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Green Synthesis of Silver Nanoparticles by using Tulasi (Ocimum sanctum)

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ABSTRACT:
Recent advances in nanoscience and nanotechnology have fundamentally changed the way we diagnose, treat, and prevent various diseases in all aspects of human life. Silver nanoparticles (AgNPs) are one of the most important and attractive nanomaterials among several metal nanoparticles used in biomedical applications. AgNPs play an important role in nanoscience and nanotechnology, especially in nanomedicine. Although several noble metals are used for various purposes, AgNPs are highlighted for their potential applications in antifungal therapy.

In this review, we discuss the synthesis of AgNPs using physical, chemical, and biological methods. The properties of AgNPs and their characterization methods are also discussed. AgNPs are characterized using UV-visible, Fourier transform infrared [FTIR]. Antifungal activity was determined by minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). AgNPs can exert antifungal activity by disrupting the structure and integrity of cell membranes. AgNPs biosynthesized using plant extract [Ocimum sanctum] have tremendous antifungal potential and can be used to treat fungal infections.

KEYWORDS: Nanotechnology, Silver Nanoparticles, Antifungal, Biosynthesis**.**

I. INTRODUCTION:

In recent years, researchers in the field of nanotechnology have discovered that metal nanoparticles have all sorts of previously unexpected benefits. They are mainly made from precious metals, namely silver, gold, platinum, and palladium, with silver nanoparticles (AgNPs) being the most commonly used (1).It is applied in various fields such as medicine, electronics, energy saving, environment, textiles, and cosmetics. Then, the antibacterial activity of the synthesized nanoparticles will be measured. Due to its unique physical and chemical properties, it has applications in fields such as medicine, food, healthcare, consumer, and industry (2). Silver and its compounds have been used for antibacterial and therapeutic applications for thousands of years. Ancient Greeks and Romans used silverware to store water, food, and wine to prevent spoilage (3).

Hippocrates used silver preparations to treat ulcers and promote wound healing. Silver nitrate was also used for wound care and disinfection of instruments. Nanomaterials (1–100 nm materials) have attracted great attention in recent decades in many fields such as biomedicine, catalysis, energy storage, and sensors due to their unique physicochemical properties compared to their bulk forms. Silver nanoparticles (AgNPs) are of special interest, especially in biomedicine (4). AgNPs are known to have broad spectrum and highly efficient antibacterial and anticancer activities. Other biological activities of AgNPs have also been investigated, such as promoting bone healing and wound repair, improving vaccine immunogenicity, and antidiabetic effects. Deciphering the biological mechanisms and potential cytotoxicity of AgNPs will facilitate better medical applications of AgNPs(4, 5).

Ocimum Sanctum: Herb Tulasi, whose systematic name is "Holy Basil", has been known for its health-promoting and medicinal properties for thousands of years. It is a key herb in Ayurveda, India's ancient traditional holistic health system. Tulasi is also known as the “one of a kind”, the “mother medicine of nature” and the “queen of herbs”. Health benefits of Tulasi include skin care, dental care, respiratory diseases, asthma, fever, lung diseases, heart disease, and stress reduction. Tulasi protects against all types of infections from viruses, bacteria, fungi, and protozoa (5).

Recent studies have shown that it can also help inhibit the growth of cells that cause HIV and cancer. AgNPs are known for their broad spectrum of activity and high antibacterial activity. They can effectively kill a variety of pathogens, even at very low concentrations, including (i) bacteria such as Escherichia coli, Klebsiella pneumonia, and Staphylococcus aureus; (ii) Fungi such as Candida albicans and Aspergillus niger. (iii) viruses such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV) (6). Additionally, several studies have shown that AgNPs have nematicidal and anthelmintic effects. Common findings regarding the antibacterial mechanisms of AgNPs include disruption of bacterial cell walls, generation of reactive oxygen species (ROS), and damage to DNA structures (7).

In contrast to the risk of antibiotic resistance, which may limit medical applications, bacterial AgNP resistance is rarely observed (8). This can be attributed to the simultaneous and diverse antibacterial mechanisms of AgNPs. Green synthesis methods have several advantages over traditional chemical methods (9). They are simple, inexpensive, generally a one-step process, and do not involve harmful chemicals or biocompatible products (10). Generally, natural products are used for the green synthesis of NPs. Various biological systems such as yeast, bacteria, fungi, and plant extracts have been used to synthesize AgNPs. The biosynthesis of AgNPs based on plant extracts from Aloe vera, Murayakoenigii leaves, Mangosteen leaves (11), Camellia leaves, Mangiferaindica leaves, Jatropha curcas, mushrooms, tansy fruits, among others, has already been reported in the literature (12). There are different types of biocompatible polymers, such as gelatin and chitosan, are also used in the synthesis of AgNPs(13).

