RESEARCH PAPER

Anti-Inflammatory Activity of Synthesized Zinc Oxide Nanoparticles Using Raphanus Raphanistrum Subsp. Sativus

Steffi Priya Jayaseelan, Anitha Anbalagan and M. Karunanithi*

Department of CARISM, SASTRA Deemed University, Thanjavur – 613401, Tamil Nadu, India
***Corresponding author:** Dr. M. Karunanithi*
Email: karuna@carism.sastra.edu

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT
The present study aimed to synthesize Zinc Oxide Nanoparticles (ZnO NPs) using Raphanus raphanistrum subsp. sativus (red radish) leaf extract and to evaluate their anti-inflammatory activity through an in vitro assay. By using aqueous leaf extract as a reducing and stabilizing agent, eco-friendly green ZnO NPs were synthesized. The synthesized ZnO NPs were characterized using UV–Visible spectroscopy, FTIR analysis, XRD analysis, and SEM analysis. The anti-inflammatory activity was evaluated by albumin denaturation assay, with the anti-inflammatory drug as a standard. UV–Visible spectroscopy confirmed the formation of ZnO NPs through characteristic absorption peaks. FTIR analysis revealed the presence of phytochemicals in nanoparticle stabilization. XRD patterns confirmed the crystalline nature of ZnO NPs, while SEM analysis demonstrated predominantly spherical morphology with nanoscale dimensions. The synthesized ZnO NPs exhibited significant concentration-dependent anti-inflammatory activity, comparable to a standard drug, indicating effective inhibition of protein denaturation. Green-synthesized ZnO NPs using Raphanus raphanistrum subsp. sativus demonstrated significant anti-inflammatory activity, highlighting its potential application as a biocompatible nanotherapeutic agent in inflammation-related disorders.

Keywords: Zinc Oxide Nanoparticles, Green synthesis, Raphanus raphanistrum subsp. sativus, Anti-inflammatory activity, Protein denaturation.
How to cite this article: Jayaseelan SP, Anbalagan A, Karunanithi M. Anti-Inflammatory Activity of Synthesized Zinc Oxide Nanoparticles Using Raphanus Raphanistrum Subsp. Sativus. Int J Drug Deliv Technol. 2026;16(34s):358-368.
DOI: 10.25258/ijddt.16.34s.44

Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Nanotechnology has rapidly emerged as one of the leading scientific domains, providing revolutionary progress across pharmaceutical, biomedical, agricultural, and materials sciences due to its ability to control substances at the nanoscale [1]. Among the various nanoscale materials, metal oxide nanoparticles have garnered significant interest due to their superior physicochemical properties, including a high surface-to-volume ratio, unique electronic characteristics, adjustable morphology, and enhanced reactivity [2, 3, 4, 5]. Zinc oxide nanoparticles (ZnO NPs) are especially recognized for their broad band gap energy of 3.37 eV and a significant exciton binding energy of 60 meV, which makes them highly suitable for optical, catalytic, biosensing, and therapeutic applications [3]. Furthermore, their capacity to form diverse nanostructures, such as nanospheres, nanorods, nanowires, and nanorings, significantly enhances their functional versatility [6, 7, 8].

Conventional methods for synthesizing ZnO NPs, such as sol–gel, co-precipitation, thermal decomposition, and microwave-assisted extraction, often require harsh reaction conditions, expensive reagents, and hazardous chemicals, which raises issues related to environmental safety and compatibility in biomedical applications [9, 10, 11]. As a result, green synthesis has become a sustainable, economical, and eco-friendly alternative. The use of biological synthesis with plant extracts replaces harmful chemicals with naturally occurring phytochemicals such as flavonoids, phenolics, proteins, and polysaccharides, which serve as reducing, capping, and stabilizing agents during the formation of nanoparticles [11,12,13]. Various plant species, such as *Calotropis procera* [14], *Ocimum basilicum L. var. Purpurascens* [15], *Corymbia citriodora* [16], *Cassia tora L.* [17], *Sageretia thea* [18], *Zingiber officinale* [19], and *Azadirachta indica* [20] have been effectively investigated for the fabrication of ZnO NPs. In numerous studies,

**Author for Correspondence: karuna@carism.sastra.edu*

nanoparticles derived from plants demonstrate improved biocompatibility and biological activity as a result of phytochemical surface functionalization [21].

