RESEARCH PAPER

Green synthesis and characterization of iron nanoparticles by using *Grewia asiatica* leaf extract and its in-vitro antioxidant and antilithiatic activities

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Abstract:

Background: Green synthesis offers an eco-friendly and sustainable alternative for nanoparticle production by minimizing the use of hazardous chemicals and energy-demanding processes. Plant-assisted synthesis has gained interest due to its biocompatibility and potential biomedical applications. However, limited information is available on the biological properties of iron nanoparticles synthesized using *Grewia asiatica* leaf extract**. Objectives:** This study aimed to synthesize iron nanoparticles using *Grewia asiatica* leaf extract through a green synthesis strategy and to assess their physicochemical characteristics, antioxidant activity, and antilithiatic efficacy**.Methods:** Iron nanoparticles were produced using an aqueous extract of *Grewia asiatica* leaves as a natural reducing and stabilizing agent. The obtained nanoparticles were characterized through UV–visible spectroscopy, Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), particle size analysis, and zeta potential measurement. Antioxidant potential was evaluated using hydrogen peroxide and DPPH radical scavenging assays, while in vitro antilithiatic activity was evaluated using the inhibition of mineralization method. **Results:** UV–visible spectroscopy showed characteristic absorption peaks at 276 and 374 nm, confirming nanoparticle formation. FT-IR analysis demonstrated the presence of functional groups responsible for nanoparticle stabilization, with Fe–O stretching vibrations confirming iron nanoparticle formation. SEM analysis showed spherical and agglomerated nanoparticles with an average particle size of 173 nm. The zeta potential value of −20.4 mV indicated moderate stability. The synthesized iron nanoparticles exhibited significant antioxidant and antilithiatic activities in comparison with the plant extract alone**. Conclusion:** The study demonstrates a simple, cost-effective, and environmentally friendly technique for preparation of stable iron nanoparticles using extract of *Grewia asiatica* leaves, with promising antioxidant and antilithiatic properties for potential biomedical applications**.**

Key Words: Green synthesis, Grewia asiatica, Iron nanoparticles, FTIR, UV-Vis.

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Introduction

Nanoparticles are defined as particulate suspensions or solid particles with dimension ranging from 10- 1000 nm[1]. They have a high surface area to volume ratio which is their key characteristic responsible for the extensive applications of Nanomaterials[2]. Owing to their extraordinary physical and chemical properties, nanoparticles are employed across various fields of science. They exhibit enhanced catalytic activities and the increased intrinsic reactivity of its surface sites. Among various nano structures, metallic nanoparticles have attracted considerable attention, and are utilized in medical, sensing, electronics, environment, and agriculture sectors[3] .Iron oxides are organic compounds consisting of iron and oxygen. The fabrication of iron oxide nanoparticles through plant materials provide several benefits of environmental safety and compatibility for diverse applications as it avoids use of

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toxic chemicals for the synthesis process. Iron oxides nanoparticles have been explored for numerous biomedical applications including drug delivery, gene transfer. magnetic resonance imaging (MRI), catalysis, detection, diagnosis, and treatment of diseases, such as neurological disease, cancer and cardiovascular conditions due to their small size, biocompatibility, strong magnetism and minimal toxicity[4].There are several reports on the development of synthesis methods to produce iron nanoparticles (INPs), including chemical reduction, vapor-solid growth methods, sol-gel techniques, chemical coprecipitation, and hydrothermal approches. However, these methods generate toxic by-products from the chemical reactions require specialized equipment and elevated temperature. This synthesis procedures negatively pose risk to both environment and human health. The disadvantages of these traditional methods could be overcome with sustainable and environmental-friendly methods by using natural precursors such as plants, bacteria, seeds, algae. Biologically-mediated synthesis of nanoparticles is a simple method. Simply a metal salt in the desired amount is taken and combined with plant extract and the reaction takes few minutes to a few hours and will complete at room temperature. Green synthesis is based on using the secondary metabolites extracted from natural initiator as reducing and stabilizing agent.[5]Grewia asiatica, a species of flowering plant belongs to Malvaceae family, is often referred as phalsa or falsa. Grewia asiatica is widely grown in south Asian regions. The name Grewia honours Nehemiah Grew, one of the founders of plant physiology, whereas asiatica denotes the Asian origin of this species[7] Grewia asiatica (leaf shown in figure 1) is a species native to south Asia from Pakistan, extending east to Cambodia, cultivated primarily for its edible fruit as well as medicinal uses. Fruits are a rich source of nutrients such as proteins, amino acids, vitamins, and minerals, and contain various bioactive constituents, like anthocyanins, tannins, phenolic compounds and flavonoids. Various parts of this plant exhibit different pharmacological activities. Leaves have antimicrobial, anticancer, antiplatelet and antiemetic effects; fruit exhibits anticancer, antioxidant, radioprotective, and antihyperglycemic activities; while stem bark shows analgesic and anti-inflammatory properties[8] .

