

# Potential of plant as a biological factory to synthesize gold and silver nanoparticles and their applications

Pei Pei Gan · Sam Fong Yau Li

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**Abstract** Green synthesis of metallic nanoparticles has become a promising field of research in recent years. Syntheses of gold and silver nanoparticles by various chemical and physical methods as well as the biosynthetic approach mediated by numerous microorganisms have been actively researched. A more scalable and economic route to produce these metallic nanoparticles would be through the plant-mediated synthetic approach. Owing to the biodiversity of plant biomasses, the mechanism by which bioconstituents of plants have contributed to the synthetic process is yet to be fully understood. Nevertheless, the feasibility of controlling the shape and size of nanoparticles by varying the reaction conditions has been demonstrated in many studies. This paper provides an overview of the plant-mediated syntheses of gold and silver nanoparticles, possible compounds and mechanisms that might be responsible for the bioreduction process as well as the potential applications of biosynthesized nanoparticles in different fields. The challenges and limitations of this plant-mediated biosynthetic approach are also discussed.

**Keywords** Biosynthesis · Plant-mediated · Nanoparticles · Gold · Silver

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P. P. Gan · S. F. Y. Li (✉)  
Department of Chemistry, National University  
of Singapore, 3 Science Drive 3, Singapore 117543,  
Republic of Singapore  
e-mail: chmlifys@nus.edu.sg

## 1 Introduction

Nanoparticles are able to exhibit unique properties that are significantly different from the bulk materials due to their large fraction of surface atoms, large surface energy, spatial confinement and reduced imperfections. These unique characteristics of nanoparticles make them useful in the field of catalysis, electronics, biomedical analysis (Wang et al. 2006; Abu Bakar et al. 2007) and even groundwater purification (Elliott and Zhang 2001). Nanoparticles are commonly synthesized by either top-down or bottom-up approaches. Top-down approach is based on the mechanical method of size reduction by breaking down the bulk materials gradually to nanoscale structures. Bottom-up approach is based on the assembly of atoms or molecules to molecular structures in nanoscale range. Both chemical and biological syntheses of nanoparticles rely on the bottom-up approach (Vijayaraghavan and Nalini 2010; Narayanan and Sakthivel 2010a).

Although existing chemical and physical methods have successfully produced well-defined nanoparticles, these processes are usually expensive and involve the use of toxic chemicals. Chemical synthesis methods might lead to the presence of some toxic chemical species being adsorbed on the surface of nanoparticles that might lead to adverse effects in medical applications. In addition, for certain biomedical applications, these nanoparticles may even have direct contact with human body in which their associated toxicity become critical. Thus, one of the

primary goals of nanotechnology is to develop an eco-friendly production method that can provide nanoparticles with low toxicity. To achieve this goal, many researchers have diverted their interest to biological synthesis of nanoparticles (Kumar and Yadav 2009; Narayanan and Sakthivel 2010a). As compared to microorganism-mediated synthesis of nanoparticles, the use of plants is more advantageous due to the ease of scaling up, less biohazards on production, and elimination of the elaborate process of maintaining cell cultures (Njagi et al. 2011). Several plants have been proven to be efficient for the rapid intra- or extracellular syntheses of nanoparticles. Extracellular synthesis by using leaf extract of *mangifera indica* (species of mango) (Philip 2010c), *syzygium cumini* (Jambul) (Kumar et al. 2010b), *aloesia citrodora* (Lemon Verbena) (Cruz et al. 2010), *geranium* (cranesbills) (Shankar et al. 2003), and coriander (Narayanan and Sakthivel 2008), as well as agricultural crops such as banana peel (Bankar et al. 2010), maize, groundnut, green and red gram (Rajani et al. 2010), coffee and tea extracts (Nadagouda and Varma 2008) were shown to produce gold (Au) and silver (Ag) nanoparticles with different dimensions and shapes. Intracellular syntheses of Au nanoparticles were also shown in living plants such as *alfalfa* (Gardea-Torresdey et al. 2002a). Besides, biosynthesis of bimetallic nanoparticles such as Au-Fe<sub>3</sub>O<sub>4</sub> by extracts of *eucalyptus camaldulensis* (River Red Gum) was also explored (Haratifar et al. 2009).

The formation of nanoparticles was proposed to occur through the ionic or electrostatic interactions between the metal complexes and the functional groups on the biomass surface. Several bioorganic compounds in plant systems such as flavonoids, terpenoids, proteins, reducing sugars and alkaloids were suggested to be involved as either reducing or capping agents during the formation of nanoparticles and their concentrations are critical in the shape directing process (Zhou et al. 2010). However, the exact roles of these constituents in the formation of nanoparticles require further experimental proof. The properties of nanoparticles strongly depend on their size, shape, crystallinity and structure (Kelly et al. 2003). For example, tadpole-shaped Au nanoparticles have unusual optical and electrical properties that can be used for the assembly of nanodevices or joints for biochips (Castro et al. 2010). It was found that several factors are important during the biosynthetic process,

such as pH, temperature, reaction time and dosage of biomass (Philip 2010a; Lukman et al. 2011; Ghodake et al. 2010). By establishing the relationship of these factors to the size and shape of nanoparticles, it is possible to produce nanoparticles with desired properties in a controlled manner.

Due to the limited capacity of plants for reducing metal ions, biosynthetic process usually works well for metal ions with large positive electrochemical potential such as Au and Ag ions (Haverkamp and Marshall 2009). In this review, we will focus on the plant-mediated syntheses of Au and Ag nanoparticles. Possible mechanisms that could be involved during the biosynthetic process as well as the potential applications of these biosynthesized nanoparticles were discussed.

## 2 Chemical and physical syntheses of metallic nanoparticles

Currently, the most common method for the synthesis of metallic nanoparticles is through chemical reduction of metal salts in solution phase (Lin et al. 2010). Depending on the condition of reaction mixture, metal ions may favor either the process of nucleation or aggregation to form small metal clusters. This synthetic method usually employs chemicals such as hydrazine, sodium borohydride and hydrogen as reducing agents (Leff et al. 1995; Pileni 1997). Synthetic or natural polymers such as natural rubber (Abu Bakar et al. 2007), chitosan (Adlim et al. 2004), cellulose (Kotelnikova et al. 2003) and copolymer micelles (Egorova and Revina 2000) have been used as stabilizers against oxidation and coalescence in nanocomposites. Due to the hydrophobicity of these chemicals, organic solvents such as ethanol, dimethyl formamide, ethylene glycol, toluene and chloroform are usually used (Vijayaraghavan and Nalini 2010). These toxic and hazardous chemicals are non-biodegradable and detrimental to the environment, which limit the scale of production. Besides, some of the toxic chemicals might contaminate the surfaces of nanoparticles and make them unsuitable for certain biomedical applications (Shankar et al. 2004a).

Physical approaches to synthesize metallic nanoparticles include ultraviolet irradiation (Kundu et al. 2007), sonochemistry (Okitsu et al. 2007), radiolysis (Meyre et al. 2008), laser ablation (Tsuji et al. 2003) and so forth. 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 nanoparticles (Egorova and Revina 2000). The main advantage of the physical approach is that nanoparticles with high purity and desired size can be selectively synthesized (Mafuné et al. 2002). However, these processes usually require complicated instruments, electrical and radiative heating as well as high power consumption, which results in high operating cost.

### 3 Biological synthesis of metallic nanoparticles by microbes

Biosynthesis of nanoparticles is an alternative way that is compatible with green chemistry principles, in which biomolecules secreted by the biomass can act as both reducing and capping agents during the reaction. Therefore, this reaction can be considered as a green chemical process that can minimize the usage of hazardous chemicals (Ahmad et al. 2010). Biosynthesis of nanoparticles by microorganisms such as bacteria (Klaus-Joerger et al. 2001; Samadi et al. 2009; Shahverdi et al. 2007), fungi (Sastry et al. 2003; Varshney et al. 2009; Shaligram et al. 2009) and yeast (Kowshik et al. 2003; Pimprikar et al. 2009) are well documented. Some famous examples of microbial synthesis of metallic nanoparticles include *Escherichia coli* DH5 $\alpha$ -mediated bioreduction of Au nanoparticles on cell surface (Du et al. 2007), *Fusarium oxysporum*-mediated synthesis of silica nanoparticles (Bansal et al. 2005), and *Candida glabrata*-mediated synthesis of monodispersed peptide-bound CdS quantum crystallites (Narayanan and Sakthivel 2010a). Another interesting example is the biosynthesis of iron oxides mediated by *tobacco mosaic virus* (TMV) (Narayanan and Sakthivel 2010a). However, there are drawbacks of microbial synthesis of nanoparticles. A major problem is the difficulty to provide good control over size distribution, shape and crystallinity of nanoparticles. The manipulation of reaction parameters such as pH and temperature might inactivate the microbes and hinder the bioreduction process. Specialized facilities and long incubation time are required for maintaining the growth of microorganisms and subsequent formation of nanoparticles. In addition, special precautions must be given when it is

necessary to handle some bacteria or viruses that might be harmful to human or pose a risk of causing an outbreak of influenza. Hence, the associated biological safety issues should also be addressed for the implementation of microorganism-mediated synthesis of nanoparticles in large scale and for commercial applications.

### 4 Biological synthesis of metallic nanoparticles by plants

In contrast to microorganism-mediated synthesis of nanoparticles, the use of plant biomasses or plant extracts is comparatively simpler and more cost-effective (Bankar et al. 2010). This is especially useful if low-value agricultural waste can be used for this purpose which is in compliance with the concept of “waste to wealth”. Plant-mediated synthesis of metallic nanoparticles has been shown to produce nanoparticles with shapes and sizes that are comparable with those produced through chemical and physical methods (Parsons et al. 2007). Biosynthesis of nanoparticles can occur either on living or inactivated plant biomasses. Gardea-Torresdey et al. (2002a, 2003) have demonstrated the possibility of using live *alfalfa* plants for the bioreduction of Au(III) to Au(0), which produced Au nanoparticles with size ranging from 6 to 10 nm. *brassica juncea* and *medicago sativa* were also shown to produce Au nanoparticles at room temperature (Bali and Harris 2010).

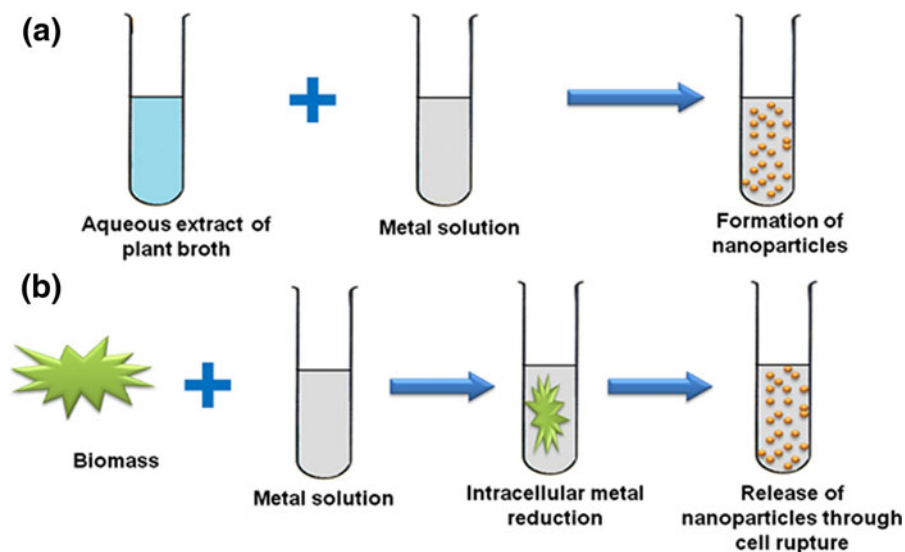
In fact, gold accumulation by plants has been documented in literature since 1970s (Girling and Peterson 1980; Girling et al. 1979; Girling and Peterson 1978). The basis of this technology is known as phytoextraction, in which plants are used to accumulate mineral species from groundwater, soil and sediments. The uptake of gold has been shown by using persimmon waste (Parajuli et al. 2007; Xiong et al. 2009) and hard shell of apricot stones (Soleimani and Kaghazchi 2008), which demonstrated the potential of agricultural waste as an alternative to remediate mining wastes. In addition, it is well-known that the ability of plants to uptake gold has made them natural bioindicator of gold deposit by mining companies (Girling et al. 1979). In view of this, there appear to be competitive processes between bioaccumulation and bioreduction of metal ions that might occur on the biomass surface.

To further facilitate the biosynthesis of nanoparticles by plant systems, the use of plant broths was introduced (Shankar et al. 2003; Shankar et al. 2004a), in which the compounds that were responsible for the reduction of metal ions were extracted and added to the reaction mixture to produce nanoparticles extracellularly. The processes of intra- and extracellular syntheses of Au and Ag nanoparticles are shown in Fig. 1. In fact, intracellular synthesis of nanoparticles is generally used for biosynthesis mediated by micro-organism such as bacteria, in which the cells could reserve their viability after crystal growth. For extracellular synthesis of nanoparticles, aqueous extracts of *mangifera indica* leaf (Philip 2010c), marine sponge *acanthella elongate* (Inbakandan et al. 2010), *geranium* leaf (Shankar et al. 2003) and *chenopodium album* (Dwivedi and Gopal 2010) have been shown to produce Au nanoparticles with size range of 2–30 nm. By adjusting the reaction parameters such as pH, metal concentration, and temperature, nanoparticles with certain size range and shape may dominate. The ability of various plant systems to produce Au and Ag nanoparticles with different morphologies has been demonstrated by different research groups in which the influences of reaction parameters to the formation of nanoparticles were demonstrated in some of the studies (Table 1). As compared to intracellular synthesis which requires additional processing steps to release the nanoparticles from the biomass, it seems that extracellular synthesis of nanoparticles using plant extract is more commonly studied due to their

greater potential in commercial applications (Song et al. 2009; Narayanan and Sakthivel 2010b).

