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"GREEN NANOTECHNOLOGY USING AZADIRACHTA INDICA: SYNTHESIS, CHARACTERIZATION AND PHARMACEUTICAL APPLICATIONS"

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Abstract:

Azadirachta indica, a medicinal plant, is well known for its variety of pharmacological properties and phytochemical richness. Green nanotechnology is a sustainable and eco-friendly approach for the preparation of nanoparticles by using plant extracts. Azadirachta indica contains bioactive compounds such as azadirachtin, nimbin, nimbidin, quercetin and various phenolic compounds which are responsible for formation and stabilization of nanoparticles. The review highlights the green synthesis of nanoparticles from different parts of Azadirachta indica viz., leaves, bark, seeds and flowers. The review additionally emphasizes their potential pharmaceutical application including antimicrobial, antioxidant, anti-inflammatory, anticancer, wound healing and drug delivery. The synthesized neem-mediated silver nanoparticles are expected to exhibit good physicochemical characteristics and enhanced biological activity, demonstrating the potential of Azadirachta indica as a natural reducing and stabilizing agent for the eco-friendly synthesis of silver nanoparticles. The study may support their future application in pharmaceutical, antimicrobial, and biomedical fields.

Keywords : *Azadirachta indica, Neem-Mediated Silver Nanoparticles, Green Synthesis, Silver Nitrate, Nanoparticle Characterization,*

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INTRODUCTION:

Azadirachta indica (A. Juss) is a species of plant native to India that is highly tolerant to hot weather and poor soil conditions and produces exceptionally bitter fruits and leaves. It has been widely planted as an ornamental and/or for its medicinal properties (JINDAL et al., 1999; GAJALAKSHMI; ABBASI, 2004; KUMAR et al., 2018). Neem is a member of the Meliaceae family. It is a deciduous tree growing throughout South and Southeast Asia. It may reach 5-20 m in height and grows in regions with an average annual rainfall of 150 mm or more in temperate and tropical areas. Azadirachta indica is propagated by seeds, which can be planted directly or transplanted when they have reached 9-12 months. The trunk is straight and rather long, and the branches are widely spreading, forming an umbrella-like crown when mature.

The bark is rough and dark brown, with long furrows splitting vertically. Leaves are pinnate, alternate, with about 5-20 serrated leaflets on either side, while leaf color becomes dark green when mature. Neem grows in loamy, sandy, and clay soils with a pH level between 5.0 and 8.0, but it is most common in 6.2 and 7.0 in sandy soils. This tree is a medium-sized tree, reaching 18 m in height, occasionally to 15-30 m, with a crown of 10-20 m in diameter (9). The leaves of this tree are light green, simple, pinnate and alternate, 20-40 cm long. The flowers are five-merous and small, white or pale yellow, sweet-scented, bisexual. The plants fruits are greenish yellow or yellow or purple. The drupes are one- or two-seeded, elliptical, 1-2 cm long at maturity, and turn green when ripe. The seeds are ovoid or spherical. The leaves, seeds, bark, roots, fruits, and oil of Neem have been the focus of attention of modern medicine and have found their way into Ayurveda, Indian Homeopathy, and Unani, where they are used to treat a large number of the diseases.

Neem (Azadirachta indica) has been long valued in traditional medicine for its numerous medicinal properties and has been found to be a natural insecticide. In Ayurvedic practices, neem extracts have been used for centuries Abu-Elala et al (2003).



Figure :1 Plant of Azadirachta indica (Neem)

Moreover, the compound is biodegradable, eco-friendly, not harmful to the human body, presenting a good alternative to conventional pesticides. However, challenges such as photo-sensitivity and quick degrading limit its effectiveness in agribusiness, requiring more frequent applications and having low systemic activity (Ahmad, 2015). Researchers have been looking into carrier systems, which have shown promising results in various health applications and offer a solution to the issues with pesticides (Ahmed, 2001). Polymeric and solid lipid nanoparticles have been found to be promising in agriculture as they have a solid matrix that protects the bioactive compounds and allows targeting and controlled release profles(Ajith et al, 2024). Polymeric nanoparticles provide additional benefits including biocompatibility, biodegradability, surface modification possibility and control over degradation and release rates by varying the polymer (Baligar, 2014). However, there are concerns about nanoparticle toxicity and isolated carrier systems impact on animals, humans, plants and the environment (Batra et al, 2022)