Fig.1 Leaf and Flower of Grewia asiatica

Phalsa plant is native to the Indian subcontinent and Southeast Asia and also distributed in the forests of central India, South India, Western Himalayas up to an altitude of 3000 ft. It is commercially cultivated mainly in Punjab and in the semi-arid regions of Maharashtra, Gujarat, Rajasthan, Andhra Pradesh, Uttar Pradesh and Haryana[9].Phalsa is a bush or medium sized tree with long slender, drooping branches and young branchlets are covered with hairs. The leaves which are widely spaced are heart shaped or may be ovate, usually apex is pointed, the base is oblique, 8 inch in length and 6 inches in width and coarsely toothed[10].

The fruits are lobed drupes. The fruit change color from green to purplish red and after fully ripened to dark purple. There are two types of fruits of phalsa large fruits contains two hemispherical, hard, buff in color seeds and small fruit bearing a single seed. Phalsa is generally a self-pollinated crop[11].Flowers are yellow in color and grown in cymes of 3-4, are 2 cm in diameter tomentose densely, ribbed[12]. Plant parts are reported to have all the essential mineral elements carbohydrates, proteins, fatty acids, and other active constituents such as flavonoids, phenols, tannins, alkaloids, steroids, triterpenoids, lignans, flavones, anthocyanins etc. [13,14]. *Grewia asiatica* contains anthocyanins mainly type cyanidin 3-glucoside vitamin C, minerals, and dietary fibers [15]. Use and cultivation of phalsa fruit have been mentioned in the ancient Indian literature and it has been utilized for various ailments in the Indian system of medicine. The unripe fruits are believed to eliminate vata, kapha and biliousness. Root bark is employed for treating rheumatism, fruit acts as astringent and stomachic and when unripe it helps to treat inflammation and is used in respiratory, cardiac and blood disorders, and in fever [16]

1. Materials and Methods

2.1. Study Design

This was an experimental in vitro study conducted to synthesize and characterize iron nanoparticles utilising *Grewia asiatica* leaf extract and to evaluate their antioxidant and antilithiatic activities.

2.2. Study Duration

The study was carried out over a period of six months.

2.3. Ethical Approval

Ethical approval was not required for this study as it did not involve human participants or
experimental animals.

2.4 Chemicals and Reagents

Grewia Asiatica from Indo Royal Nursery, Hyderabad, Methanol, Potassium dihydrogen phosphate, Sodium hydroxide, Calcium chloride dihydrate, Potassium mercuric iodide, Potassium iodide, Fehling's solution, Ferric chloride, Copper sulphate all are from S D Fine Chem. Limited, Mumbai, Ferric nitrate nano hydrate, Ascorbic acid, Bismuth nitrate, Benedict's reagent all are from Research lab chem Industries, Mumbai, DPPH, Sodium oxalate from Hi-Media laboratories, Mumbai.

2.5 Instruments

UV -visible spectrometer from Model-uv-1800, Shimadzu, Japan, Centrifuge from Remi equipment's PVT LTD, Mumbai, Analytical Balance from Shimadzu ATX224R, Japan, Water bath, Hot air oven from Bio technics India, FTIR Model-IR affinity- 1, Shimadzu, Japan, SEM from carl zeiss-6027, Germany, Particle size analyzer -Nanotrack Wave, Japan, Rotary Evaporator from Super fit laboratories.

2.6 Preparation of *Grewia asiatica* leaf extract

The plant leaves were washed with tap water and then again with distilled water to remove any residual dust and dirt. The leaves were dried and ground into a coarse powder. Aqueous leaf extract was acquired using Soxhlet extractor with distilled water which was heated for 8 hours at 50 °C and the solvent was evaporated using a rotary evaporator to obtain a gummy mass of plant in the flask. A 50 mL of water were combined with 5 g of the prepared leaf extract. Then the solution was filtered via Whatman's filter paper, the filtrate was collected and preserved at 4 °C for further analysis [1].

2.7 Preparation of Ferric nitrate solution

Ferric nitrate (0.40 g) was dissolved in 1000 mL of distilled water to produce 1 mM solution of ferric nitrate nano hydrate ($FeNO3.9H_2O$) [18].

2.8 Synthesis of Iron nanoparticles

The synthesis of iron nanoparticles was achieved through the utilization of ferric nitrate nano hydrate ($FeNO3.9H_2O$). In a conical flask, 10 mL of plant extract was combined with 100 mL of a 1-mM ferric nitrate nano hydrate solution. The mixture was then let to boil for 3 hours at 50 °C. The color of solution turned black, indicating the formation of nanoparticles shown in the figure 2 and this solution was stored under refrigeration until further analysis[18] .

Fig. 2 (a) Aqueous leaf extract (b) Leaf extract with Ferric nitrate solution (c)Synthesized INP Solution

2.9 Phytochemical Investigation

In accordance with the process described in the Khandelwal book, aqueous leaf extract was subjected to an examination of plant extract to detect different secondary metabolites.[19]

2.10 Test for Alkaloids

a) Mayer's Test: A small quantity of extract was treated with Mayer's reagent. A cream-colored precipitate signifies the presence of alkaloids.

b) Dragendroff's Test: A minute amount of extract was treated with Dragendroff's reagent. A formation of red precipitate signifies the presence of alkaloids.

2.11 Test for Flavonoids

As Lead (II) acetate solution is added to a little amount of crude extract, a yellowish- colored precipitate forms, which suggests the presence of flavonoids.

