International Journal of Advanced Research in Engineering Technology & Science
ISSN: 2349-2819
Impact Factor: 7.10
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Email: editor@ijarets.org Volume-12 Issue-1 January-2025 www.ijarets.org

BIOSYNTHESIS OF METAL NANOPARTICLES FROM MEDICINAL PLANT EXTRACTS: PHYSICOCHEMICAL CHARACTERIZATION AND ASSESSMENT OF CYTOTOXIC AND ANTIBACTERIAL PROPERTIES

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ABSTRACT

The creation of environmentally-friendly and sustainable methodologies of nanoparticle synthesis has received considerable attention over the past years. This paper will examine the green synthesis of metal nanoparticles using medicinal plant extracts, and the subsequent physicochemical characterization of the nanoparticles and cytotoxic and antibacterial activity. It is considered that plant extracts high on bioactive phytochemicals can be employed as reducing and stabilizing agents in the formation of nanoparticles. UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM) were used to characterize the synthesized nanoparticles. Cytotoxic effects were measured by cell viability assays whereas antibacterial efficacy was examined against chosen Gram-positive and Gram-negative bacterial strains. These findings indicated that stable nanoparticles with a high biological activity could be successfully synthesized, which implies that they could be used in biomedical and pharmaceutical areas.

Keywords: *Green synthesis, Metal nanoparticles, Medicinal plants, Cytotoxicity, Antibacterial activity, Characterization*

1. INTRODUCTION

Nanotechnology has become one of the most revolutionary sciences of modernity, and it has a significant impact on such disciplines as medicine, pharmaceuticals, environmental science, and material engineering. The fundamental components of this innovation, nanoparticles (often 1-100 nm in diameter), exhibit significantly different physicochemical characteristics than their bulk equivalents. These properties are a high ratio surface-to-volume, increased catalytic activity, improved optical behavior with surface plasmon resonance, and enhanced chemical reactivity. These properties render nanoparticles very appropriate in the application in drug delivery, imaging, diagnostics, antimicrobial therapy, and cancer therapy.

Traditional techniques of synthesizing nanoparticles, which include physical and chemical processes, have several downsides despite these obvious benefits. Chemical procedures may involve dangerous reducing agents and stabilizers that may produce harmful by-products, while physical approaches can necessitate complex apparatus and significant energy input. These disadvantages cast grave doubts on the sustainability of the environment, biocompatibility, and safety when nanoparticles are to find biomedical use. This has led to an increasing need of green, sustainable and cost-effective synthesis methods.

There has been a surge in interest in the green production of nanoparticles, which makes use of biological resources. Among the many biological methods, the use of therapeutic plant extracts stands out due to its simplicity, scalability, and lack of environmental impact. Many various types of phytochemicals, such as alkaloids, tannins, terpenoids, saponins, and phenolic acids, are found in medicinal plants. In addition to lowering aggregation by transforming metal ions into nanoparticles, these bioactive chemicals cap and stabilize nanoparticles, making them more stable and preventing aggregation altogether. Because the chemicals utilized are no longer hazardous, the technique is also better suited for usage in pharmaceutical and biomedical settings.

Nanoparticles made of metals, particularly silver (AgNPs), gold (AuNPs), and zinc oxide (ZnO NPs), have demonstrated impressive biological effects when synthesized in an environmentally friendly manner. Nanoparticles of both silver and gold have gained popularity for different reasons; the former for its potent antibacterial and antifungal properties, while the latter for their biocompatibility and potential use in cancer treatment and targeted drug administration. However, zinc oxide nanoparticles exhibit potent anticancer, antioxidant, and antibacterial properties. It is possible to regulate the bioefficacy of these nanoparticles by manipulating their regulated biogenesis, which in turn controls their size, shape, surface charge, and functionalization.

1.1 Green Synthesis of Nanoparticles Using Medicinal Plants

A more sustainable, environmentally friendly, and economically viable option to the traditional physical and chemical processes is the green synthesis of nanoparticles using medicinal herbs. In place of potentially harmful chemicals and high-energy procedures, this approach use plant extracts as reducing and stabilizing agents. Proteins that aid in the reduction of metal ions into nanoparticles and their subsequent stability are among the many bioactive phytochemicals found in medicinal plants. These include terpenoids, tannin, alkaloids, flavonoids, phenolic acids, and flavonoids.

