



Green Fabrication of Zero valent Iron Nanoparticles (nZVI) from *Senna auriculata* Flower Extract for Antidiabetic Activity

Veeramalli Naveen Sahith¹, J. Aravind Kumar^{2*}, D. Lakshmi³, M. Renukadevi⁴,
Vasamsetti Sai Sruthi¹ and Sorimuthu Revathi⁵

¹Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Science (SIMATS), Chennai, TN, India

²Department of Energy and Environmental Engineering, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Science (SIMATS), Chennai, TN, India

³Department of CSE, Panimalar Engineering College, Chennai, TN, India

⁴Department of CSE, Sethu Institute of Technology, Kariapatti, Virudhunagar, TN, India

⁵Department of Emergency Medicine, Saveetha Institute of Medical and Technical Science, Thandalam, TN, India

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*aravindkumarj.sse@saveetha.com



ABSTRACT

The present study aims to develop an eco-friendly and biocompatible nanoscale zero-valent iron nanoparticle system using *Senna auriculata* flower extract and to evaluate its antioxidant and antidiabetic potential for possible biomedical applications. *S. auriculata* flower extract was used as a green reducing and capping agent for the synthesis of Zero valent Iron Nanoparticles (nZVI). The presence of phytochemicals such as flavonoids, phenolics, and glycosides in the extract supported nanoparticle formation and stabilisation. The synthesised nanoparticles were characterised using UV–Vis spectroscopy, FTIR, dynamic light scattering, scanning electron microscopy, and energy-dispersive X-ray analysis to confirm their structural, functional, and morphological properties. The characterisation results confirmed the formation of spherical, nanoscale, surface-functionalised nZVI nanoparticles with good colloidal stability. In vitro analysis showed significant antioxidant activity, with DPPH radical scavenging reaching 87.2% at 500 µg/mL. The nanoparticles also exhibited notable antidiabetic activity through dose-dependent inhibition of α -amylase and α -glucosidase, with IC₅₀ values of 220 µg/mL and 235 µg/mL, respectively. The enhanced bioactivity may be attributed to the synergistic interaction between the nanoscale iron core and bioactive phytochemicals present in *S. auriculata* extract. The findings indicate that *S. auriculata*-mediated nZVI nanoparticles may serve as a sustainable and biocompatible platform for developing antidiabetic therapeutic agents. Future studies should focus on in vivo evaluation, toxicity profiling, pharmacokinetic studies, and formulation development to validate their safety, efficacy, and clinical applicability.

Keywords: nZVI; *Senna auriculata*; Green synthesis; Antidiabetic activity; Antioxidant nanomaterials.

1. INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterised by persistent hyperglycaemia, has emerged as one of the most pressing global health challenges of the 21st century. According to the International Diabetes Federation, the prevalence of diabetes is projected to exceed 700 million individuals by 2045, imposing a substantial burden on healthcare systems worldwide (Hossain *et al.* 2024). Chronic hyperglycaemia in diabetes leads to a range of complications, including neuropathy, nephropathy, retinopathy, cardiovascular diseases, and impaired wound healing, significantly reducing quality of life and life expectancy (Hussain, 2025). While conventional pharmacological interventions such as insulin and oral hypoglycaemic agents remain the primary therapeutic options, they are often accompanied by adverse side effects, patient non-compliance, and limited capacity to prevent long-term complications (García-Pérez *et al.* 2013). These challenges underscore the need for novel, safe, and

effective therapeutic strategies that can manage blood glucose levels efficiently while minimising undesirable effects (Caturano *et al.* 2024).

Traditional medicinal systems have long recognised the therapeutic potential of plants in the management of diabetes. One such plant, *Senna auriculata* (family Fabaceae), commonly referred to as “Avaram” in South India, has been extensively documented in Ayurvedic and folk medicine for its antidiabetic properties (Nambirajan *et al.* 2018). The flowers of *S. auriculata* have historically been used to treat hyperglycaemia due to their rich phytochemical composition, which includes flavonoids, phenolic compounds, tannins, and glycosides. These bioactive constituents have demonstrated potent antioxidant, anti-inflammatory, and glucose-lowering effects in both in vitro and in vivo studies (“Comprehensive Analysis of *Senna auriculata* and Its Pharmacological Biomedical

