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FOCUSING ON THE RECENT PROGRESS IN ANTIMICROBIAL POTENTIALS OF PLANT EXTRACTS-MEDIATED SYNTHESIZED SILVER NANOPARTICLES

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ABSTRACT: Using plant extracts to synthesize metal nanoparticles is one of the easiest, most convenient, cost-effective, and ecologically acceptable ways of avoiding hazardous chemicals. As a result, many environmentally acceptable methods for the fast production of silver nanoparticles utilizing aqueous extracts of plant components such as leaves, bark, and roots have been described in recent years. This study highlights and expands on recent discoveries in the green production of silver nanoparticles (AgNPs) utilizing various plant extracts and their prospective uses as antimicrobial agents. A systematic in-depth discussion on the possible influence of phytochemicals and their concentrations in plant extracts, extraction solvent, and extraction temperature, as well as reaction temperature, pH, reaction time, and precursor concentration. Extensive details of the probable mechanism of AgNPs' interaction with microbe cell walls, which leads to cell death and strong antibacterial activity, have also been developed. Furthermore, even though chemical techniques for shape-controlled synthesis are well-known, regulating the shape of biosynthesized AgNPs has numerous beneficial impacts on its activities.

INTRODUCTION: Nanotechnology is getting a lot of traction as a new field of study that deals with the creation of nanomaterials and nanoparticles (NPs) for use in a variety of areas, including catalysis, electrochemistry, biomedicines, pharmaceuticals, sensors, food technology, cosmetics and so on.

Nanoparticles (NPs) are atomic or molecular-scale solid particles with good physical characteristics compared to bulk molecules. Nanoparticles (NPs) are nanometer-sized (100 nm) atomic or molecular-scale solid particles with great physical qualities compared to bulk molecules.

Metal and metal oxide nanoparticles have been extensively investigated utilizing science and technology due to their outstanding characteristics, such as high surface-to-volume ratio, high dispersion in solution, etc. Metal and metal oxide nanoparticles have improved antibacterial capabilities due to these characteristics ¹. NPs that

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have been modified or produced are now extensively used in industrial products like as cosmetics, electronics, and textiles. Furthermore, due to the fast rise in the number of bacteria resistant to current antibiotic medications, new medicines in the form of bare NPs or in combination with existing antibiotics to exhibit a favourable synergistic effect have been widely used in a variety of medical areas. In today's world, NPs are used in molecular imaging to provide very detailed images for diagnosis. Furthermore, contrast compounds are imprinted on NPs to detect tumors and atherosclerosis ².

Additionally, since the initial FDA approval of nanotherapeutic in 1990, nanotherapeutic has been pushed all over the globe to develop various nano-based medicines. Various physical and chemical methods, including chemical reduction, milling, and others, were used to synthesize NPs and improve their efficiency around the turn of the twentieth century. Traditional methods, on the other hand, use expensive and hazardous chemicals and cannot be called ecologically friendly. Considering this, researchers are increasingly interested in the biogenic production of metal and metal oxide NPs using aqueous plant extract and microorganisms, since they are environmentally benign, stable, therapeutically adaptable, biocompatible, and cost-effective. As a result, bio-inspired nanoparticle synthesis has emerged as an important area of nanoscience and nanotechnology ³.

Many metal and metal oxide NPs have been produced thus far utilizing plant extracts, microorganisms, and other methods. Plant biomass is widely used as a catalyst for chemical synthesis and biodiesel production, owing to their broad availability, renewability, and environmentally benign character, in addition to their enormous uses in the synthesis of NPs. Silver NPs, which have a broad range of applications in microbiology, chemistry, food technology, cell biology, pharmacology and parasitology, are attracting much attention from the scientific community. Silver NPs' physical and chemical characteristics are determined by their shape. Several methods have been used to synthesize silver NPs, including sol-gel, hydrothermal, chemical vapour deposition, thermal decomposition, and microwave-assisted

combustion methods ⁴. Biogenic production of silver NPs (AgNPs) utilizing biomaterials such as plant extract and microorganisms as reducing agents has recently received much attention. Different biomolecules, such as flavonoids, ketones, aldehydes, tannins, carboxylic acids, phenolic acids, and the protein of plant extracts, oxidize Ag^+ to Ag^0 , resulting in AgNPs. The production of AgNPs may be monitored using UV-visible spectroscopy, a simple and commonly used analytical method.

