ISSN 1996-5044

Research Journal of

Nanoscience and Nanotechnology

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Research Journal of Nanoscience and Nanotechnology 1 (2): 95-101, 2011
ISSN 1996-5044 / DOI: 10.3923/rjnn.2011.95.101

Plant Mediated Synthesis of Biomedical Silver Nanoparticles by Using Leaf Extract of *Citrullus colocynthis*

K. Satyavani, T. Ramanathan and S. Gurudeeban
Faculty of Marine Sciences, Annamalai University, Parangipettai 608502, India

Corresponding Author: T. Ramanathan, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India Tel: 04144-243223 Fax: 04144-243555

ABSTRACT

Plant mediated synthesis of metallic nanoparticles is an increasing commercial demand due to the wide applicability in various areas such as electronics, catalysis, chemistry, energy, cosmetics and medicine. In the present investigation, synthesis of silver nanoparticles by using leaf extracts of *Citrullus colocynthis* (L.) Schrader and characterized by using UV spec, FT-IR and AFM. The extract incubated with $AgNO_3$ showed gradual change in the colour of the extract from greenish to reddish brown with intensity increasing during the period of incubation. Hence the leaf extract act as a reducing and capping agent. Nanoparticles were characterized by using UV visible absorption spectra, FT-IR and AFM. The synthesized silver nanoparticles were generally found to be spherical in shape and 31 nm by AFM. FT-IR peaks were in the extract ranging from 1000-4000 cm^{-1} which confirmed the presence of polyphenols with aromatic ring and bound amide region required for the synthesis and stabilization of sliver nanoparticles.

Key words: Atomic force microscope, bitter apple, *Bacillus subtillus,* silver nanoparticles

INTRODUCTION

Coastal sand dunes are formed by the sand deposited from sub tidal and intertidal regions. *Citrullus colocynthis* belongs to the family of cucurbitaceae and used for the treating mamilities, jaundice and urinary disease. The volatile composition (Gurudeeban *et al*., 2010a) and therapeutic potentials viz., antimicrobial (Gurudeeban *et al*., 2010b), antidiabetic (Gurudeeban and Ramanathan, 2010) antioxidant (Ramanathan *et al*., 2010), local anesthetic (Ramanathan *et al*., 2011) and anti-inflammatory activity reported (Rajamanickam *et al*., 2010). The sand dune vegetation had played a significant in coastal region. Also it helps in prevention of sand erosion by decreasing wind speed at ground level. *Citrullus colocynthis* is cultivated in Southeast coast of India for its edible fruits (Ramanathan, 2000) also micro propagated in our laboratory (Satyavani *et al*., 2011).

Nanobiotechnology is an emerging area of opportunity that seeks to fuse nano/micro fabrication and bio systems to the benefit of both. Nanobiotechnology is highly interdisciplinary by nature and requires close collaboration between biologists, physical scientists and engineers (Singh *et al*., 2011). Nanoparticles usually referred as particles with a size up to 100 nm (Nalwa, 2005). Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Specific surface is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles. As

specific surface area of nanoparticles is increased, their biological effectiveness can increase in surface energy (Jhan, 1999). Silver has long been recognized as having an inhibitory effect towards many bacterial strains and micro organisms commonly present in medical and industrial processes (Murphy, 2008). The most widely used and known applications of silver and silver nanoparticles are in medical industry. These include topical ointments and creams containing silver to prevent infection of burns and open wounds (Schultz *et al*., 2000). Production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. Biological methods of nanoparticles synthesis using micro organisms (Nair and Pradeep, 2002), enzyme (Willner *et al*., 2006) and plant or plant extract have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plant for nanoparticles can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell culture (Taleb *et al*., 1998). It can also suitably scaled up for large-scale synthesis of nanoparticles. If biological synthesis of nanoparticles can compete with chemical methods, there is a need to achieve faster synthesis rates. The exact mechanism of silver nanoparticles synthesis by plant extracts is not yet fully understood. Only participation of phenolics, proteins and reducing agents in their synthesis has been speculated. In the present study, we screened coastal sand dune species *Citrullus colocynthis* leaf extracts for extra cellular nanoparticles synthesis, characterized by using UV- visible spectroscopy, AFM and FT-IR.

