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Biogenic Synthesis and Characterization of Silver Nanoparticles Using *Syzygium samarangense* (Wax Apple) Leaves Extract and Their Antibacterial Activity

Nivetha Thampi and Veronica Shalini Jeyadoss

Department of Biotechnology, Jeppiaar Engineering College, Rajiv Gandhi Salai, Chennai, Tamil Nadu- 600119, India

Abstract: Nanotechnology has been effectively developed during the last decade and represents one of the most crucial dimensions in the technological developments. The silver nanoparticles (AgNPs) were synthesized from the leaves of *Syzygium samarangense* using 1mM silver nitrate solution. AgNPs were characterized by UV–Visible spectrophotometer, Fourier Transform Infrared spectroscopy, X-ray diffractometry and Field Emission Scanning Electron Microscope. The resonance plasmon peak of the AgNPs was studied by UV-Visible Spectrum analysis and the presence of primary amines that is responsible for the stabilization of the silver nanoparticles was confirmed by Fourier Transform Infrared spectroscopy. The crystalline nature and the spherical shape of the AgNPs with size ranging from 36-55nm were ascertained by X-ray diffractometry and Field Emission Scanning Electron Microscope analysis. Antibacterial activity of the silver nanoparticles was performed by well diffusion method against six bacterial pathogens and found that the highest activity was against *Staphylococcus aureus* followed by *Escherichia coli* and *Salmonella typhi*. Thus, silver nanoparticles would be equally effective as that of antibiotics and other drugs in pharmaceutical applications.

Keywords: Silver nanoparticles, *Syzygium samarangense*, Characterization, Antibacterial activity.

Introduction

The growth of nanotechnology is rapid in the areas of research and development that holds tremendous applications for the society, industry and medicine[1]. Nanotechnology deals with particles that range from 1-100nm scale known as "nanoparticles" that affords solution to technological and environmental challenges in the areas of medicine, solar energy conversion, catalysis and water treatment[2]. Due to the exclusive properties of biological molecules that undergo highly controlled assembly, makes them highly suitable for the metal nanoparticle synthesis that was found to be dependable and ecofriendly[3].

Over the past few years, metal nanoparticles have received ample attention because of their unique properties and potential applications in wide areas especially as biological sensor, in drug delivery and pharmaceutical applications[4,5]. Since the chemical procedures involved in the synthesis of nanomaterials has generated a huge amount of hazardous byproducts, the development of new chemical or physical methods resulted in environmental contaminations[6]. To diminish these disadvantages, there is a need to search for proper substitute that could be ecofriendly and non-toxic to humans and domestic animals. Thus "green nanotechnology" is a clean, safe, ecofriendly, and environmentally nontoxic method of nanoparticle synthesis that does not require the use of energy, temperature and toxic chemicals[7].

The biological methods for the synthesis of nanomaterials include the extract from plants, bacterial, fungal species, and so forth. The synthesis of nanoparticles using various plants and their extracts can be favorable over other biological synthesis methods which involves complex procedures of maintaining microbial cultures and hence plant mediated biological synthesis is hiking importance due to its simplicity and eco-friendliness[8].

Silver in its pure form was known to keep the microbes at bay. The antimicrobial property of silver is intensified if they are transformed into a nanoparticle, making it useful in effectively eliminating various microbes. As a natural material, silver is known to be safe to man and produce little to no allergic reactions when tested for curing various diseases[9]. The silver nanoparticles (AgNPs) act on a broad range of target sites both extracellular as well intracellular. In fact microbes generally have a harder time to develop resistance to silver then they do to antibiotics[10].

The cellular damages caused by the interacting with phosphorus and sulfur containing compounds, such as DNA and protein is facilitated by the deep penetration of the nanoparticles inside the cells. The bactericidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity[11]. AgNPs have wide application in biomedical science like treatment of burned patients, antimicrobial activity and used in the process of targeted drug delivery and so forth[12]. Given its broad-spectrum activity, silver nanoparticles have been the core of booming concern and are being used as a desirable candidate for therapeutic purposes.