Herbal sources provide a safe alternative to antibiotics and present an environmentally friendly option. The majority of plant-derived molecules are phytochemicals and secondary metabolites which are classified into a wide range of classes and predominantly functioning as antimicrobials like tannins, coumarins, terpenes, quinones, phenolics, polyphenols, flavonoids, lectins,polypeptides,andsaponins[13,14].and

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Neem (Azadirachta indica) is a big evergreen tree having perfumed foliage and delicious fruits. This tree's anti-inflammatory, immunological, and anti-ulcer properties had contributed to its extensive usage for various purposes [15,16]. Additionally, all parts of the

The leaves of neem tree have various medicinal values like antifungal, antibacterial, antiviral and antioxidant activities (17,18). The leaves of neem have shown antibacterial activities against Staphylococcus aureus and A. hydrophila isolated from singhi (Heteropneustes fossilis) and mrigal Cirrhinus mrigala) (19). The extract of neem leaves

was found to have antibacterial activity against A. veronii, A.hydrophila, Acinetobacter junii, A. tandoii and Pseudomonas stutzeri isolated from blackspot barb (Dawkinsia filamentosa) (20).Neem leaves extract was loaded onto chitosan nanoparticles forming nanocomposites which showed inhibitory effect against a number of Gram negative and positive pathogenic bacterial strains in biofilm (26). Our choice of title was based on the severe impact of A. sobria on health and production of Nile tilapia and antibiotics misuse in aquaculture which may cause health hazards to the consumers of treated fish. In this study we endeavored to explore the possible therapeutic effect of chitosan neem nanocapsule (CNNC) as an alternative to antibiotics against A. sobria induced hepato-renal damage, antioxidant and immunosuppressive effects in Nile tilapia.

Green synthesis of nanoparticles also has advantages over other methods of nanoparticle synthesis as it is simple, one step, cost effective, environment friendly and relatively reproducible and stable (Mittal, Batra, Singh, & Sharma, 2014). Microorganisms can also be used for synthesis of nanoparticles but the process is slow compared to plant mediated synthesis (Ahmed et al., 2015).

Major active compounds

- ✓ Azadirachtin – one of the main limonoids that exhibit insecticidal properties.
  Nimbin – possesses anti-inflammatory and antimicrobial activity.
  Nimbidin – reported activity against microbial infectious agents and inflammation.
- ✓ Nimbolide – under preliminary investigations for its potential anti-cancerous and anti-inflammatory effects.
- ✓ Salannin – insect-repellent activity.
- ✓ Quercetin – flavonoid antioxidant
- ✓ Meliantriol – inhibits feeding of various insects.
- ✓ Gedunin – limonoid exhibiting various pharmacological effects.

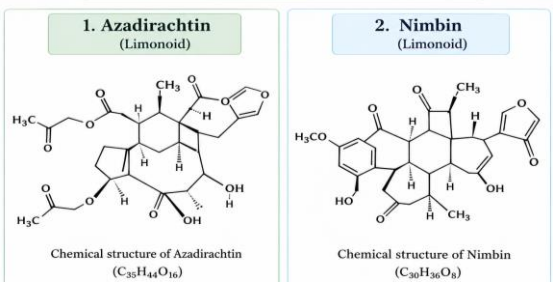


Figure 2: chemical structure of azadirachtin and nimbin

Advantages

1. **Improved stability :** Neem bioactive compounds prone to degradation by environmental factors such as light, heat, and oxidation. Nanoparticles act as protective carriers, shielding neem compounds from these detrimental factors (Rehman, 2024). By encapsulating neem bioactive within nanoparticles, their stability is enhanced, prolonging the shelf life of neem-based products and ensuring their efficacy over time. This improved stability is critical for maintaining the potency of neem compounds during storage and transportation (Sahayaraj, 2014).
2. **Enhancement of bioavailability**: Neem compounds are often poorly soluble and dispersible, limiting their bioavailability and effectiveness. Nanoparticles overcome this limitation by improving the solubility and dispersibility of neem compounds, resulting in an increased bioavailability upon administration. Enhanced bioavailability ensures that a greater portion of the active ingredients in neem can be utilized by the body, resulting in improved therapeutic outcomes and efficacy (Sahoo SK, 2007).
3. **Controlled release:** Nanoparticles allow for controlled release and prolonged delivery of neem bioactive, thus allowing for the prolonged release of therapeutic compounds over a period of time (Saraswathi, 2009). Controlled release of neem compounds maintains effective concentrations in the body, prolonging their therapeutic effects and reducing the frequency of dosing. Controlled release is particularly beneficial for chronic
4. **Targeted delivery:** Nanoparticles can be engineered to target specific tissues, cells, or organs in the body, thus enhancing their therapeutic efficacy and reducing off-target effects. Modification of the surface of nanoparticles with targeting ligands can make them selectively accumulate and deliver neem compounds to the desired site of action, such as tumor tissues or inflamed tissues (Singh, 2017) Targeted delivery increases the therapeutic efficacy of neem compounds while minimizing systemic exposure and adverse effects, enhancing the overall treatment outcomes and safety profiles (Singh, 2018). l

