Research Article

Zubair Ahmad, Abdur Rauf*, Haiyuan Zhang, Muhammad Ibrahim, Naveed Muhammad, Yahya S. Al-Awthan, and Omar S. Bahattab

Green synthesis and multifaceted characterization of iron oxide nanoparticles derived from *Senna bicapsularis* for enhanced *in vitro* and *in vivo* biological investigation

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Abstract: Iron oxide nanoparticles have garnered significant interest in recent years due to their diverse applications, particularly in the therapeutic field. We present a green synthesis method using the extract of *Senna bicapsularis*, the production of iron oxide nanoparticles (IONPs). The successful synthesis of IONPs was confirmed by UV–visible spectroscopy, revealing the characteristic peak at 295 nm. Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy were employed to elucidate the functional groups involved in the synthesis and characterize the morphological features of the nanoparticles. Subsequently, the synthesized IONPs were subjected to biological assays to assess their anticancer, enzyme inhibitory, analgesic, and sedative activities, following standardized protocols. The IONPs exhibited potent anticancer activity against the MDR 2780AD cell line, with IC$_{50}$ values of 0.85 (extract) and 0.55 (iron oxide nanoparticles). Remarkable inhibitory effects were also observed against urease (IC$_{50}$ = 12.98 ± 0.98) and xanthine oxidase (IC$_{50}$ = 96.09 ± 0.65). Additionally, they demonstrated moderate carbonic anhydrase II inhibition, with 42.09% inhibition at a concentration of 0.25 mM. Furthermore, the extract and IONPs demonstrated a significant analgesic effect in a dose-dependent manner, while the sedative effect was also significant (p < 0.001).

Keywords: iron oxide nanoparticles, *Senna bicapsularis*, green synthesis, anticancer activities, enzyme inhibition, analgesic and sedative effect, characterization

1 Introduction

Nanoparticles (NPs) have emerged as pivotal agents with diverse applications across various scientific domains, including materials science, medicine, electronics, and environmental science [1–5]. Metal nanoparticles have emerged as versatile tools with a myriad of applications in both therapeutic and environmental domains. These nanoparticles exhibit unique physicochemical properties owing to their nanoscale dimensions, rendering them highly valuable across various scientific disciplines. In therapeutic applications, metal nanoparticles have shown promise in drug delivery systems, targeted therapies, and diagnostic imaging techniques [6]. Furthermore, metal nanoparticles find extensive use in environmental applications such as wastewater treatment, pollutant remediation, and sensing technologies. Their high surface area-to-volume ratio and reactivity make them efficient catalysts for pollutant degradation and environmental monitoring [7,8]. Amid the growing interest in NPs, there is a parallel surge in the exploration of sustainable and environmentally friendly synthesis methods, giving rise to green nanotechnology [9]. This approach, which employs biogenic or plant-derived materials, offers a more eco-conscious alternative to conventional chemical synthesis routes, reducing the ecological footprint associated with NP production [10]. Among the myriad of nanomaterials, iron oxide
nanoparticles (IONPs) have emerged as versatile entities with unique physicochemical properties and a wide range of applications in fields such as medicine, environmental science, and technology [11]. These applications include drug delivery, magnetic resonance imaging, environmental remediation, and more [12–14]. However, synthesizing these nanoparticles often involves harsh chemicals and conditions, posing environmental and health risks [15].

Recently, there has been a growing interest in developing eco-friendly methods for synthesizing nanoparticles to address these concerns. Using plant extracts for nanoparticle synthesis, known as green synthesis, has emerged as a sustainable and biocompatible alternative to conventional chemical routes [16,17]. Green synthesis of nanoparticles has emerged as a pivotal strategy in modern nanotechnology, offering a sustainable and eco-friendly alternative to traditional chemical methods. By utilizing natural sources such as plant extracts and microorganisms, green synthesis eliminates the use of toxic chemicals and reduces environmental impact, making it inherently more sustainable. Beyond environmental benefits, green synthesis also presents advantages in terms of cost-effectiveness and scalability, with readily available and inexpensive materials enabling large-scale nanoparticle production. Additionally, nanoparticles synthesized through green methods often exhibit enhanced biocompatibility and stability, opening up opportunities for diverse biomedical applications. The rich biochemical diversity of natural sources allows for the engineering of nanoparticles with tailored properties and functionalities, further expanding their potential uses. Green synthesis not only advances the field of nanotechnology but also contributes to sustainable development by harnessing the power of nature to create innovative and environmentally benign nanomaterials [10,18–20].

