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ORIGINAL ARTICLE

Biosynthesis of silver nanoparticles from *Aloe vera* leaf extract and antifungal activity against *Rhizopus* sp. and *Aspergillus* sp.

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Abstract Silver nanoparticles are receiving increasing attention in the field of agriculture. This study aims at evaluating the antifungal properties of green synthesised silver nanoparticles (AgNPs) from *Aloe vera* leaf extract against two pathogenic fungus *Rhizopus* sp. and *Aspergillus* sp. Results revealed that synthesised nanoparticles showed strong absorption maximum at 400 nm corresponding to the surface plasmon resonance. The prepared nanoparticles were characterized by SEM, FT-IR and UV–Vis spectroscopy. From the scanning photograph it is clear that particles are heterogeneous in shape such as rectangular, triangular and spherical with uniform distribution. FT-IR study showed sharp absorption peaks at 1,631 and 3,433 cm^{-1} for amide and alcoholic hydroxide groups, respectively. On the other hand, synthesised silver nanoparticles showed highest antifungal activity against *Aspergillus* sp. than *Rhizopus* sp. by application of 100 µL of 1 M silver nanoparticles with maximum inhibition of the growth of fungal hyphae. However, microscopic observation revealed that synthesised nanoparticles caused detrimental effects on conidial germination along with other deformations such as structure of cell membrane and inhibited normal budding process of both the tested species. Therefore, it has been concluded that *Aloe vera* leaf extract origin silver nanoparticles have tremendous potentiality towards controlling pathogenic fungus. However, further research is needed to check the efficacy of size-dependent AgNPs on different species of fungus.

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Introduction

The term nanoparticle is used to describe a particle with size in the range of 1–100 nm (Yehia and Al-Sheikh 2014). They tend to react differently than larger particles of the same composition because of their large surface area, thus allowing them to be used in novel applications (Abou et al. 2010). Moreover, they serve as the fundamental building block of nanotechnology (Vahabi et al. 2011). Nowadays there is a wide application of nanoparticles in diverse fields including catalysis, energy, chemistry and medicine (Yehia and Al-Sheikh 2014). Nanotechnology approaches to control disease in human and plants have recently been increasing greatly and the unique physicochemical properties of nano-sized metal particles make them successful in biology and medicine (Jo et al. 2012). The current understanding of potential risks associated with the release of these materials in the environment for human and animal health is still insufficient (Wang et al. 2012). However, very recently Verano-Braga et al. (2014) reported that the toxicity of AgNPs depends upon both dosage and particle size. Metal nanoparticles show large surface to volume ratio and exhibit antimicrobial properties due to their ability to interact with cellular membranes through disruption of cell wall structure (Ahmad et al. 2013; Trop et al. 2006). Especially silver has long been known for its strong toxicity against a wide range of micro organisms including bacteria and fungi (Narayanan and Park 2014). There are numerous methods for synthesis of silver nanoparticles, but, mostly used chemical methods,

including toxic chemicals and mostly non-polar solvent. Therefore, there is tremendous need for the development of clean and biocompatible as well as cost effective and sustainable method for synthesizing silver nanoparticles. According to Bansal et al. (2011) biological synthesis of silver nanoparticles is the novel approach. Many previous researchers highlighted the green synthesis of silver nanoparticles (Vahabi et al. 2011; Mondal et al. 2014; Sukirtha et al. 2012; Huang et al. 2007). Green synthesis of silver nanoparticle has some advantages towards the reduction of metal ions and their stability (Narayanan and Sakthivel 2010). Due to the presence of a myriad of biomolecules in plant metabolites possessing bioreduction and biostabilization ability, the exploration of such molecules could facilitate control over size and morphology of metal nanoparticles (Narayanan and Park 2014).

In this article, we report the 'rapid and green' method for the synthesis of silver metal nanoparticles (SNPs) using important medicinal plant *Aloe vera* and possible mechanism on the basis of the role played by the phytochemical constituents present in the plant extract. *Aloe vera* contains several groups of chemical constituents such as steroidal lactones, alkaloids, flavonoids and tannin. The plant system, therefore, was selected for fabrication of silver nanoparticles and its antifungal activity against *Rhizopus* sp. and *Aspergillus* sp.

