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ORIGINAL RESEARCH ARTICLE

Evaluation of the anti-inflammatory and antioxidant activities of algae-mediated zinc oxide nanoparticles

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ABSTRACT

The current study investigates the anti-inflammatory and antioxidant potential of zinc oxide (ZnO) nanoparticles synthesized using an algal extract as a green, eco-friendly reducing and stabilizing agent. The biosynthesized nanoparticles were characterized using UV-Vis spectroscopy (UV, absorption peak 330 nm) XRD (24 nm crystalline size), SEM (rod shaped morphology) and FTIR (Zn-O bond at 671cm^{-1}). Anti-inflammatory activity was assessed using the bovine serum albumin (BSA) protein denaturation method showing dose dependent inhibition (28.4-88.0 % at 100- 500 ug/ml; IC_{50} = 251.64 ug/ml), comparable to ibuprofen (IC_{50} = 233.60 ug/ml). Antioxidant activity of green synthesised ZnO nanoparticles was evaluated using DPPH free radical scavenging assay (28.8-88.0% at 100- 500 ug/ml; IC_{50} = 200.06 ug/ml) attaining ~ 85% efficacy of ascorbic acid (IC_{50} = 171ug/ml). Results showed that ZnO NPs exhibited strong, concentration-dependent anti-inflammatory activity, inhibiting BSA denaturation by 28.4% to 88% over a range of 100–500 µg/mL, with an IC_{50} value of 251.64 µg/mL, closely comparable that of ibuprofen (IC_{50}=233.6 µg/mL). Moreover, the nanoparticles showed substantial antioxidant activity, with DPPH radical scavenging ranging from 28.8% to 88.9% within the same concentration range, and an IC_{50} of 200.06 µg/mL—comparable to ascorbic acid (IC_{50}=171 µg/mL). The bioactivities are likely enhanced by the phytochemicals present in *A. platensis*, such as phenolic compounds and C-phycocyanin. Thus, algae-mediated ZnO nanoparticles exhibit significant anti-inflammatory and antioxidant effects in a dose-dependent manner, suggesting their potential as bioactive agents for pharmaceutical and therapeutic applications.

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INTRODUCTION

Nanotechnology has revolutionized biomedical research, particularly after the development of nanoparticles with therapeutic potential. Among metal oxide nanoparticles, Zinc oxide (ZnO) has garnered significant attention due to its biocompatibility, chemical stability, and versatile applications. Conventional chemical synthesis methods, however, often involve hazardous chemicals and high energy consumption. In contrast, green synthesis using biological agents offers a more sustainable and environmentally safe alternative (Behra *et al*., 2013). Algae, being photosynthetic microorganisms, are rich in biomolecules such as polysaccharides, proteins, and phenolic compounds that can act as natural reducing and capping agents. These properties make algae an ideal candidate for the green synthesis of ZnO nanoparticles. Zinc oxide nanoparticles (ZnO) have attracted a lot of interest because of their wide variety of potential uses in many industries. They are now the focus of study in the technological and medical fields due to their possible medicinal qualities, which include antibacterial, anti-inflammatory, anticancer, and UV blocking agent in sunscreen (Lopez-Miranda *et al*., 2023). The complicated process of inflammation is often linked to pain and includes things like an increase in membrane permeability, and a rise in protein denaturation (Leelaprakash & Dass, 2011).

Recent studies have demonstrated the biological activities of ZnO nanoparticles, including antibacterial, anticancer, and anti-inflammatory effects (Ahmed & Othman, 2024). Zinc, an essential trace element necessary for numerous enzymes involved in preserving the equilibrium between oxidative anti-oxidative, and any interference with this might result in inflammation (Pati *et al*., 2016). Antioxidants and free radicals are balanced in a healthy metabolism. However, oxidative damage brought on by an excess of free radicals causes a number of chronic illnesses, including diabetes, inflammation and cancer (Ravipati *et al*., 2012). Consuming antioxidants offers defense against harm brought on by free radicals. However, due to their toxicity, synthetic antioxidants have not been widely used, therefore much research is needed for the natural origin antioxidant (Ramesh *et al*., 2015).