2.12 Test for phenols

A 5% aqueous ferric chloride was added to a little amount of plant extract, which results in deep blue or black color solution.

2.13 Test for carbohydrates

Benedict's reagent and Fehling A and B reagents were added to the extract and then gently heated. The presence of reducing sugars is indicated by an orange-red precipitate and brick red color respectively.

2.14 Test for saponins

In a test tube, 6 mL of water were added to 2 mL of the extract. The resultant mixture was shaken vigorously and the presence of saponins was verified by the formation of persistent foam.

2.15 Biuret test for proteins

A 3 mL of the plant extract is combined with 4% sodium hydroxide and a few drops of 1% copper sulphate solution. A noticeable appearance of violet or pink color is observed.

2.16 Braymer's tannin test

A 10% alcoholic $FeCl_3$ solution was added to 2.0 mL of the extract and results in the formation of blue or greenish color solution.

2.17 Preparation of reagent

a)Phosphate buffer solution PH 7.4*:* A 250 mL of 0.2 M

KH_2PO_4 and 393.4 mL of M NaOH were combined to form a pH 7.4 phosphate buffer solution. Distilled water was then added to the mixture to get a total volume of 1000 mL.

b)Potassium dihydrogen phosphate (0.2 M) solution: KH_2PO_4 (2.72 g) was dissolved in 100 mL of distilled water to produce a 0.2 M solution.

c)Sodium hydroxide solution (0.1 M) solution: Sodium hydroxide (0.40 g) was dissolved in distilled water to produce final volume of 100 mL.

d)Method: A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer solution (pH 7.4). H_2O_2 solution (0.6 mL) was mixed with 1mL of test compounds (10.0- 50.0 µg/mL). After ten minutes of interval, the H_2O_2 solution's absorbance at 230 nm was measured. This result was compared to a blank solution made out of phosphate buffer without H_2O_2. Furthermore, ascorbic acid was used as the reference compound and its absorbance was compared with that of the sample.

$$\% \, H_2O_2 \text{ activity} = \text{Abs}_{\text{Control}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{Control}} \times 100$$

Where, **Abs control**: Absorbance of control

Abs sample: Absorbance of sample

2.18 DPPH radical scavenging assay[20] [Maryam, S *et al.,* (2024)]

a)**Preparation of DPPH solution (0.1 mM):** Weighed 4 mg of DPPH and mixed with 100 mL of methanol.

b)**Preparation of sample:** Sample solution (extract or compound) in methanol at a suitable concentration is prepared.

c)**Method:** The free radical scavenging activity was determined by 1,1-diphenyl-2-picryl hydrazyl (DPPH). A 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of extract solution at various concentrations (10-50 µg/mL). The mixture was shaken vigorously and permitted to stand at room temperature for 30 min. Then its absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Lower absorbance values of reaction mixture Indicated Higher Free Radical Scavenging Antioxidant Ascorbic Acid. The DPPH solution without sample solution was employed as control. Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$[(A_a - A_1/A_a) \times 100]$$

Where A_a is the absorbance of control reaction (containing all reagents except the test material) and A_1 is the absorbance of sample and standard.

2.19 In Vitro Antilithiatic Activity

Inhibition of mineralization method [21]

Simultaneous flow static model was used to assess the calcium oxalate mineralization inhibition. The sample (50 mL) was taken in a beaker and two salt-forming solutions such as 0.1 M sodium oxalate (50 mL) and 0.1 M calcium chloride dihydrate (50 mL) were added dropwise using burettes to create the reservoir. The chemicals were allowed to drip into a 250 mL beaker at the same time for 35 minutes. Following 10 minutes of boiling on a heating mantle, the mixture was permitted to cool to room temperature. Little volumes were centrifuged at 2000 rpm for 20 minutes. The precipitate was collected and the supernatant was separated in a centrifuge tube that had been previously weighed. Once the precipitate filled tube had dried in a hot air oven at 120 ^{0}c, it was allowed to cool to room temperature and then weighed using a weighing balance until consistent weight was achieved. The weight of the precipitate was recorded. In this experiment set up shown in the figure 3, water was used as the blank and cyst one was used as standard. The percentage efficiency was computed using the following formula.

$$\text{Percentage inhibition} = (W_B - W_S) \, W_B \times 100\%$$

Whereas, W_B stands for the weight of the blank tube, and W_S stands for the weight of test sample tube.

Fig. 3 Set up for antilithiatic activity

3. Results and Discussion

3.1 Preparation of aqueous leaf extract of Grewia asiatica

Dried leaves of Grewia asiatica (100 g) were extracted with distilled water using Soxhlet extractor. The solvent was removed using rotary evaporator. The resulting extract was found to be 21.5 g and percentage yield was found to be 21.5 %.

$$\% \text{ yield of the extract} = \frac{\text{Amount of extract obtained}}{\text{Amount of powder used}} X100$$

$$= \frac{21.5}{100} X \, 100$$

$$= 21.5\%$$

Extract value was found to be 21.5% w/w

3.2 Preliminary phytochemical analysis

The preliminary phytochemicals analysis of *Grewia asiatica* leaf extract indicated the presence of following phytochemicals.