Materials and methods

Preparation of plant extract

Fresh leaves of *Aloe vera* were collected from the garden of the Department of Environmental Science, the University of Burdwan, Burdwan. The leaves were washed with distilled water, and after grinding, 10 g leaves was mixed with 100 ml distilled water and heated for 12 min. Then the extract was filtered through Whatman filter paper, collected and stored in refrigerator.

Preparation of metal solution

Initially 1.575 g silver nitrate was dissolved in 1,000 ml distilled water.

Synthesis of nanoparticles

10 % *Aloe vera* plant extract was mixed with silver nitrate solution in 1:9 proportion and kept at room temperature for 48 h for the development of reddish brown colour.

Characterisation of silver nanoparticles

Colour change of nanoparticles

The reduction of pure Ag^{+} ions was monitored by measuring the UV–visible spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into distilled water. UV–visible spectral analysis was done by using UV–vis spectrophotometer (Perkin Elmer, Lamda 35).

Surface morphology of nanoparticles

SEM study

The solution of *Aloe vera* leaf extract in each beaker was dried and sent for scanning electron micrograph (SEM). The SEM characterization was carried out using a scanning electron microscope (Hitachi, S-530). Infrared photograph was recorded by Fourier transform infrared spectroscopy (FT-IR) (Bruker, Tensor 27) absorbance was measured by UV–vis spectrophotometer (Perkin Elmer, Lamda 35) and fluorescent spectrophotometer (SD 1000) (Mondal et al. 2014).

FT-IR study

FT-IR analysis was carried out on Tensor-27 (Bruker) in the diffuse reflectance mode operated at a resolution of 4 cm^{-1} in the range of 400 to 4,000 cm^{-1} to evaluate the functional groups that might be involved in nanoparticle formation.

Source of organism and composition of growth media

Broth preparation

1.3 g of nutrient broth was mixed with 100 ml distilled water and two drops of antibiotic was added. The conical flask was cotton plugged and autoclaved at 15 1b/$inch^2$ pressure and 121 °C for 15 min.

Inoculation

After cooling the broth medium, fungi were (*Aspergillus* sp., *Rhizopus* sp.) inoculated with a needle from a pure culture medium to the broth medium and were kept in 30 ± 1 °C temperature in incubator for 72 h.

Medium preparation and antifungal activity test

10 g dextrose monohydrate and 14 g nutrient agar were mixed with 500 ml of potato extract (10–12 %) and boiled; 3–4 drops antibiotic was added to prevent bacterial growth

and pH of the solution was maintained between 5 and 5.6. Then the agar media was poured into sterilized petri dishes and after solidification, 50 µl fungal broth culture was spread on each plate with the help of a spreader. Then a hole was made with a hole borer in each plate. 100 µl AgNPs solution only, only leaf extract and leaf extract + salt solution were poured in each hole of plate and kept for 48 h at 30 ± 1 °C temperature for further observation.

Microscopic observation

The antifungal activity of silver nanoparticles was observed under a light microscope (Nickon Eclipse 80i, Tokyo, Japan).

Statistical analysis

Results are presented with the help of figures and tables. The basic statistics were conducted with the help of SPSS 20.

Results and discussion

UV–Visible spectra analysis and colour change

The colour of synthesised AgNPs clearly changes to reddish brown within 72 h of incubation at room temperature (Fig. 1) and corresponding UV–Visible absorption spectrum of AgNPs was recorded in Fig. 2. The spectra of AgNPs showed maximum absorption at 400 nm to the surface plasmon resonance of the formed silver nanoparticles. The colour change was due to the excitation of SPR in the production of silver nanoparticles (Narayanan and Sakthivel 2008; Xiaoming et al. 2009). Previous reports

