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Review Article

Plant-Mediated Synthesis of Silver Nanoparticles – A Critical Review

Sneha Thakur[1*], Krishna Mohan G[2], Sandhya Rani M[2]

[1]Bojjam Narasimhulu Pharmacy College For Women, Vinay nagar, Saidabad, Hyderabad.
[2]Center for pharmaceutical sciences, Institute of Science and Technology, JNTU, Kukatpally, Hyderabad.

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ABSTRACT
Nanomedicine is a revolutionary science nanoparticles of size 1 - 100nm designed for the utilization in disease diagnostics and therapeutics, targeted drug delivery of drugs which have difficulty in solubility and bioavailability and also to be applied in numerous fields. The present critical review aims at enumerating the advantages, synthesis and characterization methods, as well as diversified applications of green silver nanoparticles. Silver nanoparticles is an attractive proposition due to their distinctive physical, chemical and biological properties including a high electrical as well as thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non linear optical behavior which enables them of potential value in inks, microelectronics, and medical imaging. Plant mediated synthesis of silver nanoparticles (AgNPs) is now-a-day's gaining enormous interest as it is cost effective, ecofriendly and has less side effects since there is no addition of external stabilizing or capping agents as the plant itself acts as stabilizing or capping agent. The secondary metabolites and other plant products like proteins, metabolites like alkaloids, terpinoids, saponins, glycosides etc are known to act as external stabilizing or capping agents. The plant mediated silver nanoparticles are known to show uniform particles characteristics and morphology. The bottom to top end approach of synthesis is effective to achieve desired particles size, shape and morphology. The synthesized green silver nanoparticles are characterized by UV-Vis spectroscopy, SEM/TEM analysis, EDAX/EDS, FTIR and other methods zeta potential measurement, thermo gravimetric analysis, Raman scattering. The plant mediated synthesized silver nanoparticles (green silver nanoparticles) are effective in delivering pharmacological activities like antimicrobial, anticancer, larvicidal etc. Also green Silver nanoparticles have diverse applications acting as biosensors, as targeted drug delivery candidates, in diagnostics and therapeutics, in medical and consumer products, as anti pollutant and in agriculture. The future perspective holds promising results in green synthesis of silver nanoparticles involving plants. This review projects a collective sequence of information for synthesizing and evaluation of green silver nanoparticles and scope of its pharmacological actions.

Keywords: Silver nanoparticles, AgNPs, UV-Vis, SEM/TEM, EDAX/EDS, FTIR.

INTRODUCTION

Nanomedicine is a revolutionary science involving nanoparticles of dimensions ranging between 1 - 100nm are designed for the utilization in disease diagnostics for identifying pathology of disease with sensitivity, provide therapy at molecular level with greater degree of cell specificity and targeted drug delivery of drugs which have difficulty in solubility and bioavailability. Nanotechnology is extensively being applied to provide targeted drug therapy, diagnostics, tissue regeneration, cell culture, biosensors and other tools in the field of molecular biology[1]. During the past few years, researchers have gained enormous interest in developing the silver nanoparticles (AgNPs) due to their distinctive physical, chemical and biological properties including a high electrical as well as thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non linear optical behavior which enables them of potential value in inks, microelectronics, and medical imaging. Ag-NPs also exhibited broad spectrum bactericidal and fungicidal Activity with extraordinary defense against wide range of microorganisms and also due to the appearance of drug resistance against commonly used antibiotics. This property increased the commercial value of products in areas of plastics, soaps, pastes, food and textiles. The use of Nanosilver technologies has appeared in a variety of manufacturing processes and end products. The principle behind the use is to store the silver ions in the moisture layer released enabling them to act against bacterial/fungal pathogens[2]. The silver nanoparticles are monitored for various physico-chemical parameters and green synthesis of the silver nanoparcticles is safe and effective way to combat harmful effects of synthetic chemicals[3]. The distinct physico-chemical characteristics of AgNPs have made them applicable in various fields like biomedical[4], targeted drug delivery[5], water treatment[6], agricultural etc.[7]. AgNPs are also applied in inks, adhesives, electronic devises, pastes etc. due to high conductivity[8]. AgNPs have been synthesized by physio-chemical techniques such as chemical reduction[8], gamma ray radiation[9], micro emulsion[10], electrochemical method[11], laser ablation[12], autoclave[13], microwave[14] and photochemical reduction[15]. These methods have effective yield, but they are associated with the limitations like use

