Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268 (Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 20 | No. 2 | pp. 481-490 | 2021

Original Research Paper

https://doi.org/10.46488/NEPT.2021.v20i02.004

Open Access Journal

Greener Approach to Metallic Nanoparticles: A Review

S. Arora†*, M. Latwal*, K. D. Bahukhandi**, D. Kumar***, T. Vemulapalli**, S. Egutoori** and N. A. Siddiqui**

*Department of Chemistry, University of Petroleum and Energy Studies, Dehradun (UK), India
**Department of Health Safety & Environment, University of Petroleum and Energy Studies, Dehradun (UK), India
***Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun (UK), India
†Corresponding author: S. Arora; shefali.arora@ddn.upes.ac.in

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 05-05-2020
Revised: 21-06-2020
Accepted: 26-06-2020

Key Words:
Biosynthesis
Metal nanoparticles (MNPs)
Plant extract
Plant metabolites

ABSTRACT

Nanoscale based materials are gaining more attention due to their unique physical, chemical and thermodynamic properties. Nowadays the "Green" nanoparticle synthesis has attracted more attention as it is using environmentally acceptable solvent systems which act as eco-friendly reducing and capping agents. This review focuses on a detailed analysis of the bio-production of metal nano-particles by a biological agent, the various factors affecting the morphology, size, and yield of metal nanoparticles, the role of plant metabolites, and the experimental procedure in the synthesis of nanoparticles. This review also gives a platform for the role of natural plant biomolecules involved in the bio-reduction of metal salts during the nanoparticle synthesis, interaction of nanoparticles with various biomolecules, biological application and future directions are discussed as a step towards making a pollution-free environment.

INTRODUCTION

The "green" synthesis of metallic nanoparticles has received increasing attention due to the development of eco-friendly technology in material science. Preparation of nanoparticles via chemical procedure also produces a very high amount of hazardous by-products so the investigation of new chemical and physical methods has to be developed. Thus, there is a need for 'green chemistry' that includes a clean, cheap, nontoxic and environment-friendly method of nanoparticle synthesis (Mukherjee et al. 2001). The plants that can be used for the production of nanoparticles could be a better option in comparison to other environmentally benign biological processes as they eliminate the elaborate and conventional process of maintaining cell cultures (Gour et al. 2019). Biosynthetic methods for the production of nanoparticles would be more useful if nanoparticles were synthesized extracellular using plants or their extracts and in a controlled way in terms of their size, dispersity and shape. Biogenic synthesis of nanoparticles with controlled morphology needs more attention, as the biogenic synthesis of nanoparticles is carried out by using biological means like bacteria (Husseiny et al. 2007), fungi (Kumar et al. 2007), actinomycetes (Ahmad et al. 2003a), lichens (Shahi et al. 2003), algae (Chakraborty et al. 2009), etc. The biogenic entities are found to secrete a large amount of proteins which are found to be responsible for the metal-ion reduction and morphology control (Thakkar et al. 2010). Progress in the field of nanotechnology has been rapid and with the development of innovative synthesis protocols and characterization techniques (Sharma et al. 2009). But most of the synthesis methods are limited to the synthesis of nanoparticles in small quantities and poor morphology. Using plant extract is cheaper than microorganisms as it does not require culture preparation or maintenance of aseptic conditions. The synthesis of nanoparticles using microbes (Kathiresan et al. 2010) and plant extracts cost mainly depends on the many aspects like the metal particles and isolate the chemicals added as the precursor for the nanoparticle synthesis.

Metal nanoparticles have marvellous applications in the area of catalysis, optoelectronics, diagnostic biological probes and display devices, medicine, agriculture and industry. They have been used in the drug delivery system, biomedical devices, biosensors, optics, solar batteries, semiconductors etc. Synthesis of nanoparticles using biological entities has great interest due to their unusual optical (Lin et al. 2000), chemical (Krolikowska et al. 2003), photoelectrochemical (Ahmad et al. 2003b) and electronic properties (Chandrasekharan et al. 2000). The most effectively studied nanoparticles today are those made from noble metals, in particular Ag, Pt, Au, Zn, Cu, Fe, Ni, U, Zr, Se, Te, Ni and

Pd. Nanoscale biosynthesis of two noble metal Ag and Au is of particular interest and importance. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most efficient against bacteria, virus and other eukaryotic microorganisms at low concentrations without any side effects (Jonge et al. 2005) and gold nanoparticles produced by using phytochemicals or other extract components remain stable for a certain time (Singh et al. 2010). Moreover, plants and species mediated, stabilized or capped gold nanoparticle (AuNPs) may cross the cytotoxicity barrier which is a basic requirement in the field of biomedical application of AuNPs (Das et al. 2011). In continuation of this, we summarize the various aspects of "metal nanoparticle using plants" in this review. Here we discuss the biogenesis of nanoparticle, interaction of nanoparticles with various biomolecule, role of plant metabolites in the binding and reduction of metal ion, experimental procedure and characterization of nanoparticles and finally application and future prospects.

BIOPRODUCTION OF NANOPARTICLES

Various types of physical and chemical methods are used for the synthesis of nanoparticles. The use of these methods requires both strong and weak chemical reducing and protective agents like sodium borohydride, sodium citrate and alcohols. These agents are mostly toxic, flammable, cannot be easily disposed of due to environmental issues and also show a low production rate (Bar et al. 2009). It leads to in search of alternatives that could be eco-friendly and does not cause any harm to human and domestic animals health. One of the primary processes in biosynthesis involves bioreduction. The cell wall of the microorganisms plays a major role in the intracellular synthesis of nanoparticles. The cell wall being negatively charged interacts electrostatically with the positively charged metal ions. The enzymes present within the cell wall bioreduce the metal ions to nanoparticles, and finally the smaller sized nanoparticles get diffused of through the cell wall. Bacterial was the first species to synthesize nanoparticles and later on the use of various fungi, actinomycetes and more recently plants was also succeeded. The rate of reduction of metal ions using biological agents is found to be much faster and also at ambient temperature and pressure conditions.