Applications,” 2025). The therapeutic efficacy of *S. auriculata* flowers is primarily attributed to their ability to enhance insulin secretion, improve glucose uptake, and regulate carbohydrate-metabolising enzymes (Rahman *et al.* 2024). Despite these advantages, the direct use of plant extracts faces limitations such as poor bioavailability, instability under physiological conditions, and variable pharmacokinetics, which constrain their clinical applicability (Sanjai *et al.* 2024). Nanotechnology, the interdisciplinary science dedicated to engineering materials at the nanoscale (1–100 nm), offers transformative potential to overcome these limitations (Bayda *et al.* 2019). Nanoparticles possess unique physicochemical properties, including high surface area-to-volume ratio, tunable reactivity, and enhanced biological interactions, making them highly suitable for biomedical applications (Gujjar *et al.* 2025). Among these, Zero valent Iron Nanoparticles (nZVI) have garnered significant attention due to their strong redox reactivity, biocompatibility, and cost-effectiveness. Beyond their traditional applications in environmental remediation, nZVI are increasingly explored for biomedical purposes, including metabolic disease management (Rodríguez-Rasero *et al.* 2024). By integrating bioactive plant compounds with nZVI, it is possible to enhance therapeutic efficacy while improving stability, bioavailability, and targeted delivery of natural products (Afonso *et al.* 2024). Despite the well-documented antidiabetic potential of *Senna auriculata* and the growing interest in nZVI, limited studies have explored the integration of *S. auriculata* phytochemicals with nZVI for diabetes management. Previous research has largely focused on either crude plant extracts or chemically synthesised nanoparticles, often neglecting the synergistic enhancement of bioactivity, improved stability, and controlled delivery achievable through biogenic nanoparticles (Nille *et al.* 2021). Moreover, systematic optimisation of green synthesis parameters such as extracts concentration, pH, temperature, and reaction time to control nanoparticle size, morphology, and surface functionalisation remains underexplored (Konkal *et al.* 2026). Addressing these gaps, the present study proposes the eco-friendly synthesis of nZVI using *S. auriculata* flowers, aiming to combine the intrinsic antidiabetic properties of the plant with the unique physicochemical advantages of nanoparticles. The novelty of this work lies in its integration of traditional medicinal knowledge with green nanotechnology, providing a multifunctional, environmentally sustainable therapeutic strategy, while investigating mechanistic antidiabetic effects, enhancing bioavailability, and advancing the translational potential of plant-based nanomedicine.

Green synthesis of nanoparticles using plant extracts has emerged as an environmentally sustainable and biocompatible alternative to conventional chemical or physical methods (Saxena *et al.* 2025). Unlike chemical synthesis, which often relies on toxic reducing

agents and stabilisers, plant-mediated synthesis employs phytochemicals as both reducing and capping agents, offering a safe and cost-effective approach (Hanna *et al.* 2025). The flowers of *Senna auriculata*, rich in reducing sugars, flavonoids, and polyphenols, provide an ideal reservoir for the biogenic synthesis of nZVI. During the synthesis process, iron ions ($\text{Fe}^{2+}/\text{Fe}^{3+}$) are reduced to elemental iron (Fe^0) nanoparticles, while the phytochemicals simultaneously stabilise and functionalise the particle surfaces (Anuradha and Raji, 2022). This method not only minimises environmental hazards but also imparts bioactive molecules onto the nanoparticle surface, potentially enhancing antidiabetic activity through synergistic effects. Biosynthesised nZVI can modulate glucose metabolism via several mechanisms. These include inhibition of carbohydrate-hydrolysing enzymes such as α -amylase and α -glucosidase, stimulation of pancreatic β -cells to enhance insulin secretion, and mitigation of oxidative stress in cellular systems (Srinivasan and Rana, 2024). The nanoscale size of nZVI facilitates efficient cellular uptake, prolonged circulation, and controlled release of plant-derived bioactive compounds, thereby addressing the pharmacokinetic limitations of crude extracts. Additionally, surface functionalisation with *S. auriculata* phytochemicals can enhance antioxidant defence, reduce inflammatory responses, and improve endothelial function, all critical factors in diabetes management and complication prevention (Kamyab *et al.* 2025).

Eco-friendly fabrication of nZVI aligns with the principles of green chemistry and environmental nanotechnology. Sustainable synthesis reduces hazardous waste, minimises energy consumption, and eliminates the use of toxic solvents, making plant-mediated nanoparticle synthesis both scientifically innovative and environmentally responsible. By combining the intrinsic antidiabetic properties of *S. auriculata* flowers with the unique physicochemical characteristics of nZVI, it is possible to develop multifunctional nanomaterials that offer therapeutic efficacy, biocompatibility, and environmental sustainability (Sathish *et al.* 2024). Despite their promising potential, systematic optimisation of nZVI nanoparticle synthesis considering extract concentration, pH, temperature, and reaction time is crucial to control size, morphology, and surface chemistry. Detailed characterisation using UV-Vis spectroscopy, FTIR, DLS and SEM, alongside rigorous in vitro and in vivo evaluation, is essential to elucidate mechanistic interactions and ensure safety and efficacy. Integrating traditional medicinal knowledge with nanotechnology, *Senna auriculata* flowers provide a sustainable source for green synthesis of nZVI (Syed *et al.* 2026). These biogenic nanoparticles are expected to exhibit enhanced antidiabetic activity, combining nanoscale properties with bioactive phytochemicals, advancing environmentally responsible therapeutic strategies for diabetes.

2. MATERIALS AND METHODOLOGY

2.1 Materials

Fresh flowers of *Senna auriculata* were collected from authenticated sources in Nandyal District, Andhra Pradesh, India (Latitude: 15.4750° N, Longitude: 78.4910° E). The flowers were thoroughly washed with distilled water to remove dust and debris, then air-dried at room temperature. Analytical-grade ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was obtained from Sisco Research Laboratories Pvt. Ltd. (SRL), India, and used without further purification. Deionised water was used throughout the experiments. Reagents for *in vitro* antidiabetic assays, including α -amylase, α -glucosidase, and DPPH, were obtained from Sigma-Aldrich.