Figure 1:Tulasi(Ocimum sanctum)

- Genus: Basil
- Family: Lamiaceae.
- Kingdom:Plantae
- **Properties:**

Antimicrobial (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, anthelmintic), mosquito repellent, anti-diarrheal, anti-oxidant, anticataract, anti-inflammatory, chemopreventive, radioprotective (1, 2).

- **Uses**:

Lowers Stress and Anxiety (3,4), Stimulates and Vitalizes Your Body, Lower Your Blood Sugar (5),Lowers Cholesterol, Easing Inflammation and Joint Aches, Secures Your Stomach, Protection Against Wounds and Infections (6, 7, 8)`

II. MATERIAL AND METHODS:

Figure 2. Material and Methods (2).

A. Biological Method

Figure 3: Schematically Representation of Synthesis of AgNPs using (Ocimum sanctum L.) leaf extracts (2,3).

A1. Plant collection and identification:

Ocimum sanctumis an aromatic branched erect herb with 4 angled stems, bearded nodes, and lanceolate or ovate-lanceolate leaves. It is a common weed of open wastelands. For the study, fresh plants are to be collected from localities and brought to the laboratory in air-tight polythene bags for further processing (4).

A2. Preparation of leaf extract:

To prepare leaf extracts, fresh leaves are collected in a beaker, washed several times with water to remove dust, and finally washed with double-distilled water. 20 g of washed leaves were cut into small pieces and kneaded in 100 ml of double-distilled water using a mortar and pestle.

After grinding, the aqueous extract is placed in a 250 mL beaker and boiled for 5 min at a temperature of 80 °C. Boil on a hotplate or use induction. The plant extract was cooled to room temperature and then filtered through the Whatman filter paper. The filtrate was centrifuged at 10,000 rpm for 20–25 min, and the supernatant was collected and stored at 4°C. This filtrate was used as a stabilizing and reducing agent (4).

A3. Preparation of 1M silver nitrate (AgNO3) solution:

Silver nitrate (Central Drug House Ltd.) at a concentration of 1 M was prepared by dissolving 0.169 g of AgNO3 in 1 L of deionized water and stored in an amber bottle to prevent
Autooxidation of the silver nitrate solution (4, 5).

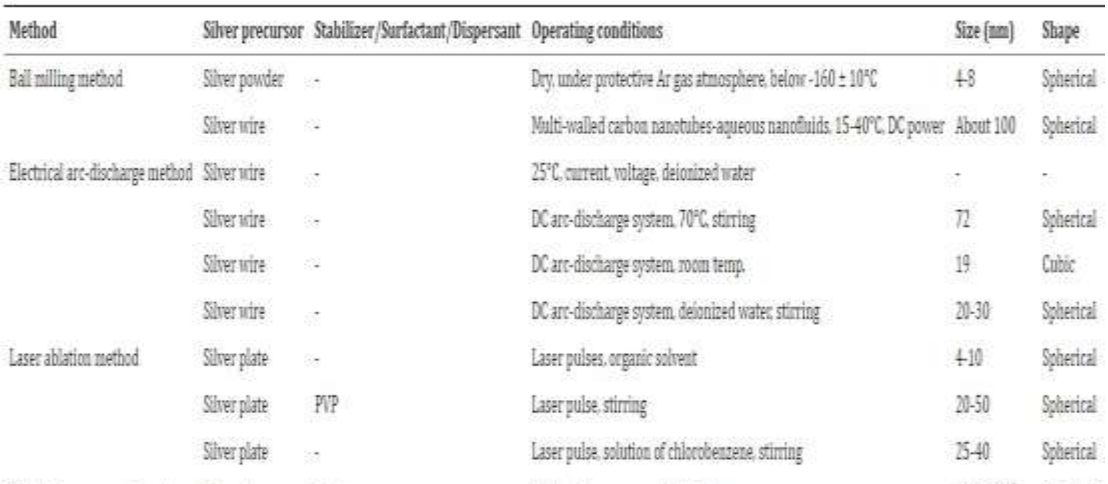
A4. Green synthesis of silver nanoparticles:

The production of silver nanoparticles (AgNPs) is a one-step synthesis. When 10 ml of Tulasi leaf extract prepared as described in section 2 above is to be added to 90 ml of silver nitrate solution, the color of the solution changes from pale yellow to brown. The synthesized Ag NPs are separated from the reaction mixture by centrifugation (5).

A5. Purification of silver nanoparticles:

To purify the nanoparticles, the synthesized AgNPs are stored in a 12 N hydrochloric acid solution for 24 h. AgNPs are isolated from the mixture by centrifugation. The mixture has to be washed with distilled water until the hydrochloric acid is completely removed (5).

