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SYNTHESIS OF ZNO NANOPARTICLES USING ALOE VERA GREEN SOURCE AND THEIR ANTIBACTERIAL PROPERTIES

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Article Info

Abstract

The green synthesis of zinc oxide (ZnO) nanoparticles (NPs) has gained significant attention due to its eco-friendly, cost-effective, and non-toxic approach. This study synthesizes ZnO NPs using Aloe vera extract as a natural reducing and stabilizing agent. The bioactive compounds in Aloe vera facilitate nanoparticle formation, eliminating the need for hazardous chemicals. The synthesized ZnO NPs were characterized using UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX) to confirm their structural, morphological, and elemental properties. The antibacterial potential of ZnO NPs was evaluated against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacterial strains using the agar well diffusion method. The results revealed significant antibacterial activity, with ZnO NPs exhibiting larger zones of inhibition compared to control samples. The mechanism of action was attributed to reactive oxygen species (ROS) generation, bacterial membrane disruption, and metal ion release, leading to cell damage and death. This study highlights the potential of Aloe vera-mediated ZnO NPs as an efficient antimicrobial agent with promising applications in biomedicine, food preservation, and agriculture. The eco-friendly synthesis method aligns with sustainable nanotechnology goals, reducing environmental toxicity while maintaining high bioactivity. Further research on optimizing synthesis parameters and evaluating long-term stability can enhance the practical applicability of these nanoparticles.

Keywords:
Green synthesis, ZnO nanoparticles, Aloe vera, antibacterial activity, reactive oxygen species, sustainable nanotechnology.

Introduction

As zinc oxide and oxygen are members of the second and sixth groups of the periodic table, respectively, ZnO is a recognized II-VI semiconductor in the field of materials science. The ZnO semiconductor has many exceptional and beneficial properties, such as good transparency, antimicrobial agents, high electron mobility, wide bandgap, high thermal and mechanical stability, and strong room-temperature luminescence [1]. Its large bandgap, or 3.37 eV, is on the borderline between ionic and covalent semiconductors [2]. The crystalline of ZnO has a wurtzite (B4) crystal structure, having a hexagonal unit cell with two lattice parameters a = 0.325 nm and c = 0.521 nm. With its hexagonal wurtzite structure, each anion is surrounded by four cations at the corners of the tetrahedron, which displays the tetrahedral coordination and hence exhibits the sp3 covalent bonding [3]. Nano-sized ZnO exhibits varying morphologies and shows significant antibacterial activity over a wide spectrum of bacterial species explored by many researchers [4]. ZnO is currently being investigated as an antibacterial agent in both microscale and nanoscale formulations. ZnO exhibits significant antimicrobial activities when particle size is reduced to the nanometre range, then nano-sized ZnO can interact with the bacterial surface and/or with the bacterial core where it enters inside the cell, subsequently exhibiting distinct bactericidal mechanisms [5]. The interactions between these unique materials and bacteria are mostly toxic, which have been exploited for antimicrobial applications such as in the food industry. Interestingly, ZnO-NPs are reported by several studies as non-toxic to human cells [6], this aspect necessitated their usage as antibacterial agents, noxious to microorganisms, and hold good biocompatibility to human cells [7]. The various antibacterial mechanisms of nanomaterials are mostly attributed to their high specific surface area-to-volume ratios [8], and their distinctive physicochemical properties. However, the precise mechanisms are yet under debate, although several proposed ones are suggested and adopted. Investigations on antibacterial nanomaterials, mostly ZnO-NPs, would enhance the research area of nanomaterials, and the mechanisms and phenomena behind nanostructured materials [9]. Aloe vera is a cactus-like plant since it is succulent, has thorns along the edge of its leaves, is covered with wax, and contains a lot of water [10]. Aloe vera contains 99%–99.5% water and the remaining solid material contains over 75 active compounds including water- and fat-soluble vitamins, minerals, enzymes, simple/complex polysaccharides, phenolic compounds, and organic acids [11]. Aloe vera belongs to the lily family of Aloe barbadensis group and has 400 species [12]. Aloe vera is also well known for its medicinal properties and has been used as a soothing agent for burns and inflammation. Furthermore, the therapeutic properties of aloe vera have been employed in the commercial applications of pharmaceutical, food, and cosmetics [13]. The extract of aloe vera plant has been used for the synthesis of gold, silver, copper oxide, indium oxide, titanium dioxide, cerium oxide [14], and tin oxide [15].

Despite the extensive research on the synthesis of zinc oxide (ZnO) nanoparticles, conventional methods often involve toxic chemicals, high energy consumption, and environmental concerns. Green synthesis using plant extracts has emerged as a sustainable and eco-friendly alternative; however, there is still a need to explore and optimize different plant sources for improved nanoparticle properties. Aloe vera, known for its rich bioactive compounds, has shown potential as a reducing and stabilizing agent, yet limited studies have investigated its role in the synthesis of ZnO nanoparticles with enhanced antibacterial activity. The present study aims to synthesize ZnO nanoparticles using Aloe vera extract as a green source, characterize their physicochemical properties, and evaluate their antibacterial efficacy against pathogenic bacteria. By focusing on a cost-effective and environmentally friendly synthesis approach, this research seeks to contribute to the development of green nanotechnology and broaden the application of ZnO nanoparticles in antimicrobial treatments.

