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Biosynthesis of gold nanoparticles using the flower extract of *Azadirachta indica* (Neem) – Characterisation and antibacterial study

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Gold nanoparticles (AuNPs) were synthesized using the aqueous extract of neem flower. The synthesis was optimized by varying the pH, temperature, extract volume and Au^{III} concentrations. UV-Visible spectral analysis, HRTEM and XRD were characteristic of AuNps. Antibacterial activity of the synthesized AuNps showed promising results.

Keywords: Neem flower, gold nanoparticle, antibacterial activity.

Introduction

Nanotechnology is primarily concerned with the synthesis of nanoparticles of variable sizes, shapes, chemical compositions, controlled dispersity and their potential use for biomedical applications[1]. Due to the expensive and potentially dangerous nature of chemical and physical methods the synthesis of nanoparticles using microorganisms, plants and algae is chosen in recent times. Through these methods the toxicity of the by-product would be lesser[2,3]. Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles and the source of the plant extract is known to influence the characteristics of the nanoparticles[4]. In a plant extract-mediated bioreduction the aqueous extract is mixed with the solution of the relevant metal salt. The reaction occurs at room temperature and is generally complete within a few minutes[5].

Gold nanoparticles (AuNPs) are a promising class of nanomaterials with many varieties of applications, which includes cancer, hyperthermia treatment, surface-enhanced Raman spectroscopy (SERS), and infrared radiation absorbing optics. Consequently, a wide variety of synthetic procedures for the formation of various shapes and sizes of AuNPs have been reported[6]. This work aims to apply a biological green technique, namely the the flower extract of *Azadirachta indica* (commonly known as Neem) a species of family Meliaceae for the bioreduction of Au^{III} ions to AuNPs. This plant is commonly available in India and each part of this tree has been used as a household remedy against various human ailments as anti-viral, antibacterial and antifungal agent[7].

Experimental

Preparation of Azadirachta indica (Neem) flower extract: The *Azadirachta indica* (Neem) flowers were washed, dried under sun shade for a week and crushed to a fine powder. 2.5 g of the crushed powder was homogenized with 100 ml de-ionized water in a 250 ml Erlenmeyer flask and heated on a hot water bath for 1 h at 80°C. The solution was cooled and filtered first through a muslin cloth and then using Whatmann No. 1 filter paper to obtain the aqueous extract filtrate. The filtrate is used immediately for the biosynthesis of AuNPs[8].

Synthesis of gold nanoparticles: The biosynthesis of gold nanoparticles was carried out using $HAuCl_4$ solution. To this solution, at optimum pH and temperature, when optimum volume of the extract was added and allowed to react, till gold nanoparticles were synthesized. The reaction mixture was maintained in sun light till the mixture colour changed from golden yellow colour to bluish purple colour. The change in colour of the mixture indicated the reduction of Au^{3+} ions to AuNPs. The absorbance of the resulting solutions was spectrophotometrically measured.

HRTEM: The samples for Transmission Electron Microscopy (TEM) analysis were prepared by drop-casting the

AuNPs solution on a carbon-coated copper TEM grid. Before casting to the grid the GNPs solution was centrifuged at 10,000 rpm for 10 min and the isolated AuNPs were dispersed in 100 µL double distilled water and sonicated for 10 min. The TEM images were recorded on a high resolution electron microscope (HRTEM: JEOL JEM 2010) operating at an accelerating voltage of 200 kV.

X-Ray diffraction: XRD studies of Neem flower reduced nanoparticles was measured by uniformly coating the dried AuNPs on XRD grid and the recording the spectrum by using Philips PW 1830 X-Ray generator operated at a voltage of 40 kV and a current of 30 mA with Cu K-1 radiation.

Antibacterial study: The AuNPs synthesized were tested for their antimicrobial activity by well diffusion method against pathogenic organisms like *Enterococcus faecalis, Proteus mirabilis, Klebsiella pneumonia* and *Staphylococcus aureus*. Amoxyclav was used as the standard antibiotic.

Results and discussion

Optimisation: The synthesis of AuNPs was optimized by varying the concentration of Au^{III}, volume of the plant extract, pH and temperature. The Surface Plasmon Resonance (SPR) band centered 540 nm confirms the formation of AuNPs in the solution (Fig. 1). The most influential pH for the synthesis of AuNPs was found to be 9. The various components present in the extract act as reducing, capping and stabilizing agent[9]. They form a layer around the nanoparticles and stabilize them at the optimum conditions namely 0.1 mM concentration and pH 9 with an extract volume of 9 ml at a reaction temperature of 50°C. No appreciable shift in the peaks was observed on changing the pH, temperature or Au^{III} concentration.

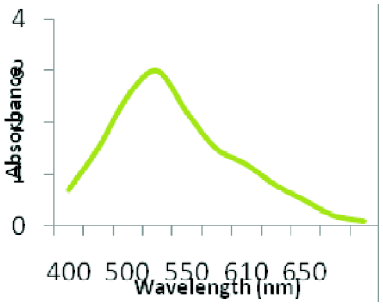

Fig. 1. UV-Visible spectra of AuNPs.