Decreased toxicity: Nanoparticle formulation can decrease the potential toxicity of neem compounds by reducing their systemic exposure and off-target effects. Controlled release and targeted delivery can reduce adverse effects while maximizing therapeutic benefits.

Literature survey

Mohamed T. (2025)The green synthesis of CNPs through methods such as microemulsion, polyelectrolyte complexation, ionic gelation, emulsification-solvent diffusion, and reverse micellization further emphasizes their eco-friendly and sustainable production. Recognized as a Generally Recognized as Safe (GRAS) material by the USA Food and Drug Administration, chitosan is widely accepted for use in biomedical applications. This review comprehensively explores the structural features, environmentally friendly synthesis strategies, and advanced characterization techniques of CNPs. Moreover, it

highlights their interdisciplinary biomedical applications, including drug delivery via ocular, oral, pulmonary, vaginal, and nasal routes, as well as their promising role in tissue engineering and cancer therapy.

Kumari A (2022) Azadirachta indica extract and evaluated for their in vitro antioxidant activity and cytotoxicity efficacy against MCF-7 and HeLa cells. The silver nanoparticles (Ag-NPs) were formed within 40 min and after preliminary confirmation by UV-visible spectroscopy (peak observed at 375 nm), they were characterized using a transmission electron microscope (TEM)compositional analysis was carried out. The cytotoxicity and antioxidant activity of various concentrations of biosynthesized silver nanoparticles, Azadirachta indica extract, and a standards.

Pushpa S. Parmar(2025) A laboratory study for synthesis and characterization of iron nanoparticles was conducted at laboratory of Department of Nanotechnology and Centre for advanced Research in Plant Tissue Culture, Anand Agricultural University,anand.The Fe nanoparticle synthesized by green method and subsequently characterized for size (Dynamic Light Scattering), functional group (FTIR) and absorbance spectra (UV-VIS Spectrophotometer).

Abhay Thakur(2026) Azadirachta indica (neem), a well-established medicinal plant, has long been known for its therapeutic value and has recently drawn attention as a sustainable biological resource for the synthesis of nanoparticles. Phyto-nanotechnology refers to a green method that uses phytochemical properties of A. indica, with the help of azadirachtin, nimbolide, quercetin, and flavonoids, acts as a capping agent to enhance the physiochemical stability and biological performance of nanoparticles such as metallic, oxides, polymeric, and lipid-based. Nanoparticles derived from neem have shown immense biomedical potential. These include Antimicrobial, antioxidant, anti-inflammatory, wound healing, anticancer, drug delivery and many more.

Elaida R(2021)study involves extracting the insecticidal properties of neem (Azadirachta indica) by fermenting air-dried leaves with rice wash. The crude fermented neem extract (FNE) was applied as a biopesticide against male B. dorsalis in an improvised olfactometer. The remaining crude extract was utilized in the green synthesis of silver nanoparticles (AgNP) and copper nanoparticles (CuNP).

Parmar P(**2025**) A laboratory study for synthesis and characterization of iron nanoparticles was conducted at laboratory of Department of Nanotechnology and Centre for advanced Research in Plant Tissue Culture, Anand Agricultural University,anand.The Fe nanoparticle synthesized by green method and subsequently characterized for size (Dynamic Light Scattering), functional group (FTIR) and absorbance spectra (UV-VIS Spectrophotometer).

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Y Nishshanka (2025) The study examined the sustained release of neem from the polymeric carrier system chitosan by varying the drug content, ionic strength of the release medium, and pH. Six different kinetic models, i.e., Korsmeyer–Peppas (KP), Peppas–Sahlin (PS), Higuchi, Hixson–Crowell, Zero order, and First order were used to investigate the drug release kinetics. Based on the R2 values, the KP and PS models were chosen from the examined models to study the drug release mechanism from the chitosan biopolymer. The values found for model parameters n and m in the KP and PS models differ noticeably, suggesting that Fickian diffusion and Case II relaxation are important components of the neem release mechanism from chitosan. At lower ionic strengths and lower pH values, neem is released from the composite mostly by Fickian diffusion.

