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BIOLOGICAL SYNTHESIS, CHARACTERIZATION AND APPLICATION OF SILVER NANO PARTICLES - A REVIEW

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ABSTRACT

Investigation of novel properties of nanoparticles and their application has become a very active area of research. Nanoparticle having one or more dimensions of the order of 100nm or less- have attracted considerable attraction due to their unusual and fascinating properties with various applications. Physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles. The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. Silver nanoparticles are being used as antimicrobial agents and they are said to show good antimicrobial action. There are many ways depicted in various literatures to synthesize silver nanoparticles includes physical, chemical, and biological methods. The physical and chemical methods are numerous in number, and many of these methods are expensive or use toxic substances which are major factors that make them not so favored methods of synthesis. An alternate, feasible method to synthesize silver nanoparticles is to employ biological methods of using microbes and plants. The new innovative ideas from various contributors for preparation, applications, and characterization methodologies followed were discussed here

Key Words: Silver nanoparticles, medical applications, characterization, synthesis.

INTRODUCTION:

Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other scientific fields, such as chemistry, biology, physics, materials science, and engineering conducted at the nanoscale, which is about 1 to 100 nanometers. Particles are further classified according to diameter. Coarse particles cover a range between 10,000 and 2,500 nanometers. Fine particles are sized between 2,500 and 100 nanometers. Ultrafine particles, or

nanoparticles are sized between 1 and 100 nanometers.

According to a recent definition suggested by British Standards Institution Nanoparticles are the particles with one or more dimensions at the nanoscale. They have defined the nanoscale as dimensions of the order of 100 mm or less. It is the critical length scale at which certain novel size related properties develop and the material start behaving differently than the molecules or bulk

material [1]. Here novel properties refer to optical magnetic and electrical properties which typically appear in materials below 100 nm size. However, many a times, besides the strictly nano (1-100 nm) all submicron colloidal particles i.e. particles with at least one dimension in the scale of 1-1000 nm. called mesoscale. are referred nanoparticles, to include organic polymers and vesicles widely used in the area of drug delivery [2,3]. At this scale the properties of the matter are different from atomic or molecular properties which are governed by the laws of quantum mechanics, or the properties of bulk materials determined by the laws of classical physics. So the dimension of 1-100 nm may be considered as an intermediate state between atomic or molecular state and bulk state where materials exhibit some unexpected and unusual new properties which cannot be defined by the classical laws of physics [4]. It is these unusual properties which have attracted immense attention of researchers from almost every field science including biology and

Investigation of novel properties of nanoparticles and their application has become a very active area of research. Nanoparticle having one or more dimensions of the order of 100nm or less- have attracted considerable attraction due to their unusual and fascinating properties, with various applications, over their bulk counterparts. Currently, a large number of physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles. Though physical and chemical methods are more popular for nanoparticle synthesis, the use of toxic compounds limits their applications. The development of safe eco-friendly methods of biogenetic production are now of more interest due to the simplicity of the procedures and versatility. Due to their amenability to biological functionalization, the biological nanoparticles are finding important applications in the field of medicine. The antimicrobial potential of metal based nanoparticles has led to its incorporation in consume, health related and industrial products . Our area of interest lies in the biological synthesis of gold silver nanoparticles.

SILVER NANOPARTICLES:

The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. Nanoparticles have been known to be used for numerous physical, biological, and pharmaceutical applications. Silver nanoparticles are being used as antimicrobial agents and they are said to show good antimicrobial action. It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganisms which include 16 major species of bacteria. This aspect of silver makes it an excellent choice for multiple roles in the medical field. Silver is generally used in the nitrate form to induce an antimicrobial effect, but when silver nanoparticles are used, there is a huge increase in the surface area available for the microbe to be exposed to. Though silver nanoparticles find use in many antibacterial applications, the action of this metal on microbes is not fully known. It has been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction. The rapid breakdown of silver nanoparticles, releases ionic silver that inactivates vital bacterial enzymes by interacting with essential thiol groups. Silver ions can inhibit bacterial DNA replication, damage bacterial cytoplasm membranes, depleting levels of intracellular adenosine triphosphate (ATP) and finally cause cell death. The high specific surfaceto-volume ratio of silver nanoparticles increases their contact with microorganisms, promoting the dissolution of silver ions, thereby improving biocidal effectiveness. The ability of silver nanoparticles to release silver ions is a key to their bactericidal activity.