MATERIALS AND METHODS

Plant material and preparation of the extract: Fresh *Citrullus colocynthis* leaves were collected from the Southeast coast of Parangipettai (Tamil Nadu) India. The specimen was certified by Botanical Survey of India (BSI) Coimbatore and documented in the Herbaria of C.A.S. in Marine Biology, Annamalai University, India, during 2010. Greenish-leaves of *Citrullus colocynthis* were collected and used to make the aqueous extract. The leaves weighing 25 g were thoroughly washed in distilled water, dried, cut into fine pieces and were crushed into 100 mL sterile distilled water and filtered through what man No.1 filter paper. The filtrate was further filtered through 0.6 μm sized filters. They were stored at 4°C and used within a week. The experimental chemicals were purchased from Sigma Chemicals (Mumbai).

Synthesis of silver nanoparticles and purification: Ten milliliter suspension of leaf extract was added to 90 mL aqueous solution of silver nitrate (1 mM) solution separately for reduction in to Ag^+ ions and incubated at room temperature (35°C) for about 24 h. The primary detection of synthesized silver nanoparticles was carried out in the reaction mixture by observing the colour change of the medium from greenish to dark brown. After 5 h of incubation the silver nanoparticles were isolated and concentrated by repeated (4-5 times) centrifugation of the reaction mixture at 10,000×g for 10 min. The supernatant was replaced by distilled water each time and suspension stored as lyophilized powder for the optical measurements.

CHARACTERIZATION OF SILVER NANOPARTICLES

Ultra violet -visible spectroscope analysis: After 5 h of incubation the silver nanoparticles were isolated and concentrated by repeated (4-5 times) centrifugation of the reaction mixture at

10,000×g for 10 min. The supernatant was replaced by distilled water each time and subjected for the optical measurements which was carried out by using UV-Vis spectrophotometer (UV- 2450 (Shimadzu) and scanning the spectra between 200-700 nm at the resolution of 1 nm.

Atomic force microscope: Purified SNP in suspension was also characterized their morphology using a VEeco diNanoscope 3D AFM (Atomic Force Microscope). A small volume of sample was spread on a well-cleaned glass cover slip surface mounted on the AFM stub and was dried with nitrogen flow at room temperature. Images were obtained in tapping mode using a silicon probe cantilever of 125 μm length, resonance frequency 209-286 kHz, spring constant 20-80 nm^{-1} minimum of five images for each sample were obtained with AFM and analyzed to ensure reproducible results.

Fourier transform infra red spectroscope: To identify silver nanoparticles associated biomolecules, the Fourier transform infra red spectra of washed and purified silver nanoparticles powder were recorded on the Nicolet Avatar 660 FT-IR Spectroscopy (Nicolet, USA) using KBr pellets. To obtain good signal to noise ratio, 256 scans of silver nanoparticles were taken in the range of 400-4000 cm^{-1} and the resolution was kept as 4 cm^{-1}

Antibacterial assay: Antibacterial activity of leaf extract derived silver nanoparticles was assessed using the Standard agar diffusion method with 6mm diameter Whatmann No.1 filter paper discs (Becerro *et al*., 1994). In this 50 μL of silver nanoparticles prepared from leaf extract was mixed in 1 mL add distilled water and applied to sterile paper discs of 6 mm diameter and standard antibiotic disc (ampicillin and tetracycline) used for control. Zobell marine agar was used for antimicrobial test. The bacteria such as *E.coli, Bacillus subtillus, Proteus mirabilis, Salmonella enteritids, Staphylococcus aureus, Streptococcus pyogens* and *Pseudomonas aeruginosa* were inoculated into Zobell marine agar plates and incubated at 27°C for 24 h. Inhibition of zone was measured after 24-48 h of incubation.