Rowida I (2023) Neem (Azadirachta indica) leaves extract was synthesized using asynthesized using a nanocapsule. neem nanocapsul (CNNC) was tested in-vitro and in-vivo against the Aeromonas sobria (A. sobria) challenge in Nile tilapia.

Rajendran R.(2012) The present study focuses on the development of herbal chitosan nanocomposite finishes for protective clothing. The medicinal plant (Azadirachta indica) was selected, and bioactive compounds were extracted. The neem chitosan nanocomposites were prepared using multiple emulsion/ solvent evaporation method. The neem chitosan nanocomposites were finished

Mohamed T. (2025)The green synthesis of CNPs through methods such as microemulsion, polyelectrolyte complexation, ionic gelation, emulsification-solvent diffusion, and reverse micellization further emphasizes their eco-friendly and sustainable production. Recognized as a Generally Recognized as Safe (GRAS) material by the USA Food and Drug Administration, chitosan is widely accepted for use in biomedical applications. This review comprehensively

explores the structural features, environmentally friendly synthesis strategies, and advanced characterization techniques of CNPs. Moreover, it highlights their interdisciplinary biomedical applications, including drug delivery via ocular, oral, pulmonary, vaginal, and nasal routes, as well as their promising role in tissue engineering and cancer therapy.

- **Material**

i. **Plant material**: fresh or dried leaves of Azadirachta indica species, preferably of uniform size and thickness, being a natural source of terpenoids, flavonoids, phenolics, etc., acting as reducing and capping/stabilizing agents.

ii. **Metal precursor**: silver nitrate (AgNO3), dissolved in water at a concentration of 1 mM to 0.1 M to give a solution of Ag+ ions.

iii. **Water**: double-distilled or deionized water for washing the leaves, preparing an aqueous solution of the leaf extract, and dissolving silver nitrate.

iv. **Equipment/apparatus**:

v. A magnetic stirrer or a heating mantle to mix and heat the leaf suspension in water.

vi. A filter paper (or a centrifuge) to remove coarse plant material and obtain a clear solution of the neem extract in water.

Glass laboratory ware including Erlenmeyer flasks and beakers.

- **Methods**

Chemicals

Silver nitrate was purchased from Merck (Mumbai, India), along with Dulbecco's modified Eagle's medium (DMEM), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), and trypsin. EDTA and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and fetal calf serum (FBS) was purchased from Gibco. Whatman filter paper no. 42 25 cm2, a 75 cm2 flask, and 96-well plates were purchased from Eppendorf, India.

Experimental Design

Typically, plant extract-mediated bio-reduction involves mixing the aqueous extract with an aqueous solution of the appropriate metal salt. The synthesis of nanoparticles occurs at room temperature and is completed within a few minutes.

Preparation of Plant Extract

A. indica (neem) leaf extract was used to prepare silver nanoparticles based on the cost effectiveness, ease of availability, and medicinal properties. Fresh leaves were collected from a greenhouse facility in Hyderabad (Telangana, Hyderabad, India). The surfaces of the leaves were cleaned with running tap water to remove debris and other contaminated organic contents, followed by washing with deionized water and air drying at room temperature. About 15 gm of finely cut leaves was kept in a 250 mL Erlenmeyer flask containing 50 mL deionized water and boiled for 25 min at 60 °C in a water bath. The extract was cooled down to room temperature, filtered with Whatman filter paper no. 42 under vacuum, and stored at 4 °C for further use.

