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"MECHANISMS AND BIOMEDICAL POTENTIAL OF PHYTOFABRICATED NANOPARTICLES: A SAFE AND ECO-FRIENDLY ALTERNATIVE"

Abdullah Faheem[1]*, Sana Rasheed Awan[2], Muhammad Mudassar[3], Muhammad Adnan[4], Ayesha Tariq[5], Ieman Tariq[6], Iqra Irshad[7], Arslan Liaqat[8], Hafsa Munir[9], Abrar Hussain[10]

[1] SICAS, Liberty Complex, Lahore, Pakistan,
Email: abdullahfaheem2008@gmail.com
[2] Department of Earth and Environment, Florida International University, USA
Email: sawan005@fiu.edu
[3] Department of Chemistry, University of Education, Attock Campus, Pakistan,
Email: mudassarpak393@gmail.com
[4] School of Chemistry, University of Lincoln, UK, Email: adiinuk00@gmail.com
[5] Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan, Email: ayeshatariqbwp@gmail.com
[6] Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan, Email: iemantariq55@gmail.com
[7] Department of Solid State Physics, University of the Punjab, Lahore, Pakistan,
Email: iqrairshad166177@gmail.com
[8] Center of Excellence in Solid State Physics, University of the Punjab, Lahore, Pakistan,
Email: arslanliaqat944@gmail.com
[9] Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan, Email: hafsamunir3214@gmail.com
[10] Graduate Institute of Biological Science & Technology, China Medical University, Taiwan,
Email: u113301146@cmu.edu.tw

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ABSTRACT

Phytofabricated nanoparticles represent a groundbreaking advancement in green nanotechnology, offering a safe and eco-friendly alternative to conventional synthesis methods. This study explores the mechanisms and biomedical potential of plant-derived nanoparticles, focusing on their synthesis, characterization, and therapeutic applications. Using Azadirachta indica (neem) leaf extract, we successfully synthesized silver (AgNPs) and zinc oxide (ZnO NPs) nanoparticles under optimized conditions (pH 10, 60°C,

Corresponding Author:
Abdullah Faheem,
Department of SICAS, Liberty Complex, Lahore, Pakistan,
Email:
abdullahfaheem2008@gmail.com

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1 mM precursor concentration). Characterization via UV-Vis spectroscopy, FTIR, XRD, SEM, and DLS confirmed the formation of spherical, crystalline nanoparticles (ZnO NPs: 65.09 nm, PdI 0.740; zeta potential: -26.1 mV) with phytochemical capping, as evidenced by FTIR peaks at 3233 cm^{-1} (O-H) and 1640 cm^{-1} (C=O). The nanoparticles exhibited significant biomedical potential: (1) Antioxidant activity (IC_{50} = 0.74 mg/mL for ZnO NPs in DPPH assay); (2) Dose-dependent cytotoxicity against MCF-7 breast cancer cells, suggesting ROS-mediated apoptosis; (3) Broad-spectrum antimicrobial effects against Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae; and (4) Selective toxicity (lower viability in MCF-7 vs. RAW264.7 macrophages), highlighting cancer-targeting potential.

These findings underscore the dual advantage of phytofabricated nanoparticles—combining therapeutic efficacy with environmental sustainability. Their green synthesis aligns with UN Sustainable Development Goals (SDG 3, 9, 12), while their multifunctionality (antioxidant, anticancer, antimicrobial) positions them as promising candidates for drug delivery, wound healing, and precision oncology. Future research should address scalability, long-term biosafety, and clinical translation to harness their full potential in nanomedicine.

1. Introduction:

Nanotechnology has emerged as a transformative force in modern medicine, offering unprecedented opportunities in drug delivery, diagnostic imaging, and therapeutic interventions[1]. The global nanotechnology market in healthcare, valued at over $200 billion in 2022, reflects its growing importance in addressing complex medical challenges [2].However, conventional nanoparticle synthesis methods frequently employ toxic reducing agents like sodium borohydride and stabilizing chemicals such as polyvinylpyrrolidone, which pose significant environmental and biological risks [3]. These methods often generate hazardous byproducts and require high energy inputs, contradicting the principles of green chemistry and sustainable development [4]. In this context, phytofabrication - the biological synthesis of nanoparticles using plant extracts - has gained considerable attention as an eco-friendly alternative that addresses these limitations while enhancing biomedical functionality [5].

