

Review

Phytosynthesis of eco-friendly silver nanoparticles and biological applications – A novel concept in nanobiotechnology

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Nano-biotechnology is an undoubtedly future generation technology which offers potential applications in multidisciplinary areas of science and technology. In the present day's production, stabilization and utilization of nanoparticles is the eliminatory division in modern science receiving immense attention of scientists engaged in different fields of research. A number of metal nanoparticles have been engineered so far; however among these, silver nanoparticles gain more attention because of their unique applications in distinctive fields of biology. This review presents an overview on phytosynthesis of silver nanoparticles; role of phytochemical constituents in reduction of silver nanoparticles, factors responsible for the synthesis of silver nanoparticles and their crucial role in control of size and shape etc. The biological applications of phyto-synthesized silver nanoparticles are given in brief which will direct a path for further biological studies in future to make the study more useful for human welfare and benefits.

Key words: Phytosynthesis, silver nanoparticles, phytochemicals, biological activities.

INTRODUCTION

In the present decade, nanotechnology has become one of the rapidly growing interdisciplinary areas of science and technology (Albrecht et al., 2006). The term nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974 to describe

precision manufacturing of materials at the nanometer level (Taniguchi, 1974). The concept of nanotechnology was given by physicist Professor Richard P. Feynman in his lecture on there's plenty of room at the bottom (Feynman, 1959). This technology was found to be the

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platform for immeasurable technological innovations in 21st century. Research and development in the field of nanotechnology is growing at speed throughout the world. Development of new materials in the nanoscale including nanoparticles (NPs) is the major output expected in the study. NPs synthesis has received great attention from Chemists, Physicists, Biologists and Engineers who wish to use them for the development of a new generation of nanodevices (Shameli et al., 2012). NPs are clusters of atoms in the size range of 1 to 100 nm. Nano is a Greek word synonymous to dwarf meaning extremely small. NPs exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology (Mukunthan et al., 2011). In the recent past, nano material fabrication and their utilization is emerging as a cutting edge technology in different fields of human welfare, because of their distinctive characteristic features, such as, catalysis, electrical, optical, magnetic, chemical, mechanical properties etc.

Recently, a number of inorganic NPs have been synthesized by employing various procedures (Rao et al., 2012). NPs of copper, zinc, titanium, magnesium, plutonium, alginate, saponin, gold, silver and their alloys have come up. However, among these silver nanoparticles (AgNPs), have wide range applications because of their remarkable physical and chemical properties obtained due to their large surface area to volume ratio and small particle size. AgNPs are excellent nanomaterials providing a powerful platform in biomedical applications like bimolecular recognition, biosensing, drug delivery and molecular imaging (Chaloupka et al., 2010; Sironmani and Daniel, 2011). These AgNPs also have good antibacterial properties which are coming up as the current interest to researchers due to the growing microbial resistance against antibiotics and the development of resistant strains (Gong et al., 2007). These AgNPs are also used to alter and improve the pharmacokinetic and pharmacodynamic properties of various drugs to set them up for effective curing of various ailments (Mohanraj and Chen, 2006).

A number of approaches have been developed previously for the synthesis of AgNPs such as physical, chemical and biological (by using bacteria, lower forms of eukaryotes etc) methods (Gan and Li, 2012). Although these techniques have been well documented with greater prospects, they have certain limitations. During physical fabrication, metallic atoms are vaporized followed by condensation on various supports, in which the metallic atoms are rearranged and assembled as small cluster of metallic NPs (Egorova and Revina, 2004). The main advantage of the physical approach is that NPs with high purity and desired size can be selectively synthesized (Pol et al., 2002). A number of physical methods have been developed such as, sonochemistry (Gottesman et al., 2011), pyrolysis, physical vapor deposition, sol-gel, lithography, microwave

irradiation (Yin et al., 2004), laser ablation (Tsuji et al., 2003), electro deposition, electrochemical (Rodriguez-Sanchez et al., 2000; Yin et al., 2003), radiolysis (Thomas et al., 2005) methods etc. Most of these methods are expensive due to continuous consumption of energy to maintain the high pressure and temperature employed in NPs synthesis and requires highly sophisticated equipments.

Chemical approaches are the popular methods for synthesis of metallic NPs (Bönnemann and Richards, 2001). Several chemical reduction methods (Micelle, precipitation, chemical vapor deposition etc.) have been used to synthesize AgNPs from silver salts. Depending on the condition of reaction mixture, metal ions may favor either the process of nucleation or aggregation to form small metal clusters (Gan and Li, 2012). During the process of chemical synthesis, chemicals like sodium dodecyl sulphate, citrate of sodium, NaOH, KOH, NH₄OH, ethylene glycol etc. and some other toxic hazardous chemicals such as sodium borohydride and trisodium citrate were used as reducing agents; whereas, organic solvents (ethanol) (Kim, 2007) and some other non biodegradable compounds (synthetic polymers) were used as stabilizing agents. With the development of chemical methods, the concern for environmental contaminations is also highlighted as the chemical processes involved in the synthesis of NPs generate a large amount of hazards byproducts. The other main disadvantage of chemical synthesis is brought about during the process of synthesis; some toxic chemicals might be attached on the surface of NPs and may lead to adverse side effects during biological applications.

Although NPs are synthesized by using physical and chemical approaches, in contrary, biological systems came up as masters of ambient condition chemistry to synthesize metal NPs. The use of microorganisms in the synthesis of NPs was developed as relatively new and exciting area of research with considerable potential for development when compared to the above methods (Mohanpuria et al., 2008). Synthesis of AgNPs by using both prokaryotic and eukaryotic microorganisms such as bacteria, cyanobacteria, algae, fungi, actinomycetes protozoan's etc. were extensively investigated by several nano biologists. Biosynthesis of AgNPs (intracellular) by using the fungus *Verticillium* with size range in 25 ± 12 nm was reported by Mukherjee et al. (2001). The enzymatic synthesis of AgNPs by using *Fusarium oxysporum* was reported by Ahmad et al. (2003). Bhainsa and D'Souza (2006) investigated extracellular biosynthesis of AgNPs using *Aspergillus fumigatus*. Bioreduction and accumulation of AgNPs on the surface of cell wall within 72 h was reported in *Aspergillus flavus* (Vigneshwaran et al., 2007).

Synthesis of metal NPs (Au, Ag, Pd and Pt) with controlled size by using common cyanobacteria (*Anabaena*, *Calothrix* and *Leptolyngbya*) as bioreactors

was studied (Brayner et al., 2007). Lengke et al. (2007) reported the successful biosynthesis of AgNPs by using *Plectonema boryanum* UTEX 485 at 25 - 100°C for up to 28 days. Synthesis of Ag, Au and Au(core)/Ag(shell) NPs by using *Spirulina platensis* (single-cell protein) were achieved within 120 h at 37°C under controlled pH of 5.6 (Govindaraju et al., 2008). Biosynthesis of AgNPs using *Oscillatoria willei* NTDM01 was reported by Ali et al. (2011). The capability of protozoan's (*Leishmania* sp.) for synthesis of Ag and Au NPs was specifically reported by Ramezani et al. (2012). Biosynthesis of AgNPs by using culture supernatant of *Staphylococcus aureus* and their significant antibacterial effect against methicillin resistant bacteria such as *S. aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*, was reported by Nanda and Saravanan (2009). Saifuddin et al. (2009) found that exposure of culture supernatant of *Bacillus subtilis* with microwave irradiation to silver ions lead to the formation of AgNPs with size in the range of 5 - 60 nm. Reddy et al. (2010) reported that there is extensive extracellular biosynthesis of AgNPs by using the same species *B. subtilis*.

Phyconanotechnology has also become one of the prominent fields of research in NPs synthesis (Narayanan and Sakthivel., 2011). Biosynthesis of AgNPs by using *Sargassum wightii* and their antibacterial effect on isolated pathogens of infected silk worms was reported (Govindaraju et al., 2009). The synthesis of AgNPs using the aqueous extract of *Gelidiella acerosa* as reducing agent and their significant antifungal effect was proved by Vivek et al. (2011). Synthesis of AgNPs from the extracts of *Sargassum plagiophyllum*, *Ulva reticulata* and *Enteromorpha compressa* and their characterization was reported by Dhanalakshmi et al. (2012). Prasad et al. (2013) reported the synthesis of AgNPs by using the extract of *Cystophora moniliformis* as both reducing and stabilizing agent. Even though the use of microorganisms (both prokaryotic and eukaryotic) in biosynthesis of AgNPs was well documented, they have certain drawbacks. It is very important to maintain controlled conditions for both microbial growth and reduction of NPs, standardization of medium composition for microbial growth and subsequent biosynthesis of AgNPs. Requirement of long duration time for growth and production of NPs is one of the main disadvantages while another disadvantage is that most of the microorganisms synthesize NPs intracellular which demands subsequent extraction, recovery steps that are more expensive.

The increasing interest in minimization of time, cost, waste etc, and implementation of sustainable process for the development of eco-friendly and simple techniques for the production of AgNPs lead to the development of photo biological approach. Photo biological approach refers to the synthesis of NPs by using plants or their extracts as reducing and stabilizing agents which is also known as green nanotechnology. Green nanotechnology is gaining much more attention due to its wide applications in pharmaceutical and biomedical fields.

Researchers are in search for biomaterials for the synthesis of AgNPs throughout the world because those synthesized through chemical and physical routes are not suitable for biomedical applications. The advantages of green nano science over traditional chemical and physical methods are that they do not use toxic chemicals during NPs synthesis, less expensive and easily scale up at the large scale. In the present review, phytosynthesis of AgNPs, factors affecting the size and morphology of AgNPs and their biological applications are discussed.

