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A Review on Green Synthetic Approach for The Production of Silver Nanoparticles

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Abstract
In recent years nanotechnology is developing very quickly and gaining more attention. In the field of green nanotechnology, the synthesis of nanoparticles using an environment-friendly approach which is the cost-efficient and lesser time required. These properties are shown by plant extract and hence they are used for the synthesis of nanoparticles. Plant extract consists of various phytochemicals like alkaloids, terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. These particles are associated with the synthesis of silver nanoparticles as it will serve as reducing and capping agents for the conversion of silver nitrate to silver nanoparticles. In the current period, the use of silver nanoparticles has been enhanced due to their development in antibiotic resistance against many pathogenic microorganisms. The silver nanoparticles have been widely used in various fields of biomedical industries as coating, cosmetics, nanodevices production, etc. The enormous potential has been shown by the medicinal plants in the evolution of the new drug molecules for different types of severe diseases. These medicinal plants are used for biosynthesis of silver nanoparticles and these can be further implemented in various other fields for various purposes.

Keywords
Green synthesis, aqueous plant extract, silver nanoparticles, Antimicrobial activity

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INTRODUCTION
Science, technology and engineering having a wide branch that is associated with one such unique and influenced branch is nanotechnology ("nanotech"). Nanotechnology is emanating as it is a hurriedly growing field in which science is used for the synthesis purpose of new particles at a smaller unit which is nanoscale level [1], which is measured in 1 to 100 nanometres. Nanoscience and nanotechnology are highly favoured for studying immensely small things among the science fields like chemistry, biology, physics, materials science, and engineering [2]. The size of nanoparticles is smaller than visible light wavelength due to its smaller size we cannot visualize nanoparticle from the optical microscope; we have to visualize it using an electron microscope. Nanoparticles can be visualized between 400nm to 700nm wavelength of light [3]. Nanoparticles are elementary units of nanotechnology and are sub-nanosized colloidal structures composed of synthetic or non-synthetic polymers [4]. Nanoparticles have remarkable and distinctive physicochemical and optoelectronic properties. Nanoparticles have in various applications such as catalysis, photonics, optoelectronics, biological tagging, pharmaceuticals, cosmetics, sensors, drug delivery, electronic devices, medical diagnostic imaging and anti-microbial activity and now extending their applications to cancer therapy [5-8]. The decrease in the size of nanoparticles leads to an increase in reactivity, making them highly reactive catalysts [9]. Metal nanoparticles are distinguished into the following types copper, zinc, titanium [10], magnesium, gold

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[11], alginate [13], silver and many more. Molecules which are metallic nano size are Molecules which are metallic nano size are peculiar and an alteration in physical, chemical and biological characteristic due to its surface to volume ratio; hence nanoparticle can be used for various purposes [14, 15]. Silver nanoparticles are taking a crucial role in the department of Nanotechnology and Nanomedicine. Silver nanoparticles are of great interest due to their unique size and shape that determines its unique optical, electrical, thermal and magnetic properties [16-19]. Factors for the biological activity of AgNPs depends on properties like surface chemistry, size, size allocation, shape, particle framework, particle constituent, coating/capping, agglomeration, and dissolution rate, particle response in solution, ability to release ion, and cell type, and the type of reducing agents used for the synthesis of AgNPs are a crucial factor for the determination of cytotoxicity [20].

