IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
August 2016 Vol.:7, Issue:1
© All rights are reserved by Karuppiah Muthu et al.

Antioxidant Activity of Biosynthesized Silver Nanoparticles: A Green Chemistry Study

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Karuppiah Muthu*

Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli - 627 012. TN, India.

Submission: 10 August 2016
Accepted: 15 August 2016
Published: 25 August 2016

HUMAN JOURNALS

www.ijppr.humanjournals.com

Keywords: *Ixora coccinea*, silver nanoparticles, spherical shape, antioxidant activity

ABSTRACT

Biosynthesis of Silver Nanoparticles (Ag NPs) was synthesized from aqueous silver ions using the reducing agent of *Ixora coccinea* L., flower extract. From the UV–Vis absorption surface Plasmon resonance band at 435nm was confirmed the synthesis of Ag NPs. X-ray diffraction (XRD) analysis of four peaks was identified the FCC crystalline nature of metallic silver. Surface morphology and sizes of Ag NPs were viewed by field emission scanning electron microscope (FESEM); the particles are spherical shapes with sizes ranging from 14 to 45 nm. Further, the Ag NPs have shown moderate antioxidant activity (DPPH, and Nitric oxide assay) against the standard ascorbic acid.

INTRODUCTION

Synthesis of metal nanoparticles (MNPs) mainly concerns the physico-chemical compositions and controlled variable sizes, shapes. NPs are extensively used in the medicines, cosmetics, electronics, sensor, catalysis, and materials sciences[1,2]. Plant extract mediated green/biosynthesis of MNPs are environmental eco-friendly, less energy expensive and high yield. In recent times, Rapeseed (*Brassica napus* L) extract mediated synthesized Ag NPs were determined the effective antioxidant activity[3]. *Alternanthera sessilis* Linn., leaves extract mediated Ag NPs have been an effective antimicrobial, antioxidant activities[4], *Ulva lactuca* (seaweed) extract used the biosynthesized Ag NPs were applied the photocatalytic degradation of methyl orange dye[5], *Gymnema sylvestre* leaf extract mediated Ag NPs and Au NPs have been noticed on *in-vitro* free radical scavenging efficacy as well as antiproliferative effect in Hep2 cells[6]. *Ixora coccinea* L., belongs to the family of Rubiaceae. This plant has been used in the Folklore and Siddha medicine as the treatment of dysentery, leucorrhoea, chronic ulcer, anti–inflammatory, hypertension, menstrual irregularities, sprains, and skin diseases[7,8]. The reported present work, biosynthesis of Ag NPs was synthesized from silver ions using the reducing agents of *I. coccinea* flower aqueous extract. Further, the Ag NPs have investigated an *in-vitro* antioxidant activity and compared with the standard ascorbic acid.

EXPERIMENTAL METHODS

Extraction of *I. coccinea* flower

Fresh *I. coccinea* flower was collected from the area of Manonmaniam Sundaranar University (MSU) campus, Tirunelveli, Tamil Nadu, India. 10 g of *I. coccinea* flower was thoroughly washed with running tap water followed by triple distilled water in order to remove the dust. The washed flower was taken in a 250 ml Erlenmeyer flask; 100 ml of distilled water was added and boiled at 80°C for 10 min. After, the extract was filtered through Whatman's No.1 filter paper and stored at 4°C for further use in the biosynthesis of NPs.