NANOPARTICLE SYNTHESIS IN BACTERIA

Many microorganisms can synthesize inorganic nanoparticles like silver, gold, magnesium, cadmium sulphide and silicon oxide nanoparticles. The resistance caused by the bacterial cell for silver ions in the environment is responsible for its nanoparticles synthesis. Previously the synthesis of nanoparticles via bacteria has enlarged comprehensively due to its immense application. *Bacillus species* has been investigated to synthesize metal nanoparticles and proved bacteria as a potent source to decrease silver and fabrication of extracellularly, consistently circulated nanoparticles, ranging from 10-20 nm size (Sunkar et al. 2012). *Lactobacillus*, a common bacterial strain present in the buttermilk, synthesizes both Au and Ag NPs of well-defined morphology under standard conditions. Nair et al. (2002) and Shahverdi et al. (2007) reported the synthesis of metallic nanoparticles of Ag using the cultural supernatants of *Klebsiella pneumonia*, *Escherichia coli* and *Enterobacter cloacae*. Shirley and co-workers reported the antibacterial activity of silver nanoparticles synthesized from a novel strain of *Streptomyces* sp. (Shirley et al. 2010). A tremendous potential antibacterial activity is shown by novel silver nanoparticles against the multi-drug resistant gram-positive and gram-negative bacterial strains were greatly established. The most widely acknowledged mechanism for the biosynthesis of silver nanoparticles is the presence of the enzyme nitrate reductase which converts nitrate into nitrite. This has been observed in *Bacillus licheniformis* which is known to secrete NADPH and NADPH-dependent enzymes like nitrate reductase that effectively converts Ag^+ to Ag^0.

Bacteria are also used to synthesize gold nanoparticles. Sharma et al. (2012) reported that complete cells of a novel strain of *Marinobacter pelagius are* responsible for the production of stable, monodisperse gold nanoparticle. Prasad et al. (2007) have reported the use of *Lactobacillus* strains to synthesize the titanium nanoparticles. Several bacterial strains have been reported for the synthesis of silver, gold, magnetite, palladium, platinum, selenium, zinc oxide, cadmium sulphide, titanium, titanium dioxide and copper nanoparticles (Ramanathan et al. 2013, Arshad et al. 2017).

NANOPARTICLE SYNTHESIS IN FUNGI

Fungi may be used to grow nanoparticles of different chemical composition and sizes. Fungi acts as a "Nanofactory" for the production of metal nanoparticles especially silver nanoparticles. Fungi produce well-defined structured nanoparticles with good monodispersity. Fungi can produce larger amounts of nanoparticles as compared to bacteria because they secrets large amounts of proteins which directly affects the higher productivity of nanoparticles (Mohanpuria et al. 2008). Fungi have a high binding capacity with the metal ions in the intracellular region, they are easy to culture on solid substrate fermentation, they can grow on the surface of an inorganic substrate during culture leading to an efficient distribution of metals as a catalyst. The main advantage of nanoparticles extracellularly from fungi is that a large quantity of enzyme which is in a pure state and free from cellular protein can be easy to apply for the simple downstream process. *Phoma glomerata* has been identified to produce silver nanoparticles, and its activity against *E.coli, S. aureus* and *P. aeruginosa* has been reported (Birla et al. 2009). Bioreduction of aqueous $AuCl^{4+}$ was done by using the fungus *Verticillium* sp. that produces gold nanoparticles with well-defined dimensions and good monodispersity. Investigations carried out on 20 different fungi reveals that fungi are extremely good candidates in the synthesis of metal and metal sulphides nanoparticles. The genus *Penicillium* seems to have a promising candidate for the silver nanoparticle synthesis, where production proceeds *via* extracellular mechanism (Sadowski et al. 2008). *Humicola sp.* has been reported to synthesize highly stable, protein capped silver nanoparticles which were non-toxic to cancer cells as well (Syed et al. 2013).

NANOPARTICLE SYNTHESIS IN ACTINOMYCETES, YEAST AND ALGAE

Actinomycetes are the class of microorganisms that has some of the properties of fungi and bacteria. Actinomycetes are now getting important for the synthesis of metallic nanoparticles because of their characteristic to produce secondary metabolites such as antibiotics. Synthesis of Au nanoparticles by using the extremophilic actinomycete, *Thermomonospora* sp which yielded polydisperse Au nanoparticles has been investigated by Sastry et al. (2003). The intracellular synthesis of Au nanoparticles by using alkalotolerant *Rhodococcus* sp. have been reported. It was observed that the concentration of nanoparticles was more on the cytoplasmic membrane than on the cell wall. This may be due to the reduction of the metal ions by enzymes present in the cell wall and on the cytoplasmic membrane but not in the cytosol (Ahmad et al. 2011).

The synthesis of cadmium nanoparticles by using *Candida glabrata* and *Schizosaccharomyce pombe* has been reported by Dameron et al. (1989). Kowshik et al. (2003) have identified yeast *Torulopsis* sp. being capable of intracellular synthesis of PbS crystallite when exposed to aqueous Pb^{2+} ions and CdS nanoparticles synthesized intracellularly by using *Schizosaccharomyces pombe* (yeast cells). The silver and gold nanoparticles biosynthesis was also investigated (Mourato et al. 2011) by taking an extremophilic yeast strain that was isolated from acid mine drainage. Algae are a diverse group in the plant kingdom that are also being explored. Hosea et al. (1986) investigated the gold nanoparticles on the algae *Chlorella vulgaris*. The rapid formation of Au nanoparticles through extracellular biosynthesis in marine alga *Sargassum wightii* was investigated. Scarano et al. (2003) reported the fabrication of phytochelatin coated CdS nanocrystals by using the phytoplanktonic alga *Phaeodactulum tricornatum*.

NANOPARTICLE SYNTHESIS IN PLANT EXTRACT

Biosynthesis reaction of nanoparticles is an important branch of bio-production of nanoparticles with the use of plant extract. As the size, dispersity and shape of nanoparticles can be controlled by the biosynthetic processes for nanoparticles when nanoparticles were produced extracellular using plants. Plants use can also be suitably scaled up for large-scale synthesis of nanoparticles. Gardea-Torresdey et al. (2002) firstly reported the preparation of gold and silver nanoparticles by living alfalfa plants. Some specific plant parts or the whole plant, specially angiospermic plants, are used for the great synthesis of nanoparticle (Kumar et al. 2014). Fabrication of inorganic nanoparticles by plants is rapid, cost-effective and eco-friendly process (Kavitha et al. 2013). The synthesis can be done by both intra and extracellular methods, such as leaf broth (Shivshankar et al. 2004, Lalitha et al. 2013, Ahmed et al. 2015, Ahmed et al. 2016), sun-dried leaves (Senthilkumar et al. 2014), fruits (Dubey et. al. 2010), seeds, bark, root etc. Metal nanoparticles prepared by chemical and biological methods use reducing agents for the reduction of metal ions and protective agents or phase transfer agents to stabilize the nanoparticle. Biosynthesis of metal nanoparticles, using plant leaf material as reductants as well as capping agent, is currently under exploitation. It is an eco-friendly, cost-effective and more efficient alternative method for large scale synthesis of metal nanoparticles.