3 Materials and methods:

Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) was utilized as the precursor for the synthesis of ZnO nanoparticles, and fresh Aloe vera leaves were gathered from the Local market in Lahore and processed

to obtain the plant extract. Deionized water was used throughout the experiment, and all chemicals were of analytical grade and used without further purification.

3.1 Preparation of Aloe Vera Extract

To get rid of any contaminants, fresh aloe vera leaves were properly cleaned with distilled water. To get a clear extract, the gel was carefully removed, mixed, and filtered. After 20 to 30 minutes of heating the extract to 60 to 80°C to optimize the release of bioactive components, Whatman filter paper No. 1 was used for filtering. For later usage, the produced extract was kept at 4°C.

3.2 Green Synthesis of ZnO Nanoparticles

An aqueous solution of zinc nitrate hexahydrate was combined with a specified volume of aloe vera extract while being constantly stirred. The mixture was heated between 70°C and 90°C until a color change suggested that ZnO nanoparticles had formed. After centrifuging the reaction mixture for 10 to 15 minutes at 10,000 rpm, the precipitate was repeatedly cleaned with ethanol and deionized water to get rid of any unreacted substances. To increase crystallinity, the resulting ZnO nanoparticles were calcined for two to three hours at 400 to 500°C after being dried in an oven at 100°C.

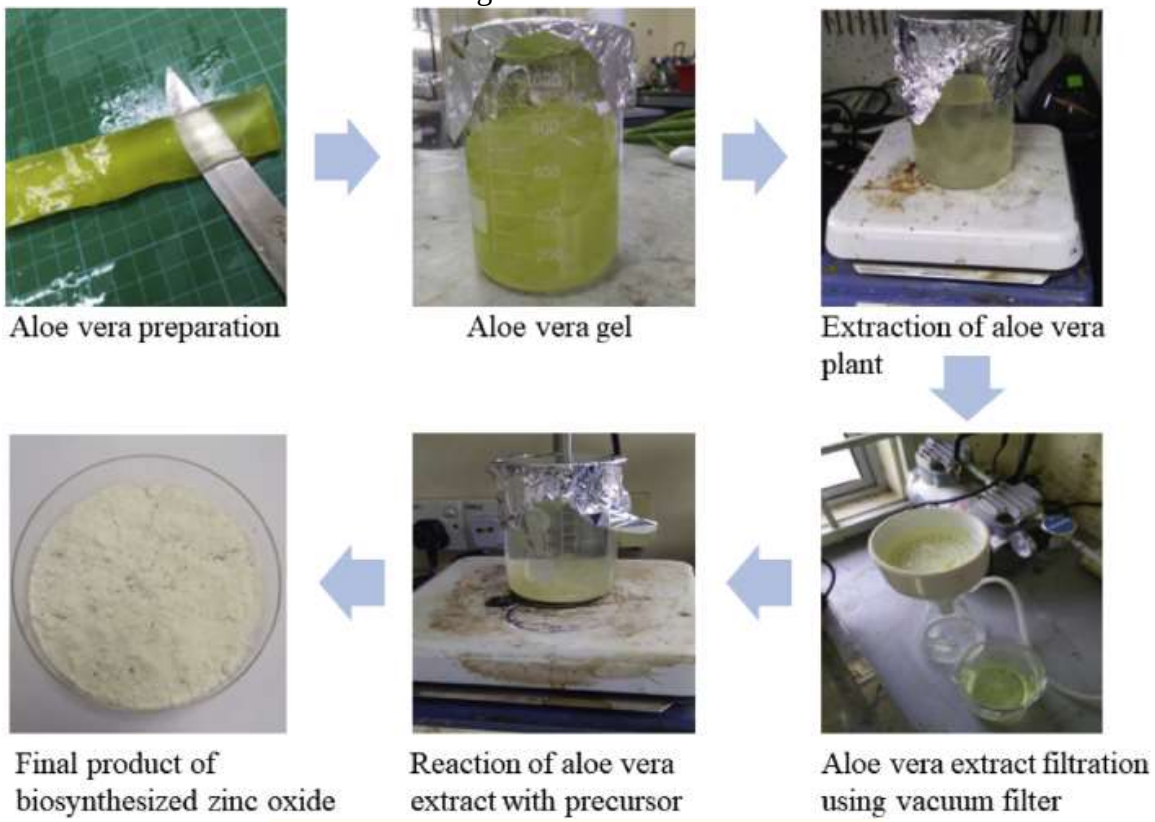

Figure 3.1: Process flow of ZnO biosynthesis.

