

Structural Characterization and Biogenic Chitosan-ZnO Nanocomposites via Bauhinia purpurea Leaf Extract: and its their Therapeutic Evaluation

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Table 1 is available in the Supplementary Files section.

Structural Characterization and Biogenic Chitosan-ZnO Nanocomposites via *Bauhinia purpurea* Leaf Extract: and its their Therapeutic Evaluation

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Abstract

The growing demand for eco-friendly nanomaterials in biomedical applications has created interest in plant-assisted nanoparticle synthesis. Here, a new green synthesis of chitosan-ZnO nanocomposites using *Bauhinia purpurea* leaf extract is presented, combining the plant's phytochemical richness with the structural advantage of chitosan and the functional diversity of zinc oxide. The synthesized nanocomposites were exhaustively characterized by UV–Vis, FTIR, XRD, SEM, EDX, and zeta potential analysis, validating stable, well-dispersed nanoparticles with high surface charge and semi-crystalline morphology. The nanocomposites exhibited remarkable antidiabetic activity through dose-dependent inhibition of α -amylase and α -glucosidase enzymes, broad antioxidant activity reflected in five radical scavenging assays, and outstanding anti-inflammatory activity established through protein denaturation and membrane stabilization methods. Interestingly, the cytotoxicity evaluation carried out through brine shrimp lethality assay revealed the least toxicity, highlighting the biocompatibility of the materials. This study for the very first time offers a synergistic nanocomposite system consisting of a medicinal plant extract and a natural polymer combined with a metal oxide to provide a safe, sustainable, and multifunctional platform for therapeutic use. The results identify this nanocomposite as a promising candidate for future development towards antidiabetic, antioxidant, and anti-inflammatory nanomedicine.

Keywords *Bauhinia purpurea*, chitosan-ZnO nanocomposites, antioxidant, antidiabetic, anti-inflammatory, biocompatibility

Highlights

- Introduces a green synthesis route for chitosan-ZnO nanocomposites using *Bauhinia purpurea* leaf extract.
- Combines phytochemicals, chitosan, and ZnO into a single multifunctional nanoplatform.
- Demonstrates simultaneous antidiabetic, antioxidant, and anti-inflammatory activities in one composite.
- Achieves high colloidal stability (+42.76 mV) and biocompatibility without toxic agents.
- Sets a precedent for plant-based nanomedicine with scalable, eco-friendly synthesis.

1 Introduction

Over the past few years, the field of nanotechnology has witnessed significant progress in biomedical and pharmaceutical fields because of the need for more complex materials with higher therapeutic efficacy and lower toxicity [1,2]. Among the array of various nanomaterials, zinc oxide nanoparticles (ZnO NPs) have attracted a lot of interest owing to their outstanding physicochemical properties like extensive surface area, bandgap tunability, and outstanding antimicrobial, antioxidant, and antidiabetic activities [3,4]. However, conventional synthetic processes for ZnO NPs are generally related to the use of toxic solvents and energy-intensive processes, which thereby limit their biocompatibility and environmental compatibility [5,6]. To counteract such limitations, green synthesis protocols utilizing plant extracts have been created as economical and environmentally friendly protocols [7,8]. Particular mention is *Bauhinia purpurea*, a medicinal plant that contains a high amount of phytochemicals [9,10]. This is a promising route towards bio-reduction and stabilization of nanoparticles. Despite the presence of many studies on the synthesis of ZnO nanoparticles through plant-mediated protocols, few of them combine this approach with the incorporation of biopolymers, like chitosan, for enhancing dispersion, stability, and functional synergism [11,12]. This study proposes a new method for green synthesis of the chitosan-ZnO nanocomposite using *B. purpurea* leaf extract, to produce a hybrid material with high antioxidant, antidiabetic, anti-inflammatory, and non-cytotoxic activity. Chitosan addition confers biocompatibility and film-forming ability, and enhances the interaction of ZnO with plant phytochemicals, to produce a stable and efficient nanoplatform [13,14]. Compared to the literature, this study makes significant advances: Green synthesis with dual-phase phytochemistry and polymer science, Improved biological activities shown in a series of assays, and Lack of cytotoxicity shown by brine shrimp lethality assays. These results demonstrate the potential of the nanocomposite as a safe and multi-purpose reagent for biomedical and pharmaceutical applications, and thus sets a new benchmark for nanotechnology from natural products.

2 Materials and Methods

2.1 Materials

Chitosan (medium molecular weight, degree of deacetylation ~85%), Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$), analytical grade, procured from Fresh leaves *Bauhinia purpurea* L.

were collected and authenticated by the Siddha Central Research Institute (CCRS), Chennai, India

2.2 Preparation of *Bauhinia purpurea* Leaf Extract

Bauhinia purpurea L. leaves involved drying them under a sunshade for half a week to one week. 10 gms of grounded dried leaf powder received 100 mL of distilled water for the preparation. The heating process of the mixture lasted 10 mins before the solution achieved room temperature. A Whatman No. 1 filter paper was used to filter the solution for removing plant debris. To extract concentration the filtrate under 5-10 mins of boiling. The cooled extraction was stored in a sterile container to continue future use.

2.3 Synthesis of Zinc Oxide Nanoparticles

zinc nitrate using different concentration was created by dissolving appropriate zinc nitrate amounts within 100 mL distilled water (0.5 mm, 1.5 mm and 2.5 mm). The mixture of *B. purpurea* leaf extract combined with 50 /mL of solution travelled through the magnetic stirrer for 1 hour. After adding the solution to a hot plate the mixture underwent magnetic stirrer heating for 2 hrs. The solution necessary for the experiment was stored within a refrigerator to rest for 24 hrs. The magnetic stirrer enabled continuous solution mixing for 2 hrs before heating and additional mixing for 4 hrs and finally allowing the mixture to rest in the refrigerator for 48 hrs. The researcher measured UV absorption of the solvent by placing it directly into the solvent after solution reconstitution at 56 hrs. The nanoparticles (pellets) were isolated from other contents through centrifugation at 8000 RPM during a 10-min. The supernatant was discarded [15]. **Fig. 1** This figure presents the overall process of synthesizing nanoparticles using *B. purpurea* leaf extract. The procedure begins with the selection and drying of fresh leaves, followed by grinding them into a fine powder. This powder is weighed and mixed with dis H_2O_2 to prepare the crude extract, which is then filtered and stored. The extract is subsequently combined with zinc nitrate and heated to facilitate nanoparticle formation. Finally, the mixture undergoes centrifugation to collect the pellet, which is heat-dried to obtain the synthesized nanoparticles.

2.4 Preparation of Chitosan Solution

1 gm of chitosan was dissolved in 1.8 mL of glacial acetic acid and diluted with 98.2 mL of dis H_2O_2 to make up a total volume of 100 mL. The mixture was stirred continuously at room

temperature for 6 hrs until a clear, homogeneous solution was obtained. chitosan solution was then used for the preparation of the chitosan-ZnO nanocomposites [16].

2.5 Preparation of Chitosan-ZnO Nanocomposites

The synthesized ZnO nanoparticles were first dispersed in distilled water using sonication for 15 mins to achieve a uniform suspension. This nanoparticle suspension was then slowly added to the prepared chitosan solution under constant stirring. The combined mixture was stirred continuously for 6 hr at room temperature to ensure thorough dispersion and effective interaction between the chitosan and ZnO nanoparticles. The chitosan-ZnO nanocomposite solution was then subjected to two different drying methods depending on the intended application. For film formation, the solution was cast into petri dishes and dried at 40°C to obtain nanocomposite films. For applications requiring a powdered form, the solution was first frozen and then subjected to lyophilization (freeze-drying) to produce a dry, porous nanocomposite powder. The final nanocomposite products - both films and lyophilized powders- were stored in airtight containers to preserve their structural and functional integrity for subsequent characterization and evaluation of their biological activities, as shown in **Fig. 2** [17,18].

2.6 Characterization Techniques

Chitosan-ZnO nanocomposites synthesized using *B. purpurea*-mediated Chitosan-ZnO nanocomposites were characterized using multiple analytical techniques to confirm their formation and assess their physicochemical properties. UV-Visible spectrophotometry performed at different concentrations (0.5 mM, 1.5 mM, and 2.5 mM) and time intervals (15, 24, 48 hours) showed a distinct absorption peak in the range of 370–380 nm, confirming the presence and stability of ZnO nanoparticles within the chitosan matrix. Fourier Transform Infrared Spectroscopy (FTIR) revealed the presence of functional groups including hydroxyl, carbonyl, and phenolic moieties from the *B. purpurea* leaf extract indicating their involvement in reducing and stabilizing the nanoparticles. X-ray Diffraction (XRD) analysis validated the crystalline nature of the Chitosan-ZnO nanocomposites showing peaks related to the hexagonal wurtzite structure with crystallite size calculated using the Scherrer equation [19,20]. Zeta potential measurements determined surface charge and colloidal stability as well-dispersed Chitosan-ZnO nanocomposites [21,22]. Scanning Electron Microscopy (SEM) provided spherical to irregular morphology in the nanoscale range, and Energy-Dispersive X-ray Spectroscopy (EDX) confirmed elemental composition as containing zinc and oxygen with

minimal impurities [19,20]. These characterization techniques proved successful green synthesis of chitosan-ZnO nanocomposite from *B. purpurea* and elucidated their structural and functional properties.

2.7 Antidiabetic Assays

2.7.1 Determination of Alpha-Amylase Enzyme Inhibition

B. purpurea-mediated Chitosan-ZnO nanocomposites against α -amylase was evaluated using the colorimetric method. An enzyme solution was prepared by dissolving 27.5 mg of α -amylase in 100 mL of d. dis H_2O_2 after. A 0.1% starch solution was also prepared as the substrate. The colorimetric reagent was a mixture of sodium potassium tartrate and 3,5-dinitrosalicylic acid (DNSA) solution (96 mM). For each assay, the starch solution was added to test samples at various concentrations (10, 20, 30, 40, and 50 μ g/mL) of Chitosan-ZnO nanocomposites. The mixtures were incubated at 25°C for 3 minutes to allow the α -amylase enzyme. Acarbose served as the positive control. The reaction was halted by the addition of DNSA reagent, and the resulting mixture was heated to develop a colour indicative of maltose production, which reduces DNSA to 3-amino-5-nitrosalicylic acid. Absorbance was measured at 540 nm using an ELISA reader. The percentage of α -amylase inhibition was calculated as:

$$I\% = 100 - \frac{As - Ab}{Ac - Ab} \times 100,$$

where I% is the inhibition percentage and As, Ab, and Ac are the average absorbance of the sample, blank, and control, respectively [23].

2.7.2 Evaluation of Alpha-Glucosidase Enzyme Inhibition

The α -glucosidase inhibitory activity was assessed for the *B. purpurea*-mediated Chitosan-ZnO material using for different (10, 20, 30, 40, and 50 μ g/mL) were mixed with a 2% starch substrate (maltose or sucrose) in the presence of 0.2 M Tris buffer (pH 8.0). The mixture was incubated for 5 mins at 37°C. Then, 1 mL of α -glucosidase enzyme solution (1 U/mL) was added and the reaction mixture was further incubated at 35°C for 40 minutes. The reaction was stopped with the addition of 2 mL of 6 N HCl. Acarbose was used as the standard reference. The absorbance of the resulting solutions was measured at 540 nm using an ELISA reader. The inhibition percentage was calculated as:

$$I\% = 100 - \frac{As - Ab}{Ac - Ab} \times 100,$$

Where I% is the inhibition percentage, and As, Ab, and Ac are the average absorbance of the sample (Chitosan-ZnO nanocomposites), blank (without enzyme or substrate), and control (with enzyme but without inhibitor, e.g., Acarbose for standard comparison), respectively [24].

2.8 In Vitro Antioxidant Activity

2.8.1 DPPH Radical Scavenging Assay

A stock solution of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared in methanol and diluted to a working concentration of 20 μ M. Different concentrations (10, 20, 30, 40, and 50 μ g/mL) of ZnO nanoparticles, chitosan, and chitosan-ZnO nanocomposites were added to 200 μ L of DPPH working solution in a 96-well plate. The mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Methanol served as the blank, and ascorbic acid was used as the standard. The percentage of radical scavenging activity was calculated using the formula:

$$\text{Inhibition (DPPH)} = [100 - (\text{ABS sample} - \text{ABS control}) \times 100] / \text{ABS control}$$

where ABS sample stands for the absorbance of the sample and ABS control stands for the absorbance of the control [25].

2.8.2 Hydrogen Peroxide (H₂O₂) Scavenging Assay

The H₂O₂ scavenging activity of Chitosan-ZnO nanocomposites, along with individual ZnO nanoparticles and chitosan, was measured by preparing a 40 mM H₂O₂ solution in phosphate buffer (pH 7.4). Varying concentrations (10, 20, 30, 40, and 50 μ g/mL) of the test samples including the nanocomposite—were mixed with 0.6 mL of H₂O₂ solution. Ascorbic acid was used as a standard antioxidant. The mixtures were incubated in the dark at room temperature for 10 minutes. Absorbance was recorded at 230 nm using a UV-Vis spectrophotometer [26]. The percentage scavenging activity was calculated using the formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

2.8.3 Ferric Reducing Antioxidant Power (FRAP) Assay

Chitosan-ZnO nanocomposites, the FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in a 10:1:1 ratio. A total of 2.3 mL of the FRAP reagent was added to 0.7 mL of each solution (including ZnO, chitosan, and Chitosan-ZnO nanocomposites) prepared at concentrations of 10–50 $\mu\text{g/mL}$. The mixtures were incubated at 37°C for 30 mins in the dark. Absorbance was measured at 593 nm. An increase in absorbance signified greater reducing power. Ascorbic acid and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were used as standards [27].

