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Design, Preparation And Characterization Of Silver Nanoparticle Containing Herbal Plant Extract Of *Campsis Grandiflora* And Its Anti-Bacterial Activity

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Abstract

Nanotechnology has emerged as the most promising fields in pharmaceutical and biomedical sciences owing to its wide range of applications in drug delivery, diagnostics, antimicrobial therapy, and tissue engineering. Among various metallic nanoparticles, silver nanoparticles (AgNPs) have gained remarkable attention because of their potent antibacterial, antioxidant, anti-inflammatory, and wound healing properties. Due to its affordability, biocompatibility, and environmental friendliness, green synthesis of silver nanoparticles utilizing extracts from medicinal plants has grown in popularity as an alternative to traditional physical and chemical processes. Campsis grandiflora is a medicinally important plant rich in phytoconstituents such as flavonoids, phenolics, tannins, alkaloids, and terpenoids, which act as natural reducing and stabilizing agents during nanoparticle synthesis. Silver nanoparticles synthesized using *Campsis grandiflora* extract exhibit enhanced antibacterial activity against several pathogenic microorganisms including Gram-positive and Gram-negative bacteria. This review article discusses the design, green synthesis, preparation methods, physicochemical characterization, and antibacterial evaluation of silver nanoparticles containing *Campsis grandiflora* extract. The article also highlights the mechanisms of antibacterial action, factors influencing nanoparticle synthesis, and potential pharmaceutical and biomedical of these nanomaterials. The synergistic interaction between silver nanoparticles and plant-derived phytochemicals makes these systems promising candidates for development of advanced antimicrobial formulations.

Keywords: Silver nanoparticles, *Campsis grandiflora*, green synthesis, antibacterial activity, herbal extract, nanotechnology, characterization, phytochemicals.

1. Introduction

The pharmaceutical and biomedical sciences' fastest-growing sectors is nanotechnology, which offers creative methods for creating new therapeutic systems with improved efficacy and decreased toxicity. The manipulation of materials at the nanoscale level, typically between 1 and 1000 nm, where distinct physicochemical and biological characteristics arise due to increased surface area and quantum effects, is called as nanotechnology. Silver nanoparticles have attracted a lot of interest among other nanomaterials because of their exceptional antibacterial, antioxidant, anti-inflammatory, and wound-healing qualities **(Malik *et al.,* 2023).**

Green manufactured nanoparticles employing extracts from medicinal plants have been developed as a result of the combination of nanotechnology and herbal medicine. Compared to traditional physical and chemical synthesis methods, plant-mediated nanoparticle synthesis is thought to be an environmentally friendly, economical, and biocompatible method. Numerous phytoconstituents found in herbal extracts, including flavonoids, phenolics, alkaloids, tannins, terpenoids, and glycosides, serve as stabilizing and reducing agents during the creation of nanoparticles **(Godeto *et al.,* 2023).**

Herbal formulations based on nanotechnology offer number of benefits, including as better stability, greater bioavailability, controlled release, targeted distribution, and more therapeutic activity. Herbal extract-derived silver nanoparticles have shown strong antibacterial activity against range of pathogenic microbes, making them attractive options for use in pharmaceutical and biomedical applications **(Simon *et al.,* 2022).**

2. Silver Nanoparticles

The main uses of silver nanoparticles are as catalysts, optical sensors of zeptomole concentration, textile engineering, electronics, optics, and—most importantly—as a bactericidal and therapeutic agent in the medical area. Dental resin composites, medical device coatings, water filters, air sanitizer sprays, pillows, respirators, socks, wet wipes, detergents, soaps, shampoos, toothpastes, washing machines, bone cement, and numerous wound dressings are just a few of the consumer goods that use silver ions as an antimicrobial agent. Although silver nanoparticles have many advantages, there is also the issue of silver's nanotoxicity. Although additional research is determine whether silver nanoparticles actually pose a threat to health and the environment, number of studies have suggested that they may **(Bouafia *et al.,* 2021).**

3. Properties of silver nanoparticles

Silver NPs have unique physical and chemical characteristics because of their small size and high surface-area-to-volume ratio, which reflect typical NP characteristics.