ZnO NPs have a wide range of biomedical uses, including antimicrobial, anti-inflammatory, antiviral, antioxidant, anticancer, wound healing, drug delivery, and biosensing functions [22, 23, 24, 25, 26]. Their antimicrobial mechanisms are well established and involve disrupting the membrane, oxidative stress induction through reactive oxygen species (ROS), electrostatic interactions, and intracellular deposition, resulting in damage to DNA and proteins [23, 27]. ZnO has been designated as "generally recognized as safe" (GRAS) by the U.S. FDA, highlighting its suitability for biomedical applications [20, 28]. ZnO NPs have demonstrated strong anti-inflammatory activity through various mechanisms, such as the reduction of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6, alongside the inhibition of COX-2 and inducible nitric oxide synthase (iNOS). They also suppress the activation of NF-κB, regulate oxidative stress, and prevent mast cell degranulation [29, 30, 31, 32, 33]. Their nanoscale size improves tissue penetration, cellular uptake, and accumulation at inflammatory sites due to the enhanced permeability and retention (EPR) effect, offering notable benefits over traditional non-steroidal anti-inflammatory drugs (NSAIDs), which often suffer from poor solubility and gastrointestinal adverse effects [34, 35, 36, 37].

Raphanus raphanistrum subsp. *sativus* (red radish) is a commonly consumed vegetable in the Brassicaceae family. It is rich in bioactive phytochemicals, including phenolics, flavonoids, glucosinolates, anthocyanins, vitamins, and minerals. These components enhance its properties, including antioxidant, antimicrobial, antiviral, anticancer, and anti-inflammatory effects [38, 39, 40, 41, 42, 43]. While radish extracts have been utilized for the synthesis of nanoparticles, major research has concentrated on the leaves and other aerial parts, with limited studies involving radish root extract for the synthesis of ZnO NPs. Preliminary findings suggest that radish-mediated ZnO NPs exhibit strong antimicrobial activity against multidrug-resistant bacteria, highlighting their therapeutic potential. Recent developments have emphasized the distinctive benefits of red radish leaves as an effective biogenic resource for nanoparticle synthesis. Compared with other leafy biomass sources, red radish leaves are significantly richer in anthocyanins, flavanols, phenolics, vitamin C, and glucosinolates, which greatly improve their efficiency in reducing and stabilizing during nanoparticle formation [44]. In comparison to the leaves of white radishes, the leaves of red radishes have a higher concentration of anthocyanins. These compounds offer significant anti-inflammatory and antioxidant properties. These bioactive compounds facilitate the formation of smaller and more stable ZnO NPs that exhibit higher biological effectiveness. It includes improved suppression of inflammatory mediators such as TNF-α, IL-6, IL-1β, COX-2, and iNOS.

Furthermore, the leaves of red radishes are a valuable agricultural by-product that is often thrown away, yet they possess a rich profile of phytochemicals, which makes them a perfect compound for the production of sustainable nanomaterials. Notably, no recent study (2022 – 2025) has reported the synthesis or anti-inflammatory evaluation of ZnO NPs derived from red radish leaves, revealing a significant research gap and providing strong novelty and relevance to the present work [45]. Due to the rising occurrence of inflammatory disorders and the limitations associated with NSAID-based therapy. Therefore, it is essential to synthesize plant-derived, biocompatible nanoformulations with improved therapeutic potential [46, 47, 48]. By using *Raphanus raphanistrum* subsp. *sativus* leaf extract, ZnO NPs were synthesized. It offers an innovative, sustainable, and biologically effective method for producing future anti-inflammatory therapeutics. Therefore, the present study aims to synthesize ZnO NPs using red radish (*Raphanus raphanistrum subsp. sativus*) leaf extract and evaluate their anti-inflammatory activity.