Studies on the silver nanoparticles signify that it can hinder the replication of the AIDS virus (HIV) and has much more impact as compared to gold nanoparticles [21]. Various studies conducted on the anti-malarial and anti-cancer activities of several plant varieties like *Callicarpa maingayi*, *Piper nigrum*, *Tribulus terrestris*, *Centella asiatica* and *Catharanthus roseus* [22]. Plant extracts are also used for medicinal purposes as well as for insecticides because of their biodegradable nature, fewer side-effects, non-toxic to non-targeted organisms and mostly effective due to synergetic and multi-targeted action [23]. Silver nanoparticles are also used in the healing of wounds like cuts, abrasion, burns, warts, fungal diseases and some skin diseases are also being treated with it [24-25], main properties of AgNPs are at a lower concentration as they show a perilous effect on the microbes whereas they are not toxic to the human cells [26]. Colloidal silver are chosen for interest because of its exceptional properties, such as good conduction capability, chemically well stable, catalytic and antibacterial effects [27]. There are various ways through which synthesis of nanoparticle are being accomplished, these technique are comprises using chemical [28], electrochemical [29], radiation [30], photochemical methods [31], Langmuir-Blodgett [22, 32] and biological techniques [33]. Biological techniques for the biosynthesis of the nanoparticle are also performed using bacteria, fungus or plants extract which appear to be simple and viable auxiliary from a more complex chemical used for the procedure to make nanoparticles, these biological agents are also used as traditional methods because of its simpler modification, high drug loading capacity, constancy, non- pathogenic, cost-effective and eco-friendly in nature [34-39], whereas they are not harmful to the human health. Nowadays the nanoparticle is the most attractive scientific field of investigation, the increasing observation towards nanoparticle synthesis and utilization for the environment protective method, this method is known as green chemistry. Green synthesis process comprises mixed-valence polyoxometalates, polysaccharides, Tollens, biological and irradiation methods having supremacy over the usual method which is chemical agents which interconnected with the environment toxicity [39]. To analyse the synthesized nanoparticles, numerous analytical technique is there which is used for the analysis, these are ultraviolet-visible spectroscopy (UV-Visible spectroscopy), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering(DLS), scanning electron microscopy(SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), etc. [40-41].

Plants are natures “Chemical Factories” which have a wide source of secondary metabolites. The use of these plants for nanoparticle synthesis can be advantageous over other biological processes, as it eliminates the maintenance of cell cultures and large scale nanoparticles can be synthesized [42]. The phytochemicals present in these plants are biologically active compounds that work as a defence mechanism against many diseases [43]. India is one of the world’s 12 biodiversity centres having over 45000 different plant species [44]. Of these about 15000-20000 plants have good medicinal value but only 7000- 7500 species are used for their medicinal values by traditional communities [45]. The Medicinal plant has several uses and is considered as a rich source of ingredients that can be used in drug development and synthesis, as an important source of nutrition and as a result of that these plants are recommended for their therapeutic values [46].

Green synthesis of the plant extracts

Recently production of the silver nanoparticles from the plants is being highly examined. The cause of its recognition is its rapid, eco-friendly, non-pathogenic, economical procedure and imparts a single-step procedure and imparts a single-step procedure for the biosynthesis processes [5]. Silver ions are reduced and stabilized by the addition of biomolecules like proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolic, saponins, terpenoids and vitamin they are already esteemed in plant extracts having medicinal utility are environmentally friendly, still chemically complex structure [47].

The procedure for the synthesis of nanoparticle comprises, collection of the plant part from the appropriate place then washing it evenly thrice with distilled water to remove dead cell and debris. For the preparation of plant extract, 20 g of green leaves were boiled to 100m of deionized distilled water. This mixture is then filtered using Whatman paper so that the unwanted particle and extracted leaves are filtered form the mixture constituent. The plant extract and $AgNO_3$ are added carefully in a test tube for the regular incubation periods. The reduction of $Ag^{(+)}$ ions to $Ag^{(0)}$ will be done by observation of data from UV-Visible spectra [47]. Silver ion reduction to silver nanoparticles by the plant extract needs certain incubation time. When the silver nanoparticles are synthesized extracellularly using aqueous leaf extract will assemble a quick, simple, economical process in contrast to chemical and microbial methods [5]. Characterization of silver nanoparticles is done using various techniques such as UV visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR) analysis, Field emission scanning electron microscope, High-resolution transmission electron microscope, X-ray diffraction, dynamic light scattering (DLS), zeta potential. Using different time intervals, varying concentrations of $AgNO_3$ and at different temperatures, these results were noted.