It is common practice to extract water from plants in order to study their leaves, stems, roots, blossoms, or seeds. The next step is to combine the extracts with solutions of metal salts, like zinc acetate, chloroauric acid, or silver nitrate ($AgNO_3$). The extract's phytochemicals function as electron donors, reducing metal ions to their nanoparticle forms. Additionally, these chemicals act as capping agents, preventing the nanoparticles from clumping together and making them more stable. It is common practice to use the color shift seen with surface plasmon resonance as a marker for nanoparticle production.

Nanoparticles made from medicinal plants have proven to be an effective therapeutic tool. Due to its high quantities of phenolic and flavonoid chemicals, which boost the antibacterial activity, Azadirachta indica (Neem) has been documented widely in the manufacture of silver nanoparticles. In addition to its strong reducing potential, Ocimum sanctum (Tulsi) has been used to create silver and gold nanoparticles that have antioxidant and antibacterial effects. The bioactive chemicals of Withania somnifera (Ashwagandha) have been increased in their cytotoxic and therapeutic activities through nanoparticle production.

Plant extract concentration, metal ion concentration, pH, temperature, and reaction time are some of the variables that determine the size, shape, and stability of plant extract-synthesised nanoparticles. Nanoparticles produced environmentally friendly tend to be between 10 and 100 nm in size and have a spherical, rod-shaped, irregular, or otherwise non-uniform shape. Nanoparticles with specific physicochemical characteristics can be synthesized by fine-tuning the optimization of these parameters.

Nanoparticles produced by green synthesis have been shown to exhibit many biological activities, such as antibacterial, antifungal, anti-oxidant, anti-inflammatory and anti-cancer. The synergistic action of metal ions and phytochemicals derived from plants on their surface is a popular explanation for their enhanced biological performance. These nanoparticles interrelate with the microbial cell membranes, generate reactive oxygen species and disrupt the cellular functions which results in inhibition of microbes. Furthermore, they have the ability to inhibit the growth of cancer cells and induce cell death in cancer cells.

1.2 Objectives

1. To synthesize metal nanoparticles using medicinal plant extracts.
2. To characterize synthesized nanoparticles using advanced analytical techniques.
3. To evaluate cytotoxic effects on selected cell lines.
4. To assess antibacterial activity against pathogenic microorganisms.

2. LITERATURE REVIEW

E. A. Moacă et al. (2022) investigated the biosynthesis of iron oxide nanoparticles and carried out a thorough physicochemical characterization of the nanoparticles using advanced analytical methods. Their results have shown that the synthesized nanoparticles have a well-defined structural and morphological property and has a significant cytotoxic effect on both healthy and tumorigenic cell lines. The present study has identified the selective biological activity of nanoparticles and has pointed out the potential positive role of nanoparticles in targeted cancer therapy and biomedical applications.

M. Sorbiun et al. (2018) analyzed how plant extracts can be used to biosynthesize metallic nanoparticles and evaluated their antibacterial efficacy against different pathogenic strains. The study found that due to their small size and high surface reactivity, plant-mediated nanoparticles effectively inhibited microbial growth. The authors proposed that these nanoparticles disturbed the cell membranes of bacteria, interfered with the cellular metabolism and ultimately caused cell death thereby establishing their potential use as effective alternatives to the conventional antibiotics and in particular in countering the problem of antibiotic resistance.

S. Sysak et al. (2023) examined the interplay between metal nanoparticles and flavonoids and the impact that each has on synthesis and functional improvement. Through their synergistic interactions, flavonoids were discovered to enhance the biological efficacy of nanoparticles in addition to their roles as reducing and stabilizing agents. Additionally, the stability of nanoparticles, biocompatibility, and therapeutic potential were enhanced by the presence of flavonoid coatings. The study highlighted that knowledge in the interaction of nanoparticle and phytochemical would be important to streamline their functioning in medical and pharmaceutical practices.

M. Shahriari et al. (2019) produced gold nanoparticles by a synthetic process involving extract from Allium noeanum leaves. Following this, they were thoroughly characterized and tested for cytotoxic, antioxidant, and antibacterial effects. The findings proved that the biosynthesized nanoparticles had high biological activity and this included effective free radical scavenging and bacterial growth inhibition. Moreover, their cytotoxic activities on cancer cells implied their suitability in anticancer therapy. The research supported the possibility of synthesis of plant-based nanoparticles as a versatile method to develop new therapeutic agents.

