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Mini Review

A review on green silver nanoparticles based on plants: Synthesis, potential applications and eco-friendly approach

Mohammadlou, M., Maghsoudi, H. and *Jafarizadeh-Malmiri, H.

Faculty of Chemical Engineering, Sahand University of Technology, Tabriz, Iran

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Abstract

Silver nanoparticles (AgNPs) are extensively used in various industries due to their unique physico-chemical and antimicrobial properties. Many natural biomolecules in plants (inactivated plant tissue, plant extracts and living plant) such as proteins/enzymes, amino acids, polysaccharides, alkaloids, alcoholic compounds, and vitamins could be involved in bioreduction, formation and stabilization of AgNPs. In this review, the role of plant based biomolecules in the synthesis of AgNPs along with their characteristics, antimicrobial activities and applications are investigated.

Introduction

Incorporation of green chemistry techniques and methodologies into nanotechnology is of great interest which has gained much attention over the past decade (Hu *et al.*, 2008). Green chemistry which is the use of chemistry principles to reduce or eliminate using of toxic reagents, has resulted to significant reduction in the amount of harmful residues to human health and environment. Green chemistry is defined as chemistry aided processes for pollution prevention which can be extended to specific areas including green analytical chemistry, environmentally friendly analytical chemistry and clean analytical methods (Melchert *et al*., 2012).

Green synthesis of nanoparticles has attracted considerable attention in recent years. In this regard, plants extracts and natural resources such as microorganisms and enzymes have been found to be good alternative reagents in nanoparticles synthesis. Utilizing green substances has several advantages including low energy consumption and moderate operation conditions (e. g. pressure and temperature) without using any toxic chemicals (Mie *et al*., 2014). Therefore, green synthesis techniques using various biological organisms such as yeast, mold, algae and bacteria, and plant extracts have been developed for nanoparticles synthesis (Kaviya *et al*., 2011).

As compared to the metallic elements in bulk state, metallic nanoparticles exhibit unusual chemical, physical, optical and thermal properties due to their high surface area to volume ratio (Caswell *et al*., 2003). Therefore, these unique properties make nanoparticles (with diameter smaller than 100 nm) favorable for many different applications (Bhatte *et al*., 2012).

It is known that silver and its based compounds are highly toxic to major species of microorganisms such as bacteria, fungus and viruses (Sukirtha *et al.*, 2012; Suman *et al*., 2013; Vadlapudi and Kaladhar, 2014). While the mechanism of bactericidal and fungicidal of silver is not fully known, it has been suggested that silver can inhibit cell transduction and also cause cell lysis (Prabhu and Poulose, 2012). Silver nanoparticles (AgNPs), due to their surface configuration and small size which in turn, increase their surface to volume ratio, exhibit amazing antimicrobial and physico-chemical properties (Thirumalai Arasu *et al*., 2010). Antimicrobial activities of silver makes it much interesting choice for application in different areas including water and air treatment, catalysis, mirrors, optics, photography, medical, dentistry, clothing, electronics, and food packaging (Prabhu and Poulose, 2012; Edison and Sethuraman, 2013).

Many different methods have been developed for synthesis of AgNPs including physical, chemical and green (biological) techniques. In all methods, stabilized nanoparticles are formed by reducing of the silver ions to silver elements using reducing agents followed by nucleation and growing processes (Chen and Yeh, 2002; Sen *et al*., 2003; Kharissova *et al*.,

*Corresponding author.
Email: *h_jafarizadeh@sut.ac.ir, h_jafarizadeh@yahoo.com*
Tel: +98-413-345-9099; Fax: +98-413-344-4355

2013). In numerous physical and chemical methods that have been applied to synthesis of nanoparticles, it is possible to obtain particles with the desired characteristics. However the use of expensive and toxic substances as reducing and stabilizing agents makes them unpromising methods (Sharma *et al*., 2009; Geoprincy *et al*., 2013).

Green synthesis of AgNPs by microorganisms and plant extracts as an alternative feasible synthesis technique which has gained much attention and application these days. Various metabolites existing in plants including sugars, alkaloids, phenolic acids, terpenoids, polyphenols, and proteins play an important role in the bioreduction of silver ions to silver nanoparticles (Makarov *et al*., 2014).

The use of *Acalypha indica* (Krishnaraj *et al*., 2010), mangrove (Gnanadesigan *et al*., 2011), *Arbutus unedo* (Kouvaris *et al*., 2012), *Tribulus terrestris* (Gopinath *et al*., 2012), *Rumex hymenosepalus* (Rodríguez-León *et al*., 2013), *Eucalyptus chapmaniana* (Sulaiman *et al*., 2013), *Eucalyptus chapmaniana* (Vadlapudi and Kaladhar, 2014) to synthesis of AgNPs, have already been reported. Polyol components, polysaccharides and water-soluble heterocyclic compounds are the main components of these plant extracts which are mainly responsible for the reduction of silver ions and the stabilization of the produced nanoparticles. In green synthesis of AgNPs using plant extracts, several factors including plant source, types of organic compounds in the crude leaf extract, concentration of initial silver ions, temperature and the type and concentration of leaf extract pigments are the key factors on the efficiency of AgNPs fabrication process (Leela and Vivekanandan, 2008). In comparison with physical and chemical methods, synthesis of nanoparticles by plant extracts is cost effective and eco-friendly method which makes the target nanoparticles safe for human therapeutic uses (Velayutham *et al*., 2013). This method can be used as an economic and valuable alternative for the large-scale production of AgNPs. Furthermore, extracts from plants may act both as reducing and stabilizing agent in nanoparticle synthesis (Makarov *et al*., 2014). Using plant extracts can also be advantageous over microorganisms for nanoparticles synthesis, by elimination of the identification, isolation, and maintaining cell cultures processes for microorganisms (Song *et al*., 2009).

The aim of this study is to (1) describe a green approach for production of AgNPs by using plant extracts; (2) evaluate its advantages and disadvantages as compared to the conventional synthesis methods, and (3) investigate the common techniques to characterize nanoparticles. The main focus is on the role of the natural plant biomolecules involved in the bioreduction of silver ions and other factors during the nanoparticle synthesis and stabilizing of the AgNPs.

Silver nanoparticles

Among the several noble metal nanoparticles, AgNPs have attracted special attention due to their unique properties including appropriate electrical conductivity, chemical stability, catalytic and antimicrobial activities (Vijay Kumar *et al*., 2014). Because of high surface to volume ratio, silver in nano-scale has demonstrated completely different properties from bulk particles made from the same material (Thirunavoukkarasu *et al*., 2013). Therefore, synthesis of AgNPs is an emerging area and interesting subject.