In the present research, *Syzygium samarangense* (Wax Apple) which belongs to the family Myrtaceae was used as a reducing and stabilizing agent for AgNPs synthesis. The leaf extract of *S.samarangense* was used to synthesize AgNPs from silver nitrate. Synthesized AgNPs were characterized using UV- Visible Spectrophotometer, Fourier Transform Infrared spectroscopy (FT-IR), X-ray diffractometry (XRD) and Field Emission Scanning Electron Microscope (FESEM). After characterization the synthesized AgNPs have been screened for their antibacterial activity against six bacterial pathogens. This study reports for the first time, the biological reduction of silver nanoparticles using the leaf extract of *Syzygium samarangense* and their antibacterial activity.

Materials and Methods

Chemicals

Silver nitrate (purity >99.99%) was purchased from Sigma–Aldrich Chemical Pvt. Ltd and media components were purchased from Hi-Media (Mumbai, India).

Collection of plant samples and bacterial strains

The leaves of *Syzygium samarangense* (Wax Apple) were collected randomly from Trivandrum, Kerala, India. Collected leaves were washed thoroughly 2-3 times in running tap water followed by sterile distilled water. Lyophilized cultures of the bacteria were procured from Marina labs, Pvt. Ltd, Chennai, Tamil nadu, India.

Preparation of plant extract

The leaves of *S.samarangense* were dried in hot air oven at 60 ºC overnight. The dried leaves were grounded well with the help of mortar and pestle and 5g of the powder was mixed with 100ml of double distilled water. This solution was boiled in the water bath at 80 ºC for one hour and after cooling at room temperature, the solution was filtered through Whatmann filter paper no.1. Filtrate was collected and was stored at 4ºC for further analysis.

Synthesis of silver nanoparticles[13]

Synthesis of silver nanoparticles was carried out in 250 ml Erlenmeyer flask containing 90 ml of 1mM silver nitrate ($AgNO_3$) and 10 ml of leaf extract. The solution was heated up to 60 ºC to 80 ºC in a water bath for one hour. The color change of the leaf extract from pale yellow to dark brown indicates the synthesis of AgNPs. The silver nanoparticles solution thus obtained was purified by repeated centrifugation at 10,000 rpm

for 15 minutes and repeated centrifugation was carried to get dried and purified silver nanoparticles. The particles obtained were used for further characterization.

Characterization of silver nanoparticles

UV-Visible Spectral analysis

The resonance plasmon of the silver nanoparticles was studied by taking small aliquot of sample in to UV–Visible spectrophotometer absorption spectra at 200-700 nm using Shimadzu UV-1800 Spectrophotometer.

Fourier Transform Infrared Spectroscopy (FT-IR) analysis

FT-IR spectral analysis was achieved using Bruker FT-IR spectrophotometric system and the spectra were recorded between 400 to 4000 cm^{-1} to study the possible functional groups for the formation of silver nanoparticles.

X-Ray Diffraction (XRD) analysis

To analyze the crystalline nature of the synthesized particles, the powder was subjected to X-Ray powder diffraction analysis by Bruker D8 Focus power X-ray diffractometer operated at 25 ºC with Cu Kα radiation in θ–2θ configurations.

Field Emission Scanning Electron Microscopic (FESEM) analysis

The surface structure and shape of the particles were analyzed by Field Emission scanning electron microscopic (FESEM) analysis. A thin film of the sample was prepared on a carbon coated copper grid by placing small amount of the sample on the grid. Then the film on the SEM grid was allowed to dry using a mercury lamp for 5 min.

Antibacterial activity - Well diffusion assay

The silver nanoparticles synthesized were tested for their antibacterial activity against pathogenic organisms like *Staphylococcus aureus, Pseudomonas aeruginosa*, *Entrobacter aerogenes*, *Salmonella typhi*, *Vibrio parahemolyticus* and *Escherichia coli* by the well diffusion method as described by Thombre et al [14]. In this, 0.1 ml of test bacterial culture (10^5 to 10^6 CFU/ml) was seeded on Nutrient agar plates. 5mm wells were made on agar surface with sterile cork borer to which 20 µl of the AgNPs was added. Plates were incubated at 37°C for 24 h and zone of inhibition was measured.