Inhibition (%) of FRAP radical = $[\text{Control (A593)} - \text{Sample (A593)}] / \text{Sample (A593)} \times 100$

2.8.4 ABTS Radical Scavenging Assay

The ABTS assay for *Bauhinia purpurea*-mediated ZnO nanoparticles (MP-ZnO NPs), chitosan, and Chitosan-ZnO nanocomposites was performed by Manoharan Janani et al. ABTS radical cation (ABTS^+) was produced by reacting 7 mM ABTS with 2.45 mM potassium persulfate and incubating the mixture in the dark for 24 hr. Before use, the ABTS^+ solution was diluted with 50% ethanol until its absorbance reached 1.0 ± 0.02 at 734 nm. For the assay, 250 μL of the ABTS^+ solution was added to 20 μL of sample solutions containing varying concentrations (10, 20, 30, 40, and 50 $\mu\text{g/mL}$) of ZnO nanoparticles, chitosan, and Chitosan-ZnO nanocomposites in a 96-well microplate. The reaction mixtures were incubated in the dark at room temperature for 10 min. The absorbance was measured at 734 nm using a spectrophotometer. Ascorbic acid was used as a positive control. The radical scavenging activity was calculated using the formula:

$$\text{Percentage of inhibition by ABTS} = [\text{C(Abs)} - \text{S(Abs)}] / \text{C(Abs)} \times 100$$

where C(Abs) is the control absorbance and S(Abs) is the sample absorbance [28].

2.8.5 Nitric Oxide (NO) Radical Scavenging Assay

Griess Illosvoy method, as described by Ebrahimzadeh et al. A 3 mL reaction mixture was prepared containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL phosphate-buffered saline (pH 7.4), and 0.5 mL of each test sample or standard (ascorbic acid) at varying concentrations (10, 20, 30, 40, and 50 $\mu\text{g/mL}$). Following incubation at 25°C for 150 minutes under diffused light, 0.5 mL of the incubated mixture was reacted with 1 mL of 0.33% sulfanilic acid in 20%

glacial acetic acid and allowed to stand for 5 minutes for diazotization. Subsequently, 1 mL of 0.1% NADPH was added, and the solution was incubated at room temperature for 30 min. The formation of a pink was measured spectrophotometrically at 540 nm. The percentage of nitric oxide radical scavenging activity was calculated using the following equation:

$$\% \text{ Antioxidant activity} = [100 - (\text{ABS sample} - \text{ABS control}) \times 100] / \text{ABS control}$$

where ABS sample is the absorbance of the sample and ABS control is the absorbance of the control [29].

2.9 Anti-inflammatory activity:

2.9.1 Bovine serum albumin denaturation assay

Green synthesized Chitosan-ZnO nanocomposites were tested for their anti-inflammatory activity using assays Bovine serum albumin denaturation. 0.45 mL of bovine serum albumin was mixed with 0.05 mL of different concentrations (10, 20, 30 ,40, 50 µg/mL) *B. purpurea* - mediated Chitosan-ZnO nanocomposites. pH was adjusted to 6.3. Then it was kept at room temperature for 10 minutes followed by incubation in a water bath at 55°C in a water bath for 30 min. Diclofenac sodium was used as the standard group while DMSO was used as control. Then, the samples were measured spectrophotometrically at 660nm [30].

The percentage of protein denaturation was determined utilizing the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

2.9.2 Egg Albumin denaturation assay

B. purpurea leaf extract was further assessed through the egg albumin denaturation assay. In this procedure, 0.2 mL of fresh egg albumin was mixed with 2.8 mL of 1X phosphate buffer (pH 6.3), followed by the addition of ZnO NCs at various concentrations (10, 20, 30, 40, and 50 µg/mL). The pH of the reaction mixture was maintained at 6.3 using 1N HCl. The samples were kept at room temperature for 10 min and then incubated at 55°C for 30 min in a water bath. Diclofenac sodium (50 µg/mL) was used as the standard anti-inflammatory drug, and dimethyl sulfoxide (DMSO) served as the negative control. After incubation, absorbance was measured at 660 nm using a UV–Visible spectrophotometer [31].

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

2.9.3 Membrane stabilization assay

The membrane stabilization activity of Chitosan-ZnO nanocomposites (ZnO NCs) synthesized using *B. purpurea* leaf extract was evaluated using the in vitro human red blood cell (HRBC) membrane stabilization assay. Fresh human blood was collected in a sterile anticoagulant tube and centrifuged at 3000 rpm for 10 min at room temperature to separate red blood cells (RBCs). The RBCs were washed three times with phosphate-buffered saline (PBS) and resuspended in 50 mM Tris-HCl buffer (pH 7.4) to prepare a 10% (v/v) RBC suspension. For the assay, 1 mL of the RBC suspension was mixed with varying concentrations (10, 20, 30, 40, and 50 µg/mL) of ZnO NPs. The samples were gently mixed and incubated at 37°C for 30 mins. After incubation, the tubes were centrifuged at 2500 rpm for 5 mins at room temperature, and the absorbance of the supernatant was measured at 560 nm using a UV–Vis spectrophotometer. membrane stabilization was calculated, with inhibition values indicating membrane-protective effects on ZnO NCs [32].

Calculate the percentage inhibition of haemolysis using the following formula:

$$\% \text{ inhibition} = \frac{(\text{OD control} - \text{OD sample}) \times 100}{\text{OD control}}$$

Where OD control is the absorbance of the RBC suspension without the test compound(s) and OD sample is the absorbance of the RBC suspension with the test.

2.10 Cytotoxic effect

2.10.1 Brine Shrimp Lethality Assay

Chitosan-ZnO nanocomposites (ZnO NCs) synthesized using *B. purpurea* leaf extract was evaluated through the brine shrimp lethality assay (BSLA), a simple and effective method for preliminary toxicity screening. Artificial sea water was prepared by dissolving 2 g of iodine-free salt in 200 mL of distilled water. Ten active *Artemia salina* nauplii were introduced into each well of a six-well ELISA plate containing 10-12 mL of the saline solution. biosynthesized ZnO-NPs were tested at concentrations of 5, 10, 20, 40, and 80 µg/mL, while one well was maintained as a control without nanoparticles. After a 24-hour incubation period at room

temperature, the number of live and dead nauplii was recorded calculated by using the following formula [33].

$$\text{Percentage Mortality of Nauplii (\%)} = \frac{\text{Number of dead nauplii}}{\text{number of dead nauplii} + \text{number of live nauplii}} \times 100$$

3 Results and Discussion

3.1 UV-Vis Spectroscopy

UV-Visible spectroscopy characterization of the 1.5 BP nanocomposite was performed to examine the optical behavior and assess the influence of exfoliation time on absorbance behavior show in Fig 3. The spectra contain a sharp peak for absorbance in the UV range (~350 nm) and a gradual decrease towards longer wavelengths into the visible region (650 nm). of the three samples, the 23 hrs exfoliated nanocomposite (orange curve) displayed the highest absorbance throughout most of the range, suggesting better dispersion or concentration of the 23 hrs exfoliated BP nanosheets. The 15-hour sample (blue curve) had a moderate absorbance, whereas the 43-hr sample (gray curve) had dramatically diminished absorbance, as could be due to over-exfoliation or re-aggregation effects that may decrease the effective optical activity. This behavior indicates that 23 hr can prove to be an ideal exfoliation time to obtain better optical properties in the 1.5 BP nanocomposite. Overall, these results the efficiency of *B. purpurea* leaf extract as a green reducing and stabilizing agent, and underscore 23 hr as an ideal synthesis time for producing well-defined chitosan-ZnO nanocomposites.

3.2. FT-IR Spectroscopy

FTIR spectroscopy was utilized to investigate the functional groups in the *Bauhinia purpurea* leaf extract-mediated chitosan-ZnO nanocomposite show in **Fig. 4**. The wide absorption band at 3279.3 cm⁻¹ is responsible for O–H stretching vibrations, showing hydroxyl groups and potential hydrogen bonding interactions. The band at 2922.7 cm⁻¹ is due to C–H stretching, whereas bands at 1562.1 and 1316.8 cm⁻¹ represent aromatic C=C stretching and amide-related vibrations, respectively. The strong peaks at 1152.5 and 1018.4 cm⁻¹ are related to C–O–C stretching and P–O–C bonds, which affirm the presence of phosphate or chitosan-related groups. Also, the bands at 823.7, 656.8, and 611.27 cm⁻¹ indicate metal–oxygen (Zn–O) vibrations, which authenticate the inclusion of ZnO nanoparticles in the matrix. These unique vibrational modes verify the effective formation of the nanocomposite as well as the strong interactions between chitosan, ZnO, and *B. purpurea* extract phytochemicals. Generally, the

FTIR results validate that the green synthesis method produces a stable and well-functionalized chitosan-ZnO nanocomposite, suggesting it as a potential material for biomedical as well as environmental applications. (**Table 1**) FTIR peak positions, corresponding vibrational modes, and functional groups identified in the *B. purpurea* leaf extract-based chitosan-ZnO nanocomposite. The presence of characteristic peaks confirms the successful incorporation of ZnO nanoparticles, chitosan, and phytochemical constituents from *B. purpurea*, indicating effective functionalization and formation of the nanocomposite.

3.3. XRD Analysis

The crystalline nature of the ZnO–chitosan nanocomposite was confirmed by X-ray diffraction (XRD) analysis, as shown in **Fig. 5**. The diffractogram exhibits a broad amorphous hump characteristic of chitosan, along with multiple sharp diffraction peaks at 2θ values of 12.640° , 16.497° , 18.871° , 22.603° , 23.693° , 25.102° , 26.586° , 27.624° , 29.553° , 30.146° , 32.520° , 33.929° , 41.421° , 41.940° , 44.420° , and 46.888° , which correspond to the characteristic reflections of the hexagonal wurtzite phase of ZnO (JCPDS No. 36-1451). Quantitative phase analysis indicates that the sample contains approximately 10.5% crystalline and 89.5% amorphous content. The high amorphous fraction is attributed to the chitosan polymer matrix, which acts as a stabilizing and dispersing agent, effectively restricting complete crystallization of ZnO nanoparticles. This semi-crystalline architecture enhances the surface reactivity and functionality of the nanocomposite, contributing to its potential in antimicrobial, antioxidant, and antidiabetic treatments.

3.4 SEM Analysis

Fig. 6(a–f) present scanning electron microscopy (SEM) micrographs of the chitosan-ZnO nanocomposite synthesized with the help of *B. purpurea* leaf extract, at different magnifications to evaluate surface morphology, particle distribution, and structural characteristics. **Fig. 6(a)** – $2,500\times$ Magnification (Scale bar: $20\ \mu\text{m}$) At this lower magnification, the composite material possesses a rough, irregular surface with bulky aggregated masses. This image gives a general impression of a stratified and clustered morphology, which suggests strong intermolecular interactions of chitosan with bio-reduced ZnO particles. The dominance of the flake-like features on the surface may be due to the polymeric nature of chitosan, which is sheet-forming. **Fig. 6(b)** – $5,000\times$ Magnification (Scale bar: $10\ \mu\text{m}$) At a more moderately enhanced magnification, individual flakes and plate-like particles are more distinguishable. The surface is more dense, with heterogeneous distribution.

This observation is corroborative of the action of *B. purpurea* phytochemicals in inducing heterogeneous nucleation of ZnO nanoparticles on the chitosan matrix. **Fig. 6(c)** – 10,000× Magnification (Scale bar: 5 µm): At this magnification, the morphology suggests smoother sheets and clear layered structures. Interface of the ZnO nanoparticles and chitosan is found to be seamless, reflecting good dispersion and integration. These layered features may also be due to intercalated or exfoliated chitosan chains that embed nanoscale ZnO. **Fig. 6(d)** – 20,000× Magnification (Scale bar: 2 µm) This higher magnification reveals the ultrafine details of the composite. The nanoparticles exist as dispersed grains in the sheets of chitosan. Some of the round ZnO particles are recognizable but agglomeration occurs in some localized areas. The polymer matrix avoids overagglomeration, showing successful encapsulation. **Fig. 6(e)** – 60,000× Magnification (Scale bar: 1 µm) The morphological characterization establishes dense packing of fine nanoparticles on the surface of chitosan. The nanoscale size of ZnO particles is established, as numerous particles are observed both dispersed within and on the surface of the polymeric film. The bioactive molecules of *B. purpurea* extract could be responsible for uniform capping and regulating the growth of the nanoparticles. **Fig. 6(f)** – 60,000× Magnification with Particle Size Marking (Scale bar: 1 µm) The fig shows particle size measurement of representative ZnO nanoparticles, with sizes ranging from about 21.5 to 54.1 nm, corresponding to features typical of nanodimensional materials. The size distribution observed is a moderate level of polydispersity, typical of green synthesis approaches. The shape of the particles primarily shows spherical to sub-spherical shapes, and their dispersion lends themselves to their applicability in different functional purposes.

3.5 EDX Analysis

The elemental composition of the chitosan-ZnO nanocomposite induced by *B. purpurea* leaf extract was confirmed by energy-dispersive X-ray spectroscopy (EDS), as observed in **Fig. 7**. The spectrum shows sharp peaks representing carbon (C), oxygen (O), and zinc (Zn), thus confirming the successful synthesis of the ZnO nanocomposite in the chitosan matrix. Quantitative analysis reveals that carbon and oxygen are the dominant components, with weight percentages of 52.56% and 36.75%, respectively, from the biopolymer chitosan and plant-based phytochemicals. The presence of zinc is observed, with a weight percentage of 10.70%, which confirms the addition of ZnO nanoparticles that were synthesized through a green chemistry route. The element distribution and the intensity of peaks confirm that zinc is uniformly dispersed in the organic matrix, with the ZAF correction matrix applied for exact

quantification. These results confirm the successful synthesis and compositional integrity of the chitosan-ZnO nanocomposite.