During the process of production of nano-particles, the plant extract is simply mixed with a solution of metal salt at room temperature. It is a quick reaction and usually takes minutes to complete. Nanoparticle properties and production time depend on various characteristics of plant extract, namely its concentration, the concentration of the metal salt, pH, temperature and contact time. Another good advantage for taking the plant extract is that the plants supplement both the reducing as well as stabilizing agents for the nanoparticles which otherwise have to be externally added in other methods. Present studies have depicted that the therapeutic effects of plants, from which the nanoparticles are being prepared, provide the perfect vehicles to act upon the site of action and eliminate the need to artificially develop a drug for that specific ailment.

FACTOR AFFECTING THE NANOPARTICLE SYNTHESIS

Several factors influence the reduction process of metal ions into NPs. Various optimum bio-reduction conditions are substrate and biocatalyst concentration, electron donor capacity, pH, exposure time, temperature, buffer strength, mixing speed, light that can be controlled. The (nano) environmental conditions determine the average size and size distribution of NPs, which is a very important feature for the technological use of such materials. However, very little has so far been elucidated for living plants.

The pH value of the medium influences the size of nanoparticles under formation in both extracts and living plants. For example, the size of gold nanoparticles was controlled by altering the pH of the medium in *Avena sativa* (Shankar et al. 2007). However, pH has a major impact on the size rather than on the shape of the nanoparticle formed. The reaction mechanism for the formation of magnetite nanoparticles has been found to be influenced by pH when co-precipitation method was followed (Armendariz et al. 2004). One of the most interesting aspects of NPs biosynthesis is the fact that this process occurs at ambient temperature. However, the temperature of the reaction medium is a critical factor that determines the nature of nanoparticles formed. For the production of anisotropic nanoparticles with the fine-tuning of the shape, size and optical properties temperature variations in reaction conditions are also an important factor. When *Cymbopogon flexuosus* was evaluated to produce gold nanoparticles at higher temperatures, the percentage of gold nano triangles relative to spherical particles were significantly reduced at high temperature, whereas low temperature mostly promoted nano triangle formation (Faiyas et al. 2010, Rai et al. 2006). The size of gold nanoparticles was shown to increase at higher reaction temperatures as explained by an increase in fusion efficiency of micelles which dissipates supersaturation (Muralidharan et al. 2011). Some other factors also play a remarkable role in nanoparticle synthesis. According to the Schikorr reaction, the size and crystallinity of magnetite nanoparticles were found to increase with increasing molar ratios of ferric/ ferrous ions during synthesis by the hydrothermal synthesis method (Mizutani et al. 2008). The polyol and water-soluble heterocyclic components were mainly responsible for the reduction of silver ions or chloroaurate ions (Huang et al. 2007). An incubation of sun-dried biomass of *Cinnamomum camphora* leaf with aqueous silver or gold precursors at ambient temperature produces both silver nanoparticles (55-80 nm) and triangular or spherical gold nanoparticles. The major difference in the shape of silver and gold nanoparticles could be ascribed to the comparative potential of protective and reductive biomolecules from leaf extracts. The concentration of the substrate also affects the size of the nanoparticle. The sizes of gold nanoparticles decrease with increasing NaCl concentrations (size ranges, 5-16 nm) than those synthesized without the addition of NaCl (size ranges 11-32 nm) (Mohamad et al. 2011). Several other factors have also been reported that affect the geometry and size of metal nanoparticles (Soni et al. 2001, Vijayaraghavana et al. 2017).

PLANT METABOLITES IN THE BINDING AND REDUCTION OF METAL ION

Various plant metabolites for e.g. alkaloids, sugars, terpenoids, phenolic compounds, polyphenols and proteins having an important role in the synthesis of nanoparticles via the bioreduction of metal ions.

Flavonoids contain various nanoparticle groups capable of nanoparticle formation (Singh et al. 2018). It has been suggested that the tautomeric transformation of flavonoids from the enol-form to the keto-form may release a reactive hydrogen atom that can reduce metal ions to form nanoparticles. Ahmed et al. (2010) reported that the formation of silver nanoparticles in *Ocimum basilicum* (sweet basil) extracts transform flavonoids, luteolin and rosmarinic acid from the enol- to the keto-form. Some flavonoids are capable to chelate metal ions with their carbonyl groups or π-electrons. For example, quercetin is a flavonoid with very strong chelating activity, because it can chelate at three positions involving the carbonyl and hydroxyls at the C3 and C5 positions and the catechol group at the C3' and C4' site. These groups chelate various metal ions such as Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+}, Pb^{2+}, and Co^{2+}. The presence of such mechanisms may help the flavonoids to be adsorbed onto the surface of a nascent nanoparticle. It has been postulated the terpenoids are often associated with nanoparticles. Terpenoids are a class of organic polymers synthesized in plants from five-carbon isoprene units, which show strong antioxidant activity (Shankar et al. 2003). Initially, it was suggested that terpenoids play a key role in the transformation of silver ions into nanoparticles in reactions using extracts from geranium leaves. The main terpenoid i.e. Eugenol, in *Cinnamomum zeylanisum* (cinnamon) extracts, was investigated to play the main role in the bioreduction of $HAuCl_4$ and $AgNO_3$ to nanoparticles (Singh et al. 2010). It was suggested that dissociation of a proton of the eugenol OH-group results in the formation of resonance structures capable of further oxidation. This process is accompanied by the active reduction of metal ions, followed by nanoparticle formation.

The sugars present in plant extracts can also act as a catalyst in the formation of metal nanoparticles. It is known that monosaccharides such as glucose-containing an aldehyde group can act as reducing agents. Monosaccharides containing a keto-group, e.g. fructose, can act as antioxidants only when they have undergone a series of tautomeric transformations from a ketone to an aldehyde. The reducing ability of disaccharides and polysaccharides depends on the ability of individual monosaccharide components to form an open-chain form within an oligomer and hence, to provide access (of a metal ion) to an aldehyde group. Glucose is able to participate in the synthesis of metal nanoparticles of different morphologies, whereas fructose facilitates the synthesis of monodispersed nanoparticles of gold and silver. It was shown (Panigrahi et al. 2004) that sucrose is not too capable to reduce silver nitrate or palladium chloride into nanoparticles. An open chain-form structured produced by the acid hydrolysis of sucrose into free glucose are responsible for the synthesis of nanoparticles with the replacement of metal by tetrachloroauric and tetrachloroplatinic acids (Makarov et al. 2014). Nowadays it is believed that the sugar aldehyde group is oxidized into a carboxyl group via the nucleophilic addition of OH-, which in turn gives to the reduction of metal ions and capable of the formation of nanoparticles.