MATERIALS AND METHODS

Materials

Red radish (*Raphanus raphanistrum* subsp. *sativs*) leaves were freshly obtained from the Kodaikanal hills in Dindigul district and authenticated. Zinc acetate, ethanol, sodium hydroxide pellets (purified, 97%), phosphate-buffered saline (PBS, pH – 6.4), and diclofenac sodium salt were purchased. Whatman No. 41 Filter Paper (qualitative, 100 circles, diameter 125 mm) was purchased. For the preparation of all the aqueous solutions, distilled water was used.

METHODS

Collection Of Plant Materials And Herbarium

Fresh red radish (*Raphanus raphanistrum* subsp. *sativs*) leaves were collected from the Kodaikanal hills in Dindigul district. The leaves were washed

with tap water, followed by distilled water, and then shade-dried at room temperature and kept for the herbarium. The herbarium was authenticated in CARISM, School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur, Tamil Nadu, with the voucher number (CARISM00198).

Preparation of Plant Extract

The leaves were cleaned, blotted dry, and extracted using the hot percolation technique. Using a mortar and pestle, 10 g of leaves were macerated with 100 mL of ethanol. Then, the solution was transferred to a beaker. It was heated for 20 – 30 min with continuous stirring, and allowed to cool at room temperature. The extract was filtered through a Whatman No. 41 filter paper into a clean beaker, which was stored at 4 °C for future use [49].

Green Synthesis of ZnO NPs

ZnO NPs were synthesized using leaf extract from *Raphanus raphnistrum* subsp. *sativus*, which is used as a reducing agent. Initially, a 0.1 M zinc acetate solution was prepared. Mix 100 mL of distilled water with 2.198 g of zinc acetate, followed by magnetic stirring for 1 h until complete dissolution. In a beaker, add 80 mL of zinc acetate solution. Then, add 20 mL of ethanolic leaf extract to it and stir the mixture continuously. Prepare a 2 M sodium hydroxide solution and gradually add into the solution. Now, the pH of the solution was adjusted to 8, 10, 12, and 14. The mixture was then heated at 70 °C for 1 h and stirred for 2 h, resulting in the formation of a white precipitate. The precipitate was washed repeatedly with distilled water until a pH of 7 was attained, filtered, and dried in a hot air oven at 60 °C to obtain the synthesized ZnO NPs [50].

Characterization of Synthesized ZnO NPs

UV – visible Spectroscopy

UV – visible spectrophotometry was employed as a primary and reliable technique to confirm the synthesis and assess the optical properties of the synthesized ZnO NPs. Due to their characteristic optical behavior, nanoparticles strongly interact with specific wavelengths of light, enabling effective monitoring of nanoparticle formation and stability. For analysis, a small quantity of the ZnO NPs powder was dispersed in approximately 10 mL of deionized water, and the resulting suspension was scanned in the wavelength range of 300 – 700 nm using a UV–Visible spectrophotometer (Shimadzu UV-1800). The absorption spectrum was recorded to determine the characteristic maximum absorbance of the ZnO NPs [49].

FTIR Analysis

Fourier transform infrared (FTIR) analysis is valuable for identifying the presence of functional groups in various phytoconstituents that are associated with the reduction, stabilization, and formation of nanoparticles. The surface binding characteristics of ZnO NPs were analyzed. The FTIR spectrum of powdered ZnO NPs was utilized to qualitatively evaluate the films for the presence of functional groups, with results observed in the wavelength range of 4000 – 500 cm^{-1} [49].

XRD Analysis

Based on the interference between X-rays and a crystalline material, X-ray Diffraction (XRD) explains the crystallographic structure, physical properties of substances, and chemical composition. The powdered sample of ZnO NPs was analyzed using the Rigaku X-ray diffractometer, with the XRD patterns recorded at a scanning speed of 0.01 ℃/sec across a 2θ range of 5 to 90 ℃ and a wavelength of 1.54 Å [51].