Table 1: Preliminary phytochemical analysis

Phytoconstituents	Results*

Alkaloids	+
Carbohydrates	+
Flavonoids	+
Phenols	+
Proteins	-
Saponin	+
Tannins	+

*'+' indicates positive, '-'indicates negative

3.3 Characterization of green synthesized Iron nanoparticles

The Iron nanoparticles were synthesized using an extract from the leaves of *Grewia asiatica*. The generation of Iron nanoparticles was confirmed with a naked eye. The color of Ferric nitrate solution changed from light brown to black. The aqueous leaf extract act as reducing agent, converting (Fe^{+}) to (Fe^{0}), which changes the color of the solution. This illustrated the beneficial plant compounds that are utilized to make Iron nanoparticles.

Fig. 4 Mechanism of Iron nanoparticles synthesis

3.4 Characterization of Iron nanoparticles by UV -Visible Spectroscopy

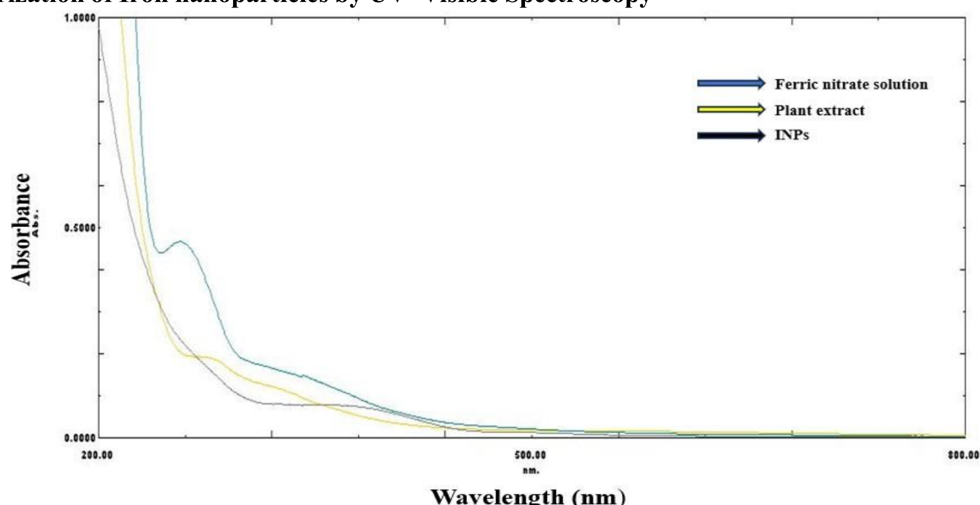

Fig. 5 UV spectrum of Ferric nitrate, Plant extract and INPs

The spectral range of 200 -800 nm has been used to study the formation of INPs. Surface plasmon resonance is a characteristic of INPs synthesis in solution. The absorbance peak for the ferric nitrate solution was found at 257 nm, for aqueous leaf extract was observed at 276 nm, while the Iron nanoparticles were detected at 374 nm, indicating that INPs have formed

