Research Article

Green Synthesis of ZnO Nanocomposites Using Combined Ziziphus Leaf Extracts: Structural Characterization and Biological Activities

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Abstract

An environmentally friendly approach was employed to synthesize chitosan/zinc oxide nanocomposites (CS/ZnO NCs) using combined leaf extracts of Ziziphus oenoplia and Ziziphus mauritiana. Phytochemicals present in the extracts facilitated nanocomposite formation and stabilization without additional chemical reducing agents. The synthesized CS/ZnO NCs were characterized using UV–Visible spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), dynamic light scattering (DLS), and zeta potential analysis. UV–Visible spectroscopy showed a characteristic absorption band near 400 nm, confirming ZnO formation. XRD revealed a hexagonal wurtzite crystalline structure with an average crystallite size of 17 nm. FTIR confirmed the involvement of plant-derived functional groups in nanocomposite stabilization. SEM showed predominantly irregular polygonal and plate-like particles with an average size of 27 nm, while DLS indicated a hydrodynamic diameter of 19 nm. The zeta potential of −28 mV suggested satisfactory dispersion stability. The CS/ZnO NCs exhibited antibacterial activity against Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, and Pseudomonas sp., with the highest inhibition observed against Pseudomonas sp. at 100 µg mL^{-1}. Antioxidant assays demonstrated concentration-dependent activity, reaching 89.84% DPPH and 87.3% hydrogen peroxide scavenging at 50 µg mL^{-1}. These findings demonstrate the potential of biogenic CS/ZnO NCs for biological applications

Keywords: Zinc oxide, Dual extract, XRD, DLS, Antibacterial, Antioxidant

Introduction

The fast development of nanoscience has changed the paradigm for designing and using materials in biologic, environmental and technological applications [1]. The properties of materials at the nanoscale can be significantly different from those of the bulk materials because of their altered surface area, particle size and surface reactivity [2]. These special properties have led to intensive research on nanoparticle based systems for diverse applications from medicine to agriculture, environmental remediation to industrial manufacturing. Zinc oxide nanoparticles/nanocomposite has been one of the most promising materials to be explored among various nanomaterials as it has versatile physicochemical and biological properties [3]. Zinc oxide is an inorganic semiconductor compound, which has been found to be stable, non-toxic, and biologically compatible [4]. Nano-sized particles of ZnO have better surface activity and functional performance, and can be used in various biomedical and pharmaceutical applications [5]. ZnO nanoparticles have been used in antimicrobial coatings, wound healing materials, cosmetic formulations, biosensors and drug delivery systems. In addition to killing a wide range of microorganisms, their interaction with reactive oxygen species has raised their interest in the health related fields [6]. Therefore, considerable efforts have been dedicated in the development of reliable, reproducible approaches to the synthesis of ZnO nanoparticles of controlled characteristics and enhanced biological activity.

Many methods have been used to synthesize ZnO nanoparticles. Due to the ability to control particle morphology and crystallinity, conventional methods such as sol–gel processing [3], co-precipitation [7], hydrothermal treatment [8] thermal decomposition [9] an chemical reduction [10] are broadly applied. Notwithstanding these benefits, some of these methods rely on high temperatures, high level of special equipment, and hazardous chemical reagents, which could cause environmental and health problems [11]. This has led to the development of alternative production strategies that are efficient and environmentally friendly due to the generation of hazardous by products and the need for extensive purification steps. Biological synthesis has been gaining in popularity in the past few years as a more environmentally friendly method to produce nanoparticles [12]. The method involves the use of biomolecules that exist in nature in the plants, microorganisms or other biological materials as a mediator for the formation of nanoparticles. This is the case of plant based synthesis which is convenient, cost-effective and not very strenuous in lab conditions [13]. There are many secondary metabolites present in plant extracts that can be used in reduction and stabilization processes in the formation of nanoparticles. Not only do these naturally occurring compounds aid in synthesis

but they may also affect the physicochemical properties and biological activity of the products, the nanoparticles. In the majority of plant mediated nanoparticle studies, extracts have been derived from a single plant source. Mixing extracts of more than one plant could, however, offer a broader spectrum of phytochemicals that could synergise during the synthesis of nanoparticles. These combinations may result in better metal ion reduction, greater stability of the nanoparticles, and affect the morphology of the nanoparticles [14]. The idea of dual extract synthesis is based on synergic effect between various phytoconstituents in the formation of nanoparticles [15]. The use of several plant extracts, therefore, could be an effective way to obtain nanoparticles with better biological and structural properties. The green synthesis of metal nanoparticles using dual plant extract for biomedical and environmental applications has been reported in several studies. The gold nanoparticles prepared by the combined plant extracts demonstrated potential biomedical activity [16] and the silver nanoparticles prepared by the dual extract method demonstrated improved antibacterial and photocatalytic activity [17]. Likewise, ZnO nanoparticles prepared by using dual extracts showed a high level of antibacterial properties against pathogenic microorganisms [18].

In recent years, biopolymer based ZnO nanocomposites have gained considerable attention in biomedical and pharmaceutical applications because of their biocompatibility, biodegradability, and low toxicity [19]. Among various biopolymers, chitosan has emerged as an important material owing to its remarkable physicochemical and biological properties, including biodegradability, biocompatibility, antimicrobial activity, and film-forming ability [20]. The incorporation of ZnO nanoparticles into a chitosan matrix using plant-derived phytochemicals provides an effective strategy for developing environmentally friendly bio-nanocomposites [21]. Surface modification of ZnO nanoparticles with chitosan can improve their stability, dispersibility, and biological performance, thereby enhancing their potential for antimicrobial, antioxidant, environmental, and biomedical applications. In addition, the synergistic interaction between chitosan and phytochemical-assisted ZnO nanoparticles may contribute to improved functional properties of the resulting nanocomposite. According to Elemike et al., chitosan-based ZnO nanocomposites synthesized through green methods exhibited significant structural and photocatalytic properties [22]. The study suggested that the incorporation of chitosan improved the stability and functional performance of the nanoparticles. In addition, plant extract mediated ZnO/chitosan nanocomposites demonstrated enhanced antioxidant activity due to the combined effects of phytochemicals, chitosan, and ZnO nanoparticles [23-24].

Ziziphus oenoplia and Ziziphus mauritiana are medicinal plant species belonging to the family Rhamnaceae [25]. These plants have long been utilized in traditional healthcare practices and are known to contain diverse phytochemical constituents, including phenolic compounds, flavonoids, alkaloids, tannins, saponins, and triterpenoids [26]. Previous investigations have associated these compounds with various biological activities such as antioxidant, antimicrobial, anti-inflammatory, and therapeutic effects [27]. The abundance of naturally occurring biomolecules within these plants makes them suitable candidates for green nanoparticle synthesis. Moreover, combining extracts from Z. oenoplia and Z. mauritiana may create a phytochemical environment capable of promoting efficient nanoparticle formation while simultaneously enhancing biological functionality [28]. The growing prevalence of microbial resistance has become a major challenge for modern healthcare systems. Numerous bacterial pathogens have developed resistance to commonly used antibiotics, reducing the effectiveness of conventional treatment strategies. This situation has intensified the search for alternative antimicrobial agents capable of suppressing microbial growth through different mechanisms. Metal oxide nanoparticles, particularly ZnO nanoparticles, have attracted attention because of their ability to inhibit both Gram-positive and Gram-negative microorganisms [29]. Their antimicrobial effects have been linked to interactions with bacterial cell structures, disruption of cellular processes, and alteration of membrane integrity. These properties suggest that ZnO nanoparticles may serve as valuable materials for antimicrobial applications. Alongside antimicrobial activity, antioxidant capacity represents another important property for biological applications. Oxidative stress resulting from excessive production of reactive oxygen species can damage cellular components and contribute to the progression of various disorders [30]. Materials capable of scavenging free radicals may therefore offer protective benefits in biological systems. Plant-mediated nanoparticles are of particular interest because phytochemicals associated with the nanoparticle surface may contribute additional antioxidant functionality. Evaluating antioxidant activity provides valuable insight into the potential biomedical relevance of biosynthesized nanomaterials [31].