Peptides, proteins and amino acids present in plant extracts probably play a very important role in determining the shape of nanoparticles and affect the overall yield of nanoparticles. It was investigated that protein molecules that help in the formation of nanoparticles from metal ions show high reducing activity and a high potential for attracting metal ions to the regions of a molecule that are responsible for reduction, but that their chelating activity is not excessive. Amino acid sequence of a protein can greatly affect the size, amount and morphology of nanoparticles. They can bind to the metal ions through the amino and carbonyl groups of the main chain or through side chains, such as the carboxyl groups of aspartic and glutamic acid or a nitrogen atom of the imidazole ring of histidine. Other side chains binding metal ions include the thiol, thioether, hydroxyl and carbonyl groups (Glusker et al. 1999). Side thiol groups and amino groups are also responsible for the reduction of metal ions. Few phenolics, lipids, terpenoids, biopolymers, ascorbic acid, hydroxypropyl starch and lignin have also been reported for the binding and reduction of several metal ions (El-Rafie et al. 2011, Kumari et al. 2016, Iravani et al. 2020).

EXPERIMENTAL PROCEDURE INVOLVED IN THE SYNTHESIS OF NANOPARTICLES USING PLANT EXTRACT

The use of plant extract for biosynthesis reaction for the production of nanoparticles was fairly investigated and described. The leaf reductants present in leaf extracts are mainly used in the preparation of metal nanoparticles. Plants act as a suitable vehicle for the synthesis of metal nano-particle and they are formed directly in living plants by reduction of the metal ions absorbed as a soluble salt. The main mechanism considered for the synthesis of nanoparticles mediated by the plants is due to the presence of phytochemicals. The major phytochemicals responsible for the spontaneous reduction of ions are flavonoids,

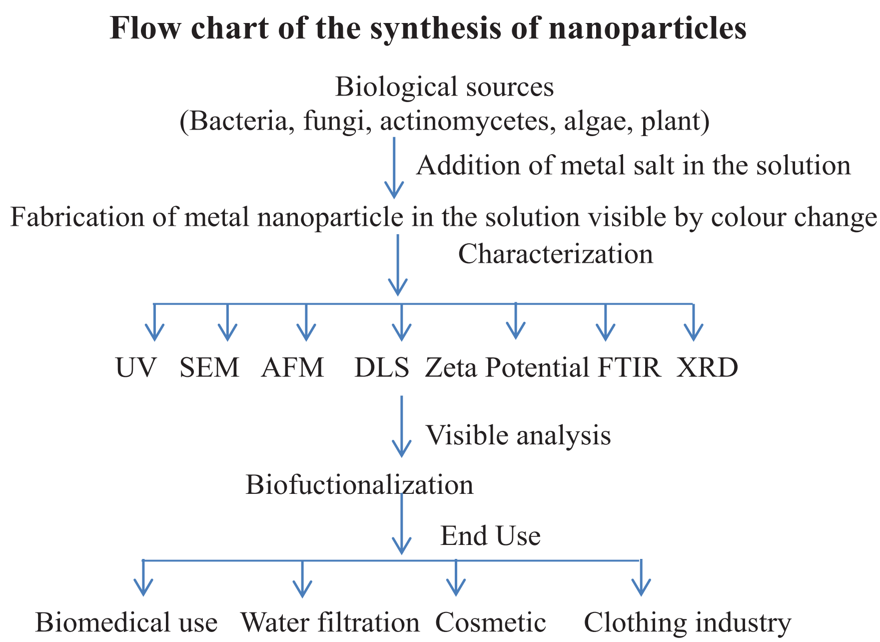


Fig 1: General procedure for synthesis of nanoparticles.

terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides. The aqueous solution of metal nitrate (mM) is treated with plant extract at ambient temperature and filtered. The supernatant is heated between 50-85°C leading to the formation of nanoparticles. The change in the colour is monitored through a UV-VIS spectrophotometer. SEM, EDAX measurements are performed to estimate the size, shape and quantity of the nanoparticles

BIOLOGICAL PERSPECTIVE OF VARIOUS METALLIC NANO-PARTICLES

The green synthesis of silver nanoparticles and its application for mosquito control has been investigated by Mondal et al. (2014). For the generation of maximum stable nanoparticles in an aqueous medium, it was found that aqueous silver ions can be reduced by aqueous root extract of *P. hysterophorus.* Larvae were exposed to varying concentration of plant extract, aqueous silver nitrate solution and synthesized silver nanoparticles for 0, 24 and 48 hours separately. Aqueous root extract depicted moderate larvicidal effects; however, the maximum efficacy (60.18%) was seen with the prepared silver nanoparticles against the larvae of *Culex quinquefasciatus*.

Zinc oxide nanoparticles have been reported to be incorporated in polymeric matrices in order to provide an antimicrobial activity to packaging material and improve packaging properties (Espitia et al. 2012). Colloidal nanoparticles give good responses to incident light, making them useful as sensors. The potential uses of nanoparticle biosensors in research and diagnosis. Recently the nanoparticles have been employed for ultrasensitive detection of cancer in human serum. Not only diagnosis but silver, gold and zinc oxide nanoparticles have been reported to show anticancer activity (Mirzaei et al. 2017). D'Britto et al. (2012) investigated the medicinal plant extracts used for blood sugar and obesity therapy and exhibit excellent inhibition of invertase activity. Extract of *Azardirecta indica, Cephalandra indica, Calotropis procera and Syzygium jambolanum,* prepared together, is an elixir for the treatment of blood sugar and obesity by homoeopathic medicine. It is established that inhibitors of ▯-glucosidases such as invertase, ▯ amylase etc, can suppress the digestion and adsorption of carbohydrates and also inhibit postprandial hyperglycemia and thus helps in Diabetes therapy. In this study, the invertase inhibitor action of the elixir was tested in order to put more light on the therapeutic mechanism of this elixir. Moreover, rapid bio-reduction of Au^{3+} and Ag^{+} ions to their respective nanoparticles was achieved using the same extract of these medicinal plants. Cytotoxicity (MTT assay) and genotoxicity (COMET assay) experiments have been performed on these nanomaterials. Invertase inhibition activity was excellent. MTT results proved the bio-compatible nature of these biochemically synthesized gold and silver nanoparticles