B. Physical Methods



Method	Silver precursor	Stabilizer/Surfactant/Dispersant	Operating conditions	Size (nm)	Shape
Ball milling method	Silver powder	-	Dry, under protective Ar gas atmosphere, below -160 ± 10°C	4-8	Spherical
	Silver wire	-	Multi-walled carbon nanotubes-aqueous nanofluids, 15-40°C, DC power	About 100	Spherical
Electrical arc-discharge method	Silver wire	-	25°C, current, voltage, deionized water	-	-
	Silver wire	-	DC arc-discharge system, 70°C, stirring	72	Spherical
	Silver wire	-	DC arc-discharge system, room temp.	19	Cubic
	Silver wire	-	DC arc-discharge system, deionized water, stirring	20-30	Spherical
Laser ablation method	Silver plate	-	Laser pulses, organic solvent	4-10	Spherical
	Silver plate	PVP	Laser pulse, stirring	20-50	Spherical
	Silver plate	-	Laser pulse, solution of chlorobenzene, stirring	25-40	Spherical
Physical vapour condensation	Silver wire	Fructose	High voltage power, rapid cooling	19.2±3.8 Ång	Spherical

Table no. 1: Synthesis of AGNPs by Physical Method (3).

B1. Ball Milling Method:

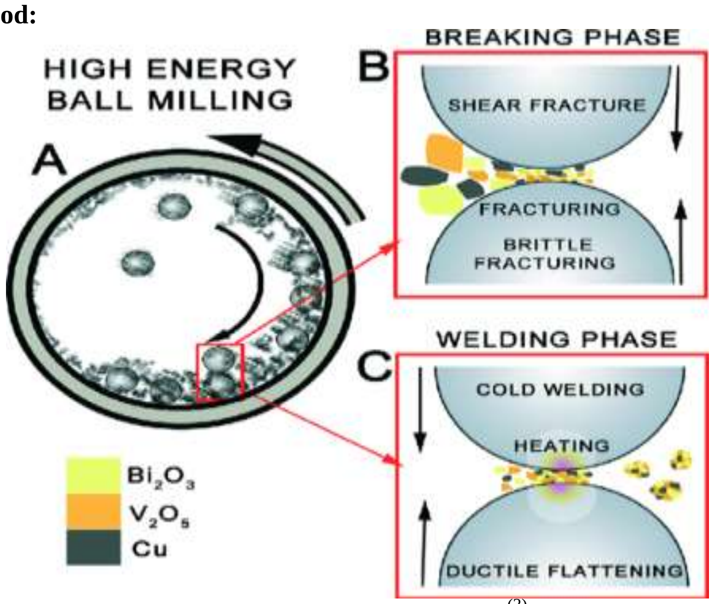


Figure 4: Ball Milling Method (3).

In mechanical ball crushing technology, grinding balls, a certain mass ratio of metal materials, and gas (air or inert gas) are placed in a container that rotates at high speed. Grinding, rotation speed, and atmospheric medium in ball milling play important roles in the morphology of metal materials. A proper diet is closely related to the production of particles of a satisfactory size. Particles preferentially agglomerate because smaller particles have higher surface energy. The temperature of the powder during the ball milling process affects the diffusivity and phase of the nanoparticles. In general, higher powder temperatures tend to result in the synthesis of intermetallic compounds, whereas lower temperatures tend to form amorphous and nanocrystalline phases (3).

B2. Electrical Arc-Discharge Method:

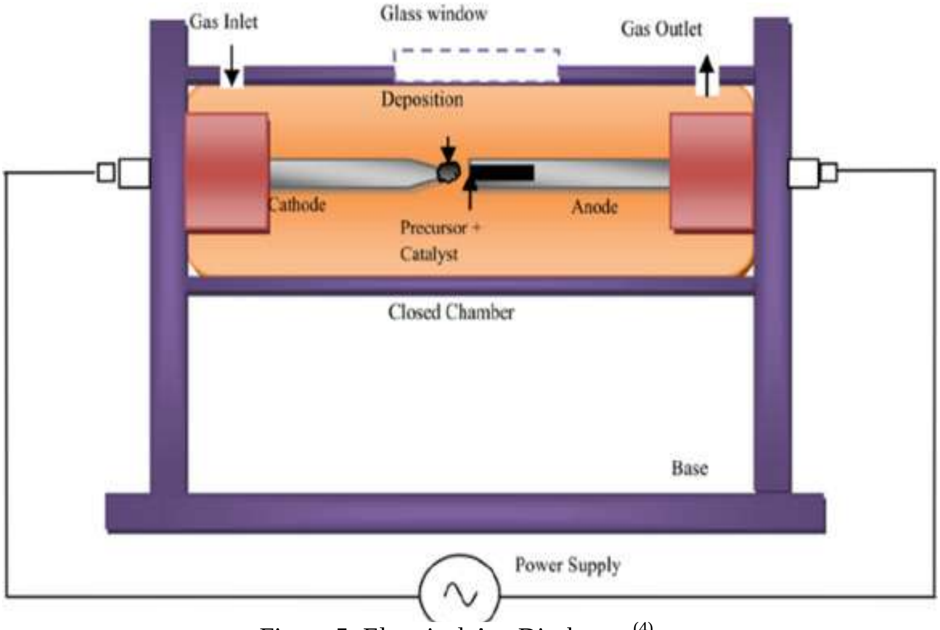


Figure 5: Electrical Arc Discharge (4).

