NCNE-2020 Special Issue

J. Indian Chem. Soc.,
Vol. 97, July 2020, pp. 1108-1114

Plant mediated green synthesis of silver nanoparticles for antimicrobial application: Present status

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Manuscript received online 15 April 2020, accepted 12 June 2020

Nanotechnology is a multidisciplinary research area owing to its various applications in medicine, pharmacy, science, engineering etc. Current research focus is in the field of green nanotechnology as it is environmentally and biologically safer. Numerous methods were used for synthesis of noble metal nanoparticles includes physical, chemical and biological methods. The conventional; chemical reduction method is not an environmentally friendly approach. Since, chemical synthesis involves various hazardous chemicals consequently; it imparts an adverse effect to living organism and on environment at later stage due to toxicity. Therefore, in last few decades' research have been focused for synthesis of metal nanoparticles using the non-toxic, clean and green methods. The green synthesis methods are simple, single step and eco-friendly in nature. In these methods some biological agent i.e. bacteria, fungus, plant etc. are used as reducing agent for synthesizing metal nanoparticles. Nevertheless, plant assisted synthesis method is the best strategy over other conventional method for noble metal nanoparticle synthesis. A range of metal like Ag, Au, Pt, Cd, Fe, etc. is being utilised for the synthesizing nanoparticles by plant extract. Among them silver nanoparticle (AgNPs) has got a special attention due to its excellent properties for dynamic applications. This review provides a brief overview about current status of the latest synthesis strategies for AgNPs using plant extract and highlights the antimicrobial applications of these nanoparticles. The synthesized AgNPs are found mostly spherical shape and have size ranging 1–100 nm. Further, the phyto-synthesized AgNPs were tested for wide range of antimicrobial action and shown major inhibitory effect against both Gram-positive and Gram-negative bacteria.

Keywords: Green nanotechnology, green synthesis, silver nanoparticles, phyto-synthesis, antimicrobial application.

Introduction

Nanotechnology and nano science are interdisciplinary field of research that provide an extensive range of advance technologies to various field including science, technology and non-science field[1]. Nanoparticles have been incorporated with nanotechnology and used as multipurpose tool in different field of science and technology because of their extraordinary physiochemical properties. Gold, silver, platinum and different metal nanoparticles are broadly used in biomedical, clinical and medicinal application. Out of them, silver nanoparticles (AgNPs) play convenient roles in the field of all discipline mention above because they have exceptional physic-chemical and optical properties and large surface to volume ratio[2]. Now a days, different methods like physical, chemical methods have been commonly used for the synthesis of AgNPs but they are time consuming, cost-effective and toxic methods. Contrary to these methods a green synthesis method is environmental-friendly and biocompatible due to use of microorganisms and plants extract[3]. Biosynthesis possess many benefits like cost-effectiveness, eco-friendliness and possibility of simple production in large scale. Also, it did not require high temperature, pressure and toxic chemical compounds. Consequently, synthesis of AgNPs using plant material is a better strategy compared to the use of microorganisms[4]. Using plants for the synthesis of AgNPs has enhanced the advantage of stable nanoparticles because of their capacity to make a broad field of bioactive secondary metabolites. Plant bio-molecules have two-fold effect such as reducing Ag ions to AgNPs and also acting as capping agent in biosynthesized nanoparticles. Compared to bacteria; plants are less susceptible to metal toxicity. So, phytosynthesis of AgNPs is a better alternate for green synthesis[5]. In recent time the infection arises due to microbes are the major concern to medical field, basically for dental and body implants. The bacterial effect on implants can result delay of recovery and leads to health hazard[6]. So, one of the widely used applications of AgNPs is based on a broad spectrum of antimicrobial activities at low concentration and facile synthesis routes. The mechanisms of bacterial inhibition by the action of AgNPs are not discovered properly. However, at least one widely accepted mechanism is that, released AgNPs react with the cell membrane of bacteria and penetrate into the cell by disrupting the cell wall, inhibit the metabolism process, damage the DNA, denature protein components; finally a microorganism leads to cell death[7]. In general the synthesis of nanoparticles followed two approaches. One is "top to bottom" and another is 'bottom to top' approach. In top to bottom approach the large particle break down to small tiny particles which is in nano range. Mostly physical methods for synthesis of naoparticles follows this approach. Again in bottom to top approach the small fine particles assembled to become bulk material in nano size. Mostly some conventional methods like chemical and biological would follow this approach[8].

