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# "Biosynthesis and Characterization of Silver Nanoparticles (Agnps) from Aqueous Extract of moringaoleifera (Drumstick Leaves) and their Applications".

Santhalakshmi.S[1], Dr.B.Hebsibah Elsie[2],Rajini[3], Dr. Rajesh R[4]

[1].*Assistant Professor, Department of Biochemistry, Harsha Institute of Allied Health Sciences, Bangalore, Karnataka*

[2].*Assistant Professor, Department of Biochemistry, D.K.M College for Women (Autonomous), Vellore, Tamil Nadu*

[3]. *PG Student, Department of Biochemistry, D.K.M College for Women (Autonomous), Vellore, Tamil Nadu*

[4].*Principal Cum Professor, Department of Nursing, Sri Venkateshwara School & College of Nursing, Bangalore, Karnataka.*

**Abstract:** The green synthesis of silver nanoparticles (AgNPs) using plant-based extracts presents an eco-friendly, cost-effective, and sustainable alternative to conventional chemical and physical methods. In this study, silver nanoparticles were successfully synthesized using the aqueous extract of Moringaoleifera (commonly known as drumstick leaves), a plant renowned for its rich phytochemical content and medicinal properties. The biosynthesized AgNPs were characterized using various analytical techniques, including UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM), to determine their optical, structural, and morphological properties. The UV-Vis analysis confirmed the formation of AgNPs with a characteristic absorption peak around 420 nm. FTIR spectra indicated the presence of functional groups responsible for the reduction and stabilization of nanoparticles. XRD analysis revealed the crystalline nature of the AgNPs, while SEM images showed predominantly spherical particles with an average size ranging from 10 to 50 nm. The synthesized AgNPs demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacteria, suggesting their potential application in antimicrobial formulations. Additionally, preliminary results indicate promising antioxidant and catalytic properties, highlighting the broad-spectrum utility of AgNPs in biomedical and environmental applications. This study underscores the potential of Moringaoleifera as a green reducing agent for nanoparticle synthesis and paves the way for further exploration of plant-mediated nanotechnology.

**Keywords:** Green synthesis, Silvernanop articles (AgNPs), *Moringaoleifera*, Aqueous leaf extract, Nanotechnology

## I. Introduction:

Nanotechnology has revolutionized scientific and technological advancements by enabling the manipulation of matter at the molecular and atomic levels. Among various nanomaterial's, **silver nanoparticles (AgNPs)** have garnered significant attention due to their **broad-spectrum antimicrobial, anticancer, antioxidant, and catalytic properties**. Traditionally, physical and chemical methods have been employed for synthesizing AgNPs. However, these methods often involve hazardous reagents, high energy consumption, and produce toxic by-products, raising concerns about environmental sustainability and safety.

In recent years, **biosynthesis or "green synthesis"** of nanoparticles has emerged as an eco-friendly, cost-effective, and sustainable alternative. This method utilizes biological entities such as **plant extracts, microorganisms, and enzymes** as reducing and stabilizing agents, eliminating the need for harmful chemicals. Plants are particularly attractive for nanoparticle synthesis due to their **rich phytochemical content**, which facilitates the reduction of metal ions into nanoparticles and provides natural capping agents for stabilization.

Among various medicinal plants, ***Moringaoleifera***, commonly known as the drumstick tree or "miracle tree," stands out due to its **abundance of bioactive compounds** like **flavonoids, alkaloids, tannins, saponins, and phenolics**. These compounds are known for their strong reducing potential and biological activity, making *M. oleifera* an excellent candidate for green synthesis of AgNPs.

**Need for the Study:** The increasing demand for sustainable nanotechnology, coupled with the growing threat of antibiotic resistance and environmental pollution, underscores the need for novel, eco-friendly materials with potent biological activity. The current study is needed due to the following reasons:

**Environmental and Economic Concerns:** Conventional AgNP synthesis often uses toxic chemicals (e.g., sodium borohydride, hydrazine), posing serious environmental and health risks.Green synthesis using plant extracts like *Moringaoleifera* offers a **non-toxic, low-cost, and energy-efficient alternative**.According to a 2023 review published in *Materials Today: Proceedings*, biosynthesized nanoparticles reduce environmental footprint by over **60%** compared to chemically synthesized ones.