The elemental composition of *B. purpurea* leaf extract-mediated chitosan-ZnO nanocomposite was validated by EDX analysis, as observed in **Fig. 8**. The spectrum clearly indicates the presence of carbon (C), oxygen (O), and zinc (Zn) with clear peaks, small peaks for magnesium (Mg), silicon (Si), phosphorus (P), and osmium (Os). Quantitative analysis revealed that carbon and oxygen are the major elements, taking up the space of 52.29 wt% and 27.58 wt%, respectively, verifying high percentages of the chitosan biopolymer and plant materials. Zinc occupied 12.40 wt%, verifying successful incorporation of ZnO nanoparticles. The trace amounts of Mg, Si, and P could be from natural materials present in the *B. purpurea* extract, whereas Os is likely added as a coating or preparation. The element distribution verifies that ZnO is uniformly distributed into the organic matrix, and the ZAF correction technique was applied to guarantee accurate measurements. These results together verify the successful green synthesis and structural stability of the chitosan-ZnO nanocomposite.

3.6 Zeta Potential Measurement

The surface charge properties and colloidal stability of the *B. purpurea* leaf extract-derived chitosan-ZnO nanocomposite were evaluated by zeta potential measurements, as depicted in **Fig. 9**. The obtained zeta potential reading was +42.76 mV, which reflects a highly positive surface charge and high colloidal stability. The high value of zeta potential reflects strong electrostatic repulsion among particles, which effectively prevents particle agglomeration and enhances the dispersion stability of the nanocomposite in aqueous media. The wall zeta potential and standard deviation derived from measurements were 28.49 mV and 5.03 mV, respectively, thus confirming the notion of a homogeneous distribution of the surface charges. Measured conductivity was 0.4438 mS/cm, and the calculated mean count rate was 4.456E+06 kcps, reflecting high-quality measurements with high signal-to-noise ratio.

3.7. Anti-Diabetic Assay

3.7.1. α -amylase Inhibition Assay

The α -amylase inhibitory property of the ZnO–chitosan nanocomposite prepared from *Bauhinia purpurea* leaf extract exhibited a robust, concentration-dependent inhibitory trend, presented in **Fig. 10**. The nanocomposite showed about 48% inhibition at 10 μ g/mL, which steadily rose to near 82% at 50 μ g/mL. These are comparable to the control drug, demonstrating

effective antidiabetic activity. This finding that the phytochemical ingredients from *B. purpurea* extract, synergistically combined with ZnO and chitosan, enhance α -amylase inhibitory activity, validating its possible claim in controlling postprandial hyperglycemia.

3.7.2. β -glucosidase Inhibition Assay

The β -glucosidase inhibitory properties of ZnO-Chitosan nanocomposites that were prepared from *Bauhinia purpurea* (BP) leaf extract at different concentrations (10–50 $\mu\text{g/mL}$) were compared with a standard inhibitor. **Fig. 11** indicates that the nanocomposite (BP NCs) had a concentration-dependent increase in enzyme inhibition. At the lowest tested concentration (10 $\mu\text{g/mL}$), BP NCs had a moderate inhibition of about 46%, which increased progressively with increasing concentration. At 50 $\mu\text{g/mL}$, inhibition was achieved at about 81%, reflecting potent inhibition of the enzyme. Interestingly, at the high concentrations (40–50 $\mu\text{g/mL}$), BP NCs got close to the standard's inhibition levels, reflecting superior enzyme inhibitory activity. *B. purpurea* leaf extract have strong β -glucosidase inhibitory activity, thus making them interesting natural antidiabetic drugs.

3.8 Antioxidant Activity Assays

3.8.1 DPPH Assay

Antioxidant capacity of ZnO-Chitosan nanocomposites prepared from *Bauhinia purpurea* (BP NCs) leaf extract was determined via the DPPH free radical scavenging assay. **Fig. 12** indicates that the BP NCs demonstrated a dose-dependent high increase in radical scavenging activity over the range of concentrations tested (10–50 $\mu\text{g/mL}$). BP NCs at 10 $\mu\text{g/mL}$ inhibited around 60%, which gradually rose to around 86% at 50 $\mu\text{g/mL}$. Although the reference antioxidant showed a slightly higher inhibition at all concentrations, BP NCs showed similar efficacy, especially at high concentrations. This confirms that the nanocomposites show notable antioxidant activity, probably due to the synergistic activity of ZnO, chitosan, and phytochemicals of *B. purpurea*.

3.8.2 H_2O_2 Radical Scavenging Assay

The antioxidant activity of ZnO-Chitosan nanocomposites prepared from *Bauhinia purpurea* (BP NCs) was measured and compared to a reference antioxidant. As indicated in **Fig. 13**, BP NCs exhibited concentration-dependent scavenging activity against H_2O_2 , validating their potent antioxidant activity. At 10 $\mu\text{g/mL}$, BP NCs revealed approximately 46% inhibition, which progressively increased to about 82% at 50 $\mu\text{g/mL}$. While slightly below standard in all

concentrations, the BP NCs showed excellent inhibition values with little variation, confirming reliable performance. The trend upwards demonstrates efficient neutralization of hydrogen peroxide radicals, which are due to the bioactive molecules of *B. purpurea* incorporated in the ZnO-Chitosan matrix. These results point out the capacity of BP NCs to counteract oxidative stress through effective H₂O₂ removal, supporting their use in antioxidant therapy.

3.8.3. FRAP Assay

The ferric reducing antioxidant capacity of *Bauhinia purpurea* (BP NCs) leaf extract-derived ZnO-Chitosan nanocomposites was determined by the FRAP assay. As shown in **Fig. 14**, BP NCs had a high and concentration-dependent reducing ability, which indicates their high antioxidant activity. The BP NCs at 10 µg/mL had approximately 65% inhibition, which increased in a progressive manner to almost 87% at 50 µg/mL. While lower than optimum, BP NCs exhibited stable and high-level antioxidant activity at all concentrations tested. The inherent ferric ion reduction ability may be due to the presence of bioactive phytochemicals present in *B. purpurea*, synergistically working within ZnO-Chitosan nanocomposite matrix. These findings validate the FRAP activity of BP NCs, justifying their use as efficient natural antioxidants in biomedical and pharmaceutical fields.

3.8.4 ABTS Assay

Fig. 15 illustrates that BP NCs produced a good and dose-dependent antioxidant activity. At a dose of 10 µg/mL, BP NCs showed about 66% inhibition, which further increased to about 85% at 50 µg/mL. While a little less than optimum, the nanocomposites of very good radical scavenging efficiency were consistently recorded at all concentration levels confirmed. The activity corresponds to the successful neutralization of ABTS radicals, which is most probably because of the synergistic action of *B. purpurea* phytochemicals with the ZnO-Chitosan nanomatrix.

3.8.5 Nitric Oxide Scavenging Activity

NO scavenging capability of *Bauhinia purpurea* (BP NCs) ZnO-Chitosan nanocomposites was determined and compared with a reference drug. As shown in **Fig. 16**, BP NCs had significant concentration-dependent inhibitory action against nitric oxide radicals. At 10 µg/mL, BP NCs displayed approximately 65% inhibition, which continuously increased to approximately 85% at 50 µg/mL. While slightly lower than the reference, the nanocomposites showed high NO scavenging activity, which suggests their potential to minimize oxidative stress caused by nitric oxide radicals.

3.9 Anti Inflammatory activity

3.9.1 Bovine serum albumin denaturation assay

Anti-inflammatory activity of chitosan-ZnO nanocomposite prepared from *Bauhinia purpurea* leaf extract (BP NCs) was evaluated using bovine serum albumin (BSA) denaturation assay, and the result is presented in **Fig. 17**. The nanocomposite inhibited protein denaturation concentration dependently, with the extent of inhibition increasing from 44% for 10 µg/mL concentration to 76% at 50 µg/mL. In contrast, the reference drug showed marginally greater inhibition, varying from 47% to 84% over the same series of concentrations. BP NCs showed good anti-inflammatory activity, especially at higher concentrations, with inhibition values comparable to the reference compound. The findings indicate that the prepared nanocomposite has good protein stabilization activity, possibly through synergistic interaction between chitosan, ZnO nanoparticles, and bioactive phytochemicals of *B. purpurea*.

3.9.2 Egg Albumin denaturation assay

The anti-inflammatory activity of the BP nanocomposites (NCs) was also confirmed by the egg albumin (EA) denaturation assay, as shown by **Fig. 18**. Inhibition of protein denaturation was concentration-dependent, and BP NCs inhibited protein denaturation by 46%, 57%, 60%, 65%, and 77% at concentrations of 10, 20, 30, 40, and 50 µg/mL, respectively. While the standard drug inhibited marginally (between 55% and 81%), the nanocomposite was found to have the same activity, particularly at higher concentrations. The results suggest that the BP NCs preserve protein structure even under inflammatory conditions due to the synergistic effect of the phytoconstituents of *B. purpurea* and the functional activities of chitosan-ZnO.

3.9.3 Membrane stabilization assay

The membrane stabilization assay additionally confirmed the anti-inflammatory property of BP NCs, as presented in **Fig. 19**. The BP NCs indicated concentration-dependent protection of erythrocyte membranes from heat-induced hemolysis, with the percentages of inhibition being 51%, 59%, 67%, 74%, and 80% at concentrations of 10, 20, 30, 40, and 50 µg/mL, respectively. The values were almost similar to the reference drug, with the range of inhibition being from 58% to 89%. The enhanced membrane-stabilizing ability of BP NCs at elevated concentrations indicates a synergistic interaction between the chitosan-ZnO matrix and bioactive molecules of *B. purpurea* responsible for their anti-inflammatory activity.

3.10 Cytotoxic effect:

3.10.1 Brine Shrimp Lethality Assay

The cytotoxic activity of the BP NCs was assessed by the brine shrimp lethality test for 48 hours. **Fig. 20** illustrates that the viability percentage of nauplii was still 100% for all tested concentrations (5–80 $\mu\text{g/mL}$) on Day 1, similar to the control group. On Day 2, minimal mortality was seen only at the highest concentration (80 $\mu\text{g/mL}$), with viability falling to 90%, whereas all other groups had 100% survival. These results show that BP NCs have zero cytotoxicity towards *Artemia salina* nauplii, even at high concentrations, which further confirms their biocompatibility and probable safety for biomedical use.

4 Discussion

The current work presents a new and green method to synthesize chitosan-ZnO nanocomposites with *Bauhinia purpurea* (BP) leaf extract, utilizing the reducing and stabilizing capability of phytochemicals and the biological compatibility of chitosan. Two-stage biosynthesis circumvents the drawbacks of chemical processes as well as enhances the structural integrity and therapeutic efficacy of the nanocomposite. UV–Vis spectroscopic studies confirmed the formation of ZnO nanoparticles and 23 h of synthesis time showed the highest absorbance—representing better dispersion and nucleation kinetics than shorter or longer synthesis times. FTIR and XRD analysis confirmed successful ZnO embedding in the chitosan matrix as well as the retention of the vital functional groups of BP extract, which supports the formation of semi-crystalline, bioactive nanocomposite architecture. SEM and EDX showed well-dispersed nanoparticles in the chitosan polymer, while zeta potential analysis (+42.76 mV) showed high colloidal stability, a critical factor for biomedical applications. Functionally, the BP-ZnO-chitosan nanocomposites obtained were highly bioactive in various in vitro assays, which surpass many of the earlier reported green-synthesis ZnO materials. The antidiabetic assays (α -amylase and α -glucosidase inhibition) showed intensive and concentration-dependent activity, almost equal to conventional drugs, which confirms the nanocomposites have the potential to modulate carbohydrate metabolism as well as decrease postprandial hyperglycemia—presumably due to synergistic interactions of ZnO, chitosan, and BP phytochemicals. In antioxidant capacity, the nanocomposites showed repeated strong radical scavenging activity in five assays (DPPH, ABTS, FRAP, H_2O_2 , and NO), confirming their potential to fight oxidative stress. This five-assay broad-spectrum antioxidant response is much superior to several similar studies, wherein only one or two assays

were undertaken. BP extract usage appears to enhance redox reactivity through the addition of flavonoids, tannins, and phenolic compounds that are electron-donating in nature. Anti-inflammatory activity, investigated through BSA, egg albumin, and membrane stabilization bioassays, further confirms the multifunctionality of the nanocomposite. At elevated concentrations, the levels of inhibition attained, and in some cases surpassed, that of the standard drug diclofenac. This indicates a significant potential for these nanocomposites in the treatment of diseases related to inflammation without the side effects of synthetic anti-inflammatories. More broadly, the brine shrimp lethality bioassay showed very low cytotoxicity even at the highest concentration tested. This is a very important observation, especially when compared to many chemically synthesized nanoparticles, which show dose-dependent toxicity. The biocompatibility observed can be explained by the natural origin of all the materials used and the lack of toxic reagents during synthesis. In conclusion, this work introduces a new paradigm in green nanotechnology whereby biomolecules of plant origin and natural polymers synergistically design a multifunctional, stable, and safe nanomaterial. The innovation is not only in the material composition but also in the demonstration of true therapeutic potential across antioxidant, antidiabetic, and anti-inflammatory dimensions with a single, sustainably synthesized nanocomposite.

5 Conclusion

This study reports a novel and eco-friendly synthesis method of *Bauhinia purpurea*-mediated chitosan-ZnO nanocomposites that combines the inherent bioactive richness of phytochemicals in medicinal plants with the biocompatibility of chitosan and the multifunctionality of ZnO nanoparticles. The green synthesis approach employed in this work circumvents the use of harmful chemicals, and therefore it adheres to sustainable development principles and reports a scalable approach to the production of biomedical materials. The resultant nanocomposite possessed strong and broad-spectrum biological activities, such as effective antidiabetic (α -amylase and α -glucosidase inhibition), antioxidant (DPPH, ABTS, FRAP, H_2O_2 , NO scavenging), and anti-inflammatory (protein denaturation and membrane stabilization) effects, with sustained excellent colloidal stability and low cytotoxicity. Such results indicate a synergistic interaction between ZnO, chitosan, and *B. purpurea* phytoconstituents and the development of a cohesive nanoplatform with promising therapeutic potential. This research presents a novel bio-nanocomposite synthesis strategy and proves its superior functional performance, indicating its potential as a next-generation therapeutic compound for the management of oxidative stress, inflammation, and diabetes. This finding presents a robust

platform for future in vivo studies, pharmacokinetics profiling, and formulation development, paving the way for plant-based nanomedicine in clinical translation.