Despite this advancement, a major research gap exists as most existing research rely on chemically synthesized nanoparticles and use of animal model to check the biological mechanism, raising ecological and ethical concern. The use of animals in scientific experiment research presents several fundamental concerns, especially when their involvement lacks strong justification—especially in cases where alternative techniques are already available or could be explored further as viable alternatives (Knight, 2011). Furthermore, the Failure to reflect analogous or identical patterns with human species renders extrapolation extremely difficult due to cross-species variations, particularly at the molecular and cellular level, where illness manifests (Balls, 2022). The excessive use of animals in testing anti-inflammatory agents from natural product, commercial products extract via activity-directed isolation is both ethically concerning and economically challenging. To solve this major issue, the Bovine Serum Albumin (BSA) assay has been proposed as an in vitro alternative that reduces reliance on live specimens. This assay evaluates the ability of compounds to inhibit heat-induced protein denaturation of BSA, a process associated with inflammatory conditions such as rheumatoid arthritis and systemic lupus erythematosus. By identifying potential anti-inflammatory agents through this method, researchers can streamline the drug discovery process while adhering to ethical standards.

Therefore, this study focuses on synthesizing of ZnO nanoparticles using algal extracts (*Arthospira platensis*) and evaluates their anti-inflammatory and antioxidant potential through established *in vitro* methods and it will contribute to the growing field of bio-nanotechnology by offering a sustainable route for nanoparticle production with promising biomedical applications.

MATERIALS AND METHODS

Materials used for the study

In this study, zinc acetate dihydrate [Zn $(CH_3COO)_2{\cdot}2H_2O$] (Sigma -Aldrich, ≥99%), methanol (Merck, HPLC grade), bovine serum albumin (BSA, HI media), DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma), phosphate-buffered saline (PBS, pH 6.4, Himedia), and ascorbic acid (SRL Chemicals) were used. All chemicals were analytical grade and used without further purification.

Synthesis of ZnO nanoparticles

The synthesis of ZnO nanoparticles referred to Kanika *et al*. (2025) using the green synthesis method, by taking *Arthospira platensis* as a reducing agent. 20 g of wet algal biomass was extracted with 200 mL of methanol/water (70:30 v/v), and the mixture was kept under dark conditions for 24 hours with constant agitation. The extract was centrifuged, and the resulting supernatant was vacuum-dried using a rotary evaporator. For nanoparticle synthesis, 100 mL of the algal extract was mixed with 2 mM zinc acetate dihydrate [Zn $(CH_3COO)_2.H_2O$] solution, stirred at 150 rpm and pH 8.4 at 25°C until a white precipitate formed. The precipitate was collected and calcinated at 200°C for 24 hours Characterization was carried out using UV-Vis XRD, FE-SEM, FTIR Zeta.

Anti-inflammatory activity

***In-vitro* anti-inflammatory activity of ZnO nanoparticles:** The anti-inflammatory potential of ZnO nanoparticles was evaluated using the Bovine Serum Albumin (BSA) denaturation assay, following the methods previously described by Williams *et al*. (2008), Gunathilake *et al*. (2018), and Harris *et al*. (2023), with slight modifications. The reaction mixture (5 ml) was made up of 2 ml of extract, 2.80 mL of phosphate buffered saline (PBS, pH 6.4), and 0.2 ml of 1% bovine albumin. After mixing the mixture, it was incubated for 15 minutes at 37 °C in a water bath before being heated for 5 minutes at 70 °C. After cooling, a UV/VIS spectrometer (Shimadzu UV-1800) was used to measure the turbidity at 660 nm. The control was phosphate buffer solution and ibuprofen as a reference drug. Figure 1 shows experimental set up for Bovine denaturation assay of the reaction mixture at different concentrations All the test was performed in triplicate and results expressed as mean ± standard deviation (SD Using the following formula, the percentage inhibition of protein denaturation was determined:

$$\% \text{ Inhibition of denaturation} = 100 \times (1 - A2/A1)$$

Where A1 = absorption of the control sample, and A2 = absorption of the test sample.