SEM Analysis

Scanning electron microscopy (SEM) was utilized to examine the characterization of both 2D and 3D materials. The surface morphology of ZnO NPs was observed using the VEGA3 TESCAN. An increasing voltage of 10 kV was applied to analyze the particles. The morphology was examined and compared at various magnifications [51].

Anti-Inflammatory Activity

The anti-inflammatory activity of *Raphanus raphnistrum* subsp. *sativus* leaf extract was evaluated using the albumin denaturation assay. The egg white was separated from a fresh hen's egg. Prepare 1% NaOH solution and gradually add it to an egg white solution, followed by thorough mixing. To obtain a clear egg albumin solution, the mixture was filtered using filter paper. The leaf extract was prepared at different concentrations ranging from 100 µL to 500 µL. A 5 mL mixture solution was prepared, consisting of 2.8 mL of PBS, 0.2 mL of egg albumin solution, and 2 mL of leaf extract solution. The solution was mixed gently and incubated for 15 min at 37 °C. Subsequently, it was heated in the water bath at 70 °C for 5 min, and allowed to cool to room temperature. The absorbance of the solution was measured at 660 nm using a UV–Visible spectrophotometer, with diclofenac sodium as a control, respectively [52]. The percentage inhibition of protein denaturation was calculated using the formula:

$$\text{Percentage of Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

RESULTS

UV – Visible Spectroscopy

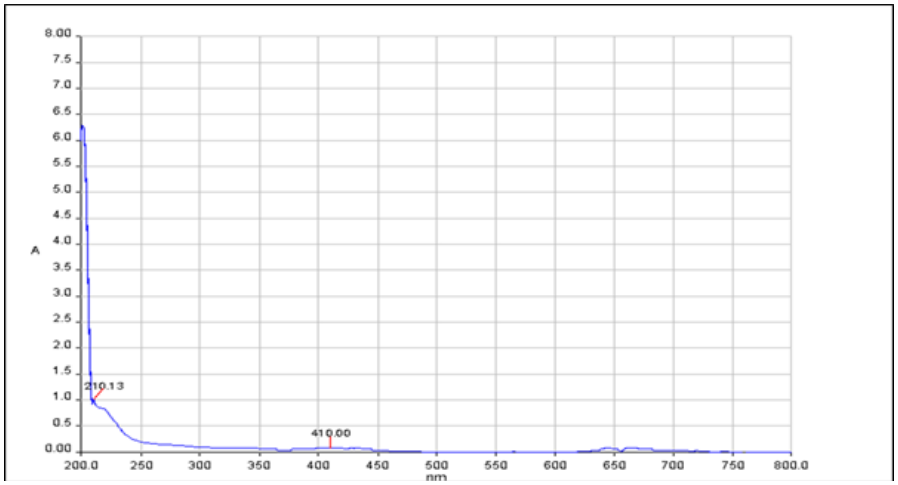

Fig. 1. UV–Visible absorption spectrum of synthesized ZnO NPs.

The synthesis of ZnO NPs was initially verified using UV–visible spectrophotometric analysis. A small quantity of the prepared ZnO NPs was suspended in 10 mL of distilled water, and the absorption spectrum was recorded over the wavelength range of 300 – 600 nm. As shown in Fig. 1, the spectrum exhibited prominent absorption bands at approximately 210 nm and 410.5 nm, corresponding to intrinsic band-gap electronic transitions from the valence band to the conduction band of ZnO NPs. The appearance of sharp and distinct absorption peaks indicates the effective bioreduction of zinc ions into ZnO NPs, as well as their good dispersion and colloidal stability. Compared with earlier studies reporting optimal absorption around 360 – 370 nm under controlled alkaline pH conditions, the present results demonstrate the effective formation of ZnO NPs without extreme pH optimization, highlighting the role of radish leaf phytochemicals in promoting controlled nucleation, stabilization, and growth of the nanoparticles [9].