PHYTOSYNTHESIS OF SILVER NANOPARTICLES IN HIGHER PLANTS

The development of reliable green process for the synthesis of AgNPs is an important aspect of current nanotechnology research (Sharma et al., 2012; Mittal et al., 2013). The role of higher plants in the synthesis of AgNPs is directly showing the relation between nanotechnology and Biotechnology. AgNPs were synthesized either by using the whole plants or plant pure compounds (glucose, starch, cellulose etc.), or by using plant dry mass or extracts etc (Song and Kim, 2008; Chandran et al., 2006; Song and Kim, 2009; Kasthuri et al., 2009). Initially the whole plants were used as biological factories for the synthesis of metallic NPs. The accumulation of AgNPs in the sprouts of alfalfa was investigated by Gardea-Torresdey et al. (2003). Haverkamp et al. (2007) reported the role of *Brassica juncea* germinating seeds in successful synthesis of Ag-Au-Cu alloy NPs; after which, Harris and Bali (2008) reported the use of whole plants to synthesize large quantities of AgNPs. They reported that it is feasible and found large uptake and reduction of silver ions and distribution of AgNPs within the cellular structures of *Brassica juncea* and *Medicago sativa*. However, the synthesis of AgNPs by using whole plants is cost effective and need to standardize the methods for further extraction and purification of NPs.

Followed by the use of whole plants for production of AgNPs, utilization of isolated pure compounds like glucose, starch, cellulose etc was extensively studied by several green nano Biologists. Park et al. (2011) explained clearly the role of polysaccharides and phytochemicals in Au and AgNPs synthesis. Raveendran et al. (2003) reported that β -D-glucose serves as the green reducing agent and starch serves as the stabilization agent for the synthesis of AgNPs. Latter *in situ* synthesis of noble metal (Ag, Au, Pt and Pd) NPs was carried out by using porous cellulose fibers (He et al., 2003). They found that the nanoporous structure and the high oxygen density of the cellulose fiber constitute an effective nano factory for *in situ* synthesis of metal NPs. Vigneshwaran et al. (2006) reported the production of stable AgNPs by using soluble starch as both the

reducing and stabilizing agents. They carried out this reaction in an autoclave at 15 psi, 121°C for 5 min. NPs thus prepared are found to be stable in aqueous solution over a period of three months at room temperature (25°C). The size of these NPs was found to be in the range of 10-34 nm.

Recently, the plant broth or extracts are being used directly for extracellular synthesis of AgNPs and it is extremely a cost effective method (Kumar and Yadav, 2009). Initially the use of *Pelargonium graveolens* leaf extract for the synthesis of metallic AgNPs were reported by Shankar et al. (2003). In continuation, the use of *Azadirachta indica* (Shankar et al., 2004), *Embelia ribes* (Ankamwar et al., 2005), *Aloe vera* (Chandran et al., 2006) and *Cinnamomum camphora* (Huang et al., 2007) were investigated for synthesis of AgNPs. Later, from the past six to seven years, extensive study was conducted on many plant species such as *Cinnamomum zeylanicum* (Sathishkumar et al., 2009), *Capsicum annuum* (Li et al., 2007), *Helianthus annuus*, *Basella alba*, *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolor*, *Zea mays* (Leela and Vivekanandan, 2008), *Mentha piperata* (Parashar et al., 2009a), *Parthenium hysterophorus* (Parashar et al., 2009b), *Pinus desiflora*, *Diopyros kaki*, *Magnolia kobus* (Song and Kim, 2009), *Jatropha curcas* (Bar et al., 2009), *Gliricidia sepium* (Raut et al., 2009), *Bryophyllum*, *Cyperus*, *Hydrilla* (Jha et al., 2009) etc. (Table 1), to standardize the commercial synthesis of AgNPs. AgNPs were synthesized by using extracts from various parts in different plant species such as leaf, stem bark, roots, rhizomes, tubers, latex, flower, fruits, seeds etc. Rajasekharreddy et al. (2010) studied production of AgNPs by using leaf extracts from different plants (*Tridax procumbens*, *Jatropha curcas*, *Calotropis gigantea*, *Solanum melongena*, *Datura metel*, *Carica papaya* and *Citrus aurantium*) by sunlight exposure method. Production of biogenic AgNPs using *Boswellia ovalifoliolata* stem bark was reported by Ankanna et al. (2010). Biomimetic of AgNPs by using *Citrus limon* juice and theoretical prediction of particle size was studied by Prathna et al. (2011). Umadevi et al. (2012) investigated on AgNPs synthesis by using various concentrations of *Daucus carota* extract and Mukunthan and Balaji (2012) extensively studied the scale up process. Efficient production of AgNPs from papaya fruit extract was reported by Jain et al. (2009). Ghosh et al. (2012) reported that tuber extract mediated synthesis of AgNPs in *Dioscorea bulbifera*. Bankar et al. (2010) found that banana peel extract was used as a novel route for the synthesis of AgNPs. Green synthesis of AgNPs by using *Jatropha curcas* latex and seed extracts were reported by Bar et al. (2009a, b). Ahmad et al. (2012) synthesized AgNPs by using peels of *Punica granatum*. Fruit extracts of *Capsicum annuum* and *Dioscorea oppositifolia* were extensively investigated to synthesize AgNPs (Anal and Prasad, 2011; Maheswari et al., 2012a). Roopan et al. (2013) investigated low cost method to synthesize AgNPs

by using *Cocos nucifera* coir.

PLANT EXTRACTS AND THEIR CHEMICAL CONSTITUENTS

Plant mediated synthesis of AgNPs is regarded as a phytosynthesis or photo biological process in which the plant biomass itself is sufficient to reduce Ag ions into AgNPs. In this process, formation of NPs was proposed to occur through either the ionic or electrostatic interactions between the metal complexes and the functional groups of biomass source. Synthesis of AgNPs requires three key components; they are the solvent medium, reducing and stabilizing agents. In phytosynthesis of AgNPs the primary and secondary metabolites themselves can act as both reducing and stabilizing agents. Aqueous medium instead of organic solvents are used for green synthesis of AgNPs which is apparently regarded as more ecofriendly. Involvement of proteins, polyphenols and carbohydrates in the synthesis of metal NPs were reported by several biologists. All these constituents are present in plants and might be responsible for the synthesis of metal NPs. However, in plants, the involvement of such constituents in NPs synthesis needs experimental proof. Isolated quercetin (natural plant pigment) and polysaccharides have been used for AgNPs synthesis.

Park et al. (2011) explained in-depth the role of polysaccharides and phytochemicals in green synthesis of Au and AgNPs. The primary metabolites (biomolecules such as reducing sugars, proteins, peptides, amino acids etc.), present in plant extracts play a vital role in reducing and stabilizing of metallic silver into AgNPs. The ability of sugars as reducing agents for the synthesis of metallic NPs was reported by Panigrahi et al. (2004). Kwon et al. (2009) reported that the aldehyde group of succinoglycan sugar was oxidized to carboxyl group by nucleophilic addition of OH⁻, which reduced Ag⁺ to Ag (0). Interaction of amino acids (as arginine, cysteine, lysine and methionine) with Ag ions was reported in earlier 1970's (Gruen, 1975). Several researchers reported that along with the bioreduction, proteins can act as stabilizing agents too. The role of peptides as bioreducing and biocapping agents were demonstrated by AgNPs synthesized by cyclic peptides in the latex of *Jatropha curcas* (Bar et al., 2009a) and targeted peptides enriched with proline and hydroxyl-containing amino acid residues (Naik et al., 2002). The effective role of proteins and enzymes in NPs synthesis was reported by Shankar et al. (2003). Proteins present in the *Nelumbo nucifera* leaf extract are essential for bioreduction of AgNPs (Santhoshkumar et al., 2010). Involvement of citric acid and some other bioorganics in the synthesis of AgNPs was reported by Prathna et al. (2011). Silver nanoparticles are formed in natural rubber matrix via photo reduction of film cast from natural rubber latex containing

Table 1. List of species and their parts used for synthesis of silver nanoparticles and their biological applications.

Species	Part used	Biological activity	Shape of nano particles	Size (nm)	Reference
<i>Acalypha indica</i> L.	Leaf	Antibacterial	-	20 -30	Krishnaraj et al., 2010
<i>Achyranthes aspera</i> L.	Leaf	-	Nano triangle	-	Venkatesh et al., 2013
	Leaf	Toxicity	Circular	20-30	Daniel et al., 2011
<i>Acorus calamus</i> L.	Rhizome	Antimicrobial	-	-	Prashanth et al., 2011
<i>Adhatoda vasica</i> Nees	Leaf	Antibacterial/anti proliferative	Spherical	16.8	Ranade et al., 2013
	Arial parts	Antioxidant, Antidiabetic and Antimicrobial	Spherical to oval	15-20	Bandi and Yasundhara,2012
<i>Aegle marmelos</i> (L.) Correa	Plant	Antimicrobial and cytotoxicity	Spherical	0.9-1.2	Lokina and Narayanan, 2011
	Leaf	-	Spherical	60	Rao and Paria, 2013
<i>Albizia adianthifolia</i> (Schum.) W. F. Wight.	Leaf	Anticancer	-	-	Govender et al., 2013
	Leaves	Cytotoxicity	-	4 - 35	Gengan et al., 2013
<i>Allium cepa</i> L.	Bulb	Antibacterial	Spherical	33.67	Saxena et al., 2010
	Bulb	Antibacterial	-	33.6	Benjamin and Bharathwaj 2011
<i>Allium sativum</i> L.	Bulb	Cytotoxicity	Spherical	4.4±1.5	White II et al. 2012
<i>Aloe vera</i> (L.) Burm. f.	Whole plant	-	Spherical	15.2±4.2	Chandran et al., 2006
<i>Ammannia baccifera</i> L.	Aerial	Larvicidal	Spherical, triangle and hexagonal	10-30	Suman et al., 2013
<i>Anacardium occidentale</i> L.	Leaf	-	Cubic	-	Sheny et al., 2011
<i>Ananas comosus</i> Merrill	Leaf	-	Spherical	12	Ahmad and Sharma, 2012
<i>Andrographis paniculata</i> Nees	Leaf	-	Cubic or hexagonal	28	Sulochana et al. 2012
<i>Andropogon muricatus</i> Retz.	Root	-	-	-	Prashanth et al., 2011
	Leaf	Larvicidal	Spherical	450	Arjunan et al., 2012
<i>Annona squamosa</i> L.	Leaf	Antibacterial	Spherical	28.47	Jagtap and Bapat , 2012
	Leaf	-	Cubic	50-200	Rajani et al., 2010
	Leaf	-	Spherical	Up to 30	Kouvaris et al., 2012
<i>Argemone mexicana</i> L.	Leaf	Antibacterial	Cubic / Hexagonal	20	Singh et al., 2010
	Leaf	-	-	-	Khandelwal et al., 2010
<i>Astragalus gummifer</i> Labill.	Gum tragacanth	Antibacterial	Spherical	13.1±1.0	Kora and Arunachalam, 2012

Table 1. Contd.