3. MATERIALS AND METHODS

The materials and experimental methodologies used to biosynthesize metal nanoparticles from medicinal plant extracts, characterize them, and assess their biological efficacy are detailed in this section. Without additional purification, all of the chemicals and reagents employed in the investigation were of analytical quality. The experimental methodology was meant to provide reproducibility, accuracy, and reliability of findings under controlled laboratory conditions. The research included preparing plant extracts, green synthesis of nanoparticles, physicochemical characterization by standard analytical methods and evaluation of their cytotoxic and antibacterial activities. All the steps were well-optimized to achieve stable nanoparticles with desired properties to be used in the biomedical field.

3.1 Research Design

The current research was done on the basis of controlled experimental laboratory-based research design. The work was systematically broken into three major phases namely: (i) green synthesis of metal nanoparticles using medicinal plant extracts, (ii) physicochemical characterization of the synthesized nanoparticles using advanced analytical tools and (iii) evaluation of their biological activities, including cytotoxic and antibacterial activities. This design guarantees the reproducibility, accuracy and reliability of the experimental results under standardized laboratory conditions.

3.2 Selection of Plant Material

The selection of medicinal plants was based on their well-documented phytochemical richness and therapeutic significance. Nanoparticle production relies on these plants because of the bioactive chemicals they contain, which work as both reducing and stabilizing agents. The fresh and healthy leaves of the plants were picked in a clean environment to prevent contamination and assure the quality of the extract.

3.3 Preparation of Plant Extract

Before being distilled, the collected plant leaves were thoroughly rinsed under running water to remove any dust or other contaminants. The heat-sensitive phytochemicals were preserved by air-drying the leaves at room temperature in a shady environment. After the leaves had dried completely, they were ground into a powder using a grinder.

In order to extract the bioactive components, the powdered material was measured and heated in distilled water for a specific duration, often 10–20 minutes. After cooling, the mixture was filtered using Whatman No. 1 filter paper to yield a clear aqueous extract. The filtrate was stored at 4^{o}C for future use in nanoparticle synthesis.

3.4 Biosynthesis of Metal Nanoparticles

The prepared plant extract was then used to prepare nanoparticles using a suitable solution of metal salt, e.g., silver nitrate (AgNO 3) to make silver nanoparticles. The solution of metal salt was prepared in a known concentration in distilled water.

Then, while stirring continuously, the metal solution was added to the plant extract in an optimized proportion. Depending on the experimental circumstances, the reaction mixture was incubated at room temperature or slightly higher. An observational change in solution color—for instance, from pale yellow to brown when silver nanoparticles were utilized—was used to visually validate the transfer of metal ions to nanoparticles. This change in color was caused by SPR.

The synthesized nanoparticles were further purified by centrifugation to eliminate the unreacted components and dried so as to conduct subsequent analysis.

3.5 Antibacterial Activity

We tested the produced nanoparticles' antibacterial capabilities against Staphylococcus aureus and Escherichia coli, two types of Gram-positive and Gram-negative bacteria. These microbes were selected due to their differing cell wall architectures and their clinical significance.

The antibacterial activity was tested using the agar well diffusion method. A sterile cork borer was used to inoculate the wells, and bacterial cultures were placed onto sterile nutrient agar plates. The different solutions of nanoparticles were added to the wells and the plates were incubated at 37C (24hours).

Following the incubation period, the area of inhibition surrounding each of the wells was measured in millimeters. Greater areas revealed a higher antibacterial activity and this is indicative of the competence of nanoparticles against the microorganisms that have been tested.

3.8 Statistical Analysis

Every experiment was repeated three times to ensure accuracy and reliability. The mean ± standard deviation (SD) format was used to present the data. The statistical difference between the two groups' results was determined using analysis of variance (ANOVA). The results that were obtained were not a consequence of random variation since any p-value below 0.05 ($p < 0.05$) was deemed statistically significant.

4. RESULTS AND DISCUSSION

Following a comprehensive analysis of the relevance of the created metal nanoparticles, this section presents the results of their biosynthesis, physicochemical characterization, and biological evaluation. We looked at the data to find out how the nanoparticles were made, what their shape and size were, and whether or not they were cytotoxic and antibacterial. To better comprehend the role of phytochemicals in nanoparticle formation and stabilization, data acquired using various characterisation approaches were compared and contrasted. Furthermore, the bioactivity results were also addressed in relation to the size, shape, and surface properties of the nanoparticles, as well as their potential applications in pharmaceutical and biomedical research.

4.1 Synthesis of Nanoparticles

As a result of successfully reducing metal ions using phytochemicals extracted from plants, the solution's color changed from light yellow to dark brown, indicating the formation of silver nanoparticles.