AgNPs are synthesized using various physical, chemical and biological techniques resulting in different shapes and sizes for use in numerous applications. These synthesis methods are categorized into two main categories namely, top-down and bottom-up. In top-down approach, the size of silver metal in its bulk form reduces mechanically to the nano-scale by using sophisticated methodologies such as lithography and laser ablation. Bottom-up approach is also known as self-assembly technique and includes of dissolution of silver salt into a solvent, reduction of silver ions to their element using addition of a reducing agent and then stabilization of the forming AgNPs using a stabilizing agents to prevent agglomeration of nanoparticles (Tolaymat *et al*., 2010). This approach leads to nanostructures with less defects, more homogenous chemical composition and better short and long range ordering (Leela and Vivekanandan, 2008). Existing top-down and bottom-up techniques for syntheses of AgNPs are summarized in Figure 1.

AgNPs with specific size, shape and morphology can be synthesized by numerous physical and chemical methods including physical adsorption, surface deposition, arc discharge, plasma polymerization, laser CVD (chemical vapor deposition), emulsion polymerization and chemical reduction, thermal decomposition in organic solvents and photo reduction in reverse micelles (Kim *et al*., 2006; Zhang *et al*., 2013). In all of these methods, organic solvents, non-biodegradable agents and toxic chemicals may be employed as reducing and stabilizing agents. These chemical reagents are potentially dangerous to the environment and biological systems. Moreover, most of these techniques require complicated controls or hard processing conditions (e.g. temperature and pressure) which make them quite expensive (Zhang

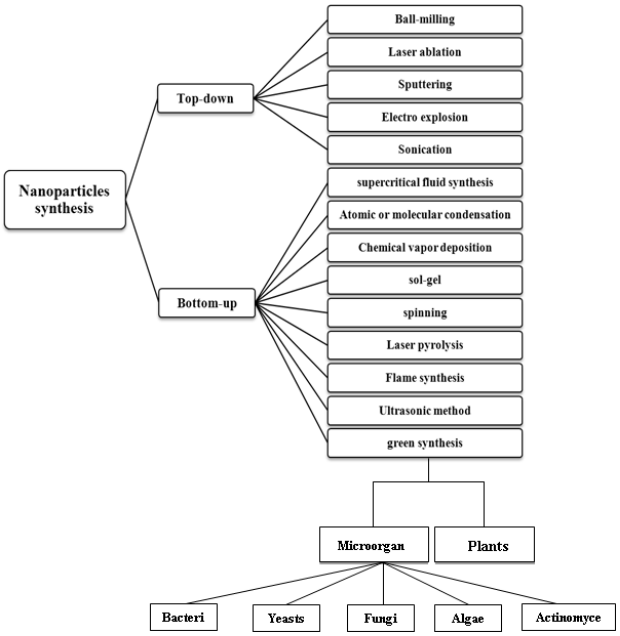

Figure1. Top-down and Bottom- up techniques used to synthesis of AgNPs

et al., 2013). Green synthesis of AgNPs using plant extracts and microorganisms provides significant advantages over the chemical and physical methods. Lower pressure, energy and temperature, non-using toxic chemicals, cost effective, environment friendly and easily scaled up for large scale applications are some of these benefits (Evanoff and Chumanov, 2005).

In green synthesis of AgNPs using plants, plant extracts can be used as reducing agent, capping agent or both (Amin *et al*., 2012; Ghaffari-Moghaddam and Hadi-Dabanlou, 2014). Additionally, AgNPs can be synthesized by several microorganisms such as the bacterial strains (*Bacillus licheniformis, Klebsiella pneumonia*) and fungi strains (*Verticillium* spp, *Fusarium oxysporum* and *Aspergillus flavus*) (Marambio-Jones and Hoek, 2010). Nitrate reductase is an important enzyme in microorganism, especially in fungi which can be act as reducing agent in AgNPs synthesis (Marambio-Jones and Hoek, 2010).

Green synthesis methods of AgNPs

Generally, AgNPs can be synthesized by either green or controlled synthesis processes. A two-step reduction process is utilized in controlled synthesis, while green synthesis involves a three-step process. In first step of controlled synthesis, a strong reducing agent, such as sodium borohydride, is added to the silver salt solution (usually $AgNO_3$) to create small silver particles. In the second step, a weaker reducing agent is applied to increase the size of these small silver particles. This two-step process is used in place of a one-step reduction process because it is easier

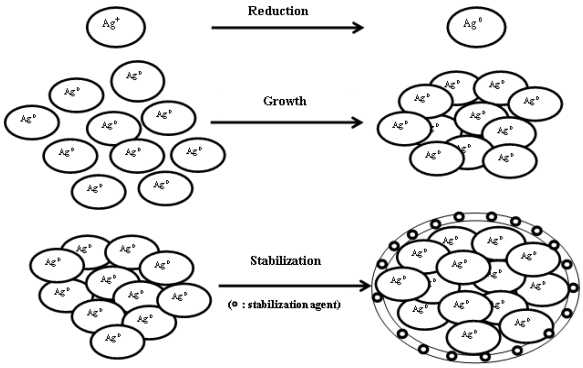

Figure 2. Mechanisms of silver nanoparticle green synthesis

to control synthesis for larger AgNPs. Nanoparticle synthesis is often accomplished in the presence of stabilizers to prevent nanoparticles aggregation.

In green synthesis, a solvent (usually water) is chosen and employed in step one. A non-toxic reducing and stabilizer agents are utilized in steps two and three, respectively. In this method, solvents, reducing, and stabilizers agents are selected from natural non-toxic and eco-friendly substances without any adverse effects on the environment (Eshleman *et al.*, 2011). Figure 2, shows the main steps in the green synthesis of metal nanoparticles.

Green synthesis of AgNPs has advantages over conventional methods involving chemical agents associated with environmental toxicity. Generally, green synthesis methods of AgNPs can be classified into polyoxometalates, polysaccharide, Tollens, irradiation, and biological methods which are described in more details in the following sections (Huang and Yang, 2004; Sharma *et al.*, 2009).