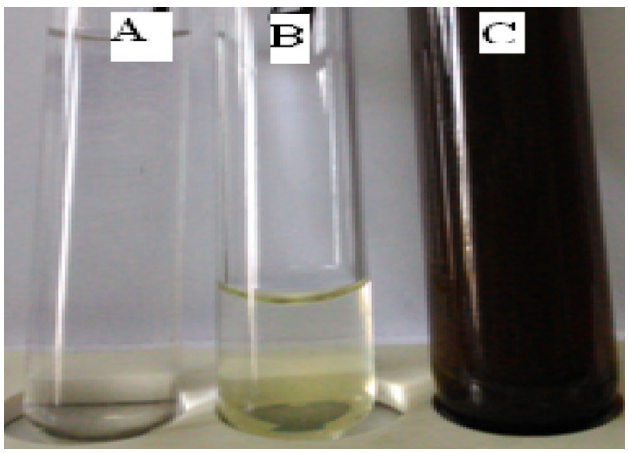

Fig. 1 Change of colour after 72 h **a** only $AgNO_3$ solution, **b** 5 % Aloe vera extract and **c** 3 mM $AgNO_3$ + 5 % Aloe vera extract

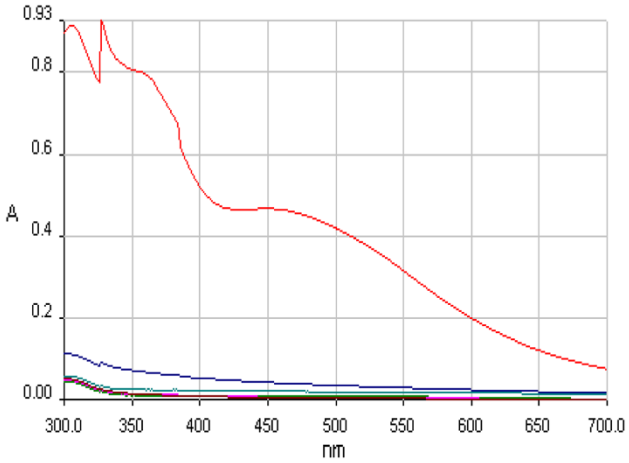

Fig. 2 UV–Visible spectra of silver nanoparticles

from Huang et al. (2007) on *C. camphora* show that silver nanoparticles may grow in a process involving rapid bioreduction and that they strongly influence the SPR in the water extract. This is accordance with the results obtained from bioreduction of silver nanoparticles using Spirulina Platensis, which showed that a SPR silver band occurred at 400–480 nm (Narayanan and Sakthivel 2008; Kasthuri et al. 2009).

Active component in *Aloe vera* plant

About 75 active components present in *Aloe vera* plant including vitamins, enzymes, lignin, saponins, salicylic acid, amino acids, sugars and minerals (Table 1) (Surjushe et al. 2008).

SEM analysis of silver nanoparticles

Scanning electron microscopy (SEM) analysis provided the morphology and size details of the nanoparticles. Figure 3

Table 1 Chemical characterization of plant extract of *Aloe vera*

Parameters	(g. 100 g^{-1} f.w)
Moisture	98.93 ± 0.06
Protein	0.12 ± 0.01
Fat	0.01 ± 0.02
Crude fibre	0.12 ± 1.20
Ash	0.16 ± 0.02
Available carbohydrates	0.66 ± 0.01
Energy (kcal.g^{-1} same)	5.84 ± 0.03
pH	4.74 ± 0.01
Acidity (% of malic acid)	0.06 ± 0.02
Glucose	25.20 ± 0.06
Fructose	9.30 ± 0.01

Mean ± SD

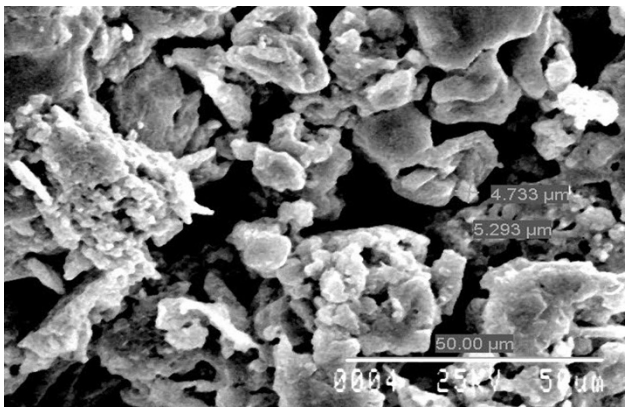

Fig. 3 SEM of synthesised silver nanoparticles

shows high density AgNPs synthesised by the plant extract of *Aloe vera* more confirmed the presence of AgNPs. The interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bond are the reason for synthesis of silver nanoparticles using plant extract (Mano et al. 2011). Figure 3 showed that silver nanoparticles are cubical, rectangular, triangular and spherical in shape with uniform distribution. However, on most occasions, agglomeration of the particles was observed probably due to the presence of a weak capping agent which moderately stabilized the nanoparticles (Nethra et al. 2012). The measured sizes of the agglomerated nanoparticles were in the range 287.5–293.2 nm; however, the average size of an individual particle is estimated to be 70 nm.