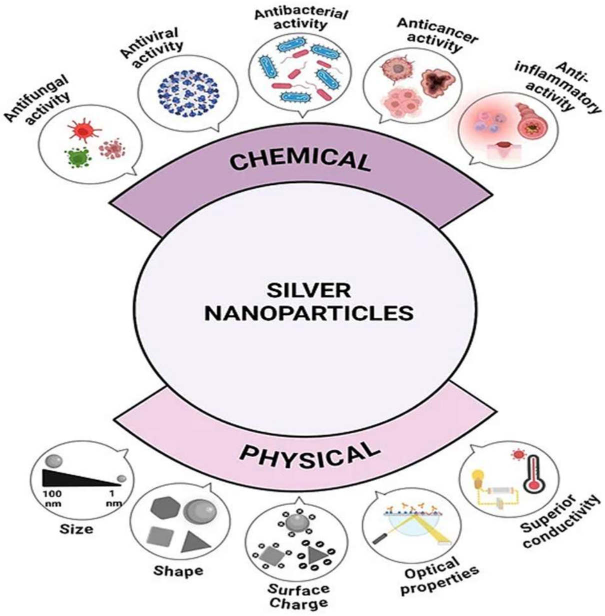

Figure 1: Illustration of physical and chemical traits of silver NPs

3.1 Size

The key elements influencing the physical, chemical, and biological characteristics of silver nanoparticles is their size. It greatly affects their conductivity, optical properties, and surface-area-to-volume ratio while also opening up potential in different disciplines. In solution or on surfaces, their tiny size enhances their reactivity and interaction with the surrounding molecules. Because of their size-dependent surface characteristics, silver nanoparticles are used as catalysts in catalysis and sensing investigations, where this enhanced reactivity is mostly beneficial **(Pareek *et al.,* 2018).**

3.2 Shape

Silver NPs can be synthesized in various shapes, including spherical, rod-shaped, triangular, cubic, wire-like, and star-shaped. Many properties, such as optical, catalytic, and electrical, are influenced by the shape of the silver NPs. For instance, the shape and size of silver NPs significantly strengthen their interactions with biological systems. Thus, they are suitable for employment in the biomedical area, especially in drug delivery and coatings. Additionally, antimicrobial activity may be effected by rate of silver ions released from silver NPs. In this manner, spherical NPs are highly favoured since they are capable of releasing silver ions efficiently due to higher surface-area-to-volume ratio in comparison to shapes, such as triangular plates and disks **(Duman *et al.,* 2024).**

3.3 Surface Charge

The surface charge is the important properties of NPs that significantly affects their stability and interactions with other molecules. Manipulating the surface charge allows for precise control of NP behavior in different environments, thus influencing their aggregation, solubility, and reactivity. In line with that, surface charge is also considered essential in biomedical applications, where modified surface charges improve targeting, uptake, and therapeutic efficiency. In addition, surface charges can be manipulated through pH adjustments as surface functionalization. Overall, these charges influence their dispersion in solvents or matrices and also their interactions with other biomolecules or surfaces **(Xu *et al.,* 2018).**

3.4 Electrical Conductivity and Melting Point

Bulk silver has the highest electrical conductivity at room temperature compared to other metals due to atomic structure and existence of free electrons in silver. Similar to other metals, silver has a single valence electron in the outermost shell that is not tightly bound to the nucleus. Therefore, it allows the electrons to move freely throughout the metals, allowing a conductive pathway. In other words, when an electric field is applied, these electrons will move easily, which allows the electric current to flow with minimal resistance. These free movements of the electrons are primary factor of silver's electrical conductivity with its utilization in conductive composite formulations with both organic and inorganic materials **(Cheng *et al.,* 2015).**

3.5 Thermal conductivity

Silver is renowned for its remarkable thermal conductivity, which is around 429 W/mK at ambient temperature. This property allows silver to easily transmit heat. Silver NPs possess exceptional heat conductivity due to high surface-area-to-volume ratio, which remains consistent even at the nanoscale. The primary cause of thermal conductivity is the effective heat transmission via electrons, which is aided by the particles' small size. This reduces scattering and resistance at the grain boundaries. Silver NPs offer superior thermal conductivity, reduced contact resistance, and oxidative resistance, making them suitable for various technological and industrial applications to their lower sintered temperatures **(Mo *et al.,* 2019).**