Polysaccharide method of AgNPs synthesis

Polysaccharide method is a simple of green synthesis technique which utilizes naturally-occurring polysaccharides as both the reducing and stabilizing agents for the synthesis of metal nanoparticles, especially, gold and AgNPs. In this method, nanoparticles are synthesized in absence of any other chemical reducing agent. As polysaccharides are completely soluble in water, it is used as a solvent in this synthesis method (Huang and Yang, 2004). Several polysaccharides such as starch, chitosan, cellulose and its derivatives are potentially applicable to use in AgNPs synthesis (Hassabo *et al*., 2015). Among them, chitosan is one of the most important polysaccharide which can be used in AgNPs green synthesis as only reducing agent or as both reducing and stabilizing agents (Huang and Yang, 2004; Travan *et al.*, 2009). The synthesized nanoparticles are highly stable and show no evidence of aggregation after several months of

storage (Huang and Yang, 2004). It is also a simple, low-cost and fast method to prepare chitosan films containing AgNPs (Hebbalalu *et al*., 2013). Starch is also used as a capping agent and β-D-glucose (starch monomer) as a reducing agent in a gently heated system for synthesis of AgNPs (Sharma *et al*., 2009). Moreover, cellulose and its derivatives and components (e. g. cellulose powder, microcrystalline cellulose, carboxymethyl cellulose, methyl cellulose and hydroxypropylmethyl cellulose) can participate as both reducing and stabilizing agents in AgNPs synthesis procedures (Hassabo *et al*., 2015).

Tollens method of AgNPs synthesis

Recently, the Tollens process has been successfully used in AgNPs preparation (Yin *et al*., 2002). This method is based on $[Ag(NH_3)_2]^+$ reduction by aldehydes in an aqueous solution in the presence of ammonia as solvent (Montazer *et al*., 2012). In the modified Tollens procedure, Ag^+ ions are reduced by saccharides (carbohydrates) in the presence of ammonia to make AgNPs (Sharma *et al*., 2009). In this method, sodium dodecyl sulfate (SDS) and polyoxyethylenesorbitane monooleate are applied as stabilizers. The main advantage of this method is production of size-controllable AgNPs in a single synthesis step (Eshleman *et al*., 2011). Due to the higher reactivity of silver compounds, synthesis of separated AgNPs with well-defined shape and well-controlled dimensions is more difficult than gold nanoparticles. The results of Tollens method have indicated that synthesized AgNPs could be very stable for a long time without adding any stabilizer or capping reagent (Le *et al*., 2010).

Irradiation method of AgNPs synthesis

In this method, bio-organisms with protein are utilized as reducing and capping agents to synthesize stable nanoparticles without a need of reducing agents (Hebbalalu *et al*., 2013). A new, easy and fast method based on electron irradiation has been developed for the synthesis of nanoparticles (Bogle *et al*., 2006). This method has been used for synthesis of AgNPs in solution with well-defined size and shape distribution. In this technique a direct laser irradiation is utilized into an aqueous solution of silver salt and a surfactant (as stabilizing agent) in the absence of reducing agents (Abid *et al*., 2002). A photosensitization technique also uses laser to synthesize AgNPs by using benzophenone. In photosensitization, low laser powers and short irradiation times lead to larger AgNPs (~20 nm) and increased laser powers and longer irradiation times produce smaller AgNPs (~5 nm) (Eshleman *et al.*, 2011).

Ionizing radiation can also reduce silver ions for AgNPs preparation. Different ionized irradiation such as γ and UV irradiations have been used for AgNPs synthesis (Mafuné *et al*., 2000; Mallik *et al*., 2001; Abid *et al*., 2002; Shin *et al*., 2004; Bogle *et al.*, 2006; Chen *et al*., 2007) .Gama irradiation with stabilizer like polyvinyl pyrrolidone (PVP) (Shin *et al*., 2004), poly-vinyl alcohol (PVA) (Bogle *et al*., 2006), acetic acid/water solutions containing $AgNO_3$ and chitosan (Chen *et al.*, 2007) have also been used. Synthesis procedures using microwave irradiation has also been employed using glutathione (Kharissova *et al.*, 2013) and a combination of culture supernatanant of *Bacillus subtilis* (Saifuddin *et al*., 2009). A microwave synthesis of AgNPs by using variable frequency microwave radiation as compared to the conventional heating method. The method increases the reaction rate and gives a higher concentration of AgNPs with the same temperature and exposure (Prabhu and Poulose, 2012). Irradiation method is not as efficient as controlled synthesis method in controlling the AgNPs size and shape.

Polyoxometalates method of AgNPs synthesis

Polyoxometalates (POMs) are usually poly anions composed of transition metals that have been widely investigated as acid and oxidation catalysts (Maayan *et al*., 2006). POMs have been extensively used as building blocks of ordered solid materials due to their unique properties such as nano-sized molecular anions, redox and acid catalysis, binding ability to various cations and thermal stability (Uchida *et al*., 2007). Water is an appropriate solvent for POM synthesis as they dissolve in water. Due to their anionic nature, POMs can reduce silver ions by donating electrons to them. They can also stabilize the AgNPs product (Keita *et al*., 2009; Sharma *et al.*, 2009). In fact, POMs can also act as both reducing and stabilizer agents (Troupis *et al*., 2002). Furthermore, POMs have potential to determine the size and shape of the nanoparticles formed in the solution. Therefore, different POMs can be selected to prepare AgNPs with varying sizes and shapes (Eshleman *et al*., 2011).

Biological method of AgNPs synthesis

In fact, synthesis of metal nanoparticles using microorganisms such as, bacteria, fungi, yeast and actinomycetes has immense potential and is environmental friendly process (Kaler *et al*., 2010). Extracts from microorganisms including enzymes, proteins, amino acids, polysaccharides and vitamins may take part in AgNPs synthesis as both reducing and capping agents. It seems that microorganisms probably play a role in providing a multitude of

nucleation centers and establish conditions for obtaining highly disperse nanoparticle systems. They have potential to immobilize nanoparticles by providing a viscous medium which in turn, prevents their aggregation. Recently, these microorganisms have been known as possible eco-friendly nano-factories. Several researchers have investigated the biosynthesis of silver and gold nanoparticles using microbial sources (Shaligram *et al*., 2009). Their results proves that biosynthesis of AgNPs using microorganisms is a cost efficient production method which needs minimum time as compared with the conventional methods. Production of metal nanoparticles by microorganisms is strongly affected by microbial growth stages. The maximum biomass is achieved in mid exponential phase (starting range of stationary phase) of culture along with the maximum synthesis of nanoparticles in the same incubation period (Natrajan *et al*., 2010). Several studies have been reported successful on biological synthesis of AgNPs using microorganisms including *Verticillium* sp. (Mukherjee *et al*., 2001; Sastry *et al*., 2003), *Aspergillus fumigatus* (Bhainsa and D'souza 2006), *Aeromonas* sp. (Fu *et al*., 2006), *Klebsiella pneumonia, Escherichia coli, Enterobacter cloacae (Enterobacteriacae)* (Shahverdi *et al*., 2007), *Aspergillus flavus* (Vigneshwaran *et al*., 2007) and *Bacillus subtilis* (Saifuddin *et al*., 2009).