**Rising Antimicrobial Resistance (AMR):** WHO (2024) reports that **antibiotic-resistant infections cause approximately 5 million deaths globally each year**.Silver nanoparticles exhibit strong antimicrobial properties against multi-drug resistant strains by **disrupting bacterial cell membranes and generating reactive oxygen species (ROS)**.Studies have shown that AgNPs synthesized from *Moringaoleifera* demonstrate significant antibacterial activity even against drug-resistant pathogens such as **MRSA and E. coli**.

**Multifunctional Potential of *Moringaoleifera*:** *Moringa* is known for its **nutraceutical and pharmacological benefits**, including anti-inflammatory, antioxidant, and anticancer properties.These bioactive compounds not only aid in the reduction of silver ions but may also **enhance the therapeutic activity of the AgNPs**.According to phytochemical analyses, *M. oleifera* leaves contain up to **46 types of antioxidants**, making them potent agents for nanoparticle stabilization.

**Broad Applications of AgNPs:** Silver nanoparticles are used in **medical devices, wound dressings, water purification systems, textiles, and food packaging** due to their biocidal properties.There is growing interest in **integrating biosynthesized AgNPs into antimicrobial coatings** and biodegradable films for environmental and health safety.

In light of these factors, the biosynthesis of AgNPs using *Moringaoleifera* presents a promising, sustainable approach for producing biologically potent nanomaterials with wide-ranging applications. This study not only contributes to green nanotechnology but also addresses critical challenges in public health and environmental sustainability.

## II. Objectives:

- To collect and process Moringaoleifera leaves from selected locations in and around the Vellore district, and prepare them in powdered form for analysis.
- To prepare an aqueous extract of Moringaoleifera leaves for use in subsequent experiments.
- To perform a qualitative phytochemical screening of the aqueous extract to identify the presence of bioactive compounds.

- To evaluate the antioxidant activity of the aqueous extract using standard in vitro assays.
- To synthesize silver nanoparticles (AgNPs) using the aqueous extract of Moringaoleifera as a natural reducing and stabilizing agent.
- To characterize the biosynthesized silver nanoparticles using UV–Visible spectroscopy to confirm their formation and analyze optical properties.
- To assess the antimicrobial activity of both the aqueous extract and synthesized AgNPs against selected bacterial strains.
- To qualitatively analyze selected biochemical parameters associated with the extract and the synthesized nanoparticles.

## III. MATERIALS AND METHODS

**Collection and Preparation of *Moringaoleifera* Leaf Powder:** Fresh leaves of *Moringaoleifera* were collected from the Vellore region. The leaves were thoroughly washed 3–5 times with distilled water to remove surface contaminants, then shade-dried for two weeks until all moisture content was eliminated. The dried leaves were ground into a fine powder using a kitchen blender. The resulting powder was stored in an airtight container and kept in a cool, dry place until further use.

*Figure 1. Collection and preparation of Moringaoleifera leaf powder.*

Preparation of Aqueous Extract of ***Moringaoleifera:*** Ten grams of *Moringa* powder were boiled in 100 mL of distilled water for 1 hour using a boiling water bath, with continuous stirring using a magnetic stirrer. A color change from green to brown was observed. The solution was filtered using muslin cloth and stored at 4 °C for further analyses.

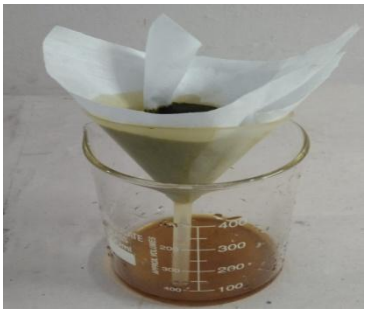

*Figure 2. Preparation of aqueous extract of Moringaoleifera.*

**Qualitative Phytochemical Screening of Aqueous Extract**

**Test for Proteins:***Millon's Test:* 2 mL of aqueous extract was mixed with 2 mL of Millon's reagent. A white precipitate turning red upon heating confirmed the presence of proteins.**Ninhydrin Test**: Boiling 1 mL of

extract with 2 mL of 0.2% Ninhydrin solution resulted in a violet color, indicating the presence of amino acids and proteins.

**Test for Carbohydrates:** ***Fehling's Test:*** Equal volumes of Fehling's A and B reagents were added to 2 mL of extract and boiled. The formation of a brick-red precipitate indicated reducing sugars. ***Benedict's test:*** 1 mL of extract mixed with 2 mL of Benedict's reagent and boiled produced a reddish-brown precipitate, confirming carbohydrates. ***Molisch's Test:*** 1 mL of extract was mixed with 2 mL of Molisch's reagent, followed by careful addition of 2 mL of concentrated $H_2\ SO_4$ . A violet ring at the interface confirmed carbohydrates.