The conducting electrons in the outermost orbital of metal NPs collectively vibrate in resonance with specific wavelengths when they come into contact with an electromagnetic field, resulting in a phenomenon known as surface plasmon resonance (SPR). Color and absorbance in a colloidal solution of AgNPs are caused by SPR excitation. SPR peaks at approximately 435 nm are often used to demonstrate silver nitrate reduction into AgNPs. Spherical NPs have just one SPR band in their absorbance spectra, while anisotropic particles have two or more SPR bands depending on the shape. In UV-Vis spectra, the lack of a peak between 335 nm and 560 nm is sometimes used to indicate that NPs are not aggregating ⁵.

This study will also reveal new ways to produce silver nanoparticles that may benefit humans using various methods. The biogenic production of silver nanoparticles utilizing different plants and their use in antibacterial activities has been thoroughly studied. The impact of the size and form of the produced silver nanoparticles on antibacterial efficacy against different harmful bacteria was also addressed. When attempting to synthesize metal NPs, remember that their success is dependent not only on their size and structure but also on their stability since NPs have the propensity to form huge aggregates that precipitate, decreasing their effectiveness.

Protocols for the Biosynthesis of AgNPs: Biogenic synthesis of AgNPs is a simple one-step procedure that produces no harsh or hazardous compounds, making them cost-effective, efficient, and environmentally benign. Plants and microorganisms have been studied intensively in recent years for the biosynthesis of AgNPs of various sizes, shapes, stability, and antibacterial

effectiveness⁶. Plant components such as leaves, roots, flowers, fruits, rhizomes, *etc.*, have all been effectively used to synthesize AgNPs. Plant components are gathered from different sources and cleaned thoroughly with common water before being distilled to remove dirt and other undesirable elements. The parts are dried and crushed to create a powder or used fresh to make extract⁷. To make the extract, the chopped pieces or powdered powder of the plant components are placed in deionized water or alcohol and heated for a few hrs at a temperature below 60°C, since prolonged high-temperature heating may cause phytochemical breakdown in the biomass extract. Plant extracts of various pH levels were added to solutions containing varying concentrations of Ag salt as a metal precursor, followed by heating at various temperatures to produce AgNPs⁸. Because biomaterials contained in the extract serve as a reducing agent and stabilizing agent for producing AgNPs, this synthesis method does not need chemical stabilizers. Visual color changes or UV-Vis spectroscopy may be used to track the production of AgNPs, with a strong peak owing to surface plasmon resonance (SPR) of AgNPs at approximately 430–450 nm visible. Following successful AgNP synthesis, the mixture is centrifuged at high rpm to separate the NPs, followed by solvent washing and drying in a low-temperature oven⁹.

Factors Affecting Nanoparticle Formation:

Although this study area has been studied for decades, effective green synthesis of AgNPs and assessment, as well as understanding antimicrobial properties, remains a difficult procedure. However, based on the literature, we may make a few assumptions that might lead to AgNPs with strong antimicrobial activity. Given the intricacy of the study on green synthesis and antibacterial activity of AgNPs, the following considerations should be made during AgNPs production:

The Chemical Make-up of the Plant Extract:

The reduction of Ag⁺ to Ag⁰ is thought to be caused by the oxidation of several biomolecules such as flavonoids, ketones, aldehydes, tannins, carboxylic acids, phenolic acids, and plant protein. Furthermore, the biomolecules serving as a capping agent determine the stability and size of the generated AgNPs. For effective AgNP production,

one must first examine the biomolecules contained in the plant extract and their capping efficiency. In general, the higher the capping activity, the more stable the NPs are and the smaller their average particle size. In certain cases, however, this is not the case. As a result, unique interactions between biomolecules and generated NPs must be considered on a case-by-case basis¹⁰.