Species	Part used	Biological activity	Shape of nano particles	Size (nm)	Reference
<i>Azadirachta indica</i> A.Juss	Leaf	Antimicrobial	Spherical	43	Gavhane et al., 2012
	Leaf	-	Spherical	50-100	Shankar et al., 2004
	Leaf	-	Nearly spherical	Below 20	Tripathy et al., 2010
	Leaf	Antibacterial	-	-	Tripathi et al., 2009
	Leaf	-	-	-	Anuradha et al., 2011
<i>Baliospermum montanum</i> (Wild.) Muell-Arg	Leaf	Antibacterial and anticancer	-	10 - 60	Renugadevi et al., 2012
<i>Basella alba</i> L.	Leaf	-	-	-	Leela and Vivekanandan, 2008
<i>Berberis aristata</i> DC.	Wood	-	-	-	Prashanth et al., 2011
<i>Boswellia ovalifoliolata</i> Bal. & Henry	Stem bark	Antimicrobial	-	-	Savithamma et al., 2011
	Stem bark	-	Spherical	30 - 40	Ankanna et al., 2010
	Leaves	Antimicrobial	-	-	Savithamma et al., 2012
<i>Brassica chinensis</i> L.	Hypocotyls	-	-	-	Tan et al., 2010
<i>Brassica juncea</i> Czern	<i>In vitro</i> cultures	-	-	-	Shekhawat and Arya, 2009
<i>Brassica napus</i> L.	Somatic embryos	-	-	-	Tan et al., 2010
<i>Brassica oleracea</i> L.	Leaf	-	-	-	Veeranna et al., 2013
	Flower	Antibacterial	Spherical	53.8	Sridhara et al., 2013
<i>Bryophyllum pinnatum</i> (Lam.) Oken.	Leaf	Antibacterial	Spherical	70-90	Baishya et al., 2012
<i>Calotropis gigantea</i> L.	Leaf	-	Spherical	< 20	Rajasekharreddy et al., 2010
	Leaf	-	-	11.8-83.7	Sivakumar et al., 2011
<i>Calotropis procera</i> (Aiton) W.T.Aiton	Flower	-	Cubic	35	Babu and Prabu, 2011
	Leaf	-	Spheroidal and some are inprisms or rods	20	Begum et al., 2009
<i>Camellia sinensis</i> (L.) Kuntze	Leaf	-	Anisotropic	200	Vilchis-Nestor et al., 2008
	Leaf	-	Cubic	20-60	Nadagouda and Varma, 2008
	Leaf	-	Cubic	4	Loo et al., 2012
<i>Capscium annuum</i> L.	Fruit	-	-	-	Li et al., 2007
	Fruit	-	Spherical	2-6	Jha and Prasad, 2011
<i>Carica papaya</i> L.	Fruit	Antimicrobial	Cubic and hexagonal	15	Jain et al., 2009
	Leaf	-	-	-	
	Callus	-	Spherical	60 - 80	

Table 1. Contd.

Species	Part used	Biological activity	Shape of nano particles	Size (nm)	Reference
<i>Cassia angustifolia</i> M. Vahl	Leaf	Antimicrobial	Spherical	21.6	Amaladhas et al., 2012
<i>Cassia auriculata</i> L.	Leaf	-	Spherical	20 - 40	Udayasoorian et al., 2011
	Flower	Antimicrobial	Spherical	10-50	Jobitha et al., 2012
<i>Cassia fistula</i> L.	Leaf		Nano wires	40-60	Lin et al., 2010
	Leaf	Antibacterial	Spherical	48-67	Mukunthan et al., 2011
	Leaf	Antiplasmodial	Cubic	35 - 65	Ponarulselvam et al., 2012
<i>Catharanthus roseus</i> (L.) G. Don	Leaf	-	-	45-70	Kannan et al., 2011
	Callus	Antimicrobial	-	-	Malabadi et al., 2012
	<i>In vitro</i> derived plants	Antimicrobial	-	-	Malabadi et al., 2012
	Dried leaves	Antimicrobial	Crystalline	27±2 and 30±2	Kotakadi et al., 2013
<i>Celastrus paniculatus</i> Willd.	Seed	-	-	-	Prashanth et al., 2011
<i>Centella asiatica</i> (L.) Urb.	Plant	Antimicrobial	-	28.4	Logeswari et al., 2012
<i>Ceratonia siliqua</i> L.	Leaf	Antibacterial	Crystalline	18	Awwad et al., 2013
<i>Chenopodium album</i> L.	Leaf	-	Quasi-spherical	12	Dwivedi and Gopal, 2010.
<i>Chromolaena odorata</i> (L.) King and Robinson	Leaf	-	Hexagonal	40-60	Geetha et al., 2012
<i>Chrysopogon zizanioides</i> (L.) Roberty	Leaf	Antibacterial, antioxidant and cytotoxic	Cubic	85-110	Arunachalam and Annamalai, 2013
<i>Cinnamomum camphora</i> (L.) J. Presl	Leaf	-	Spherical	55-80	Huang et al., 2007
<i>Cinnamon zeylanicum</i> Nees.	Bark powder/extract	Antibacterial	Quasi-spherical and small rod-shaped	31-40	Sathishkumar et al., 2009
<i>Cissus quadrangularis</i> L.	Stem	Antiphlastic	Spherical and oval	42.46	Santhoshkumar et al., 2012
<i>Citrullus colocynthis</i> (L.) Schrad.	Leaf	Biomedical	Spherical	31	Satyavani et al., 2011
	Calli	Antibacterial	Spherical	75	Satyavani et al., 2011
<i>Citrus aurantium</i> L.	Leaf	-	-	-	Rajasekharreddy et al., 2010
<i>Citrus limon</i> (L.) Burm.f.	Lime juice	-	spherical and spheroidal	50	Prathna et al., 2011
<i>Citrus sinensis</i> (L.) Osbeck	Peel	Free radical scavenging, cytocompatible	Spherical	6	Konwarh et al., 2011
<i>Citrus sinensis</i> (L.) Osbeck	Plant	Antimicrobial	-	65	Logeswari et al., 2012
<i>Clerodendrum inerme</i> (L.) Gaertn.	Leaf	skin diseases	-	Different	Farooqui et al., 2010
<i>Clitoria ternatea</i> L.	Callus and <i>in vitro</i> derived plants	Antimicrobial	-	-	Malabadi et al., 2012

Table 1. Contd.

Species	Part used	Biological activity	Shape of nano particles	Size (nm)	Reference
<i>Cochlospermum gossypium</i> DC.	Gum	antibacterial	Spherical	3	Kora et al., 2010
<i>Cocos nucifera</i> L.	coir	Larvicidal	Cubic	23±2	Roopan et al., 2013
<i>Coffea Arabica</i> L.	Seed	-	Cubic	20-60	Nadagouda and Varma, 2008
<i>Coleus aromaticus</i> Benth.	Leaf	Anti bacterial	Spherical	44	Vanaja and Annadurai., 2012
<i>Commiphora wightii</i> (Arn.) Bhandari	Resin	-	-	-	Prashanth et al., 2011
<i>Copaifera reticulata</i> Ducke	Oleoresin	Acaricidal	-	-	Fernandes and Freitas, 2007
<i>Coriandrum sativum</i> L.	Fruit	-	-	-	Prashanth et al., 2011
	Leaf	Nonlinear optics	Spherical	26	Sathyavathi et al., 2010
<i>Cuminum cyminum</i> L.	Fruit	-	-	-	Prashanth et al., 2011
<i>Curcuma longa</i> L.	Tubers	Bactericidal	Quasispherical, triangular and small rod-shaped	71-80	Sathishkumar et al., 2010
<i>Cyamopsis tetragonaloba</i> (L.) Taub.	Leaf	-	-	-	Rajani et al., 2010
<i>Cymbopogon citratus</i> (DC.) Stapf	Leaf	Antimicrobial	Spherical	32	Masurkar et al., 2011
<i>Cynodon dactylon</i> (L.) Pers.	Plant	Antimicrobial and cytotoxicity	Cubic	-	Lokina and Stephen, 2011
<i>Datura metel</i> L.	Leaf	-	-	-	Rajasekharreddy et al., 2010
	Root	-	Spherical	20	Umadevi et al., 2012
<i>Daucus carota</i> L.	Root	-	Spherical	31-52	Mukunthanand Balaji, 2012
	Root	Antimicrobial and Cytotoxicity	-	-	Lokina and Narayanan, 2011
<i>Desmodium triflorum</i> (L.) DC.	Plant	-	Spherical	5-20	Ahmad et al., 2011
<i>Dioscorea bulbifera</i> L.	Tuber	Anti microbial	Nanorods and triangles	8-20	Ghosh, et al., 2012
<i>Dioscorea oppositifolia</i> L.	Fruit	Antimicrobial	Spherical	14	Maheswari et al., 2012
<i>Diospyros kaki</i> Thunb.	Leaf	-	Au/AgNPs – cubic, AgNPs - spherical	Au/AgNPs - 50-500, AgNPS - 15-90	Song and Kim, 2008
<i>Drypetes roxburghii</i> (Wall.) Hurusawa.	Fruit	larvicidal	quasi-spherical	26.6	Haldar et al., 2013
<i>Eclipta alba</i> (L.) Hassk.	Leaf	-	Spherical	2-6	Jha et al., 2009
<i>Eclipta prostrata</i> L.	Leaf	Larvicidal	Spherical, Elongated	55 - 60	Rajakumar and Rahuman 2011
<i>Elettaria cardamomom</i> (L.) Maton	Seed	Antimicrobial	Spherical	40-70	GnanaJobitha et al., 2012
<i>Embelia ribes</i> Burm.f.	Fruit	-	-	-	Prashanth et al., 2011
<i>Emblica officinalis</i> L.	Fruit	Transmetallation	-	10-20	Ankamwar et al., 2005

Table 1. Contd.

