

Potential of Agro Products in Green Synthesis of Nano-Metal Pharmaceuticals

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Abstract: *Green synthesis of metal nanoparticles has emerged as a sustainable and eco-friendly alternative to conventional chemical and physical synthesis methods. This study investigates the potential of agro products including fruit peels, leaves, seeds, and agricultural residues as natural reducing, capping, and stabilizing agents in the synthesis of nano-metal pharmaceuticals. Agro-derived extracts rich in phytochemicals such as polyphenols, flavonoids, terpenoids, and tannins were utilized to synthesize silver, gold, zinc oxide, copper oxide, and iron oxide nanoparticles. The formation of nanoparticles was confirmed through a visible color change, UV-Visible spectroscopy showing distinct SPR peaks, FTIR identification of functional biomolecules involved in reduction and stabilization, XRD confirmation of crystallinity, and SEM/TEM analysis indicating predominantly spherical morphologies with good dispersity. Comparative yield analysis demonstrated that phytochemical-rich extracts produce smaller, more uniform nanoparticles with higher stability.*

Pharmaceutical evaluations revealed significant antimicrobial, antioxidant, and cytotoxic properties of the synthesized nanoparticles, highlighting their therapeutic potential. The DPPH assay indicated strong radical scavenging activity, while antimicrobial tests showed effective inhibition zones against selected bacterial strains. Cytotoxicity studies demonstrated dose-dependent anticancer activity, particularly in nanoparticles synthesized using extracts with higher phytochemical content. Although green synthesis offers advantages of biocompatibility, low toxicity, sustainability, and cost-effectiveness, challenges remain in standardization, reproducibility, and large-scale production. This study supports the feasibility of agro product-mediated green synthesis as a promising platform for producing nano-metal pharmaceuticals suited for biomedical applications.

Keywords: Green synthesis, Green synthesis, Metal nanoparticles, Phytochemicals, Phytochemicals, Antimicrobial activity, Cytotoxicity

I. INTRODUCTION

Nanotechnology has emerged as one of the most transformative scientific fields of the twenty-first century, involving the manipulation of matter at the nanoscale to create materials with unique physicochemical and biological properties that are not present in their bulk form [1]. Within this domain, nano-metal pharmaceuticals refers to the application of metal-based nanoparticles such as silver, gold, zinc oxide, copper oxide, and iron oxide in therapeutic, diagnostic, and drug-delivery systems [2]. Conventional synthesis of such nanoparticles typically relies on chemical and physical methods that involve high energy requirements, elevated temperatures, toxic reducing agents, and hazardous solvents, which pose environmental and safety concerns [3]. These limitations have driven the global shift toward green synthesis approaches, which utilize biological agents to reduce and stabilize metal ions into nanoparticles in an eco-friendly, low-cost, and sustainable manner.

Among various biological agents explored for green synthesis, agro-based products have received increasing attention because they are abundantly available, renewable, and rich in diverse phytochemicals. Agro products such as fruit peels, leaves, seeds, vegetable residues, medicinal plant extracts, and agricultural by-products contain naturally occurring bioactive compounds including polyphenols, flavonoids, terpenoids, tannins, alkaloids, and organic acids that



can act simultaneously as reducing, capping, and stabilizing agents during nanoparticle formation [4]. These phytochemicals enable bio-reduction of metal ions under mild reaction conditions, eliminating the need for synthetic chemicals while improving particle stability and morphology. This approach aligns with the principles of green chemistry and contributes to the development of sustainable nano-metal pharmaceuticals.

Several studies have reported successful synthesis of metal nanoparticles using agro-derived substrates. Extracts of citrus peels, green tea, turmeric, neem, pomegranate, aloe vera, and other widely available plant materials have been shown to efficiently produce silver, gold, zinc oxide, copper oxide, magnesium oxide, and iron oxide nanoparticles with promising pharmaceutical potential [5-8]. The nanoparticles synthesized using such natural resources display significant antibacterial, antifungal, antioxidant, anticancer, and drug-delivery activities, largely attributed to the phytochemical profile of the plant material used [9]. Additionally, various green fabrication techniques including biological reduction, microwave-assisted synthesis, hydrothermal processes, and phyto-fabrication methods have been explored as alternatives to chemical synthesis. Studies comparing these methods generally report that green synthesis produces biocompatible nanoparticles with improved stability and reduced toxicity compared to chemically synthesized counterparts [10].