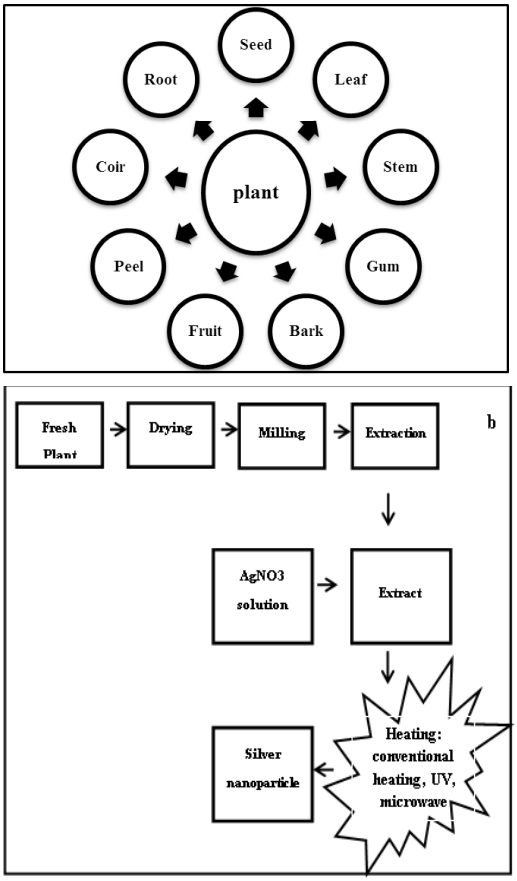

Figure 3. a) Different parts of plant use in the synthesis of AgNPs. b) Synthesis of AgNPs from plant extracts schematic

AgNPs synthesis using plants

Metal nanoparticles preparation using plant (inactivated plant tissue, plant extracts and living plant) is an important branch of biosynthesis processes. It has long been known that plants have potential to reduce metal ions both on their surface and in various organs and tissues remote from the ion penetration site (Makarov *et al*., 2014). Biomolecules existing in plant extracts including, enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids such as citrates are potentially able to reduce metal ions. In this regards, in vitro approaches have been successfully developed in recent years, in which plant extracts are used for the bioreduction of metal ions to form their nanoparticles. The extract of various parts of plants such as leaves, flowers, seeds, barks and roots (Figure 3a) have been applied for synthesis of AgNPs (Bar *et al.*, 2009; Marambio-Jones and Hoek, 2010; Velayutham *et al*., 2013). The plants extracts may work both as reducing and capping agents in AgNPs synthesis (Figure 3b). These extracts have also been reported to have antibacterial, antidiabetic, anti-inflammatory, antioxidant, anti HIV, snake venom neutralization, antifungal and larvicidal activities (Prabhu *et al*., 2013).

Plant metabolites as reducing agent

Various plant metabolites, including terpenoids, polyphenols, sugars, alkaloids, phenolic acids, and proteins play an important role in the bioreduction of metal ions to form nanoparticles (Makarov *et al.*, 2014). Terpenoids are a group of diverse organic polymers synthesized in plants from five-carbon isoprene units which display strong antioxidant activity (Makarov *et al*., 2014). Flavonoids are a large group of polyphenolic compounds that comprise several classes including, anthocyanins, isoflavonoids, flavonols, chalcones, flavones, and flavanones, which can actively chelate and reduce metal ions into nanoparticles. Various functional groups of flavonoids are capable to form nanoparticle (Makarov *et al*., 2014). In plant extracts, sugar can also use for the synthesis of metal nanoparticles. It is known that mono saccharides such as glucose, due to their free aldehyde group can act as reducing agents. Furthermore, the reducing ability of disaccharides and polysaccharides depends strongly on their type and concentration of individual monosaccharide components (Makarov *et al.*, 2014). Proteins including different amino acids are capable to reduce several metal ions, resulting their nanoparticles.

Table 1. Different biomolecules of plant extract which act as reducing agent during AgNPs green synthesis

Plant	Biomolecules involved	Experimental conditions	Size (nm)	Refs.
Jatropha curcas	proteins	5/20[1], 1[2], 80[3]	15-50	(Bar *et al.*, 2009)
Carica papaya	hydroxyflavones and catechins	10/90, 1, room temperature	15	(Jain *et al.*, 2009)
Ocimum sanctum	phenolic and flavonoid compounds, proteins ascorbic acid, Gallic acid, terpenoids,	40/10, 25, 40	Root: 10±2 Stem: 5±1.5	(Ahmad *et al.*, 2010a)
		10/90, 1, room temperature	18	(Ramteke *et al.*, 2013)
Desmodium triflorum	water-soluble antioxidative agents like ascorbic acids	5/20, 25, room temperature	5–20	(Ahmad *et al.*, 2010b)
Rosa rugosa	carboxylate content amine groups	2.5/60, 1, room temperature	12	(Dubey *et al.*, 2010)
Chenopodium album	aldehyde, alkaloids, apocarotenoids, flavonoids	2/60, 1, room temperature	10–30	(Dwivedi and Gopal, 2010)
Acalypha indica	flavonoids	12/100, 1, 37	20–30	(Krishnaraj *et al.*, 2010)
Sesuvium portulacastrum	proteins, flavones and terpenoids	5/45, 1, room temperature	5-20	(Nabikhan *et al.*, 2010)
Hibiscus rosa sinensis	carboxylate ion groups	20/25, 0.8, room temperature	13	(Philip, 2010)
Achyranthus aspera	Polyols	0.3/10, 1, room temperature	20-30	(Daniel *et al.*, 2011)
Citrus sinensis	vitamin C, flavonoids, acids and volatile oils	3/40, 1, 25 and 60	35 at 25 °C and 10 at 60 °C	(Kaviya *et al.*, 2011)
Mentha piperita	Menthol	1.5/30, 1, 28	90	(MubarakAli *et al.*, 2011)
Citrullus colocynthis	polyphenols with aromatic ring and bound amide region	10/90, 1, room temperature	31	(Satyavani *et al.*, 2011)
Anacardium occidentale	polyols and proteins	(2, 3, 4, 5 and 10)/30 0.59, 100	15.5	(Sheny *et al.*, 2011)
Zingiber officinale	alkanoids, flavonoids	5/50, 1, 37	10	(Singh *et al.*, 2011)
Piper betle	Proteins	10/190, 1, room temperature	10,20,40, 80 min ? 28,27,18, 17	(Usha Rani and Rajasekharreddy, 2011)
Solanum xanthocarpum	phenolics, alkaloids and sugars	(2–10)/10, 1, 45	10	(Amin *et al.*, 2012)
Glycyrrhiza Glabra	flavonoids, terpenoids, thiamine	7/100 ,0.5 , 27	20	(Dinesh *et al.*, 2012)
Piper nigrum	Proteins	10/ 50 ,1 , room temperature	5 - 50	(Garg, 2012)
Trianthema decandra	hydroxyflavones and catechins	(5, 10, 15)/10 , 1	36-74	(Geethalakshmi and Sarada, 2012)
Dioscorea bulbifera	polyphenols or flavonoids	5/95, 1, room temperature	8–20	(Ghosh *et al.*, 2012)
Elettaria cardamomom	alcohols, carboxylic, acids, ethers, esters and aliphatic amines	5/20, (different concentration of $AgNO_3$), 80	40-70	(Gnanajobitha *et al.*, 2012)
Leonuri herba	polyphenols and hydroxyl groups	(1, 2, 3, 4 ,5)/500, 10, 95	9.9-13	(Im *et al.*, 2012)
Morinda pubescens	hydroxyflavones, catechins	(1, 3 , 5)/10, 1, room temperature	25-50	(Jancy and Inbathamizh, 2012)
Olibanum (Boswellia serrata)	hydroxyl , carbonyl	various concentrations of gum 121	7.5 ± 3.8	(Kora *et al.*, 2012)
Agricultural waste Annona squamosa	sugars (aldoses) and terpenoids	10/80	32 ± 5	(Kumar *et al.*, 2012)
Piper betle	Proteins	50 – 150 (µl)/3, 1, room temperature	3-37	(Mallikarjuna *et al.*, 2012)
Plumeria rubra	Proteins	1/100, (100 ppm $AgNO_3$), 28	32 -220	(Patil *et al.*, 2012)
Hydrilla verticilata	Proteins	99/1, 100 , room temperature	65.55	(Sable *et al.*, 2012)
Lantana camara	carbohydrates, glycosides and flavonoids	10/90, 1, room temperature	12.55	(Sivakumar *et al.*, 2012)
Andrographis paniculata	hydroxyflavones catechins	10/90 ,1, room temperature	28	(Sulochana *et al.*, 2012)
Annona squamosa	glycoside, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic	10/90, 1, room temperature	20-100	(Vivek *et al.*, 2012)