However, it is difficult to synthesize nanoparticles with certain morphology reproducibly due to the diversely varying composition and structure of plants from different origins. Agricultural wastes or plant biomasses are always collected in a mixture which may contain other impurities as well. This will increase the difficulty to establish the process design if the production of nanoparticles with desired morphology can only be achieved when it was mediated by certain functional groups on a particular part of the plant biomass. For example, Au nanoparticles could be accumulated throughout the epidermis, cortex and vascular tissue of *Brassica juncea* (*B. juncea*). However, the size distribution of nanoparticles within the plant tissues was found to be location-dependent, with a broader range of particle sizes exhibited by the nanoparticles formed within above ground tissues as compared to those root-located nanoparticles. The particle size distribution of Au nanoparticles formed in the roots and shoots of *B. juncea* as well as the formation of differently shaped nanoparticles in the root cells after exposure to 1,000 ppm Au solution for 24 h are shown in Fig. 2. This phenomenon reveals that the growth of nanoparticles might continue during the process of metal translocation (Bali and Harris 2010). This is a critical aspect to consider if the nanoparticles to be used for certain applications have stringent requirements on their monodispersity and surface morphology. The reducing and stabilizing

**Fig. 1** The process of biological synthesis of Au and Ag nanoparticles: **a** plant extract; **b** plant biomass



**Table 1** Plants used in biological synthesis of gold and silver nanoparticles

Plants	Nanoparticles produced	Size	Morphology	Extracellular/intracellular	Possible biomolecules involved	Synthetic conditions and effects of various factors	References
<i>Mangifera indica</i> leaf	Au	20–17 nm	Spherical	Extracellular	Flavonoids, terpenoids, thiamine	Synthetic conditions: HAuCl <sub>4</sub> : 5 × 10 <sup>-4</sup> M Reaction time: 2 min Room temperature	Philip (2010c)
Banana peels ( <i>Musa pradsisiaca</i> ) extract	Au	10–20 μm	Nanoplates, microcubes, triangles and hexagon in patterned nanowire	Extracellular	Proteinaceous matter in peels	Factors affecting the color of nanoparticles studied: (1) pH pH 2.0: brown pH 3.0: purplish-pink pH 4.0: ruby red pH 5.0: dark reddish (2) Varying gold chloride concentration 1.0 mM : purplish-pink 0.5, 0.25, 0.125 mM: varying shade of purples (3) Varying biomass concentration 10 mg ml <sup>-1</sup> ; purplish pink 1.0 mg ml <sup>-1</sup> ; dark ruby 2.0 mg ml <sup>-1</sup> ; light ruby 4.0 mg ml <sup>-1</sup> ; dark reddish brown 5.0 mg ml <sup>-1</sup> ; pink (4) Temperature 40 °C: orangish-brown 60 °C: yellowish-brown 80 °C: purplish-pink 100 °C: pinkish-brown	Bankar et al. (2010)
<i>Acanthella elongate</i> extract	Au	7–20 nm	Spherical	Extracellular	Secondary metabolites from the extract of sponge <i>A. elongata</i>	Synthetic conditions: HAuCl <sub>4</sub> : 10 <sup>-3</sup> M Temperature: 45 °C	Inbakandan et al. (2010)
Sugar beet pulp	Au	20–160 nm	Irregular shapes	Extracellular	Proteins in sugar beet pulp	Reaction time: 4 h Synthetic conditions: HAuCl <sub>4</sub> : 0.5 × 10 <sup>-3</sup> M Room temperature Reaction time: 48 h	Castro et al. (2010)
					Factors affecting the color of nanoparticles studied: (1) pH pH 2.0, 4.0: blue to colorless pH 7.0, 10.0: blue to reddish		

Table 1 continued

Plants	Nanoparticles produced	Size	Morphology	Extracellular/intracellular	Possible biomolecules involved	Synthetic conditions and effects of various factors	References
Barbated skullcup herb extract	Au	5–30 nm	Spherical and triangular	Extracellular	Sugars or other biomolecules	Synthetic conditions: HAuCl <sub>4</sub> : 10 mM Room temperature Reaction time: 3 h pH 5.4	Wang et al. (2009)
Magnolia kobus and Dlopyros kaki leaf extracts	Au	5–300 nm	Spherical (95 °C) and mixture of plates with triangles, pentagons and hexagons (25, 60 °C)	Extracellular	Proteins, metabolites such as terpenoids	Factors affecting the size of nanoparticles studied: (1) Temperature 25 °C: 110 nm 60 °C: 50 nm 95 °C: 40 nm (2) Leaf broth concentration 10–50 %: Particle size decrease with increasing broth concentration	Song et al. (2009)
Foliar broth from 24 kinds of randomly selected plant leaves	Au	Not mentioned	Spherical and triangular	Extracellular	Flavanoids and reducing sugars (reductants); Proteins (protection agent)	Factors affecting the size of nanoparticles studied: (1) pH (4.1–7.6): Effect on Au(III) was not evident. (2) Flavanoids: conversion of Au(III) increase with increasing flavanoids content (3) Reducing sugars: Conversion of Au(III) increases with increasing content of reducing sugars (4) Proteins: Poor correlation with the conversion of Au(III)	Zhou et al. (2010)
Anti-maglinant guava leaf ( <i>Psidium guava</i> )	Au	27–35 nm	Spherical, hexagonal, triangular	Extracellular	Flavanoids	Synthetic conditions: HAuCl <sub>4</sub> : 10 <sup>-3</sup> M Reaction time: 5 min (>90 % yield) Temperature: 27 °C	Raghunandan et al. (2009)
Coriander leaf extract	Au	6.75–57.91 nm	Spherical, triangle, truncated triangles and decahedral	Extracellular	Proteins	Synthetic conditions: HAuCl <sub>4</sub> : 10 <sup>-3</sup> M Reaction time: 12 h Temperature: 30 °C	Narayanan and Sakthivel (2008)

**Table 1** continued

Plants	Nanoparticles produced	Size	Morphology	Extracellular/intracellular	Possible biomolecules involved	Synthetic conditions and effects of various factors	References
Pear fruit extract	Au	200–500 nm	Plate-like gold nanostructures with shapes including triangular and hexagonal	Extracellular	Alkaline-responsive phytochemicals (such as amino acids, organic acids, peptides, and/or proteins)	Synthetic conditions: HAuCl <sub>4</sub> : $2 \times 10^{-3}$ M Reaction time: 30 min Temperature: 30 °C Factors affecting the shapes of nanoparticles studied: (1) pH pH 4: triangular (7 %), partially hexagonal/spherical (32 %), others (61 %) pH 9: triangular (22 %), hexagonal (37 %), others (41 %)	Ghodake et al. (2010)
Rosa hybrid petal extract	Au	10 nm	Polydisperse nanoparticles with spherical, triangular and hexagonal shapes	Extracellular	Sugars and proteins	Synthetic conditions: HAuCl <sub>4</sub> : $2 \times 10^{-3}$ M Reaction time: <5 min Room temperature	Nouzi et al. (2011)
Live alfalfa plants	Au	6–10 nm	Icosahedrons with FCC twined structure	Intracellular	Nucleation of Au nanoparticles occurred inside living plants. Individual compounds responsible for the biosynthesis was not identified	Synthetic conditions: KAuCl <sub>4</sub> : 0, 5, 10, 20, 40, 80, 160, 320 ppm Reaction time: 2 weeks Temperature: 25/18 °C day/night	Gardea-Torresdey et al. (2002a)
<i>Nyctanthes arborrisis</i> ethanolic flower extract	Au	19.8 ± 5.0 nm	Spherical	Extracellular	Phytochemicals such as alkaloids and flavanoids	Synthetic conditions: HAuCl <sub>4</sub> : $10^{-3}$ M Reaction time: 30 min Temperature: 80 °C Factors affecting the shapes of nanoparticles studied: (1) Temperature 25 °C: Mixture of triangular, pentagonal, rod shaped and spherical nanoparticles 80 °C: predominantly spherical particles	Das et al. (2011a)
<i>Coleus amboinicus</i> Lour leaf extract	Au	4.6–55.1 nm	Spherical, triangle, truncated triangle, hexagonal and decahedral	Extracellular	Aromatic amine, amide (II) groups and secondary alcohols	Synthetic conditions: HAuCl <sub>4</sub> : $10^{-3}$ M Reaction time: 1 h Temperature: 30 °C	Narayanan and Sakthivel (2010b)

Table 1 continued

Plants	Nanoparticles produced	Size	Morphology	Extracellular/intracellular	Possible biomolecules involved	Synthetic conditions and effects of various factors	References
Oat ( <i>Avena sativa</i> )	Au	5–20 nm	Fcc tetrahedral, hexagonal platelets, irregular shaped, rod shaped, decahedral multiple twinned, icosahedral multiple twinned nanostructures	Extracellular	Functional groups present in the cell walls of the inactivated tissues of the plant such as carboxyl, amino and sulphydryl	Synthetic conditions: KAuCl <sub>4</sub> : $0.1 \times 10^{-3}$ M Reaction time: 1 h Factors affecting the sizes of nanoparticles studied: pH pH 3, 4: 5–20 nm pH 2: 25–85 nm (small quantities) pH 5: slightly larger than those obtained at pH 3 and 4	Amrendariz et al. (2004)
Sesbania seedlings	Au	6–20 nm	Spherical	Intracellular	Alkaloids or other secondary metabolites	Synthetic conditions: KAuCl <sub>4</sub> : 0–200 mg L <sup>-1</sup> Reaction time: 0–6 days pH 4.8	Sharma et al. (2007)
Korean red ginseng root ( <i>Panax ginseng C.A. Meyer</i> )	Au	16.2 ± 3.0 nm	Spherical	Extracellular	Ginsenosides, flavonones, and starch	Synthetic conditions: HAuCl <sub>4</sub> : 0.1 M Reaction time: 1–2 h (sonication) 1 g of Korean red ginseng to 1 mL of 0.1 M HAuCl <sub>4</sub>	Leonard et al. (2011)
<i>Madhuca longifolia</i> extract	Au	7 nm–3 μm	Triangular nanoplates (pH 2.0) Spherical (pH 10.0)	Extracellular	Proteins (Tyrosine)	Factors affecting the sizes of nanoparticles studied: (1) Addition of NaBH <sub>4</sub> during synthesis process With NaBH <sub>4</sub> : Aggregation with increasing pH Without NaBH <sub>4</sub> (ginseng only): No noticeable change in the pH window of 2–10 Synthetic conditions: HAuCl <sub>4</sub> : 10 mM Reaction time: formation of particles <2 h Temperature: 303 K pH 2.0 and 10.0	Mohammed Fayaz et al. (2011)
<i>Medicago sativa</i> seed exudates	Ag	5–51 nm	Spherical	Extracellular	Quercetin and other phenolic compounds (apigenin, catechin, kaempferol etc.)	Synthetic conditions: AgNO <sub>3</sub> : 10 mM Room temperature Reaction time: <50 min for 90 % yield pH 5.5 Factors affecting the size of nanoparticles studied: (1) pH pH 11.0: 11.5 nm ± 4.2 nm pH 2.0: no reaction	Lukman et al. (2011)



**Table 1** continued

Plants	Nanoparticles produced	Size	Morphology	Extracellular/intracellular	Possible biomolecules involved	Synthetic conditions and effects of various factors	References
<i>Syzygium aromaticum</i> leaf and seed extract	Ag	30–92 nm	Spherical	Extracellular	Polyphenols in plant extracts	Synthetic conditions: HAuCl <sub>4</sub> :10 <sup>-3</sup> M Room temperature Reaction time: 24 h	Kumar et al. (2010b)
Lippia citriodora leaves extract ( <i>Lemon verbena</i> )	Ag	15–30 nm	Spherical	Extracellular	Verbascoside, isoverbascoside, luteolin, and chrysoeriol-7-O-diglucuronide	Factors affecting the sizes and structures of nanoparticles studied: (1) Reaction time 3 h: 15 nm 24 h: 20 nm (saturation) (2) Leaf broth concentration Increases Ag nanoparticles (≈14 nm) conversion with increasing leaf broth concentration (3) Temperature 25 °C: less crystalline 95 °C: highly polycrystalline (higher rate of reduction)	Cruz et al. (2010)
<i>Curcuma longa</i> tuber powder and extract	Ag	71–80 nm (160 h reaction period); 21–30 nm (24 h reaction period)	Decahedral, triangular, spherical and ellipsoidal	Extracellular	Complex polyols and proteins in the biomass	Factors affecting the sizes and conversion of nanoparticles studied: (1) Reaction time Increase Ag conversion with increasing reaction time 24 h: 21–30 nm 168 h: 71–80 nm (2) Dosage of biomass/extract Increase Ag conversion with increasing dosage (3) pH (1–11) acidic pH: large-sized nanoparticles Alkaline pH: small-sized nanoparticles (4) Temperature (20–60 °C)	Sathishkumar et al. (2010)
Alfalfa Sprouts	Ag	2–20 nm	Spherical	Intracellular	Nucleation of Au nanoparticles occurred inside living plants. Individual compounds responsible for the biosynthesis was not identified	Particle size increases with increasing temperature Synthetic conditions: AgNO <sub>3</sub> : 40, 80, 160, 320 ppm pH 5.8 Reaction time: 9 days (12 h light/12 h dark) Temperature: 25/18 °C day/night	Gardea-Torresdey et al. (2003)

Table 1 continued

Plants	Nanoparticles produced	Size	Morphology	Extracellular/intracellular	Possible biomolecules involved present in the lemon juice	Synthetic conditions and effects of various factors	References
<i>Citrus lemon</i> extract	Ag	<50 nm	Spherical and spheroidal	Extracellular	Citric acid and some bio-organics present in the lemon juice	Synthetic conditions: AgNO <sub>3</sub> : 10 <sup>-2</sup> M Reaction time: 4 h Temperature: 30 °C Factors affecting the sizes and conversion of nanoparticles studied: (1) Mixing ratios between lemon juice and AgNO <sub>3</sub> solutions. Increasing ratio lead to decrease in particle size	Prathna et al. (2011)
<i>Nelumbo nucifera</i> leaf extract	Ag	25–80 nm	Spherical, triangle, truncated triangles, and decahedral	Extracellular	Protein	Synthetic conditions: AgNO <sub>3</sub> : 10 <sup>-3</sup> M Reaction time: 10 min Room temperature	Santhoshkumar et al. (2010)
Black pepper seed extract	Ag	20–50 nm	Spherical	Extracellular	Alkaloid piperine (or few basic amino acids or both)	Synthetic conditions: AgNO <sub>3</sub> : 10 × 10 <sup>-3</sup> M Reaction time: 2 h Room temperature pH 12	Shukla et al. (2010)
<i>Cinnamon zeylanicum</i> bark extract and powder	Ag	31–40 nm	Quasi-spherical and small, rod-shaped	Extracellular	Water-soluble organics	Synthetic conditions: AgNO <sub>3</sub> : 1 × 10 <sup>-3</sup> M Temperature: 25 °C Factors affecting the sizes and conversion of nanoparticles studied: (1) Reaction time: 2–168 h: conversion of Ag increased with increasing reaction time. (2) Dosage of biomass/extract Conversion of Ag increased with increasing dosage. (No marked difference in shapes observed) (3) pH (1–11) Low pH: large nanoparticles High pH: highly dispersed, small nanoparticles. (More of spherical)	Sathishkumar et al. (2009)
<i>Geranium</i> Leaf extract	Ag	16–40 nm	Spherical (small percentage being ellipsoidal)	Extracellular	Proteins/enzymes and secondary metabolites such as terpenoids	Synthetic conditions: AgNO <sub>3</sub> : 1 × 10 <sup>-3</sup> M Temperature: 25 °C Reaction time: 9 h (>90 %)	Shankar et al. (2003)

