A Green Synthesis Techniques by Silver Nanoparticles Utilizing to The Plant Components with Antimicrobial Activity

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Abstract : Nanotechnology is the investigation of particles of sizes going from 1 to 100 nanometers, which are alluded to as nanoparticles. When contrasted with their basic structures, these NPs show gigantic assortment in their optoelectronic, electrical, and physiochemical properties on account of the surface-to-volume extent. Backwoods fires, soil disintegration, dust storms, blasts of dirt minerals, volcanic ejections, and rock enduring are generally regular reasons for these small particles. AgNPs can be made through physical, synthetic, or green amalgamation methods however plant extricate nanoparticle union is among the most direct, quick, savvy, and biologically viable ways of trying not to utilize perilous synthetic substances. Accordingly, different eco-accommodating strategies for the fast structure of silver nanoparticles utilizing fluid concentrates of plant parts like the leaf, bark, and roots have been distributed lately. We make sense of the naturally amicable production of silver nanoparticles using separates from assorted plant parts, as well as their characterization and antibacterial capacities, in this audit study.

Keywords silver nanoparticles, SEM, XRD, UV-Vis spectroscopy, antimicrobial activity

1. Introduction Nanoparticles are tiny particles with at least one size of less than 100 nanometers. The production and modification of products at the nanoscale have gotten a lot of attention, resulting in unique features. Since the particles have a significant surface area per unit volume, these products have completely different properties than their bulk source. Noble metal nanoparticles, such as gold, silver, and platinum, are known among metallic nanoparticles. Silver nanoparticles have sparked a lot of curiosity, and their high solubility and other unique qualitieshave prompted a lot of research [2]. Silver (Ag) metal was once frequently employed as a colloidal substance. The synthesis, development, and characterization of innovative goods has become easier thanks to the invention and advancement of new techniques, methodologies, andtechnology. Drug delivery, biosensing, and nanomedicine are just a few of the disciplines wherethey can be used, and so are catalysis, nanodevice creation, and imaging [1]. Their antibacterial activity is influenced by the size of nanoparticles. Because the particles are smaller, there is moreroom for microbes to interact with them. [2]

The production of AgNPs can be done in a variety of ways, including chemical, physical and biological approaches. Furthermore, due to its low cost in production, simple method of manufacturing and environmentally compatible approach, the AgNPs may be made in a sustainable way. [1, 2]. In general, for the reduction of Ag^+ to Ag^0 , bacteria, fungi, algae, or plants is being widely utilized as intracellular or extracellular sources and this can promote "green" bio-synthesis of AgNPs. This reduction mechanism can be split into three primary steps:1^{st} silver ion reduction and nucleation, 2^{nd} the growth step and its aggregation, and 3^{rd} capping and stabilization in the terminal stage. Plant phytochemicals, such as sugars, polyphenols, proteins, phenolic acids, ketones, terpenoids, amides, and other secondary metabolites, constantly play a key role. Furthermore, a reducing agent derived from the plant extract is used as both a capping and stabilising agent in the majority of cases. [3] The synthesis of nanoparticlescan be done in two ways: "top-down" and "bottom-up." The first method starts with a solid massand uses any mechanical process (such as mechanical grinding) to reduce it to a smaller nanoscale particle; the particles are then fixed to the desired size. However, achieving the desired thin size in this manner is challenging. The second way begins with an atomic-scale material that is nanoscale created using chemical methods such as hydrothermal method, sol-gel method, gas phase method, and hydrolysis. Furthermore, these technologies make it difficult to manage the surface chemistry, size, and structure of nanoparticles. The bottom-up technique to creating nanoparticles is recommended in general since it starts with particles that are simpler to assemble and progresses to nanoparticles. It allows you to have more control over the shape and size of the particles that are created. The physical and chemical procedures for the production of silvernanoparticles (Ag-NPs) have certain drawbacks (Ag-NPs) such as a long preparation time, are not cost effective, need high temperatures, pressures, and energy, and are not environmentally safe. When compared to chemical and physical methods, which require more chemicals and energy and result in the formation of dangerous by-products, green synthesis has the advantages of being environmentally friendly, cost-effective, as well as using biocompatible agents to obtainthe silver nanoparticles. [2]

To monitor the creation of AgNPs, UV-visible spectroscopy is an easy and commonly adopted analytical approach. The collective vibration of the conducting electrons which are present in the outermost orbital of metal NPs in resonance with particular wavelengths when they contact with an electromagnetic field, resulting in a phenomenon known as surface plasmon resonance (SPR).The colour and absorbance observed in a colloidal solution of AgNPs is due to SPR stimulation. The reduction of silver nitrate into AgNPs is commonly confirmed by SPR peaks about 435 nm. In general, spherical NPs have only one SPR band in their absorbance spectra, whereas anisotropic particles have two or more SPR bands depending on their shape.