There are many ways depicted in various literatures to synthesize silver nanoparticles. These include physical, chemical, and biological methods. The physical and chemical methods are

numerous in number, and many of these methods are expensive or use toxic substances which are major factors that make them not so favored methods of synthesis. An alternate, feasible method to synthesize silver nanoparticles is to employ biological methods of using microbes and plants. Silver nanoparticles find use in many fields, and the major applications include their use as catalysts, as optical sensors of zeptomole textile concentration, in engineering, electronics, in optics, and most importantly in the medical field as a bactericide and as a therapeutic agent. Silver ions are used in the formulation of dental resin composites; in coatings for medical devices; as a bactericidal coating in water filters; as an antimicrobial agent in air sanitizer sprays, pillows, respirators, socks, wet wipes, detergents, soaps, shampoos, toothpastes, washing machines, and many other consumer products as bone cement and in many wound dressings to name a few. Though there are various benefits of silver nanoparticles, there is also the problem of nanotoxicity of silver. There are various literatures that suggest that the nanoparticles can cause various environmental and health problems, though there is a need for more studies to be conducted to conclude that there is a real problem with silver nanoparticles.

Extracellular biosynthesis of silver nanoparticles by Aspergillus niger isolated from soil was reported by Gade, A.K et., al. The production of silver nanoparticles was evidenced by UV-vis spectrum, showing the absorbance at 420nm (Perkin Elmer Lambda-25). The nanoparticles characterized Transmission Electron by Microscopy exhibited spherical silver nanoparticles with a diameter of around 20nm. Elemental Spectroscopy imaging showed the presence of fungal protein around the silver nanoparticles thereby increasing their stability in the suspension. The silver nanoparticles (10 µg/ml) showed remarkable antibacterial activity against gram-positive (Staphylococcus. aureus) and gram-negative (Escherichia coli) bacteria. The reduction of the silver ions might have occurred by a nitrate-dependent reductase enzyme and a shuttle quinone extracellular process. Reduction of silver ions was an extracellular and rapid process; this knowledge may lead to the development of an easy process for biosynthesis of the silver nanoparticles. Potential of fungal-mediated biosynthesis of silver nanoparticles is important for the development of effective antibacterial agents showing resistance to drugs available in the market. [1]

Biological production of silver nanoparticles by lixivium of sun-dried Cinnamomum camphora leaf in continuous-flow tubular microreactors was investigated by Jiale Huang et., al. The Properties of silver nanoparticles were examined by transmission electron microscopy (TEM), UV-vis spectroscopy, X-ray diffraction (XRD), and energy dispersive X-ray (EDX). The concentration of residual silver ions after reaction was measured by atomic absorption spectophotometry (AAS) spectroscopy. Fourier transform infrared (FTIR) spectra of C. camphora leaf lixivium were analyzed and temperature profiles along the tubes were calculated to explore the formation mechanism of silver nanoparticles. Comparison of FTIR spectra of *C. camphora* leaf lixivium before and after reaction demonstrated the polyols in the lixivium may be mainly responsible for the reduction of silver ions. According to the temperature profiles, at the inlet of microreactors at 90 °C, the soar of the fluid temperature induced the burst of silver nuclei by homogeneous nucleation. Subsequently, the nuclei grew gradually along the reactors into silver nanoparticles from 5 to 40 nm. Polydisperse particles were formed by combination of heterogeneous nucleation and Ostwald ripening along the tubes at 60 °C. [2]

