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Biosynthesis of Silver Nanoparticles from Plant Extracts and Its Antimicrobial Activity: A Review

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ABSTRACT: Synthesis of metal nanoparticles using plant extracts is one of the most simple, convenient, economical, and environmentally friendly methods that mitigate the involvement of toxic chemicals. Use of silver and silver salts is as old as human civilization but the fabrication of silver nanoparticles (AgNPs) has only recently been recognized. They have been specifically used in agriculture and medicine as antibacterial, antifungal and antioxidants. The formations of silver Nanoparticles from the extracts were identified by the colour changes. The synthesis and characterization of silver nanoparticles was confirmed by UV-Visible spectrophotometer, Fourier Transform Infrared spectroscopy (FTIR), Dynamic light scattering (DLS) and zeta potential studies.It has been demonstrated that AgNPs arrest the growth and multiplication of many bacteria such as Bacillus cereus, Staphylococcus aureus, Citrobacter koseri, Salmonella typhii, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia by binding Ag/Ag+ with the biomolecules present in the microbial cells. It has been suggested that AgNPs produce reactive oxygen species and free radicals which cause apoptosis leading to cell death preventing their replication. Since AgNPs are smaller than the microorganisms, they diffuse into cell and rupture the cell wall which has been shown from SEM and TEM images of the suspension containing nanoparticles and pathogens. AgNPs are also used in packaging to prevent damage of food products by pathogens. The present review suggests that biologically synthesized AgNPs are fairly ideal candidates for the development of new antimicrobial drugs against bacteria.

KEYWORDS: Green synthesis, Silver nanoparticles, Antimicrobial activity, UV, SEM, TEM, FTIR.

I. INTRODUCTION

Nanotechnology is the study and application of small objects which can be used across all fields such as chemistry, biology, physics, material science and engineering. The word “nano” is used to indicate one billion of a meter or 10^{-9}. It size ranges from 1-100nm. “Nano” is agreek word meaning extremely small.[8] Nanoscale material has new, unique, and superior physical and chemical properties compared to its bulk structure,due to its small size it occupies a position in various fields of nanoscience and nanotechnology. The concept of nanotechnology emerged on 9th century for the first time in 1959, Richard Feynman gave a talk on the concept of nanotechnology and described about molecular machines built with atomic precision where he discussed about nanoparticles and entitled that “There’s plenty of space at the bottom” (T.C et al., 1974). Nanoparticles of gold, silver, copper, silicon, zinc, titanium, magnetite, palladium formation by plants have been reported. [5]

Colloidal silver (Ag) nanoparticles had exhibited distinct properties such as catalytic, antibacterial (Sharma et al., 2009), good conductivity, and chemical stability. Silver nanoparticles have its application in the field of bio labelling, sensor, antimicrobial, catalysis, electronic and other medical applications such as drug delivery (Jong et al., 2008) and disease diagnosis.[2] Silver Nanoparticle is an interesting metal to be studied, especially in the field of health and medicine. Silver nanoparticle is a strong antibacterial and also toxic to cells. Silver nanoparticles have the ability to damage bacterial cell walls, inhibit bacterial cell growth, and disrupt cell metabolism because of the interaction between Silver ions with macromolecules in cells, such as proteins and deoxyribonucleic acid (DNA). A silver ion that interacts with the cell prevents protein synthesis, further decreases the membrane permeability, and eventually leads to cell death. [6]

Silver Nanoparticles can be synthesized through several methods. Generally used two methods (approach) for the synthesis of Nanoparticles such as bottom-up approach, Top-down approach it include Irradiation method, physical method, chemical method, solvothermal method,sol-gel method out of these all methods chemical reduction method are often used because they are easier and economical. The most commonly used reducing agents are sodium borohydride, hydrazine hydrate, potassium auro chlorate and sodium citrate. The reduction of various complexes with Ag^+ ions leads to the formation of silver atoms (Ag0), which is followed by agglomeration into oligomeric clusters.[2] These clusters eventually lead to the formation of colloidal Ag particles. This method is done by reducing Ag salts by reducing agents, such as sodium citrate or sodium borohydride. However, the use of chemicals in the synthesis of Ag nanoparticles results in the adsorption of toxic chemicals (reducing agents and organic solvents) on the surface of the material so that it will have adverse and harmful effects on its application. [1]