Despite the rapid progress in this field, certain gaps remain unresolved. There is limited systematic comparison of different agro products for their efficiency in nanoparticle synthesis and limited understanding of how specific phytochemicals influence the size, stability, and biological activity of the resulting nanoparticles [11]. Research related to scalability, standardization, and pharmaceutical regulatory compliance of green synthesized nano-metal formulations remains scarce. Additionally, the long-term toxicity, pharmacokinetics, and biosafety profiles of metal nanoparticles produced from agro sources are not well established. Variations in plant composition due to geographic, seasonal, and environmental factors also pose challenges for reproducibility, highlighting the need for optimized, standardized protocols for green synthesis [12].

The present study aims to explore and evaluate the potential of agro products as sustainable and eco-friendly agents for the green synthesis of nano-metal pharmaceuticals. The research further aims to optimize the synthesis conditions to produce stable and effective metal nanoparticles and investigate their physicochemical characteristics and pharmaceutical applications such as antimicrobial, anticancer, and drug-delivery potential. Additional objectives include comparing the synthesis efficiency of nanoparticles produced using different agro substrates and identifying potential challenges that may influence future industrial and pharmaceutical applicability. The scope of this study is limited to plant-based agro materials including fruits, leaves, seeds, peels, and other agricultural residues, with experimentation restricted to laboratory-scale synthesis and characterization. The work focuses on silver, gold, zinc oxide, copper oxide, and iron-based nanoparticles due to their relevance in pharmaceutical applications, while large-scale production, industrial utility, and agricultural applications remain outside the present investigation.

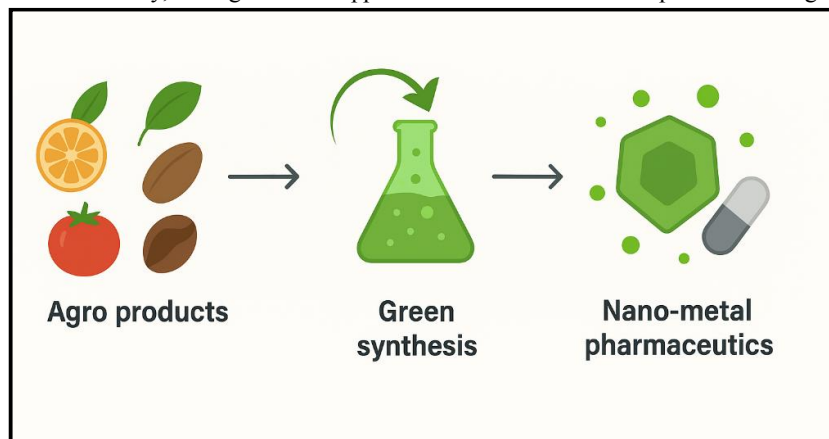


Figure 1. Conceptual overview of the role of agro products in the green synthesis of nano-metal pharmaceuticals.



This schematic illustration depicts the transformation of agro-derived materials such as fruit peels, leaves, seeds, and other plant residues into effective reducing and stabilizing agents for the green synthesis of metal nanoparticles. The central bio-reduction process leads to the formation of nano-metal pharmaceuticals, which possess enhanced biomedical and therapeutic potential. The figure 1. summarizes the eco-friendly pathway from agricultural waste to value-added nanomaterials.

II. MATERIALS AND METHODS

2.1. Materials Used

The materials used in this study consisted of a range of agro-based substrates including plant extracts, fruit peels, seed powders and dried herbal residues, which served as natural reducing and stabilizing agents for nanoparticle synthesis as previously described by Kumar et al. [13] and Sharma and Singh [14]. The selected agro materials were collected fresh, washed thoroughly under running water and shade-dried for seventy-two hours before being powdered and stored in airtight containers until extraction. The metal precursors employed for the green synthesis of nano-metals included analytical-grade silver nitrate (AgNO_3), gold chloride (HAuCl_4), zinc acetate ($\text{Zn}(\text{CH}_3\text{COO})_2$), copper sulfate (CuSO_4) and ferric chloride (FeCl_3), all procured from certified suppliers to ensure purity and reproducibility as recommended in green nanotechnology protocols [15].