2. Literature Review

We reviewed last few journals for the green approach of silver nanoparticle synthesizing.We found that free radical scavenging molecules found in medicinal plants include phenolic compounds (phenolic acids, flavonoids, catechins, proanthocyanidins, quinons, coumarins, tannins, and other secondary metabolites), nitrogen compounds (alkaloids, amines, betalains, and other secondary metabolites), vitamins, terpenoids, carotenoids, and other secondary metabolites. These phytochemicals operate as decreasing and capping agents in the system.

(Kaliyaperumal Logaranjan et al 2016) synthesized silver nanoparticles using aloe vera gel by firstly at room temperature and then by microwave heating methods which created spherical as well as octahedron-shaped particles and their antimicrobial effect was evaluated on Gram-positive and Gram-negative microorganisms. The researchers stated that the activity of the extract was increased and synergetic. This research identifies an effective SERS substrate made up of octahedron-shaped AgNP that can be made by microwave heating utilising Aloe vera gel, which is one of the probable size and shape-directing agents.

(Omid Ahmadi et al 2018) delineated that the creation of AgNPs can be improved by optimising the synthesis factors, which according to the researchers was a 360-second microwave irradiation, 9 mL $AgNO_3$ (1 mm) and 0.1 mL Aloe Vera. This examination using response surface methodology. The created AgNPs were characterised using transmission electron microscopy, UV-Vis spectroscopy, and dynamic light scattering, which reported the formation of particles of mean particle size of 46 nm, amaximum concentration of 64 ppm, and a zeta potential of +15.5 mV that are well-dispersed and spherical in shape.

(Mona S. Alwhibi et al 2021) employed Aloe Vera gel in order to develop an environmentally safe andfast method of AgNPs production. The shift in appearance from colorless to light yellow, then dark brown, indicated the reduction of Ag ions. TEM micrographs revealed spherical-shaped synthesized Ag-NPs with 50–100 nm diameters. A large absorption peak of surface plasmon resonance (SPR) was visible in the UV-Vis spectra at 450 nm. The generated Ag-NPs had an average size of roughly 82 nm and a PDI (polydispersity index) value of 0.134. Antimicrobial activity was observed in these NPs against a variety of bacterial and fungal species.

(Thanaa I. Shalaby et al, 2015) offered a cost-effective and eco-safe approach for green production of silver nanoparticles and analyze their antibacterial property using Zingiber officinal rhizome extract as a reducing and capping agent in $AgNO_3$ solution. The characterization was done by UV-vis spectrophotometer, Transmission Electron Microscopy, X-ray Diffraction and Fourier Transform Infrared Spectroscopy. Mono-dispersed silver nanoparticles with an average size of 3.1 nm were discovered by TEM analysis. The biosynthesized nanoparticles were confirmed to be crystalline silverby X-ray diffraction investigations. According to Fourier Transform Infrared spectroscopy study, the produced nano-silver was capped with bimolecular compounds due to which the reduction of silver ions occurred. These nanoparticles were tested for antibacterial activity against Staphylococcus aureus and Escherichia coli.

(Alaa H. Alkhathlan, 2020) presented an environmentally safe approach for the manufacture of silver nanoparticles by using two distinct aqueous extracts of Zingiber officinale and Nigella sativa L. seeds. The characterization of the produced particles was done by XRD, ultraviolet–visible (UV-Vis) spectroscopy, and energy dispersive spectroscopy (EDX). Particles of nearly 8nm was synthesized by the Nigella sativa L. seed extract (NSE) and that by Zingiber officinale extract was of nearly of size 12 nm, as analyzed by TEM analysis. The research showed that a high amount of extract resulted in silver nanoparticles that were less clustered and smaller in size. This paper showed that although the nanoparticles made by Nigella sativa L. seeds extract was of slightly higher quality , greater antibacterial activities was shown by NPs made with ginger extract.

(Mona S. Alwhibi et al, 2018) in this research green synthesis of AgNPs had been done using extract of fenugreek seeds. The analytic techniques used were UV-Visible spectroscopy, TEM and FTIR spectroscopy which concluded that the produced NPs displayed maximum peak of absorption at 425 nm in the UV Visible spectra, having spherical with size ranging from 10 to 15 nm when analyzed by TEM analysis and two peaks of absorption at nearly around 3250 cm^{-1} and 1635 cm^{-1} in FTIR spectroscopy. The researchers tested the antibacterial activity of the synthesized NPs against various bacteria E. coli, K. pneumonia, B. subtilis and S. aureus amongst which only it showed least effect on E. coli. The antifungal activity was also analyzed against F. equiseti and Alternaria alternata by taking different elements like for water and for methanol for both fungi.