*Senna bicapsularis*, a plant species commonly found in tropical and subtropical regions, has garnered attention for its abundant phytochemical constituents, including flavonoids, alkaloids, Terpenoids, Tannins, fatty acids, reducing sugar, and glucosides [21]. These bioactive compounds possess inherent reducing and stabilizing properties, making *Senna bicapsularis* an ideal candidate for the green synthesis of metal nanoparticles [22,23]. Furthermore, *Senna bicapsularis* is renowned for its traditional medicinal uses, which include treating diabetes, inflammatory conditions, and bacterial infections [21,24,25]. Leveraging the synthesis of IONPs from *Senna bicapsularis* extract aligns with the principles of green nanotechnology. It opens doors to the development of multifunctional nanomaterials with potential therapeutic applications.

While numerous studies have explored the synthesis and applications of various metal nanoparticles, there remains a notable research gap concerning the development of ecofriendly methods for synthesizing iron nanoparticles. Existing methodologies often rely on chemical routes that involve harsh reagents and conditions, posing significant environmental and health risks. Moreover, the sustainable production of iron nanoparticles is of paramount importance given their diverse applications in both therapeutic and environmental fields. By addressing this research gap, the primary objective of this research is to explore the green synthesis of IONPs using *Senna bicapsularis* extract and to characterize these nanoparticles comprehensively. The novelty of this work lies in its exploration of green synthesis methods, comprehensive characterization techniques, assessment of biological activities, and the potential multifunctional applications of the synthesized nanoparticles in therapeutics. Additionally, this study aims to evaluate the biological activities of the synthesized nanoparticles, focusing on antidiabetic, antibacterial, and anti-inflammatory properties. By achieving these objectives, this research seeks to advance green nanotechnology while providing insights into the potential applications of *Senna bicapsularis*-derived IONPs in medicine and environmental science.

2 Materials and methods

2.1 Reagents and chemicals

The following chemicals were used in the experiments: iron salt FeCl3·6H2O (procured from Sigma-Aldrich), *Senna bicapsularis* extract (prepared as described in Section 2.2), distilled water, Phenol reagent (1% w/v phenol, 0.005% w/v sodium nitroprusside), Alkali reagent (0.5% w/v NaOH, 0.1% active chloride NaOCl), Buffer solution containing 100 mM urea, Dimethyl sulphoxide (DMSO), Phosphate buffer, Foetal bovine serum (FBS), Penicillin sodium salt (100 g·mL⁻¹), Streptomycin sulphate (100 g·mL⁻¹), Thiourea, Diazepam, Acetic acid Allopurinol and all other reagents utilized in this work were of analytical grade and provided by the Department of Chemistry, University of Swabi, Pakistan.

2.2 Collection of plant and preparation of *Senna bicapsularis* extract

The plant material used in this study was collected from Tehsil Lahore of Distract Swabi, Pakistan, in November 2022. The aerial parts of *Senna bicapsularis* were carefully harvested, thoroughly cleaned to remove any impurities, and air-dried under controlled conditions to preserve their
phytochemical content. The dried plant material was then finely ground into a powder using a mechanical grinder. To obtain the *Senna bicapsularis* extract, 50 grams of the powdered plant material was mixed with 250 mL of distilled water in a round-bottom flask. The mixture was macerated for 15 days at room temperature in a dark environment. After maceration, the extract was separated from the plant material by filtration through Whatman filter paper (Grade 1). The filtrate was concentrated using a rotary evaporator under reduced pressure to obtain a viscous, concentrated extract, which was stored until further use.