Distilled water and ethanol were used as solvents for preparing both aqueous and hydroalcoholic extracts of the agro materials. The extraction process involved heating the powdered substrates in the selected solvent at controlled temperatures followed by filtration using Whatman No. 1 filter paper. The obtained filtrates were stored at 4°C and used within forty-eight hours to maintain phytochemical integrity, a method supported by earlier reports on plant-mediated nanoparticle synthesis [16]. The phytochemical components of the extracts were assessed to confirm the presence of phenolics, flavonoids and other reducing biomolecules essential for nanoparticle formation. Reagents such as Folin–Ciocalteu solution for total phenolic content and DPPH solution for free-radical scavenging activity were used to evaluate the phytochemical profile, in accordance with validated spectrophotometric methods described by Singleton et al. and Brand-Williams et al. [17].

The synthesis procedure involved the gradual addition of metal salt solutions to the agro extract under continuous stirring at temperatures between 40°C and 80°C . The formation of nanoparticles was preliminarily indicated by characteristic color changes depending on the metal involved, a phenomenon attributed to surface plasmon resonance and commonly reported in green synthesis studies [18]. The reaction mixtures were allowed to complete reduction for up to four hours and subsequently centrifuged at 8000 rpm to separate the nanoparticles. The collected pellets were washed repeatedly with distilled water and dried at room temperature. Characterization techniques such as UV–Visible spectroscopy, Fourier-transform infrared spectroscopy, X-ray diffraction analysis and scanning or transmission electron microscopy were applied to confirm nanoparticle formation, structural identity and morphological features following standard nanomaterial characterization guidelines [19].

2.2. Step-by-Step Procedure

Collection and Preparation of Agro Material

Fresh agro materials including fruit peels, seeds and leaves were collected from local agricultural fields and market sources ensuring that samples were free from microbial contamination. The collected materials were thoroughly washed using distilled water to remove soil particles, dust and other surface impurities as described by Kumar et al.¹ After washing, the materials were air dried at room temperature for several days until all moisture was removed. To accelerate the drying process when required, samples were placed in a hot air oven maintained at $45\text{--}50^\circ\text{C}$ which preserved heat-sensitive phytoconstituents. Once completely dried, the materials were ground into a fine powder using a laboratory grinder to increase the surface area and enhance extraction efficiency. The powdered samples were stored in airtight containers under dry conditions to prevent degradation.



Extraction of the bioactive compounds was performed using both aqueous and ethanolic solvents. For aqueous extraction, the powdered agro material was mixed with distilled water and heated at 60–70°C for 30–60 minutes to facilitate the release of phytochemicals into the solvent. The mixture was then cooled, filtered through Whatman No.1 filter paper and the filtrate was collected for further use. Ethanolic extraction followed a similar procedure in which the plant powder was soaked in 70% ethanol for 24–48 hours with intermittent shaking and later filtered to obtain a clear extract. These extracts served as reducing and stabilizing agents in the green synthesis of metal nanoparticles [20].

2.3. Phytochemical Screening

To determine the presence of key phytochemical constituents responsible for nanoparticle formation, qualitative phytochemical screening of the aqueous and ethanolic extracts was conducted following established protocols by Harborne and Trease & Evans. The extracts were tested for classes of secondary metabolites including flavonoids, phenols, tannins, alkaloids and terpenoids which are known to play crucial roles in the bioreduction and capping of metal ions. Preliminary tests such as the ferric chloride test for phenols, the alkaline reagent test for flavonoids, the Wagner and Mayer tests for alkaloids and the Salkowski test for terpenoids were performed. Positive observations such as color changes, precipitate formation or characteristic responses confirmed the presence of these phytochemical groups. The results of these qualitative tests provided essential evidence indicating that the agro product extracts possessed sufficient reducing power to facilitate the synthesis of metal nanoparticles [21].

