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**SYNTHESIS, CHARACTERIZATION AND ANTI MICROBIAL ACTIVITY
OF SILVER NANOPARTICLES BY USING PLANT SOURCES**

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ABSTARCT

Silver has been recognized as a nontoxic, safe inorganic antibacterial/antifungal agent used for centuries. Silver demonstrates a very high potential in a wide range of biological applications, more particularly in the form of nanoparticles. Environmentally friendly synthesis methods are becoming more and more popular in chemistry and chemical technologies and the need for ecological methods of synthesis is increasing; the aim is to reduce polluting reaction by-products. Another important advantage of green synthesis methods lies in its cost-effectiveness and in the abundance of raw materials. During the last five years, many efforts were put into developing new greener and cheaper methods for the synthesis of nanoparticles. The cost decrease and less harmful synthesis methods have been the motivation in comparison

to other synthesis techniques where harmful reductive organic species produce hazardous by-products. This environment-friendly aspect has now become a major social issue and is instrumental in combatting environmental pollution through reduction or elimination of hazardous materials. This research describes a brief overview of the research on green synthesis of silver nanoparticles using natural sources.

Keywords: Synthesis, Characterization Antimicrobial activity, Silver Nanoparticles

INTRODUCTION

A nanoparticle or ultrafine particle is a particle of matter 1 to 100 nanometres (nm) in diameter. The term is sometimes used for larger particles, up to 500 nm, or fibers and tubes that are less than 100 nm in only two directions. At the lowest range, metal particles smaller than 1 nm are usually called atom clusters instead [1-3].

Nanoparticles are distinguished from microparticles (1-1000 μm), "fine particles" (sized between 100 and 2500 nm), and "coarse particles" (ranging from 2500 to 10,000 nm), because their smaller size drives very different physical or chemical properties, like colloidal properties and ultrafast optical effects or electric properties.

Being much smaller than the wavelengths of visible light (400-700 nm), nanoparticles cannot be seen with ordinary optical microscopes, requiring the use of electron microscopes or microscopes with laser. For the same reason, dispersions of nanoparticles in transparent media can be transparent, [5, 6] whereas suspensions of larger particles usually scatter some or all visible light incident on them. Nanoparticles also easily pass through common filters,

such as common ceramic candles, so that separation from liquids requires special nanofiltration techniques. nanoparticles and the influence of the method on their size and morphology.

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Nanotechnology is a mindset, even though the scientific community is fascinated with the field of nanoscience, most of the ongoing discussions, definitions, and attention is focused on nanotechnology. As such, it represents a broad term which demonstrates the apotheosis of man's ceaseless urge for knowledge having practical potential. The meaning of the term nanotechnology is any technology operating on the nanoscale which has applications in the real world, that is, to employ single atoms and molecules to form functional structures.

ANTIMICROBIAL ACTIVITY

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. Antimicrobial technologies employed for preservation, disinfection, and sterilization are widely used for industrial and medical purposes in reducing or eliminating microorganisms. But in the development and application of these technologies, there are at least two major considerations: the desired antimicrobial effects (the most obvious reason behind employing these technologies) and safety requirements. Therefore, the objective of the desired end point with an antimicrobial process is important in its choice and application. This can range from the unique

control of an individual type of microorganism (eg, in a biosafety or research laboratory that may only be used for a certain type of microorganism), to the control of a range of potential pathogenic or spoilage microorganisms (eg, in environmental disinfection requirements in food production, research laboratory, general microbiology detection laboratories, and health care facilities), and to the complete eradication of all types of harmful or product degrading microorganisms in higher risk situation (as during the administration of injectable drugs or surgical intervention with devices).