Green synthesis of silver nanoparticles (AgNPs) using plant extract

Green synthesis is a noble, safe, hazard free, eco-friendly technique among all alternate methods such as chemical and physical methods for synthesizing AgNPs. This method can control the size, shape, stability and also provides simple and rapid production over other conventional methods. Mostly bacteria, plants and fungi are used as bio-resources for the synthesis of AgNPs. The bacteria and vegetations are the nonhazardous natural bio-resources which is safe to human being as well as whole environment compared to substitute methods for the synthesis of AgNPs i.e. physical and chemical methods. Recent report says that bacteria, plants and fungi have been used for the green synthesis of AgNPs. Plant assisted synthesis is advantageous over bacteria as it restricted to bacterial cell culture and preservation[11]. Plants resources are huge and easy availability in the nature is very much favor to researchers for doing research by taking plant. Almost all the plant parts such as leaves, stem, flowers, roots, fruits and barks are extensively being used for AgNPs synthesis[12]. Fig. 1 shows the schematic diagram of different plant parts used for green synthesis of AgNPs. The plant biomolecules such as protein, amino acid, enzymes, cyclic peptides, flavonoids, polyphenols and other substances contained in these plant extract helps at the time of nanoparticle synthesis. They are playing crucial role for the synthesis of AgNPs. The plant biomolecules enhance the inner properties and acting simultaneously as reducing agents, capping agents and also stabilizing agent.

Fig. 1. Plant material used for green synthesis.

The synthesis of nanoparticles (NPs) from metal precursor salt by using plant extract is a simplistic and inert atmosphere activity. The route is very simple and fast; the plant extract and metal salt are reacted homogeneously at room temperature. The reaction is finished within short times. After reduction of the metal salt by plant extract the color of the reaction solution changes. It indicates end point of the reaction and metal NPs formations. The synthesis of green NPs by plant extract can be divided in to three phases such as activation, growth and termination. Activation phase is the initial phase in which the metal salt solubilize in water medium and convert to ionic phases. The metabolites appears in the plant extract reduces the metal ion to neutral metal particle means Ag^{+} convert to Ag^{0} reduced by phytochemicals. In the second phase (growth phase) thermal stability of NPs is improved. Nucleation occurs by which small Ag^{0} particles aggregated as a result NPs forms different morphologies i.e. sphere, rods, wires. The final step is termination phase in which the NPs showing stable morphology being capped with the phytochemicals itself; with electron rich hydroxyl group on to the particles[27,30].

The plausible mechanism of green synthesized AgNPs using plant extract is displayed in Fig. 2. The characterization of sliver nanoparticles is a vital step in green synthesized AgNPs. Through characterization researchers have confirmed the formation of AgNPs. Different characteristics such as size, shape, surface morphology, stability and particle dispersal were known by performing characterization using modern sophisticated analytical techniques. Several types of techniques are used for the characterization AgNPs. These techniques are UV-Vis spectroscopy, FTIR, XRD, SEM, TEM, EDS etc.

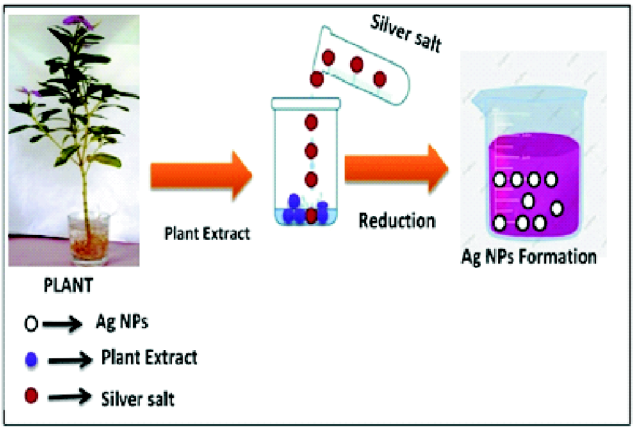

Fig. 2. Green synthesis of AgNPs.