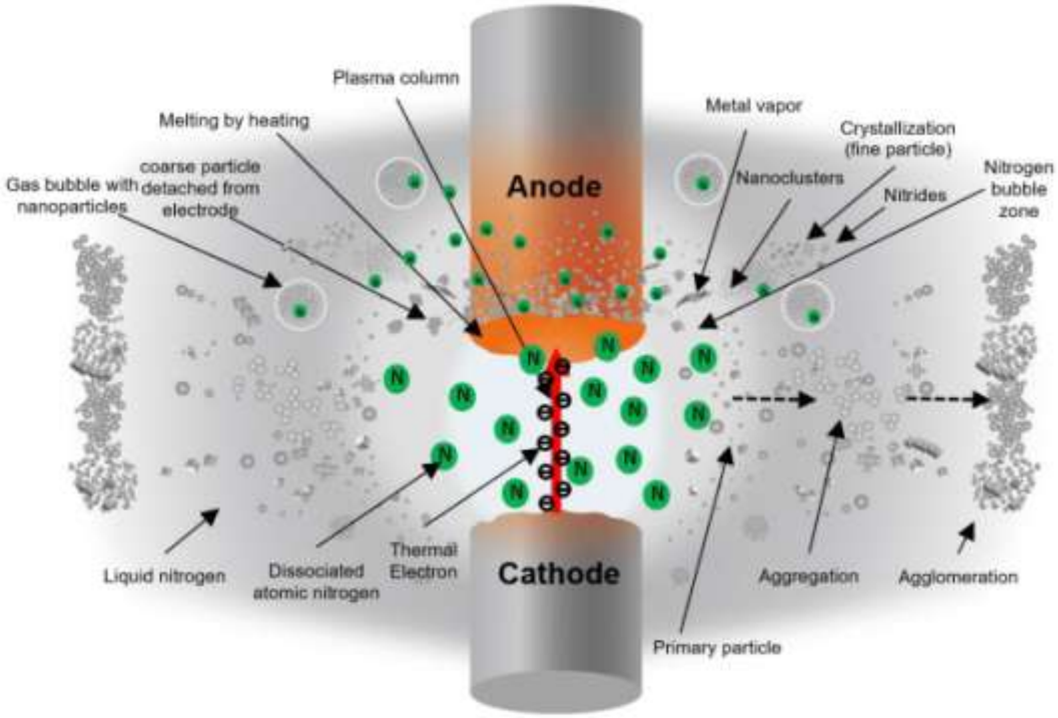


Figure 6: Electrical Arc-Discharger.

The arc discharge device consists of a direct current between two silver rods immersed in a dielectric liquid. During the arc discharge process, the silver electrode is etched into the dielectric medium, and the high temperature near the electrode evaporates the surface of the silver electrode (5). Next, the silver vapor is condensed into AgNPs and suspended in a dielectric liquid. This device can obtain pure AgNPs with a simple and inexpensive device (6).

B3. Laser Ablation Method:

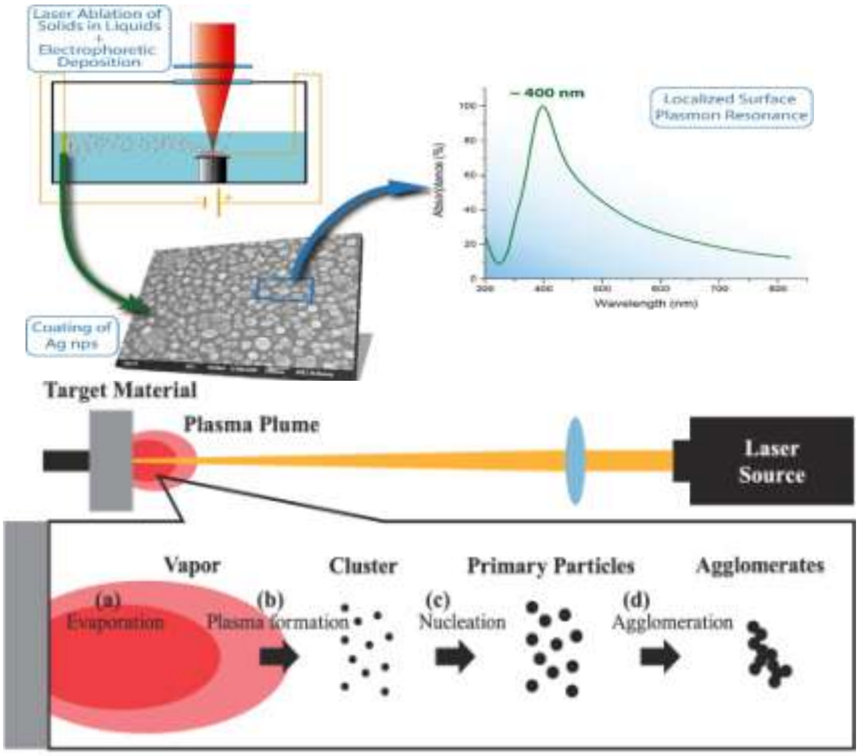


Figure 7: Laser Ablation Method (6).