(Giriraj Tailor et al, 2020) this research was done to make silver nanoparticles using the leaves of Ocimum canum Sims, a commonly used folkloric medicine. SEM and XRD characterization techniques wasused. The SEM analysis showed the morphological forms of NPs that are spherical and rod-like and the XRD showed the particles' crystal structure that had a size of 15.72 nm. The synergistic antibacterial activity of the produced silver nanoparticles was validated by the maximum Zone of Inhibition of 2.45 cmat 30 ppm against pathogenic bacterium Escherichia coli.

3. Research Methodology

Green approach for the synthesis of silver nanoparticles is a simple one-step procedure that produces no harmful or hazardous compounds, making them cost-effective, efficient, and environmentally benign. Plants & microorganisms have been studied intensively in recent decades for this purpose for these particles' various sizes, shapes, stability, and antimicrobial activities.

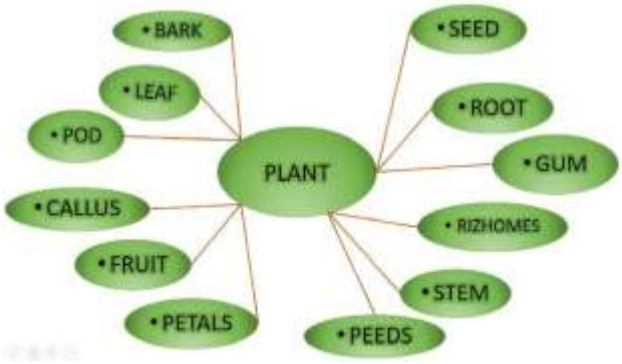

Figure 1 Different plant parts which can be used forsynthesis of AgNPs

Plant parts are obtained from varied sources and cleaned adequately with common water before being distilled to remove dirt and other undesired items. Following that, the parts are either dried and processed to form powder or used fresh to manufacture extract.

Plant extract preparation

To make the extract, the diced pieces or crushed powder of the plant components are placed in deionized water or in alcohol and heated for some time duration at a temperature below 60 degrees Celsius, as prolonged high-temperature heating might cause phytochemical breakdown in the biomass extract.

Synthesis

The synthesis of AgNPs was achieved by adding plant extracts of variable pH to solutions containing varying concentrations of Ag salt as a metal precursor, followed by heating at various temperatures. As the time progresses this mixture changes its colour which shows the creation of silver nanoparticles as it can be seen in the below images.

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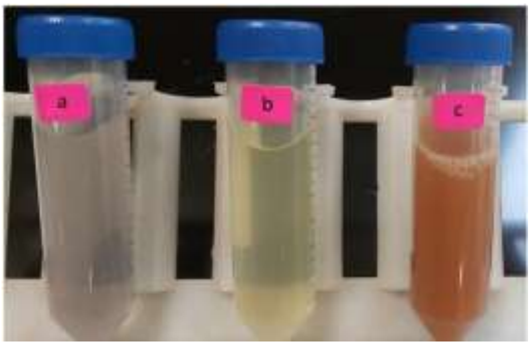

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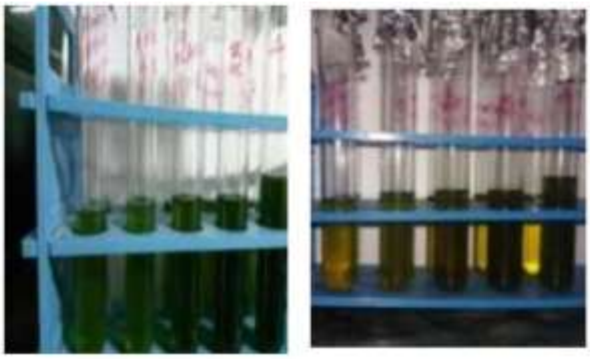

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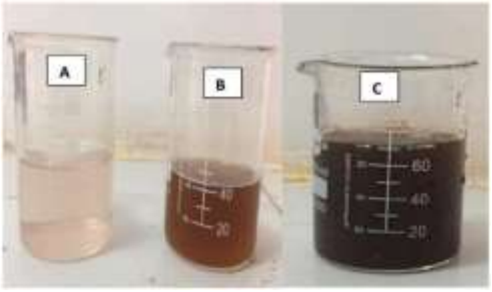