Species	Part used	Biological activity	Shape of nano particles	Size (nm)	Reference
<i>Eucalyptus chapmaniana</i> Cameron	Leaf	Antimicrobial	Cubic	-	Sulaiman et al., 2013
<i>Eucalyptus hybrida</i>	Leaf	-	Cubical	50-150	Dubey et al., 2009
<i>Euphorbia hirta</i> L.	Leaf	Larvicidal	Spherical and cubic	30 - 60	Priyadarshini et al., 2012
<i>Euphorbia hirta</i> L.	Leaf	antifungal	Spherical	40-50	Elumalai et al., 2010
<i>Euphorbia milii</i> Des Moul.	Latex	Antimicrobial	--	-	Patil et al., 2012
<i>Euphorbia nivulia</i> Buch.Ham	Stem latex	Toxicity	Spherical, psudospherical	10±2	Valodkar et al., 2011
<i>Euphorbia prostrata</i> Aiton	Leaf	Pesticidal	Rod	25 - 80 average 52.4	Zahir et al., 2012
<i>Ficus benghalensis</i> L.	Leaf	Antibacterial	Spherical	16-44	Saxena et al., 2012
<i>Ficus racemosa</i> L.	Bark	Larvicidal	-	-	Velayutham et al., 2013
<i>Garcinia mangostana</i> L.	Leaf	Antimicrobial	Spherical	35	Veerasingam et al., 2011
<i>Gliricidia specium</i> (Jacq.) Kunth ex Walp.	Leaf	Antibacterial	Spherical	27	Rajesh et al., 2009
<i>Glycine max</i> (L.) Merr.	Leaf	-	Cubic	25 - 100	Vivekanandhan et al., 2009
<i>Glycyrrhiza glabra</i> L.	Root/ Rhizome	-	-	-	Prashanth et al., 2011
<i>Helianthus annuus</i> L.	Leaf	-	-	-	Leela and Vivekanandan, 2008
<i>Hemidesmus indicus</i> R.Br.	Root	-	-	-	Prashanth et al., 2011
<i>Hibiscus cannabinus</i> L.	Leaf	Antimicrobial	Spherical	9	Bindhu and Umadevi, 2013
<i>Hibiscus rosa-sinensis</i> L.	Leaf	-	Different	~13	Philip, 2010
<i>Holarrhena antidysenterica</i> Wall.	Seed	-	-	-	Prashanth et al., 2011
<i>Hydrilla verticillata</i> (L.f.) Royle.	Whole plant	-	Spherical	65.55	Sable et al. 2012
<i>Indigofera aspalathoides</i> DC.	Leaf	Wound healing	Square	20-50	Arunachalam et al., 2013
	Leaf	-	Spherical	< 20	Rajasekharreddy et al., 2010
<i>Jatropha curcas</i> L.	Latex	-	-	10 - 20	Bar et al., 2009
	Seed	-	Spherical	15 - 50	Bar et al., 2009
	Latex	Antimicrobial	-	-	Patil et al., 2012
<i>Jatropha gossypifolia</i> L.	Latex	Antimicrobial	-	-	Patil et al., 2012
<i>Justicia genderussa</i> Burm.f.	Leaf	Cell viability	Spherical	16	Chinna and Prabha, 2012
<i>Lactuca sativa</i> L.	Leaf	Antimicrobial	Spherical	40-70	Kanchana et al., 2011
<i>Lantana camara</i> L.	Fruit	Antibacterial	Spherical	12.55	Sivakumar et al. 2012
<i>Lawsonia inermis</i> L.	Leaf	Lousicidal	spherical	59.52	Marimuthu et al., 2012
<i>Leonurus japonicas</i> Houtt.	Whole plant	Antibacterial	Spherical	9.9-13.0	Im et al. 2012
<i>Leucas aspera</i> (Willd.) Link.	Bark	Antimicrobial	-	29-45	Antony et al., 2013
<i>Lippia citriodora</i> Kunth.	Leaf	-	Spherical	15-30	Cruz et al., 2010
<i>Macrotyloma uniflorum</i> (Lam.) Verdc.	Seed	-	Cubic	~12	Vidhu et al., 2011
<i>Magnolia</i> spp.	Leaf	-	-	15 - 500	Song and Kim, 2009

Table 1. Contd.

Species	Part used	Biological activity	Shape of nano particles	Size (nm)	Reference
<i>Mangifera indica</i> L.	Leaf	-	Spherical, triangular, hexagonal	20	Philip, 2011
<i>Manilkara zapota</i> (L.) P.Royen	Leaf	Feeding deterrent activity	Spherical and oval	70 - 140	Kamaraj et al., 2012
	Leaf	Acaricidal	Spherical and oval	70 - 140	Rajakumar and Rahuman, 2012
<i>Mayaca fluviatilis</i> Aubl.	Fruit	-	-	-	Bunghez et al., 2010
<i>Medicago sativa</i> L.	Sprouts	-	Wires	Different	Gardea-Torresdey et al., 2003
<i>Melia azadirachta</i> L.	Bark	-	-	-	Prashanth et al., 2011
	Leaf	Cytotoxic	Cubical and spherical	78	Sukirtha et al., 2012
<i>Memecylon edule</i> Roxb.	Leaf	-	Square	50 - 90	Elavazhagan and Arunachalam, 2011
<i>Mentha piperita</i> L.	Leaf	Antibacterial	Spherical	90	Mubarak Ali et al., 2011
<i>Memecylon umbellatum</i> Burm.f.	Leaf	-	Spherical	15-20	Arunachalam et al., 2013
<i>Mimosa pudica</i> L.	Leaf	Parasitic	Spherical	25 - 60	Marimuthu et al., 2011
<i>Morinda pubescens</i> L.	Leaf	-	Spherical	25-50	Mary and Inbathamizh 2012
<i>Morinda citrifolia</i> L.	Leaf	Antibacterial	Spherical	27	Sathishkumar et al., 2012
	Root	Cytotoxicity	Spherical	30-55	Suman et al., 2013
<i>Moringa oleifera</i> Lam.	Whole plant	-	-	-	Shivashankar and Sisodia, 2012
<i>Morus alba</i> L.	Leaf	Antibacterial	Spherical	-	Wang et al., 2011
	Leaf	Antibacterial	Cubic	20-40	Awwad and Salem, 2012
<i>Murraya koenigii</i> (L.) Spreng.	Leaf	-	Cubodial	20-35	Suganya et al., 2013
	Leaf	-	Spherical and ellipsoidal	10 - 25	Christensen et al., 2011
<i>Murraya koenigii</i> (L.) Spreng.	Leaf	-	-	~10	Philip et al., 2011
	Leaf	Larvicidal	Spherical and cubic	20-35	Suganya et al., 2013
	Leaf	Acaricidal and larvicidal	-	50 - 150	Jayaseelan et al., 2012
<i>Musa paradisiaca</i> L.	Leaf	Antimicrobial	-	Up to 100	Bankar et al., 2010
	Peal	Antimicrobial	-	60-100	Sulaiman et al., 2013
<i>Myrtus communis</i> L.	Leaf	-	-	-	Prashanth et al., 2011
<i>Nigella sativa</i> L.	Seed	-	-	-	Prashanth et al., 2011
<i>Nelumbo nucifera</i> Gaertn.	Leaf	Larvicidal	Triangular, pentagons, hexagonal	25-80	Santhoshkumar et al., 2011
<i>Nerium oleander</i> L.	Leaf	Larvicidal	Spherical and cubic	20-35	Roni et al., 2013
<i>Nicotiana tabaccum</i> L.	Leaf	-	Crystalline	8	Prasad et al., 2011
<i>Ocimum</i> spp.	Leaf	-	-	3 - 20	Mallikarjuna et al., 2011

Table 1. Contd.

Species	Part used	Biological activity	Shape of nano particles	Size (nm)	Reference
<i>Ocimum canum</i> Sim.	Leaf	Acaricidal	Rod, cylindrical	25 - 110	Jayaseelan and Rahuman, 2012
	Leaf	Antibacterial	Spherical	18	Ramteke et al., 2013
	Roots and stem	-	Spherical	10±2 and 5±1.5	Ahmad et al., 2010
<i>Ocimum sanctum</i> L.	Leaf	-	Triangle	42	Rao et al., 2013
	Leaf	Antimicrobial	Spherical	4 - 30	Singhal et al., 2011
	Leaf	Antigenotoxic	-	50	Vijaya et al., 2013
	Leaf	Antibacterial and antifungal	Spherical	50	Rout et al., 2012
<i>Ocimum tenuiflorum</i> L.	Plant	Antimicrobial	-	28	Logeswari et al., 2012
	Leaf	Toxicity	Circular	20	Daniel et al., 2011
	Leaf	Antibacterial	-	25-40	Patil et al., 2012
	Leaf	Antibacterial	Spherical	12	Aniket et al., 2010
<i>Oryza sativa</i> L.	Leaf	-	-	-	Leela and Vivekanandan, 2008
<i>Paederia foetida</i> L.	Leaf	Antimicrobial	Spherical	4-15	Mollick et al., 2012
<i>Panicum virgatum</i> L.	Whole plant	-	Spherical, rod-like, triangular, pentagonal, hexagonal	20 - 40	Mason et al., 2012
<i>Papaver somniferum</i> L.	Seed	-	Spherical	3.2 - 7.6	Vijayaraghavan et al., 2012
<i>Parthenium hysterophorus</i> L.	Leaf	-	Various	Up to 90	Sarkar et al., 2010
	Leaf	-	-	-	Parashar et al., 2009
<i>Pedilanthus tithymaloides</i> (L.) Poit.	Latex	Antimicrobial	-	-	Patil et al., 2012
	Leaf	Anti developmental	Spherical	15-30	Sundaravadivelan et al., 2013
<i>Pelargonium graveolens</i> L. Her.	Leaf	-	Quasilinear	16 - 40	Shankar et al., 2003
<i>Pennisetum glaucum</i> (L.) R. Br	Leaf	-	-	-	Rajani et al., 2010
<i>Pergularia daemia</i> (Forssk.) Chiov.	Latex	Larvicidal	Spherical	123.50	Patil et al., 2012
<i>Persimmon</i> spp.	Leaf	-	-	15-500	Song and Kim, 2009
<i>Phyllanthus amarus</i> Schum. & Thonn	Phyllanthin	-	quasi-spherical	30	Kasthuri et al., 2009
<i>Piper betle</i> L.	Leaf	-	Spherical	3-37	Mallikarjuna et al., 2012
<i>Piper longum</i> L.	Leaf	Cytotoxicity	Spherical	17.6-41	Jacob et al., 2012
<i>Piper nigrum</i> L.	Fruits	Free radical scavenging	Spherical	40-60	Mani et al., 2012
	Leaf	-	Spherical	5-60	Mallikarjuna et al., 2012
<i>Platanus orientalis</i> L.	Leaf	-	-	-	Song and Kim, 2009