**Author for Correspondence:snehathakur2189@gmail.com*

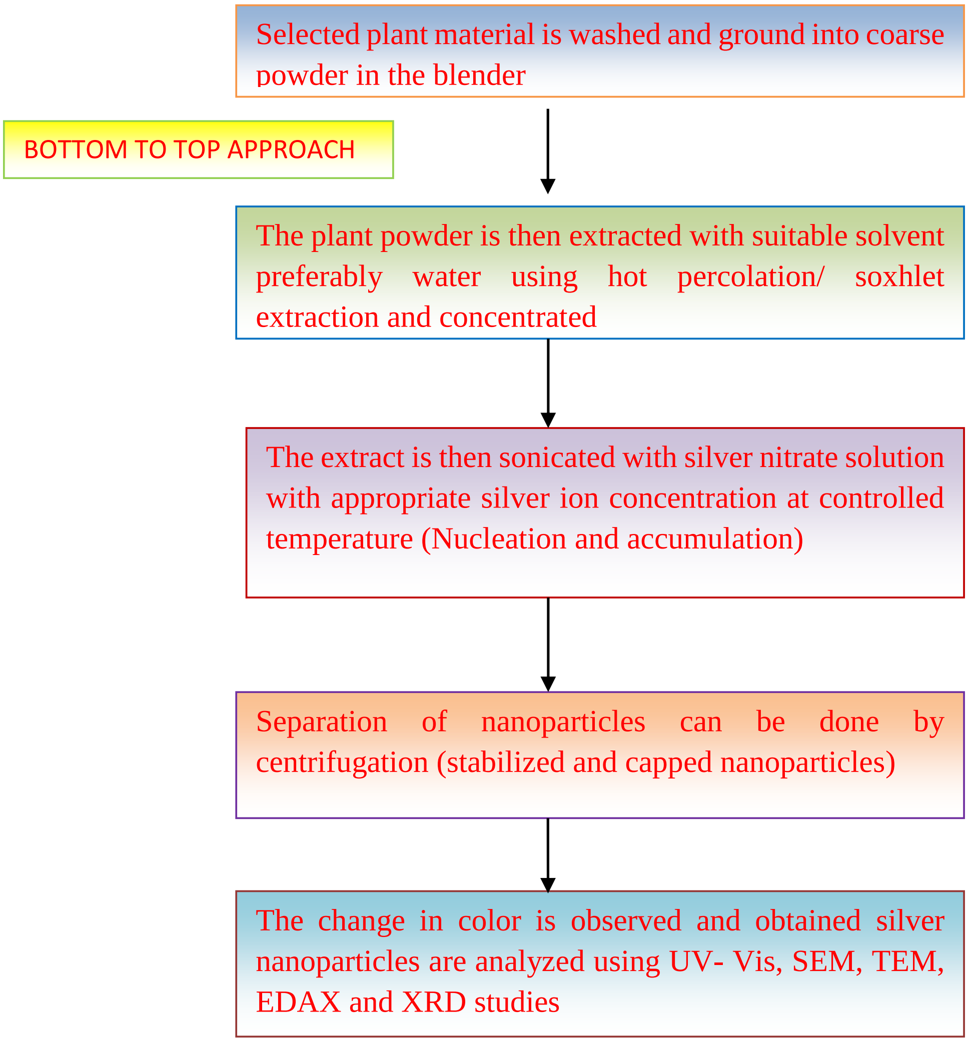

Figure 1: **S**ynthesis of plant mediated silver nanoparticles.

of toxic chemicals and high operational cost and energy needs. These physico-chemical methods though have effective yield are prone to limitations like use of toxic chemicals, high operational cost and energy needs. Hence cost-effective and energy efficient new alternative for AgNPs synthesis using natural polymers[15], plant extracts[16], animals, and microorganisms[17] as reducing and capping agents are now popular strategies. The synthesis assembly of silver nanoparticles using plants has got critical attention due to its rapid, ecofriendly, non-pathogenic, economical advantage and providing a single step technique for the biosynthetic processes. The silver ions are acted upon by stabilizing and capping agents of combination of secondary metabolites and other biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins which are chemically diverse in nature possessing medicinal values and are environmental benign[19]. The green synthesis of nanoparticles will unfold new areas of targeted therapeutics, diagnosis and conventional use[20].