The laser ablation method involves momentarily heating a target metal mass immersed in water or an organic solvent with a pulsed laser to form a plasma plume, followed by nucleation and growth of metal particles during the cooling process of the plasma plume, and eventually, metal particles grow. Formation of nanoscale clusters. During the process of laser ablation, nanoparticles may absorb photons through multiple pathways such as plasmon excitation, interbond transition, and multiphoton absorption, which are closely related to pulse time, laser wavelength, and laser fluence (6). These factors and the type of aqueous medium can influence the properties of NPs. Various synthesis conditions such as laser fluence, pulse wavelength, and solvent type can affect the size of NPs. The addition of organic stabilizers such as cetyl-trimethylammonium bromide (CTAB) and PVP can improve the dispersibility of AgNPs. However, it is difficult to control the size distribution of NPs using laser ablation methods (7, 8).

B4. Physical Vapor Deposition Method:

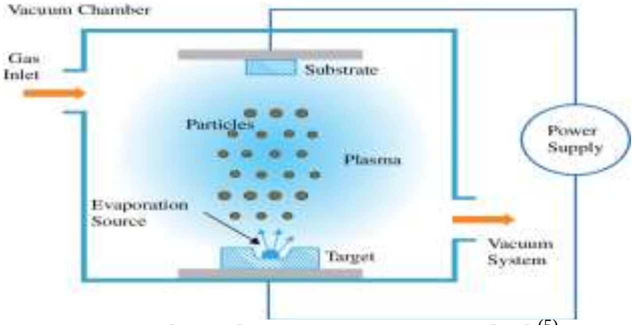


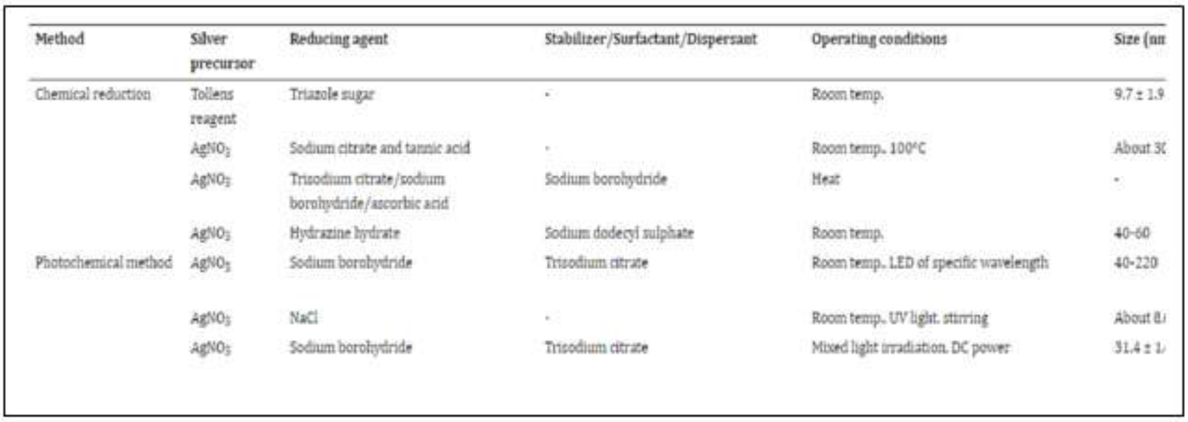
Figure 8: Physical Vapor Deposition Method (5).

The basic and most commonly used physical vapor deposition processes fall into two general categories: arc evaporation and sputtering. The former involves using a cathodic arc source in a vacuum chamber or shielding gas to obtain metal vapor and deposit it onto a target coating material to form a thin, adherent pure metal or alloy coating point. Plasma is produced by highly ionized metal vapors. The latter refers to the use of high-energy electrical charges to bombard the target coating material and deposit metal onto the substrate (5, 14).

C. Chemical Method

Currently, chemical synthesis is the most common method to synthesize AgNPs. This process involves the reduction of Ag+ (supplied by a silver salt precursor) to elemental silver (AgNP) by electron transfer under certain conditions (15, 16, 17). Chemical synthesis is generally facilitated by reducing agents such as sodium borohydride ($NaBH_4$) and sodium citrate (TSC). Chemical methods can be combined with external energy sources such as photochemical, electrochemical, microwave-assisted, and some chemical methods to produce AgNPs(18).