Table 1. Contd.

Species	Part used	Biological activity	Shape of nano particles	Size (nm)	Reference
<i>Platanus</i> spp.	Leaf	-	-	15-500	Song and Kim, 2009
<i>Plumbago rosea</i> L.	Root	-	-	-	Prashanth et al., 2011
<i>Plumeria rubra</i> L.	Latex	Larvicidal	Spherical	32 -220	Patil et al. 2012
<i>Polyalthia longifolia</i> Sonn.	Leaf	Antibacterial	Spherical	Various	Kaviya et al., 2011
	Leaves	-	Spherical	57.53	Prasad et al., 2012
<i>Pongamia pinnata</i> (L) Pierre	Leaf	Antibacterial	Spherical	38	Rajesh et al., 2010
<i>Prosopis juliflora</i> DC.	Leaf	Antimicrobial	Triangles, tetragons, pentagons and hexagons	11 -19	Raja et al.,2012
<i>Psoralea corylifolia</i> L.	Resin	-	-	-	Prashanth et al., 2011
<i>Pulicaria glutinosa</i> (Boiss.) Jaub. & Spach	Aerial parts	-	Spherical	40-60	Khan et al., 2013
	Peel	-	Spherical	21	Solgi and Taghizadeh, 2012
	Peels	-	Spherical	5 ±1.5	Ahmad et al., 2012
<i>Punica granatum</i> L.	Peels	Catalytic	Spherical	30	Edison and Sethuraman, 2013
	Leaf buds	Antibacterial	-	4 - 26	Umashankari et al., 2012
<i>Rosa damascena</i> Mill.	petals	-	Spherical	21	Solgi and Taghizadeh, 2012
	Flower	Electro chemistry	-	21	Ghoreishi et al., 2011
<i>Rosa rugosa</i> Thunb.	Leaf	-	Spherical	12	Dubey et al., 2010
<i>Rosmarinus officinalis</i> L.	Leaf	Antimicrobial and Cytotoxicity	Cubic	60	Sulaiman et al., 2013
<i>Saccharum officinarum</i> L.	Leaf	-	-	-	Leela and Vivekanandan , 2008
<i>Sesuvium portulacastrum</i> L	Callus and leaf	Antimicrobial	Spherical	5-20	Nabikhan et al., 2010
<i>Shorea tumbuggaia</i> Roxb.	Leaf	Antimicrobial	-	-	Savithramma et al., 2012
	Stem bark	Antimicrobial	-	-	Savithramma et al., 2011
<i>Shorea robusta</i> Roth.	Stem bark	Antimicrobial	Spherical	-	Savithramma et al., 2011
<i>Smilax china</i> L.	Root	-	-	-	Prashanth et al., 2011
<i>Solanum lycopersicum</i> L.	Fruit	-	Spherical	10	Umadevi et al., 2013
<i>Solanum melongena</i> L.	Leaf	-	-	-	Rajasekharreddy et al., 2010
<i>Solanum torvum</i> Sw.	Leaves	Antimicrobial	Spherical	14	Govindaraju et al., 2010
<i>Solanum trilobatum</i> L.	Plant	Antimicrobial	-	22.3	Logeswari et al., 2012
	Leaf	Antidandruff	Cubic and Hexagonal	15-20	Pant et al., 2012

Table 1. Contd.

Species	Part used	Biological activity	Shape of nano particles	Size (nm)	Reference
<i>Solanum xanthocarpum</i> L.	Berry	Antimicrobial and uriaase inhibitory activity	Spherical	10	Amin et al., 2012
<i>Sorbus aucuparia</i> L.	Leaf	-	Spherical, triangular, hexagonal	16	Dubey et al., 2010
<i>Sorghum</i> SPP.	Bran	-	Spherical	~10	Njagi et al., 2011
<i>Sorghum bicolor</i> (L.) Moench	Leaf	-	-	-	Leela and Vivekanandan, 2008
<i>Sorghum vulgare</i> Pers.	Leaf	-	-	-	Rajani et al., 2010
<i>Spinacia oleracea</i> L.	Leaf	Antimicrobial	Different	40-70	Kanchana et al., 2011
<i>Suaeda monoica</i> Forssk. ex J. Gmelin	Leaf	Cell line toxicity	Spherical	31	Satyavani et al., 2012
<i>Svensonia hyderabadensis</i> (Walp.) Mold	Leaf	Antimicrobial	-	-	Savithamma et al., 2012
	Leaf	Antimicrobial	Spherical	45	Rao and Savithamma, 2011
	Leaf	Antimicrobial	-	-	Savithamma et al., 2011
	Stem	Antimicrobial	-	-	Rao and Savithamma, 2012
<i>Swietenia mahogany</i> (L) Jacq.	Leaf	-	Spherical	20-50	Mondal et al. 2011
<i>Syzygium cumini</i> (L.) Skeels	Leaf, seed	-	Spherical	LE-30, leaf water fraction-29, SE-92, seed water fraction -73	Kumar et al., 2010
	Plant	Antimicrobial	-	26.5	Logeswari et al., 2012
<i>Tanacetum vulgare</i> L.	Fruit	-	Triangular, spherical and hexagonal	10-40	Dubey et al., 2010
<i>Terminalia chebula</i> Retz.	Fruit	antimicrobial	Spherical	-	Kumar et al., 2012a
<i>Thespesia populnea</i> (L.) Sol. ex Correa	Leaves	Antimicrobial	-	-	Savithamma et al., 2012
<i>Tinospora cordifolia</i> Miers	Leaf	Pediculicidal and Larvicidal	-	55 - 80	Jayaseelan et al., 2011
<i>Trachyspermum ammi</i> Sprague	Seed	-	Triangular	87 - 998	Vijayaraghavan et al., 2012
<i>Trianthema decandra</i> L.	Roots	Antimicrobial	Cubic	15	Geethalakshmi and Sarada, 2010
<i>Tribulus terrestris</i> L.	Plant	Antimicrobial	Spherical	16-28	Gopinath et al., 2012
<i>Tridax procumbens</i> L.	Leaf	-	Spherical	<20	Rajasekharreddy et al., 2010
<i>Trigonella foenum-graecum</i> L.	Seed	-	-	-	Prashanth et al., 2011
<i>Triticum aestivum</i> L.	Seedlings	Peroxide Catalytic Activity and Toxicology	Polydispersed spherical	10	Waghmode et al., 2013
<i>Triphala ingredients</i>	Fruit mixture	-	Spherical	59	Gavhane et al., 2012
<i>Turnera ulmifolia</i> L.	Leaf	Antimicrobial	-	-	Shekhawat et al., 2012
<i>Vigna radiate</i> (L.) Wilczek.	Leaf	Antibacterial	-	-	Rajani et al., 2010

Table 1. Contd.

Species	Part used	Biological activity	Shape of nano particles	Size (nm)	Reference
<i>Vinca rosea</i> L.	Leaf	Antimicrobial	-	-	Savithramma et al., 2012
	Leaf	Larvicidal	Spherical	25- 47	Subarani et al., 2013
<i>Vitex negundo</i> L.	Leaf	Antibacterial	Cubic	10-30	Zargar et al., 2011
<i>Wrightia tinctoria</i> (Roxb.) R.Br.	Leaf	-	-	19-68	Bharani et al., 2012
<i>Zea mays</i> L.	Leaf	-	-	-	Rajani et al., 2010
	Leaf	-	-	-	Leela and Vivekanandan , 2008
<i>Zingiber officinale</i> Roscoe.	Rhizome	-	Spherical	6-20	Kumar et al., 2012b

silver salt was reported by Bakar et al. (2007).

Several active principles (secondary metabolites) such as flavonoids, terpenoids and alkaloids were suggested to be involved as either reducing or stabilizing agents during the formation of NPs. All these constituents are present in plants and might be responsible for the synthesis of metal NPs. However, in plants, the involvement of such constituents in NPs synthesis needs experimental proof. Isolated quercetin (natural plant pigment) has been used for AgNPs synthesis (Egorova and Revina, 2000; Kundu et al., 2004). Shankar et al. (2003) reported that terpenoids in geranium leaf may be responsible for the synthesis of AgNPs.