Silver nanoparticles (AgNPs) using plant extract

Many researchers have prepared AgNPs using different plant extract. It is green and rapid synthesis method resulting mostly spherical AgNPs with nano range i.e. 1–100 nm. Different types of plants were reported are accountable for the synthesis of AgNPs. The details of plant name, plant part taken for AgNPs synthesis, particle size and shape are summarized in Table 1 and briefly elaborated in the present review. The AgNPs were synthesized by reducing Ag^+ with taking leaf extract of *Aesculushhip pocastanum*[3]; *Solanum nigurum*[10]; *Gymnema sylvestre*[11]; *Platycodon grandifloru*[13]; *Prosopis juliflora*[15]; *Veronica amygdalina*[18]; *Acalypha wilkesiana*[19]; *Cassia roxburghii*[22]; *Belgian endive*[24]; *Holoptelea integrifolia*[25]; *Parkia speciosa*[26]; *Azadirachta indica*[28]; *Cynara cardunculus*[29]; *Phyllanthus amarus*[30]; *Ricinus communis*[34]; *Artemisia vulgaris*[35] plants. The silver ion reduces to AgNPs by using fruit extract of *Crataegus microphylla*[1]; *Nauclea latifolia*[5]; *Alpinia nigra*[14]; *Ficus carica*[23]; *Garcinia indica*[33] plants. The silver ion reduces to AgNPs by using the seed extract of *Persea americana*[12] plants. The AgNPs were synthesized by using the flower extract of *Musa acuminatacolla*[21] plant.

Applications of silver nanoparticles (AgNPs)

There are various applications of green synthesized AgNPs on account of its less toxic nature. Currently, green routs are being explored for the synthesis of AgNPs in consequence of its eco-friendliness. The fascinating properties of AgNPs have increased its application in different field, such as in antimicrobial, antifungal, catalytic, bioassay, sensing etc. A brief overview on the antimicrobial applications of phytosynthesized AgNPs[9,27] have been appended below.

Antimicrobial applications of green synthesized sliver nanoparticles (AgNPs) and its mechanism of action

Owing to its antimicrobial property the silver nanoparticles (AgNPs) have find widespread applications in medical application and food packaging. The particular mechanism of antimicrobial property is not well established; however different researchers have put forward different hypothetical mechanism towards cell death. Fig. 3 shows the hypothetical antimicrobial mechanism of AgNPs (synthesized by using different plant parts). The details of plant name used for AgNPs synthesis, microorganism tested and antimicrobial activity measurement methods are mentioned in Table 2. Kup *et al*., have suggested that the antimicrobial property of green synthesized AgNPs by using leaves of *A. hippocastanum* tested by well diffusion method was studied against fourteen species of bacteria[3]. The inhibition zone's diameter is highest against Gram-positive bacteria *(P. aeuroginosa)* which is 20.0 mm and that of lowest against Gram-negative bacteria *(P. fluorescens)* which is 8 mm by using AgNPs. The mechanism reported behind the antimicrobial activity of AgNPs considered interaction of NPs with the cell membrane as a result the cell membrane ruptured and enhances the perme-

Table 1. Green synthesis of AgNPs using different plant

Sr. no.	Plant name	Plant part	Shape	Size (nm)	Ref.
1.	*Crataegus microphylla*	Fruits	Spherical	30–50	1
2.	*Aesculushhip pocastanum*	Leaves	Spherical	50	3
3.	*Nauclea latifolia*	Fruits	Irregular	10	5
4.	*Saccharum spontaneum*	Stem	Irregular	10.8	6
5.	*Handroanthus heptaphyllus*	Leaflets and petioles	Pyramidal and cubic	40	7
6.	*Handroanthus heptaphyllus*	Flower and buds	Oval	10	7
7.	*Solanum nigurum*	Leaves	Spherical	3.46	10
8.	*Gymnema sylvestre*	Leaves	Spherical	20–30	11
9.	*Persea americana*	Seed	Semi-spherical	50	12
10.	*Platycodon grandifloru*	Leaves	Spherical	19–21	13
11.	*Alpinia nigra*	Fruits	Spherical	6	14
12.	*Prosopis juliflora*	Leaves	Spherical	10–20	15
13.	*Crocus Haussknechtii*	Bulbs	Spherical	10–25	16
14.	*Senna alata*	Bark	Spherical	10–30	17
15.	*(Veronica amygdalina)*	Leaves	Spherical	2–18	18
16.	*Acalypha wilkesiana*	Leaves	Spherical	10–26	19
17.	*Berberis vulgaris*	Root and leaf	Spherical	30–70	20
18.	*Musa acuminatacolla*	Flower	Spherical	12.6–15.7	21
19.	*Cassia roxburghii*	Leaves	Spherical	15–20	22
20.	*Ficus carica*	Fruit peels	–	54–89	23
21.	*Belgian endive*	Leaves	Quasi-spherical	19–64	24
22.	*Holoptelea integrifolia*	Leaves	Spherical	32–38	25
23.	*Parkia speciosa*	Leaves	Spherical	22–43	26
24.	*Azadirachta indica*	Leaves	Spherical	20–50	28
25.	*Cynara cardunculus*	Leaves	Semi-spherical	< 45	29
26.	*Phyllanthus amarus*	Leaves	Flower like	30–42	30
27.	*Diospyros sylvatica*	Root	Spherical	10–40	31
28.	*Momordica charantia*	Leaves	Spherical	13	32
29.	*Garcinia indica*	Fruits	Spherical	< 100	33
30.	*Castor (Ricinus communis)*	Leaves	Spherical	8.96	34
31.	*Artemisia vulgaris*	Leaves	Spherical	25	35