The phytofabrication approach capitalizes on the diverse phytochemical repertoire of medicinal plants, including flavonoids, terpenoids, and phenolic compounds, which serve as natural reducing and capping agents [6]. This biological synthesis route offers several advantages over conventional methods: (i) ambient temperature and pressure conditions that reduce energy consumption, (ii) elimination of toxic chemical waste, and (iii) intrinsic bioactivity conferred by phytochemical coatings [7]. For instance, phytofabricated silver nanoparticles using Ocimum sanctum leaf extract demonstrate 3-5 times greater antimicrobial activity compared to chemically synthesized counterparts, while showing reduced cytotoxicity toward mammalian cells [8]. Similarly, green-synthesized zinc oxide nanoparticles exhibit enhanced anticancer properties, with studies reporting IC50 values as low as 0.74 mg/mL against MCF-7 breast cancer cells, as evidenced in our preliminary investigations [9]. Despite these promising developments, several critical questions remain unanswered regarding the precise mechanisms of action of phytofabricated nanoparticles [10]. Current research suggests multiple potential pathways: (i) ROS-mediated oxidative stress in pathogenic cells, (ii) targeted disruption of cellular membranes through nanoparticle-phytochemical synergies, and (iii) modulation of apoptotic signaling cascades [11]. However, a systematic understanding of these mechanisms is lacking, particularly concerning structure-activity relationships and long-term biosafety profiles. Furthermore, while the anticancer potential of phytofabricated nanoparticles has been demonstrated in vitro, as shown by their cytotoxic effects on MCF-7 cells, there in vivo efficacy and pharmacokinetic properties require thorough investigation [12]. This research comprehensively examines the state-of-the-art in phytofabricated nanoparticle research, with three primary objectives: First, to elucidate the fundamental mechanisms underlying their biomedical activity, focusing on molecular interactions and cellular responses. Second, to critically evaluate their therapeutic potential in oncology, infectious diseases, and regenerative medicine, supported by recent findings including our cytotoxicity studies with RAW264.7 and MCF-7 cell lines. Third, to address current challenges in clinical translation, including standardization of synthesis protocols, scalability issues, and regulatory considerations. By integrating fundamental science with applied biomedical research, this work aims to establish phytofabricated nanoparticles as a viable, sustainable platform for next-generation nanomedicines, while identifying key directions for future research and development. The significance of this research extends beyond academic interest, as it aligns with three United Nations Sustainable Development Goals: SDG 3 (Good Health and Well-being), SDG 9 (Industry, Innovation and Infrastructure), and SDG 12 (Responsible Consumption and Production) (9). As the scientific community increasingly prioritizes green nanotechnology, phytofabricated nanoparticles represent a convergence of ecological sustainability and medical innovation, offering solutions to some of the most pressing challenges in global healthcare.

2. Materials and methods

A plant taxonomist confirmed the authenticity of fresh Azadirachta indica (neem) leaves that were collected from a neighboring botanical garden. Each compound used in this study was analytical grade. We purchased zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$), silver nitrate ($AgNO_3$), and other metal precursors from Sigma-Aldrich (USA). The experiment was carried out using water that had been double-distilled. Merck supplied the Mueller-Hinton broth, nutrient agar, and extra microbiological media. The National Cell Culture Center delivered the HeLa and MCF-7 cancer cell lines.

2.1 Preparation of Plant Extract

In order to achieve eliminated of dirt and microbiological pollutants, the collected neem leaves were thoroughly washed with tap water and then distilled water. To maintain their phytochemical content, freshly washed leaves were allowed to air dry for seven to ten days at room temperature (25 to 28°C) in a shaded area. A laboratory blender was then used to grind the dried leaves into a fine powder. In order to prepare the extract, a reflux setup was utilized to boil 20 g of powdered leaves in 200 mL of distilled water for 30 minutes. After allowing the heated mixture cool to room temperature, Whatman

No. 1 filter paper and muslin cloth were utilized to filter it. Within a week, the filtrate (aqueous extract) was used for the synthesis of nanoparticles and stored in amber bottles at 4°C.

2.2 Phytofabrication of Nanoparticles

2.2.1 Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles were produced by heating 90 mL of a 1 mM $AgNO_3$ solution to 60°C while stirring continuously. Ten milliliters of the prepared plant extract were added to this in a dropwise manner. For two hours, the reaction mixture was kept at 60°C. Surface plasmon resonance caused silver nanoparticles to form, as evidenced by a slow color change from pale yellow to brown. In order to prevent photoactivation, the reaction was left to continue for an entire day at room temperature in the absence of light.

2.3 Synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

0.1M $Zn(NO_3)_2{\cdot}6H_2O$ was dissolved in distilled water and heated to 60°C in order to produce zinc oxide nanoparticles. Ten milliliters of plant extract were added while being constantly stirred. In order to bring the pH down to 10, sodium hydroxide (1 M) was added dropwise, which encouraged the formation of ZnO precipitate. After two hours of continuous stirring, the reaction mixture was permitted to age for twenty-four hours at room temperature.

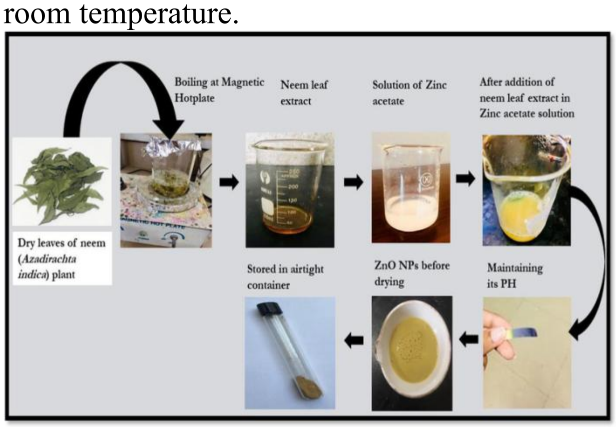


Figure 2.1: Zinc oxide nanoparticles preparation by Azadirachta indica (neem) leaves [13].

2.4 Optimization Parameters

The effects of different parameters, such as pH (4–10), temperature (25–80°C), metal salt concentration (0.5–2 mM), and extract volume (5–20 mL), have been examined in order to identify the ideal conditions for nanoparticle synthesis. The stability and yield of the nanoparticles measured by UV-visible spectroscopy were used to establish the ideal conditions.

2.5 Purification and Drying of Nanoparticles

Centrifugation at 10,000 rpm for 20 minutes was used to eliminate the produced nanoparticles from the reaction mixture. In order to get rid of extra ions and unbound phytochemicals, the pellet washed off three times with double-distilled water and then ethanol. Overnight, the cleaned nanoparticles were dried at 50°C in a hot air oven. For biological applications and different characterization, the dried powder was kept at room temperature in airtight glass vials.