2.2 Green Synthesis of nZVI Nanoparticles

nZVI nanoparticles were synthesised using a green approach with *Senna auriculata* aqueous extract as the reducing and stabilising agent. A 1 M $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution was prepared, and the plant extract was obtained by heating finely powdered dried flowers in distilled water at 80 °C for 60 minutes, followed by filtration (Rahman *et al.* 2024). The FeSO_4 solution was placed on a magnetic stirrer, and the plant extract was added dropwise using a Pasteur pipette in a 1:1 volume ratio under continuous stirring. The reaction mixture gradually changed colour from light yellow to dark black, indicating the reduction of Fe^{2+} to Fe^0 nanoparticles

(Ramadevi *et al.* 2026; Fazlzadeh *et al.* 2016). The formed nZVI particles were separated by centrifugation at 10,000 rpm for 15 minutes. The collected pellet was dried in a hot-air oven at 70 °C for 24 hours to prevent degradation of bioactive compounds and subsequently stored at 4 °C until further use (Dinsa and Mequanint, 2025). A schematic overview of the green synthesis and characterization procedure of nZVI nanoparticles is provided in Fig. 1. All experiments were performed in triplicate ($n = 3$) to ensure reproducibility.

2.3 Characterization of Synthesised nZVI Nanoparticles

The physicochemical properties of the green-synthesised nZVI nanoparticles were characterised using a combination of spectroscopic and microscopic techniques. Fourier-transform infrared spectroscopy (FTIR) was employed to identify functional groups from the *Senna auriculata* extract capping the nanoparticle surface, confirming stabilisation. Dynamic light scattering (DLS) analysis was performed to determine the hydrodynamic diameter and particle size distribution of the colloidal suspension, while zeta potential measurements were conducted to evaluate the surface charge of the particles. Scanning electron microscopy (SEM) provided morphological insights, revealing particle shape, surface texture, and aggregation patterns. UV-Vis spectrophotometry to confirm their optical properties, with absorbance measured between 200 and 800 nm.

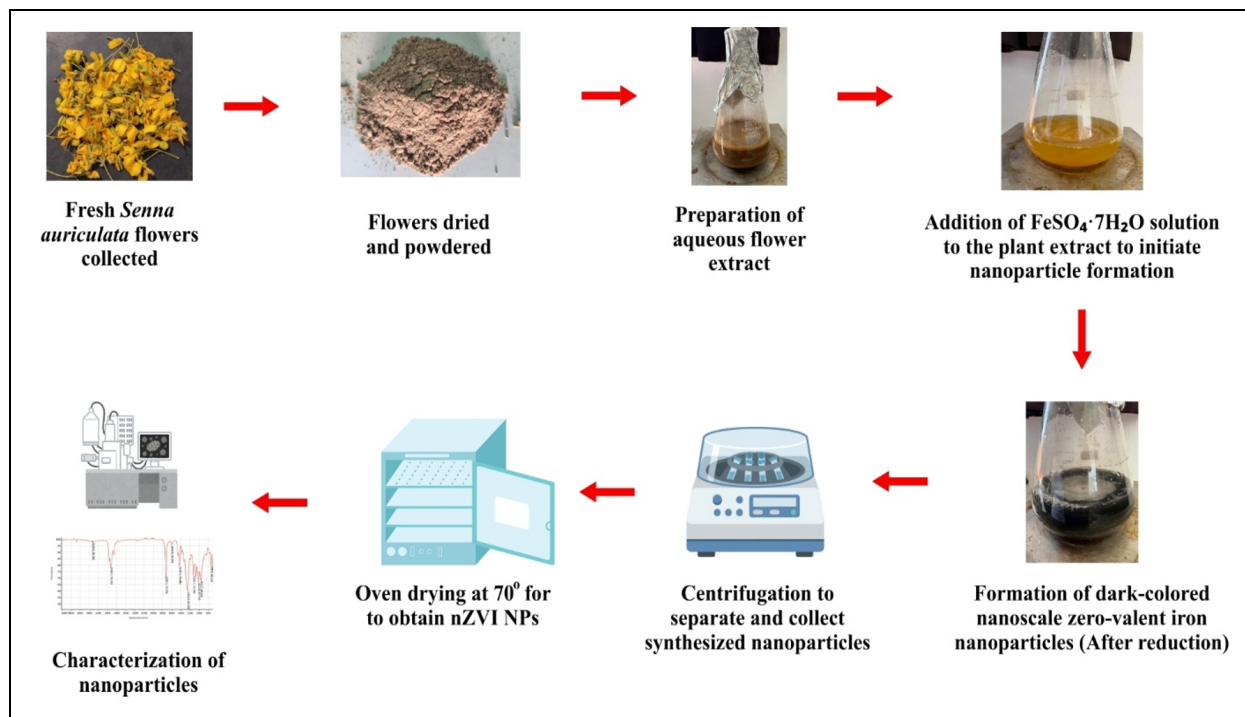


Fig. 1: Schematic illustration of green synthesis and characterization of nZVI nanoparticles using *Senna auriculata* flower extract

2.4 Evaluation of Antioxidant Potential via DPPH Radical Scavenging Assay

The antioxidant potential of green-synthesised nZVI nanoparticles was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Nanoparticle dispersions were prepared at six concentrations (10, 25, 50, 100, 250, and 500 µg/mL) in methanol. To each 1 mL of 0.1 mM DPPH methanolic solution, 1 mL of the corresponding nanoparticle dispersion was added. The mixtures were incubated at room temperature in the dark for 30 min to facilitate the scavenging reaction.

Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid served as the positive control, and methanol as the blank. The percentage of radical scavenging activity was calculated using:

$$\% \text{Scavenging} = 100 - \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}})}{\text{Abs}_{\text{control}}} \times 100$$

where $\text{Abs}_{\text{sample}}$ is the absorbance of the nanoparticle-treated DPPH solution, $\text{Abs}_{\text{blank}}$ is the absorbance of methanol, and $\text{Abs}_{\text{control}}$ is the absorbance of DPPH solution without nanoparticles (Akalya *et al.* 2026; Ramadevi *et al.* 2026).

2.5 Determination of α -Amylase Inhibitory Activity

The α -amylase inhibitory activity of nZVI nanoparticles was determined following a standard enzymatic assay. Nanoparticle dispersions were prepared at six concentrations (15.625, 31.25, 62.5, 125, 250, and 500 µg/mL) in phosphate buffer (0.02 M, pH 6.9) containing 0.006 M NaCl.

An aliquot of 200 µL of each nanoparticle dispersion was pre-incubated with 200 µL of α -amylase solution (2 U/mL) at 30 °C for 10 min. Subsequently, 200 µL of 1% starch solution was added and incubated for 3 min. The reaction was terminated by adding 200 µL of dinitrosalicylic acid (DNSA) reagent, followed by heating in a boiling water bath at 85 °C for 10 min. After cooling to room temperature, 5 mL of distilled water was added, and absorbance was measured at 540 nm. Acarbose served as the positive control, while phosphate buffer without nanoparticles was used as the negative control. The percentage inhibition of α -amylase was calculated using:

$$\% \text{Inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

2.6 Determination of α -Glucosidase Inhibitory Activity

The α -glucosidase inhibitory potential of nZVI nanoparticles was assessed using p-nitrophenyl- α -D-glucopyranoside (pNPG) as substrate. Nanoparticle dispersions were prepared at the same six concentrations as above. In each assay, 100 µL of α -glucosidase solution (1 U/mL) was pre-incubated with 100 µL of nanoparticle dispersion at 37 °C for 10 min. The reaction was initiated by adding 100 µL of 5 mM pNPG solution and further incubated at 37 °C for 30 min. The reaction was terminated by adding 1 mL of 0.1 M Na_2CO_3 . Absorbance was measured at 405 nm. Acarbose was used as the positive control, and buffer without nanoparticles as the negative control. Inhibition was calculated using the same formula as α -amylase inhibition:

$$\% \text{Inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

3. RESULTS AND DISCUSSION

The green synthesis of nanoscale zero-valent iron (nZVI) nanoparticles was successfully accomplished using *Senna auriculata* flower extract, as evidenced by the visible transformation of the reaction mixture from pale yellow to dark black.

3.1 Characterisation of Surface Functional Groups Using FTIR Spectroscopy

The FTIR spectral analysis was employed to identify the chemical functionalities present on the surface of the green-synthesised nZVI nanoparticles. A broad absorption at 3324 cm^{-1} corresponds to O–H stretching of hydroxyl groups from phenolics and flavonoids, which function as reducing agents and stabilisers (Ramu and Elangeeran, 2025). The peak at 2929 cm^{-1} indicates C–H stretching of aliphatic groups, reflecting the presence of residual organic moieties capping the nanoparticle surface (Ullah *et al.* 2025). Carbonyl C=O stretching at 1729 cm^{-1} suggests binding of biomolecules, providing surface stabilisation, while peaks at 1602 cm^{-1} and 1431 cm^{-1} are attributed to C=C aromatic stretches and O–H bending, further confirming the involvement of polyphenolic compounds. In the fingerprint region, peaks between 1254–617 cm^{-1} correspond to C–O, C–H bending, and Fe–O vibrations, indicating minor surface oxidation of Fe^0 to iron oxides (Fig 2). Collectively, these observations demonstrate that the phytochemicals in *Senna auriculata* effectively reduce Fe ions and cap the nanoparticles, yielding stable nZVI particles with surface functionalities that enhance biocompatibility and potential for environmental applications.