3.3 Characterization techniques:

The following characterization methods were used to verify the effective production of ZnO nanoparticles and assess their physicochemical characteristics.

3.4 UV-Visible Spectroscopy (UV-Vis):

The optical characteristics of the produced ZnO nanoparticles were examined using UV-Vis spectroscopy. The distinctive surface plasmon resonance (SPR) peak of ZnO nanoparticles, which is usually seen between 350 and 400 nm, was identified by recording the absorption spectra in the 200–800 nm range.

3.5 X-ray diffraction (XRD)

The crystalline structure, phase purity, and crystallite size of ZnO nanoparticles have been determined by X-ray diffraction (XRD) research. Cu-Kα radiation (λ = 1.5406 Å) was used to record the diffraction patterns, and the Scherrer equation was used to determine the average crystallite size. The hexagonal wurtzite structure was verified by ZnO-corresponding peaks.

3.6 Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups found in aloe vera extract and their function in stabilizing and reducing ZnO nanoparticles were determined using FTIR analysis. To identify distinctive peaks that corresponded to ZnO vibrations and biomolecules involved in capping and stabilization, the spectra were recorded in the 4000–400 cm^{-1} range.

3.7 Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS)

The size, shape, and surface morphology of the produced ZnO nanoparticles were examined by SEM. By identifying the existence of Zn and O without any undesirable contaminants, EDS was utilized in conjunction with SEM to verify the elemental makeup and purity of the nanoparticles.

3.8 Transmission Electron Microscopy (TEM)

To determine precisely the size, shape, and dispersion of ZnO nanoparticles, TEM examination produced high-resolution pictures. To ensure homogeneity and nanoscale dimensions, the particle size distribution and morphology were examined.

3.9 X-ray diffraction (XRD)

XRD analysis was conducted to determine the crystalline structure, phase purity, and crystallite size of ZnO nanoparticles. The diffraction patterns were recorded using Cu-Kα radiation (λ = 1.5406 Å), and the average crystallite size was estimated using the Scherrer equation. Peaks corresponding to ZnO confirmed the hexagonal wurtzite structure.

3.10 Antibacterial Activity Evaluation

Using the agar well diffusion technique, the antibacterial qualities of the produced ZnO nanoparticles were evaluated against harmful microorganisms, including *Staphylococcus aureus and Escherichia coli.* To evaluate the antibacterial effectiveness, the diameter of the inhibition zone was determined. By ensuring a thorough examination of the produced ZnO nanoparticles, these characterization approaches validated their optical, morphological, structural, and antibacterial qualities.

3.11 Method for the evaluation of Antibacterial Activity Evaluation

Using the agar well diffusion technique, the antibacterial activity of ZnO nanoparticles made from aloe vera extract was assessed against strains of bacteria that were Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli). To guarantee consistent bacterial content, the bacterial cultures were cultivated in nutrient broth for 24 hours at 37°C and adjusted to the 0.5 McFarland standard. A sterile cotton swab was used to uniformly distribute the bacterial inoculum on the surface of Mueller-Hinton agar plates. 100 µL of ZnO nanoparticle suspensions at several concentrations (25, 50, 100, and 200 µg/mL) were added to wells that were punched into the agar and had a diameter of 6 mm. Additionally included for comparison were a positive control (a common antibiotic like ampicillin or streptomycin) and a negative control (sterile water). Following a 24-hour incubation period at 37°C, the width of the zone of inhibition (ZOI) surrounding each well was measured to assess the antibacterial activity of the plates. The data were presented as mean ± standard deviation, and the experiment was carried out in triplicate. To ascertain the significance of differences, a one-way ANOVA was used in the statistical analysis; a p-value of less than 0.05 was deemed statistically significant. This technique demonstrated the green-synthesized

ZnO nanoparticles' potential as antimicrobial agents by offering a trustworthy evaluation of their antibacterial effectiveness.

4 Results and discussion:

4.1 Characterization of ZnO nanoparticles

4.1.1 Morphology and elemental analysis of ZnO

The SEM micrographs provide structural insights into ZnO nanoparticles synthesized using Aloe vera extract, highlighting their morphology and distribution at different magnifications. In the image (a), captured at low magnification (x35, scale: 1.00 mm), the ZnO nanoparticles appear as randomly dispersed, rod-like, or flaky structures, indicating minimal agglomeration and a stable synthesis process. This suggests that Aloe vera extract effectively acted as a reducing and stabilizing agent during nanoparticle formation. Image (b), taken at higher magnification (x1000, scale: 50.0 µm), reveals a highly textured, layered, and stacked morphology, with a rough and porous surface that enhances the surface-to-volume ratio, a critical factor for antibacterial activity. The sharp edges and nanoscale features observed in the high-magnification image suggest strong adhesion to bacterial membranes, facilitating bacterial inhibition through oxidative stress and Zn^{2+} ion release. The well-defined crystallinity further confirms the efficient nucleation and growth of ZnO nanoparticles during green synthesis. These structural properties contribute to the antibacterial effectiveness of the synthesized ZnO nanoparticles, making them a promising candidate for biomedical and environmental applications.