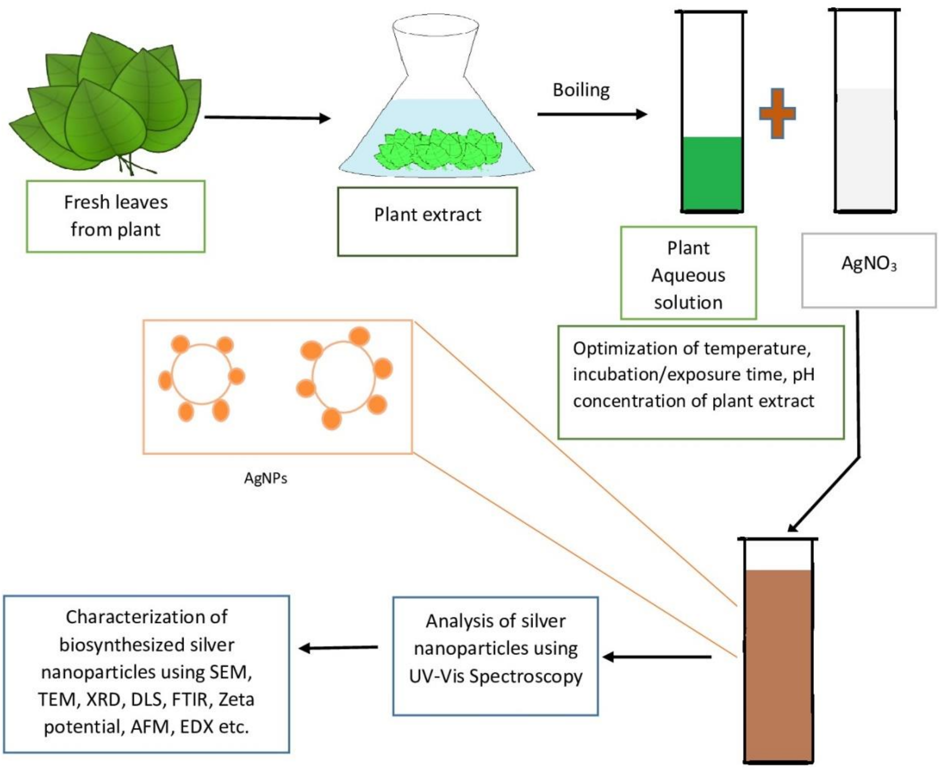

Fig.1. Green synthesis process followed while synthesizing nanoparticles and its characterization.

A wide variety of plant and plant parts are being used to explore the synthesis of nanoparticles. Plant related parts such as leaves, roots, fruits, seeds, flowers, barks and their other metabolites are used for the preparation of nanoparticles [48, 49]. These silver nanoparticles exhibit antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* and *Enterococcus faeca* [50]. de Jesús Ruíz-Baltazar et al. reported a green synthesis of nanoparticles from leaves extract of *Melissa offcinalis*. The formation of nanoparticles was confirmed by the change in the colour of the reaction mixture. An SPR band was observed at 430-433nm in 1-72 hours incubation [51]. Aqueous leaves extract of *Musa balbisiana* (banana), *Azadirachta indica* (neem) and *Ocimum tenuiflorum* (black tulsi) were used as a reducing agent for green synthesis of nanoparticles. TEM images of these leaves revealed the mixture of different shapes of nanoparticles like triangles, hexagons, pentagons and spheres. The most predominant were spherical shapes [52]. Vijay Kumar et al. also reported the green synthesis of silver nanoparticles. The reaction mixture shows the absorption spectrum at 418 nm in UV-Visible spectrum. Their XRD and TEM analysis also revealed a cubic structure of silver nanoparticles with an average size of 25 nm. They also showed the antibacterial activity against fish bacterial pathogens viz., *Aeromonas hydrophilla*, *Flavobacterium branchiophilum* and *Pseudomonas fluorescence* against the highest sensitivity towards *Flavobacterium branchiophilum* with other two bacteria [53]. Tippayawat et al. have reported the green synthesis of AgNPs from *Aleo vera* plant extract. They were also characterized by various methods like UV-Visible spectroscopy, TEM, SEM and XRD analysis. The maximum absorption spectrum of AgNPs showed at 420 nm peak. AgNPs from SEM analysis showed the spherical particles with an average size of 70.70 ± 22 to 192.02 ± 53 nm. The anti-bacterial activities were illustrated against pathogenic gram-positive *Staphylococcus epidermidis* and gram-negative *Pseudomonas aeruginosa* [54]. Green synthesis of silver nanoparticles was successfully synthesized from the aqueous leaf extract of *Piper longum* and the formation of stable silver nanoparticles at different concentrations, different temperatures, varying in pH and varying in incubation periods using UV-Vis spectroscopy. They are further characterized by XRD, SEM and TEM. The average size of SEM analysis is in the range of 25-32 nm and the average size of TEM is 28 nm [55].