FTIR Analysis

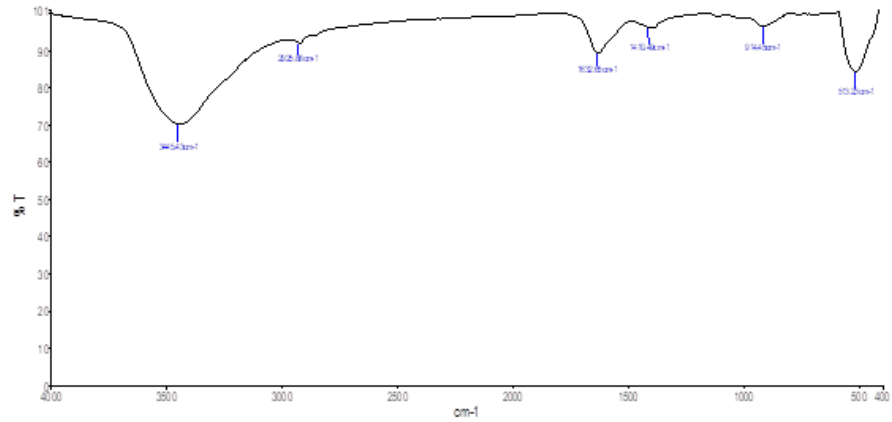

Fig. 2. FTIR spectrum of synthesized ZnO NPs.

FTIR analysis was employed to identify the functional groups associated with the surface of the synthesized ZnO NPs and to elucidate the role of biomolecules involved in their formation and stabilization. Fig. 2 represents the FTIR spectrum exhibited distinct absorption bands at 3445.43,

2925.88, 1632.65, 1410.49, 914.45, and 512.23 cm^{-1}. The broad band at 3445.43 cm^{-1} corresponds to O–H stretching vibrations of hydroxyl groups, indicating the presence of phenolic compounds and alcohols derived from the plant extract. The peak at 2925.88 cm^{-1} is attributed to aliphatic C–H stretching vibrations, while the band at 1632.65 cm^{-1} represents amide I vibrations, suggesting the involvement of proteinaceous components in nanoparticle stabilization. The absorption band observed at 1410.49 cm^{-1} is assigned to inorganic carbonate groups, and the peak at 914.45 cm^{-1} corresponds to condensed O–H bending vibrations. Importantly, the characteristic absorption band at 512.23 cm^{-1} confirms the presence of Zn–O stretching vibrations, providing direct evidence for the successful formation of ZnO NPs. Overall, the FTIR results indicate that phytochemicals present in *Raphanus raphnistrum* subsp. *sativus* leaf extract acts as an effective reducing, capping, and stabilizing agent during green synthesis. These results align with previous studies that identified similar functional groups, including hydroxyl, carbonyl, and C–O stretching vibrations, in plant-mediated ZnO NPs. Minor shifts in peak positions compared to previous studies may be attributed to differences in phytochemical composition of the plant extract and variations in synthesis conditions, further confirming the interaction between plant-derived biomolecules and the surface of ZnO NPs [53].

XRD Analysis

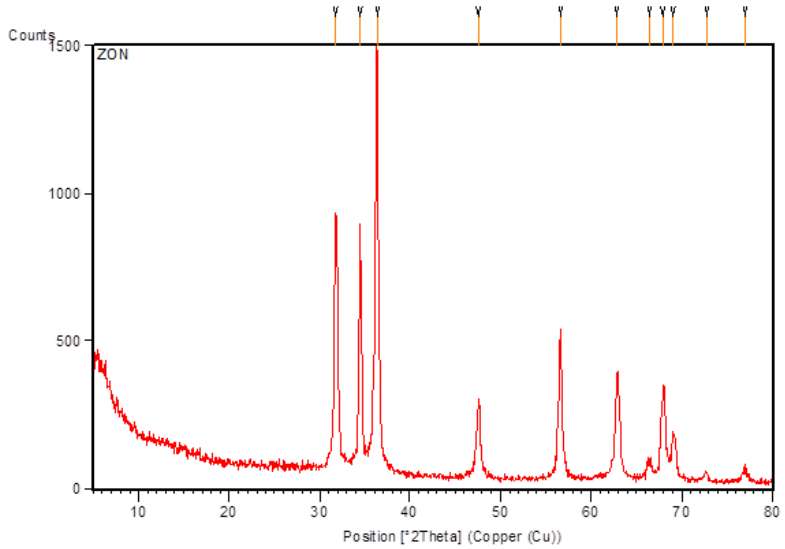

Fig. 3(a). XRD pattern of synthesized ZnO NPs.