Biosynthesis and characterization of silver nanoparticles

About 20 ml of 1 mM aqueous silver nitrate was taken in a 50 ml clean Erlenmeyer flask and added 1.0 ml of *I. coccinea* flower extract at room temperature. Initially, the colorless reaction mixture was changed to dark brown to indicate the formation of Ag NPs. This Ag NPs were recorded on Perkin Elmer Lamda 25 model UV–Vis spectrophotometer in the wavelength regions 200 to 800 nm. The obtained Ag NPs were centrifuged at 10,000 rpm speed for 10 min at 40°C. The residual part of Ag NPs was dried in hot air–oven at 60°C for 24 h. The dried Ag NPs were characterized by PANalytical X'PERT–PRO powder XRD instrument with Cu Kα radiation (1.5406Å) in the 2θ range 20-80° and the scanning speed at 0.002 min per degree. The Ag NPs capped phytochemical groups were identified by JASCO FTIR spectroscopy using KBr pellet method in the range of 4000–400 cm^{-1}. The morphology and sizes of synthesized Ag NPs were viewed by HITACHI SU-6600 model FESEM analytical instrument. The morphology of synthesized Ag NPs was viewed by HITACHI SU-6600 model FESEM with energy-dispersive X-ray spectroscopy (EDS) elemental analytical instrument to determine the particles shape and sizes.

***In-vitro* antioxidant activity of Ag NPs**

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay

The antioxidant activity of Ag NPs was determined using a DPPH radical method. 3.9 ml of (0.1 mM) DPPH was mixed with 0.1 ml of various concentrations (10, 25, 50, 75 and 100 µg/ml) of Ag NPs. The reaction mixture was allowed to stand at room temperature for about 45 min. After the absorbance of reaction mixture was determined by UV–Vis spectra with wavelength at 517 nm and against DPPH for the blank solution. The DPPH radical scavenging activity in the percentage of inhibition has calculated by the equation (1).

$$\% \text{ of inhibition} = (A_{blank} – A_{sample}/A_{blank}) \times 100 \qquad \text{-------- (1)}$$

Where A_{blank} is absorbance of the blank solution (DPPH) only. A_{sample} is the absorbance of the test sample of Ag NPs. Ascorbic acid was used as a standard positive control.

Nitric oxide (NO) radical scavenging activity

The NO radical scavenging activity of the Ag NPs was measured the standard method with some modification. Sodium nitroprusside (1.5 ml) and phosphate buffer (0.5ml of pH 7.4) were separately added to 1.5 ml of different concentrations (10, 25, 50, 75, and 100 µg/ml) of Ag NPs. All the test samples were incubated at room temperature for an hour. After the incubation period, 0.5 ml of Griess reagent was added to each test sample. The notable wavelength at 550 nm of absorbance was measured. Ascorbic acid was used as the standard. The NO radical scavenging activity was calculated by equation (2).

$$\% \text{ of I effect} = (A_0 – A_1/A_0) \times 100 \qquad \text{---------- (2)}$$

Where "% of I effect" is the percentage inhibition of NO radical, "A_0" is the absorbance of all the reagents without Ag NPs and "A_1" is the absorbance of all reagents with Ag NPs.

RESULTS AND DISCUSSION

In the green chemistry method, the colorless reaction mixture (extract and silver ions) was changed to dark brown[9] with a one hour to identify the formation of Ag NPs. The stability of synthesized Ag NPs was scrutinied by UV–Vis spectra at different time intervals as shown in Fig. 1. The UV–Vis spectral SPR sharp band at 435 nm was confirmed the synthesis of Ag NPs[9,10]. After 30 hrs, the increased UV-Vis spectra absorbance intensity was very low but did not any transfer of the wavelength region. So this result to conclude the maximum amount of aqueous silver ions was reduced to metallic silver.

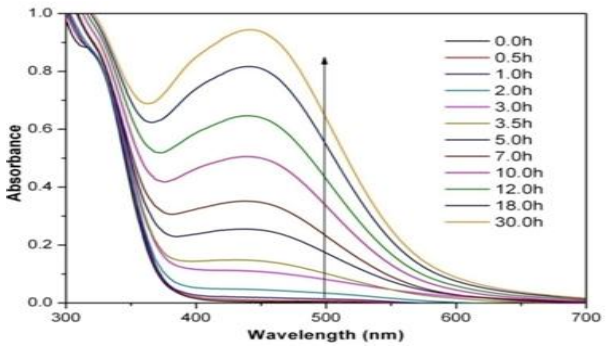

Fig. 1 UV–Vis spectra of biosynthesized Ag NPs by aqueous $AgNO_3$ in the presence of *I. coccinea* flower extract at different time intervals