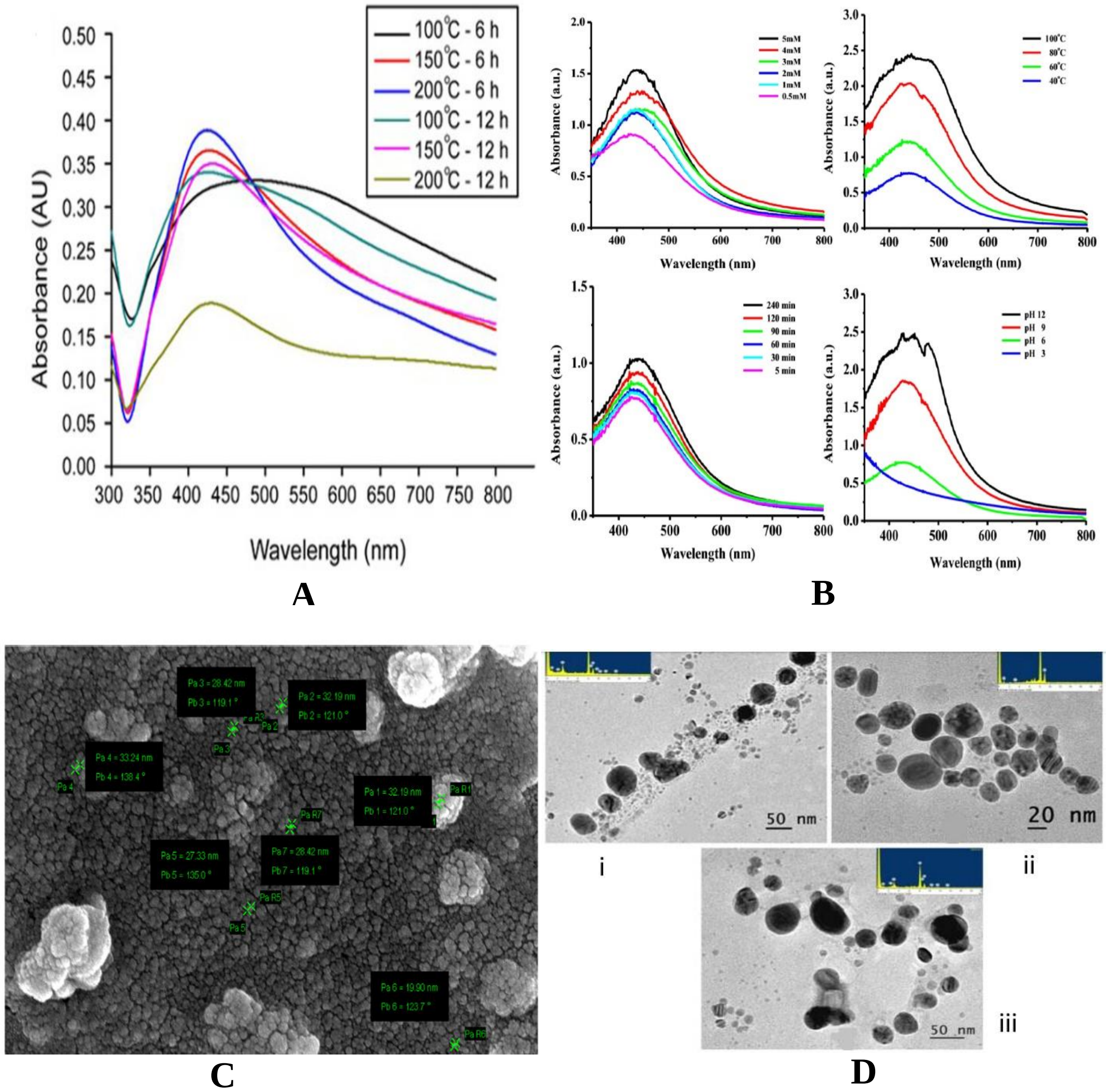

Fig.2. (A) UV-Visible spectra of *Aleo vera* plant extract with absorption spectra at 420 nm [54]. (B) UV-Visible spectroscopy with varying concentrations, temperature, incubation periods and pH [55]. (C) Morphological features of synthesized AgNPs obtained through FE-SEM correspond to 200nm of scale bar [55]. (D) TEM images of AgNPs of three different leaves extract (i) *Musa balbisiana* (ii) *Azadirachta indica* and (iii) *Ocimum tenuiflorum* [52].