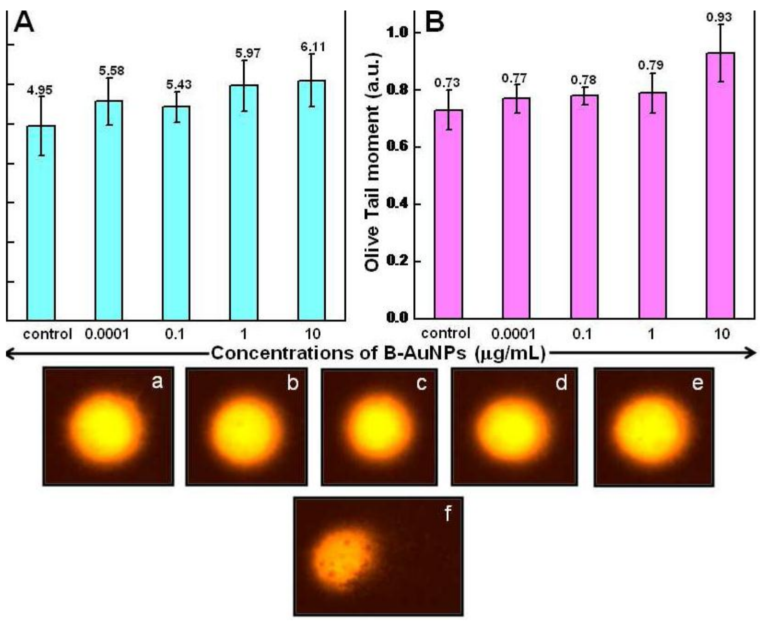


Fig. 2: COMET results obtained from 3 h exposure of HepG2 cells with different concentrations of BAuNPs. Two comet parameters, % tail DNA (A) and Olive tail moment (B) were considered as a measure of DNA damage. Figure a, b, c, d and e are images of comet showing the pattern of DNA after the exposure of HepG2 cells to 0 <g/mL, 0.0001 <g/mL, 0.1 <g/mL, 1 <g/mL and 10 <g/mL concentration of B-AuNPs respectively. Figure 'f' represents the comet pattern obtained after treatment with100 <g/mL of B-AuNPs.

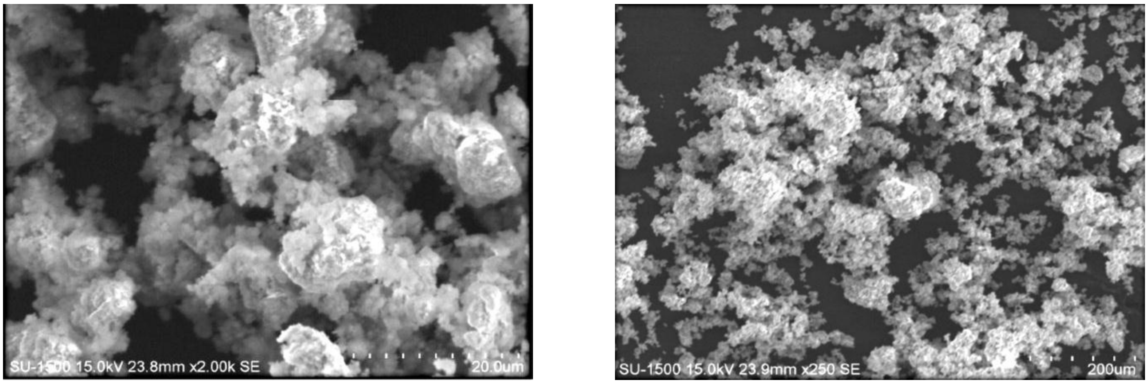
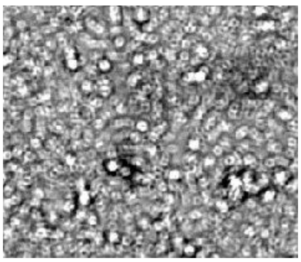
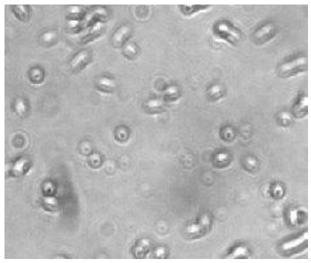
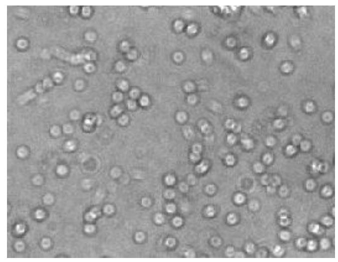
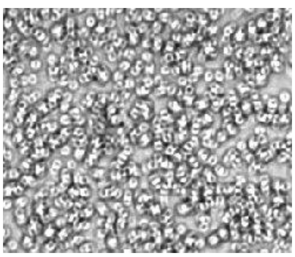
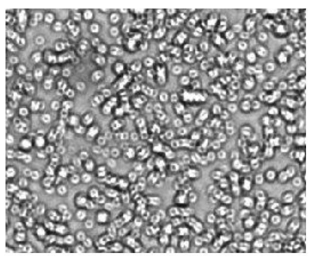
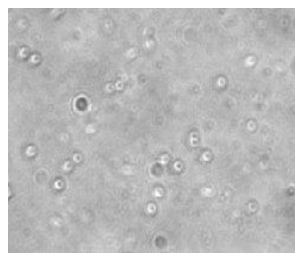


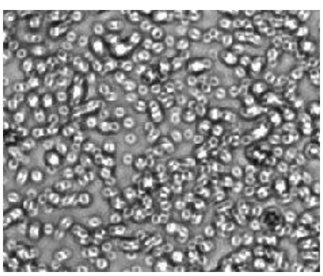
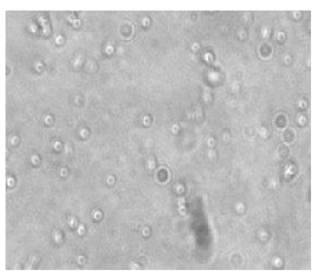
Fig. 3: SEM images of zinc oxide nanoparticles.

. (a) (b) (c)

(d) (e) (f)

(g) ***(h)***

Fig. 4: Effect of silver nanoparticles on in biofilm quenching and prevention of S.aureus biofilm formation under inverted microscope (40X) (a): Negative control (intact biofilm); (b): Positive control (Biofilm Quenched using 20% SDS); (c): Silver nanoparticles; (d): Gentamicin (10>g/mL); (e): Chloramphenicol (20>g/mL); (f): SilverNanoparticles + Gentamicin (10>g/mL); (g): 1mMsilver nitrate; (h): Silver nanoparticles in prevention of biofilm formation.

upto 10-4M concentration towards HepG2 cells. The overall study suggested that these biogenic metal nanoparticles are non-cytotoxic and non-genotoxic upto 10-4M.