4. Mechanism involved in silver nanoparticles

Not all the organisms are found to be competent for the synthesis of silver nanoparticles. As previously mentioned, those organisms which contain the “Silver resistance machinery” can synthesize silver nanoparticles provided that concentration of silver ions does not cross the “threshold limit”. The resistance mechanism differs with organisms. Extracts from bio-organisms may act both as reducing and capping agents in Ag NPs synthesis. The Ag^+ ions reduction by combinations of biomolecules found in these extracts such as enzymes/proteins, amino acids, polysaccharides and vitamins is environmentally benign, yet chemically complex. But the mechanism which is widely accepted for the synthesis of silver nanoparticles is presence of enzyme “Nitrate reductase” **(Khodke *et al.,* 2017).** Nitrate reductase is an enzyme in nitrogen cycle responsible for the conversion of nitrate to nitrite. The reduction mediated by presence of the enzyme in organism has been responsible for the synthesis. The specific enzyme a NADPH dependent nitrate reductase in the in vitro synthesis of nanoparticles is important because this would do away with the downstream processing required for use of these nanoparticles in homogeneous catalysis and nonlinear optics. During the catalysis, nitrate is converted to nitrite, and an electron will be shuttled to the incoming silver ions. This has been excellently described in the organism B. licheniformis. B. licheniformis is known to secrete the cofactor NADH and NADH-dependent enzymes, especially nitrate reductase, that might be responsible for the bioreduction of Ag^+ to Ag^0 and the subsequent formation of silver nanoparticles **(Hietzschold *et al.,* 2019).**

Although silver nanoparticles synthesis is considered as a "capability" of the organism, it is primarily considered as a defense mechanism by the organisms to the incoming very reactive silver ions. Interesting facts about silver nanoparticle synthesis can be understood when the real mechanism involved in antimicrobial activity of silver ions is known. Silver ions are very reactive and are known to bind with various vital components of the cells inducing cell death **(Khalandi *et al.,* 2017).**

5. Synthesis of silver nanoparticles

The synthesis of silver nanoparticles (AgNPs) encompasses physical, chemical, and biological (green) approaches, each offering distinct advantages and limitations based on application demands and environmental considerations.

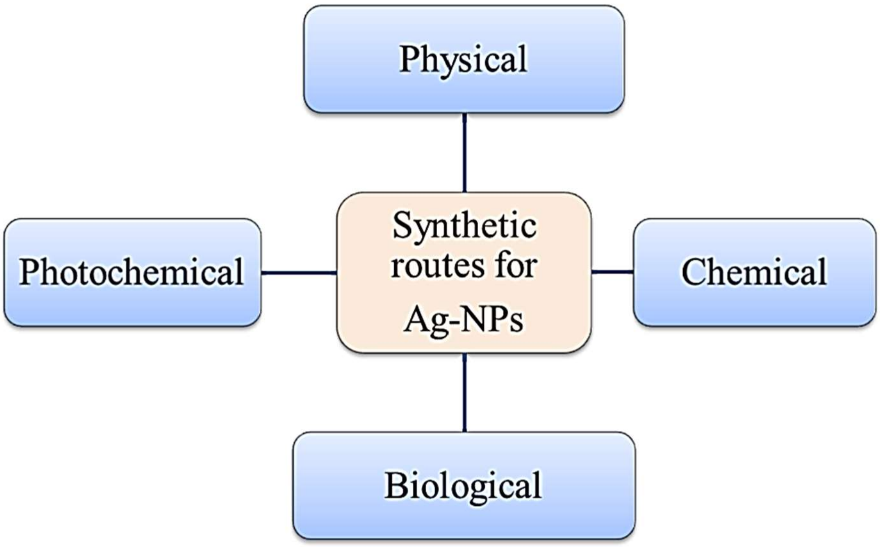

Figure 2: Synthesis of silver nanoparticle

A. Physical methods: -

Evaporation-condensation and laser ablation are the important physical approaches. The uniform distribution of NPs and absence of solvent contamination in resulting thin films are two benefits of synthesis techniques over chemical ones. There are many drawbacks to physically producing silver nanoparticles in a tube furnace at atmospheric pressure. For example, the tube furnace is large, requires a lot of energy to raise the temperature around the source material, and takes a long time to achieve thermal stability **(Hachem *et al.,* 2022).** Furthermore, to reach a constant operating temperature, a conventional tube furnace needs to warm for many tens of minutes and consume more than a few kilowatts of power. It was shown that a small ceramic heater with a local heating region may be used to create silver nanoparticles. The small ceramic heater was used to evaporate the source materials. The evaporated vapor can cool at a suitable rapid rate because the temperature gradient close to the heater surface is significantly steeper than in a tube furnace **(Li *et al.,* 2022).**