Results and Discussion

Synthesis of silver nanoparticles

During the synthesis of silver nanoparticles (AgNPs), it was visually ascertained that after the addition of the leaf extract resulted in the gradual change of the colour of silver nitrate solution from pale yellow to dark brown indicating the synthesis of AgNPs as shown in Fig 1. Silver nitrate without the extract was used as the control, which was colourless.

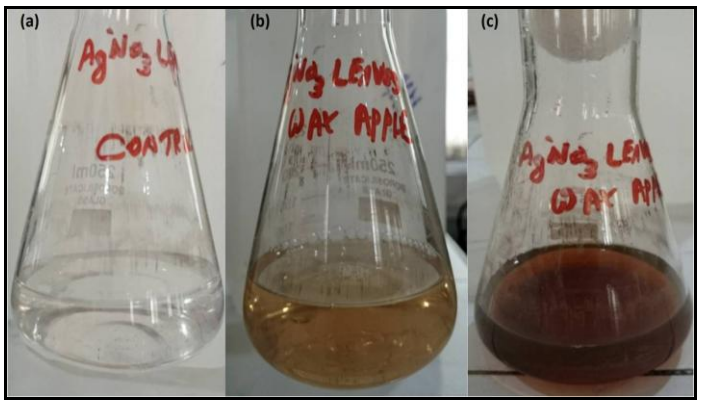

Fig 1: Biosynthesis of AgNPs (a) $AgNO_3$ solution (control) (b) *S.samarangense* (Wax Apple) leaf extracts (c) Synthesized AgNPs

Characterization of silver nanoparticles

UV-Visible Spectral analysis

UV-Visible spectroscopy is an important preliminary technique that used to examine size and shape controlled nanoparticles in aqueous suspensions. It was observed that the maximum spectrum was obtained at 418nm (Fig 2) due to the surface plasmon peak of green synthesized AgNPs from the leaves of *S.samarangense* extract.

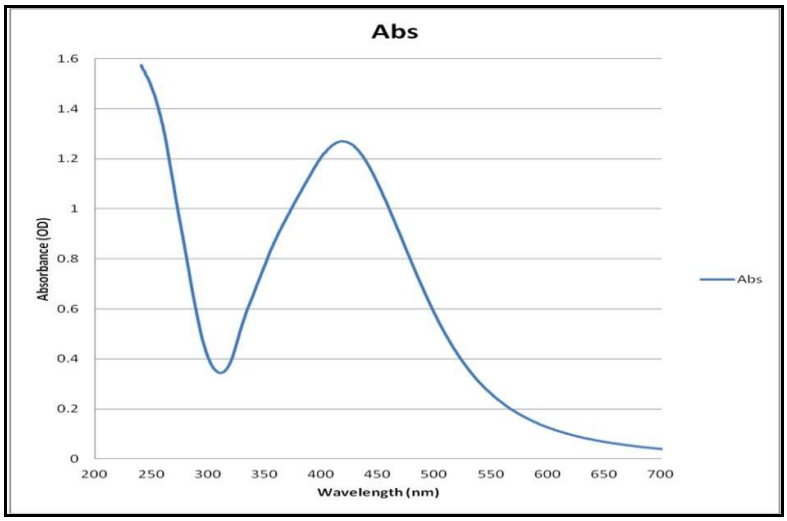

Fig 2: UV-Visible absorption spectrum of synthesized AgNPs from *S.samarangense* leaf extract