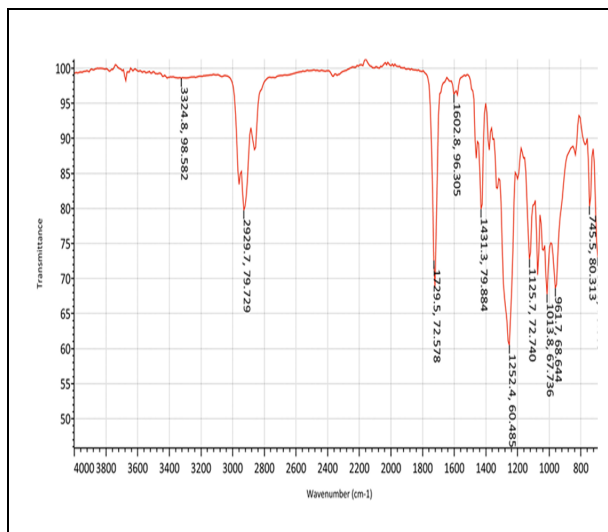


Fig 2: FTIR spectrum of nZVI nanoparticles

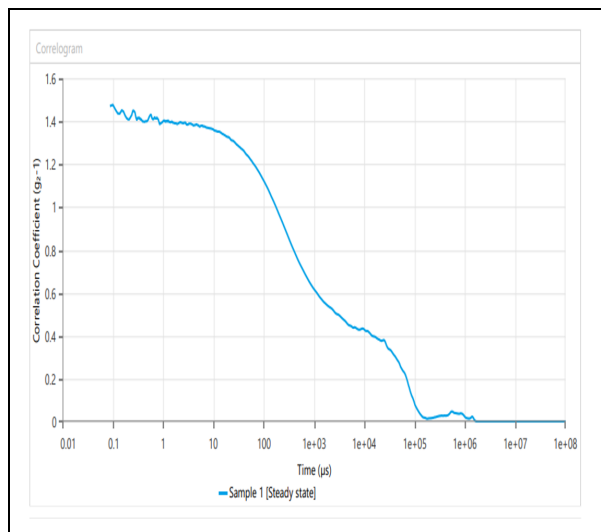


Fig. 4b: Dynamic Light Scattering (DLS) analysis graph of synthesized nZVI

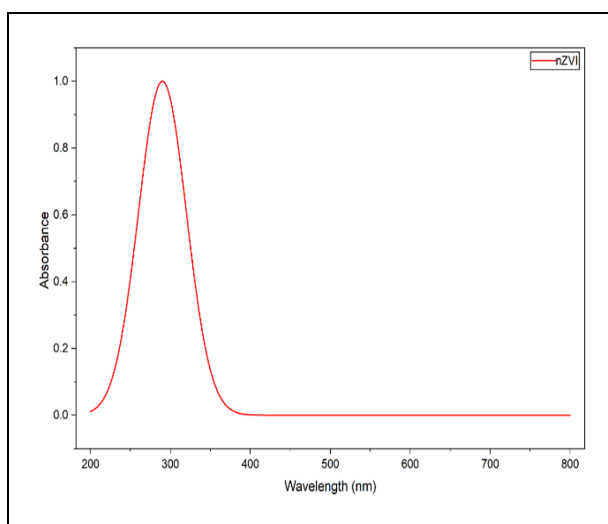


Fig. 3: UV-Vis absorption spectrum of the green-synthesised nZVI nanoparticles

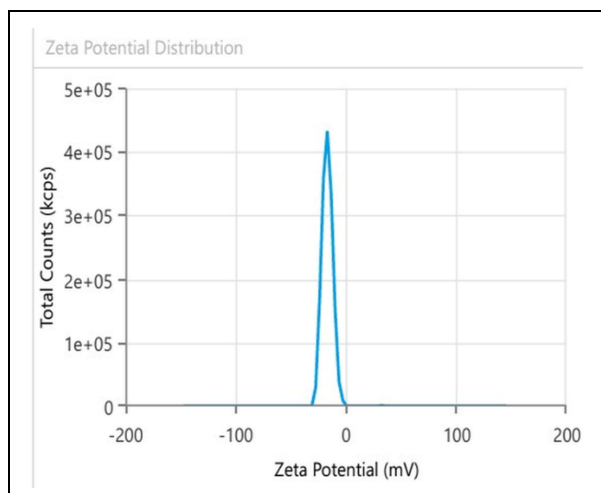


Fig. 4c: Zeta potential distribution of synthesised nZVI

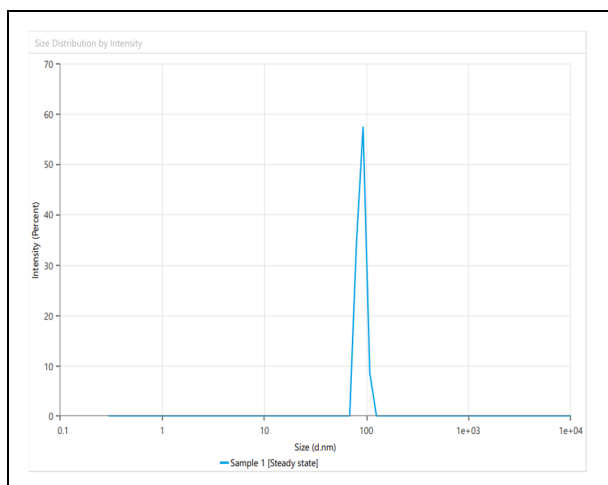


Fig. 4a: Dynamic Light Scattering (DLS) analysis graph of synthesized nZVI

3.2 UV-visible Spectroscopy

The UV-Vis absorption spectrum of the green-synthesised nZVI nanoparticles exhibits a distinct peak centred around 300 nm, as shown in Fig 3, which is consistent with previously reported green-synthesized nZVI systems (Rahman *et al.* 2023). This absorption confirms the formation of nZVI particles and indicates their nanoscale dimensions, as the SPR phenomenon arises from the collective oscillation of conduction electrons in the metallic nanoparticles when exposed to light. The sharp and intense peak suggests uniform particle distribution with minimal aggregation, reflecting effective stabilisation by the phytochemicals present in *Senna auriculata* extract. No significant secondary peaks were observed across the 200–800 nm range, which implies that the nanoparticles are relatively pure and that there are minimal oxidised iron species present in the colloidal solution (Abdussalam *et al.* 2022). The UV-Vis

analysis, therefore, corroborates visual observations of the colour change from pale yellow to dark black, confirming successful reduction of $\text{Fe}^{2+}/\text{Fe}^{3+}$ ions to Fe^0 . Overall, the spectrum demonstrates that the flower extract not only reduces iron ions but also stabilises the resulting nZVI nanoparticles, yielding a colloidal suspension suitable for environmental and catalytic applications.