Fluorescent microscope

Fluorescent microscope clearly indicates the spherical shape of silver nanoparticles with variable sizes (Fig. 4).

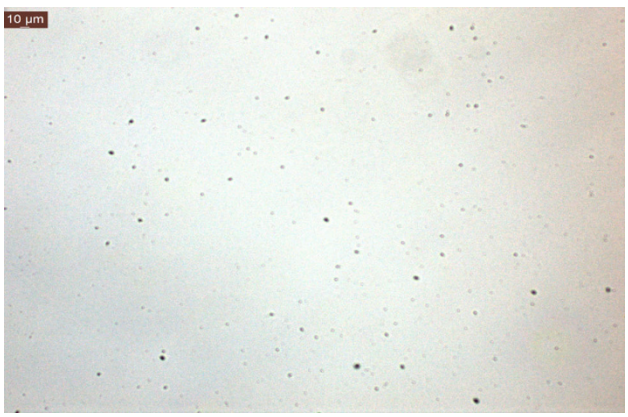

Fig. 4 Fluorescent micrograph of synthesised silver nanoparticles

FT-IR analysis

The result of FT-IR analysis for AgNPs is depicted in Fig. 5. Spectra of AgNPs showed transmission peaks at 3,355, 1,636 and 1,507 cm^{-1}. The peak at 1,636 cm^{-1} indicates primary amines, the peak at 3,355 cm^{-1} corresponds to O–H, as also H-bonded phenols and alcohols in AgNPs while the peak at 1,507 cm^{-1} in AgNPs corresponds to involvement of nitriles (–C=N) groups. Figure 5 shows the FT-IR spectra of biosynthesised silver nanoparticles and carried out to identify the possible interaction between protein and silver nanoparticles. Results of FT-IR study showed sharp absorption peaks located at about 1,631 and 3,433 cm^{-1}. Absorption band at 1,631 cm^{-1} suggested the presence of amide group, raised by the carbonyl stretch of proteins. These results indicated that the carbonyl group of proteins adsorbed strongly to metals, indicating that proteins could have also formed a layer along with the bio-organics, securing interacting with biosynthesised nanoparticles and also their secondary structure were not affected during reaction with Ag^{+} ions or after binding with Ag nanoparticles (Garg 2012). These IR spectroscopic studies confirmed that carbonyl group of amino acid residues have strong binding ability with metal suggesting the formation of layer covering metal nanoparticles and acting as capping agent to prevent agglomeration and providing stability to the medium (Baun et al. 2008).These results confirm the presence of possible proteins acting as reducing and stabilizing agents.

The synthesised AgNPs prepared from *Aloe vera* leaf extract showed antifungal activity against *Rhizopus* sp. and *Aspergillus* sp. The antifungal activity can be identified by inhibition zone formation (Figs. 6, 7). However, the antifungal activity of AgNPs depends on the types of fungus along with size of AgNPs and also closely associated with the formation of pits in the cell wall of microorganism