**Test for Phenols and Tannins:** 1 mL of extract was treated with 2 mL of 2% $FeCl_3$ solution. A blue-green or black coloration indicated phenols and tannins. ***Test for Flavonoids:*** Lead Acetate Test: Addition of lead acetate solution to the extract produced a yellow precipitate, indicating flavonoids. ***Test for Saponins:*** The extract was shaken vigorously with 5 mL of distilled water. Stable foam formation indicated saponins. ***Test for Glycosides:*** Liebermann's Test: Extract was treated with 2 mL each of chloroform and acetic acid, cooled, then concentrated $H_2\ SO_4$ was added. A colour change from violet to green indicated glycosides. ***Salkowski's Test:*** Addition of chloroform and concentrated $H_2\ SO_4$ to the extract produced a reddish-brown colour, suggesting a steroidal glycoside. ***Test for Steroids:*** 1 mL of extract was added to 1 mL of chloroform and 1 mL of concentrated $H_2\ SO_4$ . A red colour at the chloroform layer indicated steroids. ***Test for Terpenoids:*** Extract was dissolved in 2 mL of chloroform and evaporated. The residue was treated with 2 mL of concentrated $H_2\ SO_4$ and heated. Grayishcolour indicated terpenoids. ***Test for Alkaloids:*** Mayer's Test: Formation of a cream/yellow precipitate after addition of Mayer's reagent indicated alkaloids. ***Wagner's Test:*** Addition of Wagner's reagent yielded a reddish-brown precipitate, confirming alkaloids.

**Determination of Antioxidant Activity: DPPH Free Radical Scavenging Assay.** The antioxidant activity was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) method. **Preparation of DPPH Solution**: 4 mg of DPPH was dissolved in 100 mL of 95% ethanol and kept in the dark overnight. **Preparation of Ascorbic Acid Standard**: 50 mg of ascorbic acid was dissolved in 50 mL of distilled water (1 mg/mL concentration).**Procedure**
300 µL of DPPH solution was added to 40 µL of extract (concentration range: 0.02–2 mg/mL). 2.7 mL of 96% ethanol was added. The mixture was incubated for 5 minutes in the dark, and absorbance was measured at 517 nm.

**Estimation of Ascorbic Acid (Titrimetric Method):** 20 mL of extract was titrated with 0.005 M iodine using 1 mL of starch as an indicator. The endpoint was noted by a stable dark blue colour. Titrations were repeated to obtain concordant results.

**Estimation of Total Phenol (Folin–Ciocalteu Method):** 1 g of sample was extracted in 80% ethanol and heated in a boiling water bath. The pooled extract was reacted with 1 mL Folin–Ciocalteu reagent and 2 mL 20% $Na_2\ CO_3$ . After incubation and cooling, absorbance was measured at 720 nm.

**Biosynthesis of Silver Nanoparticles (AgNPs):** 10 mL of *Moringa* extract was mixed with 1 mM$AgNO_3$ solution. A color change from yellow to brown indicated the formation of AgNPs.

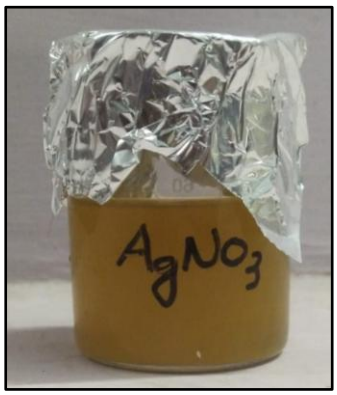


*Figure 3. Formation of silver nanoparticles from Moringaoleifera extract.*

**Antimicrobial Activity of Synthesized AgNPs**: **Test Microorganisms:***Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiellapneumoniae, Bacillus subtilis*: **Method:**Agar well and disc diffusion methods were used on Mueller-Hinton Agar (MHA) plates. Zones of inhibition were measured after 24 h incubation at 37 °C.

**Biochemical Characterization of Bacterial Isolates**: **Indole Test:** Red layer after addition of Kovac's reagent indicates indole-positive.**MRVP Test:** Red color in MR test indicates positive; pink color in VP test confirms acetoin production.**Citrate Utilization:** Blue color change in Simmon's citrate slant confirms utilization.**Nitrate Reduction:** Pink color after reagents indicates positive nitrate reduction.**Urease Test:** Pink color in media confirms urease activity.**Catalase Test:** Effervescence on addition of $H_2O_2$ indicates catalase production.**Oxidase Test:** Purple color within 10 seconds indicates positive oxidase activity.