Data availability

No datasets were generated or analysed during the current study

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Author contributions

Saranya Balasubramaniyam: Writing – Original Draft, Methodology. Thirumalaikumaran Rathinam: Data Curation. MohanaKrishnan Srinivasan: Writing And Reviewing The Manuscript. Palanisamy Arulselvan: Writing and Reviewing the Manuscript, Formal Analysis. Suresh Mickymaray: Methodology. Faiz Abdulaziz Alfaiz: Formal Analysis.

Compliance with Ethical Standards

Conflict of Interest

The authors declare no competing interests.

Funding Declaration

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Figures

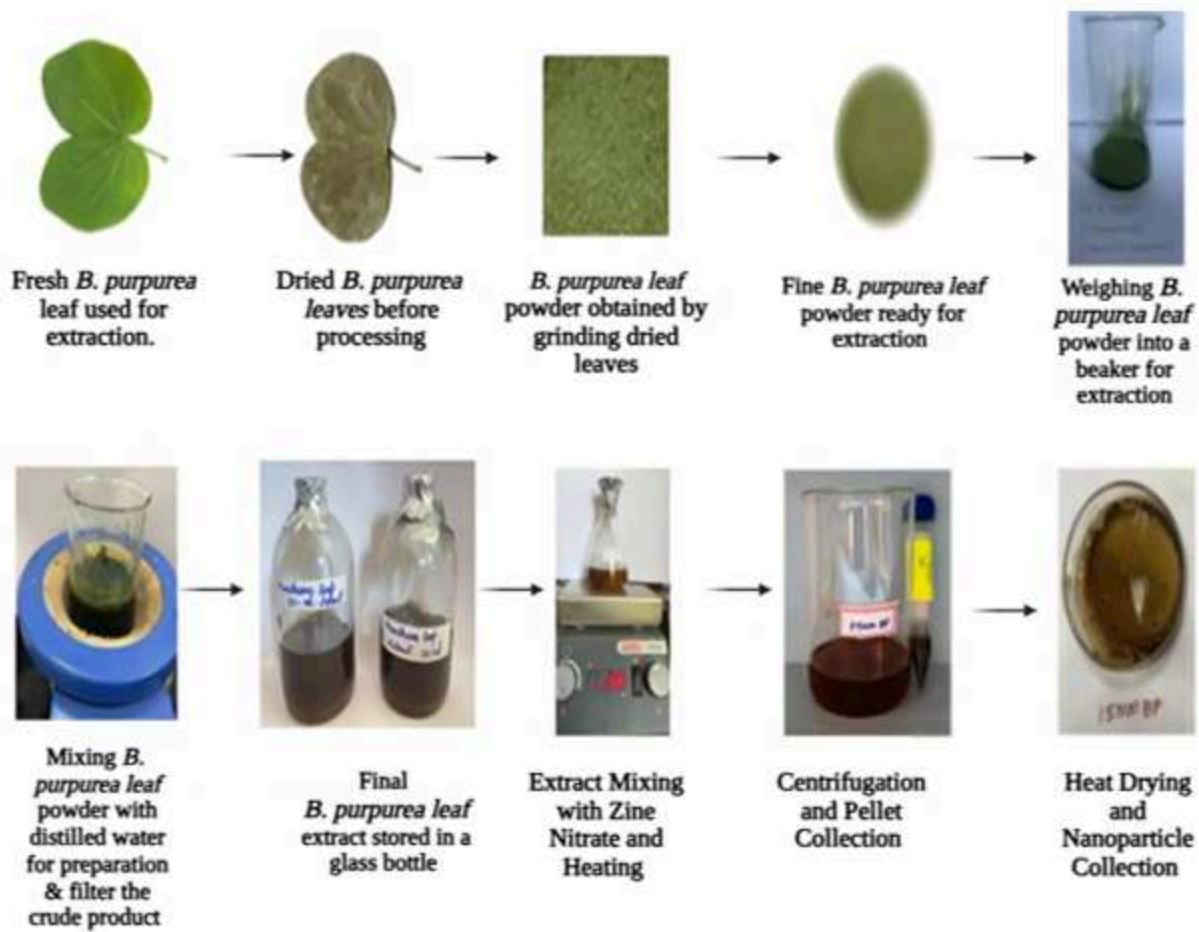


Figure 1

Stepwise process of green synthesis of nanoparticles using *B. purpurea* extract, from leaf preparation to nanoparticle collection

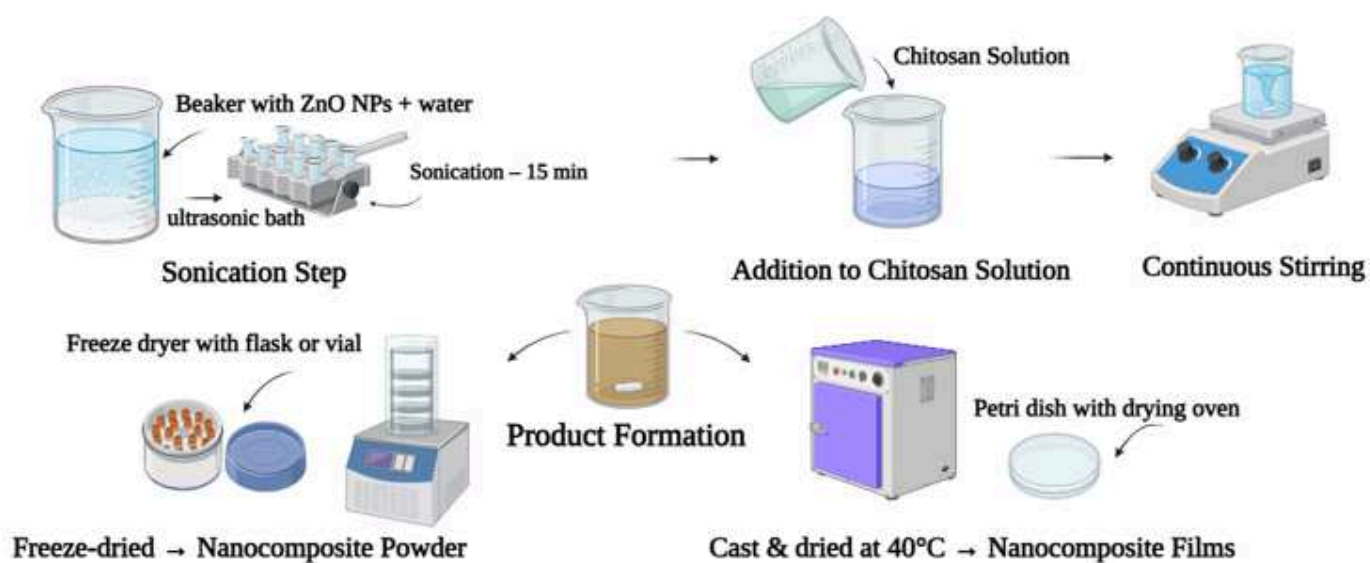


Figure 2

Schematic representation of the synthesis and processing of ZnO-chitosan nanocomposites into powder and film forms.

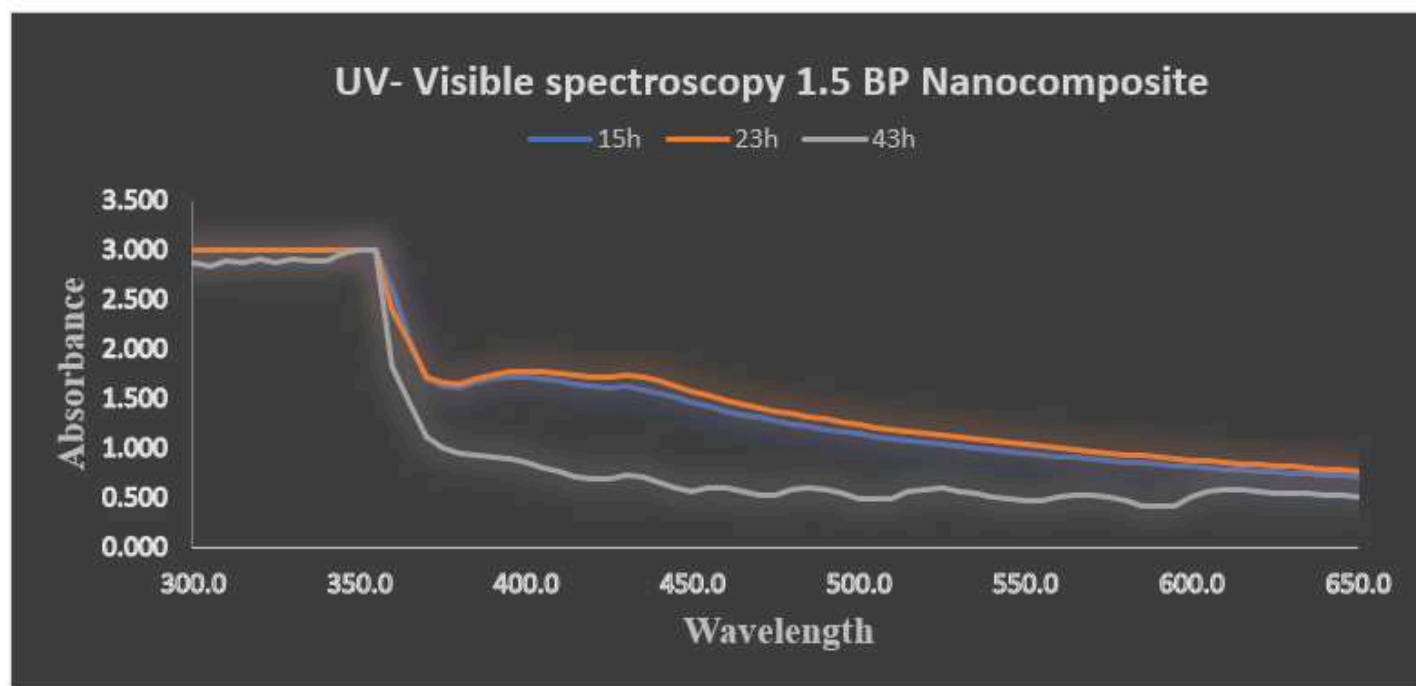


Figure 3

UV-Visible absorption spectra of 1.5 BP nanocomposite at different exfoliation times (15h, 23h, and 43h).

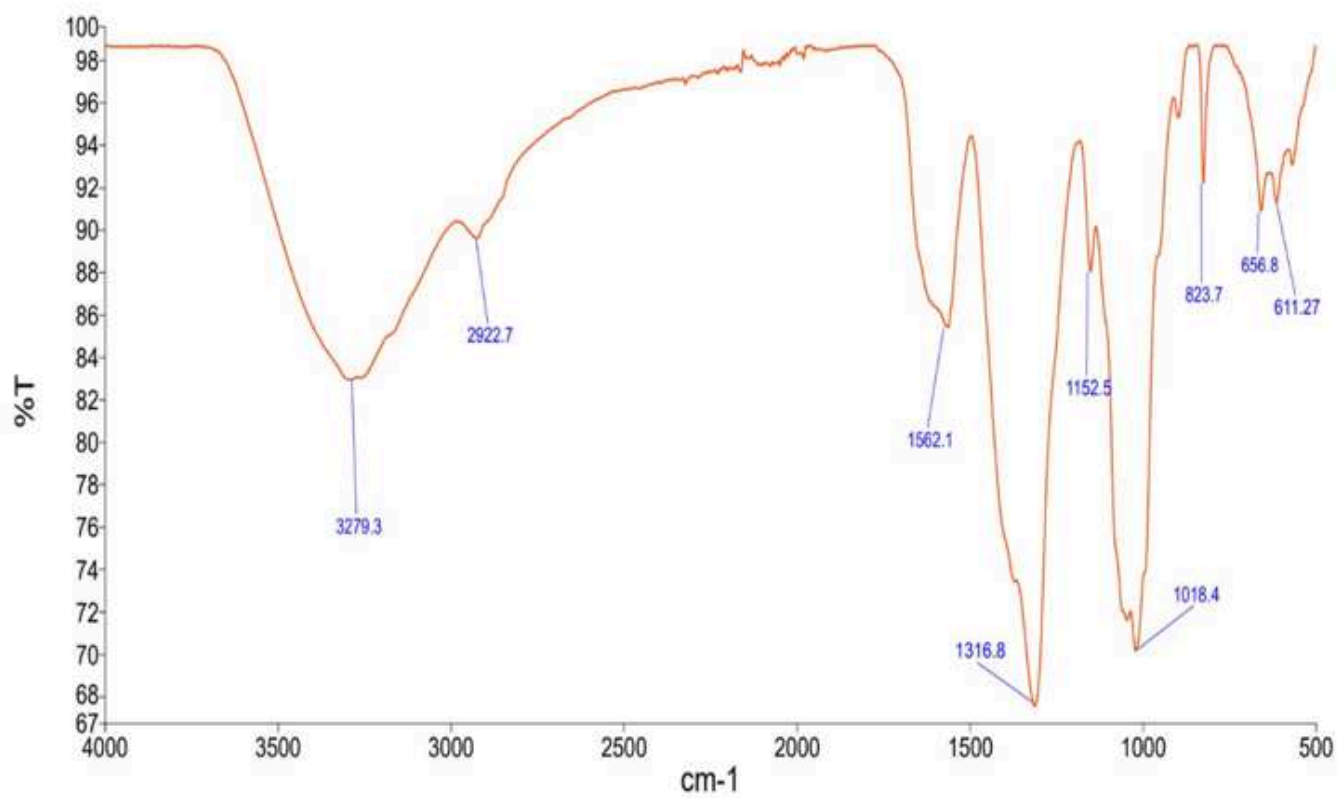


Figure 4

FTIR spectrum confirming the presence of functional groups in the *B. purpurea* leaf extract-based chitosan-ZnO nanocomposite.