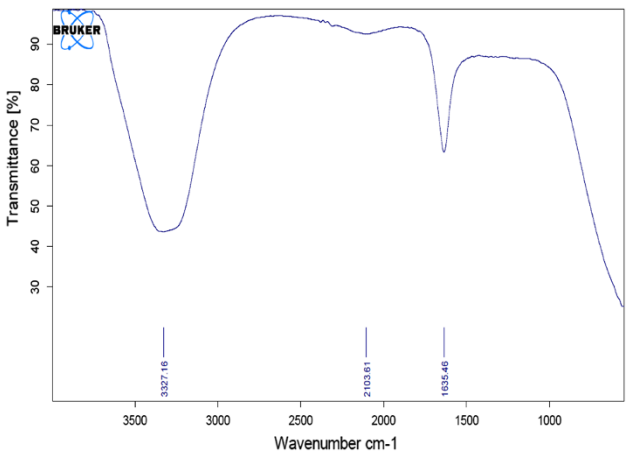

Fig. 5 FT-IR spectrum of Aloe vera mediated silver nanoparticles

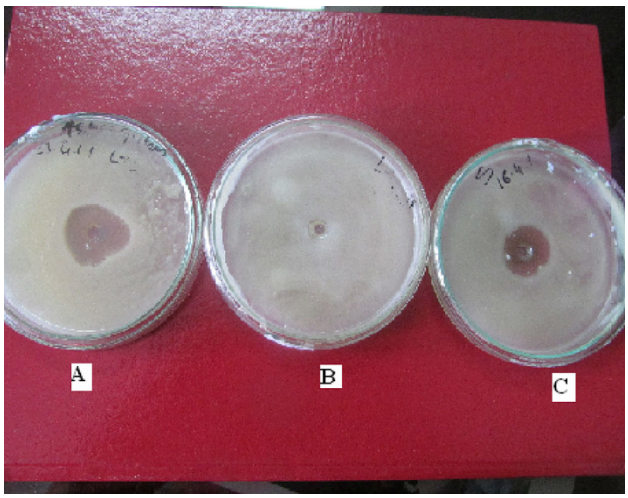

Fig. 6 Zone of inhabitation with **a** AgNPs in *Aspergillus sp*. **b** Aloe vera extract in *Aspergillus sp*. **c** $AgNO_3$ salt solution in *Aspergillus sp*

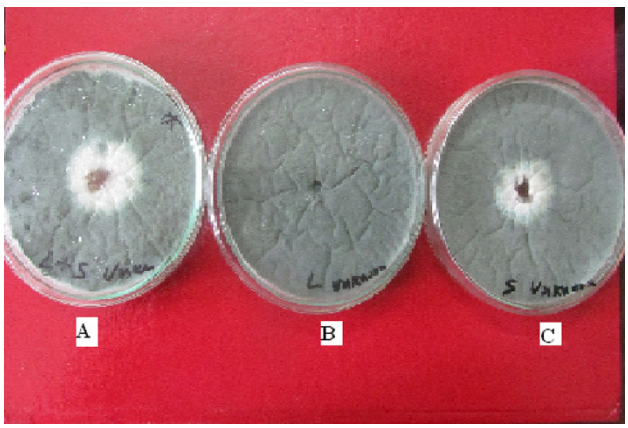

Fig. 7 Zone of inhabitation with **a** AgNPs in *Rhizopus sp*. **b** Aloe vera extract in *Rhizopus sp*. **c** $AgNO_3$ salt solution in *Rhizopus sp*

(Shafaghat 2015). According to Kim et al. (2009a), AgNPs affect fungus cells by attacking their membranes, thus disrupting the membrane potential. The biologically synthesised silver nanoparticles prepared by direct reduction method showed antifungal activity against only *Rhizopus* sp. and *Aspergillus* sp. using disc diffusion method. Control was also maintained in which no zone of inhibition was observed. The highest antimicrobial activity was observed against *Aspergillus* sp. than *Rhizopus* sp. The experimental samples with greater inhibition zones are represented in Fig. 7 (Abdeen et al. 2014). On the other hand, microscopic observation (picture not supplied) revealed that the synthesised nanoparticles caused detrimental effects not only on fungal hyphae but also on conidial germination (Lamsal et al. 2011). However, there were other deformations such as structure of the cell membrane and inhibiting normal budding process of both *Rhizopus* sp. and *Aspergillus* sp., probably due to the destruction of the membrane integrity (Narayanan and Park 2014; Kim et al. 2009b). Almost similar observation was reported by Ouda (2014) who used copper and silver nanoparticles against two plant pathogens, *Alternaria alternate* and *Botrytis cinerea*.

Green synthesis of AgNPs using *Aloe vera* plant extracts was reported to be superior to chemical synthesis in that, the former compounds offer better advantages as they are widely distributed, safe to handle, and easily available with a range of metabolites (Mulvaney et al. 1996). In the present study, silver nanoparticles were synthesised using phyto-compound aloin and the formed silver nanoparticles were characterized using UV–visible spectroscopy, SEM, fluorescent microscope technique and FT-IR analysis.