**Table 1** continued

Plants	Nanoparticles produced	Size	Morphology	Extracellular/intracellular	Possible biomolecules involved	Synthetic conditions and effects of various factors	References
<i>Cassia fistula</i> leaf	Ag	50–60 nm	Nanowires	Extracellular	Some water-soluble polyhydroxy components such as alkaloids, flavonoids and polysaccharose	<p>Synthetic conditions:  <math>\text{AgNO}_3</math>: <math>2 \times 10^{-3}</math> M                      Temperature: 30 °C                      Reaction time: 48 h                      pH 5.4</p> <p>Factors affecting the sizes and conversion of nanoparticles studied:                      (1) Reaction time:                      4–8 h: increasing intensity for peak at 2430 nm                      12–24 h: Formation of short nanowires (with diameter from 50 to 60 nm)                      48 h: long nanowires with average diameter of 60 nm</p> <p>(1) Temperature                      60 °C: only spherical nanoparticles and short nanorods were obtained</p>	Lin et al. (2010)
(a) <i>Xerophyte</i> extract ( <i>Bryophyllum</i> sp.)	Ag	2–5 nm	FCC unit cell structure	Extracellular	Metabolites (like organic acids and quinones) or metabolic fluxes and other oxido-reductively labile metabolites like ascorbates or catechol/photocatacheitic acid.	<p>Synthetic conditions:  <math>\text{AgNO}_3</math>: <math>2.5 \times 10^{-2}</math> M                      Warmed at 40 °C for 10 min and left at room temperature for 4 h</p>	Jha et al. (2009)
(b) <i>Mesophyte</i> extract ( <i>Cyprus</i> sp.)							
(c) <i>Hydrophyte</i> extract ( <i>Hydrilla</i> sp.)							
Neem ( <i>Azadirachta indica</i> ) leaf broth	Ag, Au, and bimetallic Ag–Au	Ag : 5–35 nm; Ag–Au: 50–100 nm;	Au: thin, planar structures (predominantly triangular with small percentage of hexagonal shapes) Ag: Spherical Ag–Au: Spherical (large particles capped with smaller particles)	Extracellular	Flavonones and terpenoids	<p>Synthetic conditions:  <math>\text{AgNO}_3</math>: <math>10^{-3}</math> M (Ag nanoparticles)  <math>\text{HAuCl}_4</math>: <math>10^{-3}</math> M (Au nanoparticles)                      1 : 1 <math>10^{-3}</math> M of <math>\text{AgNO}_3</math> and <math>\text{HAuCl}_4</math> (Ag–Au bimetallic nanoparticles)                      Reaction time                      Ag: 4 h (&gt;90 %)                      Au: 2 h (&gt;90 %)                      Ag–Au bimetallic: 180 min</p>	Shankar et al. (2004a)

Table 1 continued

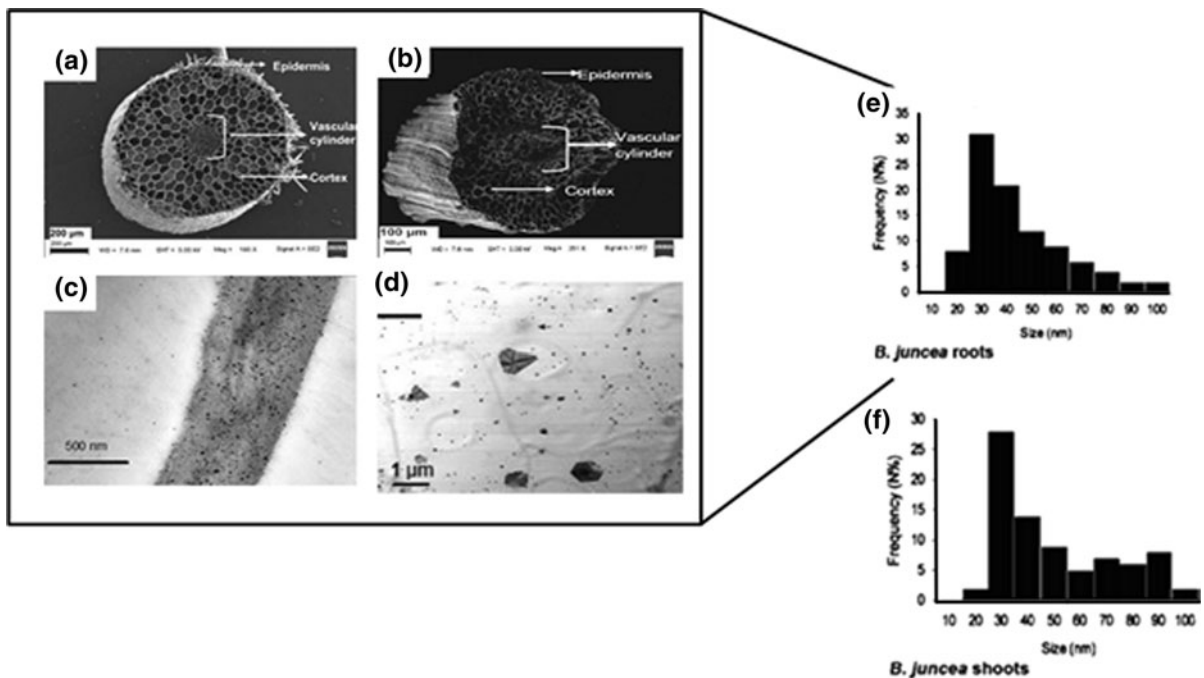
Plants	Nanoparticles produced	Size	Morphology	Extracellular/intracellular	Possible biomolecules involved	Synthetic conditions and effects of various factors	References
<i>Ocimum sanctum</i> leaf extract	Au and Ag	Au: ~30 nm; Ag: 10–20 nm	Au: hexagonal; Ag: nearly spherical	Extracellular	Flavonoids and terpenoids (biomolecules responsible for capping and stabilization of Au and Ag nanoparticles are different)	Synthetic conditions: Au nanoparticles HAuCl <sub>4</sub> : 10 <sup>-3</sup> M Reaction time: 15 min Room temperature Ag nanoparticles AgNO <sub>3</sub> : 10 <sup>-3</sup> M Reaction time: 30 min 300 and 373 K Synthetic conditions: HAuCl <sub>4</sub> : 10 <sup>-3</sup> M AgNO <sub>3</sub> : 10 <sup>-3</sup> M Reaction time: 15 min Room temperature Factors affecting the sizes and conversion of nanoparticles studied: (1) Leaf extract quantity Decrease in particle size and increase in formation of nanoparticles for both Ag and Au with increasing leaf extract quantity. (2) Metal ions concentration (10 <sup>-4</sup> –10 <sup>-2</sup> M) Increase in particle size and decrease in the formation of nanoparticles with increasing metal concentration. (1) Temperature (25–150 °C) Increase in rate of formation of nanoparticles at higher temperature. (2) Contact time (15 min–24 h) Reaction started within 15 min and increased up to 4 h. (3) pH (2–10) Au and Ag nanoparticles are stable under a wide pH range	Philip and Umni (2011)
<i>Sorbus aucuparia</i> leaf extract	Au and Ag	Au: ~18 nm; Ag: ~16 nm	Spherical, triangular and hexagonal	Extracellular	Sorbic acid and its salts	Synthetic conditions: HAuCl <sub>4</sub> : 10 <sup>-3</sup> M AgNO <sub>3</sub> : 10 <sup>-3</sup> M Reaction time: 15 min Room temperature Factors affecting the sizes and conversion of nanoparticles studied: (1) Leaf extract quantity Decrease in particle size and increase in formation of nanoparticles for both Ag and Au with increasing leaf extract quantity. (2) Metal ions concentration (10 <sup>-4</sup> –10 <sup>-2</sup> M) Increase in particle size and decrease in the formation of nanoparticles with increasing metal concentration. (1) Temperature (25–150 °C) Increase in rate of formation of nanoparticles at higher temperature. (2) Contact time (15 min–24 h) Reaction started within 15 min and increased up to 4 h. (3) pH (2–10) Au and Ag nanoparticles are stable under a wide pH range	Dubey et al. (2010)

**Table 1** continued

Plants	Nanoparticles produced	Size	Morphology	Extracellular/intracellular	Possible biomolecules involved	Synthetic conditions and effects of various factors	References
<i>Chenopodium album</i> leaf extract	Au and Ag	10–30 nm	Quasi-spherical,	Extracellular	Oxalic acids	Factors affecting the sizes and conversion of nanoparticles studied: (1) Leaf extract quantity Decrease in particle size for both Ag and Au with increasing leaf extract quantity. (2) Metal ions concentration (0.1–5 mM) Increase in particle size and decrease in the formation of nanoparticles with increasing metal concentration. (3) Temperature (25–150 °C) Increase in rate of formation of nanoparticles at higher temperature. (4) Contact time (15 min–2 h) Reaction started within 15 min and increased up to 2 h. (5) pH (2–10) Au and Ag nanoparticles are stable under a wide pH range	Dwivedi and Gopal (2010)
Edible mushroom ( <i>Volvariella volvacea</i> ) extract	Au and Ag and bimetallic Au–Ag	Au: 20–150 nm; Ag: ~15 nm	Au: triangular nanoprisms to nearly spherical and hexagonal; Ag: spherical	Extracellular	Polysaccharide/oligosaccharide and proteins/enzymes	Factors affecting the sizes and conversion of nanoparticles studied: <i>Au nanoparticles</i> (1) Leaf extract Decrease in triangular particles and increase in hexagonal and spherical shapes with increasing leaf extract quantity. (2) Temperature 313 K: Almost hexagonal 353 K: dendrite/fractal structures <i>Ag nanoparticles</i> (3) Leaf extract Increases in the formation of Ag nanoparticles with increasing leaf extract quantity. Closed chain structures were observed when high leaf extract quantity was added. Au–Ag bimetallic (4) Leaf extract Increase in the formation of Au–Ag structures with increasing leaf extract quantity	Philip (2009)

Table 1 continued

Plants	Nanoparticles produced	Size	Morphology	Extracellular/intracellular	Possible biomolecules involved	Synthetic conditions and effects of various factors	References
Natural precursor clove ( <i>Sygium aromaticum</i> )	Au and Ag	Au: triangular edge lengths of 50–70 nm	Au: triangular (mainly), various other shapes Polygonal, irregular	Extracellular	Eugenol	Factors affecting the sizes and conversion of nanoparticles studied: <i>Au nanoparticles</i> 1) Clove extract Decrease in particle size with increasing clove extract quantity. Irregular shaped, large Au nanoparticles (100–300 nm) formed when low clove extract quantity was used. <i>Ag nanoparticles</i> Decrease in particle size with decreasing clove extract	Singh et al. (2010)
<i>Hibiscus rosasinensis</i> leaf extract	Au and Ag	Au: ~ 14 nm (spherical); Ag: ~ 13 nm	Au: triangular, hexagonal, dodecahedral and spherical; Ag: spherical	Extracellular	Proteins (Au: through free amine groups; Ag: through free carboxylate groups)	Factors affecting the sizes and conversion of nanoparticles studied: <i>Au nanoparticles</i> (1) Leaf extract Decrease in triangular particles and increase in dodecahedral and spherical shapes with increasing leaf extract quantity. <i>Ag nanoparticles</i> (2) pH (6.8–8.5) pH 7.5: nearly spherical particles (~ 13 nm) Higher or lower pH: large anisotropic nanoparticles	Philip (2010a)
<i>M. piperita</i> leaf extract	Ag and Au	Ag: 90 nm; Au: 150 nm	Spherical	Extracellular	Phytochemicals in the extract	Synthetic conditions: HAuCl <sub>4</sub> : 10 <sup>-3</sup> M AgNO <sub>3</sub> : 10 <sup>-3</sup> M Reaction time: 24 h Temperature: 28 °C	MubarakAli et al. (2011)
Coffee and tea extract	Ag and Pd	5–100 nm	Mostly spherical	Extracellular	Polyphenols and caffeine	Synthetic conditions: AgNO <sub>3</sub> : 0.1 N PdCl <sub>2</sub> : 0.1 N Reaction time: 120 min	Nadagouda and Varma (2008)
Sorghum Bran	Fe and Ag	Ag: ~ 10 nm; Fe: 40–50 nm	Ag: spherical crystalline; Fe: amorphous	Extracellular	Polyphenols, policosanols, and other biomolecules such as flavonoids and phenolic acids	Room temperature Synthetic conditions: AgNO <sub>3</sub> : 0.1 M FeCl <sub>3</sub> : 0.1 M Reaction time: 1 h Room temperature	Njagi et al. (2011)



**Fig. 2** Synthesis of Au nanoparticles in the roots and shoots of *B. juncea*: **a** SEM image of root cross section of *B. juncea* showing the basic structures of 2-weeks old seedlings; **b** SEM image of *B. juncea* root after 24 h of exposure to 1,000 ppm Au salt solution; **c** TEM image of cell structures for *B. juncea* root cell walls showing presence of Au nanoparticles in epidermal *B. juncea* root cells with extensions; **d** STEM analysis showing the

presence of spherical, hexagonal, triangular and irregularly shaped nanoparticles in the *B. juncea* root cell; Particle size distribution of Au nanoparticles formed in the **e** roots and **f** shoots of *B. juncea* after 24 h of exposure to 1,000 ppm Au salt solution (Reproduced with permission from Bali and Harris 2010)

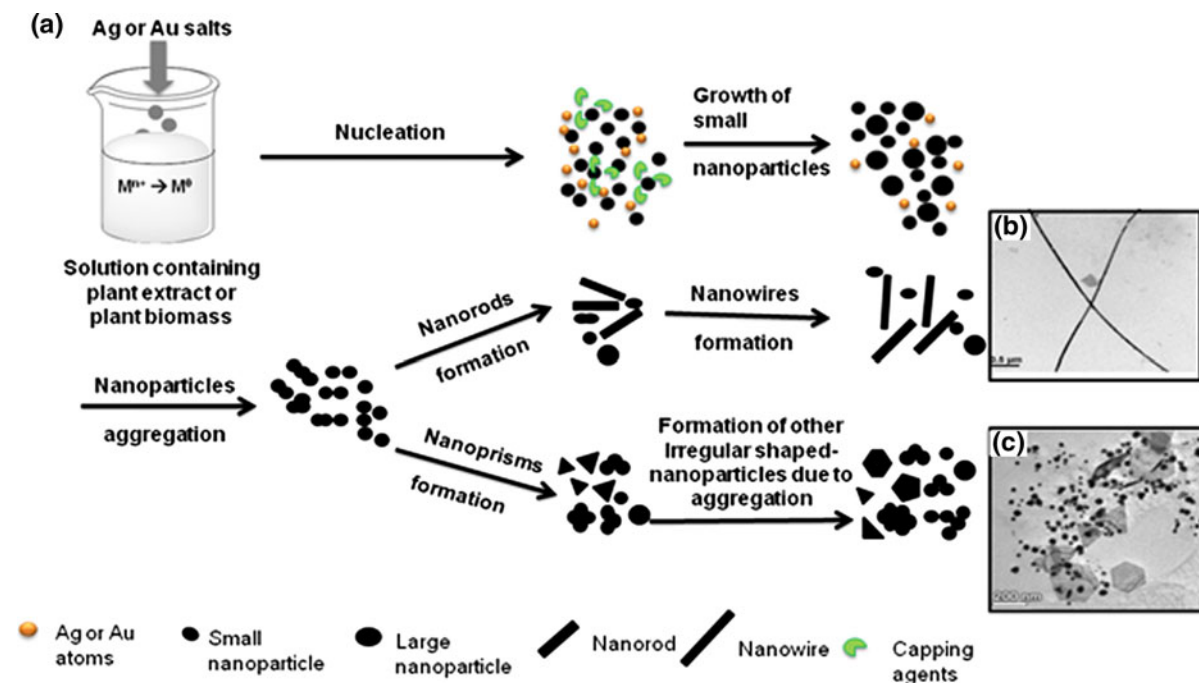
power of bioorganic compounds that are available in different parts of plant tissues has to be investigated to achieve better control over the morphology of bio-synthesized nanoparticles.