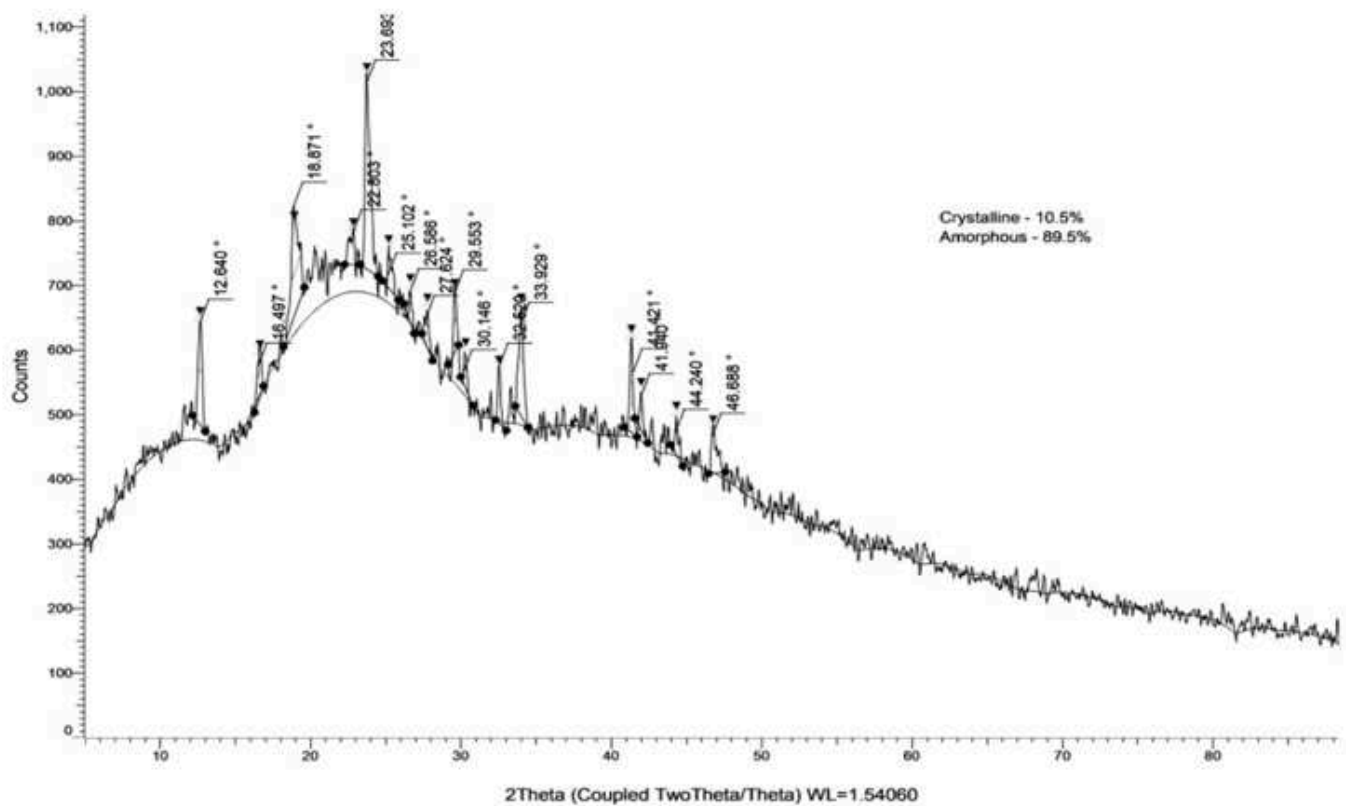


Figure 5

XRD pattern confirming the semi-crystalline nature of ZnO–chitosan nanocomposite with distinct ZnO peaks and dominant amorphous phase.

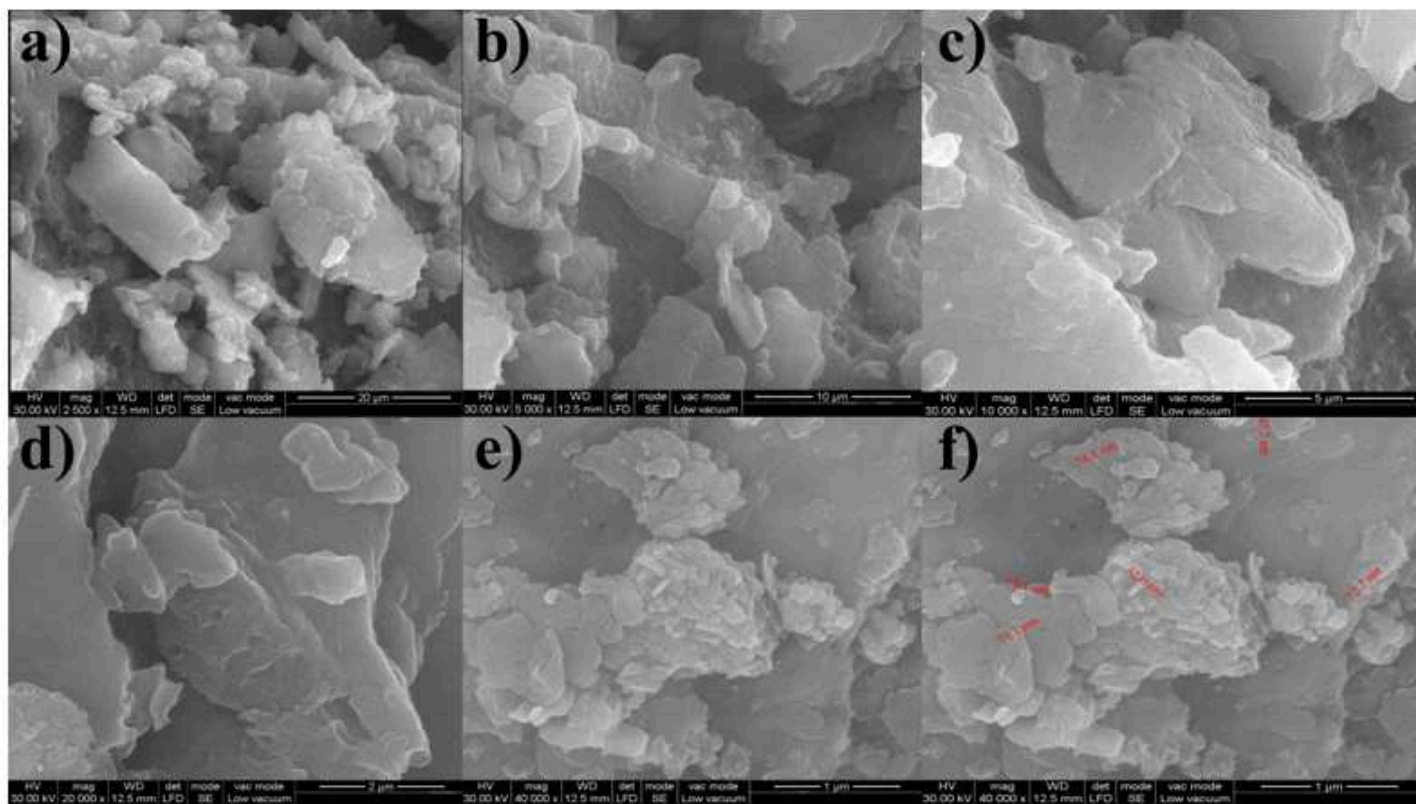
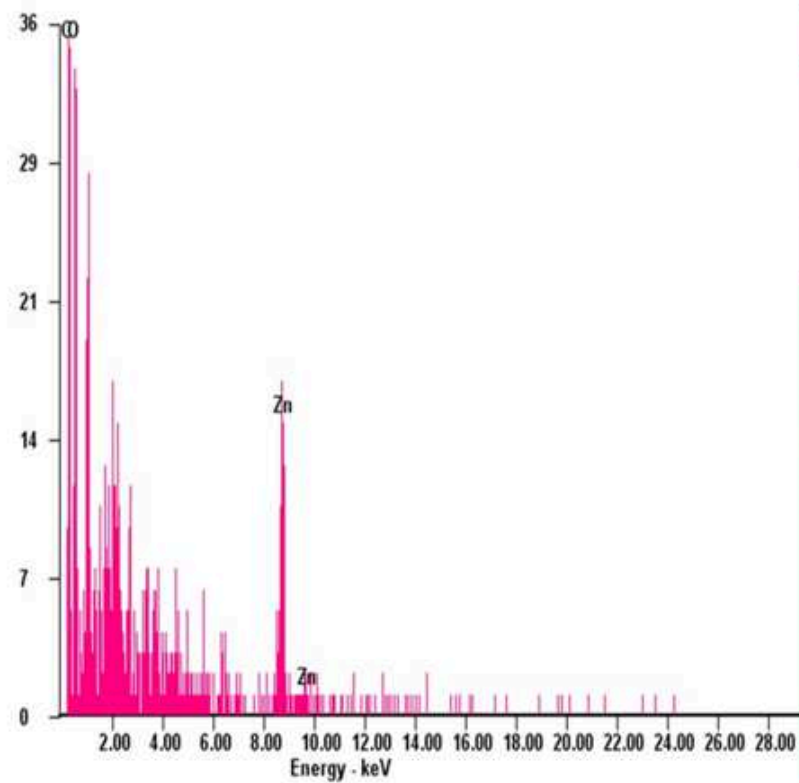
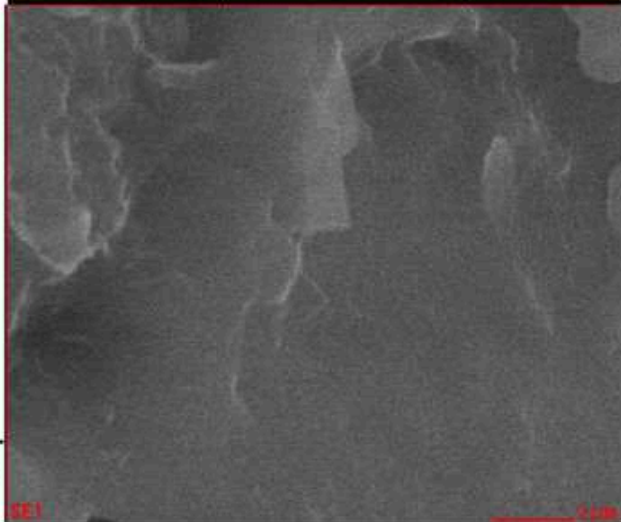


Figure 6

SEM images of *B. purpurea* leaf extract-based chitosan-ZnO nanocomposite at various magnifications showing surface morphology and particle distribution.



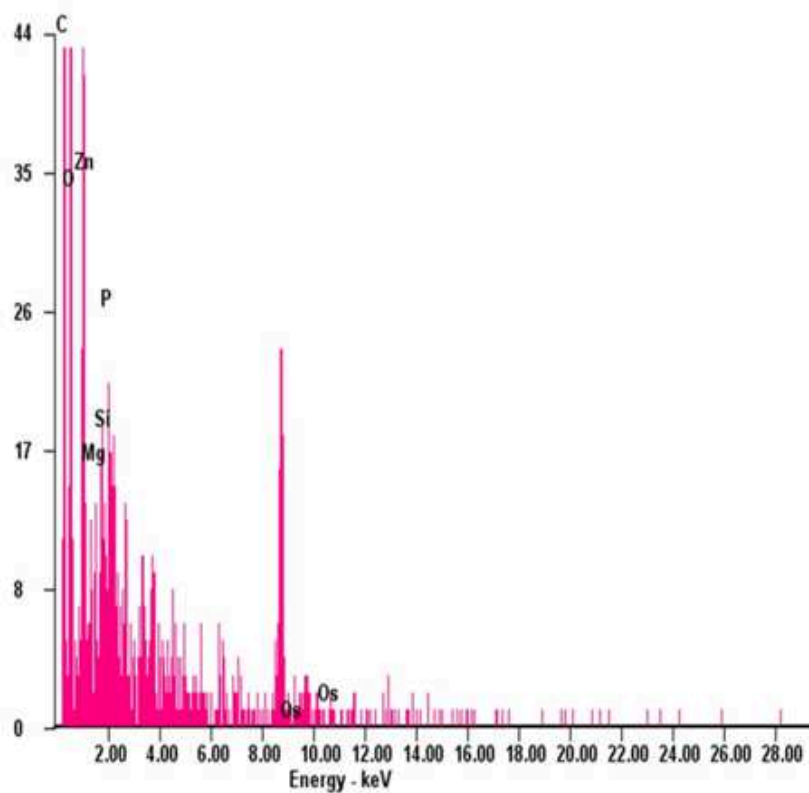
<i>Element</i>	<i>Wt%</i>	<i>At%</i>
<i>CK</i>	52.56	64.01
<i>OK</i>	36.75	33.60
<i>ZnK</i>	10.70	02.39
<i>Matrix</i>	Correction	ZAF



The image is a scanning electron micrograph (SEM) showing the surface morphology of the nanocomposite. The surface appears rough and granular. In the bottom left corner, there is a red label 'SEI'. In the bottom right corner, there is a red label '2.10kV'.

Figure 7

EDX spectrum confirming the presence of C, O, and Zn elements in the *B. purpurea* leaf extract-based chitosan-ZnO nanocomposite.



