International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.11 pp 194-199, 2015

Green Synthesis and Spectroscopic characterisations of gold nanoparticles using invitro grown hypericin rich shoot cultures of Hypericum hookerianum

Manoj L[1], Vinita Vishwakarma[2]

[1]Department of Physics, Sathyabama University, Chennai- 600119, India
[2]Centre for Nanoscience and Nanotechnology, Sathyabama University, Chennai- 600119, India

Abstract: The paper reports a biogenic approach for the synthesis of gold nanoparticles (GNPs) using in-vitro grown hypericin rich shoot cultures of Hypericum hookerianum plant. The plant extract is mixed with $HAuCl_4.3H_2O$, incubated and the synthesis of nanoparticles was studied using UV-Vis spectroscopy. The nanoparticles obtained were characterised using field emission scanning electron microscopy (FESEM) and X-ray diffraction (XRD) analyses. The results showed that the plant extract of Hypericum hookerianum is a good bioreductant for the synthesis of GNPs. The synthesised AgNPs were effective against different multidrug-resistant human pathogens such as Staphylococcus aureus (Gram positive) and Pseudomonas aeruginosa (Gram negative) species.
Keywords: Hypericum hookerianum, GNPs, Biogenic Synthesis.

1. Introduction

A new interest for gold have been found recently, when it is it is divided into miniscule grains, such as gold nanoparticles. These gold nanoparticles are the subject of substantial research due to the unique optical, electronic, and molecular-recognition properties exhibited[1-4]. Among various methods adopted for synthesis of nanoparticles, the green synthesis approach involving the reduction of Au (III) to Au(0) by plant extracts has gained profound significance in recent years due to the non-toxic and renewable nature of plant extracts[5]. More over in the green synthesis approach, plant extract itself acts as a stabilizer, and no capping agents or additional stabilizers are needed[6]. The nanoparticles derived from green process are versatile enough and can be used in many types of technological applications, from delicate electronics to revolutionary medical procedures[7-13].

Using Green Synthesis approach, several plants and their parts have been used successfully for the extracellular synthesis of metal nanoparticles[14]. Extracts of *Cinnamomum camphora*[15], *Medicago sativa*[16–18], *Pelargonium graveolens*[19], *Avena sativa*[20], *Azardirachta indica*[21], *Tamarindus india*[22], *Emblica offcinalis*[23], *Aloe vera*[24], *Coriandrum sativum*[25], *Carica papaya*[26], *Parthenium hysterophorus*[27], *Tritium vulgare*[28], *Acanthella elongata*[29], *Sesuvivm potulacastrum*[30] were successfully used as reducing agents in the production of metal nanoparticles like Ag and Au. However possibilities in plant-mediated biological synthesis of nanomaterial have to be fully explored[31].

Our previous work present a report on the synthesis of Ag nanoparticles using shoot cultures of *Hypericum hookerianum* plant[32]. *Hypericum hookerianum* belongs to the family Hyperiaceae. It is a perennial round-topped shrub distributed in Palni and Nilgiri hills of the Western Ghats in southern India. It is a well

known medicinal plant and is pharmacologically confirmed to possess wound healing[33], anti-bacterial[33], cytotoxic[34] and anti-tumour[35] properties. In our present work we report the synthesis of GNPs using shoot cultures of *Hypericum hookerianum* and the anti-bacterial activity exhibited by the synthesised GNPs was investigated.

2. Experimental

The auxin-induced, hypericin-rich shoot cultures of *Hypericum hookerianum* were raised and maintained in Murashige and Skoog (1962) medium[36] supplemented with 1.0 mg/L kinetin (KIN) and 0.2 mg/L naphthalene acetic acid (NAA) following the procedures described by Padmesh et al. [37]. These hypericin rich shoot cultures were used for the experiment. All the chemicals were obtained from Sigma-Aldrich and Milli-Q water was used throughout the experiments.

The shoot cultures obtained were thoroughly washed, shade dried and fine powdered. 10 gm of the powdered mass was mixed with 100 mL water at 70^{o}C for 15 minutes and was allowed to cool down to room temperature. The extracts were filtered using Whatman number 1 filter paper. The filtered extract was collected in a 250-mL Erlenmeyer flask and stored at 4^{o}C for further studies.