The Plant Extract's Concentration: The form and size of the produced AgNPs are determined by the plant extract concentration utilized. The production of NPs may not even occur at low concentrations, necessitating more investigation. A color shift and strong UV-Vis absorption at 430 cm⁻¹ follow AgNP formation. An increase in extract concentration causes many NPs to develop to a specific point. While a desirable quantity of extract may provide well-dispersed AgNPs with strong antibacterial activity, a high concentration of extract can lead to accumulation and massive NPs, as excess reducing chemicals can induce secondary reduction on the surface of the preformed nuclei¹¹.

AgNO₃ Concentration: The number of AgNPs grew with increasing AgNO₃ concentrations until all of the AgNO₃ salt was consumed, i.e. all Ag⁺ was converted to Ag⁰, which could be readily seen by increasing UV-Vis spectroscopy intensities. Equilibrium will be reached after all of the AgNO₃ has been used. As a result, the balance between AgNO₃ and the quantity of reducing agent contained in the extract must be examined¹².

Solvent for Extraction: Because various biochemicals in plants have varying levels of solubility in different solvents, the effectiveness of extracting the required biochemicals for AgNP production is highly dependent on the extraction solvent employed. In ethanol, methanol, and their mixtures with water (ethanol–water or methanol–water), phenolic chemicals are known to be extremely soluble. As a result, together with clean water, they are the solvents of choice for extraction¹³.

Temperature and Time of Extraction: The extraction temperature is another essential consideration for efficiently synthesizing biogenic AgNPs. It is widely known that as extraction temperature and duration rose, so did the solubility

of biochemicals. As a result, more chemicals will be extracted at a greater temperature, resulting in a powerful reducing agent. However, extracting non-reactive biochemicals or breakdown of biochemicals over a lengthy period of time at a higher temperature is a possibility¹⁴.

pH: pH may alter the electrical charges of biomolecules in plant extracts, affecting the nature of their capping and stabilising affinities and, as a result, NP development. A rise in pH typically results in a faster rate of production as well as a more uniform size distribution of NPs. However, sluggish production and aggregation occurred in acidic conditions, resulting in bigger NPs. High pH, on the other hand, may cause AgOH precipitate, which is undesirable. If an external buffer is utilized, a neutral pH of 7 is strongly suggested¹⁵.

Response Time: The size of the NPs is said to grow with time, as shown by a red-shift in UV-Vis spectrometer data. As a result, it is essential to keep a close eye on the response to ensure steady tiny NPs¹⁶.

Temperature of the Reaction: Although a high temperature is typically needed to complete the chemical reduction of AgNO₃ to AgNPs, the RT reaction is the best option from an economic and green chemistry standpoint. However, when it comes to the production of green NPs, the RT method, with a few exceptions, generally results in spherical NPs that are less sensitive to microorganisms, as previously stated. Meanwhile, synthesis of various forms of NPs for particular applications is much desired. According to a literature study, the production of cubic, pentagonal, hexagonal, triangular, rod-shape nanowire AgNPs occurs most often above RT, but additional factors such as capping agents and stabiliser concentration must be considered.

As a result, in addition to increasing reaction speed and reducing NP size with temperature, the reaction temperature must be considered when producing NPs with various shapes for a specific purpose, especially as a strong antibacterial¹⁷.

Plant-Mediated Biogenic Synthesis of AgNPs and Their Antimicrobial Activity:

***Aesculus hippocastanum*:** Special AgNPs mediated by *Aesculus hippocastanum* were shown

to exhibit the greatest antibacterial activity (ZOI 20 mm) against the Gram-negative bacteria *P. aeruginosa*. Surprisingly, although AgNPs had a strong impact on all of the bacteria examined, they have no effect on fungal strains, including *Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 13803, and *Candida krusei* ATCC 1424. The MIC and MBC of AgNPs for the bacteria examined were 0.19–12.5 mg/mL and 1.56–25 mg/mL, respectively¹⁸.

***Amaranthus gangeticus*:** In 2015, *Amaranthus gangeticus* Linn leaf extract was used to make AgNPs, which showed inhibitory efficacy against Gram-positive, Gram-negative, and fungal bacteria¹⁹.