B. Chemical methods: -

1. Chemical reduction: -

Chemical reduction, which employs both organic and inorganic reducing chemicals, is the most used method for creating silver nanoparticles. Silver ions (Ag+) in aqueous or non-aqueous solutions are usually reduced using a variety of reducing agents, including sodium citrate, ascorbate, sodium borohydride (NaBH4), elemental hydrogen, polyol process, Tollen's reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers. These reducing agents decrease Ag+, producing metallic silver (Ag0), which then agglomerates into oligomeric clusters. Eventually, these clusters result in the creation of metallic colloidal silver particles. Applying protective chemicals is essential during the production of metal nanoparticles in order to stabilize dispersive NPs and avoid NP agglomeration that could bind or be absorbed on the surfaces of the nanoparticles. Thiols, amines, acids, and alcohols are examples of surfactants that can interact with particle surfaces to stabilize particle growth and protect particles against agglomeration, sedimentation, and loss of surface properties **(Yonezawa *et al.,* 2021).**

2. Microemulsion Technique

Microemulsion techniques can be create uniform and size-controllable silver nanoparticles. The initial spatial separation of reactants (metal precursor and reducing agent) in two immiscible phases is the basis for the synthesis of NPs in two-phase aqueous organic systems. The rate at which metal precursors and reducing agents interact depends on the degree of interphase transfer between two alkyl-ammonium salts and the contact between the two liquids. Metal clusters that form at the interface are stabilized and transferred to the organic medium by the inter-phase transporter because the surface of the non-polar aqueous medium is covered with stabilizer molecules. The main drawbacks are extremely harmful organic solvents **(Dheeksha, 2021).**

3. UV-initiated photoreduction

For the synthesis of silver nanoparticles in the presence of citrate, polyvinylpyrrolidone, poly (acrylic acid), and collagen, a straightforward and efficient technique called UV-initiated photoreduction has been documented. For instance, Huang and Yang produced silver nanoparticles by photoreducing silver nitrate in solutions of layered inorganic laponite clay, which served as a stabilizing agent to prevent NPs from aggregating. The properties of the produced NPs were investigated in connection with the length of UV exposure. A size distribution and rather large silver nanoparticles were generated following three hours of UV exposure. Further irradiation was used to break up the silver NPs into smaller sizes using a single distribution mode until a reasonably stable size and size distribution were achieved. Silver nanoparticles were produced at room temperature using poly (vinyl alcohol) and the UV irradiation photoreduction technique. The concentration of poly (vinyl alcohol) and silver nitrate had a major impact on the formation of the nanorods and dendrites **(Zahoor *et al.,* 2021).**

4. Photoinduced reduction

Silver nanoparticles can be produced using a variety of photoinduced or photocatalytic reduction methods. A clean method with great adaptability, ease of use, and excellent spatial resolution is photochemical synthesis. NPs are also produced via photochemical synthesis in a range of media, including glasses, polymer films, emulsions, cells, and surfactant micelles. Using poly (styrene sulfonate)/poly (allylamine hydrochloride) polyelectrolyte capsules as microreactors, photoinduced reduction was used to create silver nanoparticles with an average size of 8 nm. Furthermore, it was demonstrated that silver nanospheres may be converted into triangular silver nanocrystals, or nano prisms, with the required edge lengths in the range of 30 to 120 nm using the photoinduced method. Dual-beam lighting of NPs regulated the particle development process. Two stabilizing agents were poly (styrene sulfonate) and citrate **(Sharma *et al.,* 2021).**

5. Electrochemical synthetic method

Silver nanoparticles are created via an electrochemical synthesis technique. By modifying the electrolysis settings, particle size may be controlled, and by altering the electrolytic solution composition, silver nanoparticle homogeneity can be enhanced. Electrochemical reduction at the liquid/liquid interface produced silver nanospheres (3–20 nm) coated with polyphenylpyrrole. This nanocompound was produced by the transfer of silver metal ions from the aqueous phase to the organic phase, where they reacted with pyrrole monomer. In a different study, depending on the extent of silver exchange of compact zeolite film modified electrodes, electrochemical reduction inside or outside of zeolite crystals created monodisperse silver nanospheroids (1–18 nm). Furthermore, spherical silver nanoparticles (NPs) with narrow size distributions in aqueous solution (10–20 nm) were easily produced using an electrochemical method. Poly N-vinylpyrrolidone was used as the stabilizer for the silver clusters **(Senila *et al.,* 2025).**

6. Microwave-assisted synthesis

According to a study, a microwave-assisted synthesis process using carboxymethyl cellulose sodium as a reducing and stabilizing agent could produce silver nanoparticles. The size was decided by the concentration of sodium carboxymethyl cellulose and silver nitrate. For two months, the generated NPs remained consistent and stable at room temperature without exhibiting any discernible alterations. Furthermore, it was noted that ethylene glycol, polyvinyl pyrrolidine, and Pt seeds may generate silver nanoparticles **(Kamal *et al.,* 2019).**