Biological synthesis of nanoparticles is a challenging concept which is very well known as green synthesis. Among the different biological agents, plants provide a safe and beneficial way to the synthesis of metallic nanoparticles as it is easily available, so there are possibilities for large scale production. In recent years, metallic nanoparticles have received great attention. Green synthesis methods for synthesizing nanoparticles using natural products can be used to address the problem by utilizing plants or microorganisms. utilization of plants in the biosynthesis of nanoparticles involves the content of secondary metabolites as reducing agents .Allegedly, biological agents act as reducers, stabilizers, or both in the process of forming nanoparticles.[3]

Biosynthesis of Ag nanoparticles has been carried out by utilizing various numbers of plants.

Liquids that use water solvents are much safer for health and the environment than using chemical solvents. Therefore in this review, they used distilled water as a solvent in medicinal plant extracts. Hence the review focused on the "Green synthesis of sliver nanoparticles from various plants extract then evaluated its antimicrobial activity". The silver nanoparticles have been characterised by UV-Vis spectroscopy, Transmission electron microscopy (TEM), Dynamic light scattering (DLS) and zeta potential studies, Fourier Transform infrared spectroscopy (FTIR).

II. METHODS FOR SYNTHESIS OF NANOPARTICLES

Many techniques for the synthesis of metallic nanoparticles are now available. Synthesis of nanoparticles generally involves either "top to bottom" approach or "bottom to up" approach. In top down method of synthesis, the nanoparticles are produced by size reduction from an appropriate starting material. Variety of physical and chemical treatments are used for the achievement of size reduction, physical approaches include techniques such as evaporation-condensation and laser ablation whereas chemical approaches include chemical reduction by use of organic and inorganic reducing agents. Top down fabrication methods introduce imperfections in the surface structure of the product and this is a major drawback because the surface chemistry and the other physical properties of nanoparticles are greatly dependent on the surface structure. In bottom up method of synthesis, the nanoparticles are built from smaller entities, for example by fusion of atoms, molecules and smaller particles. In bottom up synthesis, the nanostructured building blocks of the nanoparticles are produced first and then assembled to manufacture the final particle.The bottom up synthesis mostly achieved by chemical and biological methods. The biological methods can be used to synthesize nanoparticles without the use of any harsh, toxic and expensive chemical substances. Out of all the biological methods used for the synthesis of nanoparticles, the methods based on microorganisms have been frequently reported. Various advantages of microbial synthesis is that its readily scalable, environment friendly and compatible with the use of the product for medical applications but production of microorganisms is often more expensive than the production of plant extracts. Plant mediated nanoparticles synthesis using whole plant extract or by living plants was also reported inliterature. [7]

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Different methods for synthesis of metallic nanoparticles were depicted in Fig.1

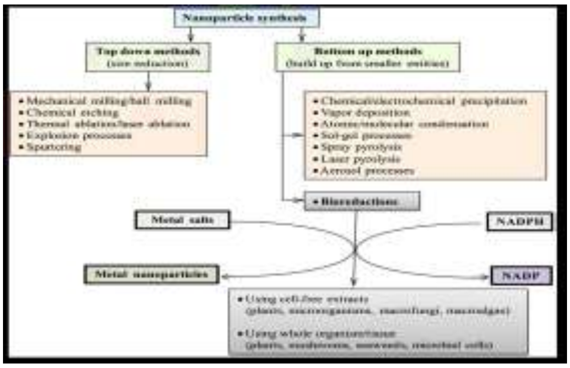

Fig.1 Different Approaches for Nanoparticle Synthesis

III. SYNTHESIS OF SILVER NANOPARTICLES (AGNPS)

The use of plants extracts in the synthesis of silver nanoparticles has drawn attention, because of its rapid, environment friendly, non-pathogenic, economical protocol and providing a rapid technique for the biosynthetic processes. Moreover plant extracts are easy and safe to handle unlike microbial cultures. The reduction and stabilization of silver ions is achieved by a combination of variety of biomolecules such as amino acids, proteins, enzymes, polysaccharides, alkaloids, phenolics, tannins, saponins, terpinoids and vitamins which are already present in the plant extracts having medicinal values and are environment friendly, yet chemically complicated structures. [7]