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
31.8071	866.30	0.3542	2.81344	61.35
34.4827	824.64	0.2362	2.60103	58.40
36.3136	1412.13	0.2755	2.47398	100.00
47.5510	264.06	0.1181	1.91227	18.70
56.6166	500.29	0.2755	1.62571	35.43
62.8997	346.90	0.3936	1.47759	24.57
66.4373	64.15	0.4723	1.40724	4.54
67.8853	278.24	0.2362	1.38071	19.70
69.0848	166.14	0.2362	1.35964	11.77
72.8327	29.57	0.2755	1.29864	2.09
77.0228	41.52	0.4723	1.23811	2.94

Fig. 3(b). Reference for refractive index of ZnO NPs.

XRD analysis was performed to determine the crystalline structure and phase purity of the synthesized ZnO NPs. The XRD pattern showed distinct diffraction peaks at 2θ values of 31.80°, 34.48°, 36.31°, 47.55°, 56.61°, 62.89°, 66.43°, 67.88°, 69.21°, 72.83°, and 77.02° (refer to Fig. 3(a) and 3 (b)). These diffraction peaks correspond to the characteristic crystal planes of ZnO, indicating the formation of a well-defined crystalline structure. The most intense diffraction peak was observed at 36.31°, confirming the highly crystalline nature of the synthesized ZnO NPs. The observed diffraction

peaks can be indexed to the hexagonal wurtzite structure of zinc oxide, confirming the successful formation of crystalline ZnO NPs. The prominent peak at 36.31°, corresponding to the (101) plane, indicates high crystallinity and structural stability of the nanoparticles, which is a desirable property for biomedical applications. The absence of additional or impurity peaks in the XRD pattern suggests high phase purity and indicates that no secondary phases were formed during the green synthesis process. These results are consistent with previously reported XRD patterns of plant-mediated ZnO NPs, further validating the effectiveness of *Raphanus raphnistrum* subsp. *sativus* leaf extract in facilitating the formation of stable and crystalline ZnO NPs [9, 54, 55, 56].