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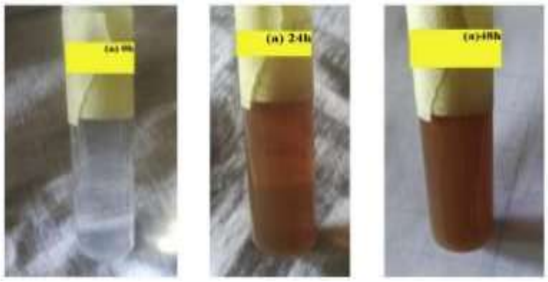
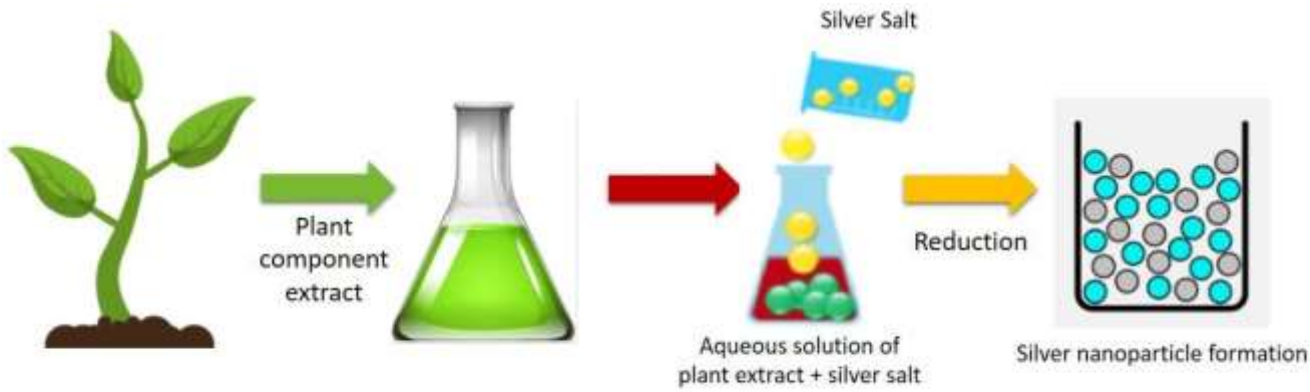


Figure 2 (i) Aloe Vera gel extract and silver nitrate mixture after (a) 10 mins (b) 45 mins; (c) final production of the nanoparticles. [8] (ii) Aquous solution of silver nanoparticles and ficus religiosa leaf extract with different concentrations in each test tube observed for 24 hours [13] (iii) Soltuion of silver nitrate and extract of leaves Ocimum canum Sims for 15 minutes of observation.[12] (iv) colloidal solution



of Acacia cyanophylla and silver nitrate at 0 th hour, 24th hour and 48th hour of preparation.[2]

Figure 3 a diagram depicting the production of silver nanoparticles

Parameters affecting shape and size of the synthesized particle:

1. **Temperature:** We learned from the literatures that the nanoparticles produced at normal temperature are spherical in shape but these particles are generated by microwave heating the processes a range of nanostructured materials with smaller sizes, narrower size distributions, and spherical, octahedron, rod, and tetrahedron shapes are developed. This kind of heating results in tiny and homogeneous in size NPs and takes less time for reaction **[6]**.
2. **Amount of AgNO3** At low concentrations of silver nitrate, the number of ions decreases, their repulsion decreases, and all free silver ions are swiftly converted to silver nanoparticles by the addition of constant extract. However, at larger concentrations of silver nitrate, the Ag ion repulsion is too strong, complicating the nucleation of free reduced Ag ions.**[7]**
3. **Source of green synthesis**
   Silver nanoparticles can be produced by using microorganisms such as fungi, bacteria yeasts etc or by plant extracts, and using membranes, virus's DNA, or diatoms.

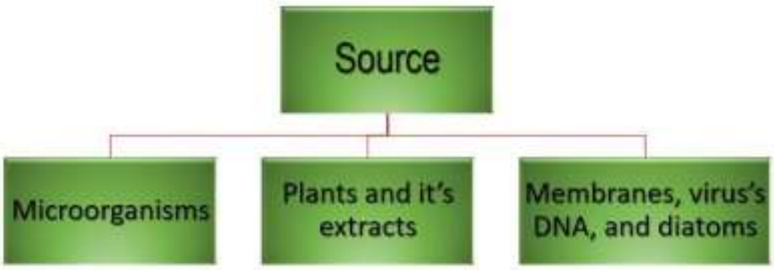


Figure 2 Classification of source of extract.