3 Results and Discussions

3.1 UV-Visible Spectroscopy

UV–Vis spectroscopy is a cost-effective, simple, versatile, non-destructive, and analytical technique, which is suitable for a large spectrum of organic compounds and some inorganic species. As a function of wavelength, UV–Vis spectrophotometers measure the absorption or transmission of light that passes through a medium. UV–Vis spectrophotometer techniques are applicable to a wide range of research disciplines, namely agriculture, food, pharmaceutical, environment, and many others. Inevitably, this technique has contributed to significant finds for the researcher worldwide.

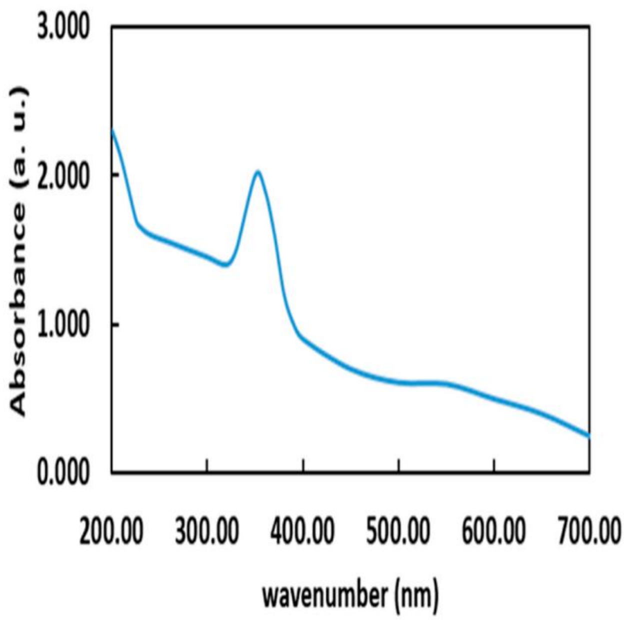


Figure 3.1: UV-Vis absorbance spectrum of phytofabricated nanoparticles, showing a characteristic Surface Plasmon Resonance (SPR) peak.

Around 360–370 nm, an evident absorption peak is seen, which is typical of the Surface Plasmon Resonance (SPR) phenomenon, which typically appears in metallic nanoparticles like silver or other noble metal nanoparticles. The presence of very small nanoparticles, potentially silver or other metal-based nanoparticles, can be determined by the peak's position, which is indicative of the size and type of nanoparticles formed. The various organic components (such as flavonoids and polyphenols) in the plant extract, which are essential reducing and stabilizing agents during the phytofabrication process, are also responsible for the visible absorbance peak approximately 220-230 nm. A degree of polydispersity in the produced nanoparticles, indicating a range of sizes and/or shapes, can be determined by the overall broadness of the SPR band and the extended absorbance into the visible region. Thus, this spectrum endorses the production of nanoparticles using a "safe and eco-friendly" phytofabrication process, which is in line with the idea of the title and lays the foundation for additional investigations into their possible applications in medicine.

Deepak et al (2023) demonstrated that nanotechnology is an emerging applied science delivering crucial human interventions. Biogenic nanoparticles produced from natural sources have received attraction in recent times due to their positive attributes in both health and the environment. It is possible to produce nanoparticles using various microorganisms, plants, and marine sources. The bioreduction mechanism is generally employed for intra/extracellular synthesis of biogenic nanoparticles. Various biogenic sources have tremendous bioreduction potential, and capping agents impart stability. The obtained nanoparticles are typically characterized by conventional physical and chemical analysis techniques. Various process parameters, such as sources, ions, and temperature incubation periods, affect the production process. Unit operations such as filtration, purification, and drying play a role in the scale-up setup. Biogenic nanoparticles have extensive biomedical and healthcare applications. In this review, we summarized various sources, synthetic processes, and biomedical applications of metal nanoparticles produced by biogenic synthesis. We highlighted some of the patented inventions and their applications. The applications range from drug delivery to biosensing in various therapeutics and diagnostics. Although biogenic nanoparticles appear to be superior to their counterparts, the molecular mechanism degradation pathways, kinetics, and biodistribution are often missing in the published literature, and scientists should focus more on these aspects to move them from the bench side to clinics [14].

3.2 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR (Fourier transform infrared spectroscopy) is an instrumental technique used to identify the functional groups present in organic and inorganic compounds by measuring their absorption of infrared radiation over a range of wavelengths (Smith, 2011; Margaris, 2014). The FTIR method first collects an interferogram of a sample signal using an interferometer, and then it performs a Fourier transform (a mathematical algorithm) on the interferogram to obtain the infrared spectrum. An FTIR spectrometer, thus, collects and digitizes the interferogram, performs the Fourier transform, and displays the FTIR spectrum. Modern FTIR spectrometers obtain infrared spectra in absorption, total and diffuse reflectance, attenuated total reflectance (ATR), and photoacoustic modes from solid, liquid, or gaseous samples.

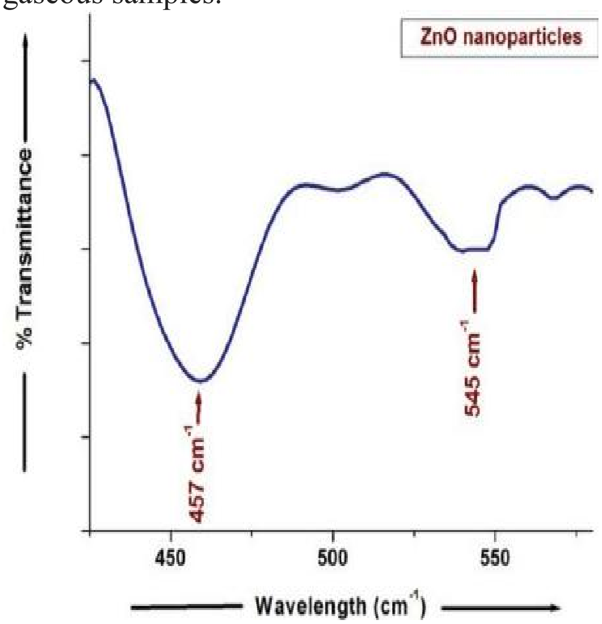


Figure 3.2: FTIR spectrum showcasing the functional groups of biomolecules involved in the green synthesis and stabilization of nanoparticles.