The present review describes the systemic description of green synthesis of nanoparticles from plants, characterization techniques and multiple facets of applications of silver nanoparticles.

Green synthesis of nanoparticles

The green synthesis of the silver nanoparticles involves the interaction of reducing biological material with the aqueous silver nitrate solution. The temperature and other conditions have to be monitored during the reaction. The biological material itself acts as the stabilizing and capping agent, hence no external stabilizers or capping agents are required in green synthesis when compared to conventional chemical methods where toxic chemicals are used as stabilizers and capping agents. The commonly used biological agents are plants, micro organisms, biopolymers and animals. The literature supports wide use of plants, micro organisms, biopolymers as biological agents and very few reports of animal sources. The plant parts like leaves, bark, root, flower, fruits, seeds etc are used in the synthesis. The microbial cell mass of both intracellular and extracellular nature is involved in the synthesis of AgNPs. The biopolymers like collagen and chitosan are widely used.

The nanoparticles prepared from the plants have well defined surface characteristics like size, shape and morphology. The solvent used in most of the cases is water also other solvents like ethyl acetate, methanol and ethanol[22-26]. The phytochemicals like secondary metabolites, chlorophyll[22] and proteins[23] of the plant are responsible to act as reducing and stabilizing agents. The green synthesis of AgNPs using the plant as biological agent is mentioned in Fig 1. Many plants with effective activities were utilized as sources for the synthesis of AgNPs. The synthesized AgNPs from the following plants, their shape and size along with their pharmacological screening are reported in Table.1 were found to have appropriate particle size shape and morphology. The synthesized AgNPs are separated as pellet or powders using ultracentrifugation or also dried[3].

The synthesis involves the reaction of the aqueous silver nitrate solution in which the Ag^+ ion has 1mm concentration (widely used by researchers) with the biological reducing agents involving extracts of plants as shown in Fig. no.1. No external stabilizing and capping agents are added generally. The major physical and chemical factors that affect the AgNPs synthesis are reaction temperature, metal ion concentration, extract contents, pH of the reaction mixture, duration of reaction and agitation. Parameters like metal ion concentration, extract composition and reaction period largely affect the size, shape and morphology of the AgNPs. The suitable conditions for synthesis of AgNPs include basic pH, 25-37 ^{0}c temperature and controlled agitation[3]. However in few cases the external chemical agents like sodium-do-decyl sulphate were used for stabilization the AgNPs[21].

Characterization of silver nanoparticles

The common characterization techniques used are UV- Vis spectroscopy, SEM, TEM, FTIR, XRD, EDAX/EDS analysis as shown in Table.1. Also analysis of zeta potential, thermal stability by thermo gravimetric analysis, circular dichroism study and concentration and conversion of AgNPs by inductive couple plasma is studied.

UV- Vis spectroscopy- The change in the color from colorless to light yellow is indicative of nanoparticles formation by many researchers and the SPR peak is generally observed at significant range of 400 - 450 nm characteristic for AgNPs[42]. The UV-Vis spectral analyses is used to analyze the dependency of pH, metal ion concentration, extract content on the formation of AgNPs and reveal the size-stability of synthesized AgNPs by exhibiting red shift in the SPR peak with increase in size of nanoparticles and blue shift for decrease in size[43].

SEM / TEM analysis- The SEM/TEM analysis is used to determine the shape of the AgNPs. Many of the plants revealed different shapes and structures of nanoparticles like cubic[27], irregular[36], spherical[39], hexagonal[44], polyhedral[45], isotropic[44], flake[46], flower[47], pentagonal[48], anisotropic[49] and rod like structures[50].