Green synthesis of ZnO nanoparticles by *Calotropis gigantea* was reported by Ca et al. (2013). The finding focused on the green synthesis of ZnO nanoparticles by utilizing the bio components of leaves extract of *Calotropis gigantean* and Zinc nitrate. The ZnO nanocrystallites of an average size range of 30-35 nm have been synthesized by a rapid, simple and eco-friendly method. Zinc nanoparticles were characterized using scanning electron microscopy (SEM) and X-ray diffraction (XRD). The particles obtained are spherical in nature and are agglomerates of nanocrystallite. The X-ray patterns show a hexagonal crystal type for ZnO. The results coincide with the literature XRD pattern for hexagonal wurtzite ZnO. The size of nanocrystallites is calculated by considering XRD data by Debye-Scherrer's Formula.

Alumina nanoparticles (AlNP) were synthesized from aluminium nitrate using extracts of tea, coffee and triphala a well known herbal plant as well as a nontoxic and eco-friendly green material (Sutradhar et al. 2013). In addition, excellent reproducibility of these nanoparticles, without the use of any additional capping agent or stabilizer will have great advantages in comparison with microbial synthesis, avoiding all the tedious and hygienic complications.

Chaudhari et al. (2012) investigated the prevention of biofilm formation, quenching and effect of biosynthesized silver nanoparticles on *Staphylococcus aureus* The synthesis of silver nanoparticles was done by using *B. megaterium* supernatant and 1 mM silver nitrate. These silver nanoparticles showed enhanced quorum quenching activity against *Staphylococcus aureus* biofilm and prevention of biofilm formation which can be seen under an inverted microscope (40 X). The synergistic effect of silver nanoparticles along with antibiotics in biofilm quenching was found to be effective.

FUTURE DIRECTIONS AND CONCLUSION

Increasing awareness towards green chemistry and biological processes has led to a desired and influenced process that is environment-friendly for the synthesis of non-toxic nanoparticles. The biological agents in the form of algae, plants and microbes have emerged as an efficient candidate for the synthesis of nanoparticles. On account of the rich biodiversity of plants, mediated nanoparticle synthesis has become a subject of interest around the globe with different plant species being rapidly explored and evaluated for synthesizing nanoparticles. Plants or their extracts can be efficiently used in the synthesis of nanoparticles as a greener route. Control over the shape and size of nanoparticles seems to be very easy with the use of plants. The synthesis of metal nanoparticles in plant extracts (plant biomasses), despite obvious limitations, has significant potential and a number of substantial advantages relative to traditional methods of nanoparticle synthesis. However, to go in for cost-effectively with nanoparticles prepared via physical and chemical methods, it is necessary to scale these methods of nanoparticle production using plant material and to develop schemes for keeping expenses in check during their synthesis. Promoting the biosynthesis of nanoparticles can influence the commercial applications of these nanoparticles in the field of pharmaceuticals and other medical sciences Nanoparticles synthesized via plants have been used for human benefit. The mechanism of plants in synthesizing nanoparticles is yet to be completely elucidated. Auxiliary research in this field can further increase the potential and scope in the biosynthesis of nanoparticles. Future research on plant-mediated biological synthesis of nanoparticles with unique optoelectronics, physicochemical and electronic properties are of great importance for applications in the areas of chemistry, electronics, electrochemical sensor, biosensors, medicine, healthcare and agriculture. Further progress is desirable in order to revolve the impression of nanoparticle technology into a rational practical approach.

REFERENCES

Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M.I., Kumar, R. and Sastry, M. 2003a. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum.* Colloids. Surf. B 28: 313-318.

Ahmad, A., Senapati, S., Khan, M.I., Kumar, R. and Sastry, M. 2003b. Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, *Thermomonospora sp*. Langmuir, 19: 3350-3553.

Ahmad, N., Sharma, S., Alam, M.K., Singh, V.N., Shamsi, S.F., Mehta, B.R. and Fatma, A. 2010. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids Surf. B. Biointerfaces., 81: 81-86.

Ahmad, N., Sharma, S., Singh, V.N., Shamsi, S.F., Fatma, A. and Mehta, B.R. 2011. Biosynthesis of silver nanoparticles from *Desmodium triflorum:* a novel approach towards weed utilization. Biotechnol. Res. Int. 2011, Article ID 454090 1-8.

Ahmed, M.J., Murtaza, G., Mehmood. A. and Bhatti, T.M. 2015. Green synthesis of silver nanoparticles using leaves extract of *Skimmia laureola*: Characterization and Antibacterial Activity. Mater. Lett., 153: 10-13.

Ahmed, S., Ahmad, M., LalSwami, B. and Ikram, S. 2016. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. J. Adv. Res., 28-17 :7.

Armendariz, V., Herrera, I., Peralta-Videa, J.R., Yacaman, M.J., Troiani, H., Santiago, P. and Gardea-Torresdey, J.L. 2004. Size controlled gold nanoparticle formation by *Avena sativa* biomass: use of plants in nanobiotechnology. J. Nanopart. Res., 6: 377-82.

Arshad, A. 2017. Bacterial synthesis and applications of nanoparticles. Nano. Sci. Nano. Technol., 11: 119.

Bar, H., Bhui, D.K., Sahoo, G.P. Sarkar, P. Pyne, S. and Misra, A. 2009. Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. Colloids Surf. A: Physicochem. Eng. Asp., 348: 212-216.

Birla, S.S., Tiwari, V.V., Gade, A.K., Ingle, A.P., Yadav, A.P. and Rai, M.K. 2009. Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Lett. Appl. Microbiol., 48: 173-179.

Ca, V., Hiremath, S., Chandraprabha, M.N., Antonyraj, M.A.L., Gopal, I.V., Jain, A.A. and Bansal, A. K. 2013. Green synthesis of ZnO nanoparticles by Calotropis Gigantea. Proceedings of National Conference on "Women in Science & Engineering" (NCWSE 2013), SDMCET Dharwad, pp. 112-116.

Chakraborty, N., Banerjee, A., Lahiri, S., Panda, A., Ghosh, A.N. and Pal, R. 2009. Biorecovery of gold using cyanobacteria and eukaryotic alga with special reference to nanogold formation – a novel phenomenon. J. Appl. Phycol., 21: 145-152.

Chandrasekharan, N. and Kamat, P.V. 2000. Improving the photoelectrochemical performance of nanostructured TiO2 films by adsorption of gold nanoparticles. J. Phys. Chem. B., 104: 10851-10857.

Chaudhari, P.R., Masurkar, S.A., Shidore, V.B., Suresh, P. and Kamble, S.P. 2012. Effect of biosynthesized silver nanoparticles on *staphylococcus aureus* biofilm quenching and prevention of biofilm formation. International Journal of Pharma and Bio Sciences, 3: 222-229.