ability and breakage of DNA present in bacteria cell ultimately leads to cell death[3]. Hekmati *et al.*, have studied the performance against bacteria of AgNPs synthesized from three different plant extract[4]. The antimicrobial activity was shown against *S. aureus* and *P. aeruginosa*. The antimicrobial effect was directly linked with the concentration of the plant extract used for preparation of AgNPs. More volume of plant extract used for synthesis of AgNPs, the NPs shows high activity against bacteria and vice-versa. The suggested mechanism behind the antimicrobial activity was the formation of oxygen radicals leading to damage of lipid, proteins, carbohydrates and DNA of the cell due to interaction of oxygen radical and cell membrane consequently, the cell death happens[4]. Melo *et al.*, have studied antimicrobial activity of AgNPs synthesized from *Handroanthus heptaphyllus* plant parts against the bacteria *E. coli* and *S. aureus*[7]. The effect against microbe is believed to be due to cell membrane disturbed by interacting AgNPs with membrane protein content and increasing penetration capability leading intracellular component comes out resulting the cell death[7]. The antimicrobial activity of AgNPs synthesized from *Gymnema sylvestre* leaf extract against *S. aureus* and *E. coli* tested

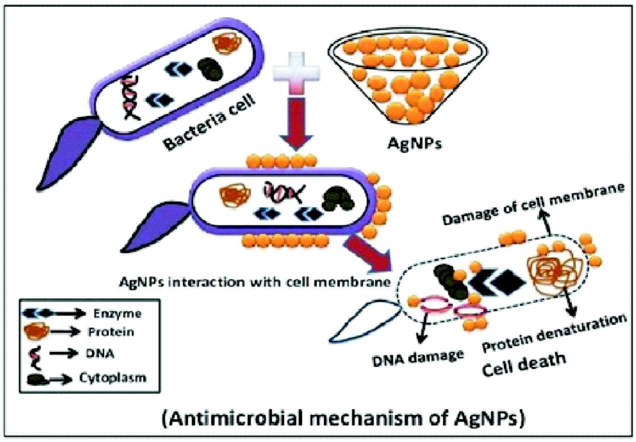

Fig. 3. Antimicrobial mechanism of AgNPs.

with well diffusion method was studied by Vijilvani *et al.*[11]. AgNPs' antimicrobial activity synthesized from *Persea americana* seed extract was studied by Giron-vajquez *et al*., against *E. coli*[12]. The synthesized NPs shows higher activity for Gram-negative bacteria in comparison with Gram-positive bacteria. The activity against microbes also varied by the particle size and with regard to the AgNPs synthesized from other extract volume. The small size of the particle attached with cell membrane, penetrates the cell membrane and disturbs the respiration process. This contact may destroy cell membrane, then the enzyme become inactive from the respiratory chain and protein denaturalization occurs. The cellular DNA gets damaged because of synthesized reactive oxygen species. Ultimately cell death occurs[11,12]. The anti-

Table 2. Antimicrobial activity of silver nanoparticles synthesized by using plant extracts