Malva parviflora	Proteins	0.4/10, 1, room temperature	19–25	(Zayed *et al.*, 2012)
Hibiscus cannabinus	ascorbic acid	1/50 ,5 , 30	9	(Bindhu and Umadevi, 2013)
Castor oil, Khat and Sun flower	proteins, phenols and flavonoids, terpenoids	1.5/30, 1, room temperature	28	(Gebru *et al.*, 2013)
Artocarpus heterophyllus	amino acids, amides group	(2, 4, 6, 8, and 10%, w/v), 1/4 ,6 ,121	10.78	(Jagtap and Bapat, 2013)
Cocos nucifera	hydrocarbon such as nonacosane and heptacosane	20/80, 1, 60	23±2	(Roopan *et al.*, 2013)
Tithonia diversifolia	proteins, polysaccharides, terpenoids	10/90, 10, room temperature	25	(Tran *et al.*, 2013)
Coleus aromaticus	flavonoids	10/90, 1, room temperature	40–50	(Vanaja and Annadurai, 2013)
Mango	aldehydes, ketone, carboxyl and hydroxyl	3/27 , 1 , 80	7–27	(Yang and Li, 2013)
Aloe	protein, alkaloids, flavonoids,	10/1+ 10.0 mL hydrazine hydrate	20	(Zhang *et al.*, 2013)
Syzygium cumini	flavonoids	(20µl to 1 mL per 50 mL) , 0.5–5, 35	10–15	(Mittal *et al.*, 2014)
Tea	amides, carboxyl, amino groups and poly phenols	14.25/750µL $AgNO_3$, 10, room temperature	20-90	(Sun *et al.*, 2014)

1. Plant extract/$AgNO_3$ solution (ml/ml)
2. $AgNO_3$ concentration (mM)
3. Temperature (°C)

Several researchers have been done to biosynthesis of AgNPs by plant extracts. Table 1 summarizes the several different plant extracts and their main biomolecules which act as reducing agent during AgNPs green synthesis.

Plant metabolites as stabilizer agent

Metal nanotriangles have high surface energy making them less stable. Therefore, resulting nanoparticles aggregate and prefer to acquire a more stable morphology, such as a truncated triangle to minimize their Gibbs free energy (Makarov *et al*., 2014). Therefore, stabilizer agents are usually utilized in nanoparticles synthesis to control the formation and dispersion stability of them (Ahmad *et al*., 2011). In this regards, it has been suggested that plant hydrocarbons such as nonacosane and heptacosane have a positive effect on stabilization of AgNPs (Roopan *et al*., 2013). The carbonyl group of amino acid such as lysine, cysteine, arginine, and methionine residues and proteins has potential to bind metal ions to form nanoparticles (e. g. capping of AgNPs) and to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of AgNPs in the aqueous medium (Tran *et al*., 2013).

Other factors affecting the AgNPs synthesis by plant extracts

Besides the nature of the plant extracts and their active biomolecules types and concentrations, several factors including pH, incubation temperature, reaction time, concentration and electrochemical potential of a metal ion can have effect on metal ions reduction process (Makarov *et al*., 2014). Temperature is an important factor which its increases improves the reaction rate and efficiency of nanoparticle synthesis. It seems that an increase in temperature elevates the nucleation rate (Makarov *et al*., 2014; Vadlapudi and Kaladhar, 2014). The pH value of the plant extracts has a great influence on the formation of nanoparticles. In fact, the charge of natural phytochemicals of the plant extracts changes by variation in pH, affects their ability to bind and reduce metal ions during nanoparticle synthesis. This may affect the shape, size, and yield of formed nanoparticles (Vijayakumar *et al*., 2013; Makarov *et al*., 2014). Furthermore, due to the limited ability of plants to reduce metal ions, the efficiency of metal nanoparticle synthesis is influenced by the electrochemical potential of an ion. For example, plant extracts may be reduced ions having a large positive electrochemical potential (e.g., Ag^+) higher than those with a low electrochemical potential such as ($[Ag(S_2O_3)_2]^{3-}$) (Makarov *et al*., 2014). The proteins existing in a plant extract can also significantly affect the formation of nanoparticles. The approaches that have recently been used for the green synthesis of metal nanoparticles combine the use of plant extracts with the exogenous supplementation of the in vitro reactions with biomatrices: peptides, and proteins, whose amino acid sequence and structure are optimized for the efficient production of nanoparticles (Makarov *et al.*, 2014).