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So, the most significant aspect is the source of extract we are using for this purpose. Plants have high availability; are very safe to use and offers a diverse range of active compounds that can help reduce Ag ions. Their biomolecules are the ones actually responsible for stabilizing and capping the nanoparticles. **[15]**

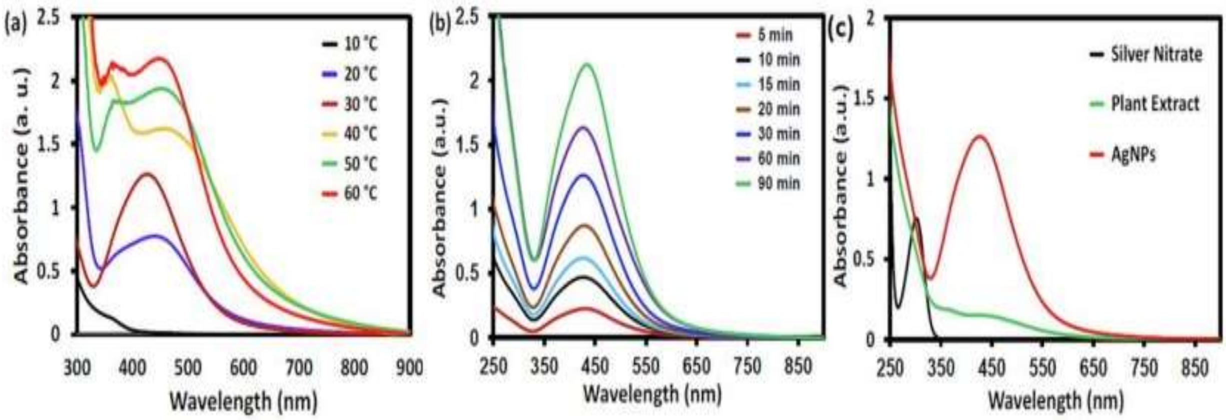
Characterization of Silver nanoparticles

Silver nanoparticles (AgNPs) are evaluated using a variety of analytical methods like

UV – Visible Spectroscopy

A highly helpful and reliable method for the initial analysis of produced nanoparticles, as well as for monitoring its production and stability. Silver nanoparticles strongly interact with specific wavelengths of light due to which, in resonance with the wave of light, its free electron collectively oscillates giving rise to surface plasmon resonance absorption bands. **[16]** For a variety of metal nanoparticles varying in size from 2 to 100 nm the identification of the spike in the absorption band, which has been allocated to a surface plasmon, has been extensively reported. **[17]**

(i)



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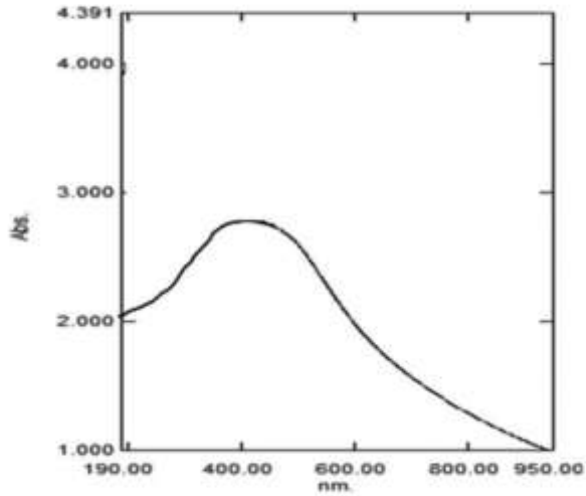

***Figure 3** UV- Vis spectra of the synthesized NPs showing absorption peaks (i) for different temperatures, time range &modified graph for AgNPs [19] (ii) AgNPs produced by extract of Aloe vera gel.[8]*

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X-ray Diffraction (XRD)

XRD is a well-known characterisation method used for measurement of the degree of crystallinity at the atomic scale, to analyse the structure of nanoparticles, particle sizes, for compounds identification, and to determine structure imperfections in the structures. The analysis is done using the diffraction patterns formed **[18].**By comparing diffracted beams to the reference database in the Joint Committee on Powder Diffraction Standards (JCPDS) library, each substance may be defined and identified. **[16]**

(i) (ii)