Avinash Ingle *et.*, *al* reported that the extracellular biosynthesis of silver nanoparticles by *Fusarium solani* (USM-3799), a phytopathogen causing disease in onion, when challenged with 1 mM silver nitrate (AgNO3). The formation of

nanoparticles was characterized by visual observation followed UV-Vis spectrophotometric analysis, which showed a peak at about 420 nm, which is very specific for silver nanoparticles. Further analysis carried out by Fourier Transform Infrared Spectroscopy (FTIR), provides evidence for the presence of proteins as capping agent, which helps in increasing the stability of the synthesized silver nanoparticles. Electron Microscopy (TEM) Transmission investigations confirmed that silver nanoparticles were formed. The synthesized silver nanoparticles were found to be polydispersed, spherical in the range of 5-35 nm with an average diameter of 16.23 nm. Extracellular synthesis of nanoparticles could be highly advantageous from the point of view of synthesis in large quantities and easy downstream processing [3].

An eco-friendly process for rapid synthesis of silver nanoparticles has been reported by Hare Krishna Bar *et.*, *al* using an aqueous seed extract of *Jatropha curcas*. The formation of stable silver nanoparticles of different concentration of AgNO3 gives mostly spherical particles with diameter ranging from 15 to 50 nm. The resulting silver particles are characterized using HRTEM, XRD and UV–vis spectroscopic techniques. XRD study shows that the particles are crystalline in nature with face centered cubic geometry. [4].

M. Sathishkumar et., al studied the exploitation of various plant materials for the biosynthesis of nanoparticles is considered a green technology as it does not involve any harmful chemicals. The study reports the synthesis of silver (Ag) nanoparticles from silver precursor using the bark extract and powder of novel Cinnamon zeylanicum. Water-soluble organics present in the plant materials were mainly responsible for the reduction of silver ions to nano-sized Ag particles. TEM and XRD results confirmed the presence of nano-crystalline Ag particles. The pH played a major role in the size control of the particles. Bark extract produced more Ag nanoparticles than the powder did, which was attributed to the large

availability of the reducing agents in the extract. Zeta potential studies showed that the surface charge of the formed nanoparticles was highly negative. The EC50 value of the synthesized nanoparticles against Escherichia coli BL-21 strain was 11 ± 1.72 mg/L. Thus *C. Zeylanicum* bark extract and powder are a good bioresource/biomaterial for the synthesis of Ag nanoparticles with antimicrobial activity. [5].

A green, low-cost and reproducible Eclipta leaves negotiated synthesis of silver nanoparticles was reported by Anal K. et., al. The synthesis is performed at room temperature. X-ray and transmission electron microscopy analyses are performed to ascertain the formation of Ag nanoparticles. Nanoparticles almost spherical in shape having a size of 2–6 nm is found. UV-visible study revealed the surface plasmon resonance at 419 nm. The lattice strain is estimated to be 0.0045 using Williamson-Hall approach. The use of Eclipta for the synthesis of silver nanoparticles offers the benefit of ecofriendliness and amenability for large scale production through scaling up. [6]

Sathyavathi et al., reported a simple and ecofriendly biosynthesis of silver nanoparticles using Coriandrum sativum leaf extract as reducing agent. The aqueous silver ions when exposed to leaf extract were reduced and resulted in silver nanoparticles whose average size was 26 nm. The silver nanoparticles were characterized by UV-Visible, X-ray diffraction (XRD), Fourier transform infra-red spectroscopy (FT-IR) and transmission electron microscopy (TEM) techniques. Nonlinear optical properties of silver nanoparticles were studied using Z-scan technique with 6 ns pulse duration at 532 nm. The nonlinear refractive index and third-order susceptibility $|\chi 3|$ were measured to be $-6.0 \times 10-13$ cm²/W and 1.38 × 10-9 esu, respectively. Silver nanoparticles were found to exhibit strong reverse saturable absorption (RSA). RSA was identified as the main mechanism responsible for optical limiting. [7]