AgNPs characterization

Nanoparticles are generally characterized by their size, shape, surface area, and dispersity (Mittal *et al*., 2013). The common techniques to evaluate

Table2. Antimicrobial properties of some of the plants

Name of the plants	Part used	Activity	Against	References
Papaya	Fruit	antibacterial	*Escherichia coli and Pseudomonas aeruginosa*	(Jain *et al.*, 2009)
Rhizophora apiculata (mangrove)	Dried leaf	antibacterial	*Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhii and Staphylococcus aureus*	(Antony *et al.*, 2011)
Citrus sinensis	peel	antibacterial	*Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus*	(Kaviya *et al.*, 2011)
Medicago sativa	seed	antibacterial	*Gram positive and gram negative bacteria*	(Lukman *et al.*, 2011)
Menth piperita	leaf	antibacterial	*clinically isolated human pathogens, Staphylococcus aureus and Escherichia coli*	(MubarakAli *et al.*, 2011)
Eclipta prostrate	leaf	larvicidal	*filariasis and malaria vectors*	(Rajakumar and Abdul Rahuman, 2011)
Euphorbia nivulia	stem latex	antibacterial	*both gram negative and gram positive bacteria*	(Valodkar *et al.*, 2011)
Tribulus terrestris	fruit	antibacterial	*Streptococcus pyogens, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Staphylococcus aureus.*	(Gopinath *et al.*, 2012)
Olibanum (Boswellia serrata)	gum	antibacterial	*Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*	(Kora *et al.*, 2012)
Acalypha indica	leaf	antifungal	*Alternaria alternata, Sclerotinia sclerotiorum, Macrophomina phaseolina, Rhizoctonia solani, Botrytis cinerea and CurvulariaLunata*	(Krishnaraj *et al.*, 2012)
Lawsonia inermis	leaf	lousicidal	*Pediculus humanus capitis and Bovicola ovis*	(Marimuthu *et al.*, 2012)
Prosopis juliflora	leaf	antimicrobial	*Gram positive and gram negative bacteria*	(Raja *et al.*, 2012)
Pithecellobium dulce	leaf	larvicidal	*Culex quinquefasciatus*	(Raman *et al.*, 2012)
Cissus quadrangularis	stem	antiparasitic	*Hippobosca maculata and Rhipicephalus (Boophilus) microplus*	(Santhoshkumar *et al.*, 2012)
Melia azedarach	leaf	anticancer	*in vitro HeLa cell lines and lymphoma mice model*	(Sukirtha *et al.*, 2012)
Cissus quadrangularis	leaf	antibacterial	*bacteria (Gram positive and Gram negative bacteria)*	(Valli and Vaseeharan, 2012)
Hibiscus cannabinus	leaf	antibacterial	*Escherichia coli, Proteus mirabilis and Shigella flexneri*	(Bindhu and Umadevi, 2013)
Pelargonium graveolens	leaf	antibacterial	*Klebsiella pneumonia, Shigella someneii, S. flexaneri, Pseudomonas aeruginosa, P. mirabilis, and Escherichia coli*	(Pandian *et al.*, 2013)
Vitex negundo	leaf	anticancer	*HCT15*	(Prabhu *et al.*, 2013)
Solanum torvum	fruit	antibacterial antioxidant	*Escherichia coli, Pseudomonas and Bacillus spp*	(Ramamurthy *et al.*, 2013)
Cocos nucifera	coir	antilarvicidal	*Anopheles stephensi and Culex quinquefasciatus*	(Roopan *et al.*, 2013)
Morinda citrifolia	root	cytotoxicity	*HeLa cell*	(Suman *et al.*, 2013)
Desmodium Gangeticum	leaf	antibacterial	*Escherichia coli*	(Thirunavoukkarasu *et al.*, 2013)
Tithonia diversifolia	leaf	antimicrobial	*Pseudomonas aeruginosa, Microbacterium foliorum, Bacillus subtilis, and Rhodococcus equi*	(Tran *et al.*, 2013)
Coleus aromaticus	leaf	antibacterial	*Bacillus subtilis and Klebsiella planticola*	(Vanaja and Annadurai, 2013)
Ficus racemosa	bark	larvicidal	*Culex quinquefasciatus and Culex gelidus*	(Velayutham *et al.*, 2013)
Artemisia nilagirica	leaf	antibacterial	*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Proteus subtilis*	(Vijayakumar *et al.*, 2013)
Mango	peel	antibacterial	*Escherichia coli, Staphylococcus aureus and Bacillus subtilis (spore forming)*	(Yang and Li, 2013)
Aloe	leaf	antibacterial	*Escherichia coli and Staphylococcus aureus*	(Zhang *et al.*, 2013)
Crataegus douglasii	fruit	antibacterial	*Staphylococcus aureus and Escherichia coli*	(Ghaffari-Moghaddam and Hadi-Dabanlou, 2014)
Caesalpinia Coriaria	leaf	antibacterial	*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylo-coccus aureus*	(Jeeva *et al.*, 2014)
Syzygium cumini	fruit	anticancer	*Dalton lymphoma cell*	(Mittal *et al.*, 2014)
Azadirachta indica	leaf	antimicrobial	*Gram positive and gram negative bacteria*	(Nazeruddin *et al.*, 2014)
Tea	leaf	antibacterial	*Escherichia coli*	(Sun *et al.*, 2014)
Boerhaavia diffusa	leaf	antibacterial	*Aeromonas hydrophila, Pseudomonas fluorescensand Flavobacterium branchiophilum*	(VijayKumar *et al.*, 2014)
Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica and Citrus sinensis	Leaf Leaf leaf leaf peel	antimicrobial	*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumonia*	(Logeswari *et al.*, 2015)

nanoparticles characteristics can be classified into two main groups namely; quantitative and qualitative. These methods include a range of various sophisticated techniques like, dynamic light scattering (DLS), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), UV–vis spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), fourier transform infrared spectroscopy (FT-IR), surface enhanced raman spectroscopy (SERS), atomic force microscopy (AFM), high angle annular dark field (HAADF), atomic absorption spectroscopy (AAS), inductively coupled plasma (ICP) and X-ray photoelectron spectroscopy (XPS) (Rajasekharreddy *et al*., 2010; Mittal *et al*., 2013). AgNPs characteristics can be studied using some of these techniques which in turn, are helpful to resolve diverse parameters such as particle size, shape, crystallinity, fractal dimensions, pores size and surface area (Ingale and Chaudhari, 2013).