The study by Beg et al. reported the formation of green AgNPs from the seeds of *Pongamia pinnata*. The change in the color of reaction from greenish-yellow to dark brown; confirmed the formation of silver nanoparticles. The SPR band of the reaction mixture was formed at 439nm. Further, the characterization was done by TEM and Zeta potential [56]. A recent study on *Pelargonium endlicherianum* Fenzl. roots extract reported the green synthesis of silver nanoparticles and its characterization. Further antimicrobial activity of AgNPs was demonstrated using bacterial strains viz. Gram-negative; *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 15442 and Gram-positive; *Staphylococcus epidermidis* ATCC 11228 for microdilution method [57]. Khan et al. reported the green synthesis of metallic nanoparticles (AgNPs) using the freshly prepared aqueous solution of *Pulicaria glutinosa* plant extract. The absorption maximum was observed in the range of 422-459 nm. The crystalline nature of Ag NPs using *Pulicaria glutinosa* was confirmed by XRD pattern analysis, FTIR analysis and TEM analysis. The average size of the XRD pattern is 42 nm. The spherical shaped AgNPs with the size range of 40-60 nm in TEM analysis. The FTIR spectra of *Pulicaria glutinosa* plant extract was demonstrated [58]. Green synthesis of *Azadirachta indica* showed the absorption maximum at 436-446 nm. TEM analysis revealed the nanoparticles are dispersed and circular with the average diameter measured using DLS histogram was 34 nm [59]. Silver nanoparticles were rapidly synthesized using leaf extract of *Acalypha indica* and the formation of nanoparticles was observed within 30 min. Characterization of silver nanoparticles was recorded by UV-Vis spectroscopy 420 nm and the average particle size was 20-30 nm confirmed in TEM analysis [60].

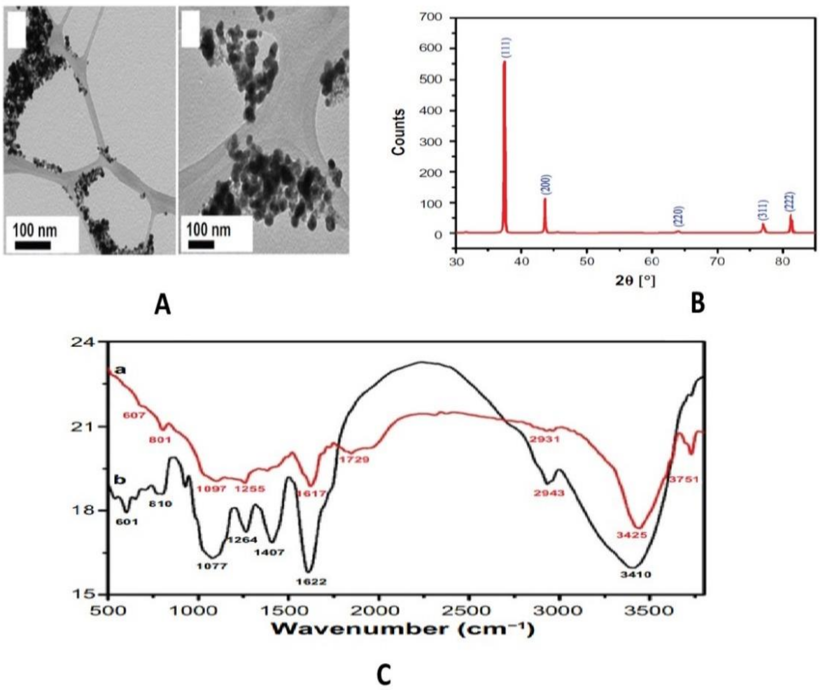

Fig.3. (A) TEM images of silver nanoparticles *Pulicaria glutinosa* with the average range of 40-60 nm [58]. (B) XRD pattern of *Pulicaria glutinosa* [58]. (C) FTIR spectra of *Pulicaria glutinosa* [58].

