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Research Article

BIOSYNTHESIS OF GOLD NANOPARTICLES USING TURNIP SEED EXTRACT AND ITS CHARACTERIZATION

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ABSTRACT

Gold nanoparticles (AuNPs) have been synthesized by single-step green method using turnip seed extract (TCE) as reducing agent. The synthesized AuNPs were characterized by UV-Vis Spectrophotometer, Transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy and Electron diffraction pattern (EDX). TEM studies revealed the spherical shape and size of gold nanoparticles ranging from 10 to 45 nm. The XRD patterns confirmed the presence of crystalline nature of AuNPs. FTIR measurements confirmed the coating of amine groups, ortho-substituted aromatic phytoconstituents and phenolic compounds on the AuNPs indicating a possible role of biomolecules for the capping and efficient stabilization of the AuNPs. This process of nanoparticle synthesis is simple, nontoxic and eco-friendly compared to the synthetic routes.

INTRODUCTION

Nanotechnology has emerged as a propitious area of technology which has given rise to the green synthesis of nanoparticles using natural substances which came to eminence only a few years ago. Nanoparticles deal with the dimensions and tolerances of particles less than 100 nm and are known to be building block of materials and devices with tuned characteristics (Philip, 2009; Philip *et al*, 2011). Metal nanoparticles particularly gold and silver nanoparticles synthesis has become a growing area of research because they possess interesting and remarkable properties like unique size and shape, large surface area and higher reactivity than their bulk metals (Ankamwar *et al*, 2005; Jiale *et al*, 2007). These metallic nanoparticles are known to exhibit tremendous capacity in biological and chemical applications.

Gold nanoparticles (AuNPs) have attracted much attention towards itself due to its application in the field of deep tissue imaging, drug delivery, cancer diagnostics, and detection of heavy metals as contaminants in the environment, electronics, biological sensors, and catalysis (Sheny *et al*, 2011). Besides medical and pharmaceutical applications they are widely applied in daily use products like soaps, toothpaste, and cosmetic products (Song *et al*, 2009). Therefore, there is need to develop fast, easy and environmentally friendly process for the synthesis of nanoparticles.

Nanoparticles production can be achieved by chemical means (sodium citrate, sodium borohydride, and other reducing agents) and chemical method facilitated by physical methods (heating, microwave, and sonication). These methods involve harsh chemicals and release of toxic substances making nanoparticles unfit for biological applications (Shankar *et al*, 2004). To conquer such issues, bio-reduction potential of plants, different micro-organisms and other green non-toxic reducing agents have been explored which have given attention to green synthesis of nanoparticles. Green synthesized NPs are (i) easy to synthesize, (ii) size controllable, (iii) easily characterizable due to its colouration,(iv) monodispersity, (v) easy functionalization, (vi) biocompatibility, and (vii) non-toxicity (Mukherjee *et al*, 2012; Sett *et al,*2016; Mishra *et al*, 2016).

Plants are the best candidates suitable for large-scale biosynthesis of nanoparticles. These phytosynthetic routes are more preferable over other biological processes as they eliminate the elaborate process of maintaining cell cultures, are cost-effective and non-hazardous (Majumdar *et al,* 2016; Bhau *et al,* 2015). Various proteins, enzymes, amino acids, vitamins, polysaccharides, and organic compounds present in plants may

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act as stabilizing and capping agents in green synthesis and are helpful in bio reduction of metals to form nanoparticles (Chanda *et al*, 2011; Ghosh *et al*, 2016; Lakshmanan *et al*, 2016). Various parts of plants like root, stem, leaves, bark, petals and seeds are being explored these days for green synthesis of nanoparticles. Many studies have reported the synthesis of gold nanoparticles using plant extracts such as *Ocimum sanctum* (Philip *et al,* 2011), *Cinnamomum camphora* (Jiale *et al,* 2007), *Emblica officinalis* (Ankamwar *et al,* 2005), Mushroom (Philip 2009), *Anacardium occidentale* Sheny *et al,* 2011), *Azadirachta indica* (Shankar *et al,* 2004), *Hibiscus sabdariffa* (Mishra *et al,* 2016), *Mimusops elengi* (Majumdar *et al,* 2016), *Nepenthes khasiana* (Bhau *et al,* 2015), *Gnidia glauca* (Ghosh *et al,* 2016), *Momordica cochinchinensis* (Lakshmanan *et al,* 2016), Coriander (Narayanan *et al,* 2008), Cardamom (Singh *et al,* 2015).