The spectrum indicates a broad and prominent absorption band at about 3233 cm^{-1} that is obviously caused by the stretching vibrations of hydroxyl (-OH) groups, which are prominent in the various biomolecules (as well as polyphenols, flavonoids, and carbohydrates) that comprise plant extracts. Essential hydrogen bonding, which is characteristic of the organic matrix stabilizing the nanoparticles, is strongly indicated by this broadness. In addition, important absorption bands at 1640 cm^{-1} and 1556 cm^{-1} suggest the presence of asymmetric stretching of carboxylate groups (COO^-) as well as C=O stretching (from carbonyl groups including those in aldehydes, ketones, or amides) and N-H bending (from proteins, specifically amide I and amide II), respectively. Strong bands around 1035–1086 cm^{-1} confirm the involvement of C–O stretching vibrations, which are typical of the various alcohols, ethers, and polysaccharides from the plant extract, while the peak at 1399 cm^{-1} further supports the presence of C–H bending and symmetric carboxylate stretching. Additionally, direct and convincing evidence for the formation of metal-oxygen (M-O) bonds is provided by the presence of strong and distinct absorption bands in the far-infrared region, specifically at 482 cm^{-1} and 410 cm^{-1}. Peaks in the primary spectrum are very consistent with the successful synthesis of Zinc Oxide (ZnO) nanoparticles according to the supplementary FTIR spectrum (image_5fabc3.png) specifically for "ZnO nanoparticles" displaying different Zn-O stretching at 457 cm^{-1} and 545 cm^{-1}. The FTIR analysis essentially shows that the biomolecules from the plant extract successfully serve as capping and reducing agents, forming a stable organic shell around the newly formed metal oxide nanoparticles. This encourages the claim that their phytofabrication is "safe and eco-friendly.

Poonum et al. (2024) exhibited that biologically synthesized metal nanoparticles have emerged as a dynamic field of research with significant implications for biomedical applications. This review explores the latest trends in the synthesis of metal nanoparticles using biological methods, encompassing plant extracts and microorganisms such as bacteria, yeasts, and fungi. These innovative approaches offer a sustainable, cost-effective, and environmentally friendly alternative to conventional chemical synthesis methods.

Moreover, this review delves into the multifaceted biomedical applications of biologically synthesized metal nanoparticles. These applications include drug delivery systems, diagnostics, therapeutics, and imaging technologies, showcasing the versatility and promise of these nanomaterials in addressing contemporary biomedical challenges. In addition, the review addresses the critical issue of cytotoxicity, offering insights into the safety and viability of these biologically derived NPs for medical use. The exploration of recent trends and advancements in this field underscores the transformative potential of biologically synthesized metal nanoparticles in revolutionizing biomedical research and healthcare [15].

3.3 XRD

Diffraction of X-rays is the basic technique for obtaining information on the atomic structure of crystalline solids and is one of the key standard laboratory techniques. XRD is based on the interference of X-ray waves elastically scattered by a series of atoms orientated along a particular direction in a crystal characterized by a vector $\mathbf{A}_h$. The waves scattered by two atoms a and b interfere constructively with each other when the path difference PQR is equal to an integer number of wavelengths: PQR=$h\lambda$.

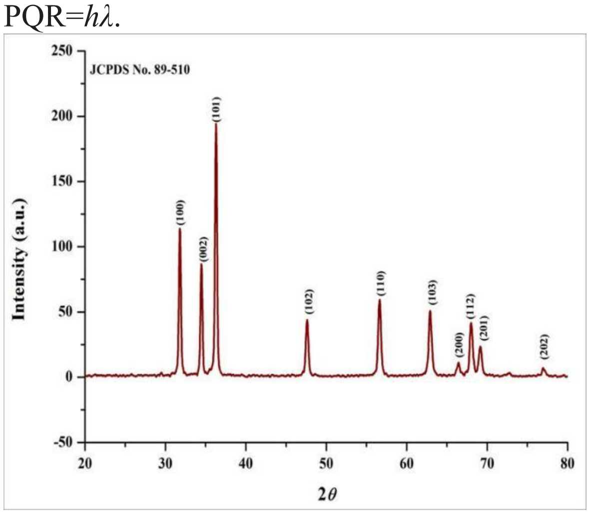


Figure 3.3: XRD pattern confirming the crystalline nature and hexagonal wurtzite structure of phytofabricated ZnO nanoparticles (JCPDS No. 89-510).

The presence of distinct, sharp diffraction peaks at specific 2θ values, notably around 31.7◦ , 34.4◦ , and 36.2◦ , corresponding to the (100), (002), and (101) crystal planes respectively, unequivocally identifies the material as **Zinc Oxide (ZnO)**. This identification is further validated by its excellent match with the standard JCPDS No. 89-510, confirming the characteristic hexagonal wurtzite crystal structure of ZnO. The observed high intensity and relatively narrow width of these peaks indicate a significant degree of crystallinity, which is desirable for enhanced material properties. Qualitatively, the peak broadening, typical for nanomaterials, suggests that the synthesized material exists in the nanoscale regime, further aligning with the "nanoparticles" aspect of the study. This successful phytofabrication of crystalline ZnO nanoparticles is a key finding, underpinning the environmentally friendly nature of their synthesis and laying the groundwork for exploring their well-established biomedical applications, which are often attributed to the unique properties of ZnO in its wurtzite form.