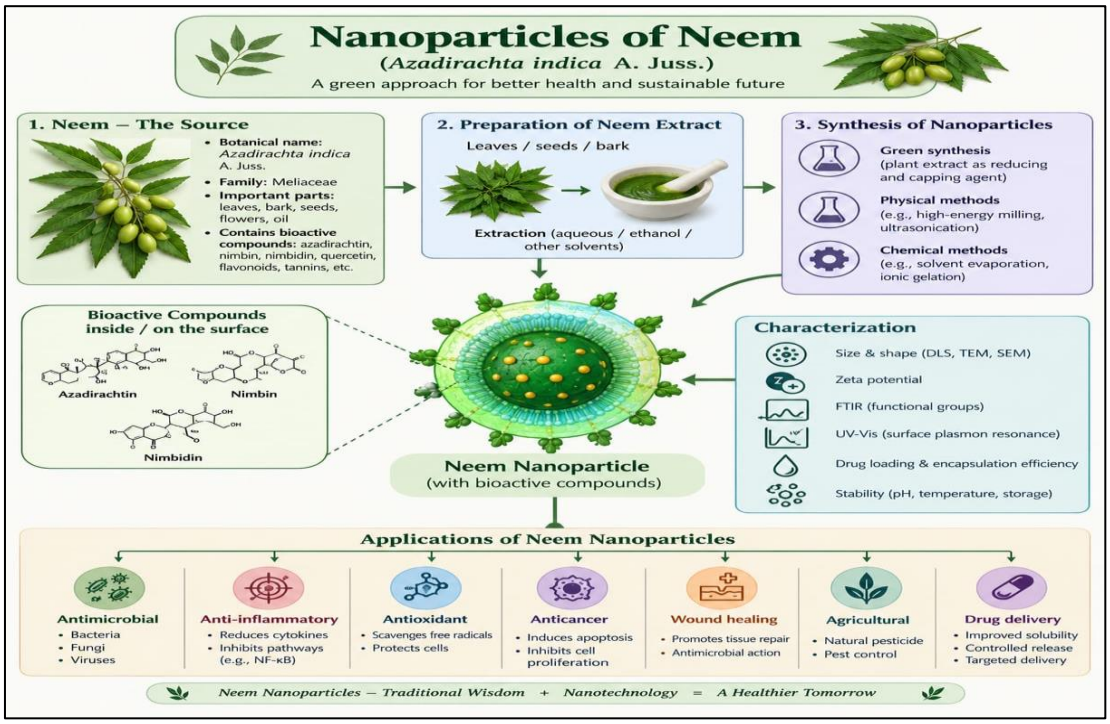


Fig. :3 Nanoparticles of Neem

➢ **Green Synthesis of Silver Nanoparticles**

- A volume of 100 mL of 1 mM solution of silver nitrate (AgNO3) was prepared in an Erlenmeyer flask. The Ag-NPs were synthesized by adding 10 mL of A. indica (neem) leaf extract to 50 mL of 1 mM aqueous AgNO3 solution at room temperature, stirring continuously for 20 min. The mixture obtained was incubated in a dark chamber at room temperature to prevent the auto-oxidation of the silver nitrate. The reduction of silver ions to silver nanoparticles was confirmed by the color change of the solution from reddish to dark brown (Figure 1), indicating the synthesis of Ag-NPs, and the formation was also confirmed using UV-visible spectroscopy.
- **Collection and Preparation of Neem** Plant Extract: Collect recently plucked neem leaves or other neem plant material. To remove any unwanted dirt or contaminants, thoroughly rinse the harvested plant material in distilled water. To stop active chemicals from degrading, dry the plant material in the shade. Make a fine powder out of the dried neem leaves or plant parts using a mortar and pestle [34]
- **Preparation of Neem Extract:** Neem powder in the amount of 10 g should be weighed and then transferred to a clean beaker. In the beaker, mix a suitable solvent (1:10) (w/v), such as distilled water or ethanol. To draw out the bioactive substances from the neem plant, agitate the mixture with a magnetic stirrer at room temperature for a predetermined amount of time (for example, 24 hours) [34].