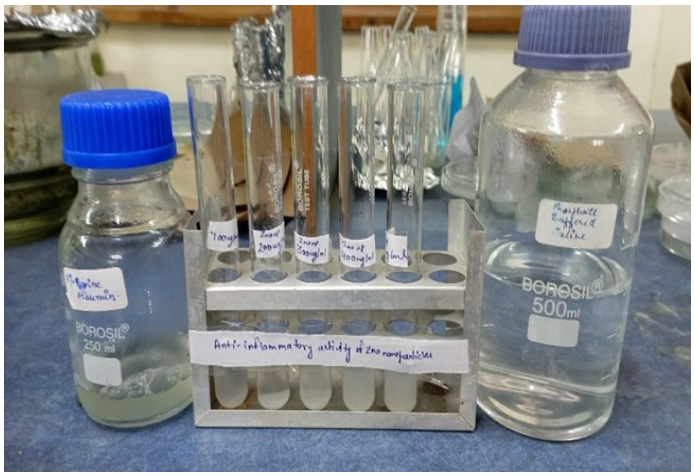

Figure 1. *Bovine denaturation assay of the reaction mixture at different concentrations.*

Figure 2. *DPPH Radical Scavenging Assay of Algae-Mediated ZnO Nanoparticles at Varying Concentrations (100–500 µg/mL).*

DPPH radical scavenging activity

The antioxidant potential of *Arthrospira platensis*-mediated zinc oxide nanoparticles (ZnO NPs) was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay using protocol done by Blois (1958) with slight modification. A 0.1 mM DPPH solution was freshly prepared in methanol and stabilized in the dark for 30 minutes. Varied concentration of ZnO NPs (100–500 µg/mL) were prepared. Each test sample was mixed with the DPPH solution (1:1 ratio) and vortexed gently and incubated in the dark at 25°C for 30 minutes. After incubation the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as the reference antioxidant (positive control) negative control (DPPH solution without nanoparticles used as negative control. Figure 2 shows DPPH Radical Scavenging Assay of Algae-Mediated ZnO Nanoparticles at Varying Concentrations (100–500 µg/mL). All the test was performed in triplicate and results expressed as mean ± standard deviation (SD). The percentage of DPPH radical scavenging activity was calculated using the formula:

DPPH scavenging (%) = (*A* control - *A* sample)/absorption of control×100

RESULTS AND DISCUSSION

Synthesis of ZnO nanoparticles

The zinc oxide nanoparticles synthesised using *A. platensis* in a previous study. The crystalline size measured using XRD was 24 nm. The previous studies reported that SEM technique produces ZnO nanoparticles in the range of 20–300 nm SEM images showed that they were rod shaped nanoparticles (Kanika *et al.*, 2025). Similar results were also confirmed by Ahmed & Othman (2024).

Anti-inflammatory activity of *A. platensis*-mediated ZnO nanoparticles using BSA denaturation assay

The anti-inflammatory potential of *A. platensis*-mediated zinc oxide nanoparticles (ZnO NPs) is evaluated using the Bovine Serum Albumin (BSA) denaturation assay (Figure 3) This assay is a well-established method to assess the anti-inflammatory activity of compounds by measuring their ability to inhibit protein denaturation, which is a key mechanism in the inflammatory response. The results demonstrated that the synthesized ZnO NPs exhibited significant anti-inflammatory activity, with inhibitory effects comparable to those of a standard anti-inflammatory drug (ibuprofen) in the BSA denaturation assay, the *A. platensis* -mediated ZnO NPs displayed inhibitory effects on BSA denaturation at various concentration, comparatively, the standard anti-inflammatory drug exhibited similar inhibitory effects on BSA denaturation. Specifically, at 100 µg/mL, the *A. platensis* mediated ZnO NPs showed 28.4 inhibition, while the standard anti-inflammatory drug displayed 33.6% inhibition. At 200 µg/mL, the percentages are 40.3% for the *A. platensis* mediated ZnO NPs and 43.9 for the standard anti-inflammatory drug; at 300 µg/mL, 58.4% and 60.69%, respectively; at 400 µg/mL, they are 70% and 69.8%, respectively; and at 500 µg/mL, they were 88% and 85.2%, respectively as shown in Table 1. These results suggest that the *A. platensis* mediated ZnO NPs exhibit anti-inflammatory potential by inhibiting BSA denaturation, with inhibitory effects comparable to those of the standard across all concentrations tested. Similar finding was shown by Varghese *et al.* (2024). The anti-inflammatory activity of ZnO NPs (IC_{50} = 251.6 µg/mL) suggests stabilization of BSA via interactions with sulfhydryl groups or hydrophobic pockets, preventing heat-induced denaturation—a similar mechanism shared with NSAIDs such as ibuprofen (Lopez-Miranda *et al.*, 2023). Protein denaturation results in the generation of autoantigens, which are inflammatory diseases that include diabetes, cancer, and rheumatic arthritis. It is therefore possible to reduce inflammatory activity by preventing protein denaturation (Curran *et al.*, 2023).