Dynamic light scattering (DLS) – The DLS is used for the measurement of average hydrodynamic parameters along with poly dispersive indexes.

FTIR analysis- FTIR analysis determines the interaction of the capping agents to achieve efficient stabilization.

X-ray diffraction analysis- XRD analysis is used to determine the lattice structure (morphology) of the synthesized nanoparticles like FCC (face centered cube), bcc (body centered cube) etc., The XRD studies of almost all the researchers reported the formation of face centered cubic (FCC) crystalline structured for AgNPs.

Energy dispersive spectroscopy EDAX/EDS-, EDS analyses the elemental composition in the nanomaterials, which is exhibited at a characteristic optical absorption band peak around 3 KeV with silver weight percentage ranging from 45% to 80%. The reported stability of synthesized AgNPs has varied from 1 day to 1 year depending upon reducing agents and other operating conditions[51,52].

Raman scattering - Raman spectroscopy detects the vibrational modes of molecules and can be used to identify vibrational signals of a variety of chemical species that are attached to the surface of nanoparticles during synthesis giving an idea about the surface morphology of the nanoparticles[53].

Pharmacological applications

Antimicrobial activity

The antigenic capacity of the microorganisms against current antibiotics projects always a need for production of new antibiotics that possess broad spectrum activity. Silver has been used since years as a effective antimicrobial agent against a wide range of over 650 microorganisms from different classes such as gram-negative and gram-positive bacteria, fungi or viruses. Silver is known to interact with the disulphide bonds of the glycoprotein/ protein contents of microorganisms such as viruses, bacteria and fungi resulting in change of three dimensional structures of proteins thus blocking the functional operations of the microorganism. The silver ions may also form reactive oxygen species which interacts and damages the ATP assembly of respiration. Both silver nanoparticles and silver ions may also accumulate inside the cells of microbes causing damage to cell and thus are effective as antimicrobial agents possibly due to this mechanism[54, 55]. The antimicrobial action may be summarized as shown in the Fig.2.

Antibacterial property

Bacterial cell lysis could be one of reason for its antibacterial property; the possible mechanism of the silver ions involves interaction of silver molecules with biological macromolecules such as enzymes and DNA through an electron-release mechanism or free radical production. The silver may bind to thiol groups (ASH) of enzymes to form stable SAAg bonds thus deactivating enzymes in the cell membrane that involve in trans membrane energy generation and ion transport. Also Ag(I) ions intercalates between the purine and pyrimidine base pairs disrupting the hydrogen bonding between the two anti-parallel strands and denaturing the DNA molecule. Gram-positive bacteria are less susceptible to $Ag+$ than gram-negative bacteria due to more peptidoglycan cell wall (negatively charged) than gram-negative bacteria, as silver ions are positively charged; more silver may get stuck by peptidoglycan in gram-positive bacteria than in gram-negative bacteria[56]. Few plants have been reported to have antibacterial activity as reported in Table 1 like *Solanum tricobactum*[36], *Syzygium cumini*[36], *Centella asiatica*[36], *Citrus sinensis*[36], *Vitis vinifera*[40] *etc.*