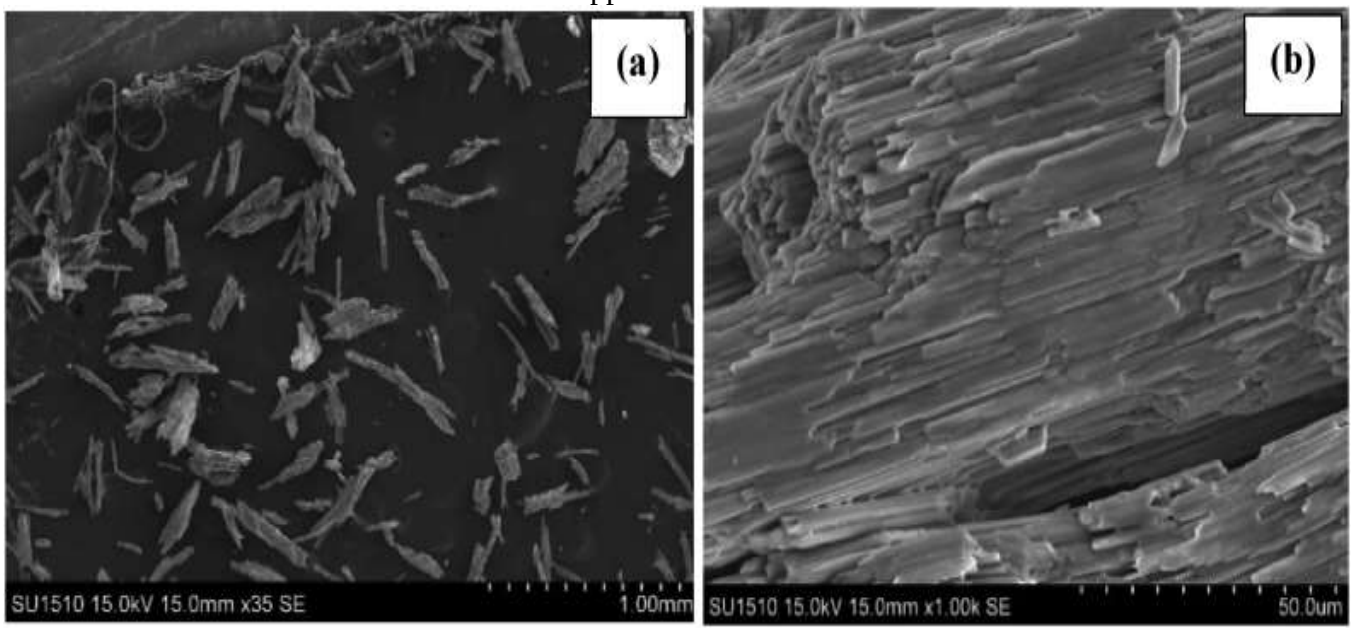

Figure 4.1: SEM images of ZnO at (a) 35× and (b) 1000× magnification levels.

4.2 EDX analysis:

The EDS spectrum confirms the elemental composition of ZnO nanoparticles synthesized using Aloe vera extract, validating the successful formation of ZnO through the green synthesis approach. The spectrum displays distinct peaks corresponding to zinc (Zn) at approximately 1 keV, 8.6 keV, and 9.6 keV, indicating the strong presence of Zn in the sample. Additionally, the oxygen (O) peak around 0.5 keV confirms the formation of ZnO nanoparticles. The absence of significant peaks for other elements suggests high purity with minimal contamination, demonstrating the efficiency of Aloe vera extract as a natural reducing and stabilizing agent. The composition of ZnO nanoparticles is crucial for their antibacterial properties, as ZnO can release Zn^{2+} ions and generate reactive oxygen species (ROS), which disrupt bacterial cell membranes and inhibit microbial growth. The EDS analysis thus verifies the elemental purity and successful synthesis of ZnO nanoparticles, highlighting their potential as an eco-friendly and effective antibacterial agent for biomedical applications.