INTRODUCTION TO SILVER NANOPARTICLES

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity, and biological properties. Due to their peculiar properties, they have been used for several applications, including as antibacterial agents, in industrial, household, and healthcare-related products, in consumer products, medical device coatings, optical sensors, and cosmetics, in the pharmaceutical industry, the food industry, in diagnostics, orthopedics, drug delivery, as anticancer agents, and have

ultimately enhanced the tumor-killing effects of anticancer drugs. Recently, AgNPs have been frequently used in many textiles, keyboards, wound dressings, and biomedical devices. Nanosized metallic particles are unique and can considerably change physical, chemical, and biological properties due to their surface-to-volume

ratio; therefore, these nanoparticles have been exploited for various purposes.

Recently, AgNPs have been shown much interest because of their therapeutic applications in cancer as anticancer agents, in diagnostics, and in probing.

MATERIALS AND METHODS:

MATERIALS	SOURCES
SILVER NITRATE	NALANDA COLLEGE OF PHARMACY
MAGNETIC STIRRER	NALANDA COLLEGE OF PHARMACY
BEAKERS AND TEST TUBES	NALANDA COLLEGE OF PHARMACY
PETRIDISHES	NALANDA COLLEGE OF PHARMACY
LEAVES OF NEEM, CITRUS SINENSIS, AMLA AND MORINGA OLEIFERA	NALANDA COLLEGE OF PHARMACY GARDEN
DISTILLED WATER	NALANDA COLLEGE OF PHARMACY

Methods: UV SPECTROSCOPIC

EXPERIMENTAL WORK:

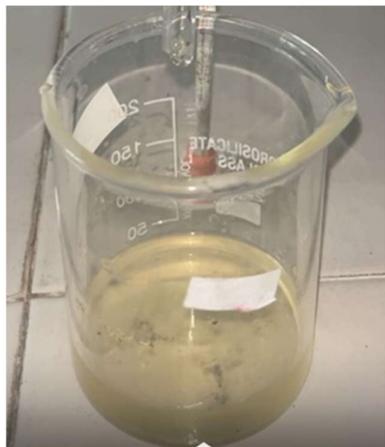
➤ **METHODOLOGY:**

PLANT EXTRACT PREPARATION

1. PREPARATION OF SILVER NANO PARTICLES BY USING NEEM, ONION AND TOMATO EXTRACT:

- Take Garden-fresh neem leaves, tomatoes and onions (5 g of each) were cut into smaller pieces and washed with distilled water thoroughly (three times).
- The tomato, onion and neem leaves were mixed in a beaker with 50ml of distilled water and boiled for 20 min.

- After boiling, the solution was cooled down for 10 min and double filtered by Whatsmann filter paper.
- 1. 1 mM solution of silver nitrate was prepared by dissolving 1.6 g of silver nitrate in 100 mL of distilled water in a beaker and kept in the airtight bottle until further use.
- Take neem extract (6 ml) and NOT [neem, onion and tomato] (6 ml) were mixed with a 10 ml silver nitrate solution in the beaker and stir it continuously.
- The colour change is seen from lime yellow to black colour due to the formation of AgNPs.



The above pictures show the colour change from lime yellow to black colour due to the formation of AgNPs.

2. PREPARATION OF SILVER NANOPARTICLES BY USING NEEM AND AMLA EXTRACT:

- Take a Garden-fresh neem leaves, amla leaves (5 g of each) were cut into smaller pieces and washed with distilled water thoroughly (three times).
- The neem leaves and amla leaves were separately mixed in a beaker with 50 ml of distilled water and boiled for 20 min.
- After boiling, the solution was cooled down for 10 min and double filtered by Whatsmann filter paper.
- 1. 1 mM solution of silver nitrate was prepared by dissolving 1.6 g of silver nitrate in 100 ml of distilled water in a beaker and kept in the airtight bottle until further use.

- Take neem extract (6 ml) and NA [neem ad amla] (6 ml) were mixed with a 10 ml silver nitrate solution in the beaker and stir it continuously.
- After the passage of time, it was observed that the colour of the solution changed from white to light brown colour due to the formation of AgNPs.