3.5 Characterization of INPs by Fourier Transform Infrared spectroscopy

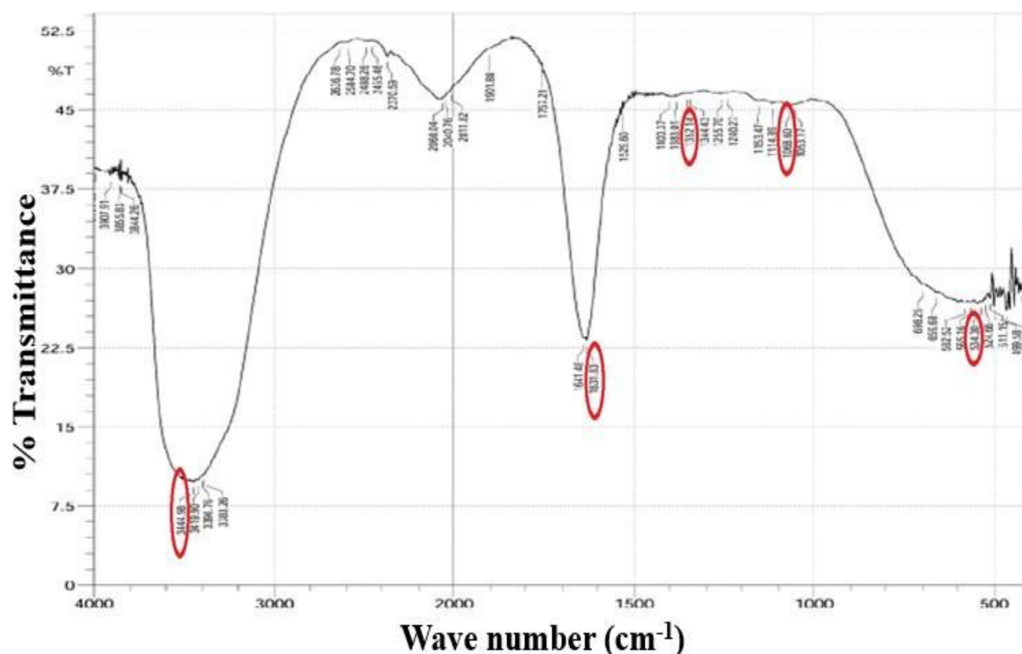

Fig. 6 FTIR spectrum of *Grewia asiatica* leaf extract

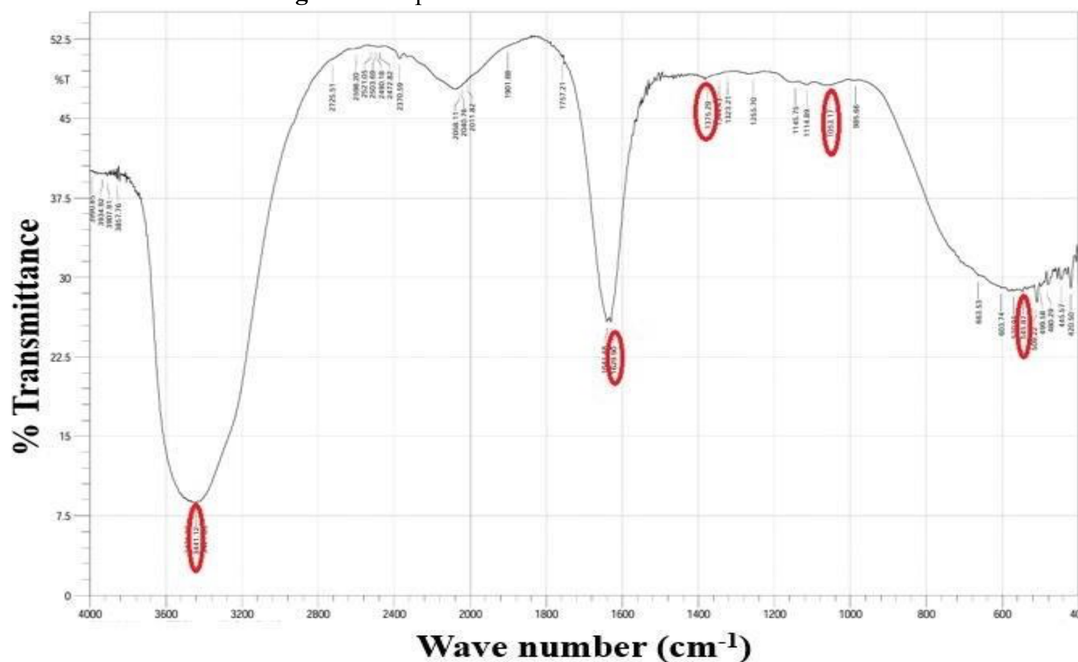

Fig. 7 FTIR spectrum of synthesized Iron nanoparticles

The functional groups which are responsible in capping of nanoparticles can be examined using FTIR technique. The wavelength range was set with in the region of 4000 cm^{-1} to 400 cm^{-1}. The difference in the spectra may serve as evidence of the transition. The FTIR spectrum for plant extract and synthesized iron nanoparticles roughly has the same pattern. Decrease in O-H group wavenumber from 3444 cm^{-1} to 3441 cm^{-1} and from 1631 to 1629 cm^{-1} of C=C stretching indicates the generation of Iron nanoparticles. The results of the FTIR studies (shown in figure 6,7) demonstrated that the extract's secondary metabolites produced strong layering on the nanoparticles.

The FTIR spectra, as illustrated in Figures 4.2 and 4.3, showed a broad absorption band in the 3471–3421 cm^{-1} region, which is associated to the O–H stretching vibrations of H-bonded polyphenols. These polyphenols have high antioxidant properties, which enable them to lower Fe^{+} ions and then adsorb on the surface of Iron nanoparticles. This provides a protective layer shields the Iron nanoparticles from contact to atmospheric oxygen, thereby improving their shelf life. Polyphenols have the potential to serve as both a capping and a reducing agent. In addition, the bands at 1631cm^{-1} and 1629 cm^{-1} (C=C ring), 1352 cm^{-1}and1375 cm^{-1} (aliphatic C-H bend), 1058 cm^{-1} and 1053 cm^{-1} (C–O–C stretching vibrations) of the plant extract and Iron nanoparticles respectively, signifying water soluble phytochemicals. Thus, the spectral analysis demonstrated that the polyphenols are an essential

constituent of the Iron nanoparticles formation in addition to adhering to their surface. Furthermore, weak band around 534 cm^{-1}, 545 cm^{-1}, 638 cm^{-1}, 663 cm^{-1} due to Fe–O stretching vibrations confirm the generation of Iron oxide nanoparticles from Grewia asiatica leaf extract.