#### 4.1 Possible mechanism for plant-mediated synthesis of nanoparticles

During the formation of metallic nanoparticles, there are three mandatory components, which are the reducing agent, stabilizing agent and a solvent medium that can solubilize the metal of interest (Vijayaraghavan and Nalini 2010). Biosynthesis of nanoparticles is regarded as a green process because the biomass itself can act as both reducing and stabilizing agents. In addition, most of the plant-mediated syntheses of metallic nanoparticles could be performed in aqueous medium instead of organic solvents, which is apparently more environmentally benign and cost-effective. It was proposed by Lukman et al. (2011) that there are three distinguishable reaction regimes which occurred

during the biosynthetic process: a short induction period, a growth phase and a termination period. The growth rate of particles is usually slower than the rapid reduction and nucleation of metallic seeds which leads to higher concentration of small particles. In the absence of other strong ligands, metallic ions could interact with the biomass through ionic binding with the bioorganic reducing agents such as flavonoids or terpenoids. It is believed that the adsorption of bioreducing agents on the surface of metallic nanoparticles is attributable to the presence of  $\pi$ -electrons and carbonyl groups in their molecular structures.

HSAB (hard and soft acid base) theory is also applicable here. Soft metal like Au(III) existing in the form of  $[\text{AuCl}_4]^-$  prefers to bind to the biomass mainly through soft ligands such as amino and sulfhydryl groups, especially when the soft ligands are positively charged at low pH (Gardea-Torresdey et al. 2002b; Shankar et al. 2004a). In fact, the formation of nanoparticles could be a result of heterogeneous nucleation and growth followed by



**Fig. 3** A proposed growth mechanism for the formation of nanoparticles with different shapes: **a** Schematic illustration of the growth mechanism of Ag and Au nanoparticles mediated by bio-reducing agents; **b** TEM image of Ag nanowires synthesized with aqueous extract of *Cassia fistula*; **c** TEM image of

spherical, triangular and hexagonal shaped Au nanoparticles synthesized with aqueous extract of *Ocimum sanctum* leaf (Reproduced with permission from Lin et al. 2010; Philip and Unni 2011)

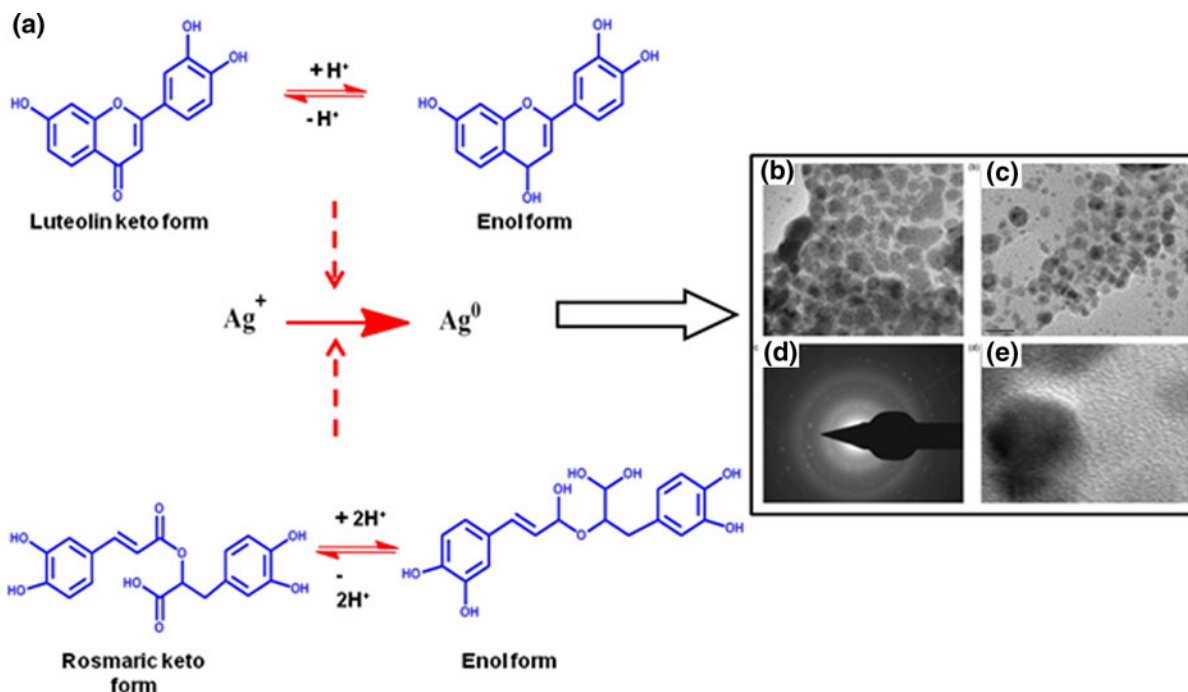
Ostwald ripening. During the biosynthetic process, extraneous catalytic materials that are either released from the plant biomass or being extracted to the plant extract could act as a scaffold for the growth of crystal. Along with the crystal growth, Ostwald ripening could take place and result in the broadening of size distribution. Several studies suggested that the mechanism of Ostwald ripening might be involved during the biosynthetic process, wherein smaller or quasi-solid nascent particles could migrate and feed into their closest neighboring particles of larger size, causing the growth of larger particles and depletion of smaller particles (Castro et al. 2010; Lin et al. 2010; Lukman et al. 2011). The excess Gibb's free energy associated with the nanoparticles could then be minimized by transformation into more energetically favorable shapes, which is directed by the bioorganic capping molecules (Fiehn et al. 2000).

To stabilize the nanoparticles in a dispersing medium, there must be sufficient repulsive forces to overcome the van der Waals force that causes coagulation (Rajani et al. 2010). The repulsive forces

can be exerted through either electrostatic or steric stabilization. Physisorbed surfactants or polymers from the bioorganic capping agents may exert steric or electrostatic barriers around the particle surface (Vijayaraghavan and Nalini 2010). For example, it was shown that Ag nanoparticles synthesized by *medicago sativa* seeds formed aggregation of hexagonal and well-defined shaped nanoparticles without signs of fusion. This could be attributed to the strong interaction between the chemically bound capping agents which counteracted the tendency of the nanoparticles to aggregate (Kumar et al. 2008). The capping of Au nanoparticles could also be achieved by secondary metabolites from marine sponge *A. elongata*, which form a coat that covers the metallic nanoparticles to prevent the agglomeration and stabilize the nanoparticles (Inbakandan et al. 2010).

Narayanan et al. (2010b) demonstrated that the nascent nanoparticles devoid of stabilizing molecules were thermodynamically unstable. At the beginning of the reaction, spherical nanoparticles were produced due to the protection by sufficient stabilizing





**Fig. 4** Synthesis of Ag nanoparticles mediated by flavonoids in *ocimum sanctum*: **a** The mechanism of the biosynthesis of Ag nanoparticles via the reactive hydrogen liberated from the keto forms of luteolin and rosmarinic acid; **b** TEM images of Ag nanoparticles derived from root and **c** stem of *Ocimum sanctum*;

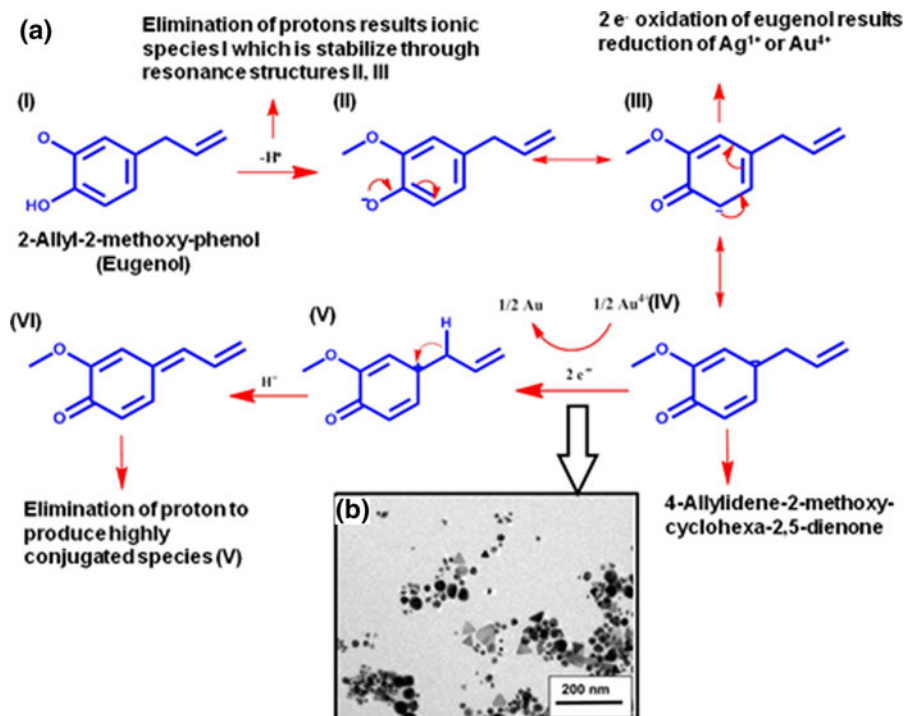
**d** selected area electron diffraction showing the characteristic crystal planes of elemental silver; **e** HRTEM image showing characteristic d spacing for the [2 0 0] plane (Reproduced with permission from Ahmad et al. 2010)

molecules. However, the particles that formed at later stage were less stable due to availability of less stabilizing molecules. The instability of the nanoparticles rendered the formation of anisotropic nanostructures like triangular nanoprisms. These nanoprisms possess high surface energy and they undergo a shrinking process in order to reduce the surface energy, resulting in the formation of truncated nanotriangles. In addition, Ag nanoparticles with short chain-shaped structures can be produced through biosynthesis mediated by *Cassia fistula* leaf. It was found that by prolonging the reaction time, the newly formed Ag atoms deposited onto the concave regions of the connected nanoparticles through capillary phenomenon, leading to the formation of long nanorods (Lin et al. 2010). A possible growth mechanism for the formation of different shapes of nanoparticles such as nanorods, nanowires, nanoprisms, hexagonal nanoparticles is illustrated in Fig. 3a. Figure 3b shows the formation of Ag nanowires synthesized with aqueous extract of *Cassia fistula* whereas Fig. 3c shows the formation of spherical, triangular and hexagonal

shaped Au nanoparticles synthesized with aqueous extract of *Ocimum sanctum* leaf (Lin et al. 2010; Philip and Unni 2011).

Depending on the reducing power and availability of bioreducing and stabilizing agents, the biosynthesized nanoparticles obtained might have higher proportion of a particular shape. It was also observed that the formation of Au nanoparticles by *alfalfa* biomass is more stable in icosahedral shape (Gardea-Torresdey et al. 1999). All these observations suggest that the formation of nanoparticles with different morphology is more likely a result of the coalescence of smaller particles. These smaller nanoparticles tend to rearrange themselves to achieve a morphology that is more stable. However, the different composition of biomolecules among individual plants and the reaction conditions used during the biosynthesis might result in the favorable formation of nanoparticles with particular shapes, which could give a higher state of stability. This also implies the necessity to study the plant-mediated synthesis of nanoparticles in a case-by-case basis.

**Fig. 5** Synthesis of Ag and Au nanoparticles mediated by terpenoids in clove extract: **a** Mechanism for the bioreduction of Au and Ag nanoparticles mediated by eugenol; **b** TEM micrographs of Au nanoparticles synthesized with AuCl<sub>4</sub> to clove extract ratio 1:1 (Reproduced with permission from Singh et al. 2010)



#### 4.2 Possible compounds responsible for the plant-mediated synthesis process

Due to the complicated nature and vast diversity of plant systems, it is a challenging task to identify a particular compound that might be responsible for the formation of nanoparticles. Some biomolecules such as terpenoids, flavonoids, polysaccharides, proteins and alkaloids are generally regarded as potential bioreducing and stabilizing agents that could render the formation of nanoparticles. It is very likely that the bioreduction process of metal ions is actually interplay of several active components present in the biomass. The details of how each of the compounds which responsible for the plant-mediated synthesis process were further discussed in the following sections.

##### 4.2.1 Flavonoids

Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms, which belong to the family of polyphenol. Flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanin and isoflavonoid. Together with carotenes, flavonoids are responsible for the coloring of fruits, vegetables and herbs (Spencer 2008). Flavonoids are usually water

soluble and were proposed to be the main components present in the aqueous extract of many plant broths, which have participated in the bioreduction process (Zhou et al. 2010). It was suggested that flavanones can be adsorbed on the surface of metallic nanoparticles through the interaction with their carbonyl groups or  $\pi$ -electrons. The internal mechanism of converting ketone group to carboxylic acid in flavonoids was suggested to play an important role in the bioreduction of metal ions. Hence, the content of flavonoids in plants could be an index for preliminary evaluations of the untapped plants in terms of biosynthesis of metallic nanoparticles (Ragunandan et al. 2009).