SEM Analysis

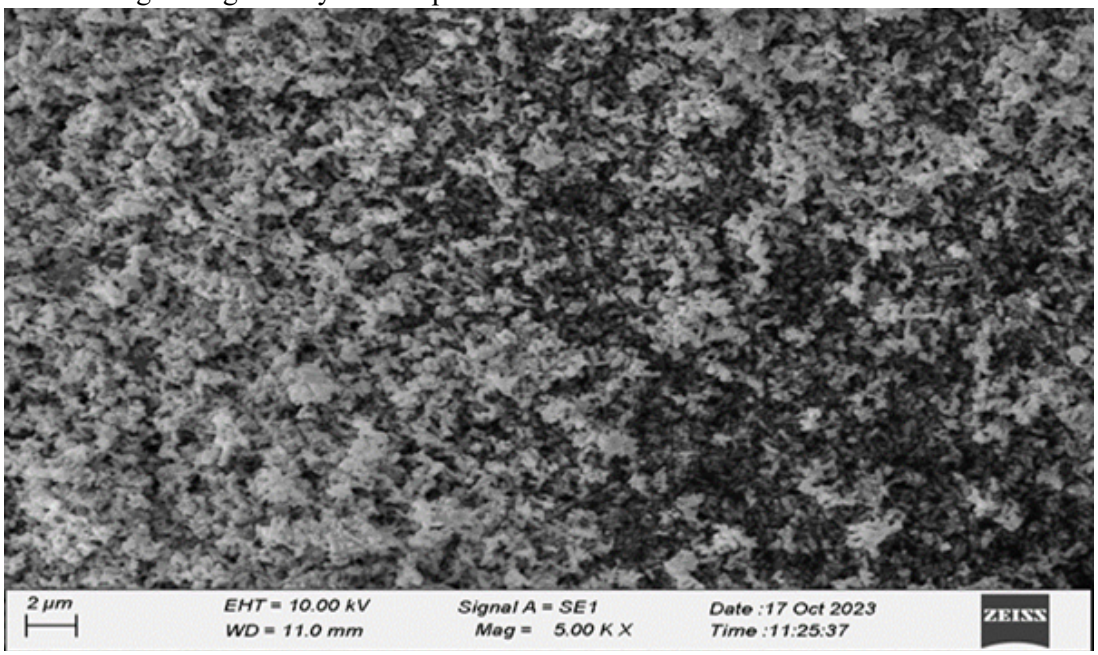

Fig. 4(a). SEM image showing surface morphology of ZnO NPs.

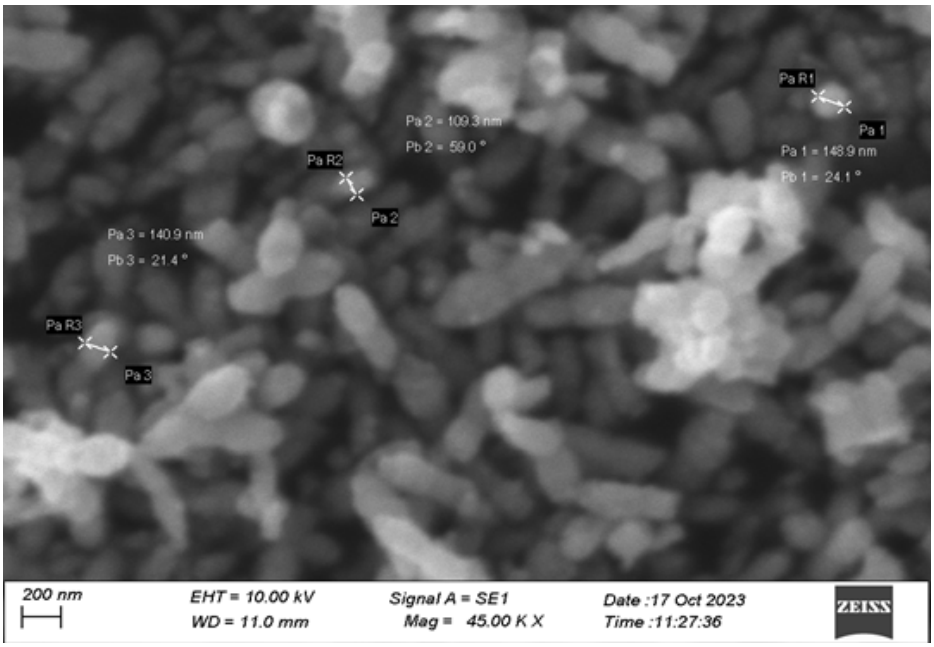

Fig. 4(b). SEM image at different magnifications of ZnO NP.

SEM analysis was used to investigate the surface morphology, shape, and aggregation behavior of the synthesized ZnO NPs. Fig. 4(a) depicts the SEM micrographs obtained at different magnifications revealed that the nanoparticles predominantly exhibit spherical to hexagonal shapes, with the sizes of 140.9

nm, 109.3 nm, and 148.9 nm at 2000X magnification, with an irregular surface morphology (Fig. 4(b)). Compared to earlier reports, the plant extract-mediated ZnO NPs showed noticeable aggregation and surface coverage by phytochemicals, which can be attributed to their high surface energy and the interaction of bioactive compounds acting as capping and stabilizing agents. Despite aggregation, the nanoparticles retained a well-defined crystalline structure, indicating effective stabilization by plant-derived biomolecules. On comparison to previous studies, these morphological characteristics of plant-mediated ZnO NPs were similar and support their potential suitability for biomedical applications [50].