3.6 Characterization of Iron nanoparticles by scanning electron microscopy

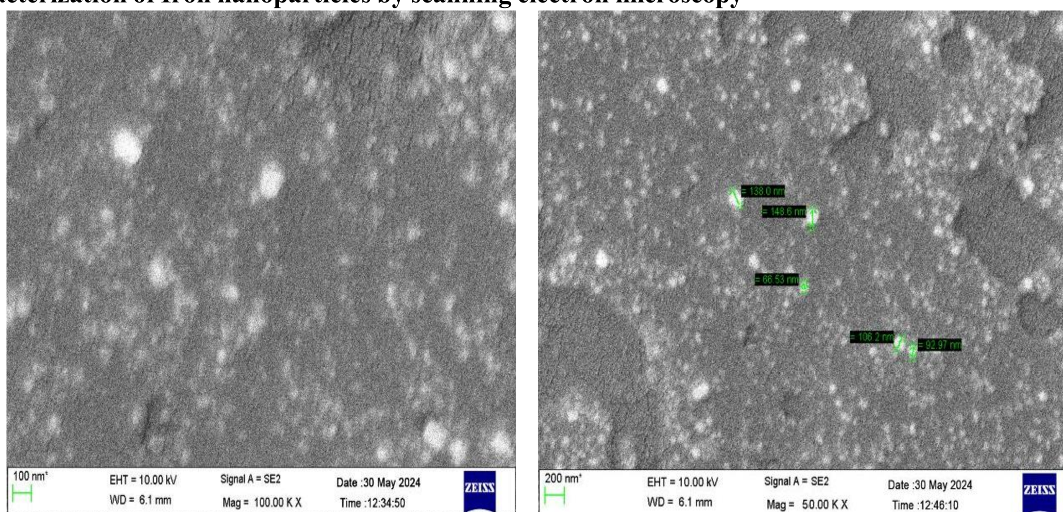

Fig. 8 SEM Images of Iron nanoparticles

The morphology, surface characteristics of the INPs determined through SEM analysis shown in figure 8. SEM was used to identify the structure of biologically produced INPs at different sizes and resolutions.
The SEM image of INPs is presented in above Figures. The evaluation of SEM image reveal that nanoparticles are clustered and tend to developed surface irregular spherical particles.
The surfaces of aggregated nanoparticles were observed to be uneven. The fact that iron nanoparticles exist in close proximity with each other is attributed to the magnetic characteristics of iron species.

3.7 Characterization of Iron nanoparticles by particle size analysis

Using a particle size analyzer, the average particle size, size distribution, and polydispersity index (PDI) of the generated iron oxide nanoparticles were calculated. The results can be seen in Figure 4.7. It was found that the Iron nanoparticles mean particle size was 173 nm. The polydispersity index was determined to be 0.233, which is a good result. The dispersion of Iron nanoparticles is more uniform and has a narrow distribution range, according to the data.

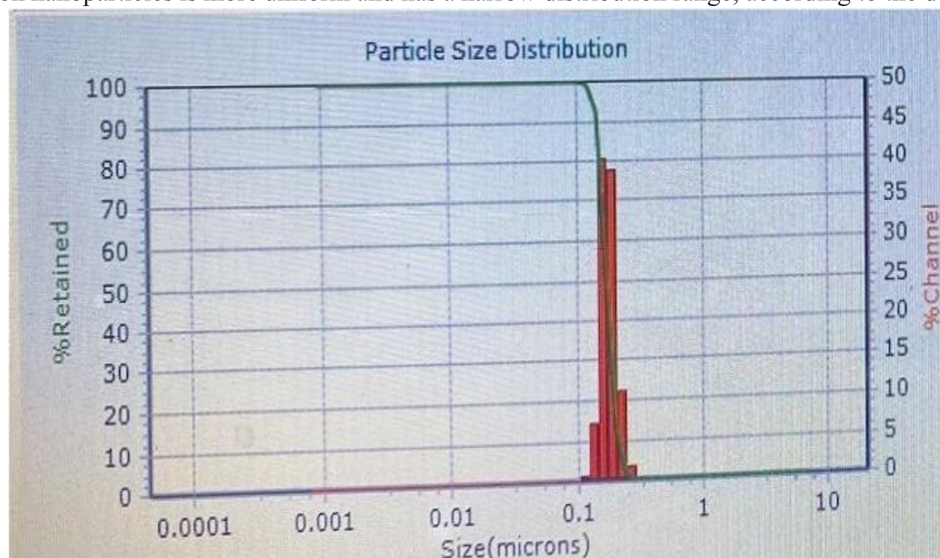

Fig. 9 Particle size distribution of iron nanoparticle solution

3.8 Characterization of Iron nanoparticles by Zeta potential

The Zeta potential of Iron nanoparticles was detected to be -20.4 mV (Figure 4.8) It demonstrates the remarkable stability of produced NPs caused by their negative surface charge.

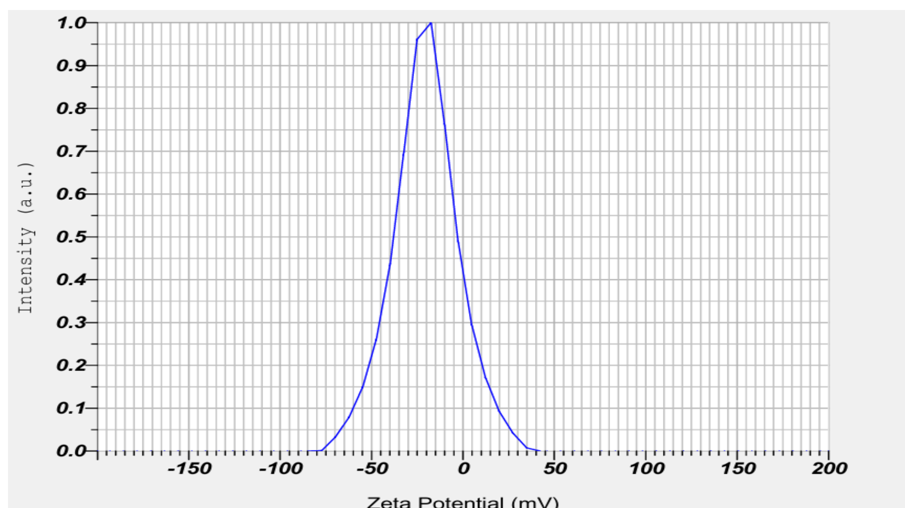

Fig. 10 Zeta potential value for Iron nanoparticles solution of G. asiatica Obtained