A large number of plants and plant extracts are reported to facilitate silver nanoparticles synthesis are mentioned in “Table 1”and are briefly discussed in the present review. The general protocol for silver nanoparticle synthesis using plant extracts involves the following steps:

1. Collection of the plant material: Various parts of plant such as leaves, roots, seeds, flowers, fruits, rhizomes are collected and washed thoroughly with tap water and followed by distilled water to exclude debris and any other unwanted materials. The fresh and clean plant part is then shed dried for 10-15 days and powdered with the help of blender. [7]

2. Plant extracts preparation: The dried powdered is mixed with desired solvent in 1:10 ratio and boiled for few minutes. The infusion is then filtered through Whatmann filter paper to remove the insoluble material from the extract. [8]

3. Precursor preparation: Precursor for silver nanoparticle synthesis is prepared as 1mM AgNO3 solution. (1.7g of Silver nitrate ($AgNO_{3)}$ was added into 100ml of distilled water and stirred continuously for 1-2min to get 1mM Silver Nitrate solution).

4. Synthesis of AgNPs: 1mM AgNO3 solution is added to few ml of the plant extract which leads to the reduction of Ag(I) ions to Ag(0) and allow to kept for 24 hours to obtain the colour change.[8]

5. Confirmation: After 24 hours green colour changed to dark brown colour. It indicates the formation of silver nanoparticles**.** Then it can be confirmed by measuring the UV–visible spectra of the solution at uniform intervals. The absorption maxima of AgNps ranges between wavelength of 400–450 nm. After synthesis the nanoparticles are characterized by using various techniques. [7]

IV. CHARACTERIZATION TECHNIQUES

Nanoparticles are generally characterized by their size, shape, surface area, and dispersity. The nanoparticles are commonly characterized by the following techniques: UV–visible spectrophotometry, scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), Powder X-ray diffraction (XRD), Dynamic light scattering (DLS) and zeta potential studies.

a) UV-Vis Spectroscopy

The UV-Visble Spectroscopy was preliminary technique to detect the presence of silver nanoparticles. The reduction of the pure Ag+ ions was monitored by measuring the UV-Vis spectrum. In particular, absorbance in the range of 420 to 500nm has been used as an indicator to confirm the reduction of Ag+ to metallic Ag. [12] [13]

b) Scanning Electron Microscopy (SEM)

Particle size and its distribution were assessed with Scanning Electron Microscope. Electron interacts with the electrons in the sample,

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producing various signals that can be detected and that contain information about surface topography and composition of the samples shows in Fig.2. SEM analysis reveals individual spherical polydispersed AgNPs as well as number of aggregates, which were irregular in shape. The size of the silver nanoparticles was found to be 5-50 nm, with an average size 15 nm. The larger silver particles may be due to the aggregation of the smaller ones. [12]

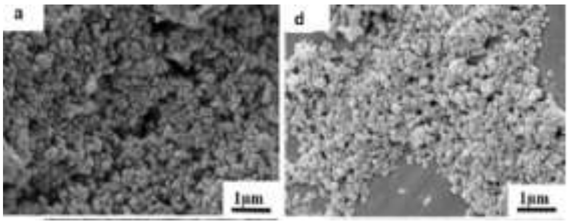

Fig.2 SEM images

c) Transmission Electron Microscopy (TEM)

The transmission electron microscopy has a 1000 times higher resolution as compared to the scanning electron microscopy as it uses more powerful beam of TEM provides greater detail at the atomic scale, such as information about the crystal structure and granularity of a sample. TEM is useful to know the exact size and shape of nanoparticles. Fig.3 images showed that the silver nanoparticles formed were spherical in shape, with an average size of around 100nm and uniformly distributed silver nanoparticles on the surface of the cells. [10]