Hemath Naveen K.S et al., Synthesised silver nanoparticles by using the filamentous fungus Penicillium Sp. The fungal culture was isolated from the soil samples collected from agriculture fields in Vellore district, Tamil Nadu, India. The purified fungal isolates were inoculated in minimal medium and incubated at room temperature for three days. For the synthesis of silver nanoparticles, 50 ml of cell filtrate was mixed with equal volume of 1mM silver nitrate [AgNO3 (1 mM)] and agitated at room temperature in the dark. The synthesis of silver nanoparticles was investigated by UV-Vis spectroscopy, Atomic force microscopy and Fourier Transform Infrared Spectroscopic analysis. Results indicate the synthesis of silver nanoparticles in the reaction mixture. Mechanism of silver nanoparticles synthesis was determined by nitrate reduction test [8].

Biosynthesis of silver nanoparticles and its activity waterborne bacterial pathogens investigated by C. Krishnaraj et al. The Silver nanoparticles were rapidly synthesized using leaf extract of Acalypha indica and the formation of nanoparticles was observed within 30 min. The results recorded from UV-vis spectrum, scanning electron microscopy (SEM), X-ray diffraction (XRD) and energy dispersive spectroscopy (EDS) support the biosynthesis and characterization of nanoparticles. high-resolution silver From transmission electron microscopy (HRTEM) analysis, the size of the silver nanoparticles was measured 20-30 nm. Further, the antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity against water borne pathogens Viz., Escherichia coli and Vibrio cholera. Silver nanoparticles 10 µg/ml were recorded as the minimal inhibitory concentration (MIC) against E. Coli and V. Cholera. Alteration in membrane permeability and respiration of the silver nanoparticle treated bacterial cells were evident from the activity of silver nanoparticles[9]. Mehrdad Forough et al., discussed the synthesis of stable silver nanoparticles by the bioreduction

method. Aqueous extracts of the manna of hedysarum plant and the soap-root (Acanthe phylum bracteatum) plant were used as reducing and stabilizing agents respectively. UV-Vis absorption spectroscopy was used to monitor the quantitative formation of silver nanoparticles. The characteristics of the obtained silver nanoparticles were studied using X-ray diffraction analysis (XRD), energy-dispersive spectroscopy (EDX) and scanning electron microscopy (SEM). The EDX spectrum of the solution containing silver nanoparticles confirmed the presence of an elemental silver signal without any peaks of impurities. The average diameter of the prepared nanoparticles in solution was about 29-68 nm. [10].

Antariksh Saxena, R.M et al ., studied the of metal and semiconductor synthesis nanoparticles is an expanding research area due to the potential applications for the development of novel technologies. Generally, nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. We have reported a fast, convenient and extracellular method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of onion (Allium cepa) extract. The characterization of nanoparticles was done by using UV- Vis Spectrophotometer and Dynamic Light Scattering (DLS). The morphology of silver nanoparticles confirmed by Transmission Electron microscopy (TEM). The antibacterial activity of these nanoparticles was studied against E. coli and Salmonella typhimurium. The bactericidal property of nanoparticles was analyzed by measuring the growth curve of bacteria and 50µg/ml concentration of silver nanoparticles was found to be effective antibacterial. [11]

Biological synthesis of silver nanoparticles using *Murraya koenigii* leaf extract was investigated by Laura Christensen1 *et al.*, and the effect of broth concentration in reduction mechanism and particle size was reported. The rapid reduction of silver (Ag+) ions were monitored using UV-visible

spectrophotometry and showed formation of silver nanoparticles within 15 minutes. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) analysis showed that the synthesized silver nanoparticle are varied from 10-25 nm and have the spherical shape. Further the XRD analysis confirms the nanocrystalline phase of silver with FCC crystal structure. From this study, it was found that the increasing broth concentration increases the rate of reduction and decreases the particle size. [12].