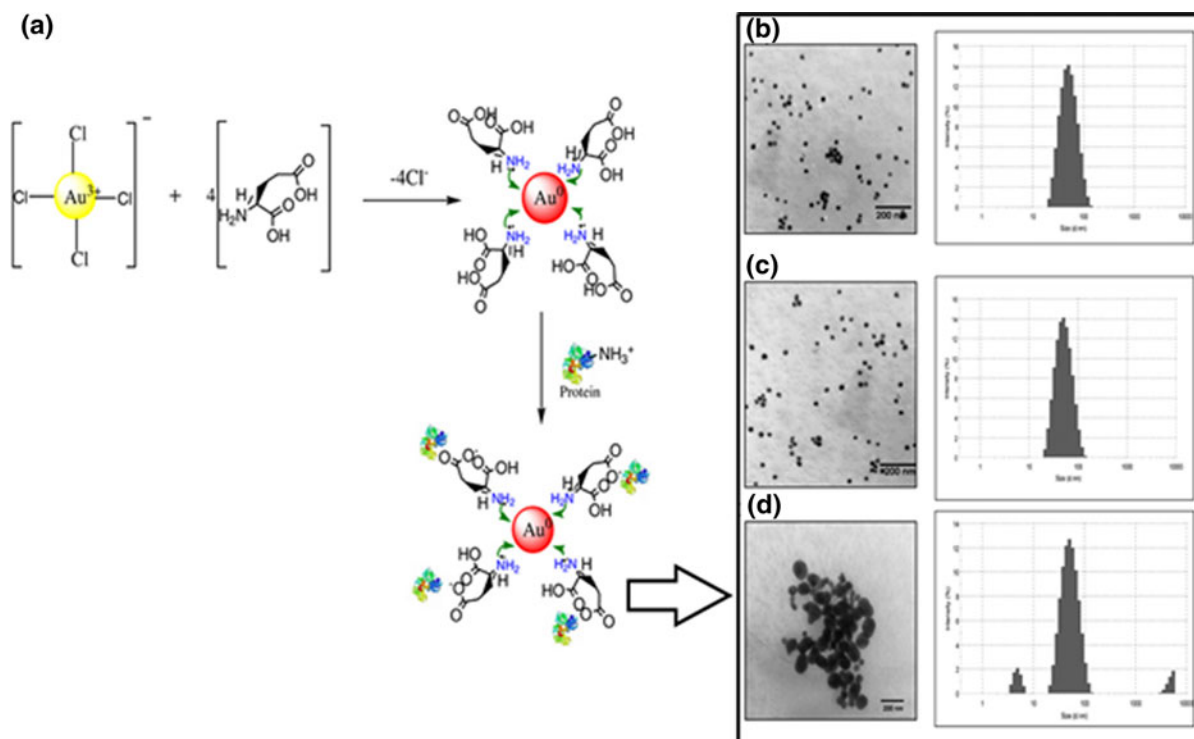
Flavonoids are able to scavenge molecular species of active oxygen directly due to their ability to donate electrons or hydrogen atoms. The capability of several flavonoids, such as quercetin to chelate various metal ions is well-documented. Biosynthesis of Ag nanoparticles mediated by basil plant was proposed to be initiated by luteolin. Luteolin is a common flavone found in the aerial parts of the plant which liberate reactive hydrogen that responsible for the conversion of Ag<sup>+</sup> to Ag<sup>0</sup>. The mechanism of the biosynthetic process is shown in Fig. 4a, in which reactive hydrogen is liberated during the conversion of enol form of luteolin to its keto form. Like luteolin,

rosmarinic acid is another natural plant flavonoid that could initiate the bioreduction of Ag ions. As shown in Fig. 4a, the presence of two hydroxyl groups on one carbon in the enol form of rosmarinic acid is less stable and hence it tends to revert back to the more stable keto form and results in the liberation of reactive hydrogens. In both cases, the liberated reactive hydrogens could participate in the synthesis of Ag nanoparticles. The resulting Ag nanoparticles derived from the root and stem of basil plant are shown in Fig. 4b, c. The selected area electron diffraction pattern and high resolution transmission electron microscopy (HRTEM) image of the as synthesized Ag nanoparticles are shown in Fig. 4d, e for the characterization of crystalline structure of the elemental Ag (Ahmad et al. 2010).

#### 4.2.2 Terpenoids

Terpenoids (also known as isoprenoids) belong to the subclass of prenyllipids (terpenes), which represent

the largest group of small molecular products synthesized by plants. Terpenoids contribute to the scent, flavor and color of some plants, such as cinnamon, cloves, and cannabis (Song et al. 2009). Shankar et al. (2003) reported that terpenoids in *geranium* leaf may be responsible for the synthesis of Ag nanoparticles. *C. 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 is able to release a proton via its  $^-\text{OH}$  group and transforms to its anionic form. Due to the inductive effect induced by the electron withdrawing groups i.e. methoxy and allyl groups present at the ortho and para positions of the  $^-\text{OH}$  group, the reducing power of eugenol is greatly enhanced. It is able to release two electrons simultaneously which are responsible for the reduction of Au and Ag ions. A possible mechanism for the reduction of Au and Ag ions is illustrated in Fig. 5a. The representative TEM micrographs of Au nanoparticles synthesized with  $\text{AuCl}_4$  to clove extract ratio 1:1 are



**Fig. 6** Synthesis of Au nanoparticles using glutamic acid as reducing agent: **a** The schematic representation of glutamic acid reduced Au nanoparticles capped with the amino acid and their subsequent binding with protein through the surface lysine

residues of protein molecules; TEM images and corresponding particle size histograms of Au nanoparticles synthesized using **b** 10 mM, **c** 15 mM and **d** 20 mM of glutamic acid as reducing agent (Reproduced with permission from Wangoo et al. 2008)

shown in Fig. 5b. In addition, different polyols contained in the plant biomass may act together as either bioreducing or capping agents during the formation of Au and Ag nanoparticles. Polyols such as terpenoids, flavones, and polysaccharides in *cinnamomum camphora* leaf were reported to be the main components responsible for the bioreduction of Ag and chloroaurate ions (Sathishkumar et al. 2009; Huang et al. 2007a).

#### 4.2.3 Reducing sugars

Reducing sugars such as monoses, dioses and oligoses are polyols with dissociated aldehyde or ketonic groups (Zhou et al. 2010). The ability of different sugars as reducing agents for the synthesis of metallic nanoparticles was demonstrated by Panigrahi et al. (2004). Their study shows that nanoparticles with uniform size can be produced if fructose was used whereas particles of variable sizes were generated if glucose and sucrose were used. The role of reducing sugars was demonstrated when sucrose as a non-reducing sugar was used. It was found that in the preparation of Ag nanoparticles by using sucrose as a reducing agent, no Ag nanoparticles were produced when  $\text{AgNO}_3$  was used as Ag precursor whereas the production of Au nanoparticles was observed when  $\text{HAuCl}_4$  was used as Au precursor. This phenomenon suggests that instead of the non-reducing sucrose, the actual compounds responsible for the bioreduction of nanoparticles were glucose and fructose produced from sucrose after being hydrolyzed by the chloroauric acid. Succinoglycan, an important acidic polysaccharide of *Sinorhizobium meliloti* consisting of one galactose and seven glucose residues, was demonstrated to be useful for the production of Ag nanoparticles in controlled pH environments. As proposed by the authors, one reducing sugar in the succinoglycan provided one electron to reduce  $\text{Ag}^+$  to  $\text{Ag}^0$  only under basic condition. The aldehyde group of reducing sugar was oxidized to carboxyl group by nucleophilic addition of  $\text{OH}^-$ , which reduced  $\text{Ag}^+$  to  $\text{Ag}^0$  (Kwon et al. 2009). Similar mechanism could also apply to the bioreduction of tetrachloroaurate ions by the reducing sugars present in the lemongrass extract as proposed by Shankar et al. (2004b). The growth of Au nanotriangles was believed to be a result of interactions between Au nanoparticles and the aldehydes or ketones groups present in the extract. The waste of

*Sccharomyces cerevisiae* was used to synthesize Au nanoparticles, and the free aldehyde groups of the reducing sugars are believed to be the main reducing agents (Lin et al. 2005).

#### 4.2.4 Proteins

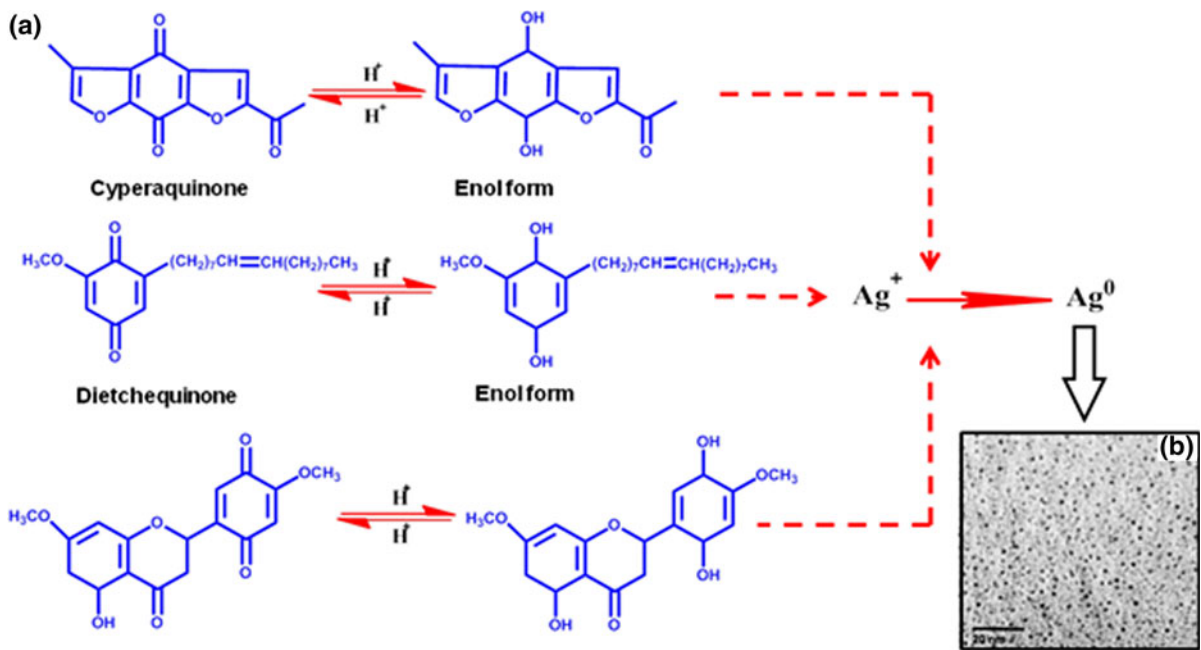
Due to the diverse structure of proteins as compared to polyols, the case of bioreduction by proteins is usually more complicated. According to the FTIR analysis of biosynthesized nanoparticles, the presence of amide I and amide II as well as the C–O stretching bands are frequently observed, which indicate the presence of protein functional groups (Philip 2010a; Raghunandan et al. 2009; Lukman et al. 2011; Narayanan and Sakthivel 2010b; Philip 2010b). This suggests that Au nanoparticles can bind to proteins through its free amine groups or carboxylate ions of the amino acid residues. In fact, amino acid residues such as arginine, cysteine, lysine and methionine are known to interact with Ag ions (Gruen 1975). Instead of bioreduction, it was proposed by some researchers that proteins could act as stabilizing agents as well (Bali and Harris 2010). Highly stable and monodispersed Au nanoparticles were obtained by electrostatic stabilization via surface-bound amino acids (Mandal et al. 2002). The roles of peptides as bioreducing and biocapping agents were demonstrated by Ag nanoparticles synthesized by cyclic peptides in the latex of *Jatropha curcas* (Bar et al. 2009) and targeted peptides enriched with proline and hydroxyl-containing amino acid residues (Naik et al. 2002). A systematic study was carried out by Tan et al. (2010), in which they have screened the reduction and binding capabilities of 20 natural  $\alpha$ -amino acids to Au ions. It was found that the reduction process was determined by the extent of complexation between the peptide and metal ions. Wangoo et al. (2008) demonstrated the preparation of Au nanoparticles in water directly by complexation with glutamic acid where the amino acid acted as both the reducing and stabilizing agents in a simple one-pot process. The reaction scheme of glutamic acid reduced and capped Au nanoparticles is shown in Fig. 6a. TEM images of Au nanoparticles synthesized using different concentrations of glutamic acid are shown in Fig. 6b–d. It can be seen that the reduction of tetrachloroaurate could occur through the transfer of electrons from the amine group of glutamic acid to the  $\text{Au}^{3+}$  ion. Subsequent nucleation and growth of the reduced  $\text{Au}^0$  resulted in

the formation of Au nanoparticles, which were then further stabilized by the amino acid.

#### 4.2.5 Organic acids and secondary metabolites from plant systems

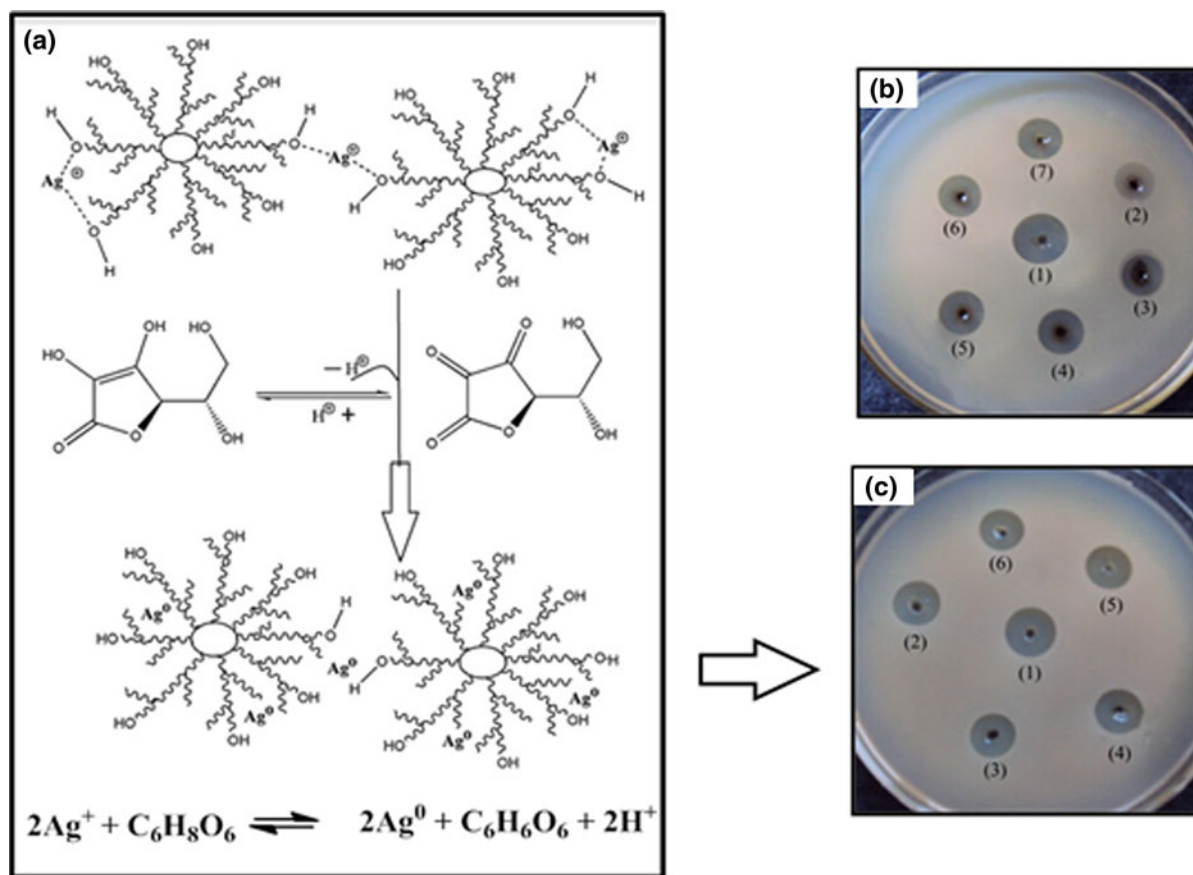
Plants are able to synthesize a variety of metabolites upon exposure to metals. These metabolites, particularly amino acids such as proline and histidine, peptides such as glutathione and phytochelatin, and amines such as spermine, spermidine, putrescine, nicotianamine, and mugineic acids were accumulated to concentrations in the millimolar range (Sharma and Dietz 2006). The content of alkaloids and secondary metabolites might also function as reducing agents for the biosynthesis of metallic nanoparticles. In xerophytes, organic acids such as malic acid and pyruvic acid generated from the redox reactions occurred in glycolytic pathway have led to the reduction of Ag ions. Along with this, succulents such as emodin (belonging to the class of *anthroquinones*) can also undergo tautomerization and lead to the reduction of Ag ions through its keto and enol forms (Goodwin and Mercer 1985; Thomas et al. 1973). In *mesophytes*, the