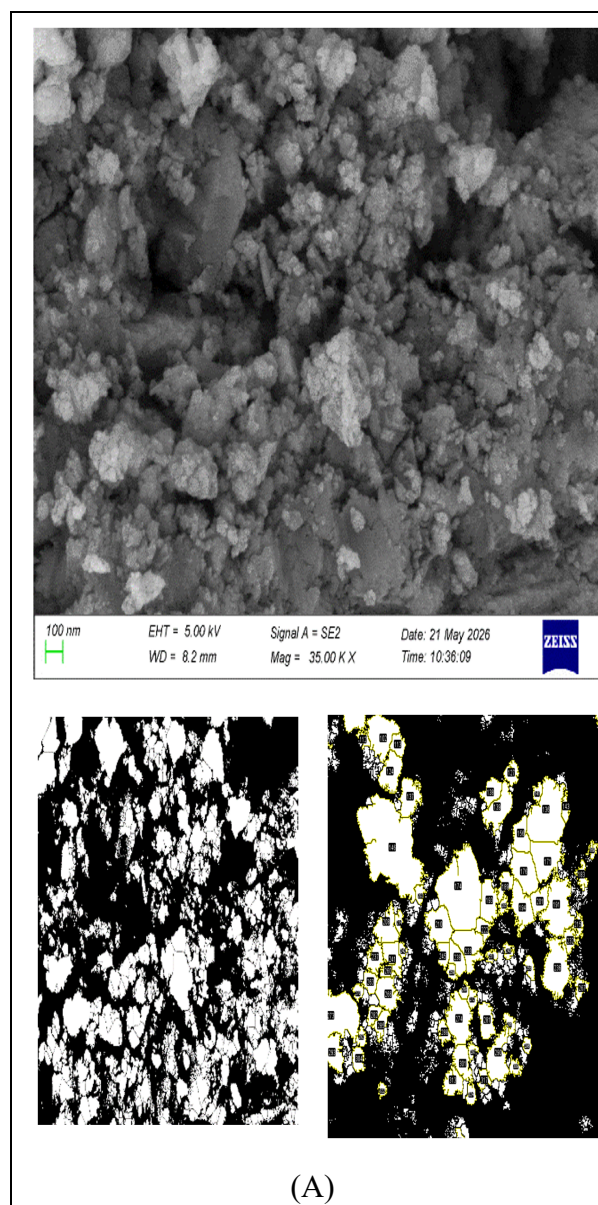
3.3 Zeta Potential and Dynamic Light Scattering

The Dynamic light scattering (DLS) analysis of the green-synthesized nZVI nanoparticles showed a dominant nanoscale particle distribution with peak intensity around 90 nm (Fig. 4a, b). The Z-average hydrodynamic diameter was 89.76 nm, indicating that the particles remained within the nanoscale range in aqueous colloidal suspension. The polydispersity index was recorded at 0.7502 suggests a broad size distribution with the presence of some larger aggregates, which may be attributed to hydration effects and slight particle association in the suspension. The smooth decay of the correlogram further indicates colloidal stability without rapid sedimentation. Zeta potential analysis showed a sharp unimodal peak in the negative region, approximately -20 mV (Fig. 4c), suggesting that the nanoparticles possessed fairly uniform surface charge characteristics. The negative surface charge indicates electrostatic repulsion among particles, contributing to dispersion stability. Overall, *Senna auriculata* phytochemicals effectively stabilized nZVI nanoparticles, although further optimization may improve long-term colloidal stability.

3.4 SEM Coupled with EDX of nZVI Nanoparticles

The SEM and EDX analysis of green-synthesised nZVI nanoparticles provides both morphological and elemental insight. SEM imaging shows that the nanoparticles are predominantly spherical with slight agglomeration, which is typical for zero-valent iron particles stabilised with phytochemicals. The surface appears relatively smooth, indicating effective capping by the bioactive compounds from *Senna auriculata*, which prevents uncontrolled growth and excessive aggregation. The SEM micrograph clearly indicated that the nanoparticles were not uniformly dispersed but existed as agglomerated masses with rough surfaces, suggesting successful nucleation and growth of iron-based nanoparticles (Fig. 5a). Particle size distribution was further analyzed using ImageJ software by thresholding the SEM image, separating particle boundaries, and measuring individual particle areas. The histogram showed that most particles were distributed within the nanoscale range, with a mean particle size of 47.79 nm and a standard deviation of 20.81 nm (Fig. 5c). The broad distribution indicates moderate polydispersity, which may be due to particle aggregation during drying

and sample preparation. EDX analysis confirmed the elemental composition of the synthesized nanoparticles. The spectrum showed prominent peaks corresponding to Fe, O, and S, indicating the formation of iron-rich nanoparticles with surface oxygenated species. Quantitative EDX analysis showed that Fe was the major element with 45.64 wt.%, followed by O with 33.21 wt.% and S with 21.15 wt.% (Fig. 5b). The high Fe content confirms the successful formation of iron-based nanoparticles, while the presence of oxygen may be attributed to partial surface oxidation of nZVI to iron oxide/hydroxide layers during synthesis or exposure to air. The sulfur signal may be associated with sulfur-containing functional groups, precursor residues, or stabilizing biomolecules attached to the nanoparticle surface. Overall, SEM-ImageJ and EDX results confirm the successful synthesis of nanosized, Fe-rich nZVI particles with an average size below 50 nm and chemically active surface composition.