RESULTS AND DISCUSSION

The leaf extract was used for the synthesis of silver nanoparticles. The reaction started with in first hour of the incubation with silver nitrate (1 mM). This was confirmed by the appearance of brown colour in the reaction mixture. The reaction rate was maximum after 25 h of incubation as indicated by the formation of silver nanoparticles. Present findings showed resemblance to the results already reported by in the case of extract of *Capsicum annum* (Li *et al*., 2007) and in case of extract of *Aloe vera* (Chandran *et al*., 2006). They reported that when the extracts of their respective test plants were challenged with silver nitrate (1 mM). They turned brown and the intensity of colour was increased with the time of incubation. In order to verify the synthesis of silver nanopartices, the test samples were subjected to the UV-Vis spectrophotometer analysis after 5 h of incubation. A peak specific for the synthesis of silver nanoparticles was obtained at 340 nm (Fig. 1). The detailed shape and size characterization of SNP was carried with AFM for leaf extract was spherical in shape and 31 nm as evident by AFM (Fig. 2), respectively. The larger size of the nanoparticles might be due to the capping of nanoparticles by polyphenols with aromatic ring and bound amide as confirmed from FT-IR analysis.

FT-IR spectra were obtained with Avatar-660 FT-IR spectroscopy using KBr pelleting. Earlier FT-IR measurements were carried out to identify the possible biomolecules responsible for the

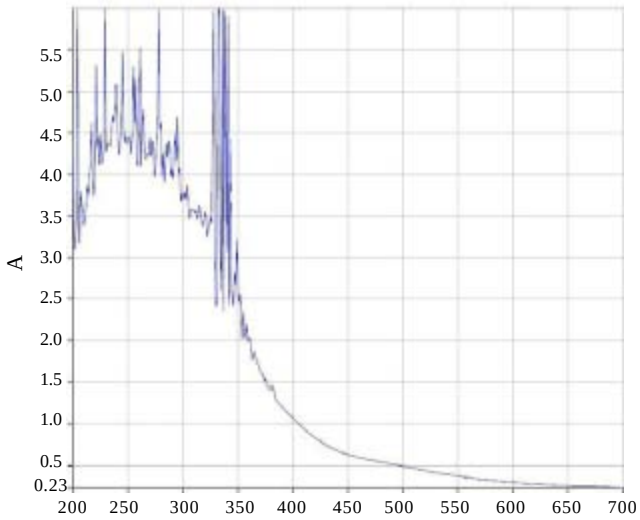

Fig. 1: Uv-visible absorpation spectra at different wave length for silver nano particals synthesized from leaf extract

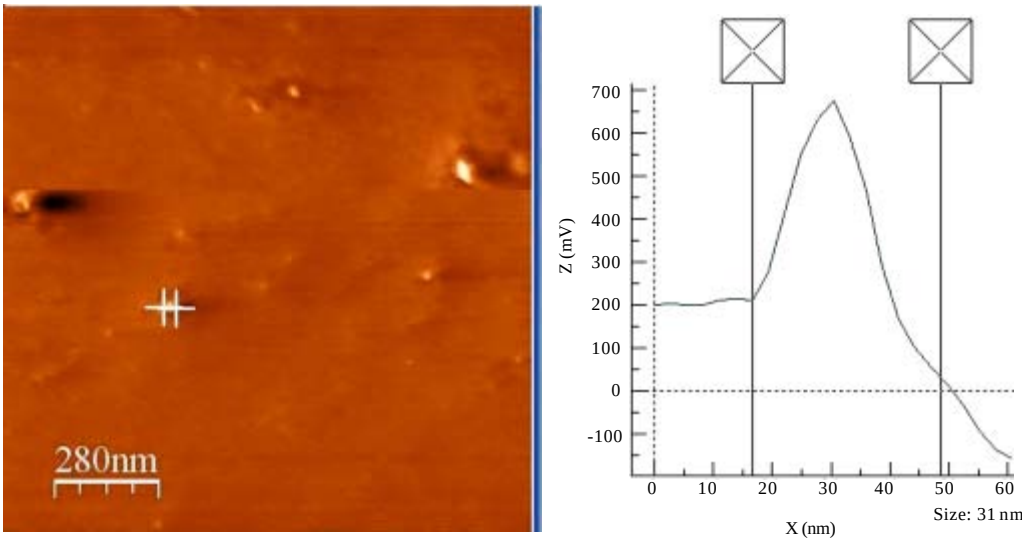

Fig. 2: AFM image of the silver nano particals formed by the reaction 1 mM AgNO3 with leafbroth