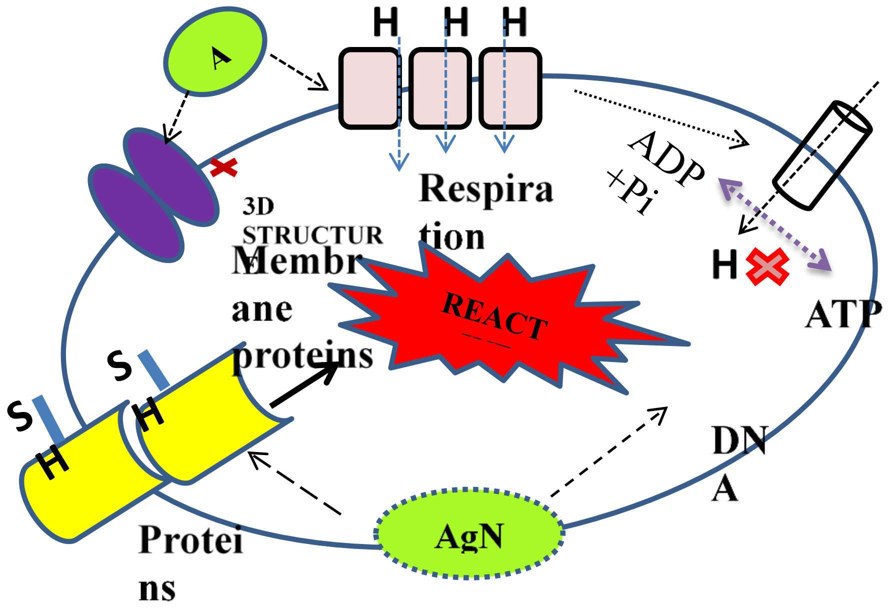

Figure 2: antimicrobial action of AgNPs.

Table 1: Plant mediated synthesis and elucidation of some plants along with pharmacological evaluation.

S no	Plant material	Spectroscopic analysis	Size, Shape and morphology	Conditions of operation	Activity studied
1.	Aqueous extract of *Alternanthera sessilis*[27]	UV-Vis TEM XRD FTIR	<100nm in size with crystalline nature		Quick reducing agent for silver ions and significant anti proliferative effect against prostate cancer cells
2.	Aqueous *Aloe* leaf extract[28]	UV-Vis TEM XRD	20 nm in size with spherical shape and FCC Structure	0.1 - 1.5 mM, 20 min, 20˚C - 40˚C, 0 to 15 ml/1 ml, Hydrazine hydrate content: 1- 15 ml, Static	Antibacterial to *E. coli* and *S. aureus*
3.	Aqueous extract of *Boerhaavia diffusa* plant powder[29]	UV-Vis FTIR SEM EDAX XRD TEM	25 nm in size with spherical shape and FCC, Cubic structure	0.1 mm, 24 hr, 100˚C, 10 ml/90 ml, Stirred	Antibacterial to fish pathogens *A. hydrophilia*, *F.branchiophilum*, *P.fluorescens*
4.	Filtered aqueous Mesocarp extract of *Cocos nucifera*[30]	UV-Vis TEM XRD	24 nm in size, spherical shape and FCC structure	1 mM, 1 hr, 60˚C, 20 ml/80 ml, Stirring, pH—2 - 11	Larvicidal nature
5.	Filtered aqueous extract of *Delphinium denudatum* root powder[31]	UV-Vis FTIR FESEM XRD	<85 nm Size, spherical Shape and FCC Structure	1 mM, 2hr, room temp. 1.5 ml/30 ml, Static, 20 min at 12000 rpm	Anti-bacterial *against S. aureus, B. cereus, E. coli and P. aeruginos* Larvicidal to *Aedes aegypti*
6.	Methanol extract and essential oil of *Eucalyptus leucoxylon* leaf[32]	UV-Vis SEM TEM XRD	50 nm in Size, Spherical Shape and FCC Structure	120 min, room temp. static	AgNP with biomedical potential