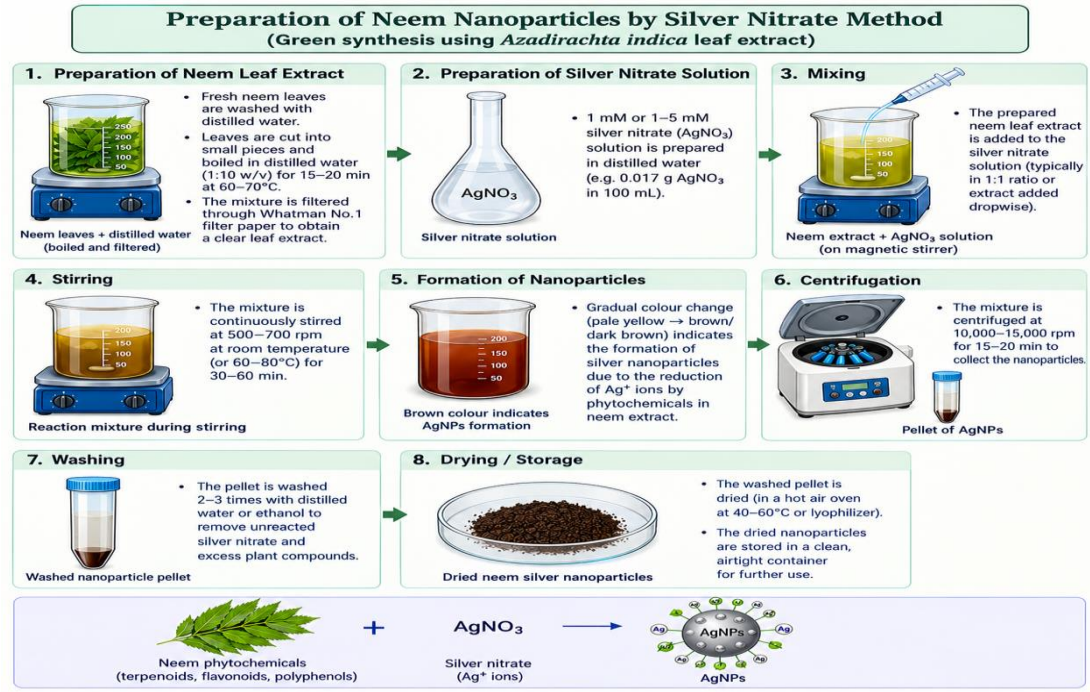


Figure:4 Preparation of Neem Nanoparticles

➢ **Synthesis of Nanoparticles**: Neem extract from step 2 is metered out (for example, 50 mL) and transferred to a clean reaction vessel (for example, an Erlenmeyer flask). To the reaction vessel, add a suitable precursor solution containing metal salts (such as silver nitrate). At a suitable temperature, such as 60°C, stir the mixture using a magnetic stirrer for a predetermined amount of time, such as two hours. Keep an eye on the reaction mixture's colour change, which shows when nanoparticles have formed. Neem plant extract is commonly used to create brownish nanoparticles [34].

- The green synthesis of neem-mediated silver nanoparticles can be described in several stages:

Neem plant material
↓
Preparation of neem extract
↓
Phytochemicals present in the extract
↓
Interaction with Ag^+ ions from $AgNO_3$
↓
Reduction of Ag^+ to Ag^0
↓
Nucleation of silver atoms
↓
Growth of nanoparticles
↓
Capping/stabilization by phytochemicals
↓
Neem-mediated silver nanoparticles (AgNPs

➢ **Factors Affecting the Formation of Neem-Mediated AgNPs**

Several parameters can influence nanoparticle formation and final characteristics.

a) Concentration of $AgNO_3$

The concentration of silver precursor can influence the availability of Ag^+ ions and may affect nucleation, particle growth and final particle size.

b) Concentration of Neem Extract

The amount of phytochemicals available for reduction and stabilization can influence the rate of nanoparticle formation and surface capping.

c) **pH**

pH can affect the ionization of phytochemical functional groups and the reduction process. Consequently, it may influence particle size and morphology.

d) **Temperature**

Temperature can influence the rate of reduction and nanoparticle formation. Different temperatures may produce differences in particle size and distribution.

e) Reaction Time

The duration of interaction between neem extract and silver ions can affect the extent of nanoparticle formation and particle growth.

f**) Plant Part**

Leaves, bark, seeds and other parts may contain different concentrations and profiles of phytochemicals. Therefore, nanoparticles produced using different plant parts may show different characteristics

➢ **Characterization of Neem-Mediated Silver Nanoparticles**

Characterization is essential for confirming nanoparticle formation and determining their physicochemical properties.

1. UV–Visible Spectroscopy : UV–Visible spectroscopy is commonly used to monitor the formation of silver nanoparticles. The characteristic optical absorption associated with the surface plasmon resonance of AgNPs can provide evidence of nanoparticle formation.
2. FTIR Spectroscopy : Fourier Transform Infrared Spectroscopy (FTIR) can be used to identify functional groups associated with phytochemicals present on or interacting with the nanoparticle surface. It can provide information regarding the possible role of plant constituents in reduction and capping.
3. X-Ray Diffraction (XRD) : XRD can be used to investigate the crystalline nature and crystal structure of silver nanoparticles.
4. SEM : Scanning Electron Microscopy provides information regarding the surface morphology and approximate particle shape.
5. TEM : Transmission Electron Microscopy can provide detailed information about nanoparticle morphology and particle size.
6. DLS : Dynamic Light Scattering is commonly used to determine hydrodynamic particle-size distribution in dispersion.
7. Zeta Potential : Zeta potential provides information about surface charge and can be useful for assessing the colloidal stability of nanoparticle dispersion

8.