Fig. 2 shows the disappearance/appearance of some characteristic functional groups in compound were present in the *I. coccinea* flower extract before and after the biosynthesis of Ag NPs. Both spectral results showed the wave numbers at 2925 and 2922 cm^{-1} of C–H stretching groups and at 1618 cm^{-1} due to carbonyl stretching of amide linkage group[11]. The vibrational bands at 3248 and 3325 cm^{-1} are free overlapping of amine (NH) and hydroxyl groups were identified by the flower extract and Ag NPs. The new absorption band at 1712 cm^{-1} is due to the carbonyl group which indicates that the hydroxyl groups of organic compounds were oxidized followed by silver nitrate was reduced to metallic silver (Ag NPs)[12]. These spectral results shows formally identified phytochemicals such as polyphenols, carbohydrates and flavonoids compound are present in the flower extract and act as good reducing agents in the biosynthesis of Ag NPs. Similarly, the plant extract used the synthesis of Ag NPs reported respectively[3, 4,13].

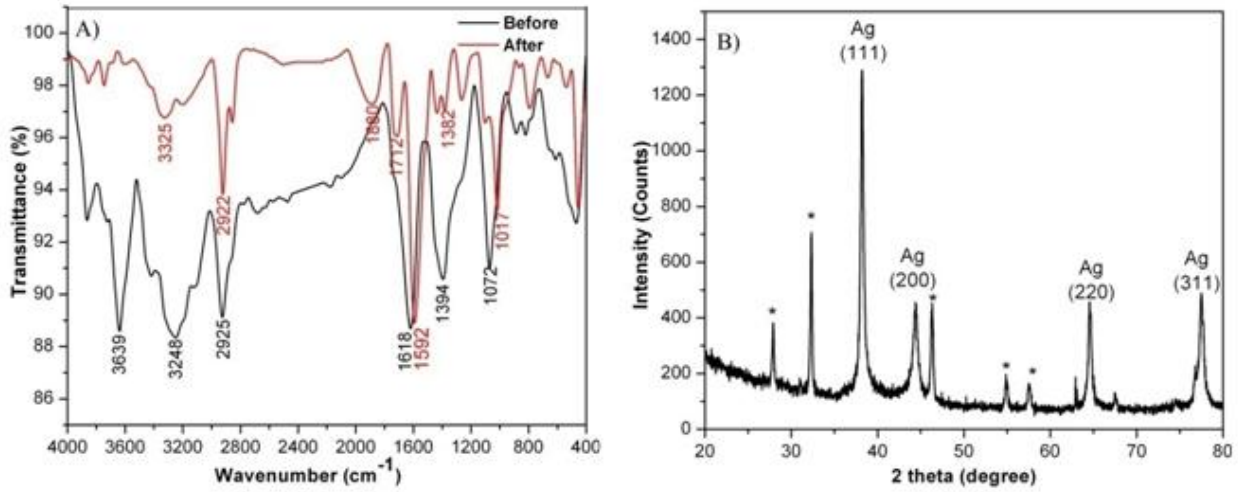

Fig. 2 Green synthesis of Ag NPs using *I. coccinea* flower extract: A) FTIR spectra;

B) XRD spectrum.