2.1. Green Synthesis of GNPs

GNPs were synthesized by adding 0.5 ml of 1% aqueous $HAuCl_4$ to 50 ml boiling H_2O. Under vigorous stirring, 2.5 ml of filtered *Hypericum Hookerianum* plant extract was added to the solution at 350 K. The process was carried out for 10 min. The bioreduction of Au^{3+} ions to Au was evident by the slow colour change of the solution, indicating the formation of colloids (S_1). The nanoparticle suspension was cooled down slowly to room temperature under constant stirring and the Au colloids (S_1) obtained were stored in a dark bottle at 4^{o}C for future use. Similarly, the experiment was repeated at 400 K and the Au colloids (S_2) were obtained and stored. A slight colour change was noticed in the colloids (S_2), indicating the increase in the rate of reduction from S_1 to S_2.

2.2. Characterisation

UV-Vis absorption spectrophotometer (JASCO V-530) was used to monitor the bio-reduction of Au^{3+} ions. X-ray Diffraction (XRD) analysis has been carried out using an X-ray diffractometer (INEL) with Co-Ka radiation. For XRD measurement, a thin film of the nanoparticles synthesized is prepared using Spin Coating machine. Field Emission Scanning Electron Microscope (FESEM – LEO SUPRA 55 – CARL ZEISS, Germany) was used to examine the morphology of the synthesized nanoparticles.

2.3. Anti-bacterial activity

The anti-bacterial activity of the synthesized GNPs was tested by using *Pseudomonas aeruginosa* (Gram negative) and *Staphylococcus aureus* (Gram positive) bacterial species. On nutrient agar medium in petridishes, the pure cultures of *Pseudomonas aeruginosa* bacteria were sub-cultured. Three wells of 6 mm diameter were dug on nutrient agar plates using gel puncture technique, as shown in Figure 4(a). In each well, 30 µL of filtered shoot extract, colloidal sample S_1 and colloidal sample S_2 were poured as indicated in Figure 4. Shoot extract was added as reference. The petri dish was incubated at 37^{o}C for 24 hours and clear zones of inhibition were noticed. The experiment was repeated with *Staphylococcus aureus* bacterial species and clear zones of inhibition were noticed.

3. Results and Discussions

3.1. Optical absorption

UV-VIS absorption spectra of the Au colloid S_1 synthesied at 350 K is recorded in the 350-750 nm wavelength regions. Figure 1 shows UV-Vis spectra of the Au colloid S_1. Optical properties of noble metal nanoparticles depend on their morphology. When the dimensions of the nanoparticles become smaller than the wavelength of the incident light, energy can be confined in small regions through the excitation of surface plasmon resonance (SPR), which is the origin of observed colour of colloids[38-39]. In the present work, the absorption maximum is at about 540 nm as shown in Figure 1. Since the concentrations of all the reagents are

constant in colloids (S_1, S_2) prepared at different temperatures, increase in the temperature increases the reaction kinetics and is expected to facilitate the nucleation process at higher temperature.

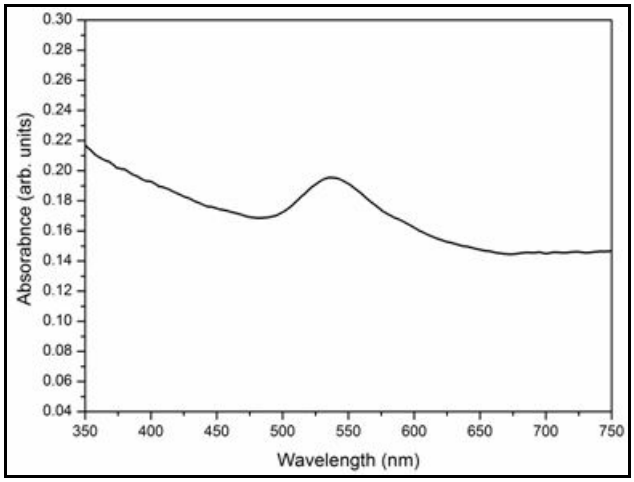

Figure 1. UV–Vis spectra of Au colloid (S_1).

3.2. FESEM studies

FESEM images of synthesised GNPs S_1 and S_2 are shown in Figure 2(a) and 2(b). Morphology of the colloids S_1 are distorted spherical shape with size ranging within 10–70 nm was observed. In colloids S_2, the particle size ranging within 10–50 nm was observed. There was a slight decrease in the size and increase in the production of GNPs from colloids S_1 to S_2 due to increase in temperature, which is a well known phenomena[40].