Sr. no.	Plant name	Test microorganism	Method	Ref.
1.	*Crataegus microphylla*	*Proteus mirabilis*	mic and mbc	1
2.	*Aesculushhip pocastanum*	*P. aeuroginosa*	Agar well diffusion and Broth microdilution	3
3.	*Nauclea latifolia*	*P. aeruginosa, E. coli* 700728, *Aspergillus niger*	Agar well diffusion	5
4.	*Handroanthus heptaphyllus*	*Escherichia coli, Staphylococcus aureus*	–	7
5.	*Handroanthus heptaphyllus*	*E. coli, S. aureus*	–	7
6.	*Solanum nigurum*	*E. coli*	–	10
7.	*Gymnema sylvestre*	*E. coli, S. aureus*	–	11
8.	*Persea americana*	*E. coli*	Disc diffusion	12
9.	*Platycodon grandifloru*	*E. coli, Bacillus subtilis*	–	13
10.	*Alpinia nigra*	*Klebsiella pneumoniae, S. aureus*	Agar well diffusion	14
11.	*Prosopis juliflora*	*E. coli, P. aeruginosa*	Disc diffusion	15
12.	*Crocus Haussknechtii*	*S. aureus, P. aeruginosa*	mic	16
13.	*(Veronica amygdalina)*	*Coliform, S. aureus*	Diffusion	18
14.	*Acalypha wilkesiana*	*E. coli, S. aureus*	Agar well diffusion	19
15.	*Berberis vulgaris*	*E. coli, S. aureus*	Disk diffusion	20
16.	*Musa acuminatacolla*	*P. aeruginosa*	Disc diffusion and microdilution	21
17.	*Belgian endive*	*S. aureus, E coli, P. aeruginosa*	Disk diffusion and mic	24
18.	*Holoptelea integrifolia*	*E. coli, S. typhimurium*	Disk diffusion	25
19.	*Parkia speciosa*	*E. coli*	–	26
20.	*Azadirachta indica*	*E. coli, S. aureus*	mbc	28
21.	*Cynara cardunculus*	*E. coli, S. aureus*	Disk diffusion	29
22.	*Phyllanthus amarus*	*Pseudomonas*	Kirby-bauer disc diffusion	30
23.	*Diospyros sylvatica*	*B. pumilis, P. aeuriginosa, B. subtilis*	Cup-plate agar diffusion	31
24.	*Momordi cacharantia*	*Pseudomonas* spp.	Kirby-bauer's disc diffusion	32
25.	*Garcinia indica*	*E. coli, B. subtilis, S. aureus, P. aeruginosa*	Well diffusion	33
26.	*Castor (Ricinus communis)*	*S. aureus, P. aeruginosa*	Well diffusion	34