Shankar et al. (2004) reported that the flavonoids and terpenoids present in the leaf broth of *A. indica* are responsible for the production of stable Ag, Au and bimetallic Ag-Au NPs. *Cinnamomum zeylanium* bark is rich in terpenoids, including linalool, eugenol, and methyl chavicol, which contribute to its distinct aroma. It was suggested by Singh et al. (2010) that eugenol plays a critical role in bioreduction of AgNPs. Furthermore, the effective role of polyphenols and caffeine present in the coffee and tea extract in the synthesis of AgNPs were reported by

Nadagouda and Varma (2008). In the case of hydrophytes, compounds such as catechol, potocatecheuic acid along with other phytochemicals in *Hydrilla* have been reported to liberate reactive hydrogen, which contribute to the reduction of AgNPs (Jha et al., 2009). In the present days, many of the researchers are investigating the phytochemicals that are involved in the bio reduction and stabilization of AgNPs and the possible mechanisms that are involved in reduction of AgNPs by using active principles (Philip, 2010; Njagi et al., 2011). In *Ananas comosus*, different types of antioxidants present in the juice synergistically reduce the Ag metal ions, as each antioxidant is unique in terms of its structure and antioxidant function (Ahmad and Sharma, 2012).

FACTORS AFFECTING THE SIZE AND MORPHOLOGY OF Ag NANOPARTICLES

The intrinsic properties of metal NPs are mainly determined by its size, shape, composition, crystallinity and structure. Normally the optoelectronic and physiochemical properties of nano-scale matter strongly depend on particle size,

whereas the particle shape contributes significantly to modulate their electronic properties. Green methods generally lead to the formation of crystalline NPs with sizes ranging in between 1 to 100 nm and with a wide variety of shapes (spheres, rods, prisms, plates, needles, leafs or dendrites).

The desired size and shape of the AgNPs can be achieved by controlling the process parameters (Chan and Don, 2013), such as the nature of plant extract and the relative concentrations of the extract and metal salts, pH, temperature, reaction time etc. The stability of produced NPs can in some cases change after a few days or the NPs can remain stable over longer periods. AgNPs were rapidly synthesized using the leaf extract of *Mimosa pudica* and the formation of NPs was observed within 6 h (Marimuthu et al., 2011). Song and Kim (2009) reported 90% formation of AgNPs within 11 min by using leaf broth of *Magnolia* at 95°C. Synthesis of AgNPs was started within 15 min and the reduction reaction was completed within 2 h by using the extract of *Panicum virgatum* (Mason et al., 2012). Sulaiman et al. (2013a) reported that when 10 ml of *Rosmarinus officinalis* extract was mixed with

90 ml of 2 mM AgNO₃ and heated at 70°C for 3 min, it resulted in rapid synthesis of AgNPs. Similarly, 10 ml of leaf extract of *Eucalyptus chapmaniana* mixed with 90 ml of 0.01 or 0.02 mM aqueous AgNO₃ and exposed for 1 h to sun light leads to synthesis of AgNPs (Sulaiman et al., 2013b). Temperature plays an important role in reduction of AgNO₃ to stable AgNPs. The effective role of temperature in NPs synthesis was well explained by Panda et al. (2011) and they found that the optimum colour intensity was achieved within 14 min at 95°C; whereas, the same reaction required 12 h at 25°C temperature. Krishnasamy et al. (2012) reported that complete reduction of silver ions observed after 48 h at 30°C under shaking condition while *Indigofera aspalathoides* leaf extract was used as reducing agent. Similar type of report was provided while *Cassia auriculata* leaf extract was used as reducing agent (Udayasoorian et al., 2011). The effect of different process parameters like the reluctant concentration, mixing ratio of the reactants etc. was studied (Prathna et al., 2011).

In this, 10⁻² M AgNO₃ solution was interacted for 4 h with lemon juice in the ratio of 1:4 and formed AgNPs with size below 50 nm which are spherical or spheroid in shape. Christensen et al. (2011) studied the biological synthesis of AgNPs using *Murraya koenigii* leaf extract and the effect of broth concentration on reduction mechanism and particle size. Huang et al. (2011) reported that increasing the AgNO₃ concentration at 30 or 65°C increased the mean size and the size distribution of the AgNPs. Singhal et al. (2011) reported that *Ocimum sanctum* leaf extract can reduce AgNO₃ into AgNPs within 8 min of reaction time. When the *Mentha piperita* extract was subjected to AgNO₃, the biosynthesis reaction was started within few minutes (Mubarakali et al., 2011). AgNPs with short chain-shaped structures were produced by using *Cassia fistula* leaf extract (Lin et al., 2010). They observed that by prolonging the reaction time, the newly formed Ag atoms deposited onto the concave regions of the connected NPs through capillary phenomenon, leading to the formation of long nanorods (Lin et al., 2010). Vivekanandhan et al. (2009) studied the effect of different *Glycine max* varieties leaf extracts on bioreduction of AgNPs. Ramteke et al. (2013) reported that when tulasi extract was used as reducing agent, more than 90% of the reaction was completed within one hour of the reaction time. It was found that AgNPs synthesized by *Medicago sativa* seeds formed aggregation of hexagonal and well-defined shaped NPs without signs of fusion. This could be attributed to the strong interaction between the chemically bound capping agents which counteracted the tendency of the NPs to aggregate (Lukman et al., 2011). Optimization studies revealed that the maximum rate of synthesis could be achieved with 0.7 mM AgNO₃ solution at 50°C in 5 h (Ghosh et al., 2012). The variation of particle size with the reaction temperature and reaction time has been reported (Sarkar et al., 2010).

PHARMACOLOGICAL APPLICATIONS

Silver products have long been known to have strong inhibitory and bactericidal effects, as well as broad spectrum of antimicrobial activities which has been used for centuries to prevent and or treat various infections. Recent research regarding green synthesized AgNPs revealed that they have wide spread biological applications like free radical scavenging, biocompatibility, antimicrobial effects etc. (Konwarh et al., 2011).

ANTIBACTERIAL STUDIES

The AgNPs show efficient antimicrobial property compared to other NPs due to their extremely large surface area, which provides better contact with microorganisms. Even though the exact mechanism involved in bactericidal activity of nanoscaled silver on bacteria was not fully understandable; the three most common mechanisms of toxicity were well explained by Jones and Hoek (2010).

Due to their nano size, AgNPs get attached to the cell membrane and penetrate easily into the bacteria. The bacterial membrane contains sulfur-containing proteins; the AgNPs interact with these proteins and diffuses into the cell. The NPs preferably attack the respiratory chain, cell division finally leading to cell death. The NPs release silver ions in the bacterial cells, which enhance their bactericidal activity. The NPs smaller than 10 nm interact with bacteria and produce electronic effects, which enhance the reactivity of NPs.

The bactericidal effect of the nano crystalline AgNPs was tested against *Escherichia coli* strain (BL 21) (Satishkumar et al., 2009). They found that different tested concentrations (2, 5, 10, 25 and 50 mg/L) exhibited different inhibition percentages (10.9, 32.4, 55.8, 82 and 98.8%), respectively. The calculated EC₅₀ value was 11 ± 1.72 mg/L and minimum inhibitory concentration (MIC), was found to be 50 mg/L. Strains with the same species were reported to possess different inhibitory effects when the same concentration of AgNPs were used (Geethalakshmi and Sarada, 2010). AgNPs exhibited significant antibacterial activity against *E. coli* and *Pseudomonas aeruginosa* showing clear inhibition zone at a concentration of 50 ppm (Jain et al., 2009). Antimicrobial assay of biosynthesized AgNPs against both Gram-negative (*E. coli*) and positive (*S. aureus*) microorganisms at different concentrations was studied (Singhal et al., 2011) and they revealed a strong dose-dependent antimicrobial activity against both the tested microorganisms. It was seen that, as the concentration of biosynthesized NPs were increased, microbial growth decreases in both the cases. Biosynthesized AgNPs were found to exhibit more antimicrobial activity on Gram-negative microorganism than Gram-positive ones. Mubarakali et al. (2011) found similar type of results while

studying antimicrobial activity of biosynthesized AgNPs and they reported that it may be due to the variation in the cell wall composition between Gram positive and Gram negative bacteria. Rajesh et al. (2010) reported that the AgNPs synthesized using dried leaves of *Pongamia pinnata* were effective against different strains of bacteria such as *E. coli* (ATCC 8739), *S. aureus* (ATCC 6538p), *P. aeruginosa* (ATCC 9027) and *Klebsiella pneumoniae* (clinical isolate). AgNPs with considerable growth inhibition of two of the well known pathogenic bacteria with zone of inhibition 11 mm (*E.coli*) and 10 mm (*S. aureus*), respectively were reported by Ramteke et al. (2013).

The inhibitory percentage of the AgNPs against *E. coli* and *S. aureus* at different concentrations shows that the higher the concentrations of NPs, the higher the inhibitory effect. The minimum inhibitory concentration for *E. coli* was 1.4 ppm; whereas, for *S. aureus*, it was 5.4 ppm (Huang et al., 2011). AgNPs synthesized by using aqueous extract of *Alium cepa* showed significant antibacterial activity (Benjamin and Bharathwaj, 2011). Kulkarni et al. (2011) reported that the AgNPs showed significant antimicrobial activity on four clinically isolated pathogens with zone of inhibitions on *P. aeruginosa* (12 mM), *E. coli* (11 mM), *Bacillus subtilis* (9 mM) and *K. pneumoniae* (8 mM), respectively. They found that with increasing the concentrations of AgNPs, there is a gradual reduction in bacterial growth of *E. coli*. The AgNPs were found to possess potent antibacterial activity against both Gram negative and Gram positive bacteria (Ghosh et al., 2012). They found that beta lactam (piperacillin) and macrolide (erythromycin) antibiotics showed a 3.6-fold and 3-fold increase, respectively in combination with AgNPs selectivity against multidrug-resistant *Acinetobacter baumannii*. Notable synergy was seen between AgNPs and chloramphenicol/vancomycin against *P. aeruginosa* and was supported by 4.9-fold and 4.2-fold increases in zone diameter, respectively. Similarly, a maximum 11.8-fold increase in zone diameter of streptomycin when combined with AgNPs against *E. coli* was found. This report provides a strong evidence for the synergistic action of a combination of antibiotics and AgNPs against multidrug resistant bacteria.