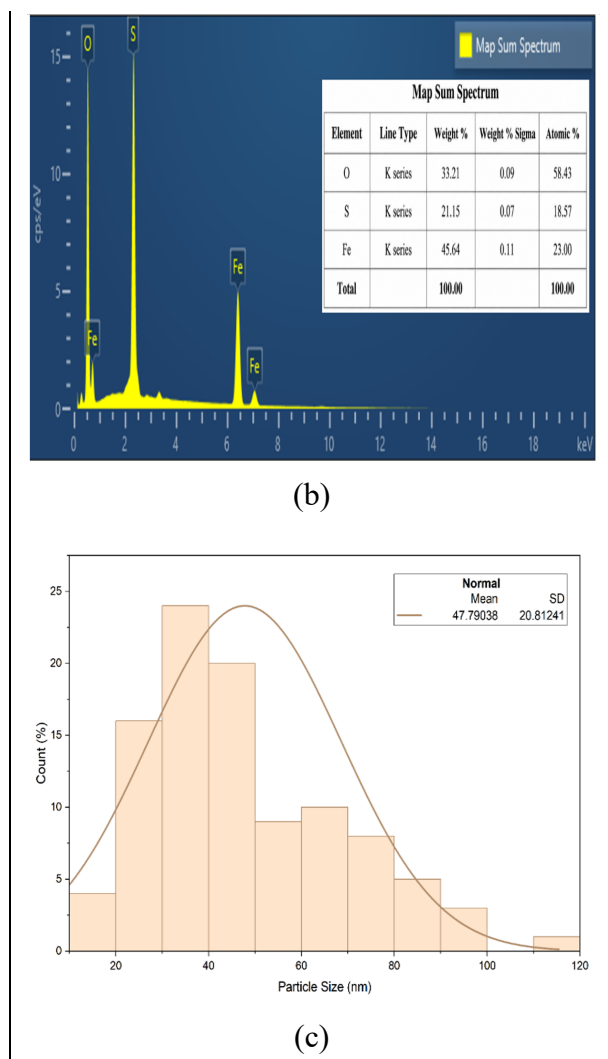


Fig. 5: (a) SEM image of nZVI nanoparticles at 3 kV magnification (b) EDX spectrum of nZVI nanoparticles and (c) Histogram representing particle size measurements derived from SEM images

3.5 Antioxidant Activity

The DPPH radical scavenging assay revealed a concentration-dependent increase in antioxidant activity for the green-synthesised nZVI nanoparticles (Table 1). At the lowest tested concentration (10 µg/mL), the nanoparticles exhibited $18.3 \pm 0.5\%$ inhibition, whereas at the highest tested concentration (500 µg/mL), inhibition reached $87.2 \pm 1.2\%$, approaching the positive control, ascorbic acid ($91.5 \pm 1.0\%$). The negative control (methanol) showed negligible activity ($2.1 \pm 0.2\%$). The potent radical scavenging is attributed to surface-bound phytochemicals from the green synthesis, which act as electron donors to neutralise DPPH radicals. The IC_{50} , estimated from the dose-response curve, was approximately 127 µg/mL, indicating moderate but significant antioxidant potential relative to ascorbic acid. Compared with *Coriandrum sativum* leaf-mediated iron oxide nanoparticles, which exhibited a lower IC_{50} of

86.68 µg/mL, our nZVI nanoparticles show slightly less potent antioxidant activity, likely due to differences in particle size, surface chemistry, and phytochemical capping density (Singh *et al.* 2020). In contrast, the study by Tonelli *et al.* 2025 using *Leucaena leucocephala* leaf-mediated nZVI reported a higher IC_{50} of 286.02 µg/mL, while BHT had an IC_{50} of 166.66 µg/mL, indicating that our nZVI nanoparticles have comparatively stronger antioxidant potential than those previously reported. Overall, these findings confirm that green-synthesised nZVI nanoparticles are effective free radical scavengers, demonstrating considerable potential for mitigating oxidative stress in biomedical applications (Baliyan *et al.* 2022).

Table 1. DPPH radical scavenging activity of nZVI nanoparticles

S. No.	Sample Concentration (µg/mL)	Ascorbic Acid Standard (%)	% of DPPH Radical Scavenging Activity (Mean $\pm$ SD)
1	10	91.5	18.3 ± 0.5
2	25	91.5	32.7 ± 0.7
3	50	91.5	49.5 ± 0.8
4	100	91.5	63.4 ± 1.0
5	250	91.5	78.6 ± 1.1
6	500	91.5	87.2 ± 1.2

3.6 Antidiabetic Activity

The antidiabetic potential of nZVI nanoparticles was assessed through α -amylase and α -glucosidase inhibitory activities, both exhibiting dose-dependent inhibition. For α -amylase, inhibition increased from $21.6 \pm 0.4\%$ at 15.625 µg/mL to $81.4 \pm 1.3\%$ at 500 µg/mL, with acarbose as the positive control showing $85.2 \pm 1.1\%$ inhibition and phosphate buffer as the negative control showing minimal activity. The IC_{50} was estimated at 220 µg/mL. Statistical analysis using one-way ANOVA followed by Tukey's post hoc test confirmed that the inhibition at all tested concentrations was statistically significant ($p < 0.05$) compared to the negative control. The observed inhibition likely arises from surface interactions between nZVI nanoparticles and the enzyme active site, hindering substrate binding, with phytochemical capping agents enhancing this effect (Devi and Ramesh, 2026).