reduction of the Ag^+ ions and capping of the bioreduced silver nanoparticles synthesized by fungal filtrate (Sastry *et al.*, 2010). Representative spectrum of the nanoparticles obtained in the present study is presented in Fig. 3. Among them, the absorption bands are observed in the region of 500-4000 cm^{-1} is 536.70, 1026.87, 1243.84, 1383.9, 1452.05, 1643.25, 2860.27, 2924.82, 3403.72, 3490.41 and 3463.01 cm^{-1}. These peaks corresponding to amide II and III aromatic rings, ether and polyphenols were present in nanoparticles synthesized by leaf extract. Result suggests that molecules attached with silver nanoparticles have free and bound amide group. These amide groups

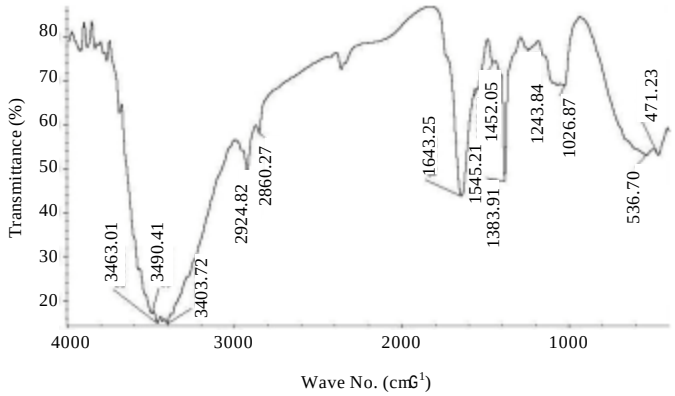

Fig. 3: FI-IR spectrum for leaf extract after treatment with 1mM silver nitrate

may also be in the aromatic rings. This concludes that the compounds attached with silver nanoparticles could be polyphenols with aromatic ring and bound amide region. The amount of polyphenols could be one of the crucial parameters determining the size and distribution of silver nano particles in *Syzygium cumini* (Sastry *et al*., 2010).

The antimicrobial properties of silver compounds and silver ions had been historically recognized and applied in the wide range of applications from disinfecting medical devices and home appliances to water treatment (Chou *et al*., 2005). Silver ion and silver based compounds are highly toxic to micro organisms, showing strong biocidal effect against microbial species. The silver nanoparticles produced by plant extracts was showed the maximum activity against *E. coli, Bacillus subtillus, Pseudomonas aeruginosa* and *Streptococcus pyogens* and also observed that it showed no activity against *Proteus mirabilis, Salmonella enteritids* and *Staphylococcus aureus*. The bioavailability of nano copper coated antidiabetic drug may help to reduce the dose of the drug and frequency of administration (Poovi *et al*., 2011).

The nanoparticles synthesized using the plant system have application in the field of medicines, cancer treatment, drug delivery, commercial appliances and sensors etc (Mude *et al*., 2009). As compared to other biological systems the plant system shows rapid and easy biosynthesis of nanoparticles. The synthesis of silver nanoparticles by the extract of *Citrullus colocynthis* may therefore, serve as a green simple, cheap and eco-friendly approach.

CONCLUSIONS

Present investigation reveals the bioreduction of aqueous Ag^+ ions by the leaf extract of the *Citrullus colocynthis* has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of fairly well-defined dimensions. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability etc. applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications make this method potentially exciting for the large-scale synthesis of other inorganic materials (nanoparticles). Though there is a report describing synthesis of silver nanoparticles using papaya callus but the present used leaf of *Citrullus colocynthis* as a source which is easily available and economic unlike callus which need extensive tissue culture facilities and expertise.

ACKNOWLEDGMENTS

The authors are gratefully acknowledge to the Dean, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India for providing all support during the study period.

REFERENCES

Becerro, M.A., N.I. Lopez, X. Turon and M.J. Uniz, 1994. Antimicrobial activity and surface bacterial film in marine sponges. J. Exp. Mar. Biol. Ecol., 179: 195-205.

Chandran, S.P., M. Chaudhary, R. Pasricha, A. Ahmad and M. Sastry, 2006. Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnol. Prog., 22: 577-583.