7.	Ethanolic extracts of *Phytolacca decandra, , Gelsemium sempervirens, Hydrastis canadensis* and *Thuja occidentalis*[33]	UV Vis SEM EDAX FTIR	90-130nm in size , spherical shape and crystalline nature	300 K temperature, 10 min time	beneficial biological action
8.	*Indigofera aspalathoides*, aqueos leaf extracts[34]	UV Vis SEM EDAX FTIR	20 – 50 nm in Size		Water-soluble organics leaf extract responsible to reduction. Wound healing applications
9.	*Morinda citrifolia* root extract[35]	UV-Vis SEM TEM XRD	30-55 nm in size, spherical shape , crystalline in nature		cytotoxic effect on HeLa cell
10.	Filtered ethanolic extracts of *Solanum tricobactum, , Syzygium cumini, Centella asiatica and Citrus sinensis* plant powders[36]	UV Vis SEM EDAX FTIR	41 - 53 nm in size, irregular Shape and FCC Structure	1 mM, 24 - 48 hr, 37˚C, 10 ml/5 ml, Additive: ammonium solution= 2.5 ml, agitated	Antibacterial against pathogenic *P. aeruginosa*
11.	Filtered aqueous extract of *Mangifera indica linn* peel[37]		7 - 27 nm in Size, spherical shape and FCC Structure	0.5 to 4 mM, 15 to 90 min, 25 to 100˚C, 0.1 to3 ml/27 ml, pH: 2 - 11. Static	Stable for 3 months, AgNPs loaded on fabrics exhibited antimicrobial property.
12.	Filtered aqueous extract of *Lonicera japonica* L leaf[38]	UV-Vis FTIR SEM TEM AFM ZP	36 - 72 nm in size, spherical shape	1 to 9 mM, 24 hr, 40˚C - 80˚C, 5% to 40% (v/v), Static, 5 min at 10000 rpm	Stability: zeta potential—41mV
13.	Filtered aqueous extract of *Terminalia chebula* fruit powder[39].	UV-Vis FTIR HR-TEM XRD EDS DLS	25 nm in size With FCC structure and phyto Capped nature	10 mM, room temp. 1 ml/25 ml, pH: 4 – 9, Static	Stabile for 10 days, AgNps showed\ catalytic activity on the reduction of methylene blue
14.	*Vitis vinifera* Extract[40]	UV-Vis SEM XRD	10 - 880 nm size, spherical shape with FCC, cubic and hexagonal structure	1 mM, 10 hr, room temp. 10 ml/90 ml, Static.15 min at 2000 rpm	Antibacterial to B. subtilis, *E. coli, P. aeruginosa* and *S. pnemoniae*
15.	*Withania somnifera* Leaf Powder[41]	UV-Vis FTIR TEM XRD EDAX	5 - 30 nm in Size, spherical. Shape and FCC Structure	100 mM, sunlight: 5min, dark room: 12hr, room temp. 100 ml/1 ml, Static	AgNPs with quasi-reversible redox behavior Anti-bacterial to *E. coli* and *S. aureus* Anti-fungal to *A. niger, A. flavus* and *C. albicans*
16.	*Abutilon indicum L. leaf extract*[63]	UV-Vis FTIR TEM EDAX	10-30 nm size, spherical shape,	2 mM, sunlight: 5min, dark room: 24hr, room temp	dose dependent free radical scavenging activity and anticancer activity against MCF-7 breast cancer cell line.

Antifungal activity

AgNPs are much effective fast-acting fungicides against a broad spectrum of common fungi including such as *Aspergillus, Candida and Saccharomyces*[57]. Silver nanoparticles of *Withania sominifera was found to exhibit antifugal activity against A. niger, A. flavus and C. albicans*[41].

Larvicidal activity

Due to the gaining population of vector borne diseases, the new type of lavicides is in demand. The AgNPs are known to show the larvicidal activity by killing the larvae of vectors at early stage. Many AgNPs have shown larvicidal activity against malarial vectors *Aedes aegypt*[31], *A. subpictus*[31],and filariasis vector *Culex quinquefasciatus*[59] and other parasites[31,60] of plants like *Cocos nucifera*[30] *Delphinium denudatum*[31] *Hibiscus rosasinensis*[5]. No attempt has been made to elucidate a possible mechanisms of larvicidal activities has been made.

Wound Healing Activity

During apoptosis, the mitochondria are activated in the cell to reduce its viability. The AgNPs are found to show wound healing activity by penetrating into the fibroblast cells and accumulating over time but do not interfere with the cell viability. The

AgNPs were found to show wound healing potential by reducing the mitochondrial activity[61]. *Indigofera aspalathoides* mediated AgNPs were tested for wound-healing applications following excision in animal models[34]. *Arnebia nobilis* root extract was found to have wound healing activity in an excision animal model exerting a positive effect due to their antimicrobial potential and provided a novel therapeutic direction for wound treatment in clinical practice[62].