microbial activity of AgNPs prepared from *Platycodon grandiflorum* leaf extract in favor of *Escherichia coli* and *Bacillus subtilis* was reported by Anbu *et al.*[13]. The AgNPs was synthesized from *Alpinia nigra* fruits extract against *Klebsiella pneumonia* and *S. aureus* measured by *aga*r well diffusion methods[14]. Most of the researchers studied about the antimicrobial activity of AgNPs approved that Gram-negative bacteria is very much vulnerable to AgNPs than Gram-positive bacteria. This is because of the cell membrane was thinner of Gram-negative bacteria as compared with Gram-positive bacteria. So, the AgNPs react with cell membrane and easily penetrate the cell and disturb intracellular activities. Moreover, the AgNPs is attracted by the negative charged cell membrane of bacteria and high affinity to sulphur and phosphorus. The sulphur was present in protein and phosphorus in DNA; collectively leads to acceleration in cell death[14]. Mosaviniya *et al.*, have studied that AgNPs synthesized from *Crocus Haussknechtii Bois* bulb extract showing antimicrobial properties against *S. aureus* and *P. aeruginosa* measured by MIC methods[16]. The mechanism of cell inhibition is supposed to be due to the formation of bond between Ag^+ and sulfydryl group of intracellular protein and denaturation of protein leading to cell death[16]. The green AgNPs was synthesized by using bark extract of *Senna alate's* plant and reportedly show promising antimicrobial properties against both Gram-positive and Gram-negative bacteria tested with Agar well diffusion method and broth micro-dilution method was reported by Ontong *et al.*[17]. The researchers have expressed the reason of inhibition of bacteria as AgNPs combined with multiple enzymes, transport proteins and nucleic, which lead to the cell death[17]. The antimicrobial activity of AgNPs synthesized from *Berberis vulgaris*'s leaf and root extract beside both *S. aureus* and *E. coli* was studied by Behravan *et al.*, using disc diffusion method[20]. It was reported that the AgNPs interact with the thiol group present in the respiratory enzyme and inhibit the respiratory process. The high affinity of nanoparticles to phosphate present in both protein and DNA of the cell. The whole metabolism system gets disturbed due to interaction affinity of AgNPs. Thus, the AgNPs shows high antimicrobial activity against both bacteria[20]. Gallucci *et al.*, have noticed that using leaf extract of *Belgian endive* preparation of AgNPs have shown prominent antimicrobial activity against *S. aureus, E coli* and *P. aeruginosa* by disc diffusion and MIC method[24]. Pethakamsetty *et al.*, suggested that AgNPs was syntesized by using the root extract of *Diospyros sylvatica* have shown prominent antimicrobial properties against *B. pumilis, P. aeuriginosa* and *B. subtilis*as measured by cup plate agar diffusion method[31]. It was observed that the microbial activity is comparatively high for phytosynthesized AgNPs than antibiotics. Phytosynthesized AgNPs have shown activity against both Gram-positive and -negative bacteria. The main reason of inhibition is adsorption of nanoparticles on cell membrane which affect the membrane. By which the porosity of cell membrane and cell respiration get badly affected. The synthesized AgNPs also apply hazardous effect on intracellular protein and DNA due to oxidative stress mechanism; causing bacterial death[31]. The green synthesized AgNPs from *Momordica charantia* leaf extract have shown prominent antimicrobial activity against *Pseudomonas* spp. As evaluated by Kirby-Bauer's disc diffusion method was reported by Ajitha *et al*. The *Pseudomonas* species is badly affected by AgNPs with the Zone of Inhibition (ZOI) 7 mm however ZOI is 5 mm for *E. coli.* The bacterial plasma membrane being ruptured and reported that denaturation of protein occurs as the strong contact of Ag^+ and bacterial cell. Thereby intracellular ATP being depleted and finally bacterial cell death occurs[32].

The preparation of AgNPs by using fruit extracts of *Garcinia indica* have shown prominent antimicrobial activity against *E. coli, B. subtilis, S. aureus* and *P. aeruginosa* measured using well diffusion method as reported by Sangaonkar *et al.*[33]. The synthesis of AgNPs by using leaves extracts of *Artemisia vulgaris* have shown prominent antimicrobial activity against *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *H. influenza* using disc diffusion method was observed by Rasheed *et al.*[35]. The AgNPs displayed major inhibition activities to all the tested bacteria and the maximum activity was shown against *S. aureus*. The highest surface area of AgNPs shows a significant role in microbial inhibition. The whole cause of inhibition is release of Ag^+ ion from AgNPs that have intracellular interaction with ribosome, different enzymes, proteins, DNA which contributed towards cell function and metabolisms[35].

Different researchers put forward so many reasons behind the antimicrobial activity of green synthesized AgNPs. However; most of them expressed that the AgNPs badly affect to Gram-negative bacteria as the cell membrane is thinner compared to Gram-positive bacteria. So, the AgNPs easily interact with the cell membrane followed by rupture. The intracellular organelles are affected and come out from cell and leads to cell death. Some researchers reported that the negative charged cell membrane has more affinity towards Ag^+. When the size of nanoparticle is small the interaction in between cell membrane and AgNPs is high. Also AgNPs have more attraction to sulfydryl group present in protein and phosphorus present in DNA of cell. As a result protein denaturation and DNA damage occurs. That finally leads to cell death.

Conclusions

Nature has provided us wide range of resources for taking step towards green nanotechnology. This review highlights the recent research activities related to phytosynthesized AgNPs and its antimicrobial application applied in diverse field. The green route means AgNPs synthesize through different renewable plant extract which is a safe, environmet-friendly, hazard free, low cost and energy saving techniques. The process should gives safer product, lesser waste and benign to environment. The plants part such as leaves, stem, flowers, roots, fruits and barks are extensively used for AgNPs synthesis. The antimicrobial property of green synthesized AgNPs has opened the doors of opportunities in several real life applications. The antimicrobial activity is shown by the AgNPs is higher for Gram-negative bacteria than Gram-positive bacteria. Due to the multifunctional properties of AgNPs, it has find potential applications in diverse area of medical, biomedical, clinical and food packaging application etc.

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