Considering these things, the present study was oriented towards the synthesis of CS/ZnO nanocomposites via green approach by using the combined extract of leaves of Ziziphus oenoplia and Ziziphus mauritiana. A dual extract system was chosen to take advantage of the different phytochemical content of both plants to explore its effect on the generation of nanoparticles. The structural and morphological characteristics of the synthesized

nanocomposites of ZnO were determined by employing proper analytical techniques. Moreover, they were tested for their antibacterial and antioxidant properties in order to assess their applicability in biological applications. The present study will help in the development of environment friendly nanomaterials and also medicinal plant resources has a great potential in making nanoparticles in sustainable manner.

MATERIALS AND METHODS

2.1 Materials

Zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$] and chitosan (C6H11NO4) obtained from Sigma-Aldrich was used as the precursor for ZnO nanocomposites synthesis. Fresh leaves of Ziziphus oenoplia and Ziziphus mauritiana were collected from the Villupuram region, Tamil Nadu, India. All experimental procedures were carried out using deionized water to avoid possible ionic interference.

2.2 Preparation of leaf extract

Freshly dried leaves of Ziziphus oenoplia and Ziziphus mauritiana were finely powdered and used for extract preparation. Equal quantities of the leaf powders (0.5 g each) were transferred into a beaker containing 100 mL of distilled water. The mixture was maintained at 60–70 °C for 20 min to facilitate the release of water soluble phytochemicals into the medium. After extraction, the suspension was allowed to cool and subsequently filtered through Whatman No. 1 filter paper to separate the liquid extract from plant debris. The clear filtrate was collected in a sterile container and preserved at 4 °C until further use in the synthesis of ZnO nanocomposites.

2.3 Synthesis of CS/ZnO nanocomposites

The CS/ZnO nanocomposites were synthesized through a green approach using aqueous leaf extracts of Ziziphus oenoplia and Ziziphus mauritiana. Initially, 50 mL of the plant extract was added slowly to 100 mL of 0.5 M zinc nitrate solution under continuous magnetic stirring at 80 °C for 30 min. Subsequently, 25 mL of chitosan solution prepared by dissolving 25 mg of chitosan in 25 mL of 1% acetic acid was introduced into the reaction mixture. The solution was further stirred continuously at 80 °C for 3 h to facilitate nanocomposite formation. After completion of the reaction, the mixture was allowed to cool and then centrifuged at 8000 rpm for 15 min. The collected precipitate was washed several times with distilled water followed by ethanol to remove residual impurities. The purified material was dried at 100 °C and finally calcined at 600 °C for 2 h to obtain crystalline CS/ZnO nanocomposites. The synthesized product was stored for further characterization and biological studies

2.4 Characterization

The synthesized nanomaterial was characterized using various analytical techniques. XRD analysis (PerkinElmer) was employed to determine the crystalline phase and structural properties. Surface morphology was examined using SEM (FEI Quanta FEG 200). FTIR spectra were recorded with a PerkinElmer Spectrum 200 instrument to identify functional groups and molecular interactions. Optical properties and photocatalytic dye degradation studies were carried out using a Shimadzu UV-1650 UV–Visible spectrophotometer. Dynamic light scattering was employed to determine the hydrodynamic diameter and size distribution of the synthesized nanoparticles. Zeta potential measurements were performed using M3-PALS technology with a 633 nm He–Ne laser and 90° detection geometry to assess the electrokinetic properties and colloidal stability of the nanoparticle dispersion.

2.5 Antibacterial activity

The antimicrobial activity of the biosynthesized ZnO nanocomposites was evaluated using the agar well diffusion method [32] against selected bacterial and fungal pathogens, namely Escherichia coli, Staphylococcus aureus, Pseudomonas sp., and Candida albicans. Bacterial strains were cultured on Mueller–Hinton agar (MHA), while the fungal strain was maintained on Sabouraud dextrose agar (SDA). Fresh microbial suspensions were prepared from actively growing cultures in sterile saline solution and adjusted to the turbidity of a 0.5 McFarland standard, corresponding to 1.5×10^8 CFU mL^{-1}. The standardized inoculum was uniformly spread over the surface of the respective agar plates using a sterile cotton swab to obtain confluent growth. After inoculation, wells of 9 mm diameter were aseptically prepared in the agar medium using sterile polystyrene pipette tips. Subsequently, 100 µL of ZnO nanocomposites suspensions at concentrations of 25, 50, and 100 µg mL^{-1} were dispensed into the wells. Amoxicillin was used as the positive control for bacterial strains, while fluconazole served as the standard antifungal agent against C. albicans. The bacterial plates were incubated at 37 °C for 24 h, whereas the fungal plates were incubated at 28 °C for 48 h. Following incubation, antimicrobial activity was determined by measuring the diameter of the inhibition zones surrounding each well using a digital Vernier caliper. The extent of microbial growth inhibition was expressed as the mean zone of inhibition (mm).

2.6 Antioxidant Activity

DPPH Assay

The antioxidant activity of ZnO nanocomposites synthesized using *Ziziphus oenoplia* and *Ziziphus mauritiana* extracts was determined by the DPPH radical scavenging assay. Briefly, 0.1 mM DPPH solution prepared in methanol was reacted with ZnO NP suspensions at concentrations ranging from 10 to 50 µg mL^{-1}. The reaction

mixtures were maintained in the dark for 30 min at ambient temperature before absorbance measurement. Ascorbic acid served as the positive control. The reduction of DPPH radicals was quantified as percentage inhibition relative to the control according to Equation (1) [33].

Inhibition (%) = ($A_{control} – A_{sample}$ /$A_{control}$) × 100

where $A_{control}$ and A_{sample} denote the absorbance values of the control and test samples, respectively.

Hydrogen Peroxide (H_2O_2) Scavenging Assay

The hydrogen peroxide scavenging activity of the biosynthesized ZnO nanocomposites (ZnO NPs) was evaluated according to a standard spectrophotometric method. A 40 mM H_2O_2 solution was prepared in phosphate buffer (pH 7.4). For the assay, 0.6 mL of the hydrogen peroxide solution was mixed with 0.4 mL of ZnO nanocomposites suspensions at different concentrations (10, 20, 30, 40, and 50 µg mL^{-1}) to obtain a total reaction volume of 1 mL. The reaction mixtures were incubated at room temperature for 10 min, after which the absorbance was measured at 230 nm using a UV–Visible spectrophotometer. Ascorbic acid, prepared at concentrations ranging from 10 to 50 µg mL^{-1}, was used as the reference antioxidant, while the reaction mixture without ZnO nanocomposites served as the control. The hydrogen peroxide scavenging capacity was expressed as percentage inhibition and calculated using Equation (2) [33]:

Inhibition (%) = (Acontrol – Asample /Acontrol) × 100

where Acontrol represents the absorbance of the control solution and Asample represents the absorbance of the reaction mixture containing ZnO nanocomposites or the standard antioxidant.