3.9 Hydrogen Peroxide Radical Scavenging Assay

Table 2 Antioxidant activity of Grewia asiatica by using hydrogen peroxide radical scavenging assay

S.no	Compounds	Concentration (µg/mL)	%Inhibition	IC50 Value (µg/mL)
1	Aqueous leaf extract of *Grewia asiatica*	10	29.22±2.87	28.49
		20	36.65±4.56	
		30	43.75±6.92	
		40	53.71±6.41	
		50	65.03±1.85	
2	Iron Nanoparticles solution	10	28.71±1.14	25.66
		20	44.42±0.81	
		30	52.36±1.03	
		40	58.61±1.31	
		50	65.87±0.15	
3	Ascorbic Acid	10	30.40±2.00	19.44
		20	41.89±1.00	
		30	49.49±1.09	
		40	55.06±0.56	
		50	63.51±0.62	

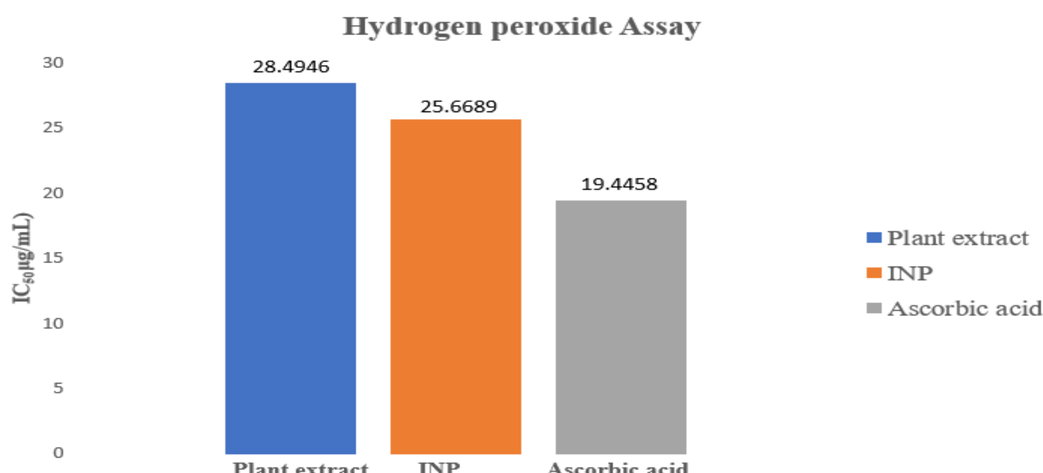

Fig. 11 H_2O_2 scavenging assay for GA and its synthesised Iron nanoparticles

An assay for H_2O_2 scavenging was used to confirm the extract's antioxidant activity. The standard antioxidant, ascorbic acid, demonstrated a considerable and dose-dependent reduction of hydrogen peroxide radicals, with an IC50 value of 19.44 µg/mL. Similarly, the IC50 values (28.49 and 25.66 µg/mL) of the leaf extract and Iron nanoparticle solution respectively, demonstrated dose-dependent supression of free radicals, which was comparable to the standard.

3.10 DPPH Radical Scavenging Assay

Table 3 Antioxidant activity of Grewia asiatica by using DPPH radical scavenging assay

S.no	Compounds	Concentration (µg/mL)	%Inhibition	IC50 Value (µg/mL)
1	Aqueous leaf extract of *Grewia asiatica*	10	19.93±0.18	
		20	29.74±0.57	
		30	34.24±0.69	
		40	39.87±0.36	33.83
		50	53.211±0.75	
2	Iron Nanoparticles solution	10	26.52±3.69	
		20	34.88±2.09	
		30	45.33±4.18	
		40	52.09±3.85	26.28
		50	58.36±5.94	
3	Ascorbic Acid	10	25.24±0.64	
		20	34.08±0.74	
		30	41.80±0.20	
		40	49.35±0.23	24.83
		50	54.82±0.44	