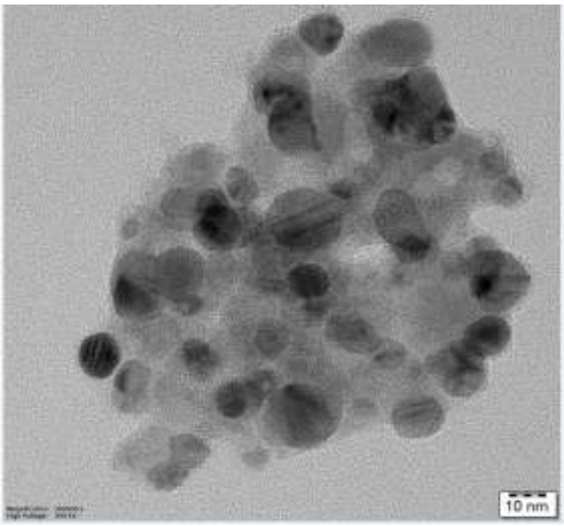

Fig.3 TEM image silver nanoparticles

d) FT-IR analysis (Fourier Transform infrared spectroscopy)

The characterization of functional groups on the surface of AgNPs by plant extracts were investigated by FTIR analysis and the spectra was scanned in the range of 4000–400 cm^{-1}range at a resolution of 4cm−1. Organic functional groups (e.g. hydroxyls, carbonyls) attached to the surface of nanoparticles and the other surface chemical residues can be detected by using FTIR. The sample was prepared by dispersing the AgNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. KBr was used as a standard in analysis of the samples. FTIR showed the structure and respective bands of the synthesized nanoparticles and the stretch of bonds. [13]

e) DLS Particle size and zeta potential analysis

The size distribution or average size of the synthesized AgNPs were determined by dynamic light scattering (DLS) and zeta potential measure the effective electric charge on Nanoparticle surface were carried out using DLS. For DLS analysis the samples were diluted 10 folds using 0.15M PBS (pH 7.4) and the measurements were taken in the range between 0.1 and 10,000 nm.[7]

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f) X-ray diffraction (XRD)

The phase identification and characterization of the crystal structure of the nanoparticles can be done by using XRD technique. X-rays penetrate deep into the nanomaterial and the resulting diffraction pattern is compared with standards to obtain structural information. [7]

V. ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING PLANT EXTRACTS

The antibacterial activities of silver nanoparticle synthesized extract were carried out by standard disc diffusion method. The antibacterial activities of the silver nanoparticle synthesized extract were tested against different pathogenic bacteria's like Bacillus cereus, Staphylococcus aureus, Citrobacter koseri, Salmonella typhii, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia. Nutrient agar (1g beef extract, 1g peptone, 0.5 g NaCl dissolved in 100 ml of double distilled water) was used to cultivate bacteria. The media was autoclaved and cooled. The media was poured in the petri discs and kept for 30 minutes for solidification. After 30 minutes the fresh overnight cultures of inoculum (100 µl) of three different cultures were spread on to solidified nutrient agar plates. Sterile paper discs made of Whatman filter paper, 5 mm diameter. The disc dipped in 50 mg/liter silver nanoparticles was placed in each plate. The cultured agar plates were incubated at 37^{o} C for 24 h. After incubation, the plates were observed for formation of clear inhibition zone around the well indicated the presence of antibacterial activity. The zone of inhibition (shown in Fig.4) was calculated by measuring the diameters of the inhibition zone around the well. [13]

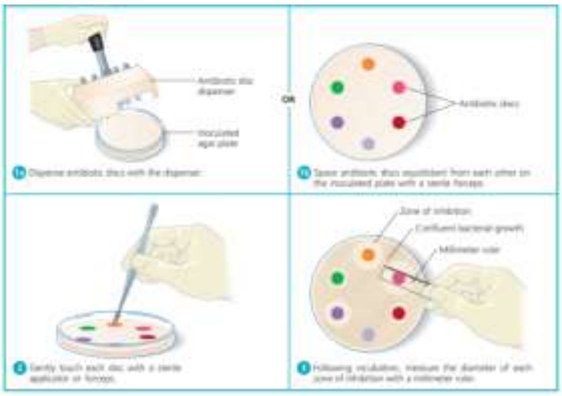

Fig.4 zone of inhibition

Table 1: Plant extract mediated synthesis of silver nanoparticles (AgNPs)