C. Bio-based methods

1. Bacteria

Silver nanocrystals with different compositions were successfully generated by Pseudomonas stutzeri AG259. The silver-resistant bacterial strain P. stutzeri AG259, which was isolated from a silver mine, accumulated some silver sulfide and 35–46 nm-sized silver nanoparticles (NPs) within its cells **(Tiquia-Arashiro and Rodrigues 2016).**

NPs were created by the bacterial cells through biosynthesis when Lactobacillus strains were exposed to silver ions. Silver ion combinations used to create silver nanoparticles have apparently been exposed to lactic acid bacteria present in buttermilk whey. Silver nanoparticles were nucleated on the cell surface by sugars and enzymes in the cell wall. After being transported inside the cell, the metal nuclei aggregated and developed into bigger particles. The bioreductive production of silver nanoparticles in L. caseisubsp. at room temperature was demonstrated by Korbekandi and associates. Researchers have shown qualitative production of silver nanoparticles by Lactobacillus sp., despite not optimizing the reaction mixture. Biosynthesized silver nanoparticles were either inside and outside of cells or attached to the surface of biomass. They were nearly spherical, solitary (25–50 nm) or in aggregates (100 nm) **(Abbas *et al.,* 2024).**

2. Fungi

Stable silver nanoparticles could be made from Aspergillus flavus. These NPs stayed stable in water for nearly three months without exhibiting any discernible aggregation due to the surface binding of stabilizing molecules generated by the fungus. Silver nanoparticles (5–25 nm) have also been produced extracellularly by Aspergillus fumigatus. The majority of NPs spherical, while few occasionally had triangular forms **(Rajput *et al.,* 2016).**

3. Algae

There are a few studies on cyanobacteria and other algae genera as biological reagents for gold accumulation. Cyanobacteria alga genera such as *Rhizocloniumheiroglyphicum*, *Lyngbya majuscule*, *Spirulinasubsalsa*, *Chlorella vulgaris*, *Cladophoraprolifera*, *Padinapavonica*, and *Sargassumfluitans* utilized as an economical method of biorecovering gold from aqueous solutions, such as creation of gold NPs40–43. Ag NPs can be produced by reducing silver ions using marine algae such as Chaetoceroscalcitrans, Chlorella salina, and Tetraselmisgracilis. Silver nanoparticles (100200 nm) have synthesized using marine cyanobacterium Oscillatoriawillei NTDM01. The cleaned marine cyanobacteria in silver nitrate solution turned yellow after 72 hours, indicating formation of silver nanoparticles. Extracellular production of spherical silver nanoparticles (7–16 nm) was seen after 120 hours at 37 °C and pH 5.6 when Spirulinaplatensis biomass was subjected to 10−3 M aqueous AgNO3 **(Parial *et al.*, 2016).**

4. Plants

Plant extracts from geranium (Pelargonium graveolens), lemongrass (Cymbopogonflexuosus), and alfalfa (Medicago sativa) have been environmentally friendly reactants in the manufacture of silver nanoparticles. Additionally, Daturametel leaf extract rapidly generated a high density of extraordinarily stable silver nanoparticles (16–40 nm) when it came into contact with silver ions **(Khodke *et al.*, 2017).**

6. Advantages

- Longer shelf-stability
- High carrier capacity
- Can be administered via different routes
- Longer clearance time
- Ability to sustain release of drug
- Can be utilized for imaging studies
- Targeted delivery of drugs at cellular and nuclear level
- Development of new medicines which are safer
- Prevent the multi-drug resistance mediated efflux of chemotherapeutic agents **(Mateo *et al.*, 2022).**

7. Herbal Plant Extract of *Campsis grandiflora*

7.1 Introduction to *Campsis grandiflora*

Campsis grandiflora, called as Chinese trumpet vine, is perennial flowering climber belonging to the family Bignoniaceae. The plant native to East Asian countries such as China and Japan and is widely cultivated as ornamental vine because of its attractive trumpet-shaped orange-red flowers. Apart from its ornamental importance, *Campsis grandiflora* has gained attention in traditional and modern medicine due to diverse pharmacological properties and rich phytochemical composition. The plant is woody climbing stems, pinnate leaves, and brightly colored flowers that bloom during warm seasons. Different parts of plant including leaves, flowers, stems, bark, and roots are for medicinal purposes. Traditionally, *Campsis grandiflora* has used in herbal medicine for treatment of inflammatory disorders, skin diseases, wounds, fever, infections, blood circulation problems, and various microbial diseases **(Killi *et al.*, 2025).**