The AgNPs synthesized by using *Argemone mexicana* showed significant antibacterial activity towards *E. coli* and *Pseudomonas syringae* at a concentration range of 30 ppm (Singh et al., 2010). Synthesis of plant-mediated AgNPs using various medicinal plant extracts and evaluation of their antimicrobial activities was studied by Prashanth et al. (2011). The antimicrobial activities of colloidal silver particles are influenced by the dimensions of the particles (Kaviya et al., 2011). They found that smaller particles lead to the greater antimicrobial effects. Finally, they concluded that the AgNPs synthesized at 60°C showed significant antibacterial effect when compared to those synthesized at 25°C because of their smaller size. Synthesis of AgNPs from stem bark extracts

of *Boswellia*, *Shorea* and leaf extract of *Svensonia* were studied (Savithramma et al., 2011). They noticed that AgNPs synthesized from bark extracts of *Boswellia ovalifoliolata* and *Shorea tumbergaia* showed toxicity towards *Klebsiella* and *Pseudomonas* species, respectively; whereas, the growth of *Pseudomonas* alone was inhibited maximumly by the AgNPs synthesized from leaf extract of *Svensonia hyderabadensis*. The antimicrobial activity of AgNPs from neem and triphala was evaluated against multiple drug resistant hospital isolates of *E. coli*, *K. pneumoniae* and *S. typhi*. They showed clear zone of inhibition against all the tested microorganisms. Zone of inhibition was found to be in the range of 11-14 mm for neem AgNPs whereas 10 - 14 mm for Triphala (Gavhan et al., 2012). Renugadevi et al. (2012) reported that the AgNPs showed significant antibacterial activity against all tested microorganisms. The maximum activity was found against *E. coli* followed by *S. aureus* and *Salmonella typhi*; whereas, least activity was found against *Vibrio cholerae*, *K. pneumoniae* and *Bacillus subtilis*. Green synthesis of AgNPs using *Bryophyllum pinnatum* and *Cassia angustifolia* and their promising antibacterial activity against *E. coli* and *S. aureus* were reported (Baishya et al., 2012; Amaladhas et al., 2012). Veeranna et al. (2013) reported that cabbage AgNPs showed significant antibacterial activity towards *S. aureus* with zone of inhibition of 13.4 mm.

The application of AgNPs as an antimicrobial agent was investigated by Sulaiman et al. (2013) against human pathogens. They found that, the antimicrobial effect was dose-dependent, and were more pronounced against Gram-positive bacteria than Gram-negative bacteria. Efficient antimicrobial activity with maximum zone of inhibition towards *E. coli* (14 mm), *S. aureus* (13 mm) and with minimum zone of inhibition towards *B. cereus* (7 mm); *K. pneumoniae* (7 mm) and *C. krusei* (6 mm) was reported by Prasad et al. (2012). The efficient antibacterial activity was noticed with stabilized AgNPs against all the tested pathogens (Malabadi et al., 2012) such as *B. subtilis*, *S. aureus*, *E. coli* and *K. pneumoniae*. Biosynthesis of AgNPs using *Ulva fasciata* extract and its significant antibacterial activity against *Xanthomonas campestris* sp. *Malvacearum* was reported by Rajesh et al. (2012). The maximum bactericidal effect of AgNPs of *Turnera ulmifolia* was against *E. coli*, *P. aeruginosa* followed by *S.aureus* and *Enterococcus faecalis* (Shekhawat et al., 2012). Antimicrobial effects of AgNPs against marine aquatic pathogens such as *Pseudomonas fluorescens*, *Proteus* spp. and *Flavobacterium* spp. was studied (Umashankari et al., 2012). They observed that the antimicrobial effect varies according to the species.

The maximum zone was recorded as 16, 14 and 14 mm for *Pseudomonas fluorescens*, *Proteus* spp., and *Flavobacterium* spp. respectively at 75 µg/µl concentration. *In vitro* anti-*Helicobacter pylori* activity of synthesized AgNPs was tested against 34 clinical isolates and two reference strains of *Helicobacter pylori* (Amin

et al., 2012). They noticed that typical AgNPs effectively inhibited the growth of *H. pylori*, indicating that the AgNPs have stronger anti-*H. pylori* activity. Finally they stated that AgNPs under study were found to be equally efficient against the antibiotic-resistant and antibiotic-susceptible strains of *H. pylori*. Rao and Savithamma (2012) found that AgNPs exhibited maximum antibacterial effect towards *Pseudomonas* spp. with zone of inhibition 18 mm among all the bacterial species tested. Sulaiman et al. (2013) has reported that 2 mM AgNPs induced 25 mm clear inhibitory zone against *S. aureus* and *S. pneumoniae* followed by 22 mm for Gram-negative bacteria such as *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Proteus vulgaris* respectively.

ANTIFUNGAL ACTIVITY

The synthesized AgNPs were generally found to be effective as antimicrobial agents against some of the important human pathogens (Maheswari et al., 2012). AgNPs of *Argemone mexicana* showed significant antifungal activity towards *Aspergillus flavus* at a concentration range of 30 ppm (Singh et al., 2010). Elumalai et al. (2010) reported that AgNPs synthesis by green route was found highly toxic against 7 clinically isolated fungal species at a concentration of 50 µg/µl; the maximum activity was observed against *Candida albicans*, *C. kefyr*, *A. niger* followed by intermediated activity towards *C. tropicalis*, *C. krusei*, *A. flavus* and *A. fumigatus*. AgNPs synthesized from bark extracts of *Boswellia ovalifoliolata* and *Shorea tumbuggaia* showed toxicity towards *Aspergillus* and *Fusarium* species, respectively; whereas, the growth of *Rhizopus* species were inhibited maximumly by AgNPs synthesized from leaf extract of *Svensonia hyderabadensis* (Savithamma et al., 2011). The antifungal activity of AgNPs from neem and Triphala was checked against hospital isolate (*C. albicans*) and found that the zone of inhibition was in the range of 15 and 16 mm, respectively (Gavhan et al., 2012). Rao and Savithamma (2012) reported that among all the tested fungi, the AgNPs showed higher activity towards *Rhizopus* with maximum zone of inhibition 14 mm. The significant antifungal activity of AgNPs with maximum inhibition towards *C. albicans* was reported by Sulaiman et al. (2013).

ANTIPLASMODIAL STUDIES

The antiplasmodial activity of AgNPs synthesized from *Catharanthus roseus* were tested against malarial parasites. Lowest parasitemia inhibition (20.0%) was found in parasites at 25 g/mL concentration of AgNPs. The parasitemia inhibitory concentration values vary with the concentration of AgNPs (20.0, 41.7, 60.0 and 75.0% for 25, 50, 75 and 100 g/ml), respectively (Ponarulselvam et al., 2012).

CYTOTOXICITY AND GENOME TOXICITY

Genotoxicity of AgNPs was assessed by using well-established *Allium cepa* assay system with biomarkers including the generation of reactive oxygen species (ROS: O₂ and H₂O₂), cell death, mitotic index, micronucleus, mitotic aberrations; and DNA damage (Panda et al., 2011). They also used other chemical forms of silver such as Ag⁺ ion, colloidal AgCl and AgNPs at doses 0 - 80 mg/L to compare with biogenic AgNPs. They found that commercial AgNPs and biogenic AgNPs exhibited similar biological effects. Both NPs (commercial and biogenic) causes lesser cytotoxicity and greater genotoxicity. It has been reported that the potential to induce cell death in root tissue of *A. cepa* of different forms of silver follows the order: silver ions > colloidal AgCl > commercial AgNPs > biogenic AgNPs. Cell death and DNA-damage induced by biogenic AgNPs were prevented by tiron and dimethyl thiourea that scavenge O₂ and H₂O₂, respectively. In another report, the toxicity of biogenic silver nanoparticles produced by *Alternaria alternata*, capped with protein, and in sizes of 25 - 45 nm, was evaluated for DNA damage in human lymphocytes using comet assay. The trypan blue dye exclusion method showed no significant changes in cellular viability on exposed cells compared with untreated control cells (up to 400 mg/ml). The *in vitro* treatment of lymphocytes using comet assay for DNA damage evaluation showed that, up to 50 mg/ml of nanoparticles, no DNA damage was observed. However, over 100 mg/ml with the increase in the concentration of silver nanoparticles, an increase in DNA damage was observed up to 300 mg/ml, as represented in terms of percentage of DNA in the tail and olive tail moment test. The values of comet parameters were ~ 5-fold higher in the positive control (100 mmol/L methyl methanesulphonate) compared with the lowest treatment dose (Sarkar et al., 2011).

Sulaiman et al. (2013) employed a time and dose dependent approach to evaluate the toxicity of the AgNPs on human acute promyelocytic leukemia (HL-60). They found that the viability of HL-60 cells considerably decreased with increasing doses and time of incubation. The mortality data obtained allow them to predict their potential not only because of the cytotoxic effect, but also in terms of the potential for tumor reduction. They stated that cytotoxic effects of AgNPs may be due to the result of active physicochemical interaction of NPs with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA. *In vitro* cytotoxic effects of AgNPs were screened against HL-60 cell line and viability of tumor cells was confirmed using MTT assay (Sulaiman et al., 2013). They found that after six hours of treatment, AgNPs at 2 mM level decreased the viability of HL-60 cells up to 44% and they also noticed that longer exposures resulted in additional toxicity to the cells that is, 80% after 24 h of incubation. Renugadevi et al. (2012) studied the *in vitro* cytotoxicity effect of AgNPs against Hep2 cell and Vero cell line at

different concentrations (20, 40, 60, 80, 100, 120 and 140 µg). They found that concentration required for 50% cell death (IC₅₀) for HEP2 and Vero cell line were 86 and 107 µg, respectively and stated that IC₅₀ value was found to be less for the Hep2 cell line than the Vero cell line. The effect of AgNPs on human epidermoid larynx carcinoma cell line exhibits a dose dependent toxicity for the cells tested (Satyavani et al., 2012) and the viability of Hep-2 cells decreased to 50% (IC₅₀) at the concentration of 500 µM. Toxicity of AgNPs on lung cancer cells (A549) and normal healthy peripheral lymphocytes (PLs) at 10 and 50 µg/ml was assessed using the MTT, ATP and lactate dehydrogenase assays (Gengan et al., 2013). They reported that A549 cells showed a 21% (10 µg/ml) and 73% (50 µg/ml) cell viability after 6 h exposure to AgNPs, whereas 117% (10 µg/ml) and 109% (50 µg/ml) cell viability in normal peripheral lymphocytes. Lactate dehydrogenase was only significantly altered at 50 µg/ml AgNPs treated cells.