Table 1. Percentage of inhibition rate of protein denaturation of ZnO nanoparticles.

Concentration	% of inhibition of ZnO	% of inhibition of standard
100	28.4±0.34	33.6±0.44
200	40.3±0.51	43.9±0.76
300	58.4±0.43	60.69±0.06
400	70.22±0.57	69.80±0.06
500	88.00±0.56	85.28±0.04
IC_{50}	251.64	233.60

Note: Each value represents the mean ± standard deviation (SD) of three independent experiments (n=3).

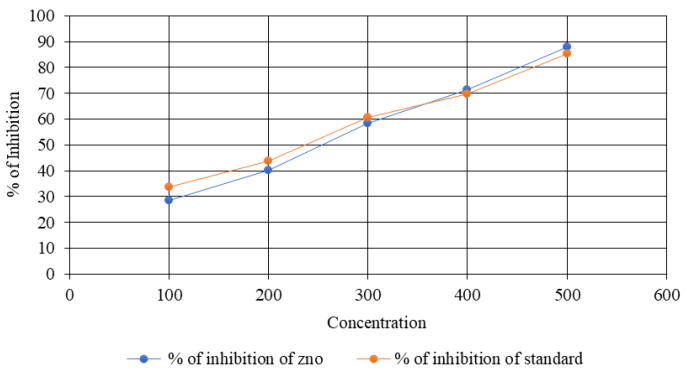

Figure 3. *Anti-inflammatory activity of algae-mediated ZnO nanoparticles and standard drug (ibuprofen) in a Dose-dependent on BSA denaturation.*

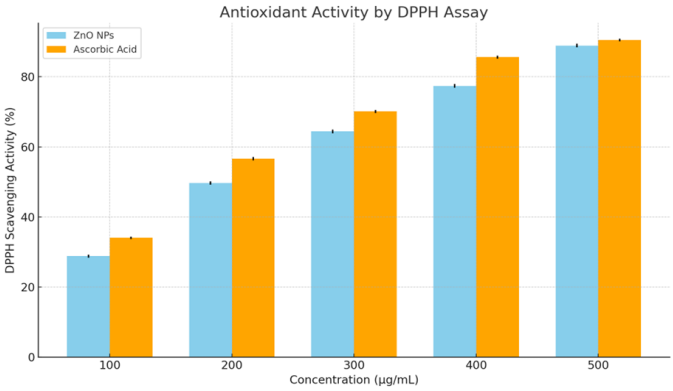

Figure 4. *DPPH radical scavenging activity of algae-mediated ZnO nanoparticles compared to ascorbic acid standard.*

DPPH radical scavenging activity

During the study, the DPPH radical scavenging activity of algae-mediated ZnO nanoparticles was measured at concentrations of 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, and 500 µg/mL (Figure 4) The antioxidant capacity of *A. platensis*-synthesized ZnO nanoparticles (NPs) are quantitatively assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. As presented in Table 2, both ZnO NPs and the reference standard ascorbic acid exhibited concentration-dependent antioxidant activity across the tested range (100–500 µg/mL). The ZnO NPs demonstrated potent radical scavenging, increasing from 28.8% at 100 µg/mL to 88.9% at 500 µg/mL. Ascorbic acid showed slightly higher activity at equivalent concentrations (34.03% to 90.5%). The IC_{50} values—calculated from dose-response curves—were 200 µg/mL for ZnO NPs and 171 µg/mL for ascorbic acid, confirming their comparable efficacy, indicating that the nanoparticles achieved ~85% of the standard's potency.