3 Results and Discussion

3.1 XRD analysis

Figure 1 illustrates the XRD pattern of CS/ZnO nanocomposites synthesized using the combined leaf extracts of Ziziphus oenoplia and Ziziphus mauritiana. The diffraction peaks detected at 2θ values of 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8°, 67.9°, and 69.1° were indexed to the (100), (002), (101), (102), (110), (103), (112), and (201) crystal planes of hexagonal wurtzite ZnO [22]. These diffraction positions are consistent with the standard crystalline structure of ZnO, confirming the successful formation of the nanocomposites without noticeable secondary phases or impurities. The intense diffraction peak corresponding to the (101) plane indicates good crystallinity of the prepared ZnO nanocomposites. The narrow and distinct peaks suggest that the synthesized particles possess a well-ordered crystalline arrangement. The average crystallite size, calculated using the Debye–Scherrer equation [34], was found to be 17 nm, confirming the nanoscale nature of the synthesized material. A comparable crystallite size has also been reported for silver nanoparticles synthesized using dual plant extracts [17]. Moreover, the obtained crystallite size was smaller than that reported for green synthesized iron oxide nanoparticles prepared using Ziziphus oenoplia extract [35].

The formation of ZnO nanocomposites is strongly influenced by the phytochemical compounds present in both plant extracts. Bioactive constituents such as phenolic compounds, flavonoids, tannins, alkaloids, and proteins present in Ziziphus oenoplia and Ziziphus mauritiana leaves play a major role in the reduction of zinc ions and stabilization of the formed nanocomposites [28]. These natural compounds act as capping agents that limit excessive particle growth and aggregation, thereby contributing to the formation of stable nanoparticles with controlled crystallite size. The use of dual leaf extracts may also provide a synergistic effect during nanocomposites synthesis by enhancing nucleation and crystal growth processes [36]. Such biologically synthesized ZnO nanocomposites are advantageous because the method avoids toxic chemicals and supports eco-friendly nanoparticle production. The obtained nanosized ZnO particles with high crystallinity are expected to exhibit improved surface-related properties, which are beneficial for antimicrobial, catalytic, and biomedical applications.

3.2 UV Visible analysis

Figure 2 presents the UV–Visible absorption spectrum of CS/ZnO nanocomposites synthesized using the combined leaf extracts of Ziziphus oenoplia and Ziziphus mauritiana. The spectrum exhibited a prominent absorption peak near 400 nm, confirming the formation of ZnO nanocomposites. This absorption behavior is mainly related to the electronic transition within the ZnO structure and is considered a characteristic feature of nanosized ZnO materials [37]. The gradual decrease in absorbance at higher wavelengths indicates good optical transparency of the synthesized material [38]. The broad nature of the absorption band suggests the formation of fine nanocomposites with uniform distribution. The observed optical response may also be influenced by naturally occurring phytochemicals present in the plant extracts, which participate in nanocomposites stabilization during the synthesis process. The use of dual plant extracts appears to improve nanocomposites formation by providing a wider range of biomolecules responsible for reduction and surface protection [39]. These compounds help in controlling particle growth and minimizing aggregation, leading to stable ZnO nanocomposites with enhanced optical properties. The obtained results demonstrate that the dual extract-

mediated green synthesis method is effective for preparing ZnO nanocomposites suitable for photocatalytic and biomedical applications.

3.3 Zeta potential and DLS analysis

The zeta potential profile of the biosynthesized ZnO nanocomposites is shown in Figure 3(a). The nanocomposites exhibited a surface charge value of −28 mV, indicating good dispersion stability in the suspension medium. The negative charge on the nanoparticle surface is mainly due to the adsorption of plant derived biomolecules during the synthesis process [3]. These naturally occurring compounds form a protective layer around the nanocomposites and reduce particle aggregation through electrostatic repulsion. The obtained value suggests that the synthesized ZnO nanocomposites possess adequate stability for further applications.

Figure 3(b) shows the particle size distribution obtained from DLS analysis. The average hydrodynamic diameter of the ZnO nanocomposites was 19 nm. The distribution pattern indicates the formation of fine nanocomposites with relatively uniform size distribution. The smaller particle size may be associated with the effective capping action of phytochemicals present in the combined leaf extracts [40]. The dual extract approach appears to enhance nanoparticle stabilization and control particle growth more effectively than single extract mediated synthesis. Biomolecules such as phenolics, flavonoids, tannins, and proteins from both plant sources contribute to the reduction and stabilization processes, resulting in stable nanocomposites with reduced agglomeration. The obtained nanoscale particle size and good colloidal stability are favorable for improving the surface related properties of ZnO nanocomposites in biomedical applications.

3.4 FTIR analysis

Figure 4 shows the FTIR spectrum of ZnO nanocomposites synthesized using the combined leaf extracts of Ziziphus oenoplia and Ziziphus mauritiana. The spectrum revealed several characteristic absorption bands associated with different functional groups present on the nanoparticle surface. A broad band observed around 3415 cm^{-1} corresponds to O–H stretching vibrations, indicating the presence of hydroxyl groups from phenolic compounds and residual moisture [3]. The peak near 2930 cm^{-1} is related to C–H stretching vibrations of organic molecules present in the plant extracts [34]. The absorption band detected at 2358 cm^{-1} may be due to adsorbed carbon dioxide molecules [41]. The peaks appearing at 1551 cm^{-1} and 1387 cm^{-1} are associated with organic functional groups such as amide, C–N, and N–H vibrations, suggesting the involvement of proteins and other phytochemicals during nanoparticle synthesis [3]. The absorption bands located at 1079 cm^{-1} and 844 cm^{-1} indicate C–O stretching and Zn–O vibrations, respectively, confirming the formation of ZnO nanocomposites [22]. The FTIR analysis clearly demonstrates that bioactive compounds present in both plant extracts participated in the reduction and stabilization processes. Phytochemicals including flavonoids, phenolics, tannins, and proteins likely acted as natural capping agents that protected the nanocomposites from aggregation. The use of dual extracts provided multiple functional groups capable of enhancing nanoparticle stability and supporting controlled ZnO nanocomposites formation.

3.5 SEM analysis

The surface morphology of ZnO nanocomposites synthesized using the dual plant extract was examined by SEM, and the corresponding micrograph is presented in Figure 5(a). The image shows the formation of well-developed particles with irregular polygonal and plate-like structures. The particles are distributed throughout the surface and exhibit partial aggregation, which is commonly observed in biosynthesized ZnO nanocomposites. Such aggregation may arise from the high surface energy of nanosized particles and the interaction between neighboring particles during drying and sample preparation [42]. Despite the presence of some clustered regions, individual particles with distinct edges and crystalline features are clearly visible, indicating successful nanoparticle formation. The observed morphology suggests that phytochemical compounds present in the dual extract played an important role during nanoparticle growth [43]. These biomolecules can act as both reducing and stabilizing agents, influencing crystal nucleation and restricting excessive particle growth. The sharp particle boundaries observed in the SEM image indicate the crystalline nature of the synthesized ZnO nanocomposites.