3. PREPARATION OF SILVER NANOPARTICLES BY CITRUS SINESIS AND MORINGA OLEIFERA:

- Take Fresh leaves of citrus and moringa oleifera (5 g of each) were cut into smaller pieces and washed with distilled water thoroughly (three times).
- The citrus and moringa leaves were mixed in a beaker with 50 ml of distilled water and boiled for 20 min. After boiling, the solution was cooled down for 10 min and double filtered by Whatman filter paper.

- 1. 1 mM solution of silver nitrate was prepared by dissolving 1.6 g of silver nitrate in 100 ml of distilled water in a beaker and kept in the airtight bottle until further use.
- Citrus extract (6 ml) and citrus and moringa leaves extract (6 ml) were mixed with a 10 ml silver nitrate solution in the beaker continuously at different pH values (5, 7 and 9) adjusted using HCl and NaOH solutions and kept for 24 h at room temperature.
- After the passage of time, it was observed that the colour of the solution changed from yellow to brown colour due to the formation of AgNPs.



The above picture shows the colour change from yellow to brown colour due to the formation of AgNPs.

4. PREPARATION OF SILVER NANOPARTICLES BY CITRUS SINESIS AND NEEM:

- Take a Fresh leaves of citrus and neem (5 g of each) were cut into smaller pieces and washed with distilled water thoroughly (three times).
- The citrus and neem leaves were mixed in a beaker with 50 ml of distilled water and boiled for 20 min.
- After boiling, the solution was cooled down for 10 min and double filtered by Whatman filter paper.
- 1. 1 mM solution of silver nitrate was prepared by dissolving 1.6 g of silver nitrate in 100 ml of distilled water in a beaker and kept in the airtight bottle until further use.
- Citrus extract (6 ml) and citrus and neem leaves extract (6 ml) were



mixed with a 10 ml silver nitrate solution in the beaker continuously at different pH values (5, 7 and 9) adjusted using HCl and NaOH solutions and kept for 24 h at room temperature.

- After the passage of time, it was observed that the colour of the solution changed from lime green to dark brown colour due to the formation of AgNPs.



The above picture shows the colour change from lime green to dark brown colour due to the formation of AgNPs.

UV SPECTROSCOPY:

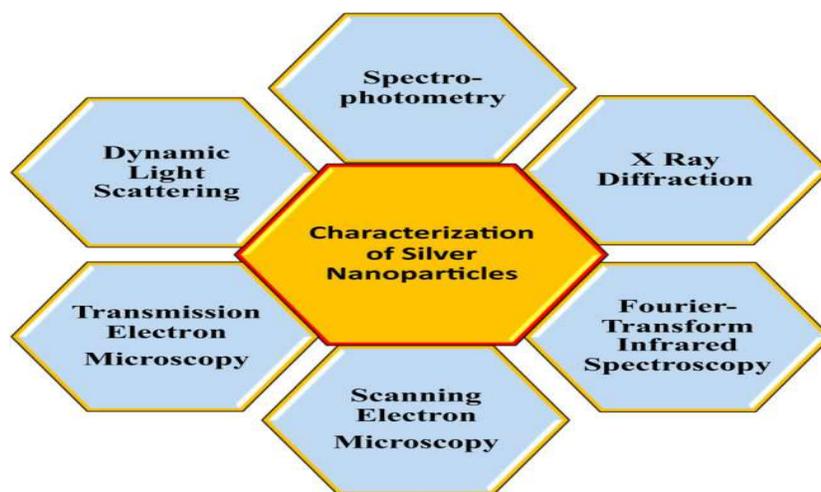


Figure 1: Various techniques used for characterisation of silver nanoparticles

This technique is most widely utilised to characterise metallic nanoparticles by monitoring their stability and synthesis. The synthesis of a metallic nanoparticle from its particular salt provides a characteristic peak

with strong absorptions in the visible region. Various studies have revealed that, in general, the absorption band at around 200–800 nm wavelength is best for the characterisation of particles in the size range

of 2–100 nm. The valence and conduction bands in silver nanoparticles are very close to each other. Electrons move freely in these bands and give rise to a surface plasmon resonance absorption band. The silver nanoparticle's absorption depends upon the chemical surroundings, dielectric medium and particle size. Examination and study of the surface plasmon peak is well known for several metal nanoparticles having a size range of 2–100 nm. Stability of silver nanoparticles produced through biological methods was examined for about 12 months and a surface plasmon resonance peak at the same wavelength was found using UV spectrophotometry.