<i>Element</i>	<i>Wt%</i>	<i>At%</i>
<i>CK</i>	52.29	67.01
<i>OK</i>	27.58	26.53
<i>ZnL</i>	12.40	02.92
<i>MgK</i>	02.05	01.30
<i>SiK</i>	02.15	01.18
<i>PK</i>	01.85	00.92
<i>OsL</i>	01.68	00.14
<i>Matrix</i>	Correction	ZAF

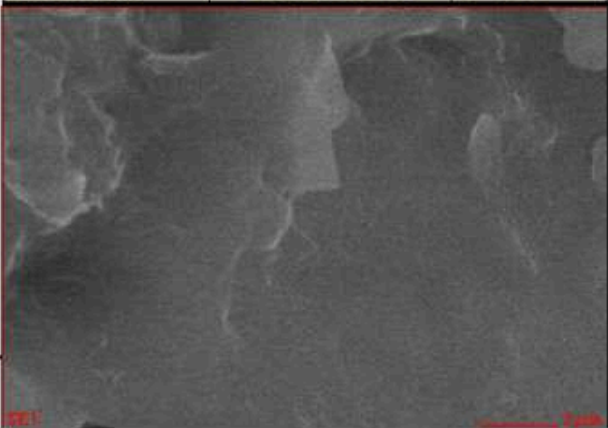


Figure 8

EDX spectrum showing elemental composition of C, O, Zn, and trace elements in the *B. purpurea* leaf extract-based chitosan-ZnO nanocomposite.

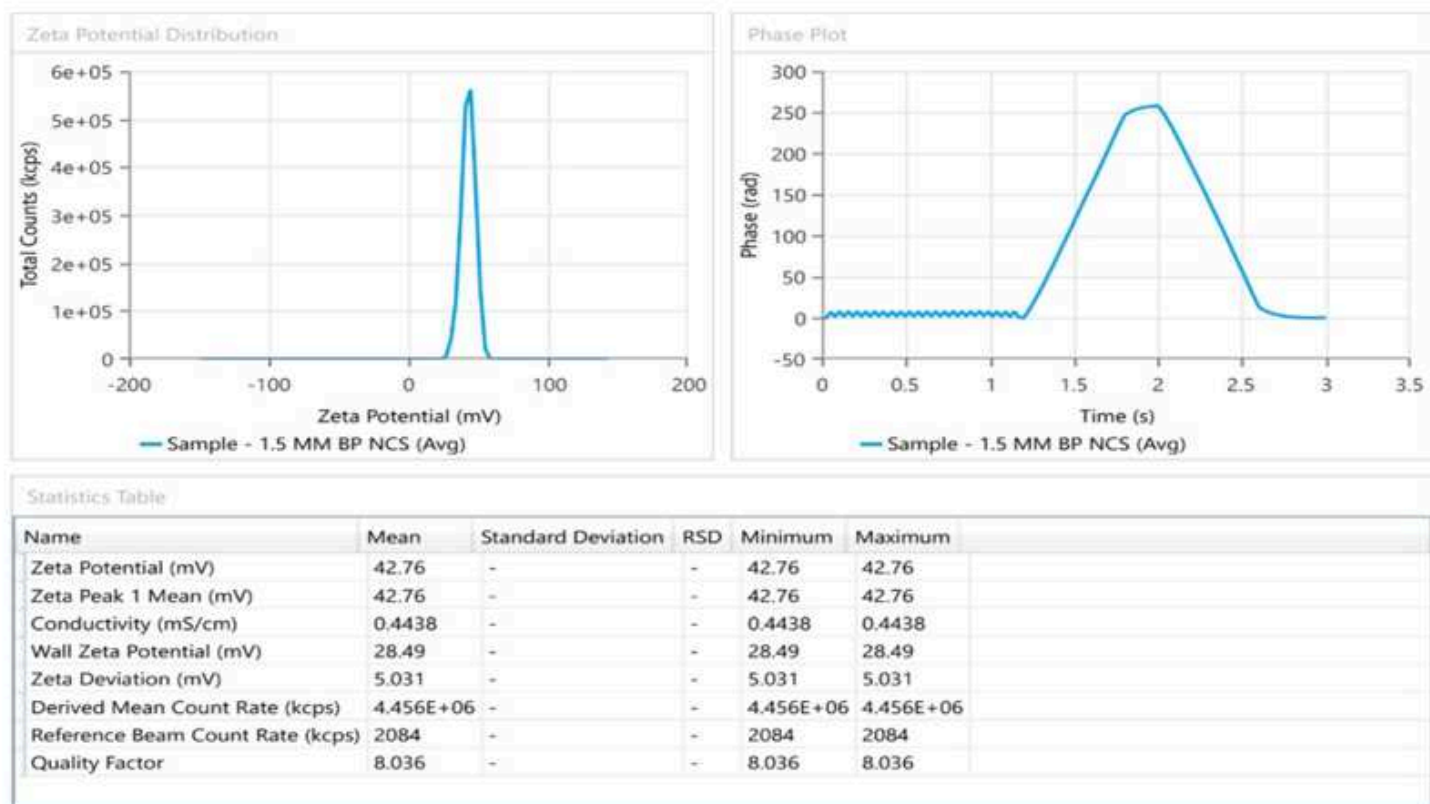


Figure 9

Zeta potential distribution showing high surface charge (+42.76 mV) and excellent colloidal stability of the *B. purpurea* leaf extract-based chitosan-ZnO nanocomposite.

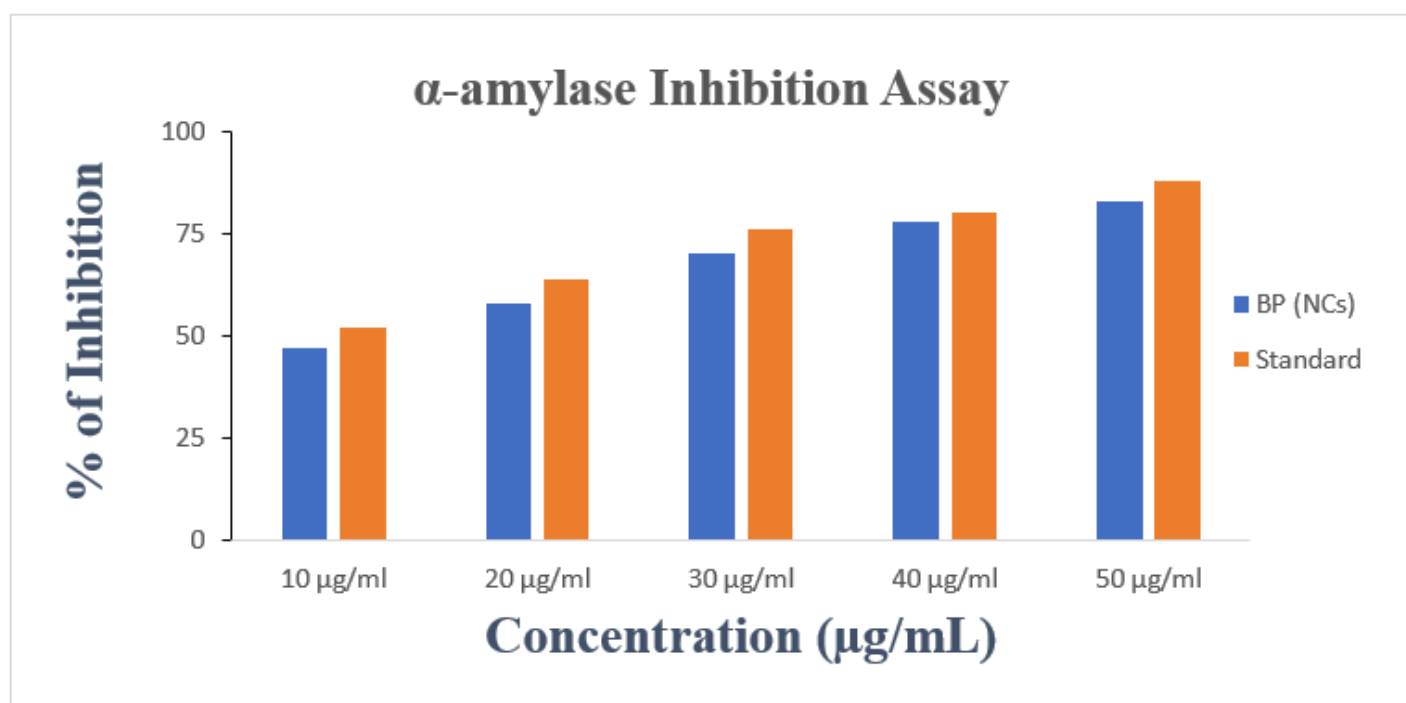


Figure 10

Concentration-dependent α -amylase inhibition by ZnO–chitosan nanocomposite synthesized using *Bauhinia purpurea* leaf extract, showing comparable activity to the standard.

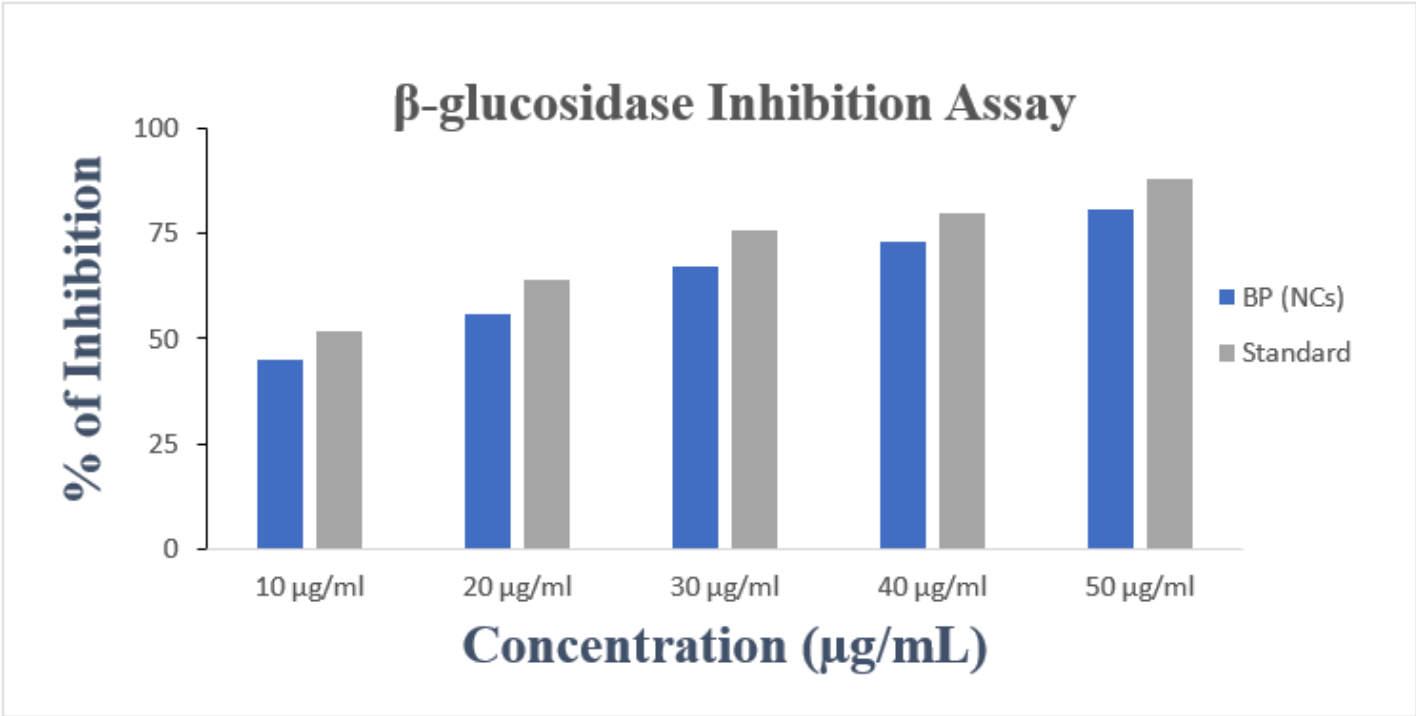


Figure 11

β-Glucosidase inhibitory activity of ZnO-Chitosan nanocomposites from *B. purpurea* leaf extract showing concentration-dependent inhibition comparable to the standard.

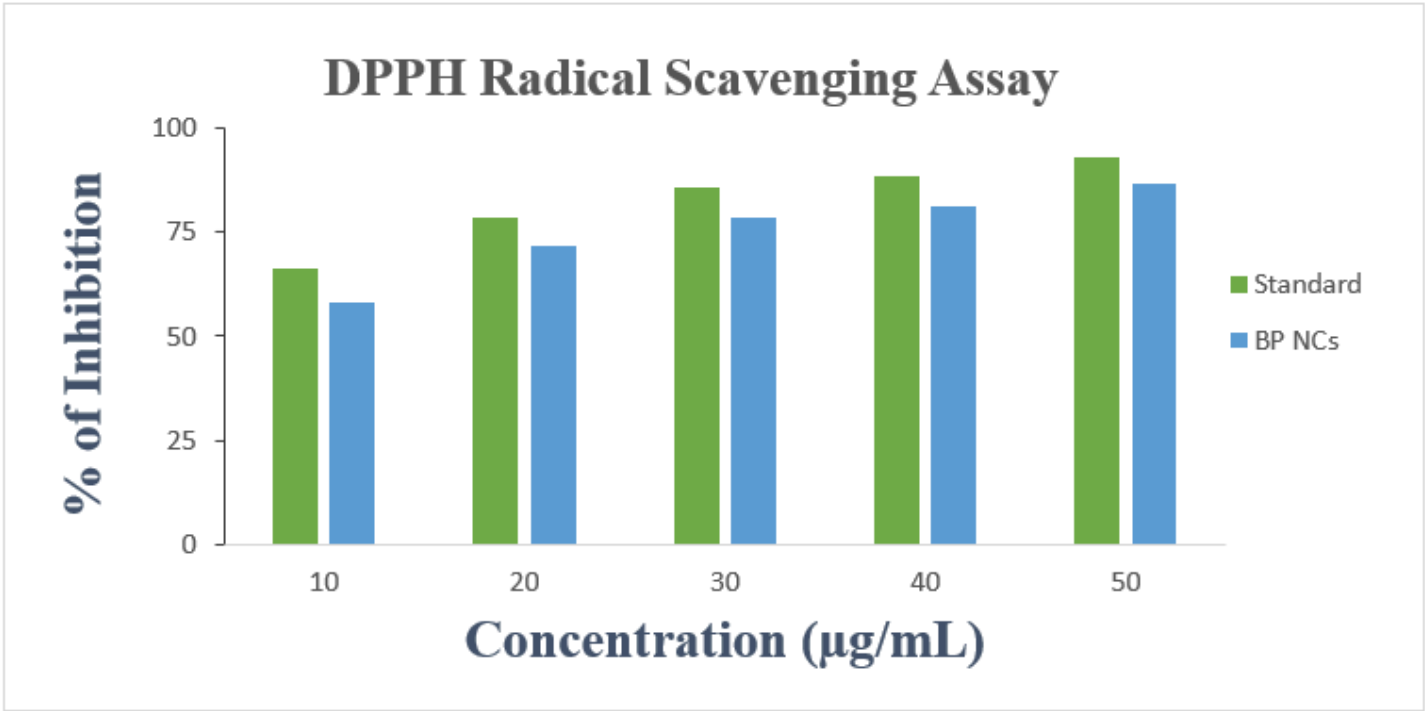


Figure 12

DPPH radical scavenging activity of ZnO-Chitosan nanocomposites from *B. purpurea* showing strong, concentration-dependent antioxidant potential.