***Andrographis echioides*:** The scientists used *Andrographis echioides* leaf extract to biosynthesize AgNPs with cubic, pentagonal, and hexagonal shapes with sizes ranging from 68.06 nm to 91.28 nm and investigated their bactericidal efficacy against a variety of microorganisms. In the case of *E. coli* (28 mm) and *S. aureus* (23 mm) in 100 mg/mL, AgNPs concentration showed a high ZOI²⁰.

***Andrographis paniculata*:** *Andrographis paniculata* leaf extract was shown to generate AgNPs with a unique cubic form. Because of the shape-dependent actions of AgNPs towards microorganisms, research on various shapes of AgNPs is of considerable interest. For Gram-negative bacteria *P. aeruginosa*, AgNPs had a high ZOI of 21.3 mm and a very low MIC of 3.125 mL mL⁻¹, indicating strong antibacterial action. Researchers noticed that other Gram-negative bacteria, such as *E. coli*, have a thicker peptidoglycan layer than *P. aeruginosa*; thus the author found a lower ZOI (16.6 mm) in *E. coli*²¹.

***Artemisia vulgaris*:** AgNPs mediated by *Artemisia vulgaris* were first reported in 2017. Antimicrobial tests showed that AgNPs had substantial inhibitory effects against the pathogens examined, with *S. aureus* having the highest value (18 mm inhibition zone)²².

***Catharanthus roseus*:** Formulators produced AgNPs from *Catharanthus roseus* in 2017. The biosynthesis of AgNPs has been confirmed using color change, UV-Vis spectrum, XRD, FTIR, and

AFM methods. After adding 2 mM AgNO₃ and heating at 70°C for 3 mins, the color of the leaf extract changes from yellowish to reddish-brown, suggesting the production of NPs. AFM shows crystalline NPs with grains ranging in size from 10 nm to 88 nm in diameter, with a mean size of about 49 nm.

The authors stated that produced AgNPs entered microorganisms' cells, causing disruption of adenosine triphosphate (ATP) synthesis and DNA replication, as well as forming reactive oxygen species (ROS) and damaging cell structures²³.

Croton bonplandianum: AgNPs produced by *Croton bonplandianum* have also been very effective against microorganisms. In the cases of *E. coli*, *P. aeruginosa* and *S. aureus*, the minimum inhibitory doses of produced AgNPs were determined to be 50, 45, and 75 µg/mL, respectively. Gram-negative bacteria with a thin cell wall, such as *E. coli* and *P. aeruginosa*, were shown to be more vulnerable to cell wall destruction than Gram-positive bacteria with a thick cell wall (*S. aureus*)²⁴.

Curcuma longa: Scientists used *Curcuma longa* leaf extract to make AgNPs and investigated their antibacterial efficacy in AgNP-coated cotton fabric. SEM examination, aided by the EDX analysis, verified the loading of AgNPs on the cotton fabric. The authors claimed that cotton fabric loaded with AgNPs resisted pathogenic microorganism growth. Thus, cotton fabric loaded with AgNPs synthesized from *Curcuma longa* can be used for various applications in medical patients and medical workers to resist microbial infection²⁵.

Jatropha curcas: The leaves of *Jatropha curcas* were gathered from the Micro model complex and utilized to make AgNPs. The diameter of NPs was determined to be in the range of 50–100 nm by SEM, while TEM examination revealed diversity in particle form and size (20–50 nm).

TEM inspection revealed that the microbial cell had been completely destroyed. The antibacterial activities of the synthesised NPs were evaluated, and the pattern of sensitivity was found in the order *E. coli* > *P. aeruginosa* > *B. cereus* > *S. enterica* = *L. monocytogenes* > *S. aureus*, based on ZOI data²⁶.

Lantana camara: Using *Lantana camara* leaf extract, researchers recently demonstrated ultrasonic-assisted production of spherical AgNPs. The use of ultrasonication in the biosynthesis of AgNPs enhances reaction conditions by decreasing reaction time and increasing reaction rate. The antibacterial activity of the produced AgNPs was found to be good against Gram-positive and Gram-negative bacteria²⁷.