The antioxidant potential of *A. platensis*-mediated ZnO nanoparticles demonstrated in this study aligns with and expands upon previous findings in the field of biosynthesized metal oxide nanoparticles. These results indicate a concentration-dependent DPPH radical scavenging activity with an IC50 of 200.06 µg/mL, which compares favourably with other green-synthesized ZnO nanoparticles reported in literature. Nagajyothi *et al.* (2015) reported an IC50 of 210 µg/mL for *Eucalyptus globulus*-mediated ZnO NPs, while Rajeshkumar *et al.* (2023) found 225 µg/mL for *Ulva lactuca*-derived ZnO NPs. The observed antioxidant activity can be attributed to multiple mechanisms. First, the phytochemical capping agents from *A. platensis*, particularly C-phycocyanin and phenolic compounds, likely contribute hydrogen donors that neutralize free radicals, as suggested by Nandhini *et al.* (2024).

Conclusion

This study illustrates the environment-friendly synthesis of zinc oxide nanoparticles (ZnO NPs) using *A. platensis* extract, which acts as both a reducing and stabilizing agent—presenting a sustainable alternative to traditional chemical methods. The resulting ZnO NPs exhibited strong, concentration-dependent anti-inflammatory activity, inhibiting BSA denaturation by 28.4% to 88% over a range of 100–500 µg/mL, with an IC_{50} value of 251.64 µg/mL, closely comparable that of ibuprofen (IC_{50}=233.6 µg/mL). Moreover, the nanoparticles showed substantial antioxidant activity, with DPPH radical scavenging ranging from 28.8% to 88.9% within the same concentration range, and an IC_{50} of 200.06 µg/mL—comparable to ascorbic acid (IC_{50}=171 µg/mL). The observed bioactivities are likely enhanced by the phytochemicals present in *A. platensis*, such as phenolic compounds and C-phycocyanin. Collectively, these studies suggest that algae-derived ZnO NPs hold strong potential for pharmaceutical use, particularly in managing inflammation and oxidative stress-related conditions. Further investigations, including in vivo validation and mechanistic studies, are warranted to assess their clinical relevance and safety.

Table 2. DPPH Scavenging Activity (%).

Concentration (µg/mL)	DPPH Scavenging Activity in % ZnO	DPPH Scavenging Activity (% Ascorbic acid)
100	28.8±0.43	34.03±0.36
200	49.7±0.49	56.6±0.47
300	64.4±50	70.1±0.45
400	77.4±0.53	85.6±0.39
500	88.9±0.53	90.5±0.34
IC_{50}	200.06	171

Note: Each value represents the mean ± standard deviation (SD) of three independent experiments (n=3). *IC50 Value: The IC50 value, represents the concentration of the sample required to scavenge 50% of the DPPH radicals.

ABBREVIATIONS

ZnO NPs: Zinc Oxide Nanoparticles; BSA: Bovine Serum Albumin; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; IC50: Half-Maximal Inhibitory Concentration; XRD: X-Ray Diffraction; SEM: Scanning Electron Microscopy; FTIR: Fourier-Transform Infrared Spectroscopy; UV-Vis: Ultraviolet-Visible Spectroscopy; PBS: Phosphate-Buffered Saline; NSAIDs: Non-Steroidal Anti-Inflammatory Drug.

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DECLARATIONS

Author contribution statement: Conceptualization: Kanika; Methodology: Kanika; Software and validation: Kanika; Formal analysis and investigation: M.K.; Resources: Kanika; Data curation: Kanika; Writing—original draft preparation: Kanika; Writing—review and editing: Kanika; Visualization: Kanika; Supervision: M.K.; Project administration: M.K. and Kanika; Funding acquisition: Kanika. All authors have read and agreed to the published version of the manuscript.

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