α -Glucosidase inhibition by the nZVI nanoparticles increased in a concentration-dependent manner from $19.8 \pm 0.3\%$ at 15.625 µg/mL to $79.8 \pm 1.2\%$ at 500 µg/mL, while the standard drug acarbose exhibited $83.5 \pm 1.0\%$ inhibition. The IC_{50} for α -glucosidase was approximately 235 µg/mL. The inhibitory effect is likely mediated through interactions of surface-bound phytochemicals with the enzyme's active site, where bioactive molecules bind to key amino

acid residues, blocking substrate access and reducing catalytic activity (Van *et al.* 2018). This inhibition may involve competitive or non-competitive interactions, depending on the affinity of the nanoparticles and associated phytochemicals for the enzyme. The nanoscale size and high surface area of the nanoparticles enhance contact with the enzyme, maximizing inhibition efficiency (Nguyen *et al.* 2025). The dual inhibition of α -amylase and α -glucosidase underscores the potential of nZVI nanoparticles to modulate carbohydrate digestion, providing a mechanistic rationale for their observed antidiabetic activity. This aligns with previously reported plant-mediated nanoparticles, where phenolic and flavonoid compounds contribute to enzyme binding and activity modulation, further supporting the therapeutic potential of the synthesized nanoparticles (Tables 2 and 3).

Table 2. α -Amylase inhibitory activity of nZVI nanoparticles

S. No.	Sample Concentration ($\mu\text{g/mL}$)	Acarbose Standard (%)	% of α -Amylase Inhibitory Activity (Mean $\pm$ SD)
1	15.625	85.2	21.6 $\pm$ 0.4
2	31.25	85.2	35.2 $\pm$ 0.5
3	62.5	85.2	48.7 $\pm$ 0.6
4	125	85.2	61.3 $\pm$ 0.9
5	250	85.2	71.5 $\pm$ 1.0
6	500	85.2	81.4 $\pm$ 1.3

Table 3. α -Glucosidase inhibitory activity of nZVI nanoparticles

S. No.	Sample Concentration ($\mu\text{g/mL}$)	Acarbose Standard (%)	% of α -Glucosidase Inhibitory Activity (Mean $\pm$ SD)
1	15.625	83.5	19.8 $\pm$ 0.3
2	31.25	83.5	34.1 $\pm$ 0.5
3	62.5	83.5	47.2 $\pm$ 0.6
4	125	83.5	60.7 $\pm$ 0.8
5	250	83.5	71.0 $\pm$ 1.0
6	500	83.5	79.8 $\pm$ 1.2

4. CONCLUSION

The present study successfully demonstrates the eco-friendly synthesis of nanoscale zero-valent iron (nZVI) nanoparticles mediated by *Senna auriculata* flower extract, highlighting a sustainable approach that integrates green chemistry principles with nanotechnology. Characterization analyses, including UV-Vis spectroscopy, FTIR, DLS, SEM, and EDX, confirmed the formation of stable, spherical nanoparticles with surface functionalization imparted by phytochemicals, which enhance colloidal stability and bioactivity. The visible colour transition from pale yellow to dark black, combined with spectral and microscopic evidence, substantiates effective reduction of $\text{Fe}^{2+}/\text{Fe}^{3+}$ to

Fe^0 and successful capping by bioactive compounds. In vitro evaluations revealed that these green-synthesized nZVI nanoparticles possess substantial antioxidant potential, with a concentration-dependent DPPH radical scavenging activity approaching that of ascorbic acid. Furthermore, the nanoparticles exhibited significant antidiabetic activity, as evidenced by dose-dependent inhibition of α -amylase and α -glucosidase, suggesting their capability to modulate carbohydrate metabolism efficiently. The synergy between nanoscale properties and bioactive phytochemicals underpins enhanced enzyme inhibition and radical scavenging, addressing limitations of conventional plant extracts such as poor bioavailability and instability. The antioxidant and antidiabetic activities observed are consistent with, or in some cases superior to, previously reported plant-mediated nZVI systems, highlighting the enhanced efficacy conferred by *S. auriculata* capping. While these in vitro results are promising, translation to clinical application should be approached cautiously, as further in vivo studies, pharmacokinetic assessments, and safety evaluations are required to validate therapeutic potential. Collectively, these findings demonstrate that *S. auriculata*-capped nZVI nanoparticles represent a promising therapeutic platform for diabetes management, combining biocompatibility, high bioactivity, and environmentally responsible synthesis. The integration of traditional medicinal knowledge with green nanotechnology not only augments therapeutic efficacy but also provides a novel and sustainable model for future biomedical nanomaterial development. Future studies should focus on in vivo validation, mechanistic elucidation, and clinical translation to harness these nanoparticles as safe and effective strategies against metabolic disorders.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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