The production of silver nanoparticles is demonstrated by the sharp peak around 400 nm for aloin-mediated silver nanoparticles in UV–vis spectrum, which indicates the availability of reducing biomolecules in aloin. Analysis of SEM image shows the formation of silver nanoparticles and indicates the agglomerated appearance with cubical, rectangular, triangular shape and size varying from 287.5 to 293.2 nm. The average size of an individual particle is estimated to be approximately 70 nm. The results of DLS technique used for the measurement of size of ANS in solution form showed a size of 67.8 nm which is in good agreement with the SEM analysis (70 nm). The results of the FT-IR studies indicated the involvement of hydroxyl, carboxyl and primary amine functional groups of aloin in the synthesis of silver nanoparticles. AgNPs showed better antifungal properties against *Aspergillus* sp. and *Rhizopus* sp. as evidenced by minimum inhibitory concentration (MIC) value 21.8 ng/mL when compared to the phyto-compound of *Aloe vera* plant extracts alone which does not show any inhibition zone. The results showed that the AgNPs were fungicidal against both the tested fungus at very low concentrations and the fungicidal activity was dependent on the tested fungus species. These results were confirmed by plating the content of each well on dextrose agar medium, and there was no growth for any of the strains resultant from the MIC point. These enhanced effects of AgNPs might be due to the antifungal properties of silver nanoparticles (Tripathu et al. 2010). Cytotoxicity studies revealed that AgNPs have no adverse toxicity and it was found to be safe. Hence, keeping in view of the economics of production, safety and efficacy of the compound, AgNPs could provide a promising alternative to the use of traditional antifungal agent.

Conclusion

In the present study, we focused on green synthesis of silver nanoparticles using aqueous leaf extract of *Aloe vera*.

The physical property of synthesised nanoparticle was characterized using relevant techniques. Further we demonstrated the possible application of AgNPs in medical field as it shows antifungal activity against plant pathogenic fungus. The data represented in our study contributed to a novel and unique virgin area of nano-materials as an alternative fungicide for future. With little uncovered mechanism in the current study, there is a wide scope for detailed investigation in the future for the application of AgNPs in the field of Agriculture for controlling the pathogen.

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References

Abdeen S, Geo S, Sukanya Praseetha PK, Dhanya RP (2014) Biosynthesis of silver nanoparticles from actinomycetes for therapeutic applications. Int J Nano Dimens 5(2):155–162

Abou El-N MM, Eftaiha A, Al-Warthan A, Ammar RAA (2010) Synthesis and application of silver nanoparticles. Arab J Chem 3:135–140

Ahmad T, Wani IA, Manzoor N, Ahmed J, Asiri AM (2013) Biosynthesis, structural characterization and antimicrobial activity of gold and silver nanoparticles. Collo Surf B: Biointerfaces 107:227–234

Bansal V, Ramanathan R, Bhargava SK (2011) Fungus-mediated biological approaches towards ''green'' synthesis of oxide nanomaterials. Aust J Chem 64:279–293

Baun A, Hartmann NB, Grieger K, Kusk KO (2008) Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. Ecotoxicology 17:387–395

Garg S (2012) Rapid biogenic synthesis of silver nano particles using black pepper (piper nigrum) corn extract. Int J Inno Biol Chem Sci 3:5–10

Huang JL, Li QB, Sun DH, Lu YH, Su YB, Yang X, Wang HX, Wang YP, Shao WY, He N, Hong JQ, Chen CX (2007) Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. Nanotechnol 18:1–11

Jo HJ, Choi JW, Lee SH, Hong SW (2012) Acute toxicity of Ag and CuO nanoparticle suspensions against Daphnia magna: the importance of their dissolved fraction varying with preparation methods. J Hazard Mater 227:301–308

Kasthuri J, Kathiravan K, Rajendiran N (2009) Phyllanthin-assisted biosynthesis of silver and gold nanoparticles: a novel biological approach. J Nanopart Res 11:1075–1085

Kim SW, Kim KS, Lamsal K, Kim, YJ, Kim et al (2009a) An in vitro study of the antifungal effect of silver nanoparticles on oak wilt pathogen *Raffaelea* sp. J Microbiol Biotechnol 19:760–764