The particle size distribution obtained from image analysis is shown in Figure 5(b). The particle diameters were found to range between 5 and 60 nm, with most particles concentrated in the 20–50 nm interval. The average particle size was determined to be 27 nm. The distribution curve demonstrates that the synthesized nanocomposites possess relatively uniform dimensions, although a moderate variation in size is evident. This variation may be associated with differences in nucleation rates and growth conditions during the biosynthesis process. The nanoscale dimensions obtained in this study are advantageous because smaller particles provide a larger specific surface area, which can enhance surface-related properties such as catalytic activity, adsorption capacity, and antimicrobial performance [44]. The average particle size of 27 nm indicates that the dual-extract-mediated synthesis route effectively controlled particle growth and produced stable ZnO nanocomposites within the nanometer range. Overall, SEM and particle size analyses confirm the successful green synthesis of ZnO

nanocomposites with crystalline morphology and nanoscale dimensions. The combination of irregular particle shapes and an average particle size of 27 nm demonstrates the effectiveness of the dual extract in promoting nanoparticle formation while limiting excessive particle enlargement.

3.6 Antibacterial activity

The antimicrobial activity of ZnO nanocomposites synthesized from the combined leaf extracts of Ziziphus oenoplia and Ziziphus mauritiana was assessed against Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, and Pseudomonas sp. using different nanoparticle concentrations. The inhibition zones obtained are shown in Figure 7. A clear variation in growth inhibition was observed among the tested microorganisms. For E. coli, the inhibition zone increased progressively from 10 mm at 25 µg mL^{-1} to 12 mm at 100 µg mL^{-1}, indicating a gradual response to increasing nanoparticle concentration. A comparable trend was recorded for S. aureus, where inhibition increased from 9 mm to 13 mm across the tested concentration range. These results suggest that increasing the amount of ZnO nanocomposites enhanced the suppression of bacterial growth for these organisms. The strongest response was observed in Pseudomonas sp. The inhibition diameter reached 22 mm at 100 µg mL^{-1}, which was substantially greater than that recorded for the remaining bacterial strains. Even at the lowest concentration, Pseudomonas sp. exhibited a larger inhibition zone than the control treatment, and this difference became more pronounced with increasing nanoparticle concentration [45]. The marked increase in inhibition indicates a greater sensitivity of this organism to the synthesized nanocomposites under the conditions employed in the present study. In the case of E. faecalis, inhibition zones of 11 mm and 12 mm were recorded at 25 and 50 µg mL^{-1}, respectively, whereas a slight decrease to 10 mm was observed at 100 µg mL^{-1}. This pattern differed from that observed for the other bacterial strains and may reflect differences in nanoparticle dispersion within the medium or variability in bacterial response at higher concentrations. Nevertheless, inhibition remained higher than that observed for the control treatment. Comparison with the control demonstrated that nanoparticle treated samples generally produced wider inhibition zones, confirming the contribution of ZnO nanocomposites to microbial growth suppression [46]. The concentration dependent increase observed for most of the tested bacteria indicates that antimicrobial performance was influenced by nanoparticle dosage. Furthermore, the nanoscale particle size obtained during synthesis may have favored interactions between the particles and microbial cells, contributing to the inhibitory effect observed in the agar diffusion assay. Among the tested organisms, susceptibility followed the order: Pseudomonas sp. > Staphylococcus aureus > Escherichia coli > Enterococcus faecalis based on the maximum inhibition zones recorded at the highest nanoparticle concentration. The findings demonstrate that ZnO nanocomposites synthesized using Ziziphus oenoplia and Ziziphus mauritiana extracts possess measurable antimicrobial activity, with particularly strong inhibition against Pseudomonas sp. The observed differences among bacterial species indicate that the effectiveness of the nanocomposites depends on the characteristics of the target microorganism as well as the concentration applied.

3.7 Antioxidant activity

The antioxidant properties of ZnO nanocomposites synthesized using the combined extracts of Ziziphus oenoplia and Ziziphus mauritiana were investigated using DPPH and hydrogen peroxide (H_2O_2) scavenging assays. The percentage inhibition obtained at different concentrations is presented in Figure 7.

DPPH Radical Scavenging Assay

The synthesized ZnO nanocomposites exhibited a progressive increase in DPPH radical scavenging activity with increasing concentration. At 10 µg mL^{-1}, the inhibition value was 63.48%, which increased to 74.57%, 82.43%, 85.71%, and 89.84% at 20, 30, 40, and 50 µg mL^{-1}, respectively. A similar pattern was observed for the standard antioxidant, where inhibition increased from 66.25% at 10 µg mL^{-1} to 93.15% at 50 µg mL^{-1}. The difference between the standard and nanoparticle treatments remained relatively small across the concentration range studied. At lower concentrations, the scavenging efficiency of ZnO nanocomposites was slightly reduced compared with the standard; however, the gap narrowed at higher concentrations. The increase in inhibition values indicates that the radical scavenging ability of the synthesized nanocomposites became more pronounced as concentration increased. The results demonstrate that the biosynthesized ZnO nanocomposites were capable of effectively reducing DPPH radicals, achieving nearly 90% inhibition at the highest concentration tested.

Hydrogen Peroxide Scavenging Assay

The H_2O_2 scavenging assay also revealed a concentration-dependent response. The inhibition percentage of ZnO nanocomposites increased from 49.6% at 10 µg mL^{-1} to 87.3% at 50 µg mL^{-1}. The corresponding values for the standard ranged from 51.1% to 89.9%. Throughout the experiment, both treatments followed a similar trend, with inhibition increasing steadily as concentration increased. At concentrations of 40 and 50 µg mL^{-1}, the scavenging activity of the nanocomposites approached that of the standard antioxidant. This observation suggests that the synthesized ZnO nanocomposites maintained considerable activity even at higher levels of

oxidative challenge. The gradual rise in inhibition values across all concentrations indicates a consistent interaction between the nanocomposites and hydrogen peroxide molecules within the assay system [47].

When the two antioxidant assays were compared, the ZnO nanocomposites demonstrated strong activity in both systems. The DPPH assay produced slightly higher inhibition values than the H_2O_2 assay at corresponding concentrations. For example, at 50 µg mL^{-1}, the nanocomposites achieved 89.84% inhibition in the DPPH assay and 87.3% inhibition in the H_2O_2 assay. Despite this difference, both methods showed the same concentration dependent pattern. The results indicate that nanocomposites synthesized from Ziziphus oenoplia and Ziziphus mauritiana extracts possess notable antioxidant capacity. The steady increase in scavenging activity with concentration and the close agreement between nanoparticle and standard values suggest that the synthesized material is capable of interacting effectively with reactive species under the conditions employed in the present study. Overall, the antioxidant assays confirmed that the biosynthesized ZnO nanocomposites exhibited substantial radical-scavenging activity, with the highest responses recorded at 50 µg mL^{-1}. The findings support the potential application of these green synthesized nanocomposites in systems where antioxidant properties are desirable.