D'Britto, V., Devi, P.P., Prasad, B.V., Dhawan, A., Mantri, V.G. and Prabhune, A. 2012. Medicinal plant extracts used for blood sugar and obesity therapy shows excellent inhibition of invertase activity: synthesis of nanoparticles using this extract and its cytotoxic and genotoxic effects. Int. J. life sci. Pharma Res., 2: 61-74.

Dameron, C.T., Reese, R.N., Mehra, R.K., Kortan, A.R., Carroll, P.J. Steigerwald, M.L. Brus, L.E. and Winge, D.R. 1989. Biosynthesis of cadmium sulphide quantum semiconductor crystallites. Nature, 338: 596-597.

Das, R.K., Gogoi, N. and Bora, U. 2011. Green synthesis of gold nanoparticles using *Nyctanthes arbortristis* flower extract. Bioprocess Biosyst. Engg., 34: 615-619.

Dubey, S.P., Lahtinen, M. and Sillanp, M. 2010. Tansy fruit mediated greener synthesis of silver and gold nanoparticles. Proc. Biochem., 45: 1065-1071.

El-Rafie, M.H., El-Naggar, M.E., Ramadan, M.A. Fouda, M.M.G. and Al-Deyab, S.S. 2011. Environmental synthesis of silver nanoparticles using hydroxypropyl starch and their characterization. Carbohydrate Polymers, 86: 630-635.

Espitia, P.J.P., Soares, N.F.F., Coimbra, J.S.R., Andrade, N.J., Cruz, R.S. and Medeiros, E.A.A. 2012. Zinc oxide nanoparticles: synthesis, antimicrobial activity and food packaging applications. Food Bioprocess. Technol., 5: 1447-1464.

Faiyas, A.P.A., Vinod, E.M., Joseph, J., Ganesan, R. and Pandey, R.K. 2010. Dependence of pH and surfactant effect in the synthesis of magnetite (Fe_3O_4) nanoparticles and its properties. J. Magn. Magne. Mater., 322: 400-404.

Gardea-Torresdey, J.L., Parsons, J.G., Gomez, E., Peralta-Videa, J., Troiani, H.E., Santiago, P. and Jose-Yacaman, M. 2002. Formation and growth of Au nanoparticles inside live alfalfa plants. Nano. Lett., 2: 397-401.

Glusker, J., Katz, A. and Bock, C.W. 1999. Metal ions in biological systems. Rigaku J., 16 : 8–16.

Gour, A. and Jain, N.A. 2019. Advances in green synthesis of nanoparticles. Artificial Cells, Nanomedicine, and Biotechnology, 47(1): 844-851.

Hosea, M., Greene, B., McPherson, R., Henzl, M., Alexander, M.D. and Darnall, D.W. 1986. Accumulation of elemental gold on the alga *Chlorella vulgari.* Inorganica Chimica Acta, 123: 161-165.

Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., Wang, H., Wang, Y., Shao, W., He, N., Hong, J. and Chen, C. 2007. Biosynthesis of silver and gold nanoparticles by novel sun dried *Cinnamomum camphora* leaf. Nanotechnol., 18: 105104-105114.

Husseiny, M.I., El-Aziz, M.A., Badr, Y. and Mahmoud, M.A. 2007. Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*. Spectrochim. Acta A: Mol. Biomol. Spectrosc., 67: 1003-1006.

Iravani, S. and Varma R.S. 2020. Greener synthesis of lignin nanoparticles and their applications. Green Chemistry, *22*(3): 612-636.

Jonge, S.H., Yeo, S.Y. and Yi, S.C. 2005. The effect of filler particle size on the antibacterial properties of compounded polymer silver fibers. J. Mat. Sci., 40: 5407-5411.

Kathiresan, K., Alikunhi, N.M., Pathmanaban, S., Nabikhan, A. and Kandasamy, S. 2010. Analysis of antimicrobial silver nanoparticles synthesized by coastal strains of *Escherichia coli* and *Aspergillus niger*. Can. J. Microbiol., 56: 1050-1059.

Kavitha, K.S., Syed, B., Rakshith, D., Kavitha, H.U., Rao, H.C.Y., Harini, B.P. and Satish, S. 2013. Plants as green source towards synthesis of nanoparticles. Int. Res. J. Bio. Sci., 2: 66-76.

Kowshik, M., Ashtaputre, S., Kharrazi, S., Vogel, W., Urban, J., Kulkarni, S.K. and Paknikar, K.M. 2003. Extracellular synthesis of silver nanoparticles by a silver- tolerant yeast strain MKY3. Nanotechnol., 14: 95-100.

Krolikowska, A., Kudelski, A., Michota, A. and Bukowska, J. 2003. SERS studies on the structure of thioglycolic acid monolayers on silver and gold. Surf. Sci., 532: 227-232.

Kumar, A.S., Ansary, A.A., Ahmad, A. and Khan, M.I. 2007. Extracellular biosynthesis of CdSe quantum dots by the fungus, *Fusarium Oxysporum.* J. Biomed. Nanotechnol., 3: 190-194.

Kumar, V., Pammi, P.P., Kollu, S.V., Satyanarayana, P. and Shameem, K.V. 2014. Green synthesis and characterization of silver nanoparticles using Boerhaavia diffusa plant extract and their antibacterial activity. Ind. Crops Prod., 52: 562-566.

Kumari, R., Singh, J.S. and Singh, D.P. 2016. Biogenic synthesis and spatial distribution of silver nanoparticles in the legume mungbean plant (*Vigna radiata* L.). Plant Physiology and Biochemistry, 110: 158-166.

Lalitha, A., Subbaiya, R. and Ponmurugan, P. 2013. Green synthesis of silver nanoparticles from leaf extract Azhadirachta indica and to study its anti-bacterial and antioxidant property. Int. J. Curr. Microbiol. App. Sci., 2: 228-235.

Lin, S.M., Lin, F.Q., Guo, H.Q., Zhang, Z.H. and Wang, Z.G. 2000. Surface states induced photoluminescence from Mn^{2+} doped CdS nanoparticles. Solid State Common, 115: 615-618.

Makarov, V.V., Love, A.J., Sinitsyna, O.V. et al. 2014. "Green" nanotechnologies: synthesis of metal nanoparticles using plants. Acta Naturae, 6(1): 35-44.

Mirzaei, H. and Darroudi, M. 2017. Zinc oxide nanoparticles: Biological synthesis and biomedical applications. Ceramics International, 43: 907-914.

Mizutani, N., Iwasaki, T., Watano, S., Yanagida, T., Tanaka, H. and Kawai, T. 2008. Effect of ferrous/ferric ions molar ratio on reaction mechanism for hydrothermal synthesis of magnetite nanoparticles. Bull. Mater. Sci., 31: 713-717.