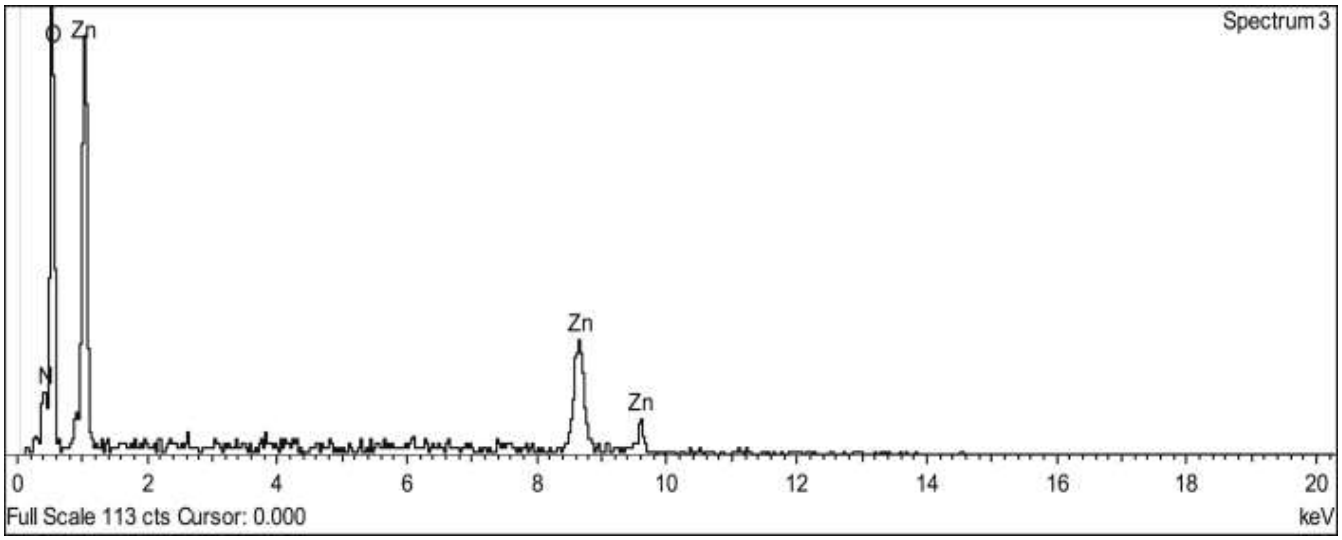

Figure 4.2: EDX spectrum of biosynthesized ZnO

4.3 FTIR analysis:

The FTIR spectra confirm the successful synthesis of ZnO nanoparticles using Aloe vera extract by identifying the functional groups involved in the process. Spectrum (a), corresponding to Aloe vera extract, shows a broad peak at 3283 cm^{-1}, indicating O-H stretching from hydroxyl groups, which are commonly present in phenols or alcohols. Additionally, the peak at 1637 cm^{-1} corresponds to C=O stretching, suggesting the presence of biomolecules such as flavonoids or amides, which act as reducing and stabilizing agents during the synthesis of ZnO nanoparticles. In contrast, spectrum (b), representing the synthesized ZnO nanoparticles, shows a peak at 3480 cm^{-1}, still indicating O-H stretching, but with a shift that suggests interaction with ZnO. The peaks at 1385 cm^{-1} and 829 cm^{-1} correspond to C-O and C-H vibrations, confirming the presence of organic compounds from Aloe vera extract. The most significant peak at 521 cm^{-1} corresponds to Zn-O stretching vibrations, providing direct evidence for the successful formation of ZnO nanoparticles. The shift and reduction in intensity of peaks in spectrum (b) compared to spectrum (a) indicate the involvement of Aloe vera phytochemicals in reducing Zn^{2+} ions and stabilizing the nanoparticles. The FTIR analysis thus validates the green synthesis of ZnO nanoparticles, demonstrating the role of Aloe vera in capping and stabilizing the particles, making them suitable for biomedical and antibacterial applications.

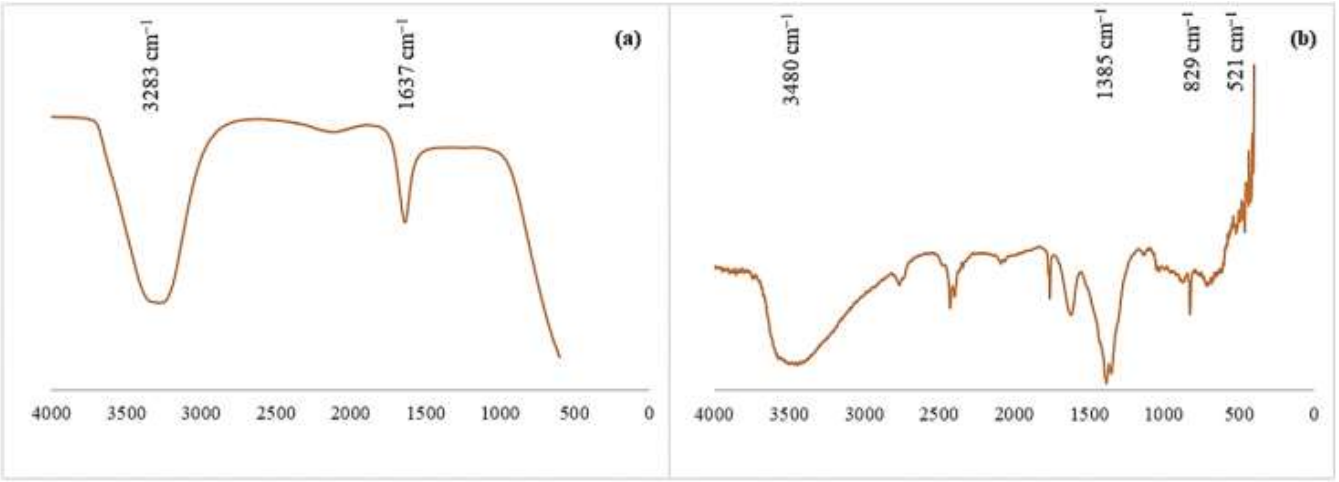

Figure 4.3: FTIR spectra of (a) pure aloe vera plant extract and (b) biosynthesized ZnO NPs.