4 Conclusion

The present investigation demonstrated that leaf extracts of Ziziphus oenoplia and Ziziphus mauritiana can be effectively utilized for the preparation of chitosan/zinc oxide nanocomposites through a simple plant mediated synthesis approach. The formation of CS/ZnO nanocomposites was achieved without the use of additional chemical reducing agents, highlighting the suitability of these medicinal plants as natural sources of biomolecules involved in nanoparticle production. The characterization results verified the successful synthesis of nanosized ZnO particles. XRD analysis confirmed the crystalline nature of the material, while UV–Visible spectroscopy, FTIR, SEM, DLS, and zeta potential analyses provided evidence of their optical properties, surface chemistry, morphology, particle size, and dispersion stability. The nanocomposites obtained were predominantly within the nanometer range and exhibited satisfactory structural characteristics for biological applications. The synthesized ZnO nanocomposites showed inhibitory effects against the tested bacterial strains, although the degree of inhibition varied among the microorganisms. The strongest antibacterial response was observed against Pseudomonas sp., indicating that bacterial susceptibility depended on the nature of the target organism. In addition, the nanocomposites displayed appreciable antioxidant activity in both DPPH and hydrogen peroxide scavenging assays. The gradual increase in scavenging activity with concentration suggests that the nanocomposites remained active over the tested concentration range and were capable of interacting effectively with free radicals. Taken together, the results indicate that the combined extracts of Z. oenoplia and Z. mauritiana provide a practical and sustainable route for the synthesis of CS/ZnO nanocomposites with promising biological properties. The study also demonstrates the value of integrating phytochemical resources from two medicinal plants to obtain stable nanomaterials with potential applications in antimicrobial and antioxidant formulations. Further work involving toxicity evaluation and application specific studies would help to establish their suitability for future biomedical and pharmaceutical use.

Ethics approval and consent to participate

This articles does not contain any studies on animals or human participant performed by any of the author

Conflict of Interest

The author of this work declare that they have no conflicts of interest

References

1. Prajapati, D., Jabborova, D., Saharan, B. S., Singh, N., Patani, A., Singh, S., & Joshi, C. (2025). Bionanotechnology: a paradigm for advancing environmental sustainability. Indian Journal of Microbiology, 65(1), 306-332.
2. Ulusoy, U. (2023). A review of particle shape effects on material properties for various engineering applications: from macro to nanoscale. Minerals, 13(1), 91.
3. Muthuvel, A., Jothibas, M., & Manoharan, C. (2020). Effect of chemically synthesis compared to biosynthesized ZnO-NPs using Solanum nigrum leaf extract and their photocatalytic, antibacterial and in-vitro antioxidant activity. Journal of Environmental Chemical Engineering, 8(2), 103705.
4. Sani, G. D., Yakubu, A., Saidu, A., Aati, R., Sahabi, S., & Abdullahi, S. (2023). A review on industrial applications of zinc oxide nanoparticles. International Journal of Advances in Engineering and Management, 5, 1031-1041.
5. Raha, Sauvik, and Md Ahmaruzzaman. "ZnO nanostructured materials and their potential applications: progress, challenges and perspectives." Nanoscale advances 4.8 (2022): 1868-1925.
6. Zhao, L., Qu, Y., Shang, G., Jiang, W., Shen, Q., Ke, J., & Bian, H. (2025). Natural pigments for microbial control in food systems: Mechanisms, preservation applications, and formulation strategies. Food Control, 111629.

7. Ghosh, S., Ghosh, A., Pramanik, S., Kuiri, P. K., Sen, R., & Neogi, S. K. (2022, September). Synthesis of ZnO nanoparticles by co-precipitation technique and characterize the structural and optical properties of these nanoparticles. In Journal of Physics: Conference Series (Vol. 2349, No. 1, p. 012014). IOP Publishing.
8. Wirunchit, S., & Koetniyom, W. (2023). ZnO nanoparticles synthesis and characterization by hydrothermal process for biological applications. physica status solidi (a), 220(10), 2200364.
9. Rathore, R., & Kaurav, N. (2022). The structural and optical properties of ZnO nanoparticles synthesized via thermal decomposition. Materials Today: Proceedings, 54, 624-627.
10. Ahmed, S., Chaudhry, S. A., & Ikram, S. (2017). A review on biogenic synthesis of ZnO nanoparticles using plant extracts and microbes: a prospect towards green chemistry. Journal of Photochemistry and Photobiology B: Biology, 166, 272-284.
11. Bose, A. K., Manhas, M. S., Ganguly, S. N., Sharma, A. H., & Banik, B. K. (2002). MORE chemistry for less pollution: applications for process development. Synthesis, 2002(11), 1578-1591.
12. Osman, A. I., Zhang, Y., Farghali, M., Rashwan, A. K., Eltaweil, A. S., Abd El-Monaem, E. M., ... & Yap, P. S. (2024). Synthesis of green nanoparticles for energy, biomedical, environmental, agricultural, and food applications: A review. Environmental Chemistry Letters, 22(2), 841-887.
13. Hano, C., & Abbasi, B. H. (2021). Plant-based green synthesis of nanoparticles: Production, characterization and applications. Biomolecules, 12(1), 31.
14. Song, T., Gao, F., Guo, S., Zhang, Y., Li, S., You, H., & Du, Y. (2021). A review of the role and mechanism of surfactants in the morphology control of metal nanoparticles. Nanoscale, 13(7), 3895-3910.
15. Siddiquee, T., Bhaskaran, N. A., Nathani, K., & Sawarkar, S. P. (2024). Empowering lung cancer treatment: Harnessing the potential of natural phytoconstituent-loaded nanoparticles. Phytotherapy Research, 38(8), 3899-3920.
16. Pratap, R., Niveria, K., Srivastava, S. K., Chaudhary, S., Sharma, P., Verma, A. K., & Parmar, A. S. (2023). Biogenic Synthesis of Gold Nanoparticles Using Dual Extract of Tulsi–Vinca for Breast Cancer Tumor Regression in Mice. Nanomedicine, 18(26), 1941-1959.
17. Mirzaei, G. H., Gheybi, F., Sabouri, Z., & Darroudi, M. (2026). Green synthesis of silver nanoparticles using Solanum melongena peel and Abelmoschus esculentus extracts: a dual study on antibacterial efficacy and photocatalytic activity. Scientific Reports.
18. Prakash, R., Saravanan, P., Kumar, P., Omer, S. N., & Rajeshkannan, R. (2025). Synergistic green synthesis of ZnO nanoparticles using Cymbopogon citratus and Nelumbo nucifera for enhanced antibacterial activity. Sustainable Chemistry One World, 100144.
19. Herin, R. F., Judit, A. S., Sebastiammal, S., Shabna, S., Dhas, S. S. J., & Biju, C. S. (2025). Functionalized ZnO NPs and biopolymers-coated ZnO NPs for drug delivery and biomedical applications—a review. Regenerative Engineering and Translational Medicine, 11(1), 165-189.
20. El-Araby, A., Janati, W., Ullah, R., Ercisli, S., & Errachidi, F. (2024). Chitosan, chitosan derivatives, and chitosan-based nanocomposites: eco-friendly materials for advanced applications (a review). Frontiers in Chemistry, 11, 1327426.
21. Ahari, H., Kalateh-Seifari, F., & Salimi Galangashi, Y. (2026). Plant-Derived Extract-Loaded Nanocarriers and Green-Synthesized Nanomaterials in Functional Foods: Applications, Challenges, and Safety Perspectives. Food and Bioprocess Technology, 19(6), 294.
22. Elemike, E. E., Onwudiwe, D. C., & Mbonu, J. I. (2021). Green Synthesis, Structural Characterization and Photocatalytic Activities of Chitosan-ZnO Nano-composite. Journal of Inorganic and Organometallic Polymers and Materials, 31(8), 3356-3367.
23. Rani, N., Kusum, & Hooda, V. (2024). Chitosan/ZnO nanocomposites for improving the growth and reducing the toxicity of Zn in Sorghum bicolor (L.) Moench plants. Acta Physiologiae Plantarum, 46(6), 67.
24. Allah, M. A. A. H., Ibrahim, H. K., & Abdulridha, A. A. (2025). Eco-friendly synthesis of ZnO/chitosan nanocomposite: detailed characterization, DFT study, docking study, adsorption kinetics, thermodynamic analysis and antioxidant properties. Journal of Molecular Liquids, 425, 127216.
25. Ammar-Amin, M. Z., Dasiman, R., Evana, K., Aida, F. M. N. A., Hamid, N., Huda, N., ... & Raseetha, S. (2026). Bioactivities and potential health benefits of Ziziphus mauritiana. Journal of Food Measurement and Characterization, 1-22.
26. Anbu, S., Boomiga, S., Suresh, A., & Padma, J. (2022). Phytochemical screening and antimicrobial activity of Ziziphus oenoplia seed extract. Research Journal of Pharmacy and Technology, 15(2), 615-620.
27. Mucha, P., Skoczyńska, A., Małecka, M., Hikisz, P., & Budzisz, E. (2021). Overview of the antioxidant and anti-inflammatory activities of selected plant compounds and their metal ions complexes. Molecules, 26(16), 4886.
28. Prakash, O., Usmani, S., Singh, R., Singh, N., Gupta, A., & Ved, A. (2021). A panoramic view on phytochemical, nutritional, and therapeutic attributes of Ziziphus mauritiana Lam.: A comprehensive review. Phytotherapy Research, 35(1), 63-77.