tautomerization of benzoquinones, such as cyperoquinone, dietchequinone, and remirin has contributed to the reduction capability of the extract of *Cyperus sp.* A mechanism of biosynthesis of Ag nanoparticles using *mesophytes* and the TEM image of the resulting Ag nanoparticles are shown in Fig. 7a, b. By applying gentle warming with subsequent incubation, it might have activated the quinone congeners, which reduced the particle size and minimized the coalescent of nanoparticles (Thomson 1976). Aqueous extract of orange peel was used to prepare starch supported nanoparticles with sizes ranging from 3 to 12 nm. The role of ascorbic acid as an effective reducing agent for the bioreduction of Ag ion in starch matrix is illustrated in Fig. 8a. These green synthesized Ag nanoparticles show free radical scavenging, biocompatibility and antimicrobial potency. Representative culture plates showing antibacterial activity are depicted in Fig. 8b, c (Konwarh et al. 2011). In the case of *hydrophytes*, compounds such as catechol, rotocatechuic acid along with other phytochemicals in *hydrilla* have been reported to liberate reactive hydrogen, which contribute to the reduction of Ag nanoparticles (Jha et al. 2009).



**Fig. 7** Synthesis of Ag nanoparticles mediated by benzoquinones in *mesophyte* (*Cyperus sp.*): **a** Mechanism of biosynthesis of Ag nanoparticles using cyperoquinone and dietchequinone;

**b** TEM photographs of Ag nanoparticles as synthesized (Reproduced with permission from Jha et al. 2009)



**Fig. 8** Synthesis of Ag nanoparticles mediated by ascorbic acid in the aqueous extract of orange peel: **a** Probable mechanism of reduction of silver ion in starch matrix using ascorbic acid as representative effective reductant present in orange peel; **b** Representative culture plate showing antibacterial test: combination of antibiotic, rifampicin (Rif) and silver nanoparticles both at different concentrations; **c** with constant concentration of the nanoparticles and increasing concentration of the antibiotic at the peripheral wells. The central well contains Rif as positive control (Reproduced with permission from Konwarh et al. 2011)

### 4.3 Influence of various factors to the formation of nanoparticles mediated by plants

#### 4.3.1 Effect of pH

In previous studies, it was shown that the size and shape of biosynthesized nanoparticles could be manipulated by varying the pH of the reaction mixtures. A major influence of the reaction pH is its ability to change the electrical charges of biomolecules which might affect their capping and stabilizing abilities and subsequently the growth of nanoparticles. This might result in the favorable formation of nanoparticles of certain shapes at a particular pH range so that a greater stability could be achieved. In the study of pear fruit extract-assisted room-temperature biosynthesis of Au nanoplates, the

authors have shown that alkaline condition appeared to be more efficient for the generation of triangular and hexagonal shaped Au nanoplates whereas at acidic pH, these nanoplate structures were hardly observed (Ghodake et al. 2010). The effect of pH on the size of Au nanoparticles biosynthesized by oat (*Avena sativa*) biomass was also studied. The results show that smaller Au nanoparticles and the higher occurrence of these were observed at pH values of 3 and 4, whereas the larger nanoparticles were observed at pH 2. The authors proposed that at pH 2, the process of aggregation of Au nanoparticles to form larger ones is favored over nucleation to form new nanoparticles. At pH 3 and 4, there could be more functional groups available for Au(III) complexes to bind to the biomass at the same time,

which allows the subsequent formation of larger amounts of nanoparticles with smaller diameters. However, at pH 5 the biomass carries an overall negative charge due to the functional groups present in the biomass such as carboxyl. Therefore the negatively charged  $[\text{AuCl}_4]^-$  do not easily approach the binding sites which prevent the binding of Au(III) and its reduction to Au(0) leading to the formation of fewer nanoparticles. The electrostatic repulsion could also prevent the growth and aggregation of small nanoparticles and hence resulted in the formation of small irregular shape nanoparticles (Armendariz et al. 2004).

The effect of pH on the size of Ag nanoparticles was demonstrated in several studies (Armendariz et al. 2004; Bankar et al. 2010; Sathishkumar et al. 2010). As reported by Sathishkumar et al. (2010) in the synthesis of Ag nanoparticles using *curcuma longa* tuber powder and extract, large Ag nanoparticles were formed at lower pH whereas small and highly dispersed nanoparticles were formed at higher pH. They proposed that at alkaline pH, large numbers of negatively charged functional groups were available for Ag binding, facilitating a higher amount of cationic Ag(I) to bind and form a large number of nanoparticles with smaller diameters due to the higher tendency of nucleation rather than aggregation. However, at acidic pH, the aggregation of Ag nanoparticles to form larger nanoparticles was believed to be favored over the nucleation to form new nanoparticles which resulted in the formation of large nanoparticles. In fact, similar change of particle size in response to the pH was also reported by the same researchers in an earlier studies regarding the synthesis of Ag nanoparticles mediated by *Cinnamon zeylanicum* bark extract and powder (Sathishkumar et al. 2009). Furthermore, in the synthesis of Ag nanoparticles by aqueous extract of *Azadirachta indica* (Neem) leaves, enhanced stability of cluster distribution was found at alkaline pH range. The authors suggest that complete charging of the clusters could be achieved at alkaline pH owing to the presence of large amount of  $\text{OH}^-$ . The repulsive electrostatic/electrosteric interactions were maximized and this resulted in the enhanced stability and decreased aggregation (Tripathy et al. 2010). This observation indicates the decreased aggregation of Ag nanoparticles under alkaline conditions which correspond to the formation of smaller particles at higher pH. The different effect of pH on the size of Au and Ag

nanoparticles could be attributed to the different form of metal species exist in the solution, in which negatively charged  $[\text{AuCl}_4]^-$  and positively charged  $\text{Ag}^+$  might interact to a different extent with the biomolecules at a particular pH.

Moreover, it was found that certain pH may promote the binding of metal ions to the biomass and resulted in higher yield of nanoparticles. As reported in the biosynthesis of gold nanoparticles by foliar broth, it was found that a stronger reducing capability upon Au(III) is favored by lower pH conditions. The functional groups of active biocompounds such as hydroxyl groups tend to undergo protonation and become positively charged at acidic pH, promoting the interaction between protonated biocompounds and the oppositely charged  $[\text{AuCl}_4]^-$  through electrostatic attraction or electrovalent bond (Zhou et al. 2010). However, there is no conclusive explanation for the effect of pH on the formation of nanoparticles. For example, the formation of Au nanoparticles mediated by *alfalfa* biomass is pH independent. This suggests that Au ions bind to the biomass in a covalent manner through electrostatic interactions (Gamez et al. 2003). In addition, *Sorbus aucuparia* leaf extract reduced Au and Ag nanoparticles were found to have high zeta potential and stable under a wide pH range (2–10) (Dubey et al. 2010). Similar phenomenon was also observed by Dwivedi and Gopal (2010) in which the *Chenopodium album* leaf extract reduced Ag and Au nanoparticles were found to be stable under a wide pH range due to their high zeta potential. Hence, despite the effect of pH is generally regarded as an important factor to control the morphology and yield of nanoparticles, it should be noted that multiple other factors varying both in quantity and quality among individual plants might regulate the conversion and formation of nanoparticles in a pattern much stronger than that of the original pH values. It is believed that highly stable Au and Ag nanoparticles could be produced through plant-mediated synthesis and these pH-resistant nanoparticles might have great potential for applications that would experience a change of environmental pH such as drug delivery systems.

#### 4.3.2 Effect of temperature

It was found in several studies that temperature control was critical in directing the shape and size of

nanoparticles during the plant-mediated synthesis process. Lukman et al. (2011) reported that *Medicago sativa* seed exudates could only produce Ag nanotriangles at temperatures higher than 30 °C due to the suppression of shape-directing agents at lower temperatures. Another example to show the crucial role of reaction temperature in the shape-directing process was demonstrated in the formation of Ag nanowires mediated by broth of *Cassia fistula* leaf. It was found that Ag nanowires were formed at room temperature whereas only spherical nanoparticles and short nanorods were obtained at 60 °C. As suggested by the authors, the interaction between biomolecules and the faces of silver might be changed at elevated temperatures and hence hinder the coalescence of nanoparticles in solution which is not favorable in the formation of Ag nanowires (Lin et al. 2010).

In the synthesis of Ag nanoparticles by *Lemon verbena*, it was demonstrated that the rate of reduction increased when the reaction temperature increased. The higher rate of reduction suggests that nucleation of nanoparticles is favored at higher temperature while the secondary reduction process on the surface of preformed nanoparticle is hindered. Additionally, highly polycrystalline Ag nanoparticles were obtained at high temperature (95 °C) as compared to the less crystalline structure obtained at room temperature (Cruz et al. 2010). In the synthesis of Au nanoparticles using *Nyctanthes arbortristis* ethanolic flower extract, it was found that mixture of Au nanoparticles of different shapes (triangular, pentagonal, rod shaped, and spherical) was obtained at low temperature (25 °C) whereas predominantly spherical particles were obtained at high temperature (80 °C). The authors suggest that the variation in shapes of Au nanoparticles with the reaction temperature could be attributed to the susceptibility of the nucleation process of metallic nanoparticles to reaction temperature. High reaction rate resulted from the higher temperature favors maximum consumption of Au ions in the formation of nuclei, which could stop the secondary reduction process on the surface of the preformed nuclei while resulting in the spherical particles. However, at low temperature, the secondary nucleation is favored which could result in the formation of different shapes of particles (Das et al. 2011a). In addition, the decreased size of Au nanoparticles synthesized by persimmon and magnolia leaf broth was observed when temperature increased.

Similarly, the authors proposed that most of the Au ions were consumed in the formation of nuclei, and the secondary reduction process on the surface of nuclei was suppressed at increasing temperature (Song et al. 2009). In accordance with these findings, it could be seen that the suppression of secondary nucleation process at high temperature might be a reason for the formation of particles with more uniform morphology.

However, it was found in most of the studies that well-defined Au and Ag nanostructures could be successfully synthesized at room temperature (Ghodake et al. 2010; Njagi et al. 2011; Nadagouda and Varma 2008). The ability of these plant species to produce metallic nanoparticles at room temperature is highly favorable because they could not only increase the ease of processing but also provide the advantage of energy-saving. Nevertheless, whether it is possible to control the shape and size of Au and Ag nanoparticles through adjustment of the synthetic temperature still requires further investigation in which the presence of temperature-sensitive phytochemicals in the plant is of relevance.

#### 4.3.3 Effect of the ratio of plant biomass or plant extract to metal concentration

As predicted by LaMer model, the formation of nanoparticles could only happen when the precursor concentration is within a suitable range for nucleation. However, it is the availability of reducing and capping agents that determines whether these metal precursors could be reduced and eventually leads to the formation of nanoparticles. Hence, the concentration of the plant biomass used during biosynthesis should not be overlooked as it determines the extent of reduction and stabilization exerted by the biomolecules which could affect the resulted shapes and sizes of nanoparticles. It has been shown by Prathna et al. (2011) in the synthesis of Ag nanoparticles by *Citrus limon* (lemon) aqueous extract that by increasing the mixing ratio between plant biomass and metal solutions, nanoparticles with smaller size were formed. As proposed by the authors, this might be due to the increased amount of electron-rich bioreducing agents when a higher dosage of plant biomass was used in the reaction medium. When the concentration of plant extract increased, it resulted in the increase of electron density as charged groups in the reductants. This would



constrain the free electrons of the metallic cluster within a small volume and increase the surface charge on the metallic clusters. The resulting surface charges exerted a repulsive force that could lead to a decrease in the particles size. Similar observation was reported by Dubey et al. (2010) in the synthesis of Ag and Au nanoparticles using aqueous leaves extract of *Sorbus aucuparia*. In addition to the increase formation of nanoparticles, decrease in the particle size for both the nanocolloids of Ag and Au was observed when the leaf extract quantity was increased.

The possibility of controlling the particle size and shape by tuning the composition of the reaction mixture was investigated by Song et al. (2009). Similarly, they also found that the size of Au nanoparticles decreased with an increase in the leaf broth concentration. However, it is worth noting that most of the particles formed at leaf broth concentration >10 % were spherical whereas those formed by using 5 % leaf broth show the presence of large plate-like structures. Shukla et al. (2010) reported that the ratio of spherical particles to triangular or hexagonal particles increased as the amount of extract increased. Similar changes in shapes and size were also observed on phyllathin-assisted and clove extract-assisted Au nanoparticle biosynthesis. The use of low concentration plant extract led to increased particle size and the formation of hexagonal or triangular Au nanoparticles (Kasthuri et al. 2009). From these findings, it could be deduced that the metallic ions preferred to form spherical nanoparticles when there were sufficient biocapping agents available at high biomass dosage. When high biomass dosage was used, the availability of excess biomolecules could result in strong interaction between the protective biocapping agents and the surfaces of nanoparticles. As a result, this could prevent the newly formed nanocrystals from sintering and result in the favorable formation of more stable spherical shaped particles. In addition, if this interaction is intensified with increasing biomass concentration, size reduction of spherical nanoparticles could be resulted. On the other hand, other shapes of nanoparticles such as hexagonal and triangular structure may be formed at lower concentrations of plant biomass. It is believed that the nanoparticles could attain a higher state of stability by transforming to other shapes or through enlargement of the particles if there are insufficient biomolecules available for reduction and stabilization.

## 5 Applications of Au and Ag nanoparticles

Several applications and the associated health effects of Au and Ag nanoparticles were discussed in the following sections. In addition, available large-scale production systems that could be used for plant-mediated synthesis of Ag and Au nanoparticles were also discussed.

### 5.1 Au nanoparticles

Since 400 years ago, Au nanoparticles have been used for the treatment of certain illness and the staining of glass and enamels (Tanaka 1999). Nowadays, Au nanoparticles are found to be useful in many applications such as biomedicine, catalysis, biosensing, electronic and magnetic devices.