The Campsis grandiflora herbal extract as stabilizing and reducing agent in creation of nanoparticles. In stabilizing the particles and preventing aggregation, the phytoconstituents in extract help reduce silver ions into metallic silver nanoparticles. This dual functionality makes the plant highly suitable for eco-friendly nanoparticle synthesis **(Chowdhury *et al.,* 2024).**

7.2 Phytochemical Constituents of *Campsis grandiflora*

The medicinal and biological activities of *Campsis grandiflora* are mainly attributed to the presence of various phytochemical constituents. The plant contains a rich of secondary metabolites contribute to therapeutic potential and nanoparticle synthesis capability.

7.2.1 Flavonoids

Flavonoids are important polyphenolic compounds widely distributed in medicinal plants. They possess strong antioxidant activity and neutralize reactive oxygen species generated during oxidative stress. Flavonoids present in *Campsis grandiflora* also contribute to anti-inflammatory and antimicrobial effects **(Hassoon *et al.,* 2022).**

7.2.2 Phenolic Compounds

Phenolic compounds exhibit potent antioxidant and free radical scavenging activities. These compounds stabilize nanoparticles and prevent oxidation and aggregation. Phenolics also contribute to the antibacterial activity of plant extract and enhance the therapeutic potential of synthesized nanoparticles **(Nishimoto-Sauceda *et al.,* 2022).**

7.2.3 Tannins

Tannins are polyphenolic called for their antimicrobial and astringent properties. They interact with microbial protein and cell membranes, leading to inhibition of bacterial growth. Tannins also assist in nanoparticle stabilization through adsorption on nanoparticle surfaces **(Huang *et al.,* 2024).**

7.2.4 Alkaloids and Terpenoids

Alkaloids and terpenoids possess diverse biological activities including antimicrobial, anti-inflammatory, and antioxidant effects. These phytochemicals contribute synergistically to medicinal properties of the plant and enhance the antibacterial effectiveness of silver nanoparticles **(Bhambhani *et al.,* 2021).**

7.3 Biological Activities of *Campsis grandiflora* Extract

The herbal extract possesses various pharmacological activities contribute to medicinal and nanotechnological applications.

7.3.1 Antioxidant Activity

The flavonoids and phenolic compounds provides strong antioxidant activity by scavenging free radicals with reducing oxidative stress **(Tumilaar *et al.,* 2024).**

7.3.2 Antibacterial Activity

The extract exhibits antibacterial activity against several Gram-positive and Gram-negative bacteria through disruption of microbial membranes and inhibition of enzyme systems.

7.3.3 Anti-inflammatory Activity

The extract suppresses inflammatory mediators and reduces tissue inflammation.

7.3.4 Wound Healing Activity

The plant promotes tissue regeneration, collagen synthesis, and wound contraction, thereby accelerating healing processes **(Shedoeva *et al.,* 2019).**

8. Characterization of silver nanoparticles

Characterization of synthesized silver nanoparticles is essential to their physicochemical properties, morphology, stability, and biological performance.

8.1 UV–Visible Spectroscopy

UV–Visible spectroscopy is confirm nanoparticle formulation by detecting surface plasmon resonance peaks characteristic of silver nanoparticles **(Ider *et al.,*2017).**

8.2 Fourier transform infrared spectroscopy

FTIR study identifies functional groups and phytochemicals involved in reduction and stabilization of nanoparticles.

8.3 Particle Size Analysis

Particle size determination is important because size significantly influences antibacterial activity, stability, and cellular uptake.

8.4 Zeta Potential Analysis

Zeta potential measures surface charge and predicts colloidal stability of nanoparticles.

8.5 Scanning electron microscopy (SEM)

SEM is used to examine surface morphology of synthesized nanoparticles.

8.6 Transmission electron microscopy

TEM provides detailed information regarding particle size and internal structure of nanoparticles.