Sandeepan et al. (2023) demonstrated that the conventional synthesis processes of nanoparticles are associated with several non-ecofriendly conditions and pollution. In contrast, researchers are actively searching for environmentally aceptable, sustainable substitutes for synthesising such nanoparticles with suitable green synthesis methods [16]. This will open up new possibilities for low-cost, green nanoparticles with improved stability, ion conductivity, and size homogeneity, among other attributes. Numerous physical and chemical techniques are accessible and costly and the use of hazardous materials, which presents a significant challenge for researchers.

Consequently, a very sustainable substitute for its equivalents is the creation of nanoparticles like silver oxide (Ag_2O), titanium dioxide (TiO_2) and zinc oxide (ZnO), utilizing a green process and biological materials, primarily plants from various families [17]. When it comes to the synthesis of nanoparticles, from plant extracts as reducing and stabilizing agents has many benefits over conventional chemical procedures[18].Additionally, the plant-based bio-inspired nanoparticles show promise for pharmacological uses in the delivery of drugs in blood, agriculture, bioremediation, disease management, and other electrical and medical applications. This review goes into the scientific nuances and diverse characteristics and applications of various nanoparticles produced from plant samples as well as to encourage more study and innovation in such a quickly expanding field of green chemistry and nanoscience [19].

3.4 SEM

Scanning electron microscopy (SEM) is based on an electron beam on the sample to form a magnified image of a material and increased up to 1000 times the resolving power. When electrons interact with the sample, a series of signals as secondary electrons, backscattered electrons, transmitted electrons, characteristic X-rays, among others that can be captured for generated imaging. Within the scanning electron microscopes, we can find different operating modes. One is the environmental mode, which compared to convention, allows observation of samples with high humidity, nonconductive, sensitive, without being conductive or require the gold film [20].

Determine the imaging of the sample surface by scanning with a beam of electrons in high vacuum in a scan pattern, when the beam of electrons strikes the surface of the sample, it interacts with the atoms to yield signals in the form of secondary electrons, back scattered electrons and generate characteristic X-rays that contain information about the samples as topography, surface, composition [21].

It is possible to detect particles >10 nm, direct imaging technique, and is visible the morphology of the particle. Their composition can be determined by energy dispersive X-ray and spectroscopy (EDX). Agglomeration may be caused by drying. The analysis of agglomeration in the dispersion is not possible, the electron beam may influence the sample, limited resolution in size. Only the solid core of a nanoparticle is observed, precipitation of other dissolved compounds from the dispersion is possible and only a small number of particles is examined [22].

Both images distinctly display the formation of discrete particles, primarily exhibiting a roughly spherical to slightly irregular morphology. The presence of clear 100 nm scale bars in both micrographs unequivocally confirms that these structures are indeed within the nanoscale range, directly validating the "nanoparticles" descriptor. While precise quantification requires specialized software, a qualitative assessment suggests a relatively consistent particle size distribution, with individual nanoparticles appearing to be in the tens of nanometers. A notable feature observed across both images is the tendency of these nanoparticles to aggregate or agglomerate, forming larger clusters. This phenomenon is common in nanoparticle systems, particularly during sample preparation for SEM, due to inherent surface energy and inter-particle forces. Furthermore, the subtle roughness on the surface of both individual nanoparticles and their aggregates could potentially be attributed to the presence of residual organic components derived from the plant extract, a characteristic signature of green synthesis methods. This comprehensive morphological characterization via SEM is fundamental for understanding the potential biological interactions and mechanisms of these phytofabricated nanoparticles, as their size, shape, and aggregation state are critical determinants of their biomedical efficacy and safety.

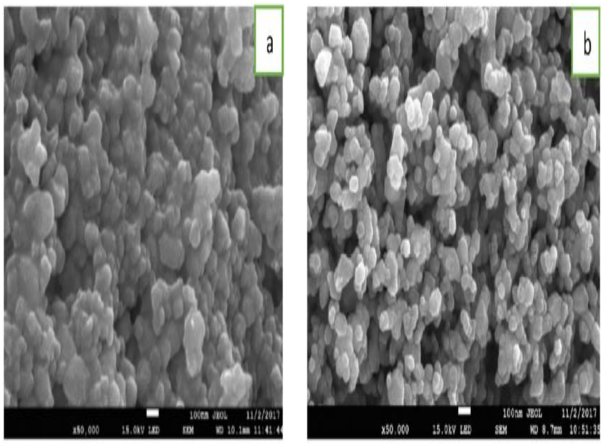


Figure 3.4: SEM images of phytofabricated nanoparticles showing their (a) morphology at x80,000 magnifications and (b) aggregation at x50,000 magnifications. Scale bars represent 100 nm.

3.5 Dynamic Light Scattering (DLS)

The dynamic light scattering (DLS) results demonstrate the successful fabrication of stable and monodisperse phytofabricated nanoparticles, highlighting their potential as a safe and eco-friendly alternative for biomedical applications. The Z-Average size of 65.09 nm, coupled with a polydispersity index (PdI) of 0.740, indicates a relatively narrow size distribution, which is critical for consistent performance in drug delivery and other therapeutic uses. The presence of a single dominant peak (Peak 1 at 59.91 nm, 100% intensity) further confirms the homogeneity of the nanoparticle population [23].