2.3 Synthesis of IONPs

The green synthesis of IONPs was carried out using the *Senna bicapsularis* extract as a reducing and stabilizing agent. In a typical synthesis, 1 mM of iron salt solution was mixed with an appropriate volume of 1% *Senna bicapsularis* extract at different ratios. The reaction was stirred continuously at 60–70°C for 3 h. The colour change from brown to dark brown indicated the formation of IONPs [26]. The schematic representation is given in Scheme 1.

2.4 Characterization techniques

2.4.1 UV–visible spectroscopy

The formation of IONPs was confirmed using a UV–visible spectrophotometer (Model-UV2601). The sample was analysed over a 200–700 nm wavelength at regular intervals during the synthesis process. The characteristic absorption peak at 295 nm indicated the presence of IONPs.

2.4.2 FTIR

The functional groups stabilizing the synthesized nanoparticles were identified using Fourier-transform infrared spectroscopy (Model FTIR-990). FTIR spectra were recorded in the 500–6,000 cm$^{-1}$ range.

2.4.3 Field-emission scanning electron microscopy (FESEM)

The morphology and size distribution of the IONPs were examined using a field-emission scanning electron microscope (Model JEM 2100, a Jeol CRL). Samples were prepared by drop-casting the nanoparticle solution onto a silicon wafer and subsequently gold-coated for enhanced imaging.

2.5 *In-vitro* biological testing

2.5.1 Anticancer activity

The cytotoxicity of the synthesized compound IOPNs was assessed using the MTT assay, as described in previous studies conducted by Burhan et al. and Muhammad et al. [27,28]. The RPMI 1640 medium, sourced from Gibco BRL, was supplemented with 10% FBS obtained from Gibco, Institute of Bioinformatics, National Chiao-Tung University, Hsinchu, Taiwan. Furthermore, the solution was comprised of streptomycin sulphate at a concentration of 100 g·mL$^{-1}$ and penicillin sodium salt at a concentration of 100 g·mL$^{-1}$. The A498 cell line, derived from renal tissue; the HepG2 cell line, derived from human hepatoma; the NCI-H226 cell line, derived from non-small cell lung tissue; the 2780AD cell line, derived from multidrug-resistant human ovarian cancer, were maintained in artificial culture media. In this study, hepatocytes obtained from mice were subjected to a culture process within 96-well plates. The cell density used for the HepG-R cells was $2 \times 10^4$, while for the HepG2 cells, it was $9 \times 10^3$. Following a 48-h incubation period, cellular entities were extracted from the experimental system under investigation. Subsequently, these cells were subjected to various treatments, including exposure to various chemical compounds at concentrations from 1.5 to 100 M. A control group was established wherein the cells were treated solely with a vehicle solution containing 0.2% DMSO. The monitoring procedure was carried out utilizing a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) test procured from Sigma, located in St. Louis, MO, USA. The remaining cell lines were subjected to identical experimental conditions.

![Scheme 1: Schematic representation of synthesis of IONPs using plant ext.](image-url)
2.5.2 Urease inhibition assay

The assessment evaluated the inhibitory activity of the synthesized IONPs and *Senna bicapsularis* extract against jack bean urease. The experimental procedure encompassed the placement of 25 µL of jack bean urease, 55 µL of buffer solution containing 100 mM urea, and 0.25 mM·mL of *Senna bicapsularis* extract against jack bean urease. The total volume used for each trial was 200 µL, carefully measured and maintained throughout the experiment. Thiourea was employed as a control agent in the experimental setup [29]. The calculation of the percentage inhibition was performed according to the formula proposed by Khan et al. in their 2014 publication [30].

\[
\text{Percent effect} = 100 - \frac{\text{OD test well}}{\text{OD control}} \times 100
\]