XRD: XRD analysis (Fig. 2) revealed four important peaks present in the (20–80) 2θ range. The diffraction peaks of 38.1° relates to (111), 44.3° relates to (200), 64.43° relates to (220), and 77.4° relates to (311) facets of the face center cubic (FCC) crystal lattice; these agree with reported values for similar gold nanostructures. The reported peak values also matched the planes and face-centered cubic structures of AuNPs prepared by other green syntheses methods[10,11].

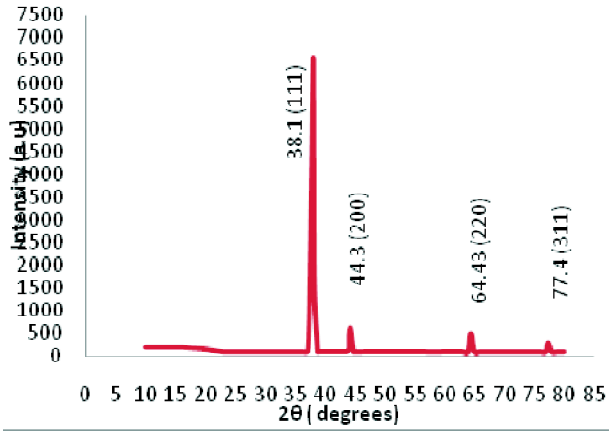

Fig. 2. XRD data of AuNPs.

HRTEM: TEM image of the AuNPs synthesised at 50°C at pH 9 shows the presence of spherical polydispersed nanoparticles around 100 nm (Fig. 3).

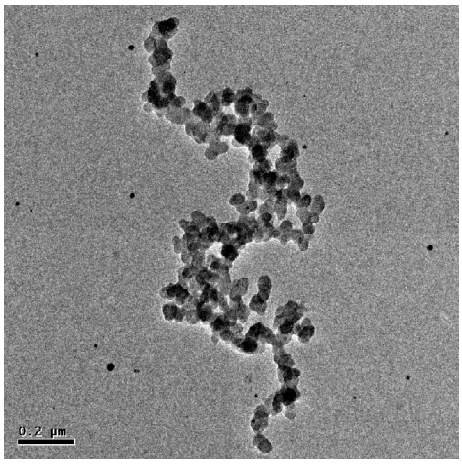

Fig. 3. HRTEM of AuNPs.

Antibacterial study: The antibacterial activity of AuNps carried out against clinically important pathogenic microor-

ganism, by disc diffusion method, showed variable degree of inhibition on all the teat organisms. Maximum activity was shown against *Enterococcus faecalis* and *Klebsiella pneumonia*. Intermediate activity was observed on *Proteus mirabilis* while *Staphylococcus aureus* shows resistance against the AuNp. All the results are tabulated in Table 1.

Table 1. Zone of inhibition obtained by disc diffusion method

Bacteria	Inhibition zone control[a] (nm)	Inhibition zone AuNp[b] (nm)	Inhibition zone AuNp[c] (nm)	Inhibition zone AuNp[d] (nm)
Ent. faecalis	20	33	34	36
Pro. mirabilis	33	25	26	20
Kleb. pneumoniae	20	35	42	50
Staph. aureus	35	15	16	17

[a]Amoxyclav – 20 μg/ml, [b]20 μg/ml, [c]30 μg/ml, [d]40 μg/ml.

Conclusion

Green synthesis of AuNPs using the aqueous extract of Neem flower was optimized at different conditions. The synthesized AuNps had good stability, pronounced antibacterial and antioxidant properties due to which they could be used for biomedical and sensor applications.

References

1. K. N. Thakkar, S. S. Mhatre and R. Y. Parikh, *Nanomedicine: NBM*, 2010, **6**, 257.
2. C. Malarkodi, S. Rajeshkumar, K. Paulkumar, G. Gnanajobitha, M. Vanaja and G. Annadurai, *J. Nanostruct. Chem.*, 2013, **3(30)**, 1.
3. M. Vanaja, G. Gnanajobitha, K. Paulkumar, S. Rajeshkumar, C. Malarkodi and G. Annadurai, *J. Nanostruct. Chem.*, 2013, **3(17)**, 1.
4. V. Kumar and S. K. Yadav, *J. Chem. Technol. Biotechnol.*, 2009, **84**, 151.
5. A. K. Mittal, Y. Chisti and U. C. Banerjee, *Biotechnology Advances*, 2013, **31(2)**, 346.
6. M. Grzelczak, J. Perez-Juste, P. Mulvaney and L. M. Liz-Marzan, *Chem. Soc. Rev.*, 2008, **37**, 1783.
7. V. U. Omoja, A. O. Anaga, I. R. Obidike, T. E. Ihedioha, P. U. Umeakuana, L. I. Mhomga, I. U. Asuzu and S. M. Anika, *Asian Pacific Journal of Tropical Medicine*, 2011, **4**, 337.
8. V. Gopalakrishnan, S. Muniraj, D. Manikandan and Nagendra Gandhi, *IJIRSET*, 2016, **1**, 11.
9. G. Narsing Rao, P. G. Prabhakara Rao and A. Satyanarayana, *International Food Research Journal*, 2014, **21(2)**, 807.
10. C. G. Yuan, C. Huo, B. Gui and W. P. Cao, *IET Nanobiotechnol.*, 2017, **11**, 523.
11. C. Karuppiah, S. Palanisamy, S. Chen, R. Emmanuel, K. Muthupandi and P. Prakash, *RSC Adv.*, 2015, **5**, 16284.