Maclura pomifera: In 2017, *Maclura pomifera* was used to produce spherical AgNPs. The ZOI of the generated NPs (0.1 mg/mL concentration) against *E. coli* was 23.4 mm, greater than Ampicillin, a well-known antibiotic medication²⁸.

Mentha aquatica: *Mentha aquatica* leaf extract was used as a reducing and capping agent in the recent syntheses of ultrasound-assisted AgNPs of size 8 nm. To our knowledge, they are the tiniest biogenic AgNPs ever discovered. Although NPs could be produced at RT, ultrasound significantly sped up the response time to just 10 minutes, compared to 1 hour at RT.

In an alkaline environment, the phenolic compounds in the *Mentha aquatica* leaf extract are oxidized to Quinone, which supplies free electrons for the reduction of the Ag⁺ ion to Ag⁰, forming the desired AgNPs, according to scientists. The AgNPs had an extremely low MIC of 2.2 mg/mL for *P. aeruginosa*, owing to their ultra-small size, demonstrating their excellent effectiveness against the tested microorganism²⁹.

Mukia maderaspatana: For the biosynthesis of AgNPs with a size range of 58 nm–458 nm, *Mukia maderaspatana* leaf extract was used. The antibacterial efficacy of the synthesized nanoparticle conjugated to the drug ceftriaxone was tested against human pathogens such as *B. subtilis*, *K. pneumonia*, *S. typhi*, and *S. aureus*, and compared to the pathogen inhibitory effectiveness of the free nanoparticle and antibiotic. In comparison to the other AgNPs, the AgNPs conjugated with ceftriaxone exhibited the greatest inhibitory activity³⁰.

Prosopis farcta: At room temperature (RT), researchers used *Prosopis farcta* extract to biosynthesize AgNPs with an average size of 10.8 nm. The antibacterial activity of produced AgNPs

was evaluated using the disc diffusion technique against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) microorganisms and compared to the control. The findings revealed that for each pathogen examined, the inhibitory diameter increased, indicating that produced AgNPs cause cellular harm to bacteria and therefore may be utilized as nanoantibiotics³¹.

***Skimmia laureola*:** A variety of leaf extracts have been used in the biosynthesis of AgNPs to date. *Skimmia laureola* was shown to be capable of producing spherical AgNPs with a diameter of 38 nm, which were tested against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. vulgaris*, and *S. aureus*³².

***Solanum nigrum*:** AgNPs with an average size of 3.46 nm were recently created utilizing *Solanum nigrum* plant leaf extract. This is one of the tiniest biogenic AgNPs yet discovered, with NPs as tiny as 1.74 nm. SPR bands verified the production of AgNPs at 442 nm in UV-visible spectroscopy. Surprisingly, AgNPs outperformed AuNPs and PdNPs in terms of antibacterial activity. This may be attributed to the more efficient capping of AgNP nanoparticles than Au or PdNPs, resulting in well-dispersed tiny AgNPs with less agglomeration, as revealed by HRTEM. The scientists believe that the polyphenols in *Solanum nigrum* extract produce a negative environment surrounding the particles, overcoming the van der Waals force of attraction and preventing AgNP agglomeration. At a concentration of 10 mg/mL, AgNPs had a ZOI of 22 mm, whereas Au NPs and Pd NPs had ZOIs of 20 mm and 19 mm, respectively, against *E. coli*. Although the authors attribute AgNPs' greater antibacterial activity to their efficient capping, it may also be owing to their lower size (3.46 nm) as compared to Au (9.39 nm) and PdNPs (21.55 nm)³³.

***Trichoderma viride*:** Another intriguing study on the shape-dependent activity of biogenic AgNPs was published using *Trichoderma viride* extract. The authors found that pentagonal and hexagonal NPs had greater antibacterial properties and activity than spherical NPs when their sizes were comparable. Physical factors, temperature, pH, and reaction time were used to create various shapes of

AgNPs, including pentagonal, hexagonal, and spherical. Under all reaction conditions, spherical NPs were observed at neutral pH. Rectangular and pentagonal/hexagonal NPs were produced at 40°C after 72 hrs of incubation at pH 5.0 and 9.0.