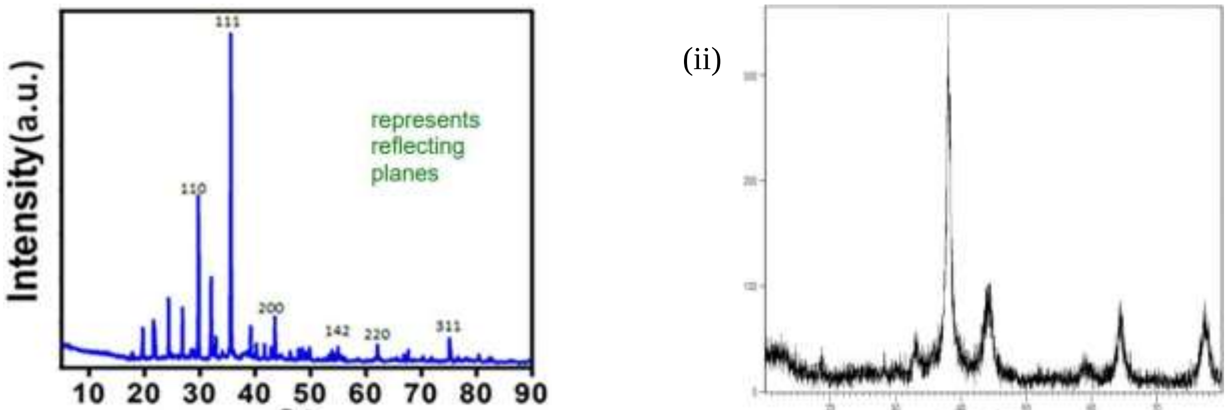

Figure 4 XRD patterns of synthesized AgNPs (i) using Conocarpus Lancifolius fruit extract [19] (ii) Zingiber officinale rhizome extract; the peaks shows the lattice planes by which the shape of the silver nanoparlicles crystalline structure can be determined [20].

SEM (Scanning Electron Microscopy)

At the micro and nanoscales, SEM may be used to investigate diverse particle sizes, size distributions and surface morphology of manufactured particles. **[16]** Sometimes a light, fine coating can be seen all around the silver nanoparticles which indicates the particles' capping by substances found in plants like protein compounds and flavonoids helps in avoiding agglomeration. This can be further verified by other analytical techniques such as EDEX & FTIR. **[21]**

(i) (ii) (iii)

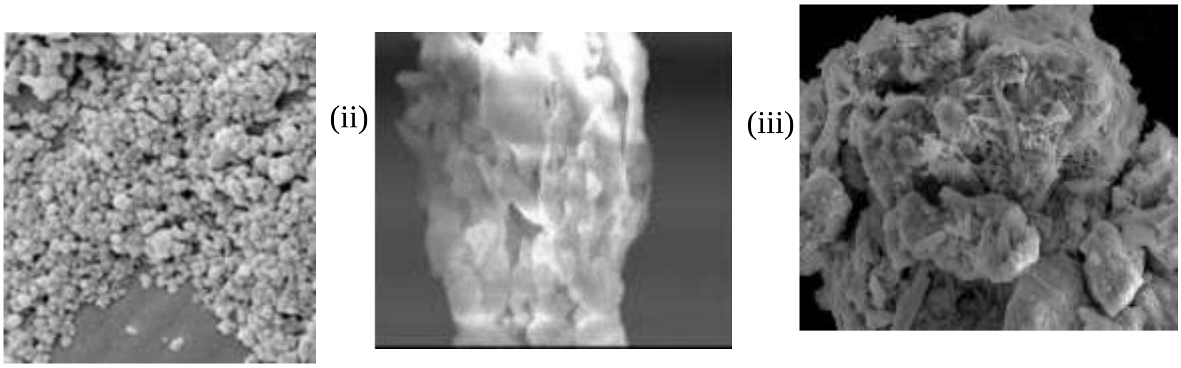

Figure 5 SEM images of synthesized AgNPs (i) using Aloe Vera gel extract and incubated for 12h for 100 °C[22]

(ii)using Ficus religiosa leaves [13] (iii) Tulsi leaves extract at magnification of 10000.[12]

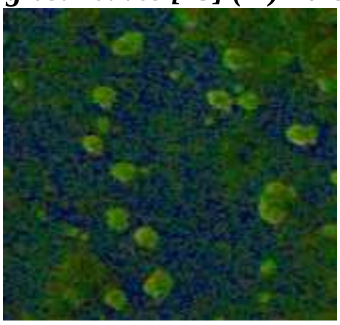

Figure 6 SEM image of AgNPs capped with garlic extract [21]

The different strengths and limits of each approach challenge the selection of appropriate method, while a combinational characterisation technique is sometimes required. Various characterisation approaches are categorised based on the concept/group of the technique, the information it may give, or the materials it is intended for.