Mohamad, M.F., Kamarudin, K.S.N., Fathilah, N.N.F.N.M. and Salleh, M.M. 2011. The effects of sodium chloride in the formation of size and shape of gold (Au) nanoparticles by microwave-polyol method for mercury adsorption. World Acad. Sci. Eng. Technol., 74: 691-695.

Mohanpuria, P., Rana, K.N. and Yadav, S.K. 2008. Biosynthesis of nanoparticles: Technological concepts and future applications. J. Nanopart. Res., 10: 507-517.

Mondal, N.K., Chowdhury, A., Dey, U., Mukhopadhya, P., Chatterjee, S., Das, K. and Datta, J.K. 2014. Green synthesis of silver nanoparticles and its application for mosquito control. Asian Pac. J. Trop. Dis., 4: 204-210.

Mourato, A., Gadanho, M. Lino, A.R. and Tenreiro, R. 2011. Biosynthesis of crystalline silver and gold nanoparticles by extremophilic yeasts. Bioinorg. Chem. Appl., Article ID 546074, 8.

Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S.R. Khan, M.I., Parishcha, R. Ajaykumar, P.V., Alam, M., Kumar, R. and Sastry, M. 2001. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. Nano. Lett., 1: 515.

Muralidharan, G., Subramanian, L., Nallamuthu, S.K., Santhanam, V. and Kumar, S. 2011. Effect of reagent addition rate and temperature on synthesis of gold nanoparticles in microemulsion route. Ind. Eng. Chem. Res., 50: 8786-8791.

Nair, B. and Pradeep, T. 2002. Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* strains. Crystal Growth and Design, 2: 293-298.

Panigrahi, S., Kundu, S., Ghosh, S., Nath, S. and Pal, T. 2004. General method of synthesis for metal nanoparticles. J. Nanoparticle Res., 6: 411-414.

Prasad, K., Jha, A.K. and Kulkarni, A.R. 2007. *Lactobacillus* assisted synthesis of titanium nanoparticles. Nanoscale Res. Lett., 2: 248-250.

Rai, A., Singh, A., Ahmad, A. and Sastry, M. 2006. Role of halide ions and temperature on the morphology of biologically synthesized gold nanotriangles. Langmuir, 22: 736-741.

Ramanathan, R., Field, M.R., O'Mullane, A.P., Smooker, P.M., Bhargava, S.K. and Bansal, V. 2013. Aqueous phase synthesis of copper nanoparticles: a link between heavy metal resistance and nanoparticle synthesis ability in bacterial systems. Nanoscale, 5(6): 2300-2306.

Sadowski, Z., Maliszewska, I.H., Grochowalska, B., Polowczyk, I. and Kozlecki, T. 2008. Synthesis of silver nanoparticles using microorganisms. Materials Science-Poland, 26: 419-424.

Sastry, M., Ahmad, A., Khan, M.I. and Kumar, R. 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr. Sci., 85: 162-170.

Scarano, G. and Morelli, E. 2003. Properties of phytochelatin-coated CdS nanocrystallites formed in a marine phytoplanktonic alga (*Phaeodactylum tricornutum, Bohlin*) in response to Cd. Plant Science, 165: 803-810.

Senthilkumar, S.R. and Sivakumar, T. 2014. Green tea (Camellia Sinensis) mediated synthesis of zinc oxide (ZnO) nanoparticles and studies on their antimicrobial activities. Int. J. Pharm. Pharm. Sci., 6: 461-465.

Shahi, S.K. and Patra, M. 2003. Microbially synthesized bioactive nanoparticles and their formulation active against human pathogenic fungi. Rev. Adv. Mater. Sci., 5: 501-509.

Shahverdi, A.R., Minaeian, S., Shahverdi, H.R., Jamalifar, H. and Nohi, A.A. 2007. Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteriaceae: A novel biological approach. Process Biochem., 42: 919-923.

Shankar, S.S., Ahmad, A., Pasricha, R. and Sastry, M.J. 2003. Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. Mater. Chem., 13: 1822-1846.

Sharma, N., Pinnaka, A.K., Raje, M., Fnu, A., Bhattacharyya, M.S. and Choudhary, A.R. 2012. Exploitation of marine bacteria for production of gold nanoparticles. Microb. Cell. Fact., 11: 86.

Sharma,V.K., Yngard, R.A. and Lin, Y. 2009. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv. Colloid Interface Sci., 145: 83-96.

Shirley, A., Dayananda, B., Sreedhar, B., Syed, G. and Dastager, C.C. 2010. Antimicrobial activity of silver nanoparticles synthesized from novel *Streptomyces* species. Dig. J. Nanomater. Biostruct., 5: 447-451.

Shivshankar, S., Ahmed, A., Ankamwar, B., Sastry, M., Rai, A. and Singh, A. 2004. Biological synthesis of triangular gold nanoprisms. Nature, 3: 482-488.

Singh, A.K., Talat, M., Singh, D.P. and Srivastava, O.N. 2010. Biosynthesis of gold and silver nanoparticles by natural precursor clove and their functionalization with amine group. J. Nanopart. Research, 12: 1667-1675.

Singh, J., Dutta, T., Kim. K.H., Rawat, M., Samddar, P. and Pawan Kumar. 2018. 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation. Journal of Nanobiotechnology, 16: 84.

Soni, N. and Prakash, S. 2011. Factors affecting the geometry of silver nanoparticles synthesis in *Chrysosporium Tropicum* and *Fusarium Oxysporum*, Amer. J. Nanotechnol., 2: 112-121.

Sunkar, S. and Nachiyar, C.V. 2012. Microbial synthesis and characterization of silver nanoparticles using the endophytic bacterium *Bacillus cereus*: a novel source in the benign synthesis. Global Journal of Medical Research, 12: 43-49.

Sutradhar, P., Debnath, N. and Saha, M. 2013. Microwave-assisted rapid synthesis of alumina nanoparticles using tea, coffee and triphala extracts. Adv. Manuf., 1: 357-361.

Syed, A., Saraswati, S., Kundu, G.C. and Ahmad, A. 2013. Biological synthesis of silver nanoparticles using the fungus *Humicola sp.* and evolution of their cytotoxicity using normal and cancer cell lines. Spectrochim. Acta. A Mol. Biomol. Spectrosc., 114: 144-147.

Thakkar, K.N., Mhatre, S.S. and Parikh, R.Y. 2010. Biological synthesis of metallic nanoparticles. Nanomed., 6: 257-262.

Vijayaraghavana, K. and Ashokkumar, T. 2017. Plant-mediated biosynthesis of metallic nanoparticles: A review of literature, factors affecting synthesis, characterization techniques and applications. Journal of Environmental Chemical Engineering, 5: 4866-4883.