➢ **Application**

➢

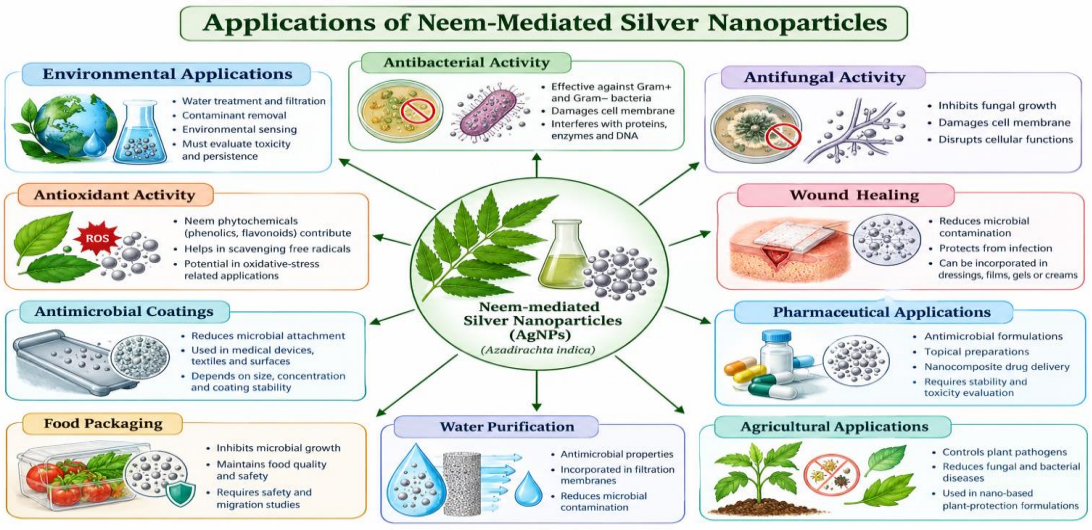


Figure :5 Application of Neem Mediated Silver Nanoparticles

1. Antibacterial Application: Inhibition of Gram-positive and Gram-negative bacteria through antimicrobial effects on cell membranes, proteins, enzymes, and DNA.
2. Antifungal Application: Potential inhibition of fungal growth and disruption of fungal cellular functions.
3. Wound Dressing: Incorporation into wound dressings, hydrogels, films, and topical preparations for antimicrobial protection.
4. Pharmaceutical Applications: Potential use in antimicrobial formulations, topical preparations, and nanocomposite-based drug delivery research.
5. Agricultural Applications: Potential use in controlling plant-pathogenic bacteria and fungi in crop protection.
6. Water Purification: Incorporation into filtration membranes and nanocomposites to reduce microbial contamination in water.
7. Food Packaging: Development of antimicrobial packaging materials to inhibit microbial growth and help maintain food quality.
8. Antimicrobial Coatings: Incorporation into coatings for medical materials, textiles, and laboratory surfaces to reduce microbial growth.
9. Antioxidant Application: Investigation of antioxidant properties associated with neem-derived phytochemicals and nanoparticle composition.
10. Environmental Applications: Potential use in antimicrobial water treatment, filtration systems, contaminant removal, and environmental sensin

Evaluation Parameters of Neem-Silver Nanoparticles (AgNPs)

The evaluation of Neem mediated AgNPs is carried out for confirmation of nanoparticle formation, their characterization in terms of physicochemical properties, and for evaluation of pharmaceutical activities.

1. Physical appearance

The prepared dispersion is observed for color, clarity, and sedimentation. Formation of silver nanoparticles is usually indicated by change in color, from pale yellow to brownish or dark brown, due to formation of silver nanoparticles.

2. UV-Visible spectroscopy

UV-Visible spectroscopy is used for preliminary confirmation of formation of silver nanoparticles. Silver nanoparticles show the characteristic surface plasmon resonance (SPR) absorption band, usually appearing in the range of 400-500 nm. The position and intensity of the peak can provide information about nanoparticle formation and aggregation.

3. Particle size

Particle size is an important parameter as it influences surface area, dissolution, cellular interaction, biological activity, and stability of nanoparticles. It can be measured using Dynamic Light Scattering (DLS) method.

4. Polydispersity Index (PDI)

This represents the degree of particle size heterogeneity of the nanoparticle dispersion. Lower PDI indicates a more homogeneous nanoparticle preparation, whereas high PDI indicates a large range of particle sizes.