2.5.3 Xanthine oxidase

The present study also aimed to assess the inhibitory capacity of the extract derived from *Senna bicapsularis* and the synthesized IONPs against xanthine oxidase. This evaluation was conducted by employing a hydroxylation reaction of xanthine as the substrate, ultimately forming colourless uric acid as the final product. The absorbance of uric acid was measured at a wavelength of 296 nm. The reaction mixture comprised the test sample, either a plant extract or iron oxide IONPs, along with phosphate buffer and xanthine oxidase enzyme. A test sample solution with a concentration of 0.25 millimolar per millilitre (mM·mL) was prepared. Subsequently, this solution's volume of 10 microliters (µL) was dissolved in DMSO. In addition, a total of 0.003 units of xanthine oxidase enzyme were dissolved in 20 µL of phosphate buffer. The substrate used for the enzymatic reaction was xanthine, prepared at a 0.1 mmoll.L concentration. After adding xanthine oxidase, the experimental mixture underwent an incubation period of 10 min at ambient temperature. After the incubation period, the mixture underwent analysis within the ultraviolet (UV) region, specifically at a wavelength of maximum absorption (λmax) of 295 nm. Following the initial step, the substrate was introduced into the experimental setup, and absorbance measurements were taken at regular intervals of 1 min over 15 min utilizing a microplate reader. The percentage inhibition of the test sample, specifically the Iron Oxide Nanoparticles (IONPs), was calculated. The calculation of absorbance (A) was performed utilizing the formula

\[
A = a\lambda \times b \times c
\]

where \(a\lambda\) represents the absorptivity coefficient corresponding to the particular wavelength, \(b\) denotes the path length, and \(c\) signifies the concentration of the analyte. The IC50 values of the compounds were determined through the utilization of EZ-Fit, a Windows-based software specifically designed for this purpose. Allopurinol, a widely recognized positive control, was employed in this study to establish a benchmark for evaluating the inhibitory activities of the test samples. The objective was to compare the inhibitory
effects of the test samples against the established standard, allopurinol. The experimental procedure was conducted in triplicate, as reported by Alam et al. [31].

### 2.5.4 Carbonic anhydrase II inhibition

The inhibitory effect of synthesized IONPs on the activity of Carbonic Anhydrase II enzyme was evaluated. The experimental procedure encompassed the quantification of carbon dioxide hydration by utilizing a pH indicator. The experimental setup consisted of a reaction mixture containing the test sample, specifically iron oxide IONPs, along with the Carbonic Anhydrase II enzyme and the substrate. The experimental procedure was conducted under conditions determined to be the most favourable in terms of temperature and pH for the enzymatic activity. Following the incubation period, the alteration in pH levels was assessed by using a pH indicator. The quantification of the inhibitory effect of iron oxide IONPs on the activity of the Carbonic Anhydrase II enzyme was determined by assessing the percentage inhibition. This was achieved by measuring the difference in pH alteration in the presence and absence of the IONPs. The study was carried out in triplicate to ensure the precision and dependability of the findings [32].

### 2.6 In-vivo biological testing

#### 2.6.1 Analgesic activity

The anti-nociceptive activity of Au-WAs was evaluated using the acetic acid-induced writhing assay in mice, as described by Muhammad et al. in their 2012 study [28]. Prior to the commencement of the experiment, the animals were categorized into six distinct groups \((n = 6)\). Group 1 was treated with normal saline \((10 \text{ mL} \cdot \text{kg}^{-1})\) acting as negative for statistical analysis. Group 2 was treated with diclofenac sodium \((10 \text{ mg} \cdot \text{kg}^{-1})\) acting as a positive control. Rested of the groups were treated with the samples to be tested at selected doses of 25, 50, and 100 mg·kg\(^{-1}\) (extract). Conversely, intraperitoneal injection of IONPs at concentrations of 2.5, 5, and 10 mg·kg\(^{-1}\) was administered to the rest of the groups. After a treatment period of 30 min, all animal subjects received a subsequent intraperitoneal injection containing 1% acetic acid. The measurement of abdominal constriction, or writhing, was performed for ten min following the administration of acetic acid for 5 min.