29. Azam, A., Ahmed, A. S., Oves, M., Khan, M. S., Habib, S. S., & Memic, A. (2012). Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. International journal of nanomedicine, 6003-6009.
30. Jomova, K., Raptova, R., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Archives of toxicology, 97(10), 2499-2574.
31. Jain, A., Jangid, T., Jangir, R. N., & Bhardwaj, G. S. (2024). A Comprehensive Review on the Antioxidant Properties of Green Synthesized Nanoparticles: in vitro and in vivo Insights. Free Radicals & Antioxidants, 14(2).
32. Venkataraju, J. L., Sharath, R., Chandraprabha, M. N., Neelufar, E., Hazra, A., & Patra, M. (2014). Synthesis, characterization and evaluation of antimicrobial activity of zinc oxide nanoparticles. Journal of Biochemical Technology, 3(5), 151-154.
33. Muthuvel, A., Adavallan, K., Balamurugan, K., & Krishnakumar, N. (2014). Biosynthesis of gold nanoparticles using Solanum nigrum leaf extract and screening their free radical scavenging and antibacterial properties. Biomedicine & Preventive Nutrition, 4(2), 325-332.
34. Manimaran, M., Moorthy, N., Gurumoorthy, M., Muthuvel, A., Alhomaidi, E., & Alhabardi, S. (2025). Sustainable Biogenic Synthesis of Europium Oxide Nanoparticles from Durian Leaf Extract for Antibacterial and Antioxidant Application. Russian Journal of General Chemistry, 95(8), 2072-2080.
35. Selvam, K., Sudhakar, C., Senthilkumar, B., Sakthivel, V., Ragu Prasath, A., & Sangameshwaran, V. (2024). Green synthesis and characterisation of iron oxide nanoparticle using ziziphus oenoplia fruit extract: A biomedical and environmental potential. Waste and Biomass Valorization, 15(11), 6415-6429.
36. Kalimuthua, N., Subashchandrabose, S., & Meganathan, C. (2026). Dual-Extract Driven AgCl Nanoparticles (AgCl-NP): Mechanistic Insights into Bioactivity, Ecotoxicity, and Targeted Molecular Interactions. Applied Nanoscience, 16(2), 24.
37. Wang, L. Y., Shi, B. Y., Yao, C. B., Wang, Z. M., Wang, X., Jiang, C. H., ... & Song, Y. L. (2023). Size and morphology modulation in ZnO nanostructures for nonlinear optical applications: a review. ACS Applied Nano Materials, 6(12), 9975-10014.
38. Sangeetha, G., Rajeshwari, S., & Venckatesh, R. (2011). Green synthesis of zinc oxide nanoparticles by aloe barbadensis miller leaf extract: Structure and optical properties. Materials Research Bulletin, 46(12), 2560-2566.
39. Singh, H., Desimone, M. F., Pandya, S., Jasani, S., George, N., Adnan, M., & Alderhami, S. A. (2023). Revisiting the green synthesis of nanoparticles: uncovering influences of plant extracts as reducing agents for enhanced synthesis efficiency and its biomedical applications. International journal of nanomedicine, 4727-4750.
40. Kanimozhi, K., Jayakumari, T., Muthuvel, A., & Sumethra, R. (2025). Sustainable Synthesis and Characterization of Cerium Oxide Nanoparticles Using Camphora Leaf Extract for Efficient Dye Degradation. Russian Journal of General Chemistry, 95(10), 2949-2959.
41. Liao, L. F., Lien, C. F., Shieh, D. L., Chen, M. T., & Lin, J. L. (2002). FTIR study of adsorption and photoassisted oxygen isotopic exchange of carbon monoxide, carbon dioxide, carbonate, and formate on TiO2. The Journal of Physical Chemistry B, 106(43), 11240-11245.
42. Vollath, D., Fischer, F. D., & Holec, D. (2018). Surface energy of nanoparticles–influence of particle size and structure. Beilstein journal of nanotechnology, 9(1), 2265-2276.
43. Jothibas, M., Paulson, E., Srinivasan, S., & Kumar, B. A. (2022). The impacts of interfacing phytochemicals on the structural, optical and morphology of hematite nanoparticles. Surfaces and Interfaces, 29, 101734.
44. Merzah, Z. F., Shanan, Z. J., Abdalameer, N. K., & Fakhry, S. (2025). Innovative jet plasma-assisted green synthesis of TiO2@ Ag core–shell nanoparticles using chard leaf extract for antibacterial applications. Plasmonics, 1-14.
45. Fadwa, A. O., Alkoblan, D. K., Mateen, A., & Albarag, A. M. (2021). Synergistic effects of zinc oxide nanoparticles and various antibiotics combination against Pseudomonas aeruginosa clinically isolated bacterial strains. Saudi journal of biological sciences, 28(1), 928-935.
46. Fontecha-Umaña, F., Ríos-Castillo, A. G., Ripolles-Avila, C., & Rodríguez-Jerez, J. J. (2020). Antimicrobial activity and prevention of bacterial biofilm formation of silver and zinc oxide nanoparticle-containing polyester surfaces at various concentrations for use. Foods, 9(4), 442.
47. Magar, H. S., Hashem, M. S., & Sobh, R. A. (2024). Design of metal oxide nanoparticles-embedded polymeric nanocomposites for hydrogen peroxide chronoamperometric sensor. Polymer Composites, 45(4), 3653-3665.