4.4 Compound identification and crystallinity

The X-ray diffraction pattern of the synthesized ZnO nanoparticles exhibits distinct peaks at various 2θ values, corresponding to specific crystallographic planes such as (100), (002), (101), (012), (110), (103), (020), (021), (022), and (023). These peaks confirm the presence of a hexagonal wurtzite ZnO structure, which is the most stable and widely observed phase of ZnO nanoparticles. The sharp and well-defined peaks indicate a high degree of crystallinity in the synthesized nanoparticles, while the absence of any additional peaks suggests phase purity, ensuring that no significant impurities or secondary phases are present. The intensity and sharpness of these peaks further suggest that the ZnO nanoparticles possess excellent crystallinity, which is a crucial factor for their optical, electronic, and antibacterial properties. The broadening of certain peaks can be attributed to the nanoscale dimensions of the ZnO particles, as smaller crystallites tend to exhibit peak broadening due to lattice strain and size reduction. The average crystallite size can be determined using Scherrer's equation, which establishes a relationship between peak broadening and particle size, further confirming the nanoscale nature of the synthesized ZnO.

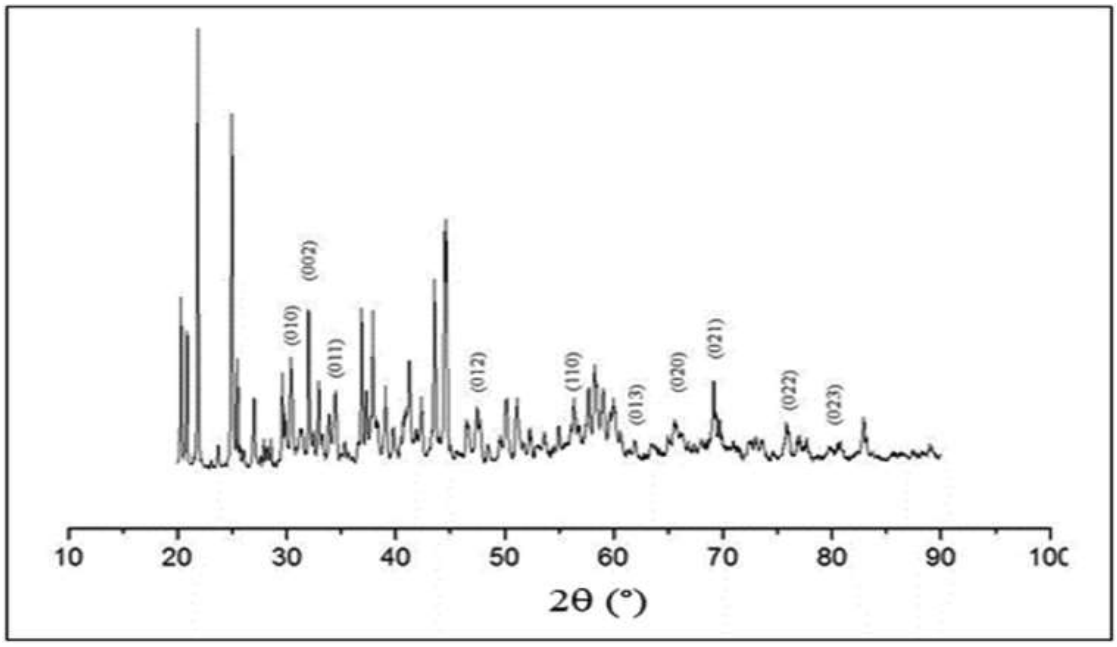

Figure 4.4: XRD spectrum of biosynthesized ZnO

4.5 Antibacterial activity:

The given image appears to represent an antibacterial activity assay, possibly performed using the disc diffusion method to evaluate the inhibitory effect of ZnO nanoparticles synthesized using Aloe vera extract. The image shows a circular zone surrounding a disc, with measured values for circumference (C), area (A), and radius (r) for two distinct regions, labeled as DC0 and DC1. The larger zone (DC0) has a circumference of 4.468 mm, an area of 1.589 mm², and a radius of 0.711 mm, indicating a greater zone of inhibition, while the smaller zone (DC1) has a circumference of 1.823 mm, an area of 0.264 mm², and a radius of 0.290 mm, representing a weaker antibacterial effect. The difference in inhibition zones suggests varying effectiveness of ZnO nanoparticles against bacterial growth, likely influenced by nanoparticle concentration, diffusion rate, and bacterial susceptibility. The presence of a well-defined inhibition zone supports the antimicrobial potential of ZnO nanoparticles, making them promising candidates for biomedical applications such as antimicrobial coatings, wound healing, and pharmaceutical formulations.