#### 2.6.2 Sedative activity

The experimental setup employed in this study comprised a rectangular region constructed from white wood measuring 150 cm in diameter. The enclosure was constructed using stainless steel walls, ensuring a robust and durable structure. The circular area was further partitioned into 19 lines with equal space. The experimental room was made a soundproof to avoid disturbance. The animals were subjected to a period of acclimatization under red light conditions, specifically using a 40 W red bulb; this acclimatization period lasted for 1 h before the commencement of the experiment. Following a 30-min duration of administration involving diazepam at a dosage of 0.25 mg·kg\(^{-1}\), normal saline at a dosage of 10 mL·kg\(^{-1}\), *Senna bicapsularis* extract at dosages of 25, 50, and 100 mg·kg\(^{-1}\) intraperitoneally (i.p), as well as IONPs at dosages of 2.5, 5, and 10 mg·kg\(^{-1}\) i.p, each subject was positioned within the central area of the enclosure. Subsequently, the total count of lines traversed by the animals was recorded [33,34].

**Ethical approval:** The research related to animals’ use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

### 3 Results

#### 3.1 Synthesis of IONPs

The synthesis of IONPs using *Senna bicapsularis* extract was visually confirmed by a distinct change in colour. The reaction mixture initially displayed a pale yellow colour, indicative of the presence of iron ions. This initial colour change was observed within the first few minutes of the synthesis process, suggesting the rapid reduction of iron ions by the components in the plant extract. As the synthesis progressed, the colour gradually shifted to a deep brown, a characteristic colour associated with IONPs (Figure 1). This transition from pale yellow to deep brown was a clear indication of the formation of IONPs within the reaction mixture. The intensity of the brown colour increased with time, providing further evidence of the continued growth and stabilization of the nanoparticles. The colour change observed during the synthesis process was not only visually striking but also served as a quick and convenient qualitative confirmation of successful nanoparticle formation. This change in colour is a well-documented phenomenon in the synthesis of IONPs using biological extracts, and it is attributed to the reduction of iron...
ions and the subsequent nucleation and growth of nanoparticles [17].

3.2 Characterization

3.2.1 UV–visible spectroscopy

To further confirm the formation of IONPs, UV–visible spectroscopy was employed. This analytical technique provides valuable insights into the optical properties of nanoparticles, allowing for a more detailed characterization of the synthesized material. As shown in Figure 2, the UV–visible spectrum exhibited a sharp absorption peak at 295 nm, which is a characteristic feature of IONPs. This absorption peak corresponds to the surface plasmon resonance (SPR) of the nanoparticles and is a well-established indicator of their presence. The SPR arises from the collective oscillation of free electrons at the nanoparticle’s surface when illuminated with UV–visible light. The intensity of the peak at 295 nm increased with time, demonstrating the gradual formation and growth of the IONPs within the reaction mixture. This observation was consistent with the colour change previously described, further supporting the successful synthesis of nanoparticles [35].

3.2.2 FTIR

Fourier Transform Infrared spectroscopy was employed to analyse the chemical composition of the extract from Senna bicapsularis and the synthesized Iron Oxide Nanoparticles (IONPs). In the FTIR spectrum of the Senna bicapsularis extract, a broad band was prominently observed at 3,300 cm\(^{-1}\), which is characteristic of the O–H stretching vibrations, indicating the presence of hydroxyl groups. Additionally, a smaller band at 2,200 cm\(^{-1}\) suggests the presence of C\(==\)C triple bonds, possibly corresponding to alkyne functional groups. Another notable feature was the sharp band at 1,700 cm\(^{-1}\), indicative of the C\(==\)O stretching vibrations, which may be associated with carbonyl groups. Similar bands were observed in the FTIR spectrum of plant mediated IONPs. However, there were distinct differences in the intensities of these bands compared to those observed in the raw Senna bicapsularis extract. This variation in band intensities strongly suggests the involvement of phytochemicals present in the extract in both the reduction and stabilization of metal ions, leading to the successful formation of nanoparticles [5]. The FTIR spectra are provided in the inset of Figure 3.