- ✓ Samples (1 mL) of the suspension were collected periodically to monitor the completion of bioreduction of Ag⁺ in aqueous solution,
- ✓ followed by dilution of the samples with 2 ml of deionized water and subsequent scan in UV spectra, between wave lengths of 200 to 700 nm in a spectrophotometer (SHIMADZU-1601) having a resolution of 1 nm.
- ✓ UV spectra were recorded at intervals of 0 min, 15 min, 30 min, 45 min, 60 min and 24 h.
- ✓ Absorption spectra of AgNPs formed in the reaction media has absorption maxima in the range of 425 to 475 nm due to surface plasmon resonance of AgNPs.

- ✓ The UV spectra recorded, implied that most rapid bioreduction was achieved. This was denoted by broadening of the peak which indicated the formation of poly dispersed large nanoparticles due to slow reduction rates. The formation of AgNPs occurred rapidly within the first 15mins only and the AgNPs in solution remained stable even after 24 h of completion of reaction.

ANTI MICROBIAL ACTIVITY:

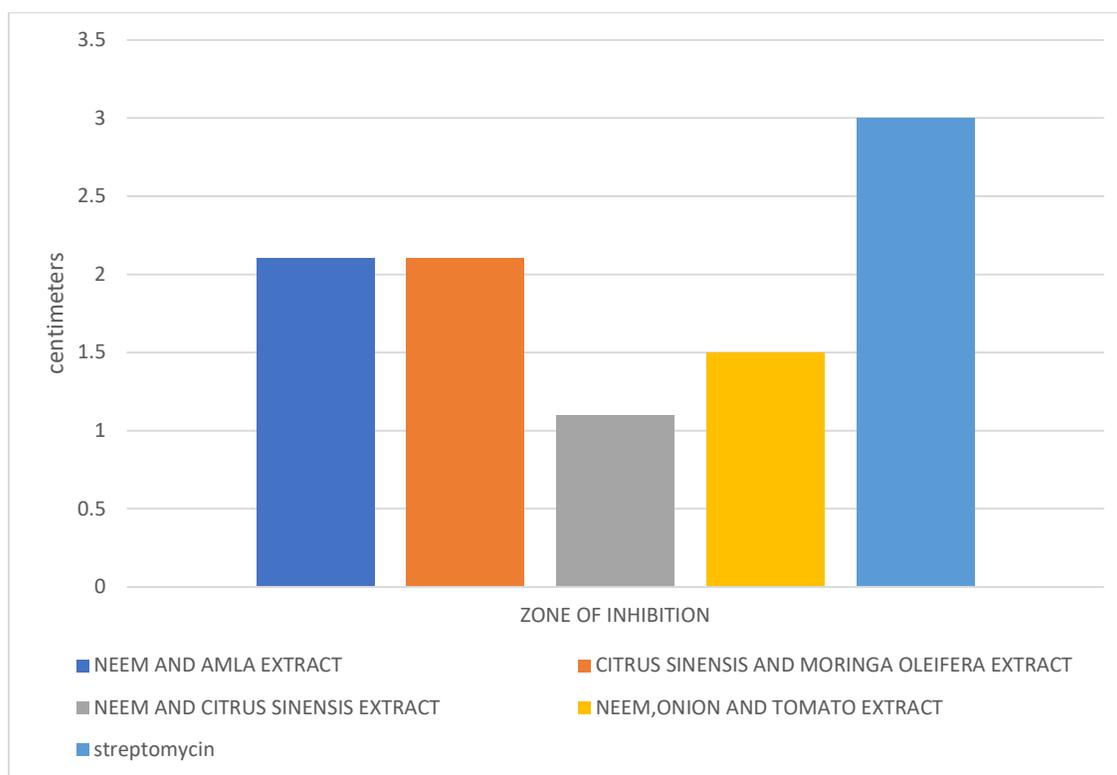
➤ PREPARATION OF CULTURE MEDIA:

- A bacterial or fungal strain of interest is grown in pure culture.
- Using a sterile swab, a suspension of the pure culture is spread evenly over the face of a sterile agar plate.
- The antimicrobial agent is applied to the center of the agar plate (in a fashion such that the antimicrobial doesn't spread out from the center).
- A hole can be bored in the center of an agar for a liquid substance.
- The agar plate is incubated for 18-24 hours (or longer if necessary), at a temperature suitable for the test microorganism.
- Streptomycin is used as standard solution and plant extracts are used as test solution.
- If antimicrobial agent leaches from the object into the agar and then exerts a growth-inhibiting effect, then a clear

zone (the zone of inhibition) appears around the test product.

- The size of the zone of inhibition is usually related to the level of antimicrobial activity present in the sample or product – a larger zone of inhibition usually means that the antimicrobial is more potent.

- The two plant extracts (Neem & Amla) and (Moringa oleifera & citrus sinensis) shows the 2.1 cm of zone of inhibition.
- The extract (Neem, onion and tomato) shows 1.5 cm of zone of inhibition.
- The extract (citrus sinensis and neem) shown 1.1 cm of zone of inhibition.
- The standard drug (streptomycin) shows 3cm of zone of inhibition.



Graphical Representation

Table 1:

EXTRACTS	ZONE OF INHIBITION (in cms)
Neem and amla extract	2.1cm
Citrus sinensis and moringa oleifera	2.1 cm
Neem and citrus sinensis	1.1 cm
Neem, onion and tomato	1.5 cm
Streptomycin (standard drug)	3 cm

MEASURES OF ZONE OF INHIBITION:

- The zone of inhibition is measured using a ruler, calipers or a template

- Its size is measured in millimeters and is usually rounded off to the closest millimeter.

- The diameter of the antibiotic disk is also included in the measurement.
- These measurements are done by the naked eye without the help of any instrument.

RESULT AND DISCUSSION:

The standard drug Streptomycin shows more Anti-Microbial activity as compared to control (aqueous). The two plant extracts (Neem and Amla) and (Moringa oleifera and citrus sinensis) shows the 2.1 cm of zone of inhibition. And the two extracts (Neem, onion, tomato) and (citrus sinensis and neem) shows less zone of inhibition as compared to other two plant extracts. The results demonstrated that synthesis of silver nanoparticles occurred by reduction of Ag⁺ ions. During this process, the colour of the reaction mixture was converted from Yellow to brown indicates the presence of silver nanoparticles. The wavelength absorbed at 300 to 500nm.

Silver Nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical applications. AgNPs play an important role in nanoscience and nanotechnology, particularly in Nanomedicine.

CONCLUSION:

This research, we presented a detail overview about NPs, their types, synthesis, characterizations, physiochemical

properties and applications. Through different characterization techniques such as UV Spectrophotometer that NPs have size ranges from few nanometer to 500 nm. While the morphology is also controllable. Due to their tiny size, NPs have large surface area, which make them suitable candidate for various applications. Beside this, the optical properties are also dominant at that size, which further increase the importance of these materials in photocatalytic applications. Synthetic techniques can be useful to control the specific morphology, size and magnetic properties of NPs. Though NPs are useful for many applications, but still there are some health hazard concerns due to their uncontrollable use and discharge to natural environment, which should be consider for make the use of NPs more convenient and environmentally friendly. The plant extracts of (Neem and amla) and (citrus sinensis and moringa oleifera) has shown greater Anti-microbial activity compared to others. Synthesis of nanoparticles by using plants like neem, amla, citrus sinensis, moringa oleifera are useful and these plant shows good anti-microbial actions.

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