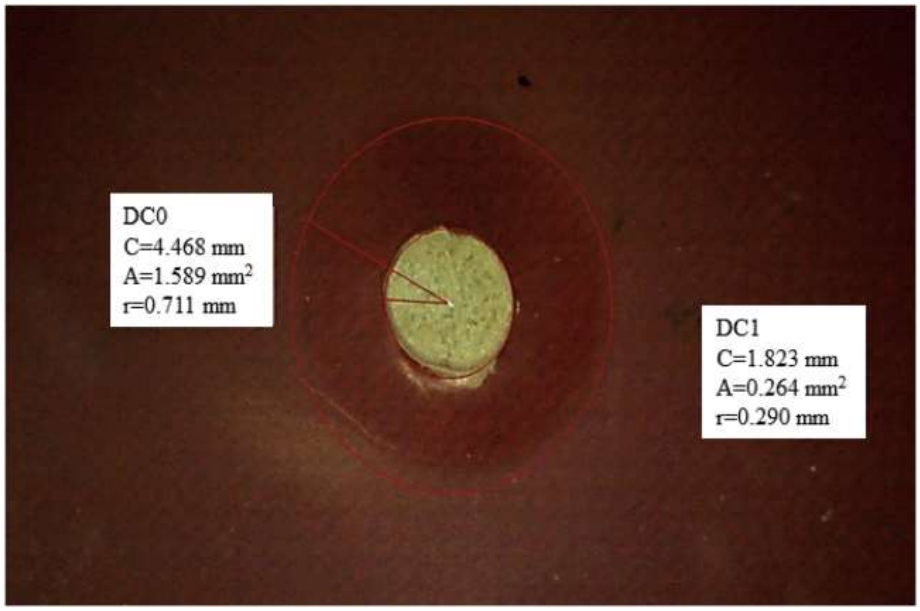

Figure 4.5: Inhibition zone of the synthesized ZnO using E. coli.

4.6 Mechanism of Antibacterial Activity of ZnO-NPs

The antibacterial mechanism of ZnO nanoparticles synthesized using Aloe vera extract primarily involves three key pathways: Reactive Oxygen Species (ROS) Generation, Cell Membrane Disruption, and Metal Ion Release & Protein Inhibition. ZnO nanoparticles induce oxidative stress in bacterial cells by generating ROS, such as hydroxyl radicals (•OH), superoxide anions (O_2^-), and hydrogen peroxide (H_2O_2). These ROS damage essential cellular components, including proteins, lipids, and DNA, leading to bacterial cell death. Additionally, ZnO nanoparticles interact with bacterial cell membranes, causing structural disruptions and increasing membrane permeability, ultimately leading to leakage of intracellular contents and cell lysis. Furthermore, the release of Zn^{2+} ions play a crucial role in bacterial inhibition by interfering with enzymatic activities and protein functions necessary for bacterial survival. These combined mechanisms contribute to the strong antibacterial activity of ZnO nanoparticles, making them highly effective against a wide range of bacterial pathogens, particularly in biomedical and antimicrobial applications.

ZnO causes morphological changes in bacteria, which researchers analyze using SEM or FESEM to quantify the various pathways. Although ZnO-NPs' antibacterial activity has been linked to a variety of problems, the precise toxicity mechanism is still unclear and up for debate since some of the questions within the antibacterial activity spectrum need in-depth answers. The following are some of the unique processes that have been proposed in the literature: direct interaction between ZnO-NPs and cell walls, which destroys the integrity of bacterial cells [2], the release of antimicrobial ions, primarily Zn2+ ions [16], and the production of reactive oxygen species. In addition to the physicochemical characteristics of ZnO-NPs, the species of dissolved zinc may vary depending on the medium components, therefore the toxicity mechanism changes in different media [17]. Abdullah et al. (2024) describe that ZnO and Cu-doped ZnO nanoparticles (NPs) are synthesized using a biosynthesis process utilizing Aloe barbadensis (Aloe vera) leaf extract. The investigation thoroughly explores the structural, morphological, antibacterial, optical, and photocatalytic properties exhibited by ZnO and Cu-ZnO NPs. Both NPs formed a hexagonal wurtzite phase, and the crystallite sizes of ZnO and Cu-ZnO NPs are 21.28 nm and 17.55 nm, respectively. From the morphology, it has been observed that the particle size of Cu-ZnO is 15-20 nm whereas it is 20-50 nm for undoped ZnO. The antibacterial assessment was conducted through the agar well diffusion

method, employing Klebsiella pneumonia, Escherichia coli, and Bacillus zhangzhownsis bacterial mediums. The highest bacterial growth inhibition observed for Cu-ZnO against Klebsiella pneumonia is 21 mm, 35% higher than undoped ZnO. It is found that the band gap for Cu-ZnO (3.22 eV) has reduced compared to the band gap of undoped ZnO (3.37 eV). The photocatalytic capabilities of both nanoparticles were evaluated through the degradation assessment of Methylene Blue (MB) dye and natural blackberry dye. Nevertheless, improved photocatalytic activities were observed in Cu-ZnO nanoparticles (the highest value is 83.4%) compared to the un-doped ZnO [18].