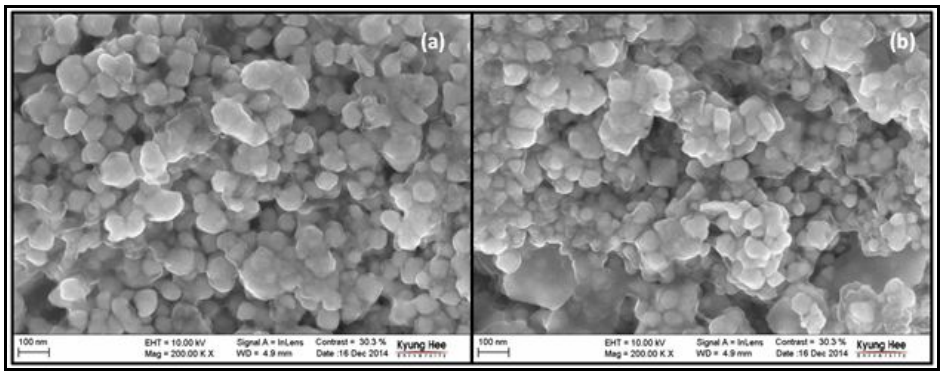

Figure 2. (a) FESEM image of colloid S_1. (b) FESEM image of colloid S_2.

3.3. XRD analysis

X-ray diffraction (XRD) analysis was performed to confirm the crystalline nature of GNPs. Sample for XRD analysis was prepared by drop coating the GNPs solution onto a glass slide. The study was performed using INEL X-ray diffractometer. The diffraction pattern was recorded by Co-Kα_1 radiation with λ of 1.78 Å in the region of 2θ ranging within 40°–100° at 0.02/min and the time constant was 2s.

Figure 3 shows the XRD pattern of the colloid S_1. X-ray diffraction pattern showed different peaks corresponding to the (111), (200), (220), (311) and (222) planes, which are consistent with face centered cubic (FCC) structure of the Au (JCPDS File: 04-0784). The average particle size is estimated by using Debye–Scherrer formula as 40 nm and it was done by using the width of the (111) Bragg's reflection which was in consonance with the size of the particle calculated from FESEM analysis. From the broadening of XRD peaks, it can be said that nanosized particles are getting formed. As the temperature increases, there was no change in the size of the particles.

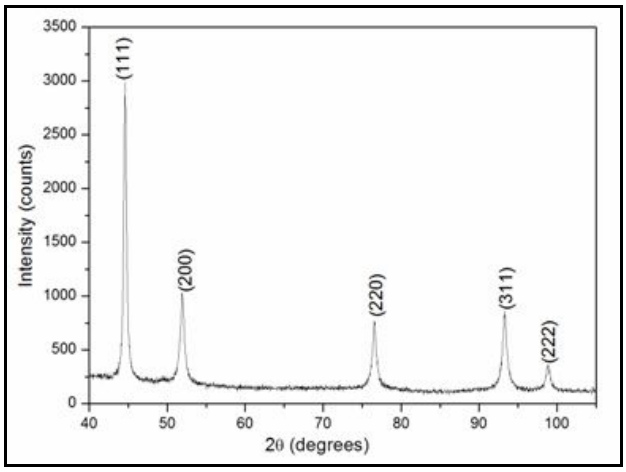

Figure 3. XRD pattern of Au nanoparticles obtained from colloid S_1.

3.4. Anti-bacterial study

The study is focused on testing the effect of anti-bacterial activity exhibited by synthesised GNPs. The colloids S_1 and S_2 exhibited good anti-bacterial activity against *Pseudomonas aeruginosa* (Gram negative) and *Staphylococcus aureus* (Gram positive) bacterial species. The synthesised GNPs restricted bacterial growth, which can be evidenced by the formation of clear zone with restricted bacterial growth around the cavity as shown in Figure 4(a) and 4(b). The results show that both the colloids S_1 and S_2 exhibited greater anti-bacterial activity with *Staphylococcus aureus* (Gram positive) bacterial species than *Pseudomonas aeruginosa* (Gram negative), which can be evidenced from the increase in the diameter of zone of inhibition.

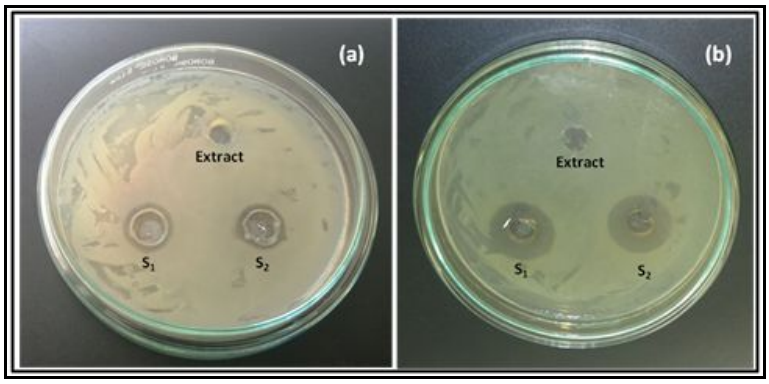

Figure 4. Appearance of inhibitory zones on agar plates with Au colloids S_1 and S_2 against (a) *Pseudomonas aeruginosa*; (b) *Staphylococcus aureus*.