The longer the reaction, the larger the NPs, while a greater temperature always results in a smaller NP. It was also discovered that triangular shape AgNPs had better antimicrobial activity than spherical and rod-shaped AgNPs because they have a higher percentage of facet (1 1) with a high atomic density, which increases Ag binding efficiency to sulfur-containing components. In contrast, spherical and rod-shaped particles have a high percentage of (1 0 0) facets³⁴.

***Urtica dioica*:** In 2016, a surprising study on the production of AgNPs using *Urtica dioica* leaf extract was published, demonstrating a good synergistic impact with recognized antibacterial medicines. Surprisingly, the produced AgNPs, in addition to having strong antimicrobial activity against a variety of microorganisms, also had a good synergistic effect with antibiotics and had a higher antibacterial impact than AgNPs alone. The synergistic effect of AgNPs was shown by a 17.8 fold rise in ZOI when amoxicillin was combined with AgNPs against *S. marcescens*³⁵.

Future Perspectives: Because of the increased surface area in contact with the microbial cell, the smaller the NPs are, the greater their antibacterial properties are. Antimicrobial activity of AgNPs in the same size range is in the following order: triangular > pentagonal, hexagonal, cubic, nano-rod > spherical³⁶. The most activity was seen in the triangular one, owing to superior edge fitting due to the sharp edge and majority stable (1 1 1) facet. When compared to triangular form NPs, hexagonal, cubic, nano-rods have a bend edge, which may diminish their effectiveness against microorganisms³⁷. The antibacterial effects of spherical shape NPs with no sharp edge and mainly (1 0 0) facets were the least. Several studies have shown that Gram-positive bacteria (e.g., *S. aureus*) are less sensitive to AgNPs than Gram-negative bacteria (e.g., *E. coli*), which have a cell wall made up of lipopolysaccharides on the outside and a peptidoglycan layer (7–8 nm) beneath. However, this is not always the case³⁸. In light of this,

researchers should investigate the function of lipopolysaccharides in Gram-negative bacteria, which may have served as a shield against certain AgNPs, as well as the synergetic impact of AgNPs and biomolecules, which may have altered the manner of NPs' interaction with the cell wall. As a result, deciphering the interaction's underlying mechanism remains a problem³⁹. AgNPs' antibacterial effectiveness may be significantly boosted by their synergistic interactions with a variety of well-known antibiotics. This offers up a whole new world of possibilities in the fight against a slew of recently developed, highly infectious, multidrug-resistant bacteria⁴⁰. As a result, despite its youth, this study area has become a "hot" issue in recent years. To better understand the mechanism of AgNPs' interaction with medicines, as well as the change in mode of attack owing to the synergetic interaction with microorganisms, must be thoroughly understood and empirically verified⁴¹.

CONCLUSION: Given the many advantages of green synthesis of AgNPs using plant extracts, as well as their outstanding antibacterial properties, whether used alone or in combination with antibiotic medicines, there is little question that this area of study will continue to draw a lot of attention in the coming years. Various biogenic techniques for producing AgNPs utilizing phytochemicals, nontoxic, cheap, and environmentally acceptable routes have been thoroughly explored in this paper. The antibacterial susceptibility of the AgNPs generated has also been emphasized against various harmful microorganisms. Although plant extract-based fast and green synthetic techniques, have shown significant promise in AgNPs synthesis, the mechanism by which phytochemicals from these plants are engaged in synthesis and the manner of antimicrobial inhibition is yet unknown. Furthermore, even though chemical techniques for shape-controlled synthesis are well-known, regulating the shape of biosynthesized AgNPs, which has numerous beneficial impacts on its activities, has remained largely unaddressed till now. This issue may be caused by the vast number of phytochemicals contained in the plant extract, making systematic control of the interaction with the generated AgNPs challenging. As a result, gaining a greater knowledge of each phytochemical, its amounts, and how they interact

would pave the road for shape-selective biogenic NP production.

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