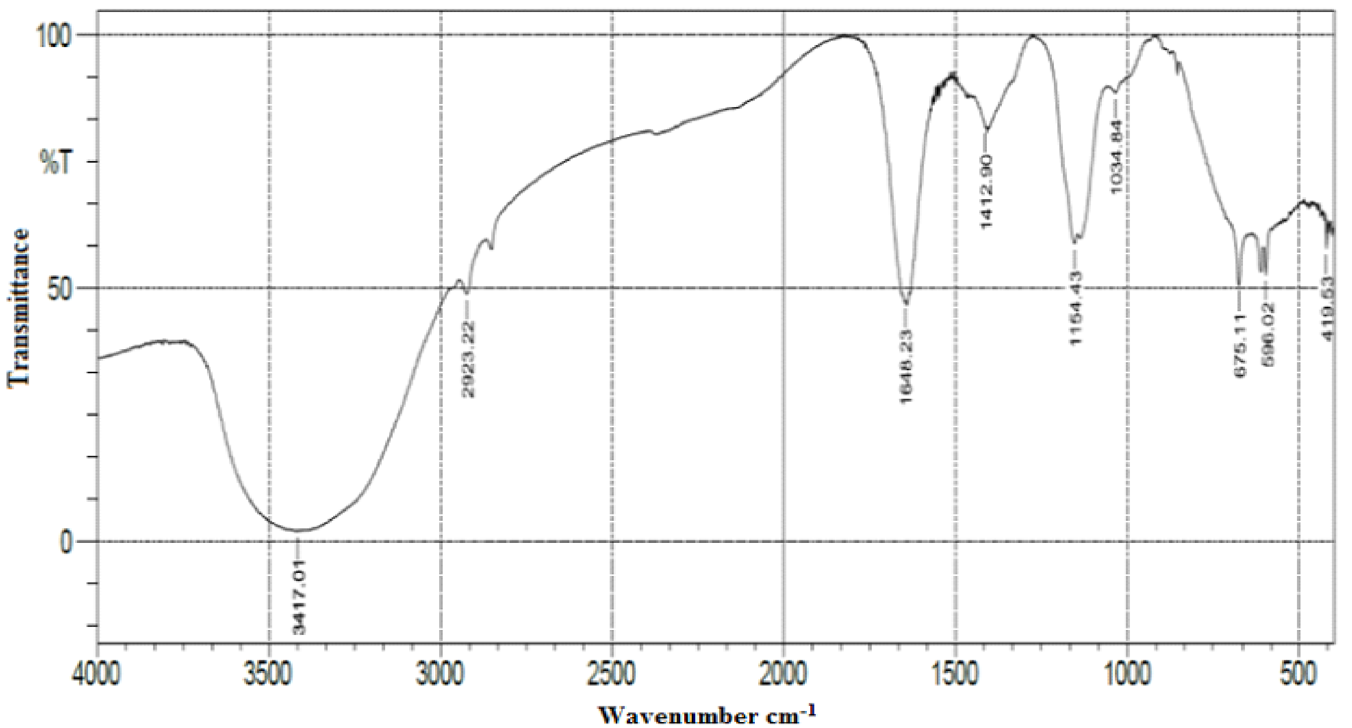

Figure 2: FT-IR Spectrum of Ferrous Nanoparticles Synthesised from *Psidium guajava* Leaf Extract

4.1. Discussion

Researchers synthesized iron nanoparticles using an environmentally friendly method that involved guava (*Psidium guajava*) leaf extract. This extract acted both as a reducing agent helping convert iron ions and a stabilizer for the nanoparticles, all at room temperature (25°C). When the leaf extract was mixed with a solution containing ferric salts, the color changed rapidly from pale yellow to dark brown or black, indicating the immediate formation of nanoparticles due to swift reaction kinetics (Malik et al., 2014). Analysis through UV-Vis spectroscopy confirmed the presence of nanoparticles, with a strong absorption peak observed at 360 nm (Behera et al., 2012). This peak corresponds to surface plasmon resonance (SPR), a phenomenon unique to metallic nanoparticles where collective oscillations of electrons at the particle surface interact with light to produce distinct optical properties. The SPR band also reflects the reduction of iron from Fe^{3+} to Fe^{2+}, facilitated by the bioactive compounds in the guava leaves (Devatha *et al.*, 2016). Altogether, these findings show that phytochemicals in *P. guajava* effectively enable both the formation and stabilization of iron nanoparticles in a clean and sustainable way.

FTIR spectroscopy was employed to identify functional groups associated with iron nanoparticles (FeNPs) synthesized using *Psidium guajava* leaf extract. The spectrum revealed key absorption bands indicative of bioactive compounds involved in nanoparticle formation and stabilization. A broad peak at 3417.01 cm^{-1} was attributed to O–H stretching vibrations, suggesting the presence of hydroxyl groups from polyphenols and carboxylic acids. These groups are known to facilitate hydrogen bonding, enhancing nanoparticle hydrophilicity and stability (Sivakami et al., 2021; Drozdz et al., 2020). The 2923.22 cm^{-1} band corresponded to C–H stretching, typically found in aliphatic chains, implying the involvement of terpenoids or fatty acids in the synthesis process (Fazlzadeh et al., 2017). A signal at 1648.23 cm^{-1} was linked to C=C stretching, likely originating from aromatic compounds such as flavonoids. Between 1412.90 and 1034.84 cm^{-1}, peaks associated with C–O stretching and C–H bending were observed, further supporting the presence of alcohols and aromatic structures. These functional groups are consistent with plant-derived capping agents that stabilize nanoparticles. While additional signals were detected in the fingerprint region, only those central to the synthesis mechanism were emphasized. Collectively, the FTIR data confirm that phytochemicals in *P. guajava* notably polyphenols, flavonoids, and terpenoids played a dual role: reducing Fe^{3+} ions and capping the resulting nanoparticles to prevent aggregation (Kanagasubbulakshmi & Kadirvelu, 2017; Devatha et al., 2016; Pan et al., 2020). These findings align with previous studies demonstrating the efficacy of plant-based molecules in nanoparticle synthesis and colloidal stabilization (Kalainila et al., 2014; Abdullah et al., 2020).

5. Conclusion

This study used *Psidium guajava* leaf extract as a stabilizing and reducing agent to demonstrate the environmentally friendly synthesis and characterization of ferrous nanoparticles (FeNPs). The eco-friendly approach employed aligns with the principles of sustainable nanotechnology, offering a cost-effective, non-toxic, and environmentally benign alternative to conventional chemical and physical synthesis methods. Based on the surface chemistry and functional groups identified particularly the presence of hydroxyl, carboxyl, and aromatic compounds these ferrous nanoparticles exhibit promising properties for practical applications. Their hydrophilic nature and stability in aqueous media make them suitable candidates for water treatment, where they can be used to adsorb heavy metals or catalyze the degradation of organic pollutants. Additionally, the biocompatibility conferred by plant-derived capping agents suggests potential biomedical applications, such as targeted drug delivery, antimicrobial therapies, and imaging contrast agents. These dual capabilities highlight the versatility of green-synthesized ferrous nanoparticles and their potential role in advancing both environmental remediation and healthcare technologies.

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