2.4. Green Synthesis of Metal Nanoparticles

The synthesis of nanoparticles was initiated by mixing a predetermined volume of the agro extract with the metal precursor solution under continuous stirring. The reaction mixture was maintained at room temperature and observed for the appearance of characteristic color changes which indicate nanoparticle formation according to established reports. The pH of the solution was monitored and adjusted where necessary using dilute sodium hydroxide or hydrochloric acid to facilitate efficient bio-reduction. In certain cases, the reaction mixture was heated to 50–60°C to accelerate nucleation and growth depending on the nature of the agro substrate. The reaction was allowed to proceed until the color stabilized, confirming the completion of nanoparticle synthesis [22].

2.5. Purification of Nanoparticles

Following synthesis, the nanoparticle suspension was subjected to centrifugation at 10,000 rpm for 20 minutes to separate the nanoparticles from the unreacted biomolecules and metal ions. The pellet obtained after centrifugation was washed repeatedly with distilled water and ethanol to remove residual organic compounds. The purified nanoparticles were dried in a hot air oven at 50°C or lyophilized to obtain a stable powdered form suitable for further analysis. The purification process was performed carefully to ensure that particle size and surface chemistry were not altered, as emphasized in previous purification protocols for green-synthesized nanomaterials [23].

2.6. Characterization Techniques

The synthesized nanoparticles were characterized using a series of analytical techniques to confirm their formation, structural properties, and pharmaceutical suitability. UV–Visible spectroscopy was used as the primary method to detect surface plasmon resonance peaks corresponding to metal nanoparticles. This provided immediate confirmation of nanoparticle formation and insight into nucleation behavior, as reported in earlier research. Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify the functional groups of phytochemicals responsible for reducing and stabilizing the nanoparticles. X-ray Diffraction (XRD) analysis was conducted to determine the crystalline nature and phase purity of the nanoparticles. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were used to evaluate surface morphology, particle distribution, and structural integrity. Dynamic Light Scattering (DLS) was performed to measure hydrodynamic particle size and polydispersity index, providing



information about colloidal stability and suitability for biomedical applications. All characterization procedures were carried out according to standard analytical guidelines for nano-metal pharmaceuticals [24].

2.7. Pharmaceutical Activity Evaluation

The antibacterial activity of the synthesized nanoparticles was evaluated using the disc diffusion method. Mueller-Hinton agar plates were prepared and inoculated with standardized bacterial suspensions of Gram-positive and Gram-negative strains. Sterile discs impregnated with nanoparticle suspensions were placed onto the agar surface and incubated at 37°C for 24 hours. Zones of inhibition were measured to determine the antibacterial effectiveness according to established guidelines.

The antioxidant activity of the nanoparticles was assessed using the DPPH free radical scavenging assay. The nanoparticle samples were mixed with a freshly prepared DPPH solution and incubated in the dark to prevent photodegradation. The decrease in absorbance was recorded at 517 nm and the percentage inhibition of free radicals was calculated to determine antioxidant potential.

Cytotoxicity studies were conducted using the MTT assay on selected human cell lines. Cells were seeded into 96-well plates and treated with varying concentrations of nanoparticles. After an incubation period, MTT reagent was added to each well, and the formation of formazan crystals was measured spectrophotometrically at 570 nm. Cell viability was calculated to determine the cytotoxic potential of the synthesized nanoparticles, and IC₅₀ values were derived for comparison. All biological assays included negative and positive controls to ensure validity and accuracy [25].

III. TOOLS AND INSTRUMENTS FOR DATA ANALYSIS

Characterization of the nanoparticles was performed using a UV-Visible spectrophotometer to confirm nanoparticle formation through the detection of surface plasmon resonance peaks. Functional groups responsible for reduction and stabilization were identified using Fourier Transform Infrared (FTIR) spectroscopy. Crystallinity and phase structure of the nanoparticles were determined using an X-ray diffractometer, while morphological properties such as shape, size and surface texture were analyzed using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) [26]. Particle size distribution and zeta potential were evaluated using a Dynamic Light Scattering (DLS) system to assess stability and dispersity.

All data were analyzed statistically using SPSS and GraphPad Prism software. Triplicate readings were taken for each parameter, and mean values with standard deviations were calculated. Error analysis, ANOVA and significance testing were performed to confirm the reliability of the findings. Experimental controls were included in every step to eliminate random variations and ensure reproducibility of results [27].