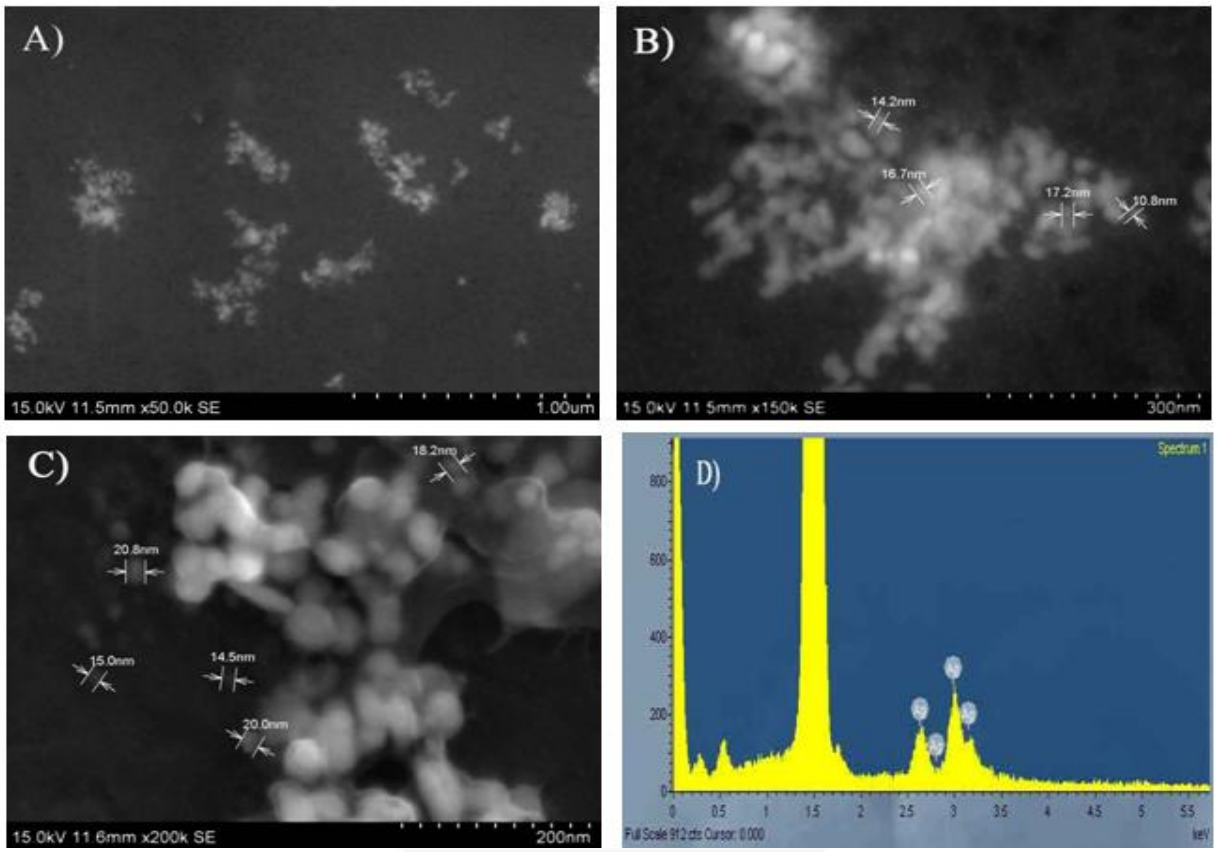

Fig. 3 FESEM image of the synthesized Ag NPs; A) Low magnification image; B) & C) High resolution image; D) EDS spectrum of the presence of silver.

The XRD spectrum (Fig. 2B) of Ag NPs shows four major Bragg reflection at 2θ peak values and corresponding lattice planes at 38.14° (111), 44.27° (200), 64.51° (220) and 77.56° (311) has to confirm the metallic silver as face centered cubic (FCC) structure. The XRD patterns were corroborated with the database of Joint Committee on Powder Diffraction Standards (JCPDS) file No. 89–3722. Few unassigned peaks (asterisk *) were identified the crystallization of bioorganic compounds that occurs on the surface of NPs respectively reported[9,14]. FESEM analysis of biosynthesized Ag NPs (Fig. 3) were identified by shapes and sizes. Morphology of the particles has been confirmed by poly-dispersed spherical shape and sizes range from 14 to 45 nm (Fig. 3B & 3C) and into slightly agglomerated to the surface. The purity of biosynthesized Ag NPs was determined by Energy dispersive X-ray diffraction (EDX) analysis as shown in Fig.

3D. The spectrum reveals that the strong elemental signals of silver were confirming the synthesized Ag NPs and some other biomass peaks were identified[15,16].