Size and shape are two of the most important criteria addressed in the characterisation of NPs. We may also study the surface chemistry and estimate the size distribution, level of agglomeration, surface charge, and surface area. Other features and applicability of NPs may be determined by their size, size distribution, and chelates on their surfaces. Some of the analytical techniques and their functions are mentioned in table 1

. *Table 1 Functions of different characterization techniques*

Analytical techniques	**Functions**	**Ref.**
UV-Visible spectroscopy	Evaluate the stability and characteristics of AgNPs	23
XRD	Measure the degree of crystallinity at the atomic scale. Used to analyse the structure of nanoparticles, particle sizes, for compounds identification, and to determine structure imperfections in the structures. The analysis depends on the formation of diffraction patterns	18
TEM	Measure of particle size, morphology, and size distribution. Provide better spatial resolution compared to SEM	24
ZETA POTENTIAL	stability of colloidal dispersions	25
FTIR	Characterize various chemical bonding in nanomaterials.	14
SEM	size, shape and surface morphology of the nanoparticles	13
DLS	Measures the hydrodynamics of nanoparticle size in an aqueous solution comprising a metallic core, ions, and biological macromolecules bound to the NPs' surface.	23

Action Mechanism

Nanoparticle size determines the mode of action; the smaller the nanoparticles, the lower the minimal inhibitor concentration (MIC). Antibacterial characteristics of AgNPs include the following: 1. Adherence to the surface membranes of microbes. 2. AgNPs penetrate cells, alter biomolecules, and cause interior destruction. 3. Generating oxidative stress in cells and triggering cellular toxicity by creating ROS. 4. Enabling cellular signal transduction pathways to be disrupted **[26].**The below figure demonstrates the action mechanism.

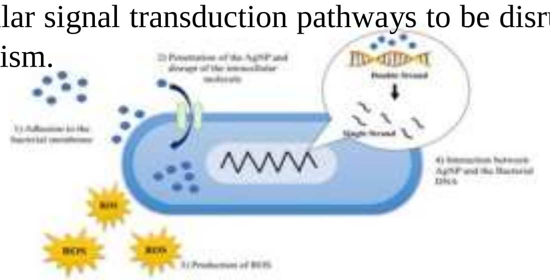

Figure 7 Action silver nanoparticles onto a bacteria [26]

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Table 2 Showing Zone of Inhibition for different microbes [6]

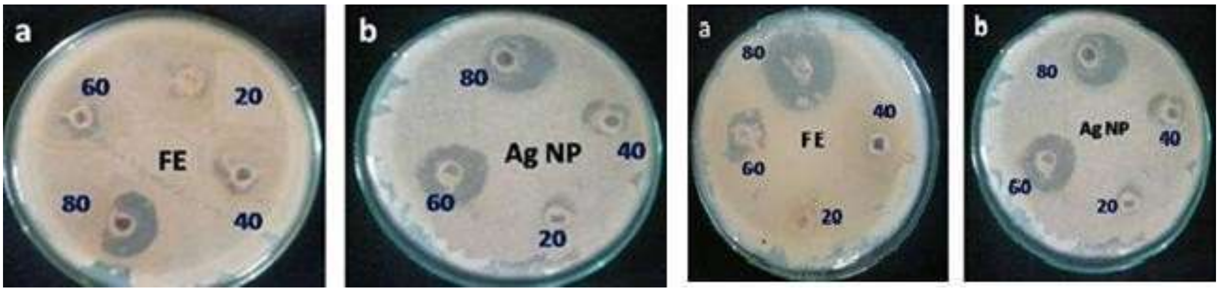
Microorganisms	*Aloe vera* extract	AgNP using *Aloe vera* extract (biological synthesis)	AgNP using chemical method (non-biological synthesis)	$AgNO_3$
	Zone of inhibition (mm)			
Staphylococcus aureus	15	43	26	20
Bacillus cereus	13	38	22	18
Micrococcus luteus	19	33	13	15
Escherichia coli	26	42	14	22
Klebsiella pneumoniae	19	38	15	17

Antimicrobial activity

To study the antimicrobial activity the method of diffusion is generally followed. **[27]**. It can be seen in figure 9

.The zone of inhibition increases according to the effectiveness of the antimicrobial agent being tested. Theinteraction of AgNPs depends on its size & shape**. [28]**

(i)



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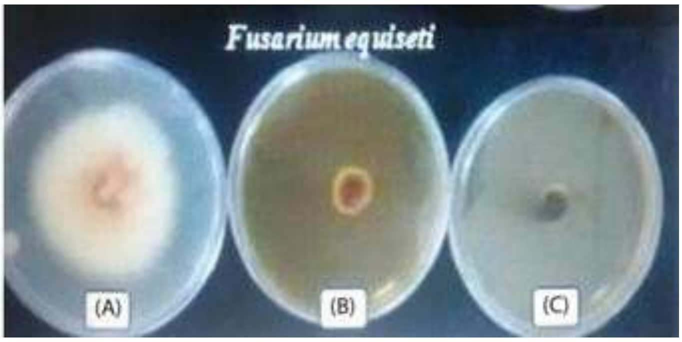