Chou, W.L., D.G. Yu and M.C. Yang, 2005. The preparation and characterization of silver-loading cellulose acetate hollow fiber membrane for water treatment. Polymer. Adv. Tech., 16: 600-607.

Gurudeeban, S. and T. Ramanathan, 2010. Antidiabetic effect of *Citrullus colocynthis* in alloxan induced diabetic rats. Invent. Rapid Ethno Pharmacol., 1: 1-112.

Gurudeeban, S., K. Satyavani and T. Ramanathan, 2010a. Bitter apple (*Citrullus colocynthis*): An overview of chemical composition and biomedical potentials. Asian J. Plant Sci., 9: 394-401.

Gurudeeban, S., E. Rajamanickam, T. Ramanathan and K. Satyavani, 2010b. Antimicrobial activity of *Citrullus colocynthis* in gulf of mannar. Int. J. Curr. Res., 2: 78-81.

Jhan, W., 1999. Chemical aspects of the use of gold clusters in structural biology. J. Struct. Biol., 127: 106-112.

Li, S., Y. Shen, A. Xie, X. Yu, L. Qiu, L. Zhang and Q. Zhang, 2007. Chemical markers for the quality control of herbal medicines: An overview. Green Chem., 9: 825-825.

Mude, N., A. Ingle, A. Dade and R. Mahendra, 2009. Synthesis of silver nanoparticles using Callus extract of *Carcia papaya*-A first report. J. Plant Biochem. Biotechnol., 18: 83-86.

Murphy, C.J., 2008. Sustainability as an emerging design criterion in nanoparticle synthesis and applications. J. Mater. Chem., 18: 2173-2176.

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

Nalwa, H.S., 2005. Handbook of Nanostructured Biomaterials and Their Applications in Nanobiotechnology. American Scientific Publishers, Los Angeles, pp: 1-2.

Poovi, G., U.M.D. Lekshmi, N. Narayanan and P.N. Reddy, 2011. Preparation and characterization of repaglinide loaded chitosan polymeric nanoparticles. Res. J. Nanosci. Nanotechnol., 1: 12-24.

Rajamanickam, E., S. Gurudeeban, T. Ramanathan and K. Satyavani, 2010. Evaluation of anti-inflammatory activity of *Citrullus colocynthis*. Int. J. Curr. Res., 2: 67-69.

Ramanathan, T., 2000. Studies on medicinal plants of Parangipettai Coast (Southeast coast of India). Ph.D. Thesis, Annamalai University, India.

Ramanathan, T., S. Gurudeeban and K. Satyavani, 2010. Antioxidant and radical scavenging effect of *Citrullus colocynthis*. Invent Rapid Nutraceuticals, 1: 38-38.

Ramanathan, T., S. Gurudeeban and K. Satyavani, 2011. Local anesthetic effect of *Citrullus colocynthis* on rana hexadactyla. Res. J. Med. Plant, 5: 338-342.

Sastry, K., C.Y. Subhash and K.Y. Sudesh, 2010. *Syzygium cumini* leaf and seed extract mediated biosynthesis of silver nanoparticles and their characterization. J. Chem. Technol. Biotechnol., 85: 1301-1309.

Satyavani, K., T. Ramanathan and S. Gurudeeban, 2011. Effect of plant growth regulators on callus induction and plantlet regeneration of bitter apple (*Citrullus colocynthis*) from stem explants. Asian J. Biotechnol., 3: 246-253.

Schultz, S., D.R. Smith, J.J. Mock and D.A. Schultz, 2000. Single-target molecule detection with non bleaching multicolor optical immunolabels. Proc. Natl. Acad. Sci., 97: 996-1001.

Singh, M., S. Manikandan and A.K. Kumaraguru, 2011. Nanoparticles: A new technology with wide applications. Res. J. Nanosci. Nanotechnol., 1: 1-11.

Taleb, C., M. Pettai and P. Pileni, 1998. Nano particles and nano structured films preparation. Characterization Appl. Chem., 22: 1203-1203.

Willner, I., R. Baron and B. Willner, 2006. Growing metal nanoparticles by enzymes. Adv. Mater., 18: 1109-1120.