Among these methods, the production process of AgNPs can be divided into two parts: nucleation and growth. The monomer concentration in solution quickly rises above the critical value of supersaturation, causing “burst nucleation” and precipitation (19). Precipitation of monomers forms the germ, and repeated germination processes promote the continued formation of new seeds. During nucleation, the monomer concentration falls below the critical value of supersaturation. After nucleation, increasing the amount of monomer added induces the growth of the nuclei, forming NPs with larger sizes. During the synthesis process, stabilizers such as PVP and CTAB are commonly used to stabilize and disperse AgNPs. Although the chemical method for AgNPs is a reliable, high-yielding, time-saving, and controllable method, it should be noted that the chemicals used in this method can cause environmental pollution is important (20).



Method	Silver precursor	Reducing agent	Stabilizer/Surfactant/Dispersant	Operating conditions	Size (nm
Chemical reduction	Tollens reagent	Triazole sugar	-	Room temp.	9.7 ± 1.9
	$AgNO_3$	Sodium citrate and tannic acid	-	Room temp., 100°C	About 3
	$AgNO_3$	Trisodium citrate/sodium borohydride/ascorbic acid	Sodium borohydride	Heat	-
	$AgNO_3$	Hydrazine hydrate	Sodium dodecyl sulphate	Room temp.	40-60
Photochemical method	$AgNO_3$	Sodium borohydride	Trisodium citrate	Room temp., LED of specific wavelength	40-220
	$AgNO_3$	NaCl	-	Room temp., UV light, stirring	About 8
	$AgNO_3$	Sodium borohydride	Trisodium citrate	Mixed light irradiation, DC power	31.4 ± 1

Table no. 3: Chemical Methods to Synthesize of AgNPs(4).

III. CHARACTERISATION:

The physicochemical properties of nanoparticles are important for their behavior, biodistribution, safety, and efficacy. Therefore, the characterization of AgNPs is important to evaluate the functional aspects of the synthesized particles. Characterization is performed using a variety of analytical techniques, including (2, 3, 4).

3.1 UV-vis Spectroscopy (1)
3.2 X-ray Diffractometry (XRD) (2, 3, 4)
3.3 Fourier Transform Infrared Spectroscopy (FTIR) (5)
3.4 Dynamic Light Scattering (DLS) (6)
3.5 Scanning Electron Microscopy (SEM) (6, 7)
3.6 Transmission Electron Microscopy (TEM) (8)
3.7 Atomic Force Microscopy (AFM) (10)

3.1UV-vis Spectroscopy:

UV-vis spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles and is also used to monitor the synthesis and stability of AgNPs(1). 1 AgNPs have unique optical properties that cause strong interactions with light at specific wavelengths. Moreover, UV-vis spectroscopy is fast, simple, easy, sensitive, selective for different types of NPs, requires short measurement time, and finally, it is easy to use calibrators for particle characterization of colloidal suspensions is not required. In AgNPs, the conduction and valence bands are very close, allowing electrons to move freely (2, 3). These free electrons give rise to surface plasmon resonance (SPR) absorption bands formed by collective oscillations of the silver nanoparticle's electrons in resonance with the light waves. The absorption of AgNPs depends on the particle size, dielectric medium, and chemical environment. The observation of this peak associated with surface plasmons is well documented because of a variety of metal nanoparticles ranging in size from 2 to 100 nm. The stability of AgNPs prepared by biological methods was observed over 12 months, and SPR peaks were observed at the same wavelength using UV-vis spectroscopy (4).

3.2 X-ray diffractometry (XRD):

X-ray diffraction (XRD) is a common analytical technique used to analyze molecular and crystal structure, qualitatively identify various compounds, quantitatively separate chemical species, and measure crystallinity, isomorphic substitution, and particle size. XRD measurements were recorded using a RirakuMiniflex II X-ray diffractometer. For XRD measurements, AgNPs were oven-dried at 60 °C, and the dried powders were further analyzed by XRD for phase structure and precise material identification. The α line (Å) of Cu was selected and diffractograms were obtained in the 2θ range of 10–60 degrees. When X-ray light reflects off a crystal, many diffraction patterns are formed, and the patterns reflect the physicochemical properties of the crystal structure (5).

3.3 Fourier Transform Infrared Spectroscopy (FTIR):

The binding properties of AgNPs synthesized by Tulasi leaf extract were studied by FTIR analysis. FTIR measurements were performed on a Bruker Vertex 70. The dried and powdered AgNPs were palletized with potassium bromide (KBr) (ratio 1: 10).Spectra were recorded in the wavenumber range 450–2500 and analysed by subtracting the spectrum of pure KBr(3, 5, 11).