S.C.G. Kiruba Daniel et al., stated that the Silver nanoparticles of different size and shape were synthesized using Ocimum tenuiflorum leaf extract. The nanoparticles were characterized by UV- visible, TEM, XRD and FTIR measurements. The toxicity of the silver nanoparticles was evaluated against zebra fish Danio rerio using direct exposure to silver nanoparticles and indirectly through food chain via feeding silver nanoparticles exposed chironomous larva) There was no toxicity developed against Ocimum tenuiflorum stabilized silver nanoparticles and it could penetrate all tissues including the brain through BBB Lifetime protection can be given to healthy young ones at a very low concentration (160ug) by simple bathing method. [13].

Green nanoparticle synthesis has been achieved by Sampath Marimuthu et al., using environmentally acceptable plant extract and eco-friendly reducing and capping agents. The present study was based on assessments of the antiparasitic activities to determine the efficacies of synthesized silver nanoparticles (AgNPs) using an aqueous leaf extract of Mimosa pudica Gaertn (Mimosaceae) against the larvae of the malaria vector, Anopheles Grassi, filariasis vector subpictus quinquefasciatus Say (Diptera: Culicidae), and Rhipicephalus (Boophilus) microplus Canestrini (Acari: Ixodidae). Parasite larvae were exposed to varying concentrations of aqueous extract of M. pudica and synthesized AgNPs for 24 h. AgNPs were rapidly synthesized using the leaf extract of M. pudica and the formation of nanoparticles was

observed within 6 h. The results recorded from UV-vis spectrum, Fourier transform infrared, X-ray diffraction, scanning electron microscopy, and transmission electron microscopy support the biosynthesis and characterization of AgNPs. The maximum efficacy was observed in synthesized AgNPs against the larvae of *A. subpictus, C. quinquefasciatus*, and *R. microplus* (LC50 = 13.90, 11.73, and 8.98 mg/L, r 2 = 0.411, 0.286, and 0.479), respectively. This is the first report on antiparasitic activity of the plant extract and synthesized AgNPs. [14]

Development of green nanotechnology generating interest of researchers toward ecofriendly biosynthesis of nanoparticles. Singhal et al., studied the biosynthesis of stable silver nanoparticles using Tulsi (Ocimum sanctum) leaf extract. These biosynthesized nanoparticles were with help of characterized the UV-vis spectrophotometer, Atomic Absorption Spectroscopy (AAS), Dynamic light scattering X-ray diffraction (DLS), (XRD), Fourier transform infrared spectroscopy (FTIR), Transmission electron microscopy Stability of bioreduced silver nanoparticles was analyzed using UV-vis absorption spectra, and their antimicrobial activity was screened against both gram-negative and gram-positive microorganisms. It was observed that O. The sanctum leaf extract can reduce silver ions into silver nanoparticles within 8 min of reaction time. Thus, this method can be used for rapid and ecofriendly biosynthesis of stable silver nanoparticles of size range 4-30 nm possessing antimicrobial activity suggesting their possible application in the medical industry. [15]

Silver nanoparticles (AgNPs) have gained great interest in nanotechnology, biotechnology and medicine. The green synthesis of nanoparticles has received an increasing attention because of its maximize efficiency and minimize health and environmental hazards as compared to the other conventional chemical synthesis. U. B. Jagtap *et al.*, reported biosynthesis of AgNPs by aqueous