Applications of silver nanoparticles

Silver nanoparticles' antimicrobial properties are also utilized for medicine as well as for home-usage. One of its usages is the prevention of burnt infection using Silver sulfadiazine creams. Various types of plants are also used for the preparation of the silver nanoparticles, various parts are also utilized for the production of silver nanoparticles. It was observed that the higher level of steroids, sapogenins, carbohydrates and flavonoids act as reducing agents and phytoconstituents as the capping agents which provide stability to silver nanoparticles.

Silver nanoparticles are known for their significant catalytic activities in the area of dye reduction and their removal, the reduction of methylene blue by arsine influenced by the presence of the silver nanoparticles [61]. The catalytic activities are also studied using phenosafranine dye reduction [62]. Antimicrobial application of silver nanoparticles is examined by growing E. coli on agar plates and in liquid LB medium and these were supplemented with silver nanoparticles [63]. To investigate the membrane transport in living microbial cells single silver nanoparticles (P. Aeruginosa) are applied immediately [64]. Lithographically triangular silver nanoparticles fabricated by nanosphere and these were generally used as sensitive and selective nanoscale affinity biosensors. These nanosensors keep all the beneficial characteristic of Surface Plasmon Resonance (SPR) spectroscopy having fundamental principle behind numerous color based biosensor applications and by changing nanoparticles size and shape, these nanosensors load with two distinctive properties: (i) modest refractive sensitivity and (ii) a short-range, sensing length scale resolute by its property of decay length of the local electromagnetic field. These aspects unite to yield an area of mass sensitivity of ~100–1000 pg/mm^2. These factors are 100 poorer than the best propagating SPR sensitivities [65]. In study toxicity of starch-coated silver nanoparticles using normal human lung fibroblast cells (IMR-90) and human glioblastoma cells (U251). The toxicity was analyzed using variation in cell morphology, cell viability, metabolic activity, and oxidative stress. These constructed nanoparticles having ATP which emphasis the damaging effect on the mitochondria increased production of reactive oxygen species (ROS) in a dose-dependent manner. The damage in DNA can be measured using single-cell gel electrophoresis (SCGE) and cytokinesis blocked micronucleus assay (CBMN), was also dose-dependent and more notable in the cancer cells [66]. Electrical behaviour of highest frequency of nanosilver is up to 220 GHz [67]. Antiviral activity of silver nanoparticles shows relevant effect against HIV-1 at non-cytotoxic concentrations, yet the mechanism underlying HIV-inhibitory activity hasn't clarified. The silver nanoparticles are estimated for clarifying antiviral action against HIV-1 using a panel of different *in vitro* assays [68]. This mechanism concentrate on the development of nanoscale devices and platforms and these were utilized for single-molecule characterization of nucleic acid, DNA or RNA, and protein having increase rate over traditional techniques [69].

Antimicrobial activity and mechanism of silver nanoparticles

Silver metals are highly used for different purposes in our society; these uses are in form of jewellery, ornamentation and fine cutlery. Health benefits are also associated with the silver and hence they are used in jewellery, wares and cutlery. In ancient times silver has been used as anti-microbial for prevention of contamination of microbes back to around the Phoenicians who used silver as a natural biocide to coat milk bottles [5]. Around 650 microorganisms from different classes such as gram-negative and gram-positive bacteria, fungi or viruses, Silver is known for its antimicrobial effects against them. In the prehistoric medicinal approach (Ayurveda), silver is known for its therapeutic agent for many diseases. Among all metals showing antimicrobial properties, silver comes out is the most effective anti-bacterial action and they have a minimal toxic effect on animal cells [5]. In World War I the wounded soldiers are treated with treatment silver to prevent microbial growth [70]. From around 2000 years medicinal properties of silver are recognized [71]. Silver nitrate is utilized for prompting antimicrobial effect but when silver nanoparticles are utilized it enlarges the surface area available for the microbe exposure. Silver nanoparticles are synthesized using plant extract; these are taken to examine antimicrobial property using different microbes.