3.4 Dynamic Light Scattering (DLS):

Physicochemical characterization of prepared nanomaterials is an important element for the analysis of biological activity using radiation scattering techniques. DLS can study the size distribution of small particles ranging from sub-micrometers to 1 nanometer in solution or suspension (6). Dynamic light scattering is a method based on the interaction of light and particles (12, 14). This method can be used to measure narrow particle size distributions, especially in the range 2 to 500 nm. Among nanoparticle characterization techniques, DLS is the most commonly used (15). DLS measures the scattered light of a laser transmitted through a colloid and is primarily based on Rayleigh scattering of suspended nanoparticles (16).

3.5 Scanning electron microscopy (SEM):

Recently, the fields of nanoscience and nanotechnology have been a driving force behind the development of various high-resolution microscopy techniques to learn more about nanomaterials, using high-energy electron beams to study objects at very fine scales. Among various electron microscopy techniques, SEM is a surface imaging method that can completely resolve different particle sizes, size distributions, shapes of nanomaterials, and surface morphologies of synthetic particles at the micro- and nanoscale. SEM allows you to manually measure and count particles or use specialized software to inspect particle morphology and derive histograms from the images (17).

3.6 Transmission electron microscopy (TEM):

TEM is a valuable and important technique commonly used for the characterization of nanomaterials, allowing quantitative measurements of particle and/or particle size, size distribution, and morphology. The magnification in TEM is mainly determined by the ratio of the distance between the objective and the sample and the distance between the objective and its image plane (16, 17).

3.7 Atomic force microscopy (AFM):

Generally, AFM is used to study the size, shape, adsorption, structure, as well as dispersion and aggregation of nanomaterials (17). Three different scanning modes are available, including contact mode, non-contact mode, and intermittent sample contact mode. AFM can also be used to characterize interactions between nanomaterials and supported lipid bilayers in real-time, which is not possible with current electron microscopy (EM) techniques (18, 19).

IV. APPLICATION OF AGNPs:

Due to their unique properties, AgNPs are widely used in household products (21), healthcare (22, 23), food preservation, environmental, and biomedical applications (24).

Several reviews and book chapters are devoted to different application areas of AgNPs. Here, we would like to focus on the application of his AgNPs in various biological and biomedical applications, such as antibacterial, antifungal, antiviral, anti-inflammatory, anticancer, and antiangiogenic effects (24, 25). Here, we specifically review the groundbreaking studies published to date and conclude with recent updates. A schematic diagram illustrating the various applications of AgNPs is shown in the figure.

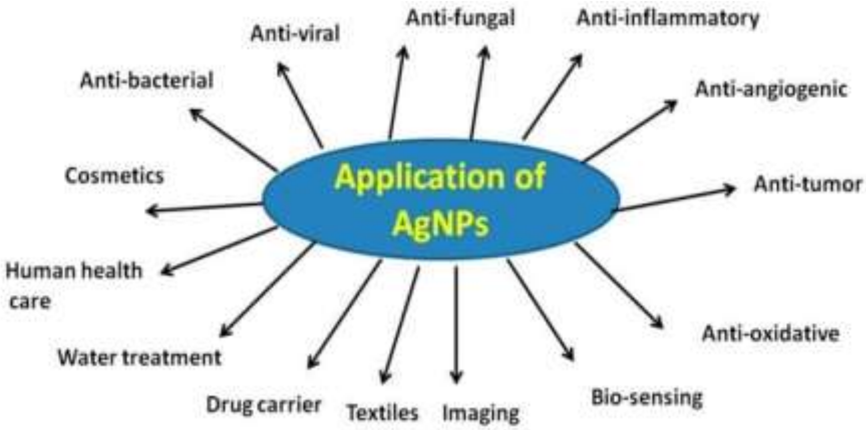


Figure 9: Applications of AGNPs (4, 5).

4.1 Antimicrobial Mechanisms of AgNPs:

The antibacterial effect of AgNPs has been extensively studied, and its mechanism is currently under investigation. AgNPs have been observed to immobilize and penetrate bacterial membranes, subsequently causing cell membrane disruption and leakage of contents (20, 21). The antibacterial mechanism of AgNPs is shown in the figure. AgNPs also exhibit excellent bactericidal activity against drug-resistant bacteria by affecting cellular targets involved in drug resistance and pathogenesis.

For example, Venkatraman et al. showed that AgNPs may influence drug susceptibility by acting on multiple cellular targets in Candida albicans, including fatty acids such as oleic acid, which are important for hyphal morphogenesis involved in pathogenesis (22).

It was shown that some studies speculate that AgNPs can saturate and adhere to fungal hyphae and ultimately inactivate the fungi. AgNPs can block cell contact and entry steps or directly inactivate viruses, including herpes simplex virus (HSV), human parainfluenza virus type 3, vaccinia virus, chikungunya virus, and respiratory syncytial virus (23, 24).

It can be used to prevent virus infections against multiple viruses (25).

These studies suggest that AgNPs can be used as novel and promising virucides(26).