5. Zeta potential

The surface charge of nanoparticles is measured by Zeta potential. It is a useful indicator of the physical stability of the nanoparticle formulation. A sufficiently high positive or negative zeta potential generally indicates better stabilization of the nanoparticles.

6. Morphological study

The shape and surface morphology of nanoparticles are studied using techniques like:
SEM – Scanning Electron Microscopy
TEM – Transmission Electron Microscopy
AFM – Atomic Force Microscopy
These methods help determine if the nanoparticles are spherical, irregular, or aggregated.

7. FTIR analysis

The FTIR (Fourier Transform Infrared Spectroscopy) helps identify the functional groups present in the neem extract and nanoparticles. It provides information on the role of neem phytochemicals like phenols, flavonoids, proteins, etc., in the reduction and stabilization of silver nanoparticles.

8. X-Ray Diffraction (XRD)

XRD is used to determine the crystalline nature and crystal structure of the silver nanoparticles. Characteristic diffraction peaks can confirm the formation of crystalline metallic silver.

9. Energy Dispersive X-Ray Analysis (EDX) or EDS (Energy Dispersive Spectroscopy)

EDX is generally performed along with SEM. It helps determine the elemental composition of the nanoparticles and confirm the presence of silver in the formulation.

10. Dynamic Light Scattering (DLS)

This technique measures the hydrodynamic particle size and the particle size distribution in the dispersion. It is particularly useful in determining the dispersion stability of nanoparticles.

11. Total phenolic content

Neem contains several phenolic components that can contribute to antioxidant and biological activity. Total phenolic content can be estimated to determine the amount of phenolic constituents present in the formulation.

12. Total flavonoid content
Flavonoids are an important phytoconstituent in neem. The content can be estimated to evaluate the contribution of flavonoid compounds to the biological properties of the nanoparticles.
13. Encapsulation/loading efficiency
If neem extract or a particular neem constituent is encapsulated in nanoparticles, loading efficiency or encapsulation efficiency can be determined. This indicates how much active material is successfully encapsulated or associated with the nanoparticles.
14. In-vitro drug/active constituent release
The release of neem phytoconstituents from the nanoparticle formulation can be studied using an appropriate diffusion or membrane-based method. The release profile can help determine if the formulation provides rapid, sustained, or controlled release.
15. Antimicrobial activity
Neem-silver nanoparticles can be evaluated against selected bacterial or fungal strains. Some common methods are:
Agar well diffusion method
Disc diffusion method
Minimum inhibitory concentration (MIC)
The activity can be assessed by the zone of inhibition and determining the minimum concentration required to inhibit microbial growth.
16. Antioxidant activity
The antioxidant potential can be evaluated using assays such as:
DPPH radical-scavenging assay
ABTS assay
FRAP assay
These tests help determine the ability of the formulation to neutralize free radicals.
17. Cytotoxicity study
For pharmaceutical usage, the nanoparticles can be evaluated for their effect on suitable cell lines. Cytotoxicity assays can help determine their concentration-dependent effects and give preliminary information on cellular safety.
18. pH determination
The pH of the nanoparticle dispersion is measured to determine its chemical stability and suitability for the intended pharmaceutical application.
19. Solubility and dispersibility
The ability of the nanoparticles to remain uniformly dispersed in the selected medium is studied. Good dispersibility helps reduce aggregation and gives more consistent dosing.
20. Stability study
The change in properties such as color, particle size, PDI, zeta potential, pH, and biological activity can be monitored at specific intervals when stored under designated conditions.
21. Antibacterial mechanism studies
As a part of the comprehensive research study, additional studies can be performed to investigate various mechanisms of antibacterial action, such as membrane damage, oxidative stress, and interaction of nanoparticles with microbial cells.
22. Overall pharmaceutical evaluation
The effectiveness of the nanoparticle formulation in the intended application, such as antimicrobial, antioxidant, anti-inflammatory, wound-healing, or drug-delivery, can be evaluated based on the objective of the study.

CONCLUSION:

Green synthesis of neem-mediated silver nanoparticles using Azadirachta indica leaf extract and silver nitrate ($AgNO_3$) is a promising and eco-friendly approach in nanotechnology.
Characterization techniques such as UV–Visible spectroscopy, FTIR, XRD, SEM, TEM, DLS, and zeta potential help evaluate their properties.

Conflict of interest:
The Author declares that there is no conflict of interest.

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