#### 5.1.1 Applications of Au nanoparticles in biomedicine

For the applications in biomedicine, Au nanoparticles have emerged as a new delivery strategy for transporting drug and gene through nanosized drug-delivery systems due to their inherent low toxicity, high surface area and tunable stability. The applications of Au nanoparticles in tumor-targeted drug delivery systems have been successfully demonstrated in several studies. Paciotti et al. (2005) found that the efficiency of an anticancer protein tumor necrosis factor increased when it was attached to Au nanoparticles. Photothermal effect of Au nanoparticles in therapy was demonstrated by Huang et al. (2007b) who use citrate-stabilized Au nanoparticles (30 nm core diameter) coated with anti-EGFR (epidermal growth factor receptor) to target HSC3 cancer cells (human oral squamous cell carcinoma). The resulting enhanced efficacy of photothermal therapy by 20 times was probably due to the local heating effect induced by the Au nanoparticles when irradiated with light. Gibson et al. (2007) successfully functionalized Au nanoparticles with 2 nm core diameter with a chemotherapeutic drug, paclitaxel. TGA analysis reveals that about 70 molecules of paclitaxel could be attached to one Au nanoparticle. In addition, the tunable size and functionality of Au nanoparticles allow them to function as a useful scaffold for the delivery of large biomolecules such as nucleic acids like DNA or RNA (Yeh and Perricaudet 1997), as well as peptides and proteins (Verma et al. 2004; Bhumkar

et al. 2007). Au nanoparticles attached with VEGF antibodies have been used in treating B-chronic lymphocytic leukemia (Mukherjee et al. 2007). In view of the various applications of Au nanoparticles in biomedicine, the cytotoxicity of Au nanoparticles is a major issue to consider. It was suggested in some studies that the toxicity of Au nanoparticles was more likely affected by the ligand chemistry (Connor et al. 2005; Pan et al. 2009). Au nanoparticles synthesized by natural bioreducing and stabilizing agents may offer an exciting alternative that is worth exploring due to their inherent low toxicity and biocompatibility. In Sect. 5.3, the health effects posed by Au and Ag nanoparticles are discussed.

### 5.1.2 Applications of Au nanoparticles in catalysis

Gold is also an interesting catalytic material that plays an important role in green chemistry. The unique properties of Au nanoparticles and their ability to catalyze selective reactions at low temperature, such as water gas shift reaction, selective oxidation of CO (Andreeva 2002; Grisel et al. 2002; Hutchings and Haruta 2005), and hydrogenation (Sinha et al. 2004; Claus 2005; Hughes et al. 2005) have attracted a lot of research interest (Hutchings and Haruta 2005; Christensen and Nørskov 2010; He et al. 2009). Glycerol, as a major by-product from the manufacture of biodiesel could be oxidized to glycerate with 100 % specificity using of Au/C as catalyst (Carrettin et al. 2002). Direct synthesis of H<sub>2</sub>O<sub>2</sub> was also achieved when catalyzed by Au–Pd alloys supported on alumina (Landon et al. 2002; Landon et al. 2003). In addition, the catalytic activity of Au nanoparticles was shown to be size and shape-dependent. For example, Au nanospheres were shown to catalyze the reduction of aromatic nitro compounds with the fastest rate as compared to Au nanoprisms and nanorods (Kundu et al. 2009). The rate constant, *k*, for the reduction of aromatic nitro compounds was found to be proportional to the size of the Au nanoparticles (Panigrahi et al. 2007). It was shown in a previous study that *sesbania* seedlings-assisted synthesized Au nanoparticles were able to reduce the toxic pollutant, 4-nitrophenol, to 4-aminophenol (Sharma et al. 2007). This shows the potential of using biosynthesized Au nanoparticles in the field of catalysis. The ability of some bio-assisted synthesis approaches that can produce various shapes of Au

nanoparticles could be advantageous in certain catalysis reactions.

### 5.1.3 Applications of Au nanoparticles in chemical and biosensing

The unique physical and optical properties of Au nanoparticles have fueled a growing field of research in chemical and biosensing. Krasteva et al. (2002) have shown the ability of a thin film resistor composing of gold and different organic-based dendrimers for the detection of various organic vapors. The aggregation phenomena of Au nanoparticles functionalized with different chemical groups is a useful technique for the detection of various analytes. Enhanced optical sensing of anions was achieved by using Au nanoparticles which were surface-functionalized with amide ligands (Watanabe et al. 2002). Chemical sensing of metal cations such as potassium (Lin et al. 2002), lithium (Obare et al. 2002) as well as toxic heavy metals like mercury, lead and cadmium (Kim et al. 2001) have been demonstrated by using surface engineered Au nanoparticles. In addition, Au nanoparticles can also be used for the detection of organic compounds such as pesticides. An extremely sensitive chemical sensor based on the reductive enlargement of Au nanoparticles controlled by the concentration of the substrate and activity of enzyme acetylcholine esterase, AChE, was developed as a pesticide sensor through measurement of electrochemical current (Pavlov et al. 2005). In addition, salt-induced aggregation of Au nanoparticles was employed for the detection of pesticides in drinking water at low concentration (Burns et al. 2006). Au nanoparticles have also been used in the *Carter-Wallace* home pregnancy “First Response” kit. When the latex microparticles and Au nanoparticles derivatized with a hormone released by pregnant women were mixed, the micro- and nanoparticles coagulated and resulted in pink-colored clumps (Bangs 1996). Orendorff et al. (2005) reported that bio-detection sensitivity derived from spherical nanoparticles was not strong enough to trace the interaction of biomolecules. Instead, irregular shaped nanoparticles were thought to display novel properties and might improve biological detection sensitivity greatly. It was found that some plant-mediated synthesis of nanoparticles could produce irregular shaped nanostructures in a one pot process due to the complexity of the bioreducing and capping

agents (Lukman et al. 2011). This sheds light on the potential of these plant-mediated synthesized nanoparticles to be utilized in the applications that require irregular shaped nanostructures.

It is well-known that collective oscillations of conduction electrons with the incident light could cause electric field amplification near the noble metal surface. This activity could be utilized in absorption spectroscopy such as Surface-Enhanced Raman Spectroscopy (SERS), which is useful for the detection of some organic or inorganic species at low concentrations. Ultrasensitive chemical analysis of organic pollutants was achieved by using an enhanced Raman detection induced by humic acid-protected Au nanoparticles. It was found that the vibrational profiles of noble metal nanoparticles were very sensitive to the presence of heavy metal ions. Useful nanoprobe based on SERS detection for  $\text{Hg}^{2+}$  and  $\text{Cd}^{2+}$  were made by using trimercaptotriazine-modified Au nanoparticles (Alvarez-Puebla et al. 2007).

#### 5.1.4 Applications of Au nanoparticles in water treatment

Au nanoparticles were also found to be useful in water purification. The interactions of noble metallic nanoparticles with organochlorine and organophosphorus pesticides such as endosulfan, malathion and chlorpyrifos show the potential of noble metallic nanoparticles for the mineralization of pesticides to metal halides and amorphous carbon. Au nanoparticles are also useful for the removal of heavy metals through the formation of alloys with varying composition such as  $\text{Au}_3\text{Hg}$ ,  $\text{AuHg}$ ,  $\text{AuHg}_3$  phases, and thus could be used for the removal of Hg ions in water (Pradeep and Anshup 2009). The application of plant-mediated synthesized Au nanoparticles in water purification is worth exploring because it is not only more affordable for poorer countries but also helps to reduce the discharges of agricultural waste by adding value to them.

## 5.2 Silver nanoparticles

Owing to the excellent antimicrobial activity, incorporation of Ag nanoparticles in medical field as well as air and water purification will continue to be a growing trend. This increasing use of Ag nanoparticles in consumer products will provide stimulus for the

development of an eco-friendly production method which is not only cost-effective but also able to retain its antimicrobial activity.

#### 5.2.1 Applications of Ag nanoparticles in biomedicine

Ag nanoparticles are well-known for their anti-fungal, anti-inflammatory, and anti-viral activities. Ag nanoparticles are known to inactivate microbes by interacting with their enzymes, proteins or DNA to inhibit cell proliferation (Singh et al. 2010). It was found that the interaction of Ag and thiol-containing compounds appeared to play an important role in bacterial inactivation (Liau et al. 1997). The anti-bacterial activity of Ag nanoparticles was found to be shape-dependent. Pal et al. (2007) have compared the antibacterial properties of differently shaped Ag nanoparticles through their interactions with the gram-negative organism, *Escherichia coli* (*E. coli*). Their study revealed that truncated triangular nanoplates displayed the strongest activity as compared with spherical particles and nanorods. Ag nanoparticles were found to be useful in controlling HIV infection. It was demonstrated by in vitro study that Ag nanoparticles with size ranges from 1 to 10 nm could interact with the glycoproteins of HIV-1 preferentially and inhibited the virus from binding to the host cells (Elechiguerra et al. 2005). The toxicity of plant latex capped Ag nanoparticles towards human lung carcinoma cells was studied in vitro and it was concluded by the authors that these nanoparticles is toxic to A549 cells in a dose dependent manner. It was suggested that plant latex could solubilize Ag nanoparticles in water and act as a potential biocompatible vehicle for transport of Ag nanoparticles to target tumor cells (Valodkar et al. 2011). However, there is lack of comprehensive study to compare the similar response of chemically synthesized Ag nanoparticles which could give us a better insight into the advantage of biocompatibility of these biosynthesized Ag nanoparticles as claimed in this study.

The anti-bacterial properties of Ag nanoparticles can be utilized in imparting disinfection properties to the materials and devices used in biomedical applications. Extracellularly synthesized Ag or Au nanoparticles were shown to be incorporated in several kinds of materials such as cloths and some medical devices used in hospital to prevent infection with pathogenic bacteria (Silver 2003). In addition, Ag nanoparticles

could also be used in topical ointments and creams, which prevent infection of burns and open wounds (Becker 1999). A comparative study of bactericidal effect induced by biologically and chemically synthesized Ag nanoparticles on gram-negative and gram-positive bacteria shows that biogenic-Ag has higher toxicity compared to colloidal-Ag for all three strains tested. The authors suggest that different chemical and biological coatings on the nanoparticles could significantly affect their toxicity which could be a result of interactions between the capping agents with the bacteria (Suresh et al. 2010). This case study shows that biosynthesized Ag nanoparticles might have higher anti-bacterial activity due to the stronger surface interactions with the bacteria which is rendered by their biological capping agents.

### 5.2.2 Applications of Ag nanoparticles in catalysis and water treatment

Excellent catalytic activity of Ag for a number of partial oxidation reactions such as the oxidation of methanol to formaldehyde and ethylene to ethylene oxide was widely known and practiced in industry worldwide. The catalytic oxidative power of Ag is due to its ability to chemisorb oxygen in atomic form. The atomic oxygen could fit into the octahedral holes of Ag and cause the accumulation of oxygen within the bulk of Ag (Nagy and Mestl 1999; Wachs and Madix 1978). The ability of Ag to adsorb atomic oxygen has also enabled the development of two water purification systems i.e. the *Katadyn* and *Ionic*s systems. *Katadyn* filter was made by incorporating 0.28 % by weight of metallic Ag in a ceramic fired, which created a pore size of 0.2  $\mu\text{m}$  that can filter out living organisms mechanically. Bacteria trapped in the pores could be destroyed by the adsorbed oxygen atoms on the Ag surface. For *Ionic*s system, more than 1 % by weight of metallic Ag was deposited on activated carbon. The dissolution of Ag during the catalytic process prevented the buildup of bacteria throughout the activated carbon filter (Davies and Etris 1997). In addition to water filters, the coating of Ag nanoparticles to activated carbon fibers could impart antimicrobial functions to air filters as well. Removal of hazardous gaseous pollutants was achieved by inhibition of the survival of microorganisms (Yoon et al. 2008).

The anti-bacterial properties of Ag nanoparticles have also made a significant improvement to a number

of membrane technologies (Basri et al. 2011; Son et al. 2004). The addition of Ag nanoparticles on the membrane matrix has not only solved the problem of biofouling as frequently encountered in membrane technologies, but also imparted antimicrobial ability to the membrane. The antibacterial activity against *E. coli* and *Staphylococcus aureus* of a Ag-loaded cellulose acetate hollow fiber membrane remained even after immersing in water bath for 180 days (Chou et al. 2005). Plant-mediated synthesized Ag nanoparticles (20–30 nm) using *A. indica* leaves extract were proved to exhibit excellent antimicrobial activity against water borned pathogens, namely *E. coli* and *Vibrio cholera*, through the alteration in membrane permeability and respiration of bacterial cells (Krishnaraj et al. 2010). The use of these biosynthesized Ag nanoparticles for reducing microbial load in water treatment appears to be more advantageous due to its lower production cost and also lower risk of causing secondary pollution resulted from the release of left-over hazardous chemicals from the synthetic process.

### 5.2.3 Applications of Ag nanoparticles in chemical and biosensing

Ag nanoparticles have also been utilized for SERS-based detection. The detection of perchlorate and cyanide anions using Ag nanoparticles functionalized with amino and amide groups was reported to reach detection limits of 8 and 7 ppb, respectively (Tan et al. 2008). Recently, it was reported that SERS detection of arsenate (As(V)) with a detection limit of 5  $\mu\text{g L}^{-1}$  was achieved by using multilayer Ag nanofilms (Han et al. 2011). A highly sensitive and robust sensor that allowed naked eye colorimetric detection for isomers of aromatic compounds was developed using  $\beta$ -cyclodextrin modified Ag nanoparticle probes (Chen et al. 2010). In addition to chemical sensing, Ag nanoparticles can be used in biosensing as well. Rapid detection of *E. coli* based on surface plasmon resonance (SPR) was demonstrated by using silica particles (200 nm) coated antibodies-conjugated Ag nanoshells (20 nm) (Kalele et al. 2006).

### 5.3 Health effects posed by Au and Ag nanoparticles

Due to the unique properties exhibited by nanomaterial, the behavior of nanoparticles cannot be

extrapolated from their larger bulk counterparts. Hence, toxicity studies specific to nanoparticles are required to understand the potential hazards to human health prior to their widespread application. Undesirable adverse interactions between nanoparticles and cells could lead to toxicity, induction of oxidative stress, cellular dysfunction and inflammation, etc. (Sperling et al. 2008). In general, factors determining the degree of toxicity include the chemistry of nanoparticles (Bar-Ilan et al. 2009), particle size (Semmler-Behnke et al. 2008), and specificity to cell types (Soto et al. 2007). In addition to the composition of nanoparticle itself, ligand chemistry is also one of the important parameters that affect the biocompatibility of nanoparticles (Pan et al. 2009).