Annona squamosa L. LeaJagtapact and its characterization by UV-visible spectroscopy (UVvis), Field emission gun scanning electron microscopy (FEG-SEM), X-ray energy dispersive spectroscopy (EDX), Transmission electron microscopy Selected-area (TEM), electron diffraction (SAED) and Fourier transforms infrared spectroscopy (FTIR). The results indicated that AgNPs formed were spherical in shape with size ranging from 14 to 40 nm with an average diameter 28.47 nm. Furthermore, it was observed that the AgNPs exhibited an antibacterial activity against different Gram positive and Gram negative microorganisms. Our report confirmed that the ALE is a very good eco-friendly and nontoxic bioreductant for the synthesis of AgNPs and opens up further opportunities for fabrication of antibacterial drugs, medical devices and wound dressings. [16]

Naheed Ahmad et al., presented a simple and ecofriendly biosynthesis of silver nanoparticles using Pomegranate peel extract as the reducing agent. Peel extract of Pomegranate was challenged with silver nitrate (AgNO3) and chloroauric acid (HAuCl4) solution for the production of silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs), respectively. The reaction process was simple for the formation of highly stable silver and gold nanoparticles at room temperature by using the biowaste of the fruit. The morphology and crystalline phase of the NPs were determined from UV-Vis spectroscopy, transmission electron microscopy (TEM), selected area electron diffraction (SAED) and X-ray diffraction (XRD) spectra. TEM studies showed that the average particle size of silver nanoparticles was 5 ± 1.5 nm whereas the gold nanoparticles were found to be 10 ± 1.5 nm. An effort has also been made to understand the possible involved mechanism for the biosynthesis of the NPs. Presumably biosynthetic products or reduced cofactors play an important role in the reduction of respective salts to nanoparticles. [17].

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. In this work deals with the synthesis and characterization of silver nanoparticles using Cassia auriculata flower and evaluation of their antioxidant activity. Synthesized nanoparticles were characterized by using UV-Vis absorption spectroscopy and SEM analysis. The synthesized particles were evaluated its in vitro antioxidant activity. The reaction mixture turned to a brownish gray color after 5 hours of incubation and exhibits an absorbance peak around 450 nm characteristic of Ag nanoparticle. Scanning electron microscopy (SEM) analysis showed silver nanoparticles was pure and polydispersed and the size were ranging from 10-40 nm. Biosynthesized nanoparticles exhibited strong antioxidant activity. The approach of green synthesis seems to be cost efficient, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis. The powerful bioactivity demonstrated by the synthesized silver nanoparticles leads towards the antioxidant agent by Velavan. S. et al.,

Metallic nanoparticles have been traditionally synthesized using wet chemical techniques by S. Rajesh et al., where the chemicals used are quite often toxic and flammable. They presented a simple and ecofriendly biosynthesis of Ag nanoparticles using Ulva fasciata crude ethyl acetate extract as reducing and capping agent. The bionanoparticle characterized bv UV-vis Spectroscopy, FTIR, XRD, SEM and EDX. Characterization reveals that the nanoparticles are crystalline in nature, spherical in shape and polydispersed with size ranging from 28 to 41 nm. The alkyne group (3424.30 cm-1) of *U. fasciata* crude ethyl acetate extract shifted and reduced the AgNO3. GC-MS analysis revealed the presence of 1-(Hydroxymethyl) –2, 5, 5, 8A-tetramethyl decahydro-2- napthalenol as reducing agent and hexadecanoic acid was found to be a stabilizing

agent. *Ulva fasciata* based bionanoparticles inhibited the growth of Xanthomonas campestris pv. malvacearum (14.00±0.58 mm Zone of inhibition), with a Minimum Inhibitory Concentration of 40.00±5.77µg/ml. The study shows that *U. fasciata* crude ethyl acetate extract could be used as a reducing agent for the simple ecofriendly synthesis of silver nanoparticles. However, more studies are essential before recommending them for disease management. [19] The cost effective and eco-friendly technique for synthesis of silver nanoparticles from the extract of seed of P. granatum was discussed by Shalini Chauhan et al., The reduction process was simple and convenient to handle and was monitored by UV-vis spectroscopy. So the green synthesis of silver nanoparticles from 1mM AgNO3 solution was prepared through the extraction of P. Granatum seed which acts as a capping and reducing agent. The morphology and uniformity of silver nanoparticles were investigated by TEM and EDX. The functional group of protein molecule was identified by using FTIR. Increase in temperature leads increase in size of silver nanoparticles. [20]