Figure

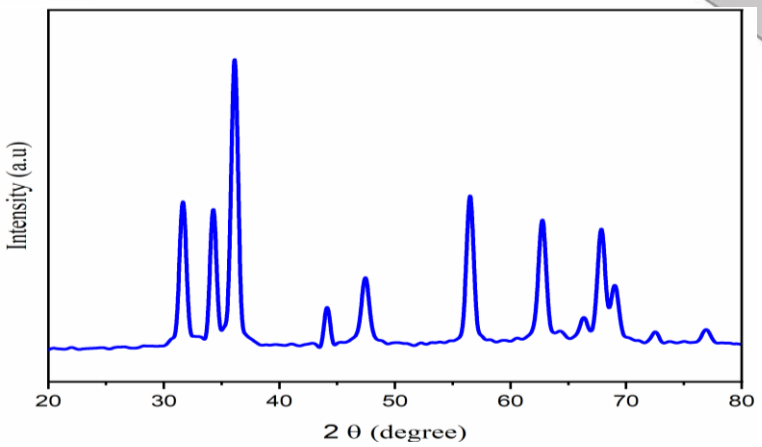


Figure 1 XRD paten of biosynthesized CS/ZnO nanocomposites

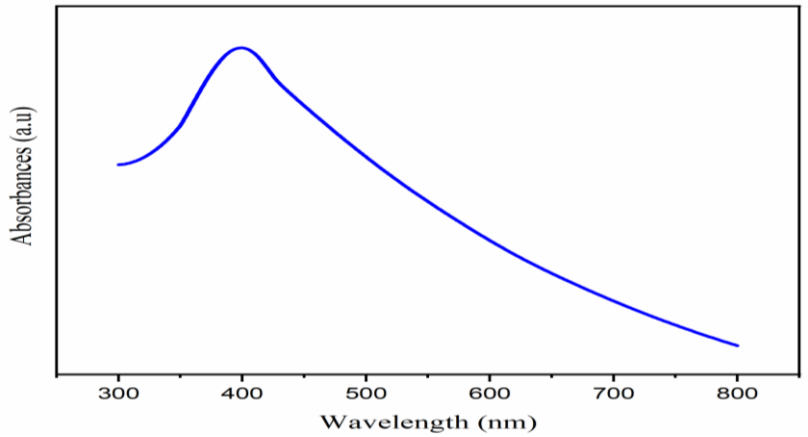
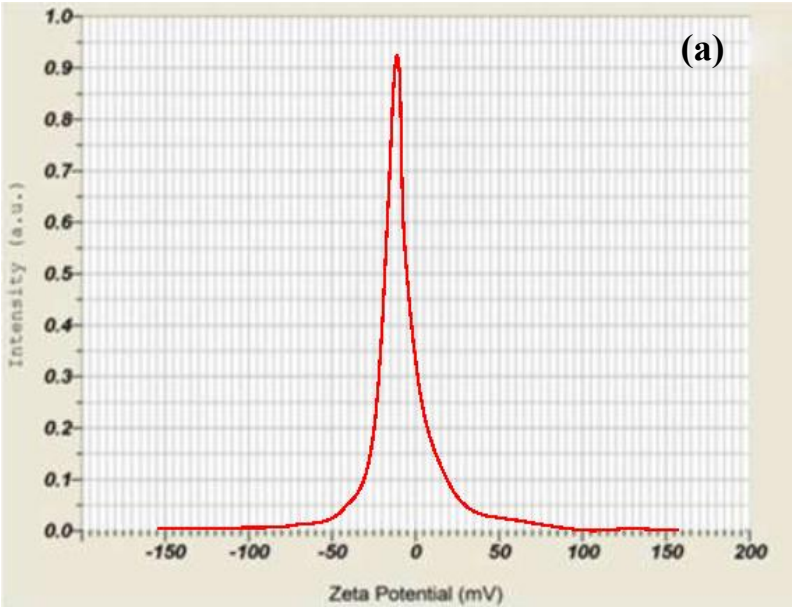