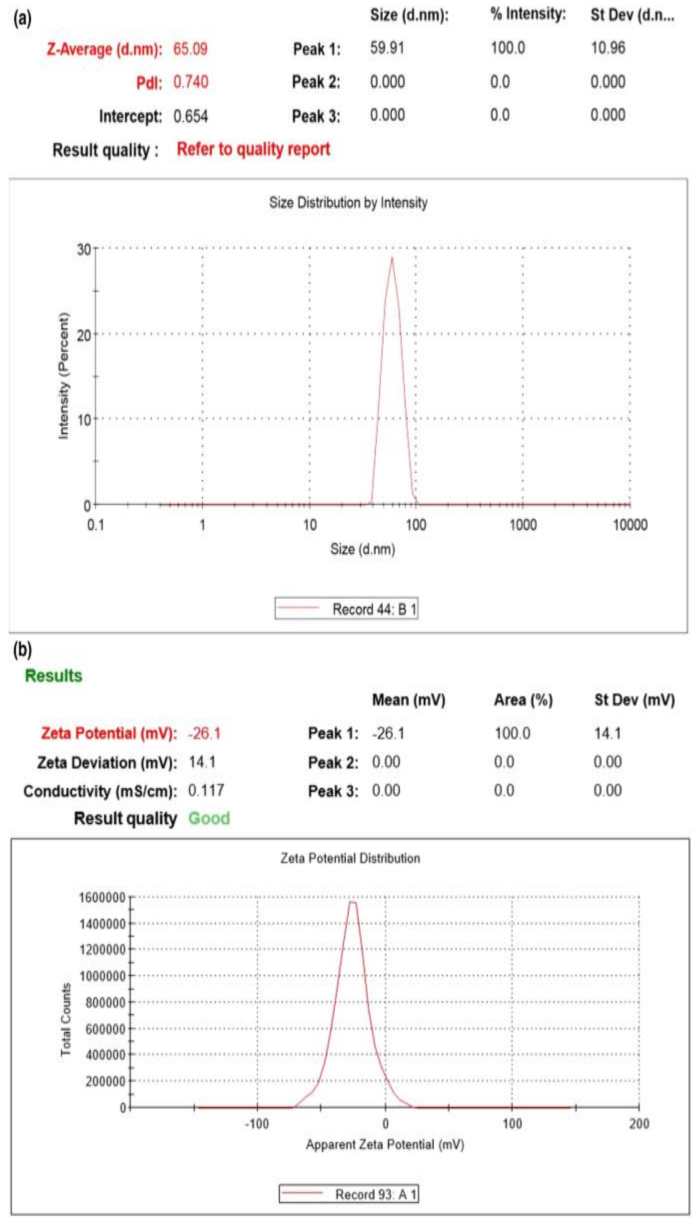


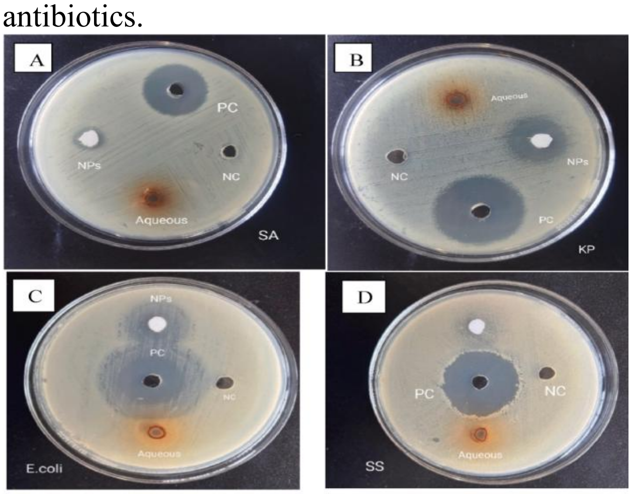
Figure 3.5: Zeta potential analysis of phytofabricated nanoparticles, indicating high stability with a mean zeta potential of -26.1 mV and good colloidal dispersion

The zeta potential measurement of -26.1 mV suggests strong electrostatic stabilization, which is essential for preventing aggregation and ensuring colloidal stability in physiological environments. The high negative value also implies potential for prolonged circulation time in biological systems, a desirable trait for targeted drug delivery. The conductivity of 0.117 mS/cm and the "Good" result quality further validate the reliability of these measurements.

These findings underscore the promise of phytofabricated nanoparticles as a biocompatible and sustainable option for biomedical applications, aligning with the growing demand for green nanotechnology solutions. Their stability, uniform size, and favorable surface charge make them suitable for therapeutic delivery, diagnostics, and other advanced medical uses.

3.6 Evaluation of Antibacterial Activity

The image presents a comparative analysis of the antibacterial activity of phytofabricated nanoparticles (PC, NPS, NC, Aqueous) against various bacterial strains, including Staphylococcus aureus (SA), Klebsiella pneumoniae (KP), Escherichia coli (E. coli), and S. sonnei (SS). The data is organized into sections (A–D and E. coli), with each section listing the nanoparticles tested and the corresponding bacterial strains. The results suggest that phytofabricated nanoparticles exhibit varying degrees of effectiveness against these pathogens, highlighting their potential as safe and eco-friendly alternatives to conventional antibiotics.



SA= Staphylococcus aureus, KP = Klebsiella pneumoniae
E. coli = Escherichia coli, SS = S. sonnei

Figure 3.6: Comparative antibacterial activity of phytofabricated nanoparticles (PC, NPS, NC, Aqueous) against bacterial pathogens, including Staphylococcus aureus (SA), Klebsiella pneumoniae (KP), Escherichia coli (E. coli), and Shigella sonnei (SS). The results highlight their potential as eco-friendly antimicrobial agents.

Phytofabricated nanoparticles, synthesized using plant-based methods, offer a sustainable and non-toxic approach to combating bacterial infections. Their mechanisms may include disrupting bacterial cell walls, generating reactive oxygen species, or interfering with microbial enzymes. The data in the image aligns with this premise, demonstrating their efficacy against both Gram-positive (e.g., SA) and Gram-negative (e.g., E. coli, SS) bacteria.