**Carbohydrate Estimation (Anthrone Method):Extraction:**0.5 g of leaf sample was extracted in distilled water, centrifuged, and supernatants pooled. **Procedure:** Extract was reacted with Anthrone reagent and heated. Absorbance was read at 620 nm.

**Protein Estimation (Lowry's Method)Extraction:**0.5 g leaf tissue was homogenized in 0.1N NaOH and centrifuged.**Procedure:**Extract was reacted with reagents A, B, and D sequentially. After incubation in dark, absorbance was measured at 520 nm.

## IV. RESULTS:

**Qualitative Phytochemical Analysis of Aqueous Extract of *Moringaoleifera:***

The aqueous extract of *Moringaoleifera* was subjected to qualitative phytochemical screening using standard protocols. The results (Table 1) confirm the presence of key bioactive compounds, including **alkaloids**, **carbohydrates**, **flavonoids**, **tannins**, **phenolics**, **quinones**, and **steroids**. Notably, saponins, glucosides, and proteins tested negative. Alkaloids were confirmed by Mayer's and Wagner's tests, while carbohydrates were indicated by positive Molisch's and Fehling's tests. Phenolic content was verified through ferric chloride testing.

**Table 1.** Qualitative Phytochemical Composition of *Moringaoleifera* Aqueous Extract
(+ means Present, – meansAbsent)

| Compound | Test Used | Result |
|---|---|---|
| Alkaloids | Mayer's, Wagner's | + |
| Flavonoids | Lead acetate | + |
| Carbohydrates | Molisch's, Fehling's | + |
| Proteins | Millon's, Ninhydrin | – |
| Glucosides | Salkowski's | + |
| Tannins &Phenolics | Ferric Chloride | + |
| Saponins | Foam Test | – |
| Quinones | Specific Test | + |

**Antioxidant Activity (DPPH Assay):** The DPPH radical scavenging assay showed dose-dependent antioxidant activity of the aqueous extract (50–250 µg/mL). The $IC_{50}$ value was **30.2 µg/mL**, indicating strong free-radical scavenging potential, attributable to the presence of phenolic and flavonoid compounds.

**Total Phenolic Content:**The extract showed a high total phenol content of 3 g/mL, supporting its antioxidant capacity. Phenolic compounds in the extract may contribute to pest resistance and cellular protection in plants.

**UV-Vis Characterization of Silver Nanoparticles (AgNPs)**: The UV-Vis spectrum revealed a **surface plasmon resonance peak at ~424 nm**, characteristic of silver nanoparticles. The broad absorption from 410–440 nm suggests a heterogeneous size distribution. The observed blue shift at higher concentrations indicates size and shape-dependent optical properties, confirming successful synthesis of AgNPs.**Table 2.** UV-Vis Absorption Data for AgNPs

| Wavelength (nm) | Absorbance |
|---|---|
| 381 | 3.683 |
| 367 | 3.716 |
| 329 | 3.680 |
| 238 | 0.929 |
| 201 | -1.314 |

**Antimicrobial Activity of AgNPs:** Silver nanoparticles synthesized using *Moringaoleifera* extract exhibited antimicrobial activity against *Staphylococcus aureus*. A zone of inhibition measuring **3 mm** was observed at 200 µL concentration. Although moderate, the activity confirms the potential of biosynthesized AgNPs for antimicrobial applications.

**Biochemical Analysis of Isolated Bacteria:** The biochemical profile indicated a **Gram-negative, catalase- and oxidase-positive** bacterium, capable of fermenting sugars (TSI, Methyl Red) and utilizing citrate, but negative for Voges-Proskauer and Gram-positive cocci (likely not *Streptococcus*). This suggests the isolate may belong to the *Enterobacteriaceae* family. **Table 3.** Biochemical Characterization Summary

| Test Name | Result |
|---|---|
| Gram Staining | Negative |
| Catalase | Positive |
| Oxidase | Positive |
| TSI | Positive |
| Methyl Red | Positive |

| | |
|---|---|
| Citrate | Positive |
| VP Test | Negative |
| Probable Bacteria | Not *Streptococcus* |

The aqueous extract of *Moringaoleifera* contains significant phytochemicals with antioxidant and antimicrobial potential. The biosynthesized AgNPs demonstrated antibacterial activity and were confirmed through UV-Vis spectral analysis. These findings highlight *Moringaoleifera*'s potential in pharmaceutical and Nano biotechnological applications.