Fourier Transform Infrared Spectroscopy (FT-IR) analysis

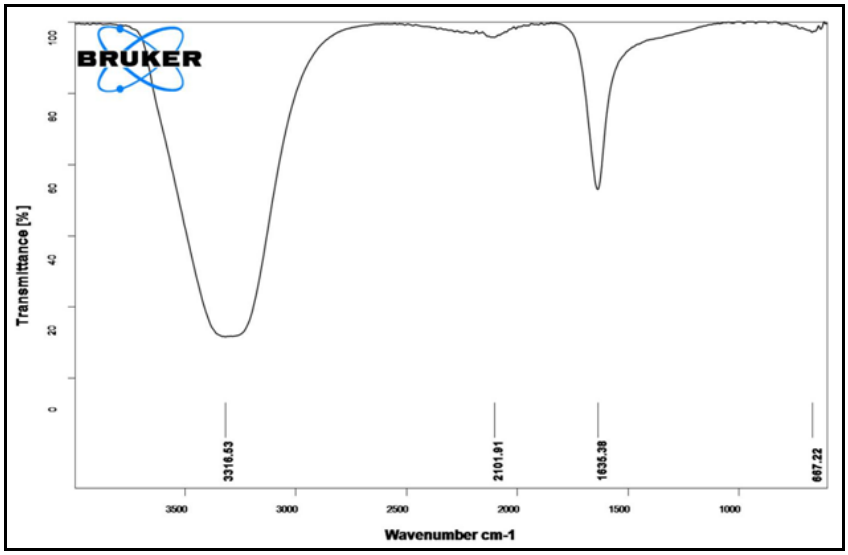

Fig 3: FT-IR spectrum of AgNPs synthesized from *S.samarangense* aqueous leaf extract

FT-IR spectral analysis was carried out to identify the possible biomolecules responsible for the reduction of the Ag+ ions and capping of the bioreduced silver nanoparticles synthesized by he leaves of *S.samarangense* extract. The FT-IR spectrum of AgNPs is shown in Fig 3. The peak value at 3316.53 cm^{-1} corresponds to phenols (O-H). The peak value at 2101.91 cm^{-1} corresponds to carboxylic acids and their derivatives (C=O). The peak value 1635.38 cm^{-1} corresponds to primary amine groups (N-H). Finally peak value at 667.22 cm^{-1} corresponds to alkynes group (C-H).

X-Ray Diffraction (XRD) Analysis

X-Ray Diffraction (XRD) analysis was used to ascertain the crystalline nature of the silver nanoparticles. The XRD pattern showed the number of Bragg's reflections that may be indexed on the basis of the face centered cubic structure of silver. The diffracted intensities were recorded from 30º to 80º at 2 theta angles (2θ). The XRD graph shows two peaks at degree (2θ), 32.16 and 38.05 corresponding to two diffraction facets of silver (Fig 4). The mean size of silver nanoparticles was calculated using the Debye-Scherre's equation and the average size of the AgNPs synthesized by *S.samarangense* was found to be 46 nm with size ranging from 36 – 55 nm.

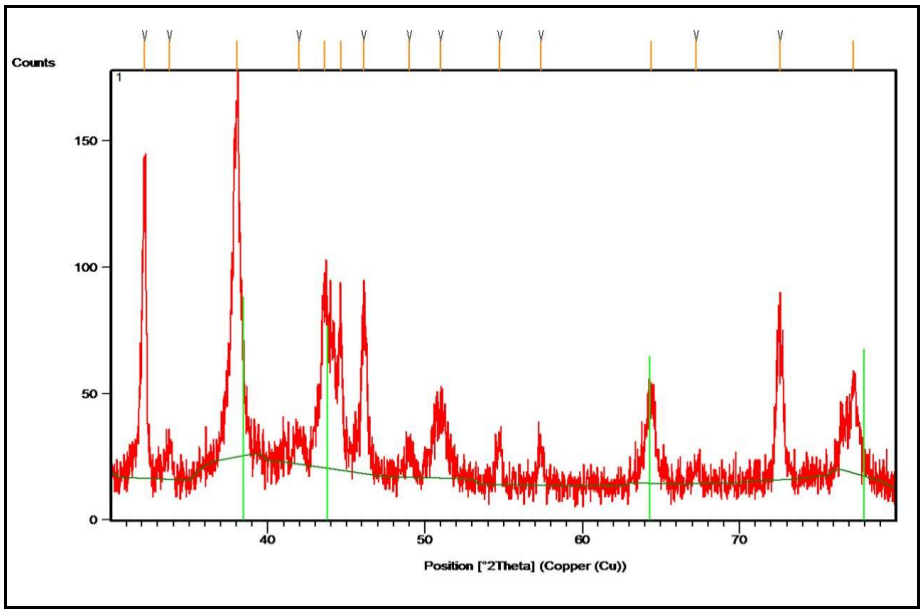

Fig 4: XRD pattern of AgNPs synthesized using *S.samarangense* leaf extract

Field Emission Scanning Electron Microscopic (FESEM) analysis

Field Emission Scanning Electron Microscopy (FESEM) analysis was used to confirm the size, shape and morphology of the synthesized AgNPs. FESEM provided further insight into the structural and size details of the AgNPs. The FESEM image showed the presence of silver nanoparticles that are predominantly spherical shaped and polydispersed (Fig 5). The arrows in the figure show presence of AgNPs in the range approximately from 36-55 nm in diameter.