Currently, there is an increasing commercial demand for nanoparticles due to their wide applicability in various markets, such as medicine, catalysis, electronics, chemistry and energy. M. Vijayakumara et al., reported that a simple and eco-friendly chemical reaction for the synthesis of silver nanoparticles (AgNPs) from Artemisia nilagirica (Asteraceae) has been developed. Silver nitrate was used as the metal precursor and hydrazine hydrate as a reducing agent. Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDX) were used to characterize the nanoparticles obtained from A. nilagirica. The morphology of the AgNPs was determined by SEM and the average diameter of the particles was determined as 70-90 nm. The EDX analysis of the nanoparticles dispersion, using a range of 2-4 keV, confirmed the presence of elemental silver, with no other impurity peaks detected. In addition,

the characterized AgNPs has the potential for various medical and industrial applications. The results showed that microbial susceptibility to AgNPs is different for each microorganism. [21] The green synthesis of silver nanoparticles from 1mM AgNO3 solution was prepared by Shalini Chauhan et al., through the extraction of *P. Granatum* seed which acts as a capping and reducing agent. The morphology and uniformity of silver nanoparticles were investigated by TEM and EDX. The functional group of protein molecule was identified by using FTIR. [22]

The biosynthesis of nanoparticles have been proposed as a cost effective and environmentally friendly alternative to chemical and physical methods. Plant mediated synthesis of nanoparticles is a green chemistry approach that intercom- nests nanotechnology and plant biotechnology. Naheed Ahmad et al., discussed on synthesis of silver nanoparticles (AgNPs) or (Green-Silver) and demonstrated using extracts of Ananas comosus reducing aqueous silver nitrate. The AgNPs were characterized by Ultraviolet-Visible (UV-vis) Spectrometer, Energy Dispersive X-ray Analysis (EDAX), Selected Area Diffraction Pattern (SAED) and High Resolution Transmission Electron Microscopy (HRTEM). TEM micrographs showed spherical particles with an average size of 12 nm. The XRD pattern showed the characteristic Bragg peaks of (111), (200), (220) and (311) facets of the face center cubic (fcc) silver nanoparticles and confirmed that these nanoparticles are crystalline in nature. The different types of antioxidants presented in the pineapple juice synergistic- Cally reduce the Ag metal ions, as each antioxidant is unique in terms of its structure and antioxidant function. The reaction process was simple for formation of silver nanoparticles and AgNPs presented in the aqueous medium were quite stable, even up to 4 months of incubation. This work proved the capability of using biomaterial towards the synthesis of silver nanoparticle, by adopting the principles of green chemistry. [23]

Silver nanoparticles were prepared by treating a solution of AgNO3 dissolved in deionized water with Vitamin C rich fruits (Guava, grape and tomato) as reducing agent by Natthawan Phuphansri *et al.*, The factors that affect the synthesis of silver nanoparticles (reaction time, temperature and concentration of silver nitrate) were investigated. The nanoparticles were characterized by UV-vis spectrophotometer, X-ray diffraction and Fourier transform infrared (FT-IR). [24]

Silver nanoparticles (Ag NPs) were synthesized by using aqueous leaf extracts of *Euphorbia prostrata* as a simple, non-toxic and ecofriendly green material. A. Abduz Zahir *et al.*, discussed on assessments of the pesticidal activity to determine the efficacies of aqueous leaf extracts of *E. Prostrate*, silver nitrate (AgNO3) solution (1mM) and synthesized Ag NPs against the adult of *Sitophilus oryzae* L. The synthesized nanoparticles were characterized by UV- visible spectroscopy, X- ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscope (SEM) analysis. [25].

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