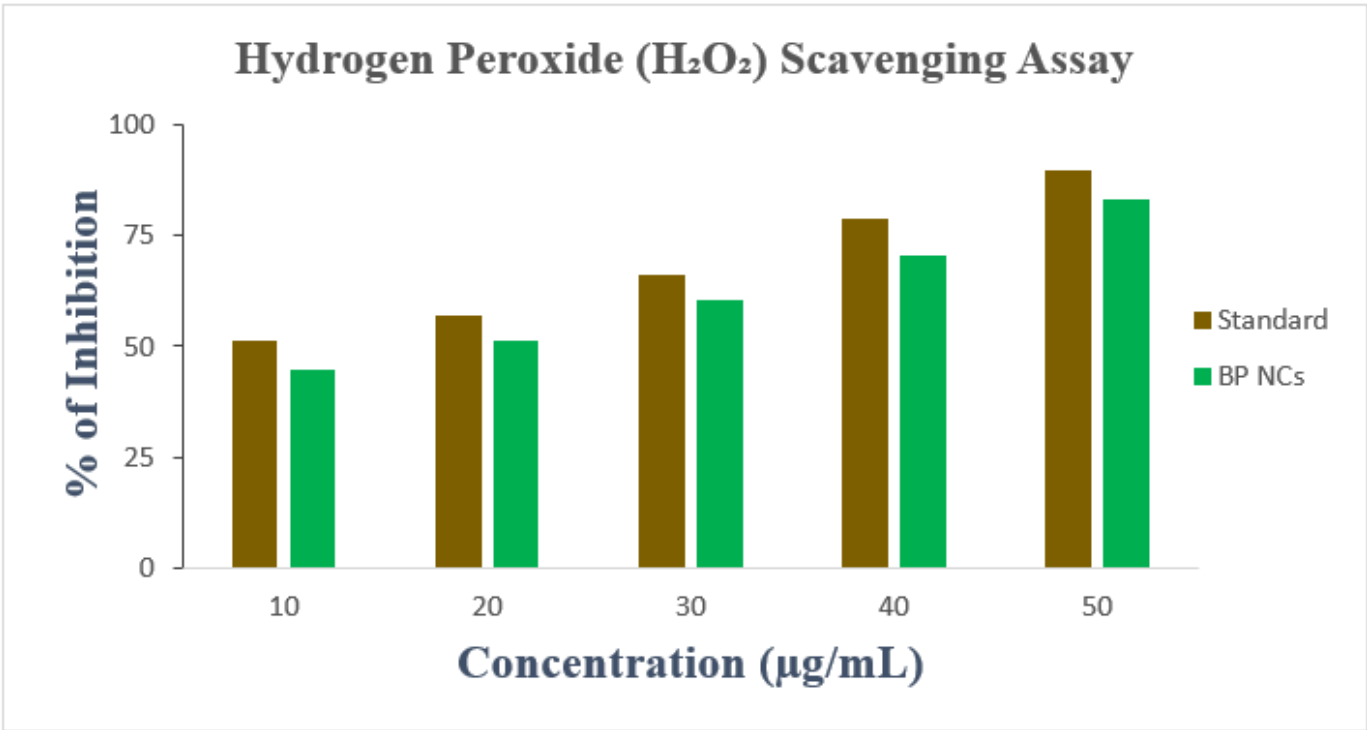


Figure 13

H₂O₂ scavenging activity of ZnO-Chitosan nanocomposites from *B. purpurea* demonstrating potent, dose-dependent antioxidant capacity.

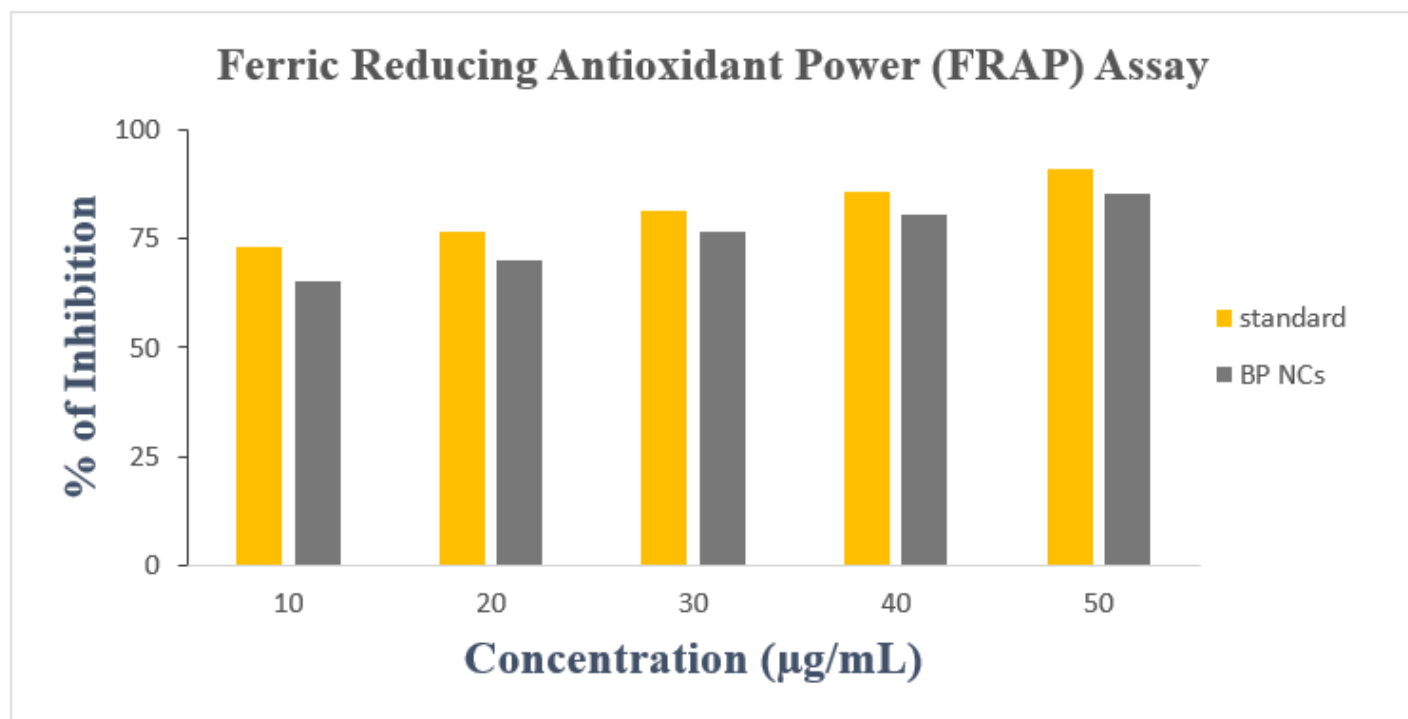


Figure 14

FRAP assay showing strong, dose-dependent antioxidant potential of ZnO-Chitosan nanocomposites from *B. purpurea* compared to the standard.

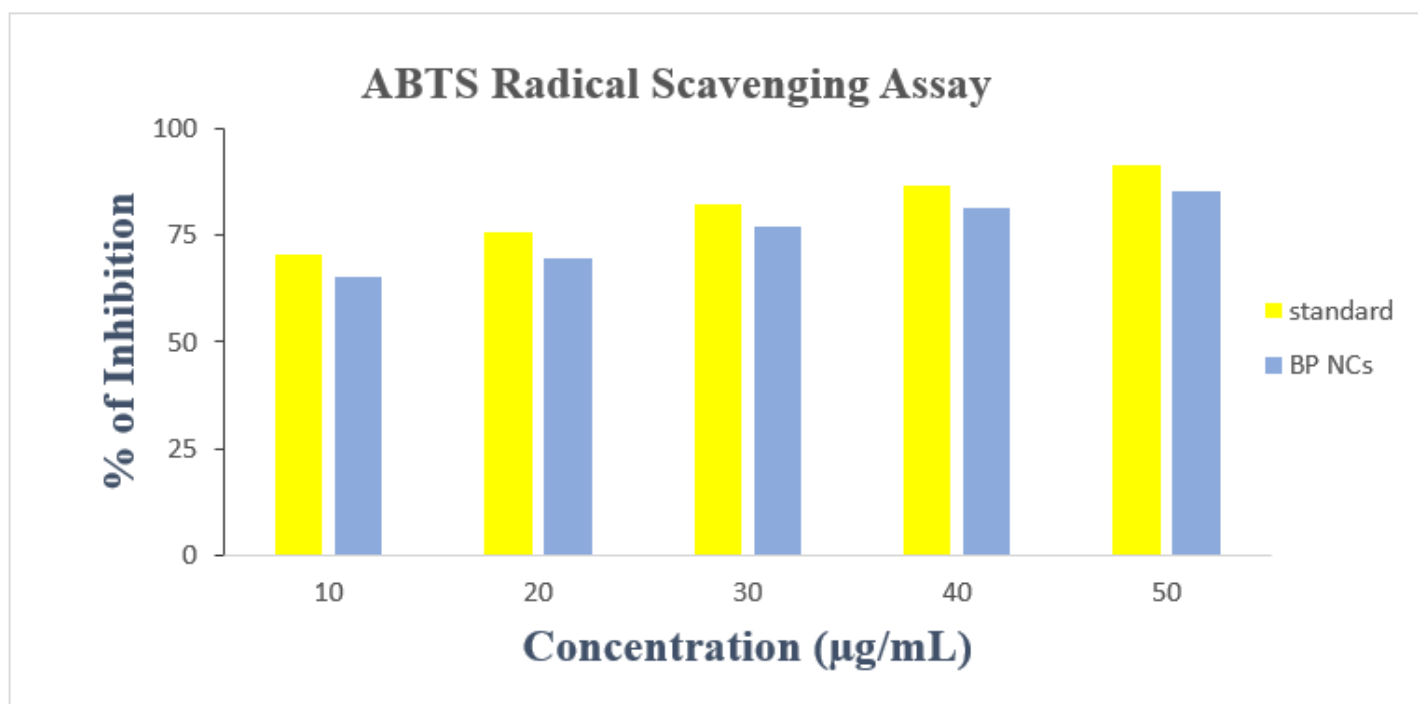


Figure 15

ABTS radical scavenging activity of ZnO-Chitosan nanocomposites synthesized from *B. purpurea* leaf extract, showing effective and dose-responsive antioxidant potential.

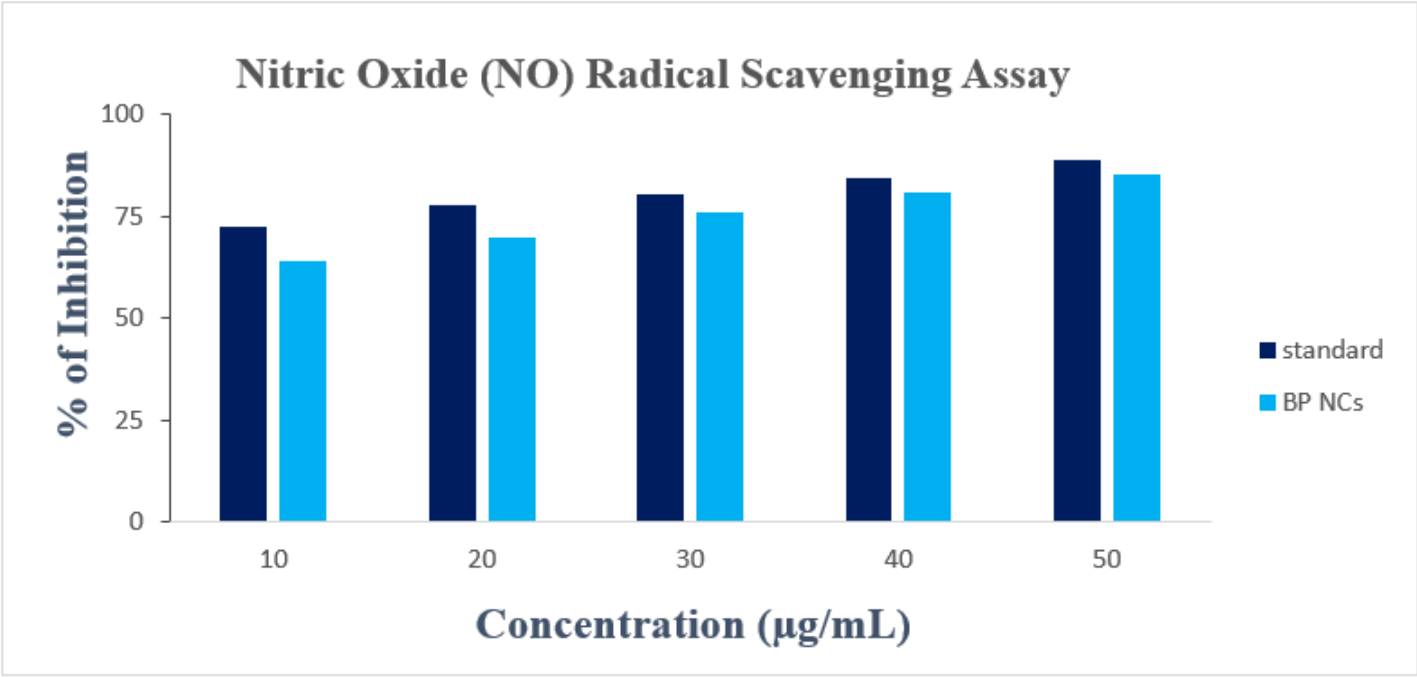


Figure 16

Nitric oxide radical scavenging activity of ZnO-Chitosan nanocomposites synthesized using *B. purpurea* leaf extract, showing potent, concentration-dependent inhibition