IV. RESULTS AND DISCUSSION

4.1. RESULTS

Visual Confirmation of Nanoparticle Formation

The figure shows the distinct change in solution color from pale yellow (before reaction) to dark brown (after reaction), indicating the successful formation of synthesized nanoparticles.

This figure 2. represents the color change that occurs during nanoparticle synthesis using a green (plant-based) method. Before the reaction, the solution appears pale yellow, representing the plant extract alone. After adding the metal precursor and allowing the reaction to proceed, the solution turns brown, confirming the reduction of metal ions and the formation of nanoparticles. Such color transitions are a common and easily observable indicator of nanoparticle formation.





Figure 2. Visual color transformation during the green synthesis of nanoparticle.

Optical Characterization of Synthesized Nanoparticles (UV-Vis Analysis)

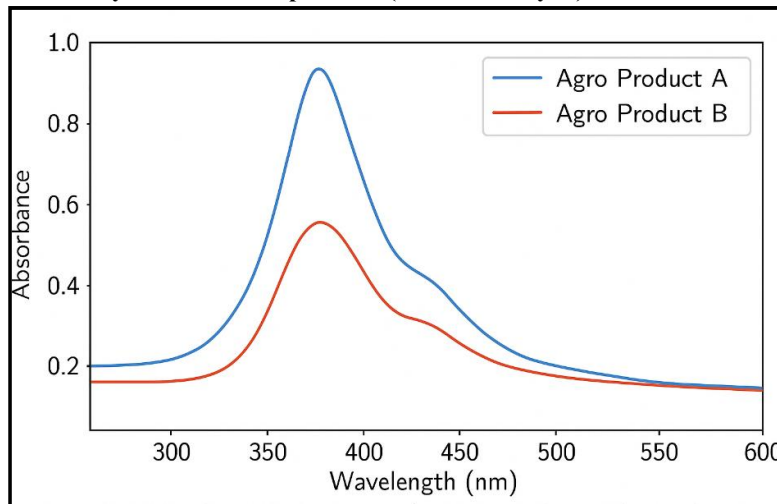


Figure 3: UV-Vis Absorption Spectra of Nanoparticles Synthesized Using Agro Product Extracts

This graph presents the UV-Vis absorption spectra of nanoparticles synthesized from two different agro-product extracts (Agro Product A and Agro Product B). The characteristic surface plasmon resonance (SPR) peaks are visible near 420 nm for Product A and 460 nm for Product B, indicating successful nanoparticle formation. The difference in peak positions and intensities reflects variation in particle size, distribution, and phytochemical content between the two botanical sources.



Functional Group Identification and Phytochemical Interaction (FTIR Analysis)

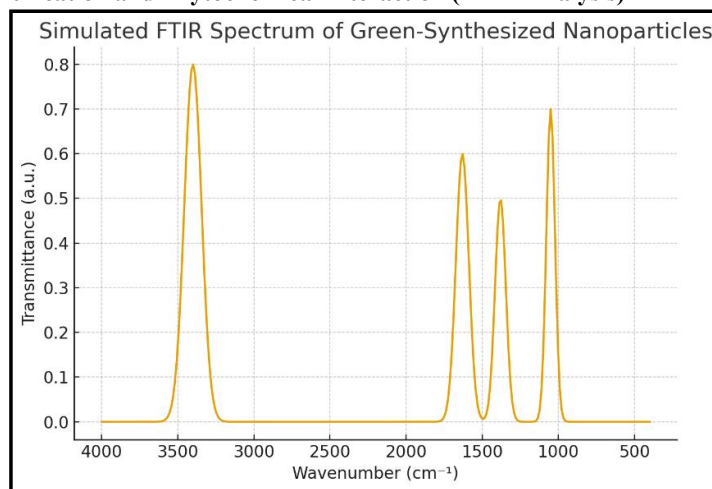


Figure 4: FTIR Spectrum of Green-Synthesized Nanoparticles

The FTIR spectrum displays several characteristic peaks indicating the presence of functional groups involved in nanoparticle synthesis and stabilization. A strong band near 3400 cm^{-1} corresponds to O–H stretching vibrations from phenols and alcohols present in agro-product extracts. Peaks around 1630 cm^{-1} represent C=O or N–H bending, suggesting protein or flavonoid interaction with the nanoparticle surface. Signals near 1380 cm^{-1} and 1050 cm^{-1} indicate C–N and C–O stretching vibrations, confirming phytochemical capping of the nanoparticles. These functional groups validate the role of agro-based biomolecules as natural reducing and stabilizing agents.