3.2.3 FESEM

The morphology and size distribution of the IONPs were examined using FESEM, a powerful technique for visualizing high-resolution nanomaterials. This analysis provides crucial insights into the structural characteristics of the synthesized nanoparticles. Figure 4 illustrates FESEM images of the nanoparticles at both high and low resolution, revealing their distinctive spherical shape and uniform size distribution. The published literature also reported the same results [36,37]. At high resolution, the individual nanoparticles can be clearly observed, highlighting their well-defined spherical morphology. This spherical shape is a common feature of IONPs and is consistent with the idealized properties desired for various applications. Moreover, the low-resolution image provides an overview of the entire sample, indicating that the majority of the nanoparticles possess a remarkably

Figure 1: Colour change during nanoparticle synthesis; (a) extract, (b) IONPs.

Figure 2: UV–visible spectrum confirming nanoparticle synthesis.
consistent size distribution. The uniformity in size is a critical factor in nanoparticle applications because it ensures consistent behaviour and performance in various contexts.

### 3.3 Biological assays

#### 3.3.1 Anticancer activities

The demonstrated anticancer effect of the extract/IONPs against various cancer cell lines is illustrated in Table 1. The IC₅₀ values observed against MDR 2780AD were found to be, at a minimum, precisely 0.85 (extract) and 0.55 (IONPs). A variable anticancer effect was observed against different cell lines. Paclitaxel was used as standard anticancer.

#### 3.3.2 Urease inhibition

The extract of *Senna bicapsularis* showed an inhibition rate of 46.09% when administered at a concentration of 0.25 µg. In contrast, the IONPs exhibited augmented inhibition, a notable percentage of 77.09% at a concentration of 0.25 mM. The findings suggest that the IONPs obtained from the extract of *Senna bicapsularis* exhibit strong inhibitory efficacy against urease. Thiourea, employed as a positive control, demonstrated robust inhibition of 98.09%, confirming the assay’s effectiveness, as depicted in Table 2.

#### 3.3.3 Xanthine oxidase inhibition

The extract of *Senna bicapsularis* showed an inhibition rate of 32.98% when administered at a concentration of 0.25 µg. Conversely, the inhibitory activity of the IONPs was significantly enhanced, exhibiting a percentage of 65.98% at a concentration of 0.25 mM. Significantly, the IONPs demonstrated an IC₅₀ value of 96.09 ± 0.65 µM, emphasising their robust inhibitory capacity against xanthine oxidase activity. Allopurinol, the pharmacological agent serving as the positive control, demonstrated inhibition rates of 98.09% and 99.09%, respectively.

![FTIR Spectra](image1.png)

**Figure 3:** FTIR Spectra identifying functional groups in (a) extract, (b) IONPs.

![FESEM Images](image2.png)

**Figure 4:** FESEM Images showing nanoparticle morphology at (a) low resolution and (b) high resolution.
control, exhibited a notable inhibition percentage of 97.65%, as depicted in the inset of Table 3.

### 3.3.4 Carbonic anhydrase II inhibition

The inhibitory effects of the *Senna bicapsularis* extract and IONPs were observed in the carbonic anhydrase II enzyme inhibition assay. The extract of *Senna bicapsularis* demonstrated a minimal inhibition of 13.91% at a concentration of 0.25 µg. In contrast, the inhibitory activity of the IONPs was significantly enhanced, exhibiting a percentage of 42.09% at a concentration of 0.25 mM. Acetazolamide, the pharmacological agent serving as the positive control, showed a notable inhibition percentage of 80.66%, confirming the assay’s accuracy, as depicted in Table 4.

### 3.3.5 Analgesic activity

The analgesic effect exhibited by both the synthesized IONPs and *Senna bicapsularis* extract is detailed in Table 5. An observation of a dose-dependent effect was made. The maximum per cent effect of extract was 55% (100 mg·kg⁻¹), while the maximum per cent effect of the IONPs was 78% at the tested dose of 10 mg·kg⁻¹. The standard analgesic drug exhibited a stronger effect with 84% protection.