To develop safe and effective antibacterial agents, the still-unexplored mechanisms of the antibacterial properties of AgNPs need to be further investigated (27).

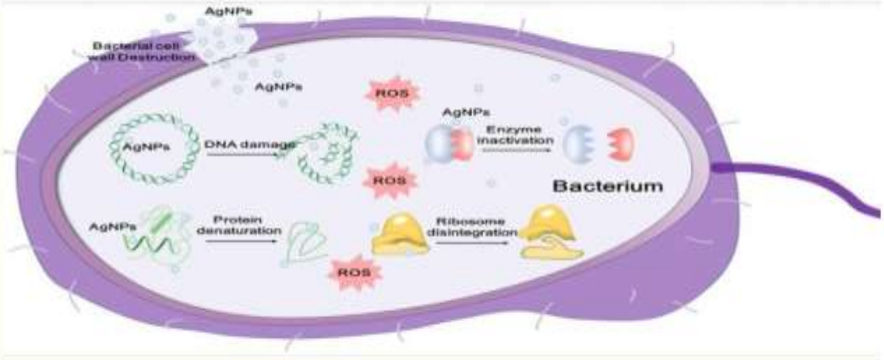


Figure 10: Schematic Representation of the Mechanisms of AgNPs against Bacteria Candida albican(4).

4.2 Antifungal and Antiviral Activities of AgNPs(3):

Some studies confirm that AgNPs have good antifungal properties against Colletotrichum coccodes, Moniliniasp, Candida spp., and various plant pathogenic fungi in size- and dose-dependent manners. Some studies also point out that the type of culture media used in their experiments may also affect the inhibition activity (4, 5).Besides, AgNPs also show good antiviral activity against hepatitis B virus (HBV), human parainfluenza virus (HPIV), herpes simplex virus (HSV), and influenza A (H1N1) virus. AgNPs with less than 10 nm size exhibit good antiviral activity, which may be due to their large reaction area and strong adhesion to the virus surface (6, 7).

For example, AgNPs can bind to the glycoprotein knobs and inhibit the reverse transcriptase (RT) of HIV-1 and interact with the virus in a size- and dose-dependent manner. To develop AgNPs for antimicrobial applications, the detailed mechanism needs to be further studied (7).

4.3 Diagnostic, Biosensor, and Gene Therapy Applications of AgNPs:

The headway in clinical advancement is expanding. Utilizing nanoparticles to enhance or replace current treatments is a topic of intense interest (4). Because they can be engineered to have particular properties or behave in a particular manner, nanoparticles offer advantages over current treatments (5, 6). The use of nanoparticles in the creation of novel and efficient medical diagnostics and treatments is one recent innovation in nanotechnology (7, 8). The capacity of AgNPs in cell imaging in vivo could be extremely helpful for concentrating on aggravation, growth, resistant reaction, and the impacts of immature microorganism treatment (10), where, in contrast, specialists were formed or embodied to nanoparticles through surface change and bioconjugation of the nanoparticles (10, 11). Silver assumes a significant part in imaging frameworks due to its more grounded and plasmon reverberation (13, 14). By increasing the acoustic reflectivity, AgNPs are primarily utilized in diagnostics, therapy, and combined therapy and diagnostic approaches due to their smaller size (15). This ultimately results in an increase in brightness and the production of a clearer image (16). Nanosilver has been seriously utilized in a few applications, including conclusion and therapy of malignant growth and as a medication transporters(17, 18). Nanosilver was utilized in blending with vanadium oxide in battery cell parts to further develop the battery execution in cutting-edge dynamic implantable clinical gadgets (19, 20). As of late, silver has been utilized to foster silver-based biosensors for the clinical identification of serum p53 in head and neck squamous cell carcinoma (21,22). Moreover, it has been investigated for the area of disease cells and can ingest light and specifically annihilate designated malignant growth cells through photothermal treatment (25).

V. CONCLUSION:

In conclusion, it can be said that green synthesis of silver nanoparticles is a promising and environmentally friendly approach using natural extracts or plant materials. This method not only produces silver nanoparticles that can be used in a wide range of applications but is also consistent with sustainable practices for minimizing environmental impact. The synthesized nanoparticles show potential in various fields such as medicine, catalysis, and electronics. As research in this field advances, green synthesis approaches are emerging as a viable and socially responsible method for producing silver nanoparticles·

Additionally, this environmentally friendly synthesis method offers advantages such as cost-effectiveness, scalability, and avoidance of harmful chemicals commonly associated with traditional synthetic routes· The use of plant extracts or bio-based reducing agents adds a layer of biocompatibility to the produced silver nanoparticles, increasing their potential for biomedical applications. As the scientific community continues to explore and refine green synthesis techniques, we expect to see further development of silver nanoparticles as versatile and sustainable materials with far-reaching implications in various scientific and industrial fields.

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