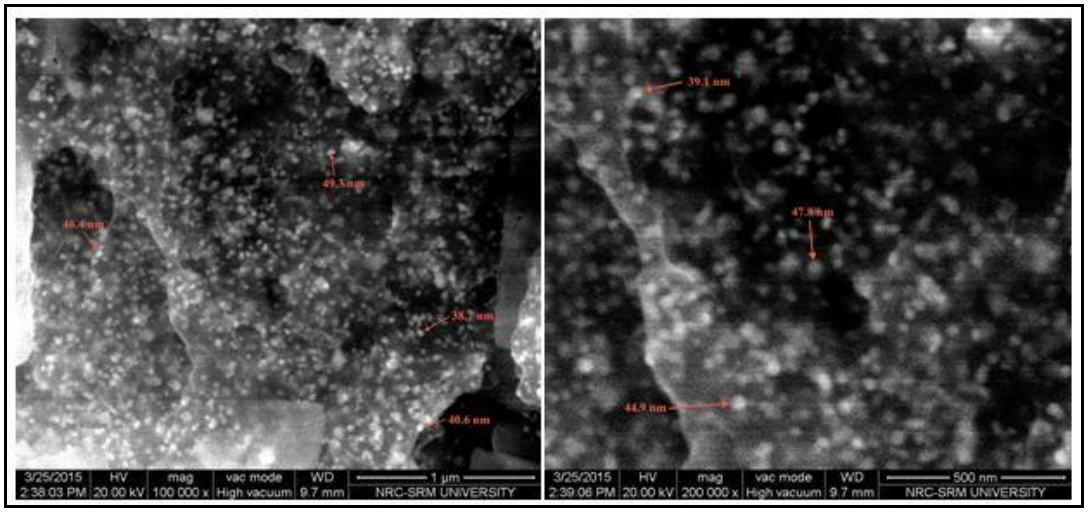

Fig 5: FESEM images showing spherical AgNPs synthesized from *S.samarangense* aqueous leaf extract

Antibacterial activity - Well diffusion assay

In the antibacterial activity assay, the various zones of inhibition (in mm) produced by the green synthesized AgNPs against different pathogens have been tabulated in Table 1 and was compared with controls like two standard antibiotics - gentamycin and amikacin as well as silver nitrate. The maximum inhibitory activity of AgNPs was against *Staphylococcus aureus* with a zone of inhibition of 17mm followed by *Escherichia coli* and *Salmonella typhi* with 16mm zone of inhibition. The plates displays the inhibitory effects of synthesized AgNPs against the six bacteria were shown in Fig 6.

Table 1: Antibacterial activities of synthesized AgNPs from *S.samarangense* leaves against different bacterial pathogens

Test Organism	Zone of Inhibition (in mm)			
	Synthesized AgNPs	AgNPs	Gentamycin	Amikacin
E.coli	16	14	20	0
E.aerogenes	15	13	14	18
S.aureus	17	12	19	18
S.typhi	16	12	18	14
P.aeruginosa	15	15	18	15
V.parahemolyticus	15	15	15	20