The antimicrobial properties of silver nanoparticles depend on:

1. Size and environmental conditions (size, pH, ionic strength).
2. Capping agent

The precise mechanism of antimicrobial or toxicity activities of silver nanoparticle is still under study and highly discussed area. The positive charge present on Ag ions is essential for antimicrobial activity. The ionized form of silver is having antimicrobial properties. In ionized form silver ion are stable but when they come in contact with moisture, they release silver ions [72]. Silver containing compounds with the presence of antimicrobial properties are anyhow the source of the silver ions (Ag^+), silver ions integrated into the substance and slowly with time as with silver sulfadiazine or silver ions can approach through ionizing the surface of a solid piece of silver as with silver nanoparticles [73, 74]. Some literature mentioned the electrostatic attraction between positively charged nanoparticles and negatively charged bacterial cells [75] and they are recommended as appropriate bactericidal agents [76, 77]. It was suggested that Ag (I) ion penetrate and intercalates between the purine and pyrimidine base pairs disrupting the hydrogen bonding between the two anti-parallel strands and denaturing the DNA molecule [5]. Antibacterial property is one of the causes of bacterial cell lysis. Nanoparticles regulated phosphotyrosine profile of bacterial peptide that can influence signal transduction and inhibit the growth of microorganisms. The antibacterial effect is dose-dependent and is an independent collection of resistance by bacteria against antibiotics. E. coli cells, when treated with silver nanoparticles, observe to be piling up in the bacterial membrane which outcome in the rise in permeability and death of a cell. Gram-positive bacteria are less resistant to Ag^+ than gram-negative bacteria. Hence the gram-positive bacteria cell wall composed of peptidoglycan molecule and has better peptidoglycan than gram-negative bacteria [5]. Gram-positive cell wall is thicker because of negatively charged and silver ions are positively charged, more silver may get attached by peptidoglycan in gram-positive bacteria compare to gram-negative bacteria [5]. Lesser accountability of gram-positive bacteria easily describes by the verity that the cell wall of gram-positive bacteria is thicker than that of gram-negative bacteria [70]. The interaction mechanism of silver molecules with biological macromolecules like enzymes and DNA by an electron-release mechanism [78] or free radical production [70] has submitted. The inhibition of cell wall synthesis as well as protein synthesis exhibit to be convinced by silver nanoparticles and recommended by several works of literature with the proteomic data having evidence of aggregation of envelope protein precursor or destabilization of the outer membrane, which at last leads to ATP leaking [79]. Nanosilver is a much efficacious and fast-acting fungicide against a broad spectrum of common fungi including genera such as *Aspergillus*, *Candida* and *Saccharomyces* [80].

Silver is anciently known for its use as an antiseptic and disinfectant and they can interact with disulphide bonds of the glycoprotein/ protein contents of microorganisms such as viruses, bacteria and fungi. Three-dimensional structure of proteins by interfering with disulphide bonds and block the functional operations of the microorganism can be modified by silver nanoparticles and silver ions [81-83]. The implementation of environmentally benign materials like bacteria, fungi, plant extracts and enzymes for the synthesis of silver nanoparticles gives a number of benefits of eco-friendly and compatibility for pharmaceutical and other biomedical applications as they do not require toxic chemicals for the synthesis protocol.

Conclusion

The various research field has been explored which justifies the use of production of silver nanoparticles in various applications. The field of nanotechnology has given immense applications in various fields of physics, chemistry, biotechnology and medical biotechnology.

The process of green synthesis using plant or plant extracts is environmentally friendly, harmless, cost efficient, protecting human health and production of eco-friendly products.

Silver nanoparticles are extensively produced that show a wide range of various activities like, anti-microbial, anti-fungal, anti-inflammatory, anti-viral, anti-cancer and many more. The biological methods of green synthesis of silver nanoparticles using plant extract are simple, non-hazardous, environmentally friendly and affordable. The modern world is now relying on nanoparticles for the treatment of various diseases like leprosy, tuberculosis, bronchitis, and asthma, as they have an advantage of organ targeting, enhanced bioavailability and many more.

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