The product of Ag NPs were investigated by *in-vitro* antioxidant activity of DPPH assay, and Nitric oxide assay[4,18]. The DPPH activity of Ag NPs shows the disappearance of purple color which indicates the presence of the antioxidant property. Fig. 4a illustrates an increase in Ag NPs concentration as well as increases the radical scavenging activity[19]. The NO radical scavenging activity was determined by the different concentration of Ag NPs using the nitrating agent of sodium nitroprusside and Griess reagent. An increase in concentration of NPs, increases the NO radical scavenging activity (Fig. 4b). This result reveals that the Ag NPs shows superior scavenging activity as compared to the standard with similar such inhibition was respectively reported[20].

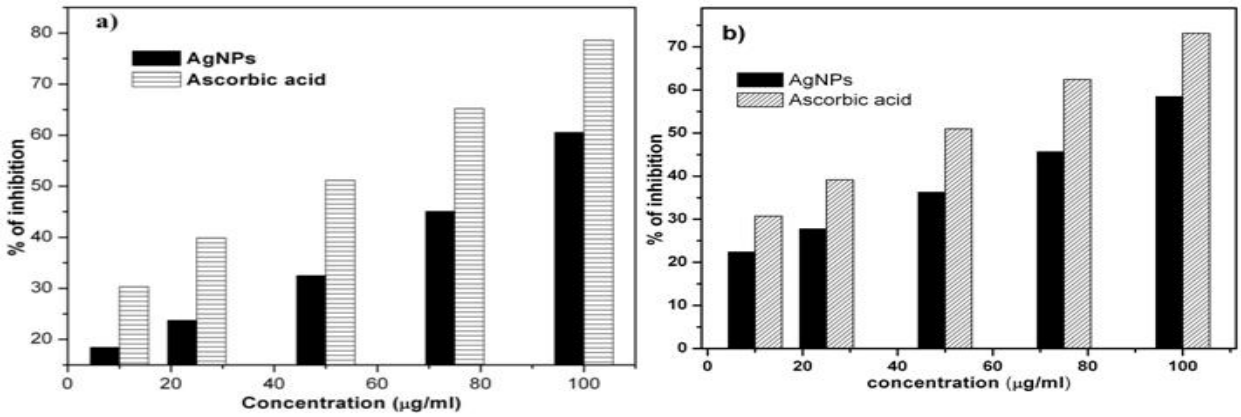

Fig. 4 *In–vitro* antioxidant activity of Ag NPs and standard Ascorbic acid; a) DPPH assay; b) NO radical assay.

CONCLUSION

In the *I. coccinea* flower extract mediated biosynthesized Ag NPs was eco-friendly, environmentally simple one step, nontoxic, low-cost, and high yield of NPs. The color and UV–Vis SPR band at 435 nm was confirmed the production of Ag NPs. The Ag NPs surface morphology and sizes were clearly identified by FESEM. The Ag NPs was showed moderate antioxidant activities of DPPH and NO radical assays. Nowadays the plant biosynthesized MNPs

have been used in the various biological investigations of antimicrobial, antioxidant, anti–HIVand anticancer activities in the modern medicine.

ACKNOWLEDGEMENT

The author would like to acknowledge the Director, National Centre for Nanoscience and Nanotechnology, University of Madras, Guindy Campus, Chennai, Tamil Nadu for the FESEM analysis, and the Head, Department of Physics, MSU, Tirunelveli for XRD analysis.