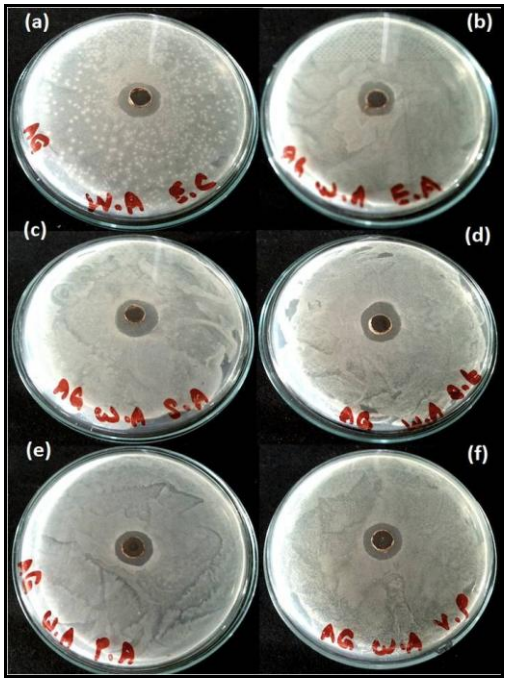

Fig 6: Antibacterial activity of synthesized AgNPs against (a) *Escherichia coli* (b) *Entrobacter aerogenes* (c) *Staphylococcus aureus* (d) *Salmonella typhi* (e) *Pseudomonas aeruginosa* (f) and *Vibrio parahemolyticus*

It is well known that AgNPs produces a yellowish-brown colour in solution due to excitation of Surface Plasmon Resonance (SPR) vibrations that in turn is due to the presence of free electrons[15]. It was studied that the frequency and width of surface plasmon absorption depend on the size and shape of nanocolloids in an aqueous suspension[16]. The broadening of peak and splitting of Surface Plasmon Resonance (SPR) is probably due to the dampening of the SPR and particle size distribution in colloidal solution[17].

The FT-IR results thus indicate that the secondary structure of the proteins is not affected as a consequence of reaction with the Ag+ ions or binding with the silver nanoparticles. This result suggests that the biological molecules could possibly be involved in a function for the formation and stabilization of AgNPs in an aqueous solution. It is well known that proteins can bind to AgNPs through free amine groups in the proteins[18].

The presence of silver metal as well as its crystalline nature was confirmed by XRD analysis. The intense peaks observed in the spectrum are in agreement with the Braggs's reflection of silver nanocrystals[19,20]. It has been found that circular nanoparticles can be effectively used as a medicine for treatment of various ailments. The SEM images of silver nanoparticles were synthesized from plant extract were assembled on to the surface due to the interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bound to the Ag nanoparticles[21].

Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles [22]. AgNPs were preferentially bound to the cytoplasmic membrane and disturb the cell membrane protein thereby it kill the bacteria 9. Ag+ ions uncouple the respiratory chain from oxidative phosphorylation or collapse the proton-motive force across the dependent on the size and shape of nanoparticles [23]. As per our results, similar reports were produced which suggested that the surface cell walls of *S.aureus* and *E.coli* were disrupted by silver nanoparticles [24].

Plants and their extracts can be effectively used in the synthesis of silver nanoparticles as a more safer and eco-friendly route. Shape and size control of nanoparticles can be easily understood with the use of plants. The nanoparticles synthesized from the various sources of plants are used in many applications for the benefits of human beings. The most assuring area of research includes the elucidation of the mechanism of plant-mediated synthesis of silver nanoparticles.

Conclusion

To summarize, the present study reveals that the leaves of *S.samarangense* (Wax Apple) is a good source of potential reducing and stabilizing agent for synthesis of silver nanoparticles at a faster rate. The characterization of the bio-synthesized AgNPs by UV-Visible spectroscopy and FT-IR made it possible to analyze the presence of AgNPs in the aqueous medium. The XRD and FESEM results showed the synthesis of polydisperse, spherical, crystalline nanoparticles of the size range 36-55 nm. The biosynthesized silver nanoparticles displayed effective antibacterial activity against all the tested bacteria which confirms that the silver nanoparticles are capable of rendering the infectious bacterial growth which provides an active platform to make it as a value added source of alternative drugs in the fields of pharmaceutics and medicine. However, further in-vivo studies are essential to fully characterize the antibacterial potential of the green synthesized AgNPs that would prove to analyze whether this would be a novel approach for accelerating antibacterial potentiality.