S.No	**Plant**	**Plant's Part**	**Size (nm)**	**Shape**
1	Aloe vera	Leaves	70nm	Spherical
2	Acorus calamus	Rhizome	31.83nm	Spherical
3	Allium sativum	Leaves	4-22nm	Spherical
4	Acalypha indica	Leaves	20-30nm	Spherical
5	Abutilon indicum	Leaves	7-17nm	Spherical
6	Brassica rapa	Leaves	16.4nm	-
7	Boerhaavia diffusa	Whole plant	25nm	Spherical
8	Calotropis gigantean	Leaves	6-12nm	Spherical
9	Citrus sinensis	Peel	10-35nm	Spherical
10	Carica papaya	Leaves	25-50nm	-
11	Cinnamon	Leaves	50-100nm	Spherical

	zeylanicum			
12	Chenopodium album	Leaves	12nm	Spherical
13	Carambola	Fruit	12-16nm	Spherical
14	Calotropis procera	Flower	35nm	Cubic
15	Datura metel	Leaves	16-40nm	Quasilinear superstructures
16	Desmodium triflorum	Leaves	5-20nm	Spherical
17	Eucalyptus hybrid	Leaves	50-150nm	Spherical
18	Eucalyptus globulus	Leaves	5-25nm	Spherical
19	Euphorbia hirta	Leaves	40-50nm	Spherical
20	Embelia ribes	Seeds	20-30nm	Crystalline and spherical
21	Ficus carica	Leaves	13nm	-
22	Garcinia mangostana	Leaves	35nm	-
23	Hibiscus rosa-sinensis	Petals	18.79nm	Spherical
24	Mentha piperita	Leaves	90nm	Spherical
25	Melia dubia	Leaves	35nm	Spherical
26	Moringa oleifera	Leaves	57nm	Spherical
27	Millettia pinnata	Leaves	15nm	Spherical
28	Pongamia pinnata	Seeds	5-30nm	Spherical
29	Plumeria alba	Flower	36.19nm	Spherical
30	Pongamia pinnata	Leaves	15-35nm	Spherical
31	Salvinia molesta	Leaves	12.46nm	Spherical
32	Syzygium alternifolium	Fruit	4-48nm	Spherical
33	Solanum indicum	Leaves	10-50nm	Spherical
34	Tea extract	Leaves	20-90nm	Spherical
35	Tribulus terrestris	Fruit	16-28nm	Spherical
36	Tectona grandis	Leaves	26-28nm	Spherical
37	Trachyspermum ammi	Seeds	87,99.8nm	Spherical
38	Vitis Vinifera	Fruit	30-40nm	Spherical
39	Ziziphus jujuba	Fruit	25.75nm	Spherical
40	Ziziphora tenuior	Leaves	8-40nm	Spherical

VI. MECHANISM OF ACTION OF SILVER NANOPARTICLES AGAINST BACTERIAL CELLS

- ❖ Attachment to the bacterial cell wall and changing the permeability of the cell membrane.
- ❖ Production of Reactive oxygen species and damage the cell membrane. It shows in Fig.6
- ❖ Bind to DNA and leads to problem in DNA replication.[9] [11] it shows in Fig.5