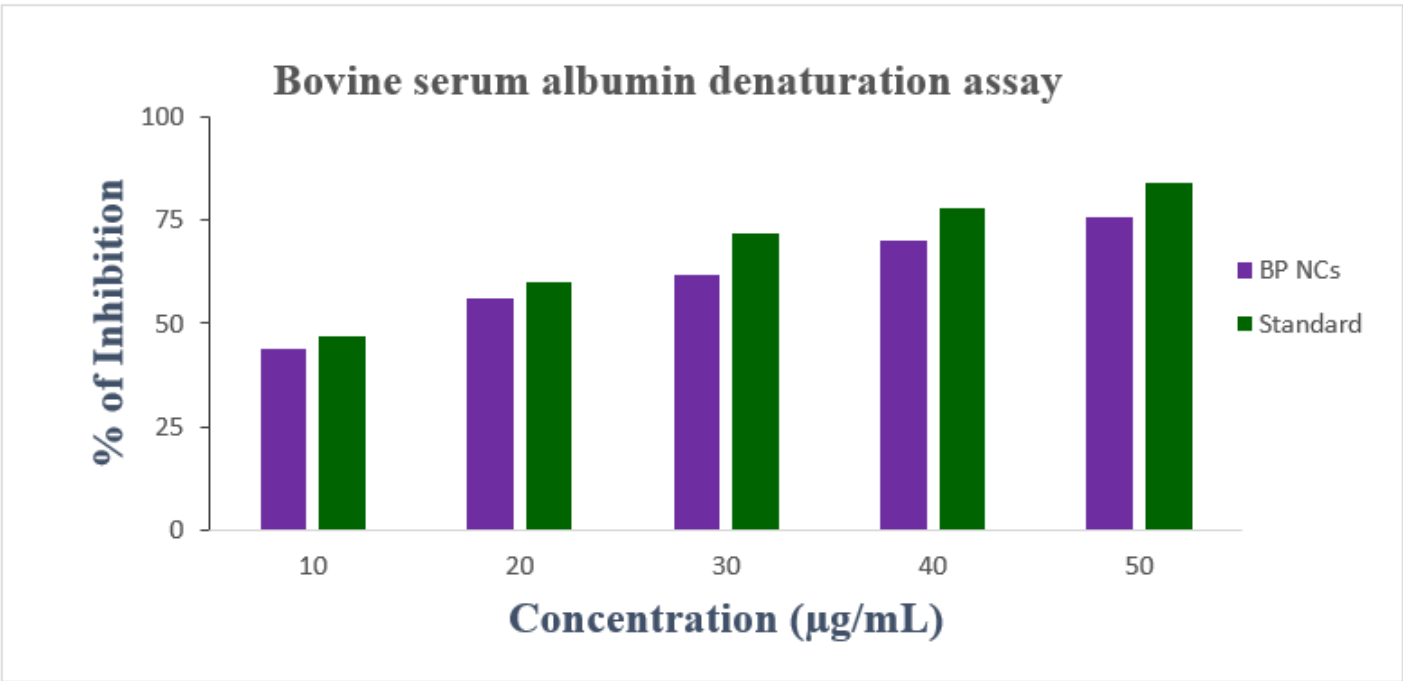


Figure 17

BSA assay showing dose-dependent anti-inflammatory activity of BP NCs compared with the standard drug.

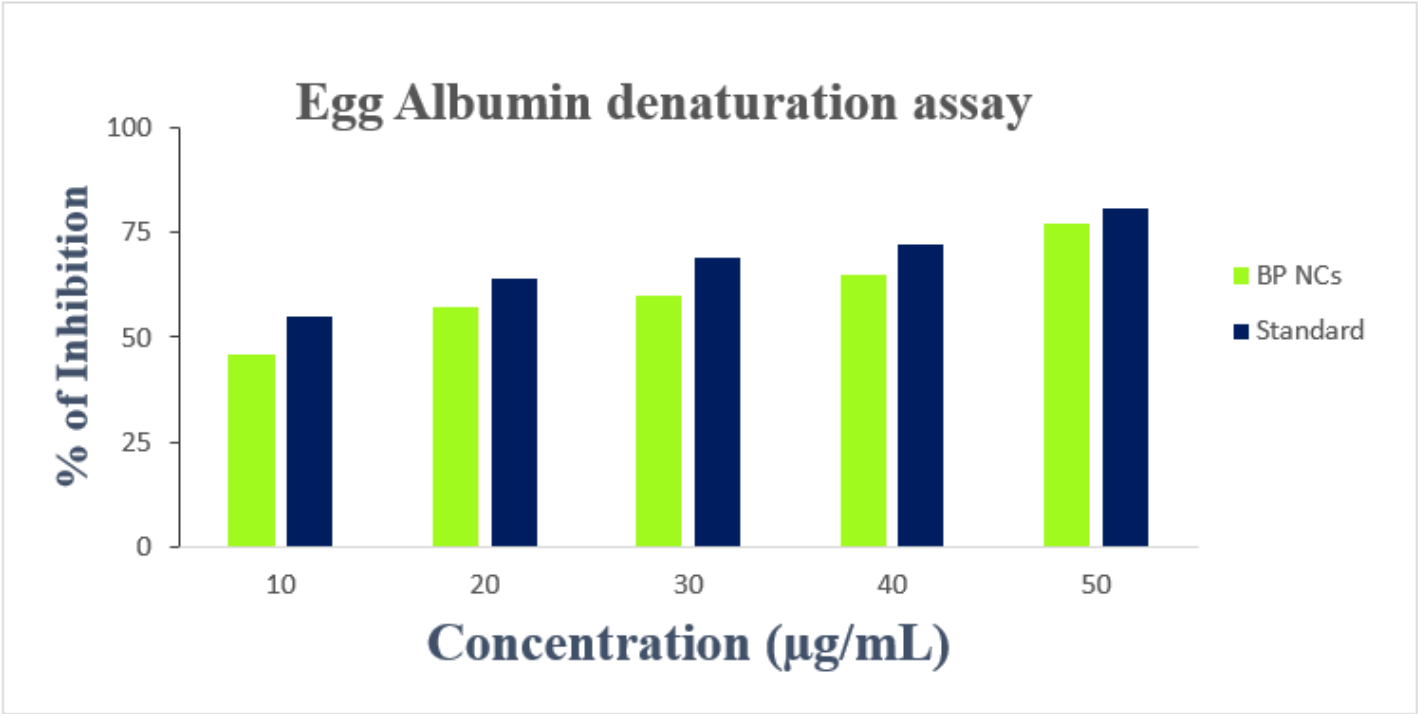


Figure 18

EA assay demonstrating dose-dependent anti-inflammatory activity of BP NCs compared to standard drug.

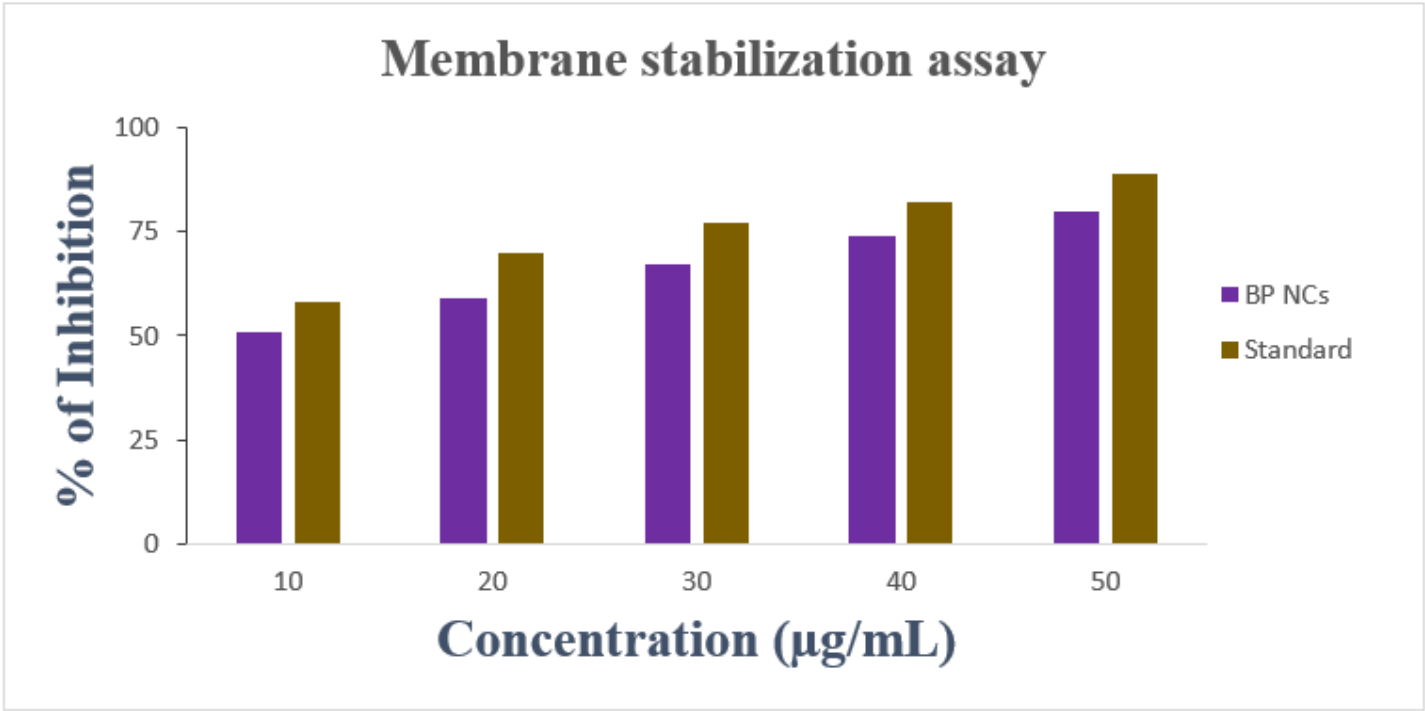


Figure 19

Membrane stabilization assay showing dose-dependent inhibition of hemolysis by BP NCs compared to standard.

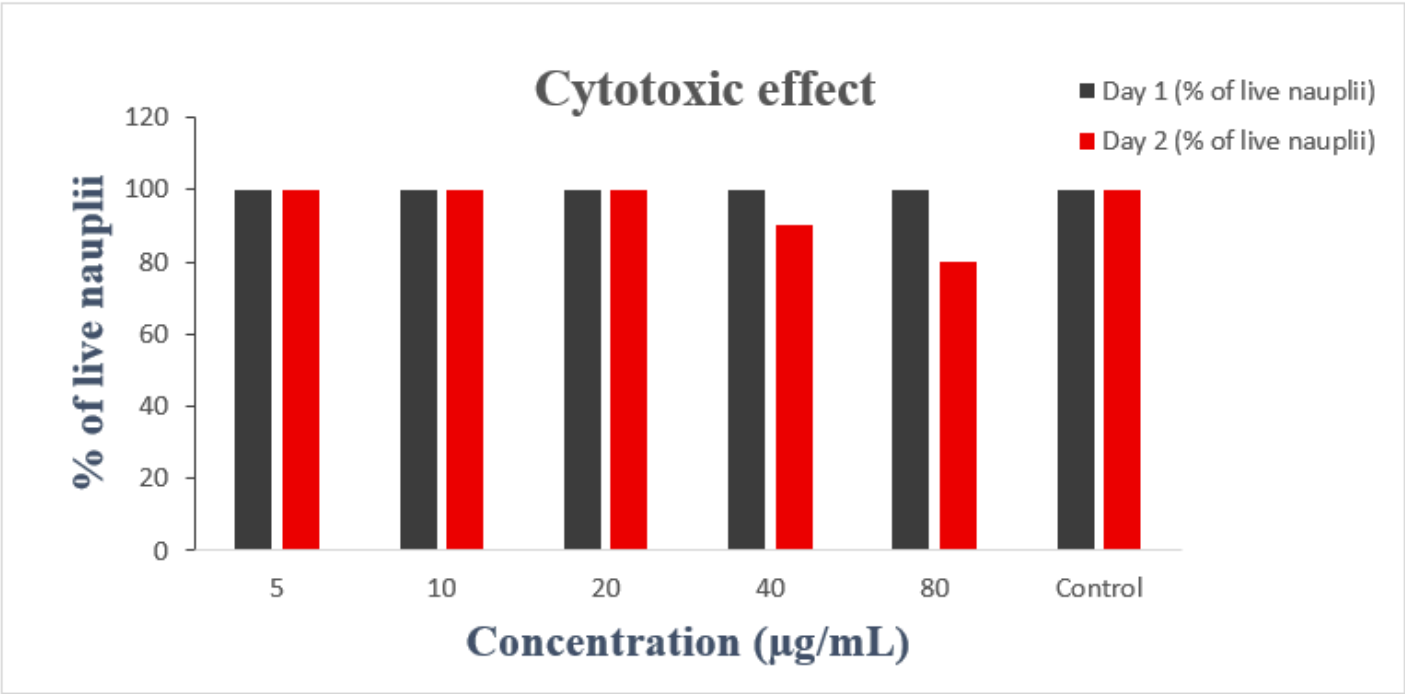


Figure 20

Cytotoxic effect of BP NCs at various concentrations against *Artemia salina* nauplii over 48 hours, indicating minimal toxicity.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TABLE.docx](#)
- [GA.png](#)