8.7 X-Ray diffraction (XRD)

XRD analysis confirms crystalline nature and structural characteristics of silver nanoprticles **(Chouhan, 2018).**

9. Antibacterial activity

9.1 Importance of Antibacterial Nanoparticles

The rapid emergence and spread of multidrug-resistant bacterial strains have become a serious global health concern and pose significant challenges in treatment of infectious diseases. Excessive and inappropriate use of conventional antibiotics has led to development of bacterial resistance, reducing effectiveness of many commonly used antimicrobial drugs. Resistant microorganisms are surviving antibiotic therapy through various mechanisms such as enzymatic drug degradation, modification of drug targets, reduced membrane permeability, and active efflux of antibiotics. As result, infections caused by resistant bacteria lead to prolonged illness, and higher mortality rates. Therefore, the antimicrobial agents capable of overcoming microbial resistance has become extremely important in modern biomedical and pharmaceutical research **(Medina and Pieper 2016).**

Herbal extract-mediated silver nanoparticles have demonstrated potent antibacterial activity against pathogenic gram positive and gram negative bacteria like Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Klebsiella pneumoniae. These microorganisms are commonly associated with various infections such as wound infections, respiratory tract infections, urinary tract infections, gastrointestinal disorders, and hospital-acquired infections. Some of bacteria possess strong resistance mechanisms against multiple antibiotics, making treatment difficult using conventional antimicrobial therapy **(Wahab *et al.,* 2021).**

The antibacterial activity is mainly attributed to disrupt bacterial cell membranes, generate reactive oxygen species, interfere with protein synthesis, damage bacterial DNA, and inhibit essential metabolic enzymes. Silver ions released from nanoparticles interact with sulfur-containing proteins and phosphorus-containing biomolecules within bacterial cells, leading to cellular dysfunction and death. Additionally, the small particle size allows nanoparticles to penetrate bacterial membranes more effectively and produce rapid bactericidal action **(Khalandi *et al.,* 2017).**

9.2 Mechanism of Antibacterial activity

The antibacterial activity containing Campsis grandiflora extract is attributed to multiple mechanisms that collectively interfere with the structure, function, and survival of microbial cells. The nanosized dimensions and large surface area of silver nanoparticles enable close interaction with cell membranes, resulting in enhanced antimicrobial efficacy against Gram positive and Gram negative bacteria. In addition, phytochemicals present in herbal extract contribute synergistically to the antibacterial action, making the nanoparticle system highly effective against microorganisms. One of primary mechanisms involves disruption of bacterial cell membrane integrity. Silver nanoparticles to bacterial cell wall with membrane through electrostatic interactions, causing structural damage and increased membrane permeability. This interaction disturbs the normal arrangement of membrane lipids, leading to weakening of cell membrane. As membrane integrity compromised, essential cellular functions such as nutrient transport, respiration, and maintenance of osmotic balance become impaired, resulting in bacterial cell death **(Madhusudanan *et al.,* 2025).**

Another important antibacterial mechanism is the generation of reactive oxygen species. Silver nanoparticles induce production of reactive molecules like hydroxyl radicals, superoxide radicals, and hydrogen peroxide within bacterial cells. These reactive oxygen species cause oxidative stress, which harms vital biomolecules in cells, including proteins, lipids, and DNA. Oxidative damage leads to membrane lipid peroxidation, enzyme inactivation, and nucleic acids, thereby contributing significantly to bacterial destruction. Inhibition of enzyme systems is another major mechanism responsible for antibacterial activity. Silver nanoparticles interfere with respiratory enzymes and other essential enzymes involved in energy and cellular metabolism. Inactivation of these enzymes reduces ATP production and disrupts normal physiological functions, eventually leading to bacterial cell death **(Flores-López *et al.,* 2019).**

The antibacterial effectiveness of silver nanoparticles by *Campsis grandiflora* extract is further enhanced by synergistic action of phytochemicals present in plant. Bioactive compounds including flavonoids, phenolics, tannins, alkaloids, and terpenoids possess inherent antimicrobial and antioxidant properties. These phytoconstituents may enhance nanoparticle stability, improve interaction with bacterial cells, and contribute additional antimicrobial effects through membrane disruption and inhibition of microbial enzymes **(Tufail *et al.,* 2022).**

9.3 Evaluation of Antibacterial activity

Antibacterial activity is an essential step in determining therapeutic potential and effectiveness of silver nanoparticles containing Campsis grandiflora extract against microorganisms. Antibacterial studies help assess ability of synthesized nanoparticles to inhibit or kill bacterial cells and provide important information regarding their spectrum of antimicrobial action. Various microbiological techniques are evaluate antibacterial efficacy, including agar well diffusion method, disc diffusion method, minimum inhibitory concentration, and minimum bactericidal concentration (MBC) studies **(Oselusi, 2025).**

9.3.1 Agar Well Diffusion Method

The agar well diffusion method is the commonly used techniques for evaluating antibacterial activity of nanoparticle formulations and plant extracts. In this, sterile nutrient agar or Mueller–Hinton agar plates are prepared and inoculated uniformly with bacterial suspension using sterile cotton swabs. Wells of specific diameter are then created in the agar medium using a sterile cork borer. Different silver nanoparticle formulation are carefully introduced into the wells, while standard antibiotics and solvents are positive and negative controls respectively. The inoculated plates are incubated at appropriate temperature, usually 37°C, for 18–24 hours to allow bacterial growth **(Mahboob *et al.,* 2019).**