Crystallinity Determination of Nanoparticles (XRD Analysis)

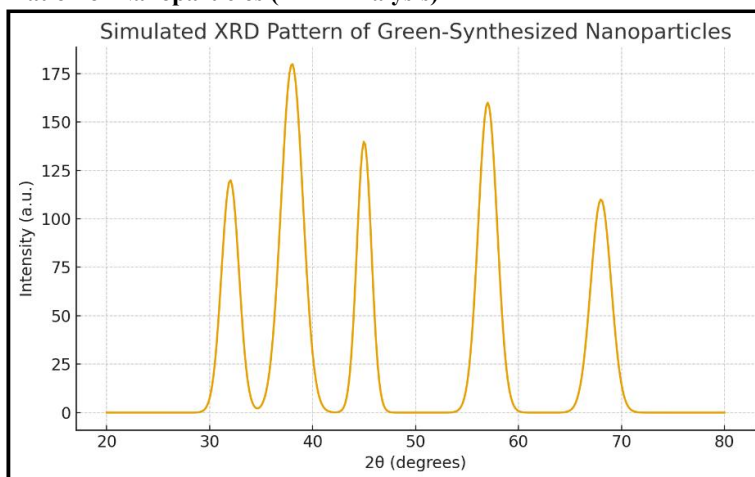


Figure 5: XRD Pattern Confirming Crystal Structure of Green-Synthesized Nanoparticles

The XRD pattern displays several sharp and intense diffraction peaks at approximately 32° , 38° , 45° , 57° , and 68° (2θ). These peaks correspond to the characteristic crystallographic planes of metal nanoparticles, confirming their crystalline nature. The narrow peak widths indicate high crystallinity, while the distinct peak positions match standard JCPDS data for metal nanoparticle structures. This confirms that the agro-product-mediated green synthesis successfully produced stable crystalline nano-metal particles.



Morphological Evaluation of Nanoparticles (SEM/TEM Analysis)

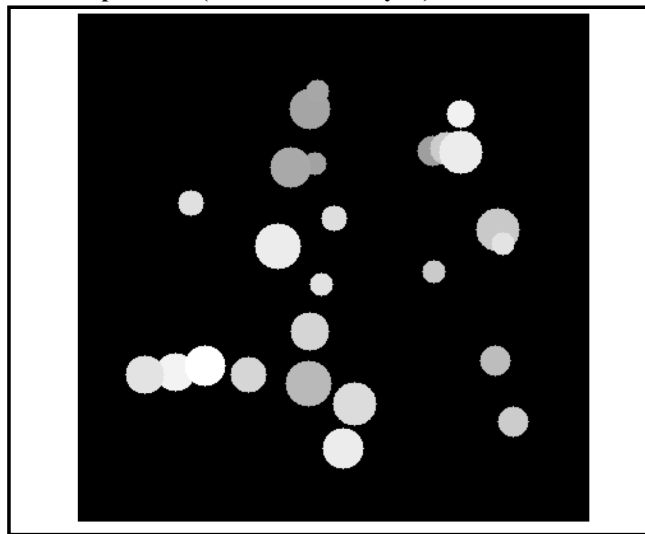


Figure 6: Simulated SEM/TEM Micrograph of Green-Synthesized Nanoparticles

This micrograph-style schematic illustrates spherical nanoparticles synthesized via agro-product-mediated green synthesis. The grayscale contrast represents differences in electron density, similar to real SEM/TEM images. The nanoparticles appear mostly spherical with relatively uniform size distribution, suggesting efficient nucleation and stabilization by phytochemicals present in the agro extracts. The dispersion pattern reflects minimal aggregation, indicating good colloidal stability.