Kim KJ, Sung WS, Suh BK, Moon SK, Choi JS, Kim JG, Lee DG (2009b) Antifungal activity and mode of action of silver nanoparticles on Candida albicans. Biometals 22:235–242

Lamsal K, Kim SW, Jung JH, Kim YS, Kim KS, Lee YS (2011) Application of silver nanoparticles for the control of Colletotrichum species in vitro and pepper anthranose disease in field. Mycobiology 39:194–199

Mano PM, Karunai SB, John PJA (2011) Green synthesis of silver nanoparticles from the leaf extracts of *Euphorbia Hirta* and *Nerium Indicum*. Dig J Nanomater Biostruct 6(2):869–877

Mondal NK, Chowdhury A, Dey U, Mukhopadhya P, Chatterjee S, Das K, Datta JK (2014) Green synthesis of silver nanoparticles and its application for mosquito control. Asian Pac J Trop Dis 4(Suppl 1):S204–S210

Mulvaney P (1996) Surface plasmon spectroscopy of nanosized metal particles. Langmuir 12:788–800

Narayanan KB, Park HH (2014) Antifungal activity of silver nanoparticles synthesized using turnip leaf extract (*Brassica rapa* L.) against wood rotting pathogens. Eur J Plant Pathol doi:10.1007/s10658-014-0399-4

Narayanan KB, Sakthivel N (2008) Coriander leaf mediated biosynthesis of gold nanoparticles. Mater Lett 62:4588–4590

Narayanan KB, Sakthivel N (2010) Biological synthesis of metal nanoparticles by microbes. Adv Colloid Interface Sci 156:1–13

Nethra DC, Sivakumar P, Renganathan S (2012) Green synthesis of silver nanoparticles using *Datura metel* flower extract and evaluation of their antimicrobial activity. Int J Nanomater Biostruct 2(2):16–21

Ouda SM (2014) Antifungal activity of silver and copper nanoparticles on two plant pathogens, *Alternaria alternate* and *Botrytis cinerea*. Res J Microbiol 9(1):34–42. doi:10.3923/jm.2014.34.42

Shafaghat A (2015) Synthesis and characterization of silver nanoparticles by phytosynthesis method and their biological activity. Synth React Inorg Metal-Org Nano-Metal Chem 45:381–387

Sukirtha R, Priyanka KM, Antony JJ, Kamalakkannan S, Thangam R, Gunasekaran P, Krishnan M, Achiraman S (2012) Cytotoxic effect of green synthesized silver nanoparticles using Melia azedarach against in vitro HeLa cell lines and lymphoma mice model. Process Biochem 47:273–279

Surjushe A, Vasani R, Saple DG (2008) Aloe Vera: a Short Review. Ind J Dermatol 53(4):163–166

Tripathu S, Pradhan D, Anjan M (2010) Anti-inflammatory and antiarthritic potential of *Ammania baccifera* Linn. Int J Pharma Biol Sci 1:1–7

Trop M, Novak M, Rodl S, Hellbom B, Kroell W, Goessler W (2006) Silver-coated dressing acticoat caused raised liver enzymes and argyria-like symptoms in burn patient. J Trauma Injury, Infect Crit Care 60:648–652. doi:10.1097/01.ta.0000208126.22089.b6

Vahabi K, Mansoori G, Karimi V (2011) Biosynthesis of silver nanoparticles by fungus Trichoderma reesei. Insci J 1(1):65–79

Verano-Braga T, Miethling-Graff R, Wojdyla K, Rogowska-Wrzesinska A, Brewer JR, Erdmann H, Kjeldsen F (2014) Insights into the cellular response triggered by silver nanoparticles using quantitative proteomics. ACS Nano 8(3):2161–2175

Wang H, Wu L, Reinhard BM (2012). scavenger receptor mediated endocytosis of silver nanoparticles into J774A.1 macrophages is heterogeneous. ACS Nano 6(8):7122-7132

Xiaoming S, Liming Z, Songhua H (2009) Amplified immune response by ginsenoside-based nanoparticles (ginsomes). Vaccine 27:2306–2311

Yehia RS, Al-Sheikh H (2014) Biosynthesis and characterization of silver nanoparticles produced by *Pleurotus ostreatus* and their anticandidal and anticancer activities. World J Microbiol Biotechnol DOI 10.1007/s11274-014-1703-3