Figure 2 UV Visible spectra of biosynthesized CS/ZnO nanocomposites



(b

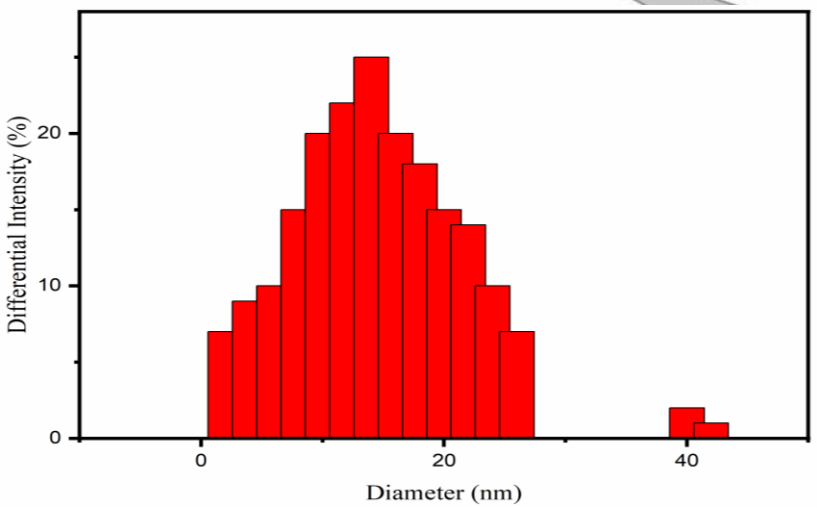


Figure 3 (a) Zeta potential (b) DLS of biosynthesized CS/ZnO nanocomposites

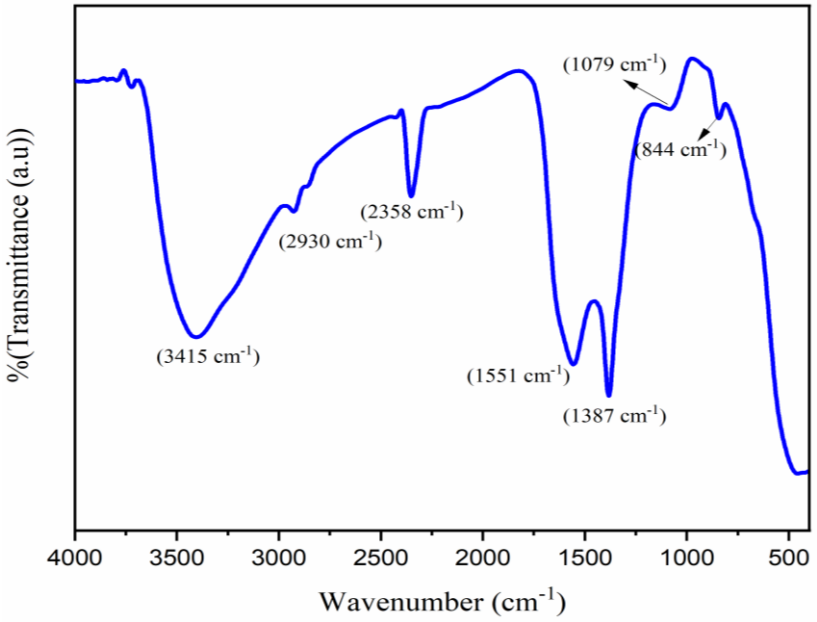


Figure 4 FTIR spectra of biosynthesized CS/ZnO nanocomposites

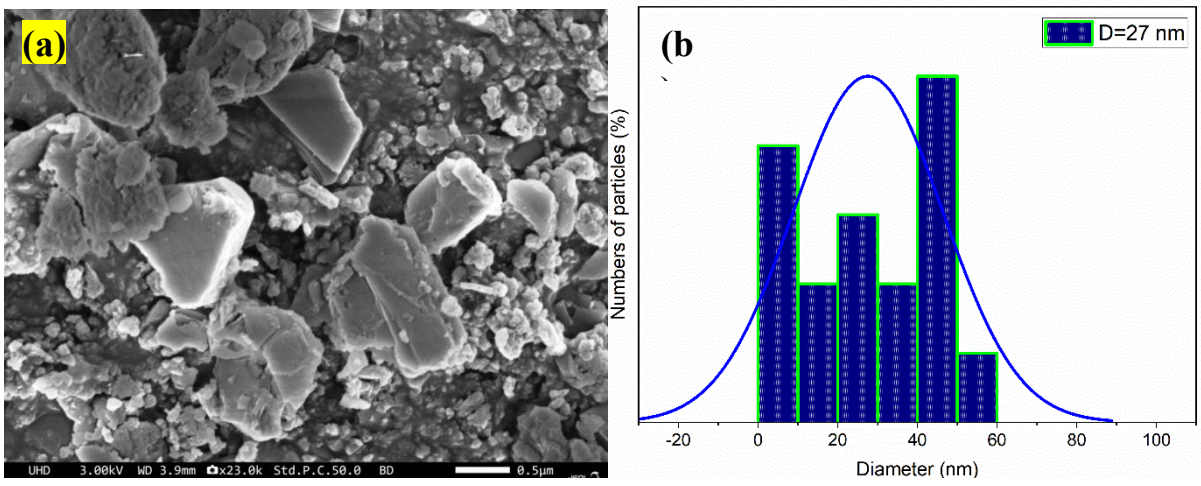


Figure 5 (a) SEM image (b) particle size of biosynthesized CS/ZnO nanocomposites

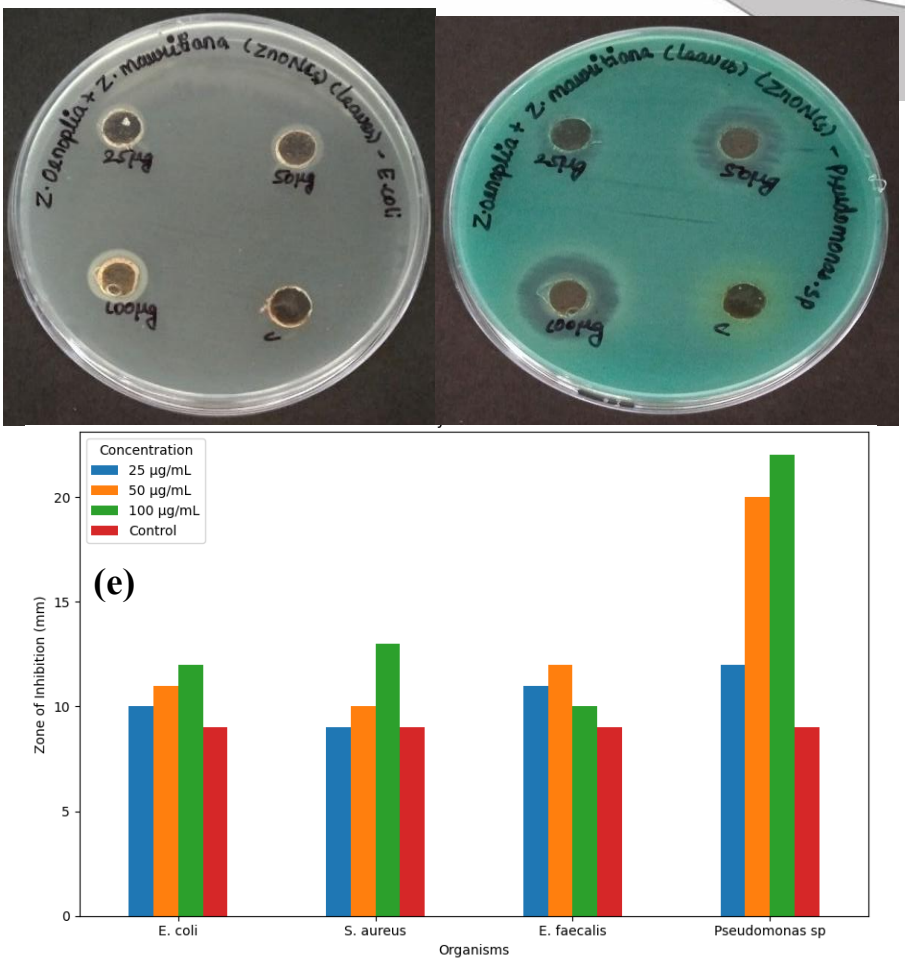


Figure 6 (a-d) disc image (e) bar diagram Antibacterial activity of biosynthesized CS/ZnO nanocomposites

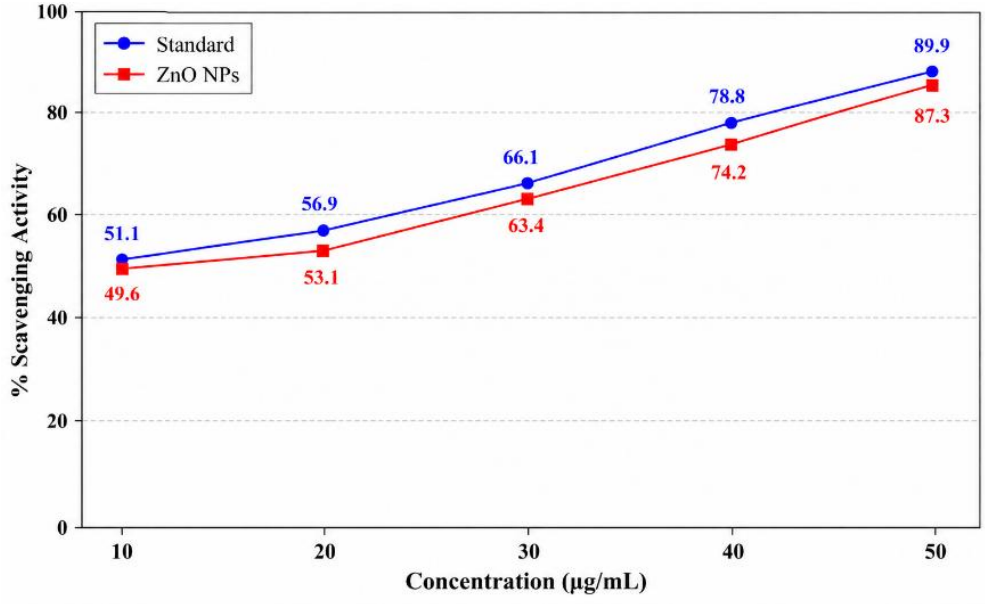


Figure 7 (a) DPPH (b) H2O2 assay of biosynthesized CS/ZnO nanocomposites