Anti cancer activity

The need for potent anticancer agents is always a topic of interest. The potential targets nad need for effective therapy with less side effects is matter of concern. Green silver nanoparticles are cost effective, ecofriendly and have less side effects when compared to conventional chemotherapy, and synthetic drugs. The silver nanoparticles were found to have antiproliferative, cytotocic activities. Silver nanoparticles synthesized using *Acalypha indica* Linn show only 40% cell inhibition against human breast cancer cells (MDA-MB-231)[64]. The differences in their level of anticancer activity against A375 skin melanoma cells was observed for the AgNPs synthesized using the ethanolic extracts of *Phytolacca decandra, Gelsemium sempervirens, Hydrastis canadensis,* and *Thuja occidentalis showed beneficial biological action*[33]. The cell death (100%) of the HeLa cell line was observed with 100 μg of AgNPs synthesized using the root of *Morinda citrifolia*[35]. The piperidine, piperlongumine, and piperlonguminine present in Piper longum may be responsible for the synthesis of silver nanoparticles and exhibited a significant cytotoxic effect (94.02%) at 500μg/mL on HEp-2 cell lines[65]. The aqueous extract of *Alternanthera sessilis*[27]was quick reducing agent for silver ions and was found to have significant anti proliferative effect against prostate cancer cells.

Miscellaneous applications

These nanoparticles have been used in a diverse range of applications such as biosensors[66], targeted drug delivery candidates[67], diagnostics and therapeutic agents[68], cancer treatments[69], pesticides[70], and antimicrobials[71], water treatment[72], anti fouling agents[73]. Medical and consumer products

Water treatment

Stable AgNPs synthesized using *Anacardium occidentale* fresh leaf extract at 80^{o}C bud as a novel probe for sensing chromium ions [Cr^{+6}] in tap water.

Anti fouling agents[73]

The AgNPs may be used to treat many environmental concerns like; air disinfection, water disinfection, ground water and biological water disinfection and surface disinfection.

Medicinal Textiles and Devices- Extract of *Curcuma longa* tuber powder capped silver nanoparticles exhibited minimum bactericidal concentration for Escherichia coli BL-21 strain at 50mg/L. The immobilization onto the cotton cloth using sterile water is reported to show better bactericidal activity when compared to polyvinylidene fluoride immobilized cloth[74]. The incorporation of *Azadirachta indic*a synthesized silver nanoparticles into cotton cloth results in antibacterial effect against E. coli[75].

Catalytic activity

Filtered aqueous extract of *Terminalia chebula* fruit powder[39] showed catalytic activity on the reduction of methylene blue. Extract of *Acacia nilotica* pod mediated AgNPs modified glassy carbon electrode showed greater catalytic activity on the reduction of benzyl chloride compared to those of glassy carbon and metallic Ag electrode[76].

Agriculture

Emerging application of Ag nanoparticles particularly is in crop protection and the management of agricultural plant diseases[77,78].

CONCLUSION

The present review focuses on plant mediated synthesis of silver nanoparticles and its advantages with multi-access of its applications- an emerging area of nanotechnology. There is a steady increase in the publication scenario regarding the current topic. The angiosperm species has been widely used in comparison with the other sources of plants like gymnosperms, ferns etc. There are several characterizations methods for confirmation and simplest bottom to top approach of synthesis is advantageous due to its cost effectiveness, ecofriendly nature and fewer side effects as there is no additional stabilizing or capping agent added in plant mediated synthesis. The AgNPs synthesized using biological reducing and capping agents have shown wide variation in shape and size. The synthesized plant mediated silver nanoparticles have diverse applications like the anti-microbial action, anticancer activity, larvicidal activity, wound healing activity. Also plant mediated silver nanoparticles were found to have miscellaneous applications in fields of diagnostics and therapeutics, medicinal and consumer products, targeted drug delivery areas, agriculture and as ecofriendly The results, however, are conflicting and there is a need for more focus to resolve new arenas in this field. The potential of AgNPs for their use as drug carriers in cancer therapy, as biosensors for metabolites and pollutants, as catalyst etc. is quite high and requires intensive and integrated research activity for harnessing it. Finally, we hope that this critical review may help scientists, researchers, and teachers in the field of materials science and nanotechnology.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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