Qualitative analysis

FT-IR

FT-IR is a molecular vibrational spectroscopy that dissects chemical functional groups in different absorbance regions between 4000 and 400 cm^{-1} (Meng *et al*., 2014). FT-IR measurements are carried out to identify the possible biomolecules responsible for reduction, capping and efficient stabilization of AgNPs and the local molecular environment of the capping agents on the nanoparticle (Chanda, 2013).

UV–vis spectrophotometry

UV-vis spectroscopy refers to absorption spectroscopy in the UV-vis spectral region. Light wave lengths in the 300–800 nm are generally used for characterizing various metal nanoparticles in the size range of 2 to 100 nm (Mittal *et al*., 2013). UV–vis spectroscopy is an important technique to determine the formation and stability of AgNPs in aqueous solution (Bar *et al.*, 2009; Philip *et al*., 2011). Spectrophotometric absorption measurement in the wavelength ranging from 400 to 450 nm is used to characterize AgNPs (Mittal *et al*., 2013; Mittal *et al*., 2014).

SEM

SEM is a technique that uses electrons instead of light to form an output image (Klein *et al*., 2012). The SEM analysis is employed to characterize the size, shape, morphology and distribution of synthesized AgNPs (Chanda, 2013). The SEM micrographs also indicate the purity and polydispersity of resulting AgNPs (Mittal *et al.*, 2013).

XRD

XRD is a useful tool in obtaining information about the atomic structure of materials. XRD is not only usually used for qualitative identification of minerals in geological samples by fingerprinting approach but also is used for the quantification of mineralogical data (Al-Jaroudi *et al*., 2007). XRD is a valuable characterization tool to prove the formation of AgNPs, determine the crystal structure and calculate the crystalline nanoparticle size (Philip, 2010; Chanda, 2013).

AFM

The shape, size and surface area of the synthesized AgNPs are studied using AFM (Mohan Kumar *et al*., 2012). The improvement of AFM over conventional microscopes such as SEM and TEM is that AFM technique makes three dimensional images so that particle height and volume can be intended (Ingale and Chaudhari, 2013).

SERS

The Raman spectrum of the nanoparticle solution is recorded to detect the possible functional groups of capping agents participated in stabilization of the nanoparticles (Kora *et al*., 2012). Surface enhanced Raman spectroscopy is a popular technique in bioanalytical chemistry and a potentially powerful enabling technology for *in vitro* diagnostics. In fact, SERS combines the excellent chemical specificity of Raman spectroscopy with the good sensitivity provided by enhancement of the signal that is observed when the analyzed molecule lies over (or very close to) the surface of metal nanoparticles (Morasso *et al*., 2014).

The modern modification of Raman spectroscopy, SERS, utilizes generation of very strong electromagnetic field resulting from exciting of the localized surface plasmons in the metallic nanoparticles. SERS spectrum is observed if a molecule is in a close contact with a SERS-active support. Nowadays, SERS has been widely used in detection, identification and monitoring various biochemical processes since this technique has fast, label-free and non-invasive nature together with its high molecular specificity and sensitivity. First of all, SERS provides valuable information on the adsorption mechanism of a (bio) molecule on a metallic surface pointing what functional groups or atoms participate in metal–adsorbate interactions (Jaworska and Malek, 2014).

Color

Usually change in color of the aqueous salt

solution of a metal is indicative of metal nanoparticle formation. Distinct color change of the silver nitrate solution from colorless to gray color after reduction process indicates formation of AgNPs (Chen *et al*., 2009).

Quantitative analysis

TEM

TEM is a useful real-space analysis method and helps to observe the particle size of a material in nano-scale and to study the crystal structure meticulously with highest resolution (Tanaka, 2008). TEM measurements are conducted in order to estimate the particle size and size distribution of the synthesized AgNPs (Chanda, 2013).

DLS

Dynamic light scattering (DLS) technique as a diagnostic tool for particle size distribution (PSD) profile of AgNPs in solution or colloidal suspensions has been widely used in science and industry (Li *et al*., 2014). DLS is defined as a technique by changing the scattering light intensity fluctuation to obtain the sample average hydrodynamic diameter. Real-time monitoring nanoparticles size can be realized by DLS because the measurement process of DLS is rapid and sensitive solution phase detection. Currently, this technique has been applied to detect metal ions and cancer biomarkers (Ma *et al.*, 2014).

HAADF

Several electron microscopy techniques such as high angle annular dark field (HAADF) are used to study the mechanism by which AgNPs interact with bacteria. The size distribution of the nanoparticles interacting with each type of bacteria was obtained from the HAADF images (Morones *et al*., 2005). HAADF is a powerful technique for analysis of biological samples, such as proteins, and bacteria interfaced with inorganic nanoparticles. HAADF images are mainly formed by electrons that have undergone Rutherford backscattering. As a result, image contrast is related to differences in atomic number with intensity varying as ~ Z^2 (Elechiguerra *et al*., 2005).

ICP

The detection capabilities of single particle inductively coupled plasma-mass spectrometry (SPICPMS) with respect to particle size and number concentrations can be investigated for the case of AgNPs (Tuoriniemi *et al*., 2012). Ag concentrations in the deionized and the original AgNPs solutions can be determined by ICP spectrometry (Kim *et al*., 2009). The resulting silver concentration is measured by either inductively coupled plasma (ICP) emission spectroscopy (ES) or ICP mass spectrometry (MS) (Pal *et al*., 2007).

XPS

The X-ray photoelectron spectroscopy (XPS) measurements have been carried out to clarify the surface chemical states of the nanoparticles (Ashida *et al*., 2007). AgNPs are investigated by XPS to characterize the nature of the surfactant chemisorbed to the surface (Wilson and Langell, 2014).It is used to examine the valence of the resulting AgNPs while it also provides further information regarding the structure of the AgNPs encapsulated in the organic network (Xiong *et al*., 2013).

Antimicrobial properties of AgNPs

It is well known that silver-based compounds are highly toxic to microorganisms showing strong biocidal effects on more than 16 species of bacteria including *Escherichia coli, Bacillus subtilis, Vibria cholera, Pseudomonas aeruginosa, Syphilis typhus,* and *Staphylococcus aureus*. Moreover, it has proven to be active against several types of viruses such as hepatitis B virus and herpes simplex virus (Sondi and Salopek-Sondi, 2004; Galdiero *et al*., 2011). It is suggested that silver's mode of action depends strongly on Ag^+ ions which intensely prevent bacterial growth through suppression of respiratory enzymes and electron transport components and through interference with DNA functions (Galdiero *et al.*, 2011). The results of many different studies have shown that the membrane permeability and respiratory function can be affected by attaching silver ions to cell surface. Another probable phenomenon is that silver not only have a close interaction with the surface of the membrane, but can also penetrate deep inside the bacteria. Furthermore, it is believed that DNA loses its replication ability in the presence of silver ions (Sondi and Salopek-Sondi, 2004). Several studies have indicated that the silver has relatively higher antimicrobial activity against gram negative bacteria than gram positive bacteria, which may be attributed to the thinner peptidoglycan layer and the presence of beta barrel proteins called porins in their cell wall structure (Geoprincy *et al*., 2013).