### 5.3.1 Health effects of Au nanoparticles

In view of the various biomedical applications of Au nanoparticles, it is essential to understand their potential toxicity and health impact before they can be used in real clinical settings. Au nanoparticles were variously described as non-toxic or toxic (Pan et al. 2007). Toxicity assessment of multisized Au and Ag nanoparticles in zebrafish embryos shows that colloidal Au is significantly less toxic than colloidal Ag, which produces less than 3 % mortality as compared to 100 % mortality after colloidal Ag treatments (Bar-Ilan et al. 2009). Due to the inert chemistry of Au nanoparticles, it is used extensively in drug delivery (Dhar et al. 2008), gene therapy (Bhattarai et al. 2008), biosensing (Chuang et al. 2010) and as contrast agents for cell imaging (Peng et al. 2012). In a study conducted by Connor et al. (2005), they suggest that Au nanoparticles are taken up by human cells but do not cause acute cytotoxicity after investigating the toxic response of human leukemia cells (K562) towards spherical Au nanoparticles with average diameters from 4 to 18 nm that contain a variety of surface modifiers.

However, size-dependent toxicity of Au nanoparticles (stabilized with triphenylphosphine derivatives) to epithelial cells (HeLa), phagocytes (J774A1), melanoma cells (SK-Mel-28) and fibroblasts (L929) was demonstrated by Pan et al. (2007) by using nanoparticles with size ranging from 0.8 to 15 nm. The authors suggest that 1.4 nm Au nanoparticles could be more toxic than smaller or larger Au nanoparticles with similar chemical composition. This study shows the

importance of particle size in determining the toxicity of Au nanoparticles but further investigations are needed to verify this observation due to the unlikely difference in toxicity response induced by particles with only small size variation (1.2 vs. 1.4 nm). In addition, the possible differences in their preparation procedure should also be taken into considerations. Sonavane et al. (2008) has investigated the permeation efficiency of Au nanoparticles with larger size range (15, 102, and 198 nm) through rat skin and rat intestine and found that smaller particles had a higher efficiency for penetration. The ease of permeation for small Au nanoparticles has enabled them to be a potential carrier for transdermal and oral delivery but it should not be overlooked that particles with decreasing dimensions has larger surface area which could become very reactive in the cellular environment. Specifically, Au nanoparticles with smaller size could penetrate through the cell membrane and get stored in either the perinuclear compartment or even inside the cell nucleus. The interaction between Au nanoparticles and DNA inside the nucleus could lead to conformational changes within the structure of DNA which could induce genotoxicity or block transcription (Goodman et al. 2006).

Several biosynthetic methods which are believed to be able to produce Au nanoparticles that are more biocompatible have been actively researched such as biosynthesis of Au nanoparticles using microorganisms (Suresh et al. 2011), fungus (Sheikhloo et al. 2011) and plant extract (Dwivedi and Gopal 2011). It is believed that Au nanoparticles produced without using exterior toxic chemicals or surfactants could work better for biomedical applications due to their inherent biocompatibility. Toxicity of Au nanoparticles synthesized by using aqueous extract of *Calotropis procera* latex was tested on three cell lines, HeLa, A549 and BHK21. As compared to the varying degrees of cytotoxicity exhibited by a vast majority of Au(I) and Au(III) compounds, it was found that cells exposed to biosynthesized nanoparticles showed excellent viability even up to 150  $\mu\text{M}$  concentrations of Au nanoparticles. The lack of toxicity of the biosynthesized Au nanoparticles as compared to other gold compounds suggesting their better biocompatibility which might be a result of the total elimination of synthetic reducing and capping agents in the synthetic procedure (Das et al. 2011b). The use of traditional medicinal plants such as Korean red ginseng root in the biosynthesis of Au nanoparticles was also explored

and the synthesized Au nanoparticles was demonstrated to be non-toxic through cytotoxicity assays performed on normal cervical cells lines. In addition, the ginseng-mediated synthesized Au nanoparticles are also found to be resistant to aggregation caused by pH variation and high ionic strength environment which is important characteristic for utilizing colloidal nanoparticles in biomedical applications (Leonard et al. 2011). On the other hand, Au nanoparticles synthesized by using chemical methods might not only contain the core material (Au), surface-bound stabilizing ligands, but also potential left-over chemicals resulted from the synthetic process. Hence, any of these components could contribute to the observed toxicity from Au nanoparticles. One of the examples regarding the toxic effects induced by the left-over chemicals from chemical synthesis is the toxicity exerted by CTAB-capped nanoparticles. In this study, it was found that the CTAB alone could exhibit similar toxicity with the CTAB modified Au nanoparticles. In order to determine whether the unbound CTAB or the CTAB-modified nanoparticles was accounted for the observed toxicity, the authors have purified the CTAB-modified nanoparticles from the unbound CTAB precursors by repeated centrifugation and washing. Their results revealed that the washed CTAB-modified nanoparticles are actually non-toxic under the tested conditions (Connor et al. 2005). Despite further experiments are necessary to confirm the role of surface functional groups on affecting the observed toxicity, it shows that toxicity of Au nanoparticles could be a result of the inadequate purification during the preparation procedure which is sometimes unavoidable. However, this could be eliminated if biological synthesis method is used in which the inherent less toxic biomolecules could act as both reductants and surfactants. However, at this current stage, there is still lack of enough toxicity studies regarding the use of biosynthesized nanoparticles in various applications. It is suggested that more *in vivo* and *in vitro* studies are necessary to study the different toxic profile of biosynthesized Au nanoparticles which could prove their lower toxicity as compared to chemically synthesized Au nanoparticles.

### 5.3.2 Health effects of Ag nanoparticles

Among various metal nanoparticles, toxicity of Ag nanoparticles has received the greatest attention due to

the highest degree of commercialization of Ag nanoparticles incorporated products (Johnston et al. 2010). The antibacterial activity of Ag nanoparticles has rendered it useful in the production of more than 200 consumer products (Usha Rani and Rajasekharreddy 2011). However, it is worthwhile determining whether the same properties that cause lethality to microbial cells could be toxic to eukaryotic cells as well. Likely routes of human exposure to Ag nanoparticles include ingestion, inhalation, and dermal penetration. To assess the potential risk of Ag nanoparticles, it is necessary to consider whether these particles are able to pass from their exposure site into the blood and become distributed within the body.

In a clinical study conducted by Vlachou et al. (2007), 30 burn patients were treated with Acticoat burn wound dressings that contain Ag nanoparticles. It was observed that the increased serum silver level returned to normal 6 months after the treatment was stopped. Since there were no haematological or biochemical indicators of toxicity associated with the silver absorption observed in this study, the authors considered this dressing is safe for use on burns. In line with this, systematic availability of silver following dermal contact with an Acticoat wound dressing was demonstrated by Trop (2006). This case study shows that silver is able to stimulate liver toxicity and skin discoloration. These findings show the potential toxicity of Ag nanoparticles incorporated wound dressings in which the Ag nanoparticles might induce toxic response upon biologic interactions with human cells.

On the other hand, exposure via ingestion of Ag nanoparticles is often encountered within health products. The ingestion of colloidal silver as an alternative therapy for diabetes has been demonstrated to cause argyria (blue-gray hyperpigmented skin due to silver deposition after prolonged exposure), which is more likely a result from the absorption of Ag nanoparticles via gastrointestinal tract followed by subsequent distribution to the skin (Chang et al. 2006). Moreover, the potential harmful effects of Ag nanoparticles on reproductive health have been demonstrated in a recent study. The result shows that Ag nano- and submicron-particles are cytotoxic and cytostatic, which caused apoptosis, necrosis and decreased proliferation of Ntera 2 (NT2, human testicular embryonic carcinoma cell line) in a concentration- and time-dependent manner (Asare et al.

2012). In addition, inhalation experiment of Ag nanoparticles was done on rats by exposing them to a spark-generated silver particles environment. The authors suggests that inhaled Ag nanoparticles could be translocated from the lung to other organs such as liver, kidney, spleen, brain and heart through blood circulation and being cleared due to their solubilization (Takenaka et al. 2001).

In view of the above mentioned toxicity of Ag nanoparticles upon different routes of exposure, it is necessary to evaluate the possible benefits that could be obtained from the alternative use of biosynthesized nanoparticles. The cytotoxicity and genotoxicity of Ag nanoparticles synthesized by using broth prepared from the aromatic spath of male inflorescence of screw pine was studied and the results revealed that the biosynthesized Ag nanoparticles could induce cell death and DNA damage in a dose-dependent manner. However, the associated less cytotoxicity exhibited by biosynthesized Ag nanoparticles was found and the authors suggest that it might be due to the difference in their mode of preparation rather than to the difference of their size or diameter (Panda et al. 2011). Due to the lack of studies on the toxicity of biosynthesized nanoparticles as compared to the chemically synthesized ones, the safer use of biosynthesized nanoparticles in human applications could not be concluded at this current stage.

Since most of the Ag nanoparticles incorporated products will be eventually disposed and enter to the environment. It is of relevant to determine the associated ecotoxicity of these biosynthesized nanoparticles due to their possible implications to human health. The acute toxicity of Ag nanoparticles synthesized by using *Piper Betle L.* leave extract against aquatic organism, *Daphnia magna* was evaluated and comparison was made between the toxicities exerted by chemical synthesized Ag nanoparticles. It is concluded that apart from being superior over chemical synthesized nanoparticles, the biogenic nanoparticles are safer to the environment as they are less toxic to non-target organisms (Usha Rani and Rajasekharreddy 2011). Although there is lack of enough toxicity studies to evaluate the environment impacts of these biosynthesized nanoparticles, this case study sheds light on the benefits of using biosynthesized nanoparticles over chemical synthesized nanoparticles which could be less harmful to the environment.

#### 5.4 Possibility for large-scale biosynthesis of Au and Ag nanoparticles mediated by plants

Traditional methods for mass production of metal nanoparticles are more likely derived from batch process that was extended from bench-top batch process. Usually, they are synthesized by using large-scale hotplates, beakers and condensers which require well-trained personnel and labor intensive processes (Yang et al. 2010). The feasibility of using these production methods is usually limited by the traditional reaction vessel which includes inefficient micromixing, non-uniformity of temperature throughout the reactor, and non-idealities in flow rates which could eventually lead to broad particle size distributions. In addition, these processes usually consume a larger amount of reagents and are also more time consuming due to the required cleaning procedure after each batch to avoid contamination (Kumar et al. 2010a). The formation of metallic nanoparticles consists of two essential steps which are the nucleation and subsequent particle growth. In most conventional synthetic methods both these processes are inevitably occur concurrently throughout the process and hence resulted in the formation of particles with uneven size distributions. In order to overcome this drawback, it is necessary to develop a system that could allow the nucleation to occur in short period of time under efficient mixing and at the same time allow additional reagents to be added downstream in a controlled manner so that the particle size generated could be tunable by varying the process parameters such as reagent dosage, pH and temperature. In view of this, microfluidic devices which provide manipulation of fluids in microchannels with greatly reduced consumption of reagents and demonstrate intrinsically efficient heat and mass transfer due to high surface-area-to-volume ratios are considered as an attractive alternative. In addition, efficient mixing and rapid chemical reaction could be easily achieved through continuous automation system in nanoscale which allows microfluidic devices to better control the synthesis parameters and thus the quality of nanoparticles produced (Hung and Lee 2007). All these advantages of using microfluidic reactors have satisfied the requirements of large-scale production of nanomaterials. Although it seems that the instantaneous reaction volumes that are typically used in the range of nanolitre to low-microlitre are too low to be

applied in mass production, it should be noted that those systems are operated with automated continuous flow and can be run in parallel for extended periods of time so that mass production could be achieved. Several studies were done to demonstrate the application of microreactors in the synthesis of Au and Ag nanoparticles (Luty-Blocho et al. 2010; Singh et al. 2009; Sugano et al. 2010) in which the great potential of using these microreactors for the biosynthesis of nanoparticles was shown. An example of using the microfluidic reactors in biosynthesis of Ag nanoparticles mediated by lixivium of sundried *Cinnamomum camphora* leaf was demonstrated by Huang et al. (2008) through the use of continuous-flow tubular microreactors. This study has shown the potential ease of scaling up for the biosynthetic process using continuous-flow systems which could be exploited for mass production of nanoparticles by plant extract. Therefore, it is believed that microfluidic reactors will play an important role in manufacturing biosynthesized nanoparticles which allow fast and precise control of various process variables such as temperature, flow rate, reagent concentration etc. Furthermore, the utilization of natural plant biomass without adding other toxic chemicals will also serve as an environmentally benign production method that produces less hazardous waste and safer working environment for the labors involved. We believe that among other biosynthesis methods such as those using microorganisms and fungi, plant-mediated synthesis of nanoparticles which does not require specialized operation systems for maintaining cell cultures and growth of microorganisms has the greatest potential of scaling up for mass production in terms of its economic and environmental viability.

## 6 Challenges and limitations

Despite the advantages of plant-mediated synthesis of nanoparticles, there are still a lot of challenges to be overcome before it can be applied practically. One significant challenge is the control of size, shape and crystallinity of nanoparticles under a given set of biosynthetic conditions, in which the biosynthesis mechanism is not fully understood. The diversity of plant systems makes individual purification and determination of various biocompounds a challenging task. Bioprotocol for the production of metallic

nanoparticles may vary greatly among different plants. Hence, it is necessary to have a better understanding of the biosynthetic process by different plant systems. In addition, the attachment of some biomolecules from plant biomass onto the surface of biosynthesized nanoparticles might involve irreversible binding of the functional groups which could hinder the subsequent functionalization of the nanoparticles.

The stability of biosynthesized nanoparticles is another key issue to consider. It is important to ensure that the biosynthesized nanoparticles remain stable upon storage with no significant changes of morphology before they are used in practical applications. More experiments are required to study the stability of these biosynthesized nanoparticles in which comparison against chemically synthesized nanoparticles is of relevance. In addition, toxicity profiles and environment impacts of these biogenic nanoparticles as compared to chemically synthesized nanoparticles are also important aspects to be considered. However, the utilization of noble metallic nanoparticles such as Au and Ag nanoparticles in various human applications still largely depends on the economical viability of using those precious metals in future.

## 7 Conclusions

Nature provides a rich source of crude plants, marine organisms and microorganisms. The potential of plant systems as a biological factory to produce nanoparticles has been shown in many studies. Biosynthesis of nanoparticles is more beneficial to human use due to the elimination of hazardous chemicals and the reduced capital cost involved in the production. The major advantage of green synthesis of nanomaterials is their important role in protecting the environment which is the also the ultimate goal of other green and clean technologies. Control over the shape and size of nanoparticles was shown to be feasible by varying the reaction conditions. However, biosynthesis of nanoparticles by plant systems is still at its infant stage and currently still underexploited. Biomolecules such as flavonoids, terpenoids, reducing sugars, proteins and alkaloids are believed to play crucial roles during the bioreduction or the shape and size-directing processes. Due to the complex nature of plant systems, more experiments are necessary to



elucidate the biosynthesis mechanisms so that a better process design could be implemented.

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