HIV-1 INHIBITION

It was noticed that AgNPs undergo size dependent interaction with HIV-1. NPs within the size range of 1 to 10 nm readily interact with the HIV-1 virus via preferential binding to gp 120 glycoprotein knobs. The specific interaction of the AgNPs inhibits the virus from binding to host cells (Elechiguerra et al., 2005). This provides evidence that AgNPs can prevent and control HIV infections.

INSECTICIDAL AND LARVICIDAL ACTIVITIES

The larvicidal effect of *Mimosa pudica* aqueous leaf extract, silver nitrate solution and synthesized AgNPs against the larvae of malaria vector (*Anopheles subpictus*) and filariasis vectors (*Culex quinquefasciatus* and *Rhipicephalus microplus*) was studied (Marimuthu et al., 2011). Parasite larvae were exposed to varying concentrations extracts for up to 24 h and found that the highest mortality rate was with AgNPs. The lethal concentrations of *A. subpictus*, *C. quinquefasciatus* and *R. microplus* larvae were (LC₅₀=13.90, 11.73, and 8.98 mg/L, r²=0.411, 0.286 and 0.479), respectively. The acaricidal and larvicidal activity of synthesized AgNPs utilizing aqueous leaf extract of *Musa paradisiaca* was tested against the larvae of *Haemaphysalis bispinosa* and larvae of hematophagous fly (*Hippobosca maculata*) and the fourth-instar larvae of malaria vector (*Anopheles stephensi*), Japanese encephalitis vector (*Culex tritaeniorhynchus*) (Jayaseelan et al., 2012). The green synthesized AgNPs of *M. paradisiaca* showed significant effect towards all the parasite vectors with the LC₅₀ and r² values against *H. bispinosa*, (1.87 mg/L; 0.963), *H. maculata* (2.02 mg/L; 0.976), and larvae of *A. stephensi* (1.39 mg/L; 0.900), against *C. tritaeniorhynchus* (1.63 mg/L; 0.951), respectively.

The larvicidal activity of AgNPs synthesized using *Euphorbia hirta* leaf extract against malarial vector (*Anopheles stephensi*) was studied (Priyadarshini et al., 2012). AgNPs show the significant larval mortality against the first to fourth instar larvae and pupae and with the lethal concentrations (LC₅₀ = 10.14, 16.82, 21.51, 27.89 and 34.52 ppm, and LC₉₀ = 31.98, 50.38, 60.09, 69.94 and 79.76 ppm) respectively. Kamaraj et al. (2012) investigated feeding deterrent activity of AgNPs synthesized using *Manilkara zapota* leaf extract against *Musca domestica*. Adult flies were exposed to different concentrations of synthesized AgNPs for 1, 2 and 3 h and found that 100% of mortality within 3 h of duration at a concentration of 10 mg/L. The LD₅₀ and LD₉₀ values of AgNPs towards *M. domestica* were 3.64 and 7.74 mg/ml, respectively. The larvicidal potential of AgNPs synthesized using aqueous leaf extract of *Nelumbo nucifera* against fourth instar larvae of *A. subpictus* and *C. quinquefasciatus* were investigated (Santhoshkumar et al., 2011). The maximum efficacy was found against the larvae of *A. subpictus* (LC₅₀= 0.69 ppm; LC₉₀=2.15 ppm) followed by the larvae of *C. quinquefasciatus* (LC₅₀= 1.10 ppm; LC₉₀= 3.59 ppm), respectively. AgNPs synthesized by using aqueous leaf extract of *Ocimum canum* were assessed for antiparasitic activity against the larvae of *Hyalomma anatolicum* and *Hyalomma marginatum*. The maximum efficacy was noticed against *H. anatolicum* followed by *H. marginatum* with LC₅₀ and LC₉₀ values of 0.78 and 1.00 and 1.51 and 1.68 mg/L, respectively (Jayaseelan and Rahuman, 2012).

Synthesized AgNPs from *Pedilanthus tithymaloides* were investigated for their efficacy against the dengue vector (*Aedes aegypti*) by exposing the larvae to varying concentrations of AgNPs for 24 h (Sundaravadivelan and Nalini, 2012). AgNPs showed 100% mortality from first to fourth instars and pupae of *A. aegypti* at 0.25%. Lethal concentrations (LC₅₀) values of AgNPs against the larval and pupal stages were found to be 0.029, 0.027, 0.047, 0.086 and 0.018%, respectively. The bioactivity of latex of *Pergularia daemia* as well as synthesized AgNPs against the larval instars of *Aedes aegypti* and *Anopheles stephensi* was determined. The LC₅₀ and LC₉₀ values of AgNPs-treated against first, second, third and fourth instars of *A. aegypti* (LC₅₀ =4.39, 5.12, 5.66, 6.18; LC₉₀ = 9.90, 11.13, 12.40, 12.95 ppm) and *A. stephensi* (LC₅₀ = 4.41, 5.35, 5.91, 6.47; LC₉₀ = 10.10, 12.04, 13.05, 14.08 ppm) were found many fold lower than crude latex treated (Patil et al., 2012). The pediculicidal and larvicidal activity of synthesized AgNPs and aqueous leaf extract of *Tinospora cordifolia* against human head louse (*Pediculus humanus*) and fourth instar larvae of malaria vector (*A. subpictus*) and filariasis vector (*C. quinquefasciatus*) was studied. The maximum mortality was found with AgNPs against louse than *A. subpictus* and *C. quinquefasciatus* with lethal concentration (LC₅₀=12.46, 6.43 and 6.96 mg/L; r²=0.978, 0.773 and 0.828), respectively. 33% of mortality at 5 min, 67% at 15

min and 100% after 1 h were observed (Jayaseelan et al., 2011). Larvicidal activity of synthesized AgNPs utilizing aqueous extract from *Eclipta prostrata* was investigated against fourth instar larvae of filariasis vector (*C. quinquefasciatus*) and malaria vector (*A. subpictus*). The maximum efficacy of AgNPs was noticed towards *C. quinquefasciatus* (LC₅₀ = 4.56 mg/L; LC₉₀ = 13.14 mg/L) followed by *A. subpictus* (LC₅₀ = 5.14 mg/L; LC₉₀ = 25.68 mg/L) respectively (Rajakumar and Rahuman, 2011). The Larvicidal activity of *Murraya koenigii* ethanol leaf extract and AgNPs synthesized were studied by Suganya et al. (2013). The maximum mortality was found with synthesized AgNPs, in both *A. stephensi* (LC₅₀ values of 10.82, 14.67, 19.13, 24.35, and 32.09 ppm and LC₉₀ values of 32.38, 42.52, 53.65, 63.51, and 75.26 ppm) and *A. aegypti* (LC₅₀ values of 13.34, 17.19, 22.03, 27.57, and 34.84 ppm and LC₉₀ values of 36.98, 47.67, 55.95, 67.36, and 77.72 ppm), respectively.

PESTICIDAL ACTIVITY

The efficacies of all the agents (aqueous leaves extracts of *Euphorbia prostrata*, silver nitrate (AgNO₃) solution (1 mM) and synthesized AgNPs were tested against the adult *Sitophilus oryzae* for 14 days to study their pesticidal activity. The LD₅₀ values of aqueous extract, AgNO₃ solution and synthesized AgNPs were found to be 213.32, 247.90, 44.69 mg/kg⁻¹; LD₉₀=1648.08, 2675.13, 168.28 mg/kg⁻¹, respectively (Zahir et al., 2012). *E. prostrata* synthesized AgNPs against the adult *H. bispinosa* showed LC₅₀ at 2.30 ppm and LC₉₀ value at 8.28 ppm where as against *H. maculata* they showed LC₅₀ at 2.55 ppm and LC₉₀ at 9.03 ppm respectively. Mortality of 100% was found in synthesized AgNPs at a concentration of 10 mg l⁻¹ (Zahir and Rahuman, 2012).

CONCLUSION

Recent research works regarding the photo-biological approach for the synthesis of silver nanoparticles were summarized in the present review. Nature itself is a nano-factory; in nature, a number of plant species have potentiality to produce nanoparticles. In this review also, research work regarding the mechanisms involved in the phytosynthesis of silver nanoparticles is elucidated. Studies regarding the phytochemicals and factors that are responsible for the synthesis and stabilization of nanoparticles were briefly summarized. It is important to understand the complete mechanism involved in the biosynthesis (using plants) of silver nanoparticles for scaling up the process. Several biologists proposed numerous hypotheses regarding the mechanisms involved in plant mediated synthesis; it may be due to complex nature of plant materials. Therefore, further studies would be required to understand the particular mechanism involved in a specific plant species.

In the present decade interest in AgNPs applications has increased mainly because of the important antimicrobial activities of these nanoparticles, allowing their use in several industrial sectors. However, together with these applications, there is increasing concern related to the biological impacts of the use of silver nanoparticles on a large scale, and the possible risks to the environment and health. So, the investigation of potential inflammatory effects and diverse cellular impacts of silver nanoparticles is important. Another important issue related to nanoparticle toxicity is damage to the genetic material, because AgNPs are able to cross cell membranes and reach the cellular nucleus. Little is known about the genotoxicity of AgNPs and their effects on the DNA of organisms; up to the present literature, available biogenic silver nanoparticles are generally less cyto/genotoxic *in vivo* compared with chemically synthesized nanoparticles. However, further studies would be required to carefully analyze nanoparticles toxicity in order to decrease the possible discrepancies related to final conclusions.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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