Among noble metal nanoparticles, AgNPs have gained wide applications in different fields due to their strong antimicrobial activities (Valli and Vaseeharan, 2012; Ghaffari-Moghaddam and Hadi-Dabanlou, 2014; Jeeva *et al*., 2014; VijayKumar *et al.*, 2014). The high specific surface to volume ratio of

AgNPs increases their contact with microorganisms, promoting the dissolution of silver ions and hence improving biocidal effectiveness. The bactericidal activity of AgNPs is achieved by the ability of AgNPs to release silver ions (Vijaykumar *et al*., 2013).

The surface of AgNPs can easily form a layer of water and thus many silver ions can be released from AgNPs into the water. On the other hand, the main composition of bacteria cell membrane is phospholipid bilayers and protein molecules having negative electricity which make the whole cell membrane negatively charged. Therefore, the silver ions with positive electricity have the ability to attach to bacteria cell membrane quickly, which alters or damages the structures of bacteria. Moreover, Ag^+ ions can be attracted to the sulfhydryl group (SH) of bacterial enzymes (respiratory enzymes), making the enzymes inactivated and even died out (Zhang *et al*., 2013).

The AgNPs antimicrobial activity depends strongly on several factors including type of microorganisms, temperature, pH and $AgNO_3$ concentration (Marambio-Jones and Hoek, 2010). It is inversely proportional to the Ag^+ concentration. This can be attributed to the fact that smaller particles have larger surface area available for interaction and will give more bactericidal effects than the larger particles (Chanda, 2013). Antimicrobial activities of the synthesized AgNPs can be evaluated using the standard micro-dilution method, determining the minimum inhibitory concentration (MIC) which leads to inhibition of bacterial growth. The minimum bactericidal concentration (MBC) can be characterized as the minimum concentration of the sample required to achieve irreversible inhibition, such as killing the bacteria after a defined period of incubation (Panáček *et al*., 2006). Table 2, shows some of the plants and their antimicrobial properties.

Potential applications of AgNPs

Nowadays, many industries utilize the specific and unique properties of silver materials in their products such as clothing, respirators, household water filters, antibacterial sprays, cosmetics, detergent, dietary supplements, cutting boards, sox, shoes, cell phones, laptop keyboards and children's toys (Jeeva *et al*., 2014). Furthermore, AgNPs have been widely incorporated in surgical instruments, wound dressings, bond prosthesis and heart valves, electronics, and biosensing. In addition, AgNPs are applied in or on the surface of various textiles, laundry additives, room sprays, water cleaners, nano-device manufacture and food storage containers (Vivek *et al.*, 2012; Roopan *et al*., 2013).

Topical ointments and creams containing silver to avoid infection of burns and open wounds are examples in the field of medical industries where the most widely used and known applications of silver materials and AgNPs are seen (Song and Kim, 2009). AgNPs have potentially antimicrobial effects against infectious organisms such as *Escherichia coli* (Thirunavoukkarasu *et al*., 2013). Additionally, in the case of drug delivery applications, the noble metal nanoparticles such as AgNPs are powerful and promising tools due to existing functionalized surface. In other words, the unique properties of AgNPs such as large surface to volume ratio, absorption in the visible range, surface functionalization, and controlled drug release make them valuable in human life studies (Mittal *et al*., 2014). AgNPs ensure safety of food and preserve food for longer periods by killing the microorganisms when they are used in the packaging of them (Das *et al.*, 2008). Additionally, packaging films and coatings having AgNPs are able to adsorb and decompose ethylene which is a natural plant hormone produced during ripening process. Removing ethylene from a package environment helps the fresh produce like fruits and vegetables to have a longer shelf life (Silvestre *et al*., 2011).

In addition to human life related applications, the unique chemical and physical properties of nanoparticles make them extremely suitable for other high-tech applications such as designing new and improved sensing devices especially electrochemical sensors and biosensors. In this regard, AgNPs and certain core-shell metal nanoparticles have also been utilized in electro analysis by the labeling of biomolecules. In the literature, it has been revealed that an electrochemical DNA biosensor based on AgNPs label could sense the target oligonucleotides at levels as low as 0.5 pM (Luo *et al*., 2006). AgNPs have good electrical conductivity and hence they can also act as the electron transfer enhancer between proteins and electrodes. Furthermore, researches obtained results indicating that AgNPs can also utilized as the electrical bridge for the electron transfer between cytochrome c and the electrode (Liu *et al*., 2003; Luo *et al*., 2006).

Future of green AgNPs based on plants

Chemical and physical methods for synthesis of AgNPs have been followed over the decades. The use of expensive procedures and various toxic chemicals in their synthesis processes makes the biological synthesis the more preferred alternative (Prabhu and Poulose, 2012). Microbial synthesis is readily scalable, environmentally benign and biocompatible. However, production of microorganisms is often

more expensive than the plant extracts. Results of several studies have indicated that AgNPs synthesized by plants have more stability in comparison with those produced by microorganisms. Shape and size of nanoparticles could be controlled with the use of plants. However, AgNPs synthesis mechanism by plants is quite complex to understand (Geoprincy *et al.*, 2013). Additionally, plant extracts are able to reduce metal ions faster than fungi or bacteria (Iravani, 2011). Furthermore, these processes can also be easily scaled up (Vadlapudi and Kaladhar, 2014). The AgNPs synthesized based on plants are utilized in many applications beneficial to humans. The use of environmentally benign materials like plant extracts in the synthesis of AgNPs offers numerous pharmaceutical and biomedical applications due to the absence of toxic chemical in the synthesis procedures. Therefore, AgNPs applications in drug delivery systems might be the future thrust in the field of medicine.

Conclusion

Natural sources have the potential to reduce silver ions into AgNPs. It is understood that the variety of natural compounds that are present in plant extracts can act as both reducing and stabilizing agents for synthesis of AgNPs. Plants mediated AgNPs are stable due to the presence of natural capping agents such as proteins which prevent the particles from aggregation. Green synthesis of AgNPs using plant extracts has several advantages such as eco-friendliness, biocompatibility and cost-effectiveness. It is concluded that due to these unique properties, AgNPs will have a key role in many of the nanotechnology based processes.

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