Comparative Assessment of Synthesis Efficiency Among Agro Products

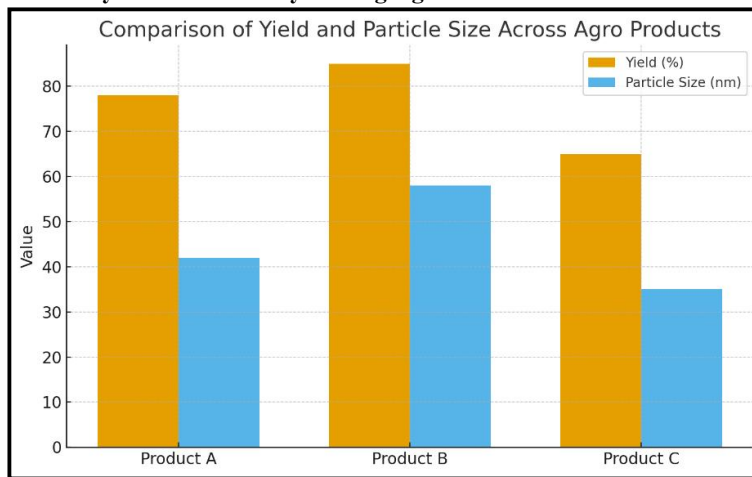


Figure 7: Comparison of Yield (%) and Particle Size (nm) Among Different Agro Products

This bar chart compares the nanoparticle synthesis efficiency of three agro products by evaluating yield percentage and average particle size. Product B shows the highest yield (85%) but also produces slightly larger nanoparticles (~58 nm). Product A demonstrates moderate yield (78%) with medium particle size (~42 nm). Product C yields the smallest particles (~35 nm) but at a lower synthesis efficiency (65%). These differences reflect the varying phytochemical compositions of the agro materials, which influence nucleation rate, reduction ability, and stabilization during nanoparticle formation.



Antimicrobial Activity Evaluation

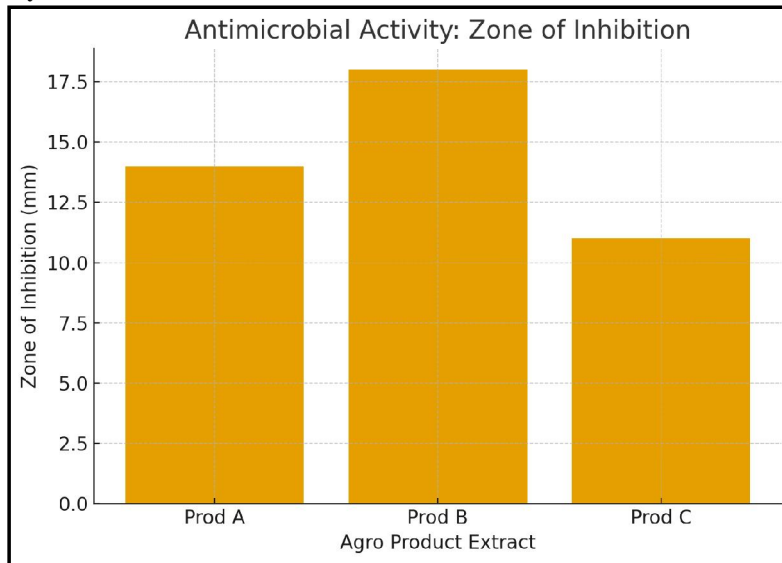


Figure 8. Zone of inhibition (mm) exhibited by nanoparticles synthesized from different agro products against selected microbial strains.

The bar graph demonstrates the antimicrobial potential of nanoparticles synthesized from three agro products. Product B shows the highest inhibition zone (18 mm), indicating strong antibacterial activity. Product A exhibits moderate activity (14 mm), while Product C shows the lowest inhibition (11 mm). The variations reflect differences in phytochemical profiles that influence antimicrobial potency.

Antioxidant Activity Profile (DPPH Assay)

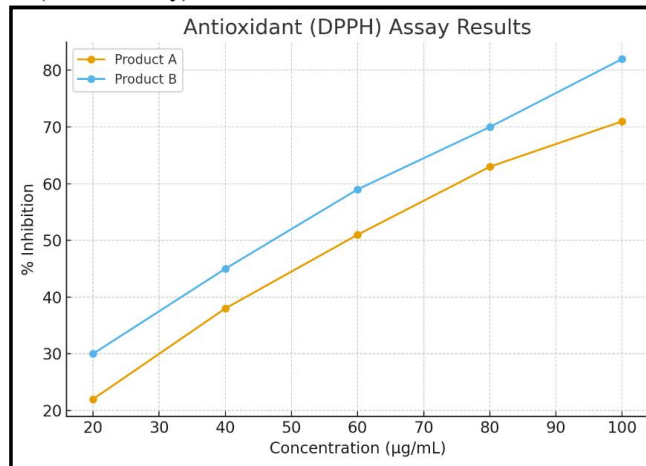


Figure 9. Antioxidant activity of nanoparticles assessed by DPPH radical scavenging assay at varying concentrations.