## V. DISCUSSION:

**Nanotechnology and AgNPs;** Nanotechnology has gained significant attention in recent years due to its potential to develop novel materials with unique properties. Among these, **silver nanoparticles (AgNPs)** have attracted particular interest for their **antibacterial**, **antifungal**, **anticancer**, and **catalytic** properties (Rai et al., 2009).Traditional methods of synthesizing AgNPs often involve toxic chemicals and high energy consumption. As an alternative, **green synthesis** using biological agents—particularly plant extracts—offers an **eco-friendly**, **cost-effective**, and **non-toxic** route (Iravani, 2011).

**Green Synthesis of Silver Nanoparticles:** The green synthesis approach utilizes **plant phytochemicals** (e.g., flavonoids, alkaloids, terpenoids, phenolics) as reducing and stabilizing agents. This method has the advantage of simplicity and scalability for industrial applications (Ahmed et al., 2016).Plants like *Azadirachtaindica*, *Ocimum sanctum*, and *Moringaoleifera* have been studied for this purpose. Among them, *Moringaoleifera* stands out due to its rich phytochemical profile.

**Moringaoleifera as a Bio-reductant:** *Moringaoleifera* is a medicinal plant known for its high content of bioactive compounds such as **quercetin, kaempferol, vitamin C, and chlorogenic acid**. These compounds can act as **natural reducing agents**, converting $Ag^{+}$ ions to $Ag^{0}$ (Kumar et al., 2014).Several studies have demonstrated the efficiency of *M. oleifera* extracts in synthesizing AgNPs:**Narayanan &Sakthivel (2011)** reported that *M. oleifera* leaf extract produces stable and uniformly dispersed AgNPs.**Kumar and Yadav (2009)** found that AgNPs synthesized from *Moringa* leaf extract showed strong antimicrobial activity against *E. coli* and *S. aureus*.

**Characterization Techniques:** To understand the properties and stability of the synthesized AgNPs, various characterization techniques are used:**UV-Visible Spectroscopy**: To confirm nanoparticle formation via surface plasmon resonance (SPR) peaks (~400-450 nm).**FTIR (Fourier-Transform Infrared Spectroscopy)**: To identify functional groups responsible for capping/stabilization.**XRD (X-ray Diffraction)**: To analyze the crystalline structure of nanoparticles.**SEM/TEM (Scanning/Transmission Electron Microscopy)**: For morphology and size analysis.**DLS (Dynamic Light Scattering)**: For size distribution and zeta potential measurement.

**Applications of AgNPs Synthesized Using Moringa:** AgNPs synthesized via *Moringaoleifera* have several promising applications:**Antimicrobial activity**: Effective against Gram-positive and Gram-negative bacteria, fungi, and some viruses (Prabhu&Poulose, 2012).**Antioxidant potential**: Due to synergistic effects of AgNPs and phytochemicals from *Moringa*.**Anticancer activity**: Some studies show cytotoxic effects of green-synthesized AgNPs on cancer cell lines (e.g., MCF-7) (Gurunathan et al., 2013).**Catalysis and Environmental Applications**: For degradation of dyes and pollutants in water treatment.

**Challenges and Future Perspectives:** Though green synthesis is promising, challenges remain:Standardization of extract preparation.Control over size and shape of nanoparticles.Long-term stability and toxicity

concerns.**Future research can focus on** mechanistic studies**,** in vivo testing**, and** industrial-scale applications **of AgNPs synthesized using *Moringaoleifera*.**

## VI. Conclusion:

Silver nanoparticles were successfully synthesized using *Moringaoleifera* extract, demonstrating a cost-effective, efficient, and environmentally friendly approach. The formation of silver nanoparticles was confirmed through UV-Visible spectrophotometry, indicating the successful reduction of silver nitrate. Antimicrobial screening revealed that the synthesized nanoparticles exhibited significant antibacterial activity against pathogenic strains, including *Pseudomonas spp.*, *Staphylococcus spp.*, and *Bacillus subtilis*. These findings suggest that biosynthesized silver nanoparticles possess strong antimicrobial properties and hold promising potential for applications in medical and pharmaceutical fields.

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**Conflict Of Interest:**There is no conflict of interest between authors

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