Cuiling Wu et al. (2024) explained that using plant extracts to synthesize zinc oxide nanoparticles is recognized as one of the most environmentally friendly approaches. This study focuses on the green synthesis of zinc oxide nanoparticles (ZnO NPs) using Aloe vera leaf extract (ALE) as reducing and capping agents. Additionally, the immersion phase-conversion method prepared an antimicrobial regenerated-cellulose composite film (RCF) incorporating ZnO NPs (ZnO/RCF). The structure and morphology of ALE-ZnO NPs and ZnO/RCF were characterized, and the antimicrobial properties of ZnO/RCF were assessed using the inhibition zone method. UV and FTIR spectra confirmed the presence of the Zn–O bond stretching mode. HR-TEM and XRD results indicated that ALE-ZnO NPs were formed in a pure crystalline state, exhibiting sphere-like shapes with an average crystallite size of approximately 25 nm. SEM images and EDS mapping of ZnO/RCF demonstrated that ALE-ZnO NPs were well dispersed and stabilized in the cellulose matrix. Furthermore, ZnO/RCF exhibited increased antimicrobial activity against Kosakonia Cowanii and Alternaria alternate strains with higher content of ALE-ZnO NPs. The maximum zone of inhibition was observed for ZnO-10/RCF against Kosakonia Cowanii (22 ± 0.4 mm). Additionally, ZnO/RCF displayed antioxidant activity as a DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenger. This study suggests that ZnO/RCF holds potential applications in industries such as food packaging, pharmaceuticals, agriculture, and cosmetics [19].

Murtaza Hasan et al.(2024) explained that Biological and green synthesis of nanomaterial is a superior choice over chemical and physical methods due to nanoscale attributes implanted in a green chemistry matrix, have sparked a lot of interest for their potential uses in a variety of sectors. This research investigates the growing relevance of nanocomposites manufactured using ecologically friendly, green technologies. The transition to green synthesis correlates with the worldwide drive for environmentally sound procedures, limiting the use of traditional harsh synthetic techniques. Herein, manganese was decorated on ZnO NPs via reducing agent of With Ania-extract and confirmed by UV-spectrophotometry with highest peak at 1:2 ratio precursors, and having lower bandgap energy (3.3 eV). XRD showed the sharp peaks and confirms the formation of nanoparticles, having particle size in range of 11–14 nm. SEM confirmed amorphous tetragonal structure while EDX spectroscopy showed the presence of Zn and Mn in all composition. Green synthesized Mn-decorated ZnO-NPs screened against bacterial strains and exhibited excellent antimicrobial activities against gram-negative and gram-positive bacteria. To check further, applicability of synthesized Mn-decorated Zn nanocomposites, their photocatalytic activity against toxic water pollutants (methylene blue (MB) dye) were also investigated and results showed that 53.8% degradation of MB was done successfully. Furthermore, the installation of green chemistry in synthesizing nanocomposites by using plant extract matrix optimizes antibacterial characteristics, antioxidant and biodegradability, helping to build sustainable green Mn decorated ZnO nanomaterial. This work, explains how biologically friendly Mn-doped ZnO nanocomposites can help reduce the environmental impact of traditional packaging materials. Based on these findings, it was determined that nanocomposites derived from biological resources should be produced on a wide scale to eradicate environmental and water contaminants through degradation [20].

5. Conclusion:

Aloe vera extract offers a sustainable, economical, and environmentally beneficial substitute for traditional chemical and physical synthesis techniques in the green production of ZnO nanoparticles. Aloe vera's bioactive substances function as organic stabilizing and reducing agents, which promotes the development of very stable ZnO nanoparticles with improved antibacterial capabilities. XRD, SEM, EDX, FTIR, and UV-Vis spectroscopy are among the characterization methods that verify the nanoparticles' effective production, high crystallinity, and purity. Reactive oxygen species (ROS) production, cell membrane rupture, and metal ion release are the mechanisms by which Aloe vera-mediated ZnO nanoparticles exhibit antibacterial action against a variety of harmful bacteria. These mechanisms work together to prevent bacterial development. Aloe vera's extensive phytochemical profile gives it clear benefits over other plant-mediated synthesis techniques, including enhanced stability, regulated shape, and more potent antibacterial properties. These results open the door for further study into the medicinal and industrial uses of ZnO nanoparticles produced from aloe vera, highlighting their potential uses in environmental remediation, agriculture, and medicine.

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