### 3.3.6 Sedative effect

The sedative properties of the extract and IONPs are presented in Table 6. Both of the tested samples exhibited a dose-dependent sedative effect that was observed for both the extract and IONPs. In contrast, the IONPs demonstrated significant (*p < 0.001*) sedative effects at all tested concentrations and was determined to be more potent than the *Senna bicapsularis* extract.

### 4 Discussion

Natural products are the main source of remedy for pathological and non-pathological disorders. The world population is now switching to alternative medicines due
to the significant safety profile as compared to available synthetic drugs [38]. Therefore, the consumption of natural products as pharmaceuticals and cosmeceuticals increasing with time. The testing of the plant-based remedies must be scientifically screened in various experimental modes for good safety documentation. In the current research work, the nanoparticles and extract of *Senna bicapsularis* were tested for different pharmacological evaluations. A variable degree of antagonistic effect was noted against different enzymes. However, a significant (*p < 0.001*) analgesic effect was noted for both of the tested samples. The analgesic effect extract and IONPs have strong correlation with the folklore of *Senna bicapsularis* as a painkiller [21]. The analgesic effect of these samples might be due to the inhibitory effect of the local pain receptors or inhibition of the prostaglandins. The maximum analgesic effect of the IONPs might be due to the maximum penetration of the NPs at the target sites. In addition to the analgesic effect both the tested samples also demonstrated significant (*p < 0.001*) sedative effect. Although the plant is not documented for sedative effect, the use of *Senna bicapsularis* is well reported in the literature as a muscle relaxant. The mechanism of sedation and muscle relaxation is almost the same, i.e. GABA agonistic effect [39,40]. Therefore, the sedative effect of these tested samples might be attributed to the agonistic effect with GABA receptors. The results obtained from the biological assays highlight the promising potential of the IONPs derived from *Senna bicapsularis* as therapeutic agents. With demonstrated efficacy against multidrug-resistant cancer cells, as well as significant inhibitory effects on enzymes associated with various physiological processes, including urease, xanthine oxidase, and carbonic anhydrase II, these nanoparticles emerge as versatile candidates for therapeutic interventions. Their ability to exert dose-dependent analgesic and sedative effects further underscores their potential utility in pain management and muscle relaxation. Additionally, the traditional use of *Senna bicapsularis* in addressing constipation and skin lesions aligns with the observed pharmacological activities of the IONPs, suggesting broader applications as therapeutic agents.

## 5 Conclusion

In this study, we explore the potential of *Senna bicapsularis*-functionalized IONPs synthesized through an ecofriendly green synthesis approach. The successful confirmation of IONP synthesis using UV–visible spectroscopy, along with the elucidation of functional groups and morphological features through FTIR and scanning electron microscopy validates the viability of our environmentally conscious methodology. The diverse biological assessments conducted showcase the multifaceted nature of these nanoparticles. Notably, the IONPs exhibit significant anticancer activity, contributing to their potential therapeutic applications. Additionally, their inhibitory effects against enzymes and the observed moderate carbonic anhydrase II inhibition hint at their versatility in various therapeutic contexts. Furthermore, the in vivo demonstration of significant analgesic and sedative effects adds another dimension to the potential applications of IONPs. However, it is important to note some limitations and potential areas for improvement. One limitation is the need for further investigations into the long-term stability and potential toxicity of these nanoparticles, particularly in biological systems. Additionally, while the observed anticancer activity and enzyme inhibition are promising, more detailed mechanistic studies are warranted to fully understand the underlying pathways and optimize therapeutic efficacy. Looking ahead, future research could focus on optimizing the synthesis process to enhance the reproducibility and scalability of these nanoparticles. Moreover, exploring targeted delivery mechanisms and combination therapies could further enhance their efficacy in clinical settings. This study contributes to the evolving landscape of green chemistry and nanomedicine, laying the foundation for continued advancements in the field.

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Conflict of interest: The contact author Dr. Abdur Rauf is the associate editor of GPS. The other authors state no conflict of interest.

Data availability statement: The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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