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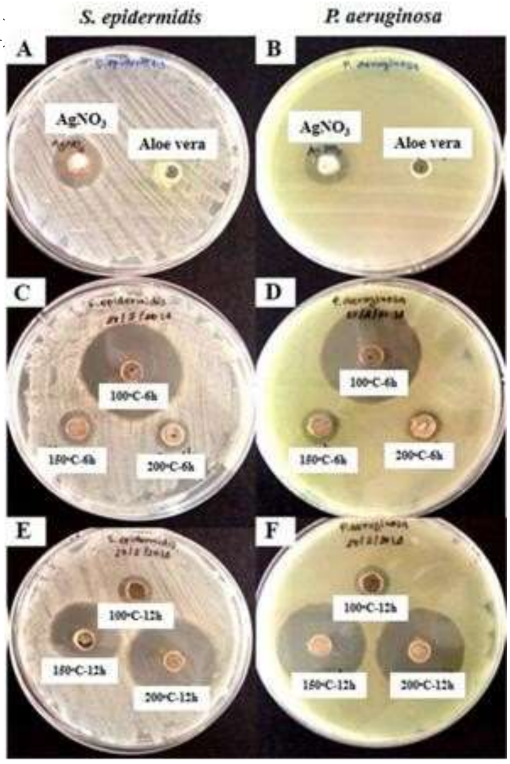

Figure 8 (i) Antimicrobial activity of (a) only fruit extract (b) AgNPs against E.coli and B.subtilis respectively [28] (ii) antifungal activity of AgNPs against F. equiseti [11] (iii) antibacterial activity against S. epidermidis and P. aeruginosa at different temperatures for different duration of incubation [22]

Table 3 showing the antimicrobial activity of AgNPs and the colour change observed for different components of the plants

Plants	Plant part	Shape and size	Colour change observed	Microrganisms tested	Ref.
Fenugreek	Seed	spherical 10 to 15 nm	Yellowish brown	E. coli, K. pneumonia, B. subtilis and S. aureus, F. equiseti and Alternaria alternata	[11]
Acacia cyanophylla	aerial parts like leaves and flowers.	Spherical 88.11 nm	Light yellow to reddish brown	E. coli	[2]
Aloe vera	leaves	spherical 50–100 nm	Light yellow to brown	Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus, E. coli	[8]
FICUS RELIGIOSA	leaves	spherical 20 nm to 50 nm	Green -> Brown	-----	[13]
Ocimum canum	leaves	Spherical and rod like 15.72 nm	Yellow -> Brown	Escherichia coli	[12]
Zingiber officinale	root	Spherical 12 nm	Light yellow to reddish brown	Staphylococcus aureus and Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa	[10]
Nigella sativa L.	seeds	Spherical 8 nm	Light yellow to reddish brown	Staphylococcus aureus and Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa	[10]
Zingiber officinale	rhizome	Spherical3.1 nm	yellow ->yellowish brown	Staphylococcus aureus and Escherichia coli	[9]
Conocarpus Lancifolius	leaves	Spherical 5 & 30 nm	light yellow to dark yellow	Streptococcus pneumonia and Staphylococcus aureus; Rhizopusus stolonifera and Aspergillus flavus	[19]

Conclusions and Prospects

This study examines a variety of investigations on silver nanoparticles in order to acquire a better knowledge of its synthesis using plant extracts, factors that affects its physical dimensions, functioning of its antimicrobial activity and the methods used for its characterisation. This technique to produce silver nanoparticle offers advantages like simple procedure, financial feasibility, and so on. We emphasized on the usage of plants because of its easy availability and medicinal values it carry. At first glance, the presence of AgNPs can be confirmed by colour changes which occurs in the aqueous solution. Then the colloidal solution is taken for UV-Visible spectroscopy where it is generally observed that the absorption peaks has a range 300-600 nm which is due to the surface Plasmon vibrations in silver nanoparticles being excited. **[2, 11]** .

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Likewise with each characterization technique, different properties of NPs can be determined. The antimicrobial of these silver nanoparticles can be seen against a wide range of microorganisms due to which it can used in many fields like medical, textile industries or for controlling environmental pollution by monitoring their cytotoxicity. To reduce the bacteria like E. coli and B. subtilis which are found in waste water, these nanoparticles can be impregnated onto cotton fibres or nylon fibres, which can be further used as a mesh in filters. This could be an easy and cost-effective way of preparing filters for waste water treatment **[29,30]** and for that more research work has to be done to understand its limitations also.

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