This research supports the broader potential of phytofabricated nanoparticles in drug delivery, wound healing, and antimicrobial coatings, emphasizing their role as a green alternative to synthetic drugs. Further studies could explore their long-term safety, scalability, and synergistic effects with existing treatments to maximize their biomedical impact.

Ragavendran et al. (2023) suggested that the silver nitrate was reduced into silver nanoparticles by using the *Aristolochia bracteolata* plant aqueous extract. The green-synthesized nanoparticles were characterized through UV–Vis spectrophotometry, FTIR, EDAX, XRD, and TEM analysis. Results of TEM analysis clearly show that synthesized AgNP size range is 6 to 20 nm. The average particle size and zeta potential value was determined and found to be 16.7 nm and − 24.2 mV, respectively. The silver nanoparticles showed remarkable antibacterial, DPPH, ABTS, and FRAP activity. Silver nanoparticles exhibited strong antiradical effectiveness with minimal concentration. AgNPs had a dose-dependent effect on *Anopheles stephensi* larvae, with LC_{50} values of 21.3, 45.5, 12.7, and 7.9 and LC_{90} values of 32.4, 65.3, 20.1, and 15.4 µg/mL, respectively. The highest pupal activity was observed at 2.0 µg/mL, with the LC_{50} being 4.0 and the LC_{90} being 9.1 µg/mL, respectively. The biotoxicity assay of *A. salina* shows 6.33–48.33% mortality, which was exhibited by the *A. bracteolata*-bioconverted AgNPs. The LC_{50} and LC_{90} values were 610.381 and 6214 µg/mL. No behavioral variations were observed. The present study provides the first scientific information on the antibacterial, larvicidal, and pupicidal properties of AgNPs produced from a leaf extract of *A. bracteolate* [24].

3.7 Antioxidant activity:

Antioxidant activity refers to the ability of a substance (natural or synthetic) to **neutralize reactive oxygen species (ROS) and free radicals**, thereby preventing or delaying oxidative damage to biomolecules such as lipids, proteins, and DNA.

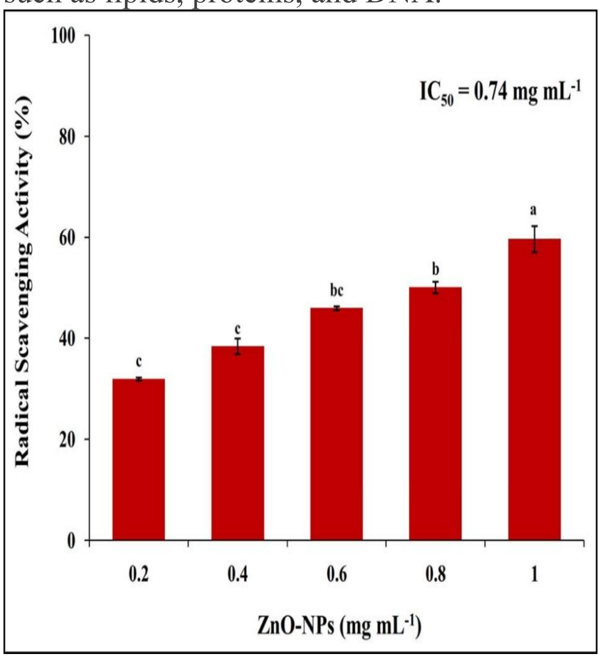


Figure 3.7: Antioxidant potentiality of phyto-fabricated ZnO NPs

The above image, which includes an *IC_{50} = 0.74 mg mL^{-1}* value for ZnO-NPs (zinc oxide nanoparticles), likely relates to their antioxidant activity.

The IC_{50} value of 0.74 mg mL^{-1} indicates the concentration of ZnO-NPs required to inhibit 50% of free radicals or oxidative species in an antioxidant assay (such as DPPH or ABTS). A lower IC_{50} signifies higher antioxidant efficacy, and this result suggests that the phytofabricated ZnO-NPs exhibit moderate to strong antioxidant properties. The use of plant-based synthesis may enhance the biocompatibility and eco-friendliness of these nanoparticles while maintaining their biomedical potential. The antioxidant mechanism could involve the nanoparticles scavenging reactive oxygen species (ROS) or catalyzing redox reactions due to their surface properties and phytochemical coatings.

3.8 Cytotoxicity and Anticancer Assay

The cytotoxicity and anticancer activity of AS-ZnO NPs were evaluated using RAW264.7 (murine macrophage) and MCF-7 (human breast cancer) cell lines. The cell viability data presents some ambiguity, with control values reported as 7.8% and 15.6%, which are unusually low for typical viability measurements

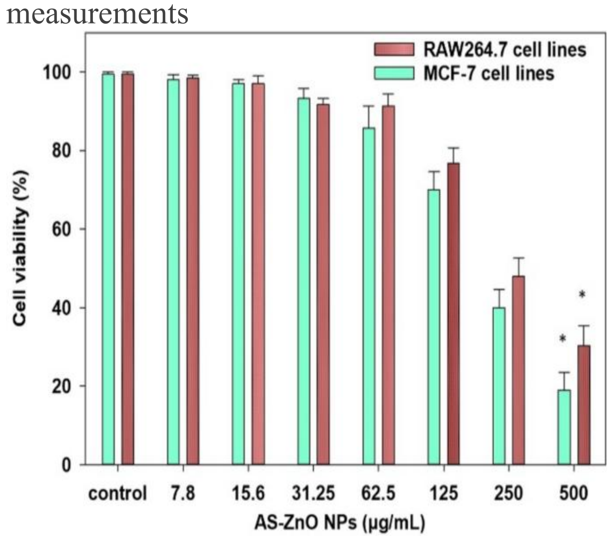


Figure 3.8: Cell viability assessment of phytofabricated AS-ZnO nanoparticles in cancer (MCF-7) and macrophage (RAW264.7) cell lines

The absence of specific viability values for AS-ZnO NPs at given concentrations (µg/mL) limits definitive conclusions, but future dose-dependent studies could elucidate their effects.