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Reference

1. Seeman NC, Belcher AM. Emulating biology: building nanostructures from the bottoms-up. Proc. Natl. Acad. Sci. U.S.A., 2002, 99: 6451–6455.
2. Hutchison JE. Greener nanoscience: a proactive approach to advancing applications and reducing implications of nanotechnology. ACS Nano., 2008, 2: 395–402.
3. Bar H, Bhumi DK, Sahoo GP, Sarkar P, Sankar PD. Green synthesis of silver nanoparticles using latex of J*atropha curcas*. Coll. Surf. A., 2009, 39: 134-139.
4. Mirkin CA, Letsinger RL, Mucic RC, Storhof JJ. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. Nature Nanobiotechnol., 1996, 382: 607-609.
5. Bhumkar DR, Joshi HM, Sastry M, Pokharkar VB. Chitosan reduced gold nanoparticles as novel carriers for transmucosal delivery of insulin. Pharma Res., 2007, 24: 9257-9.
6. Zhang M, Liu M, Prest H, Fischer S. Nanoparticles secreted from ivy rootlets for surface climbing. Nano Lett., 2008, 8: 1277–1280.
7. Savithramma N, Linga RM, Rukmini K, Suvarnalatha DP. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. Int. J. ChemTech Res., 2011, 3: 1394–1402.

8. Sastry M, Ahmad A, Islam NI, Kumar R. Biosynthesis of metal nanoparticles using fungi and *actinomycetes*. Current Sc., 2003, 85: 162-170.
9. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ. The bactericidal effect of silver nanoparticles. Nanotechnol., 2005, 16: 2346-2353.
10. Landsdown AB, Williams A. Bacterial resistance to silver in wound care and medical devices J. Wound Care., 2007, 16-15.
11. Amarendra DD, Krishna G. Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. Coll. Surf. A., 2010, 369: 27-33.
12. Singh A, Jain D, Upadhyay MK, Khandelwal N, Verma HN. Green synthesis of silver nanoparticles using *Argemone mexicana* leaf extract and evaluation of their antimicrobial activities. Dig J Nanomat Bios., 2010, 5: 483–489.
13. Anuradha G, Sundar BS, Ramana MV. *Ocimum americanum L.* leaf extract mediated synthesis of silver nano particles: A noval approach towards weed utilisation. Arch. Appl. Sci. Res., 2014, 6: 59-64.
14. Thombre R, Leksminarayanan P, Hegde R, Parekh F, Francis G, Mehta S, Patil N, Zunjarrao R. A facile method for green synthesis of stabilized silver nanoparticles and its *in vitro* antagonistic applications. J. Nat. Prod. Plant Resour., 2013, 3: 36-40.
15. Jae YS, Beom SK. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst. Eng., 2009, 32: 79-84.
16. Rai A, Singh A, Ahmad A, Sastry M. Role of halide ions and temperature on the morphology of biologically synthesized gold nanotriangles. Langmuir., 2006, 22: 736-741.
17. Tripathy A, Ashok M, Raichur, Chandrasekaran N, Prathna TC, Mukherjee A. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta indica* (Neem) leaves. J Nanopart Res., 2010, 12 : 237–246.
18. Gole A, Dash C, Ramachandran V, Mandale AB, Sainkar SR, Rao M, Sastry M. Pepsin-gold colloid conjugates: preparation, characterization, and enzymatic activity. Langmuir., 2001, 17: 1674–1679.
19. Nabikhan A, Kandasamy K, Raj A, Alikunhi NM. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum L*. Coll. Surf. B., 2010, 79: 488–493.
20. Kuber CB, D'Souza SF. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. Coll. Surf. B., 2006, 47:160–164.
21. Priya MM, Selvi KB, Paul JA. Green synthesis of silver nanoparticles from the leaf extracts of *Euphorbia hirta* and *Nerium indicum*. Dig J Nanomat Bios., 2011, 6: 869-877.
22. Panacek A, Kvitek L, Prucek R, Kolar K, Vecerova R, Pizurova N, Sharma VK, Nevecna T, Zbori R. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. J Phy Chem., 2006, 110: 16248-16253.
23. Kim KJ, Sung WS, Moon SK, Choi JS, Kim JG, Lee DG. Antifungal effect of silver nanoparticles on dermatophytes. J Microbiol Biotechnol., 2008, 18: 1482–1484.
24. Cho KH, Park JE, Osaka T, Park SG. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochimica Acta., 2005, 51: 956-960.

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