4.2 Characterization Findings

The following table shows the UV-VIS spectroscopy of the prepared silver nanoparticles (AgNPs). The absorption spectrum was measured in the range of wavelengths between 300700 nm to observe the typical optical properties of the nanoparticles.

Table 1: UV–Visible Spectral Analysis of Synthesized Nanoparticles

Sample	Wavelength Range (nm)	Absorption Peak (nm)	Interpretation
AgNPs	300–700	430	Surface plasmon resonance confirms nanoparticle formation

The appearance of a conspicuous absorption peak at ca. Surface plasmon resonance (SPR) provides evidence of silver nanoparticle production at 430 nm. The appearance of this peak suggests that the reduction of silver ions to nanoparticles has been successful, and it's safe to infer that the particles are evenly distributed and stable in the solution.

The functional groups found in the plant extract and utilized in nanoparticle synthesis are listed in this table. They were identified using FTIR.

Table 2: FTIR Analysis – Functional Groups Identified

Peak (cm^{-1})	Functional Group	Possible Compound	Role in Synthesis
3420	–OH Stretching	Phenols/Alcohols	Reduction of metal ions
1635	C=O Stretching	Proteins	Stabilization
1384	C–N Stretching	Amines	Capping agent

Plant compounds such phenols, proteins, and amines are crucial to the synthesis process, as shown by the presence of peaks for -OH (hydroxyl), C=O (carbonyl), and C -N (amine) groups. Both the reducing agent (which transforms metal ions into nanoparticles) and the stabilizing/capping agent (which keeps nanoparticles from clumping together) are present in these chemicals.

The morphological characteristics of the produced nanoparticles, as identified by SEM, are summarized in this table.

Table 3: SEM Analysis

Parameter	Observation
Shape	Spherical
Size Range	20–50 nm
Distribution	Uniform
Aggregation	Minimal

The findings of the SEM show that the nanoparticles are mainly spherical shaped with a size range of 20-50 nm. The homogeneous distribution and low aggregation suggest that the biosynthesis technique is efficient in the production of stable and well dispersed nanoparticles. This target morphology has been sought-after because it increases biological activity such as antibacterial activity, cytotoxic activity.

4.3 Cytotoxic Activity

Cell viability, as assessed by the MTT assay, was examined in the following table for different quantities of produced nanoparticles. Using concentrations ranging from 10 to 100 µg/mL, the percentages of cell viability and corresponding inhibition were calculated.

Table 4: Effect of Nanoparticles on Cell Viability (MTT Assay)

Concentration (µg/mL)	**Cell Viability (%)**	**Inhibition (%)**
10	92	8
25	78	22
50	60	40
100	35	65

The results clearly indicate a dose-dependent cytotoxic effect of nanoparticles. With the concentration, cell viability decreases by 92 to 35 and inhibition increases by 8 to 65. This indicates that the greater the concentration of nanoparticles, the greater the cytotoxic activity, which could be due to an increase in contact with cellular components and the induction of oxidative stress or apoptosis.

The graphical relationship between nanoparticle concentration and cell viability percentage is shown in Figure 1, and reveals the trend of decreasing viability with increasing concentration.

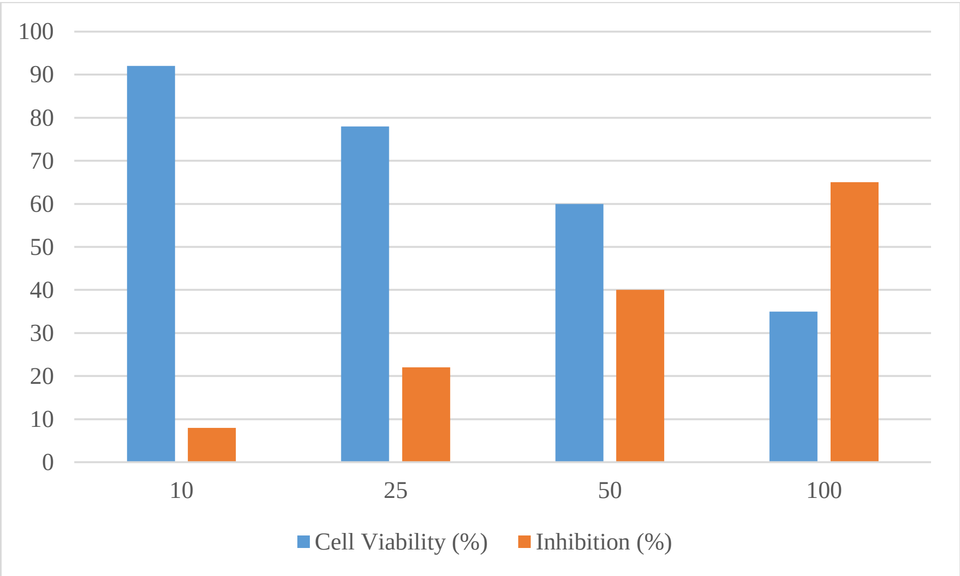

Figure 1: Graphical Representation of Effect of Nanoparticles on Cell Viability (MTT Assay)