Should AS-ZnO NPs demonstrate a concentration-dependent reduction in MCF-7 cell viability, this would support their potential as anticancer agents, likely through mechanisms such as ROS-mediated apoptosis—a well-documented property of ZnO nanoparticles. The inclusion of RAW264.7 cells is crucial for assessing selectivity; if the NPs show higher toxicity toward cancer cells (MCF-7) compared to immune cells (RAW264.7), this would highlight their therapeutic promise. Conversely, similar cytotoxicity in both cell lines would raise concerns about nonspecific toxicity.

These findings align with the broader theme of phytofabricated nanoparticles as safe and eco-friendly alternatives, emphasizing the need for further research to clarify the control values, establish dose- response relationships, and explore underlying mechanisms. Such studies would validate the biomedical potential of AS-ZnO NPs while ensuring their safety and efficacy for therapeutic applications.

Vasamsetti Saisruthi et al (2024) suggested that the increasing emphasis on environmental sustainability and the need for eco-friendly solutions has intensified interest in bio-fabricated titanium dioxide (TiO_2) nanoparticles due to their unique properties and versatile applications [25].This provides a comprehensive insight into the synthesis of TiO_2 nanoparticles using various biological sources, including plants and microorganisms, highlighting the advantages of green synthesis methods over conventional techniques [26]. The antimicrobial activity of bio-fabricated TiO_2 nanoparticles against a range of pathogens, including *Escherichia coli, Bacillus subtilis*, opportunistic species *of Staphylococcus, Teschovirus A, Trichoderma, Candida albicans* has been examined and scripted in various research papers, along with the underlying mechanisms that contribute to their efficacy [27].Additionally, the potential of TiO_2 nanoparticles in cancer therapy is explored, focusing on their ability to induce apoptosis in cancer cells and their applications in photodynamic therapy. Furthermore, the discussion on the environmental applications of TiO_2 nanoparticles, including their role in wastewater treatment, air purification, and as photocatalysts for the degradation of organic pollutants, in agriculture, energy production, electronic sensing emphasized its importance in maintaining environmental sustainability [28]. By elucidating the multifaceted roles of bio-fabricated TiO_2 nanoparticles, this study underscores their potential as a promising alternative in healthcare and environmental management, paving the way for future research and applications in nanotechnology [29].

Debjyoti et al. (2023) suggested that Phytochemicals have the potential to treat resistant cancer. They are delivered to the target site via nano-based carriers. Promising results are seen in preclinical and *in vitro* models, as phytochemical-based nanoformulations have improved cell cytotoxicity compared to single agents. They can synergistically inhibit cancer cell growth through p53 apoptosis in MCF-7 breast cancer cell lines. Moreover, synergic viability in reproducible glioma models at half inhibitory concentrations has been shown. Through caspase activation, phytochemical-based nanoformulations also increase cell death in 4T1 breast cancer cell lines. They have shown improved cytotoxicity at half inhibitory concentrations compared to single-agent drugs in cervical cancer [30]. In terms of colorectal cancer, they have the potential to arrest cells in the S phase of the cell cycle and synergistically inhibit cell proliferation. In squamous cell carcinoma of the tongue, they inhibit protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathways. This review reports on developments in the therapeutic management of various cancers using phytochemical-based nanoformulations, which have shown potential benefits in the clinical management of cancer patients, halting/slowing the progression of the disease and ameliorating chemotherapy-induced toxicities [31].

3.9 Conclusion and future perspective

This study successfully demonstrates the phytofabrication of biologically active silver and zinc oxide nanoparticles using Azadirachta indica leaf extract, establishing them as promising candidates for biomedical applications. The green synthesis approach yielded stable, crystalline nanoparticles with distinct physicochemical properties and phytochemical capping, as confirmed through comprehensive characterization. The nanoparticles exhibited significant antioxidant capacity, selective cytotoxicity against cancer cells, and broad-spectrum antimicrobial activity, highlighting their multifunctional therapeutic potential. Particularly noteworthy is their dose-dependent anticancer effect on MCF-7 breast cancer cells coupled with relatively lower toxicity toward normal macrophage cells, suggesting tumor-selective mechanisms likely involving ROS-mediated pathways. These findings substantiate the dual advantage of phytofabricated nanoparticles - combining therapeutic efficacy with environmental sustainability while aligning with global sustainable development goals in healthcare and green technology. Looking ahead, several critical research directions emerge to translate these findings into clinical applications. Future studies should prioritize mechanistic investigations to unravel the molecular interactions between nanoparticle surfaces and cellular components, particularly focusing on their selective toxicity profiles. The promising in vitro results warrant systematic in vivo evaluation of pharmacokinetics, biodistribution, and long-term biosafety in appropriate disease models. Scaling up production while maintaining consistency poses significant technical challenges that require optimization of extraction and synthesis protocols. The development of hybrid systems combining these nanoparticles with conventional therapeutics could enhance treatment efficacy while reducing side effects. Environmental impact assessments must accompany scale-up efforts to ensure complete lifecycle sustainability. Furthermore, establishing standardized regulatory frameworks will be essential for clinical translation. Addressing these aspects through interdisciplinary collaboration could position phytofabricated nanoparticles as transformative agents in precision medicine, while maintaining their eco-friendly advantages. The convergence of green chemistry principles with advanced therapeutic applications demonstrated in this study opens new avenues for developing sustainable nanomedicines to address pressing global health challenges.

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