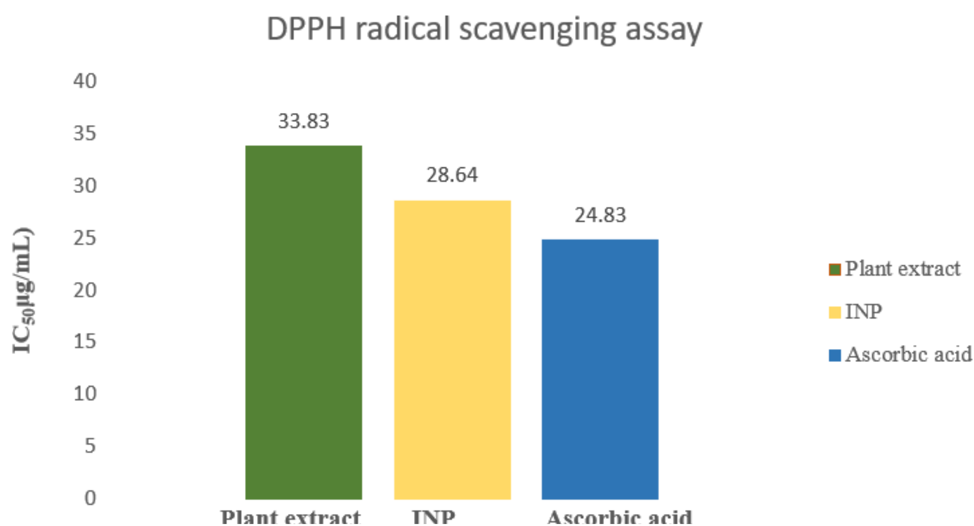

Fig. 12 DPPH scavenging Assay for GA and its synthesised iron nanoparticles

The results of the DPPH scavenging assay are shown in Fig. 12 as percentage supression values at different concentrations. According to the findings, Aqueous leaf extract had a DPPH radical scavenging potential of 33.83 µg/mL, whereas INPs had a value of 26.28 µg/mL. The ascorbic acid (standard) was detected to have a radical scavenging potential of 24.83 µg/mL. It has been detected that when the concentration increases, the percentage of supression increases. The INPs exhibited significant scavenging activity, and their results were compared to that of standard.

3.11 Invitro antilithiatic activity

In vitro antilithiatic activity was assessed using the Inhibition of mineralization method. This technique demonstrated 49.58% inhibition of calcium oxalate for the standard drug cyst one, 36.53% inhibition for the leaf extract, and 41.07%inhibition for the solution containing iron nanoparticles. The inhibitory effect of the iron nanoparticle solution was greater than that of the leaf extract.

Table 4 In-vitro antilithiatic activity of G. asiatica

S.no	Compound	% Inhibition
1	Aqueous leaf Extract of *Grewia asiatica*	36.53
2	INP	41.07
3	Standard (Cystone)	49.58

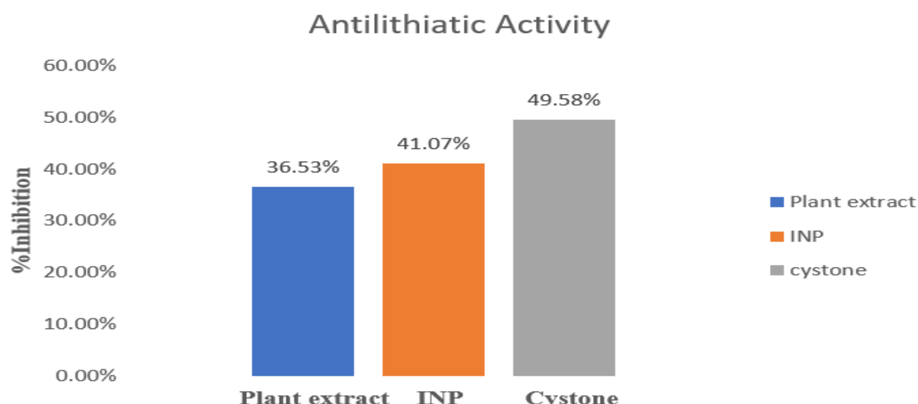

Fig.13 Inhibition of mineralization by Aqueous leaf extract of *Grewia asiatica* and its synthesized INP.

4. CONCLUSION

In conclusion, iron nanoparticles (INPs) have been successfully synthesised by a green synthesis method using *Grewia asiatica* leaf extract. This economical, reliable, low toxic, environmentally friendly approach to synthesis has the potential to enhance the ecofriendly production for the reduction of Fe^{+} into Fe^{0} and the stabilization of INP's.

UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR), Scanning electron microscope (SEM), particle size distribution, zeta potential were employed to characterize the produced INPs. UV-visible spectroscopy exhibited an absorption peak at 374 nm for iron nanoparticles. The presence of a functional groups was verified by FTIR spectrum analysis. The development of spherical and uniform nanoparticles was demonstrated by SEM analysis. The particle size analyser indicated a particle size of 173 nm and zeta potential value was -20.4 mV. notable antioxidant and antilithiatic activity have been shown by Iron nanoparticles. This environmentally friendly technique was simple to carryout for develop highly potent iron nanoparticles which is considered alternative to conventional chemical synthesis.

Zeta potential is an important tool for evaluating the condition of a nanoparticle's surface and predicting whether the particles distributed in a solution will remain stable over time. The stability, dispersion, and surface charge of the nanoparticles are explained by the zeta potential. The capping activity of the biomolecules in the *G. asiatica* leaf extract may be the cause of the negative potential value. Particle concentration, electrical conductivity, and pH all have a influence impact on the zeta potential of INPs. The biosynthesized INPs possess a high level of stable antioxidant activity, as indicated by zeta potential studies.

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Conflict of Interests

The authors declare no conflict of interest.

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