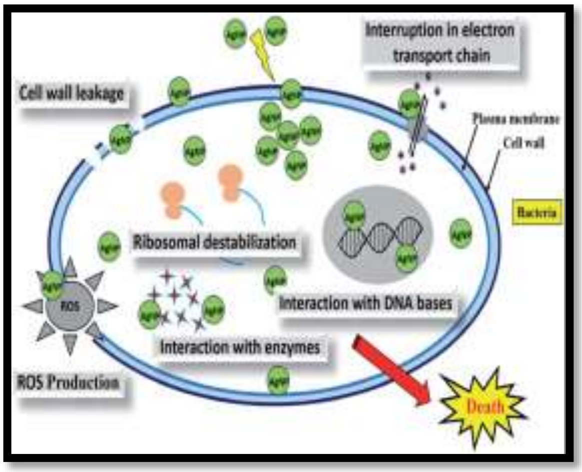

Fig.5 Mechanism of AgNPs

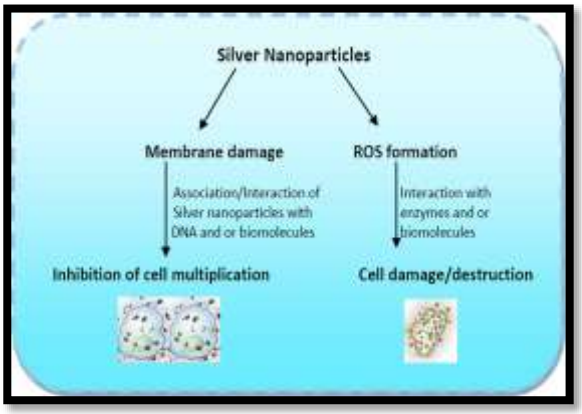

Fig.6 Mechanism of AgNPs

VII. APPLICATIONS OF SILVER NANOPARTICLES

1. Scientific Applications

Due to the surface Plasmon resonance (SPR) and surface enhanced raman scattering (SERS) properties of silver nanoparticles, they have many applications such as sensing applications including detection of DNA sequences.laser desorption/ionization mass spectrometry of peptides, colorimetric sensors for Histidine , enhanced IR absorption spectroscopy, biolabeling and optical imaging of cancer.biosensors for detection of herbicides and glucose sensors for medical diagnostics.[4]

2. Medical Applications

Nanosilver is used for coating such as incorporated in wound dressings, diabetic socks, scaffolds, sterilization materials in hospitals, medical textiles etc. One website claims that "the number of people using colloidal silver as a dietary supplement on a daily basis is measured in the millions". [4]

3. Industrial Applications

Catalysis

The silver nanomaterials and silver nanocomposites are used as catalyst in many such as CO oxidation, benzene oxidation to phenol, photodegradation of gaseous acetaldehyde and the reduction of the p-nitrophenol to p-aminophenol. To catalyze the reduction of dyes by sodium borohydride (NaBH4), silver nanoparticles immobilized on silica spheres. [4]

Electronics

Nanosilver has high electrical and thermal conductivity along with the enhanced optical properties leads to various applications in electronics. In nanoelectronics, silver nanowires are used as nanoconnectors and nanoelectrodes. Other applications include, optoelectronics, nanoelectronics (such as single-electron transistors and electrical connectors), data storage devices, the preparation of active waveguides in optical devices high density recording devices, intercalation materials for batteries, making micro- interconnects in integrated circuits (IC) and integral capacitors etc.[4]

Applications of silver nanoparticles in pharmaceutics, medicine, and dentistry
Pharmaceutics & Medicines
❖ Treatment of dermatitis; inhibition of HIV-1 replication
❖ Treatment of ulcerative colitis & acne
❖ Antimicrobial effects against infectious organisms
❖ Remote laser light-induced opening of microcapsules
❖ Silver/dendrimer nanocomposite for cell labeling
❖ Molecular imaging of cancer cells
❖ Enhanced Raman Scattering (SERS) spectroscopy
❖ Detection of viral structures (SERS & Silver nanorods)
❖ Coating of hospital textile (surgical gowns, face mask)
❖ Additive in bone cement
❖ Implantable material using clay-layers with starch-stabilized Ag NPs
❖ Orthopedic stocking
❖ Hydrogel for wound dressing
Dentistry
❖ Additive in polymerizable dental materials Patent
❖ Silver-loaded SiO_2 nanocomposite resin filler (Dental resin composite)
❖ Polyethylene tubes filled with fibrin sponge embedded with Ag NPs dispersion.

VIII. CONCLUSION

The green synthesis of silver nanoparticles using plants extracts have many advantages over other methods as they are cost effective, easily scaled up and environment friendly. It is especially suited for making nanoparticles that must be free of toxic contaminants as required in therapeutic applications. Green synthesized silver nanoparticles have noteworthy aspects of nanotechnology through unmatched applications and synthesis of nanoparticles using plants can be beneficial over other other biological methods because plant products are easier and safer to handle, widely distributed and easily available. In this review conclude that biologically synthesized AgNPs are fairly ideal candidates for the development of new antimicrobial drugs against bacteria.

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