Anti-Inflammatory Activity

Table 1: In vitro anti-inflammatory activity of leaf extract by protein denaturation assay

Samples	Absorbance	Percentage of Inhibition (%)
Control	0.6690	–
100 µL	0.1483	77.83
200 µL	0.2770	58.46
300 µL	0.0166	97.51
400 µL	0.5944	11.15
500 µL	0.4906	26.66

The anti-inflammatory activity of *Raphanus raphnistrum* subsp. *sativus* leaf extract, mediated ZnO NPs were evaluated using the egg albumin denaturation assay. The nanoparticles exhibited concentration-dependent inhibition of protein denaturation, with maximum inhibition (97.51%) observed at 300 µL, followed by 77.83% at 100 µL and 58.46% at 200 µL, while lower inhibition was noted at higher concentrations, possibly due to aggregation effects (refer to Table 1). The comparatively reduced inhibition at 200 µL may be attributed to non-linear dose response behaviour, transient nanoparticle aggregation, and suboptimal nanoparticle protein interactions, which can temporarily reduce the effective surface area available for protein stabilization. The strong inhibitory activity indicates significant anti-inflammatory potential, which may result from the synergistic action of ZnO and plant-derived bioactive compounds, such as flavonoids, phenolics, and anthocyanins. At 300 µL, which plays a crucial role in maximizing anti-inflammatory activity.

DISCUSSION

The research study emphasizes the effective green synthesis of ZnO NPs using *Raphanus raphnistrum* subsp. *sativus* leaf extract, confirmed through multiple physicochemical characterization techniques. UV–visible spectroscopic analysis demonstrated characteristic absorption peaks associated with intrinsic band-gap transitions of ZnO, indicating effective bioreduction of zinc ions by plant phytochemicals, as reported in previous studies on plant-mediated ZnO NPs [9]. FTIR analysis revealed the presence of hydroxyl, aliphatic, amide, and carbonate functional groups, confirming the requirement of phenolics, proteins, and other biomolecules from the leaf extract in the reduction, capping, and stabilization of ZnO NPs [53]. These functional groups are essential in enhancing nanoparticle stability and biological activity. XRD results confirmed the crystalline hexagonal wurtzite structure of ZnO NPs with high phase purity, aligning with standard JCPDS data and earlier findings on biosynthesized ZnO NPs [9, 54, 55, 56]. The prominent diffraction peak corresponding to the (101) plane indicates high crystallinity, which is advantageous for biomedical applications. SEM analysis displayed predominantly spherical to hexagonal nanoparticles with moderate aggregation, attributed to phytochemical capping and high surface energy, a common feature in green-synthesized metal oxide nanoparticles [50]. The synthesized ZnO NPs exhibited significant in vitro anti-inflammatory activity by effectively inhibiting protein denaturation. This activity may be attributed to the synergistic effect of ZnO NPs and bioactive compounds such as flavonoids, phenolics, and anthocyanins present in red radish leaves, which are known to suppress inflammatory mediators and stabilize protein structures. The slight decrease in activity at higher concentrations could be due to aggregation of nanoparticles, which leads to a reduction in effective surface area. In summary, the results suggest that ZnO NPs synthesized through *Raphanus raphnistrum* subsp. *sativus* could serve as a safe and effective agent for anti-inflammatory applications.

CONCLUSION

In conclusion, ZnO NPs were successfully synthesized using an eco-friendly green synthesis approach employing *Raphanus raphanistrum* subsp. *sativus* extract as a natural reducing and stabilizing agent. Comprehensive characterization confirmed the

formation of crystalline, stable ZnO NPs with nanoscale morphology. The ZnO NPs exhibited significant in vitro anti-inflammatory activity, demonstrating effective inhibition of protein denaturation. The enhanced biological activity can be attributed to the synergistic effects of ZnO NPs and plant-derived bioactive compounds. These results suggest that *Raphanus raphanistrum* subsp. *sativus* mediated ZnO NPs are a promising compound for the development of biocompatible anti-inflammatory nanotherapeutics. Further *in vivo* studies and toxicity assessments are warranted to explore their clinical potential.

CONFLICT OF INTEREST

No conflict of interest

ETHICAL APPROVAL

Not applicable

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