4. Conclusion

GNPs have been synthesised by reduction method using hypericin rich shoot cultures of *Hypericum hookerianum* as reducing agent. UV/ Vis study gives an absorption peak at 540 nm due to SPR. FESEM images show that the GNPs synthesised are in distorted spherical shape with size ranging from 10-70 nm. XRD study confirms the FCC structure of prepared nanoparticles. The synthesised GNPs were found to exhibit good anti-bacterial activity with both Gram positive and Gram negative bacterial species. The anti-bacterial activity exhibited was more in Gram positive than that of Gram negative bacterial species.

References

1. Alkilany AM, Lohse SE, Murphy CJ. The gold standard: gold nanoparticle libraries to understand the nano bio interface. Acc. Chem. Res., 2013; 46: 650–661.

2. Zhang Y, Cui X, Shi F, Deng Y. Nano-gold catalysis in fine chemical synthesis. Chem. Rev., 2012; 112: 2467–2505.
3. Murphy CJ, Gole AM, Stone JW, Sisco PN, Alkilany AM, Goldsmith EC, Baxter SC. Gold nanoparticles in biology: beyond toxicity to cellular imaging. Acc. Chem. Res., 2008; 41: 1721–1730.
4. Thomas KG, Kamat PV. Chromophore-functionalized gold nanoparticles. Acc. Chem. Res., 2003; 36: 888–898.
5. Anastas PT, Kirchhoff MM. Origins, current status, and future challenges of green chemistry. Acc. Chem. Res., 2002; 35: 686–694.
6. Nadagouda MN, Varma RS. Green synthesis of silver and palladium nanoparticles at room temperature using coffee and tea extract. Green Chem., 2008; 10: 859–862.
7. Mohanpuria P, Rana NK, Yadav SK. Biosynthesis of nanoparticles: technological concepts and future applications. J Nanopart Res., 2008; 10: 507–517.
8. Jain PK, Huang X, EI-Sayed IH, El-Sayed MA. Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. Acc Chem Res., 2008; 41: 1578–1586.
9. Alanazi FK, Radwan AA, Alsarra IA. Biopharmaceutical applications of nanogold. Saudi Pharm J., 2010; 18: 179–193.
10. Pradeep T, Anshup. Noble metal nanoparticles for water purification: a critical review. Thin Solid Films., 2009; 517: 6441–6478.
11. Philip D, Gopchandran KG, Unni C, Nissamudeen KM. Synthesis, characterization and SERS activity of Au-Ag nanorods. Spectrochim Acta A., 2008; 70: 780–784.
12. Zheng B, Qian L, Yuan H, Xiao D, Yang X, Paau MC, Choi MM. Preparation of gold nanoparticles on eggshell membrane and their biosensing application. Talanta., 2010; 82: 177–183.
13. Hu J, Wang Z, Li J. Gold nanoparticles with special shapes: controlled synthesis, surfaceenhanced Raman scattering. Sensors., 2007; 7: 3299–3311.
14. Mohanpuria P, Rana N K, Yadav S K. Biosynthesis of nanoparticles: technological concepts and future applications. J. Nanopart. Res., 2008; 10: 507-517.
15. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, He. N, Hong J, Chen C. Biosynthesis of Silver and Gold Nanoparticles by Novel Sundried Cinnamomum camphora Leaf. Nanotechnology., 2007; 18: 105104-105115.
16. Gardea-Torresdey J L, Gomez E, Perlata-Videa J R, Parsons J G, Troiani H, Jose-Yacamen M. Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. Langumir., 2003; 19: 1357-1361.
17. Schabes-Retchkiman P S, Canizal G, Herrera-Becerra R, Zorrilla C, Liu HB, Ascencio J A. Biosynthesis and characterization of Ti/Ni bimetallic nanoparticles. Opt. Mater., 2006; 29: 95-99.
18. Lukman A L, Gong B, Marjo C E, Roessner U, Harris A T. Facile synthesis, stabilization, and anti-bacterial performance of discrete Ag nanoparticles using Medicago sativa seed exudates. J. Colloid Interface Sci., 2011; 353: 433-444.
19. Shankar S, Ahmad A, Sastry M. Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnol. Prog., 2003; 8: 1627-1631.
20. Armendariz V, Herrera I, Jose R, Videa P, Yacaman M J, Troiani H, Santiago P, Jorge L, Torresdey G. Size controlled gold nanoparticle formation by Avena sativa biomass: use of plants in nanobiotechnology. J. Nanopart. Res., 2004; 6: 377-382.
21. Shanker S S, Rai A, Ahmed A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J. Colloid Interface Sci., 2004; 275: 496-502.
22. Shanker S S, Rai A, Ankamwar B, Singh A, Ahmed A, Sastry M. Biological synthesis of triangular gold nanoprisms. Nat. Mater., 2004; 4: 482-488.
23. Ankamwar B, Damle C, Ahmed A, Sastry M. Biosynthesis of gold and silver nanoparticles using Emblica officinalis fruit extract, their phase transfer and transmetallation in an organic solution. J. Nanosci. Nanotechnol., 2005; 5: 1665-1671.
24. Chandran S P, Chadudhary M, Pasricha R, Ahamad A, Sastry M. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnol. Progr., 2006: 22: 577-583.
25. Badrinarayanan K, Sakthivel N. Coriander leaf mediated biosynthesis of gold nanoparticles. Mater. Lett., 2008; 62: 4588-4590.
26. Mude N, Avinash I, Aniket G, Mahendra R. Synthesis of silver nanoparticles using callus extract of Carica papaya. J. Plant Biochem. Biotechnol., 2009; 18: 83-86.