The graph shows that there is a negative correlation between the concentration of nanoparticles and the viability of the cells. This tendency proves the cytotoxic effect of the synthesized nanoparticles and supports its potential use in cancer therapy, where controlled inhibition of cells is needed.

This table demonstrates the distribution of the level of antibacterial activity (low, moderate, high) that was observed in the tested samples, their frequency, and percentage.

Table 5: Frequency Distribution of Antibacterial Activity

Activity Level	Number of Samples	Percentage (%)
Low	1	25%
Moderate	1	25%
High	2	50%
Total	**4**	**100%**

The results show that 50% of the samples were highly active against bacteria, 25% were moderately active and 25% were weakly active. This implies that most synthesized nanoparticles have high antibacterial capacity which means that they are effective against the microbial strains.

Figure 2 is a graphical (bar or pie chart) display of the frequency distribution of the levels of antibacterial activity of the samples.

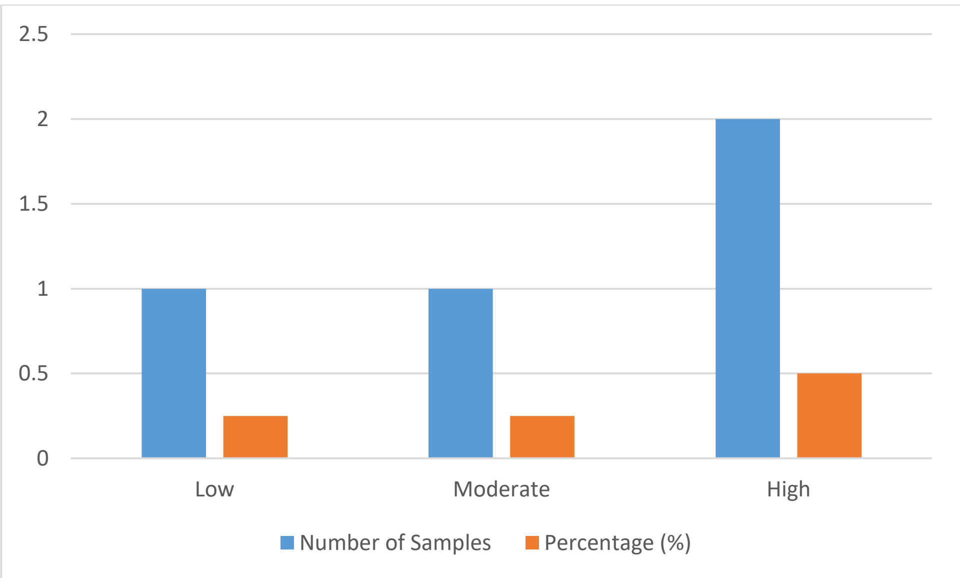

Figure 2: Graphical Representation of Frequency Distribution of Antibacterial Activity

As the visual portrayal makes abundantly evident, the nanoparticles' efficacy is supported by their significant antibacterial activity. Nanoparticles made from plant extracts are promising antibacterial agents, and one benefit is that their activity levels can be more easily compared.

5. CONCLUSION

This study has shown that, compared to the conventional method, the green production of metal nanoparticles using medicinal plant extracts is more sustainable and less harmful to the environment. Physical and chemical characterization methods, including SEM, Fourier transform infrared spectroscopy (FTIR), and ultraviolet visible spectroscopy (UV spectroscopy), validated the role of phytochemicals as natural reducing and stabilizing agents in the synthesis of stable nanoparticles. Not only did the biological test show significant antibacterial activity, particularly against Gram-negative bacteria, but it also showed large dose-dependent cytotoxic effect, which could be useful in cancer treatment. Generally speaking, the results show that plant-mediated nanoparticles are good for nanomedicine, drug delivery, and antimicrobial treatment because of their physicochemical and biological properties. However, it is clear that more in vivo trials and large-scale development are needed to make these nanoparticles practical.

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