REFERENCES

1. Brust M, Bethell D, Kiely CJ, Schiffrin DJ. Self-assembled Gold nanoparticle thin films with nonmetallic optical and electronic properties. *Langmuir* 1998;14:5425–5429.
2. Xia Y, Xiong Y, Lim B, Skrabalak SE. Shape-controlled synthesis of metal nanocrystals: simple chemistry meets complex physics? *Angew Chem Int Ed*. 2009;48:60–103.
3. Szydlowska-Czerniak A, Tulodziecka A, Szlyk E. A silver nanoparticles based method for determination of antioxidant capacity of rapeseed and its products. *Analyst* 2012;137:3750–3759.
4. Niraimathi KL, Sudha V, Lavanya R, Brindha P. Biosynthesis of silver nanoparticles using *Alternanthera sessilis* (Linn.) extract and their antimicrobial, antioxidant activities. *Colloids and Surf. B: Biointerfaces* 2013;102:288–291.
5. Kumar P, Govindaraju M, Senthamilselvi S, Premkumar K. Photocatalytic degradation of methyl orange dye using silver (Ag) nanoparticles synthesized from *Ulva lactuca*. *Colloids and Surf. B: Biointerfaces* 2013;103:658–661.
6. Nakkala JR, Mata R, Bhagat E, Sadras SR. Green synthesis of silver and gold nanoparticles from *Gymnema sylvestre* leaf extract: study of antioxidant and anticancer activities. *J Nanopart Res*. 2015;17:151–166.
7. Sastri BN. The Wealth of India – Rawmaterials. Reprinted, New Delhi (India): Vol. V. Council of Scientific Industrial Research; 2001:275.
8. Saha MR, Alam MdA, Akter R, Jahangir R. *In-vitro* free radical scavenging activity of *Ixora coccinea* L. *Bangladesh J Pharmacology* 2008;3:90–96.
9. Karuppiah M, Rajmohan R. Green synthesis of silver nanoparticles using *Ixora coccinea* leaves extract. *Materials letter* 2013;97:141–143.
10. Gopinath V, MubarakAli D, Priyadarshini S, Priyadharsshini NM, Thajuddin N, Velusamy P. Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: A novel biological approach. *Colloids and Surf. B: Biointerfaces* 2012;96:69–74.
11. Santhoshkumar T, Rahuman AA, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C, Zahir AA, Elango G, Kamaraj C. Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. *Parasitol Res*. 2011;108:693–702.
12. Vidhu VK, Aromal SA, Daizy P. Green synthesis of silver nanoparticles using *Macrotyloma uniflorum. Spectrochim. Acta Part A: Mol and Biomol Spect*. 2011;83:392–397.
13. Huang J, Zhan G, Zheng B, Sun D, Lu F, Lin Y, Chen H, Zheng Z, Zheng Y, Li Q. Biogenic silver nanoparticles by Cacumen platycladi extract: synthesis, formation mechanism, and antibacterial activity. *Ind. Eng. Chem. Res*. 2011;50:9095–9106.
14. Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. *J Nanopart Res*. 2011;13:2981–2988.
15. Raghunandan D, Mahesh BD, Basavaraja S, Balaji SD, Manjunath SY, Venkataraman A. *J Nanopart. Res*. 2011;13:2021-2028.

16. Arunachalam R, Dhanasingh S, Kalimuthu B, Uthirappan M, Rose C, Mandal AB. *Colloids and Surf. B: Biointerfaces* 2012;94:226–230
17. Rajeswari A, Sujatha D, Balasaraswathi K, Mani U, Rose C, Mandal AB. Phytosynthesis of silver nanoparticles using *Coccinia grandis* leaf extract and its application in the photocatalytic degradation. *Colloids and Surf. B: Biointerfaces* 2012;94:226–230.
18. Inbathamizh L, Ponnu TM, Mary EJ. *In vitro* evaluation of antioxidant and anticancer potential of *Morinda pubescens* synthesized silver nanoparticles. *J Pharmacy Research* 2013;6:32–38.
19. Dipankar C, Murugan S. The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from *Iresine herbstii* leaf aqueous extracts. *Colloids and Surf. B: Biointerfaces* 2012;98:112–119.
20. Shanmugasundaram T, Radhakrishnan M, Gopikrishnan V, Pazhanimurugan R, Balagurunathan R. A study of the bactericidal, anti-biofouling, cytotoxic and antioxidant properties of actinobacterially synthesised silver nanoparticles. *Colloids and Surf. B: Biointerfaces* 2013;111:680–687.

HUMAN