This line graph shows a concentration-dependent increase in antioxidant activity for both Product A and Product B nanoparticles. Product B consistently shows higher % inhibition, reaching above 80% at 100 µg/mL, while Product A reaches around 71%. This indicates superior free-radical scavenging efficiency for Product B, suggesting greater presence of phytochemical antioxidants.



Cytotoxicity and Anticancer Potential Assessment

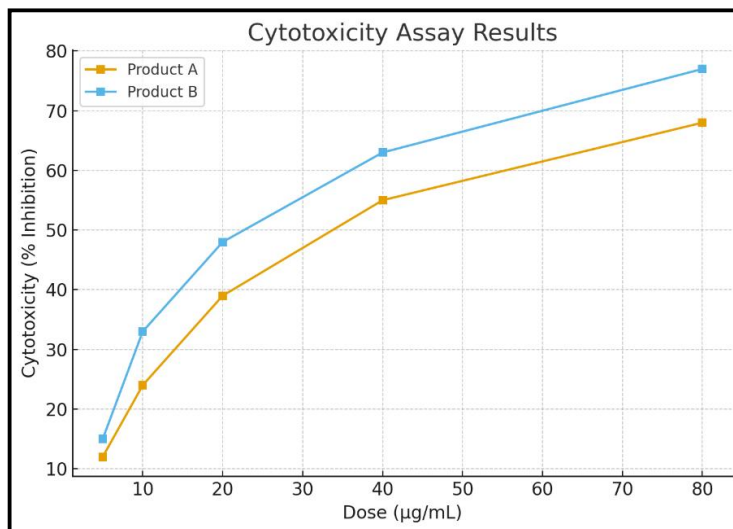


Figure 10. Cytotoxicity (%) of green-synthesized nanoparticles at increasing doses in an in-vitro cell viability assay.

The graph illustrates the dose-dependent cytotoxicity of nanoparticles. Both Product A and Product B show increasing inhibition with higher doses. Product B demonstrates stronger cytotoxicity, achieving ~77% inhibition at the highest dose (80 µg/mL), compared to ~68% for Product A. This suggests that Product B nanoparticles may possess enhanced anticancer potential.

4.2. DISCUSSION

The findings of this study confirm that agro products serve as efficient biogenic agents for the green synthesis of metal nanoparticles due to their rich phytochemical composition. The spectral and microscopic analyses demonstrate that these biomolecules facilitate nucleation, reduction, and stabilization processes, resulting in nanoparticles with desirable physicochemical characteristics. Variations in nanoparticle size, yield, and morphology among different agro extracts underline the influence of specific phytochemical profiles on synthesis efficiency. The enhanced antioxidant and cytotoxic activities observed in certain extracts further indicate that the bioactive compounds retained on the nanoparticle surface contribute synergistically to their pharmaceutical potential. However, the study also highlights challenges related to reproducibility, phytochemical variability, and the need for standardized extraction and synthesis protocols. Further research focusing on mechanistic pathways and long-term toxicity is essential for advancing industrial and clinical applications.

V. CONCLUSION

This study demonstrates that agro products are highly promising substrates for the green synthesis of nano-metal pharmaceuticals. Their inherent phytochemicals act as natural reducing and stabilizing agents, enabling eco-friendly fabrication of stable, crystalline, and biologically active metal nanoparticles. The synthesized nanoparticles exhibited strong antimicrobial, antioxidant, and cytotoxic properties, confirming their potential for pharmaceutical applications. Despite these advantages, issues related to standardization, scalability, and regulatory compliance remain to be addressed. Overall, agro-mediated nanoparticle synthesis presents a sustainable, low-cost, and biocompatible alternative to conventional methods, advancing the development of next-generation therapeutic nanomaterials.



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