27. Parashar V, Parashar R, Sharma B, Pandey A C. Parthenium leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. Digest J. Nanomater. Biostruct., 2009; 4: 45-50.
28. Armendariz V, Parsons J G, Lopez M L, Peralta-Videa J R, Yacaman M J, Jorge L, Torresdey G. The extraction of gold nanoparticles from oat and wheat biomasses using sodium citrate and cetyltrimethylammonium bromide, studied by X-ray absorption spectroscopy, high-resolution transmission electron microscopy, and UV-visible spectroscopy. Nanotechnology., 2009; 20: 105-607.
29. Inbakandan D, Venkatesan R, Ajmal Khan S. Biosynthesis of gold nanoparticles utilizing marine sponge Acanthella elongate. Colloids Surf. B., 2010; 81: 634-639.
30. Nabikhan A, Kandasamy K, Raj A, Alikunhi N M. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, Sesuvium portulacastrum L. Colloids Surf. B., 2010; 79: 488-493.
31. Ghodake G S, Deshpande N G, Lee Y P, Jin E S. Pear fruit extract-assisted room-temperature biosynthesis of gold nanoplates. Colloids Surf. B Biointerf., 2010; 75: 584–589.
32. Manoj L, Vinita V, Subhranshu S S, Sooriamuthu S. Green synthesis of silver nanoparticles using hypericin-rich shoot cultures of Hypericum hookerianum and evaluation of anti-bacterial activities. Journal of Experimental Nanoscience., 2013; 10: 181-188.
33. Mukherjee PK, Saritha GS, Suresh B. Antibacterial spectrum of *Hypericum hookerianum*. Fitoterapia., 2001; 72: 558–560.
34. Vijayan P, Vinod KS, Badami S, Mukherjee PK, Dhanaraj SA, Suresh B. Selective in vitro cytotoxicity of *Hypericum hookerianum* towards cancer cell lines. Int J Orient Pharm Exp Med., 2003; 3: 141–146.
35. Dongre SH, Badami S, Godavarthi A. Antitumor activity of *Hypericum hookerianum* against DLA induced tumor in mice and its possible mechanism of action. Phytother Res., 2008; 22: 23–29.
36. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant., 1962; 15: 473–479.
37. Padmesh P, Seeni S, Reji JV, Nair GM. A novel process for the production of hypericin from shoot and callus cultures of *Hypericum hookerianum*. Indian Patent. 2008; 239540.
38. Guozhong C, Nanostructures & Nanomaterials, Synthesis, Properties & Applications. Imperial College Press, London, UK, 2004.
39. Kreibig U, Vollmer M, Optical Properties of Metal Clusters, Springer, Berlin, 1995.
40. Park J, Joo J, Kwon GS, Jang Y, Hyeon T. Synthesis of monodisperse spherical nanocrystals. Angew Chem Int Ed., 2007; 46: 4630–4660.

*****