Following incubation, antibacterial activity is determined by measuring the zone of inhibition surrounding the wells. The zone of inhibition represents the clear area where bacterial growth has been prevented due to antimicrobial action of nanoparticles. Larger inhibition zones indicate stronger antibacterial activity and greater effectiveness against the tested microorganisms. The agar well diffusion method is widely preferred because of its simplicity, cost-effectiveness, and suitability for preliminary screening of antimicrobial activity **(Balouiri *et al.,* 2016).**

9.3.2 Disc Diffusion Method

The disc diffusion method is another widely employed technique for antibacterial evaluation. In this, sterile filter paper discs impregnated with nanoparticle formulation are placed on agar plates previously inoculated with bacterial cultures. The plates are incubated under suitable conditions to permit diffusion of nanoparticles into the agar medium and growth of microorganisms. After incubation, clear zones surrounding the discs are observed and measured in millimeters. The inhibition zone reflects susceptibility of bacterial strains to the nanoparticle formulation. Greater zone diameters indicate higher antibacterial potency. Standard antibiotic discs are generally used as reference standards for comparison of antimicrobial effectiveness. The disc diffusion method is simple, reproducible, and useful for comparing antibacterial activity among different formulations and concentrations **(Hossain *et al.,* 2022).**

9.3.3 Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial agent required to inhibit visible growth of microorganisms after incubation. MIC determination provides quantitative information regarding the potency of silver nanoparticle against bacterial strains. MIC studies are usually performed using broth dilution or microdilution techniques. In this, serial dilutions of nanoparticle formulation are prepared in nutrient broth containing bacterial inoculum. The samples are incubated under suitable conditions for a specified period. After incubation, the tubes or microplates are examined for turbidity or visible bacterial growth. The concentration at which no visible growth is observed is considered the MIC value. Lower MIC values indicate stronger antibacterial activity and higher effectiveness of nanoparticles against the tested organisms. MIC studies are important for determining optimum therapeutic concentration and evaluating antimicrobial potency of synthesized nanoparticles **(Parvekar *et al.,* 2020).**

9.3.4 Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration (MBC) refers to minimum concentration of antimicrobial agent required to kill bacterial cells completely rather than merely inhibiting their growth. MBC determination is for distinguishing bactericidal activity from bacteriostatic activity. Following MIC determination, samples from tubes no visible bacterial growth are subcultured to fresh agar plates without antimicrobial agent and incubated again. The concentration at no bacterial colonies appear on the agar surface is considered the MBC value. MBC studies provide valuable information regarding the killing efficiency of silver nanoparticles that help assess their suitability for treating severe microbial infections **(Rodríguez-Melcón *et al.,* 2021).**

9.3.5 Zone of Inhibition Measurement

Zone of inhibition is the important parameters used in antibacterial evaluation. It represents the clear circular area around wells or discs where bacterial growth has been inhibited due to antimicrobial action of nanoparticles. The inhibition zone is measured in millimeters using a ruler or digital caliper. Larger zones indicate stronger antibacterial potency and greater diffusion of nanoparticles through agar medium **(Balouiri *et al.,* 2016).**

Several factors influence zone of inhibition including:

- Concentration of nanoparticles
- Particle size
- Diffusion capacity
- Type of bacterial strain
- Incubation conditions
- Composition of growth medium

10. Conclusion

Silver nanoparticles using Campsis grandiflora herbal extract represent an innovative and eco-friendly nanotechnological approach for developing potent antibacterial agents. The phytochemical constituents present in plant extract act as natural and stabilizing during nanoparticle synthesis and contribute synergistically to antimicrobial activity. Proper design, silver nanoparticles preparation essential for obtaining stable nanoparticles with desirable physicochemical and biological properties. Characterization method like UV–Visible spectroscopy, FTIR, SEM, TEM, XRD, particle size analysis, and zeta potential measurement provide valuable information regarding nanoparticle formation and stability.

The remarkable antibacterial activity demonstrated by these nanoparticles against pathogenic microorganism highlights their significant therapeutic potential in pharmaceutical and biomedical applications. Therefore, silver nanoparticles containing Campsis grandiflora extract may serve as promising candidates for development of advanced antimicrobial formulations and nanomedicine-based therapies.

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