Turnip belongs to the family Brassicaceae and has nutritional value. It has anti-oxidant property due to the presence of phenols, phenolic acids, hydroxycinnamic acid and flavonoids, which instigated us to synthesize AuNPs in an ecofriendly manner (Romani *et al,* 2006; Cartea *et al,* 2011). In the present study, we have attempted to synthesized biocompatible and non-toxic AuNPs in aqueous medium by employing single-step phytosynthesis approach using aqueous seed extract of *Brassica rapa* (turnip).

Experimental procedures

Materials

All reagents were of analytical grade. Chloroauric acid ($HAuCl_4$) was procured from SRL, Turnip seeds (*Brassica rapa L.*) were purchased from local market, Varanasi (India). All aqueous solutions were prepared using double distilled water, experiments were performed in triplicate.

Preparation of aqueous turnip seed extract (TSE)

Preparation of TSE was done by the method of (Awasthy *et al,* 2012) with slight modification. Briefly, 20 g of turnip seeds were washed with distilled water, added to 100 ml of Milli Q water and boiled at 100°C for 20 min in a water bath. The solution was allowed to cool and filtered through Whatman Filter Paper No. 1 and filtrate was collected. The filtrate was directly used for synthesis of the AuNPs or stored at 4°C as stock solution for further experiments.

Biosynthesis of Gold Nanoparticles (AuNPs)

To optimize the concentration of the seed extract, the experiment was carried out by varying the concentration of the extract against a fixed concentration of gold chloride. With the optimized volume of TSE and 1mM $HAuCl_4$ aqueous solution, the reaction mixture was incubated at 50°C, under continuous stirring condition. The synthesis of gold nanoparticles was monitored by visual inspection in the colour change within 15 minutes and was further subjected to spectroscopy. Reaction mixture was left for 4h at room temperature for complete reduction. The resultant mixture was centrifuged at 10,000 rpm for 10 min, and resulting pellets were washed three times to remove unbound proteins and other impurities if present. The purified gold nanoparticles were suspended in Milli Q water and stored at 4°C for further characterization.

Characterization of Gold Nanoparticles

UV-Visible spectrometric analysis

The resultant gold colloids were visually monitored through color change. UV-Vis spectroscopy is primary confirmatory tool for the detection of the surface plasmon resonance (SPR) property of AuNPs. The UV-Vis spectrum of synthesized gold nanoparticles was recorded as a function of wavelength using a UV-Vis spectrophotometer **(**Biotek Synergy H1Microplate reader), and was measured periodically at 200-800 nm. A spectrum of gold nanoparticles was plotted with the wavelength on *x*-axis and absorbance on the *y*-axis.

X-ray diffraction (XRD) analysis

The x-ray diffraction pattern of nanoparticles was recorded on Bench Top X-ray Diffraction (RigakuMiniflex 600 Desktop X-ray Diffraction System, Rigaku Corp).

Fourier Transform Infrared (FTIR) analysis

FTIR measurements of gold nanoparticles as well as turnip seed extract were carried out to identify the major functional groups present and involved in the reduction of gold. FTIR measurements were carried out using Perkin Elmer FTIR by employing KBr pellet technique. The FTIR spectra were collected in the transmission mode at 4000-400 cm^{-1}.

Transmission electron microscope (TEM) and energy dispersive x-ray spectroscopy (EDX)

The size and morphology of gold nanoparticles were determined by transmission electron microscopy. The micrographs were obtained using TEM (Tecnai G2 20 TWIN) microscope operated at an accelerating voltage of 200 kV. For the preparation of samples, the synthesized particles were suspended in Milli Q water and dispersed with ultra-sonication. A drop of sonicated gold nanoparticles was deposited on carbon-coated copper grid and allowed to dry in desiccator, prior to measurements. The grid was also analyzed by EDX for elemental analysis.

RESULTS AND DISCUSSION

Reduction property of turnip seed extract and UV-Vis Spectroscopy

Reduction of gold ion into Au particles during exposure to the plant extract was visually evident from color change. Stable ruby-red color indicated the formation of AuNPs as shown in Figure 1.

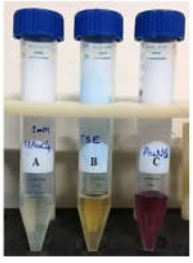

Figure 1 Synthesis of gold nanoparticles (A) 1mM $HAuCl_4$, (B) TSE, and (C) Synthesized AuNPs

Synthesis of the AuNPs was confirmed by scanning the absorption maxima of the reacted mixture at the wavelength between 200-800 nm using UV-Vis Spectrophotometer. UV-

Vis absorption spectrum of gold nanoparticles is shown in Figure 2. Spectroscopic scanning of coloured solution exhibited surface plasmon resonance (SPR) band with an absorption peak centered at 530 nm, characteristics of spherical Au nanoparticles (Mulvaney, 1996; Shankar *et al,* 2003; Hvolbaek *et al,* 2007; Shukla *et al,* 2008].

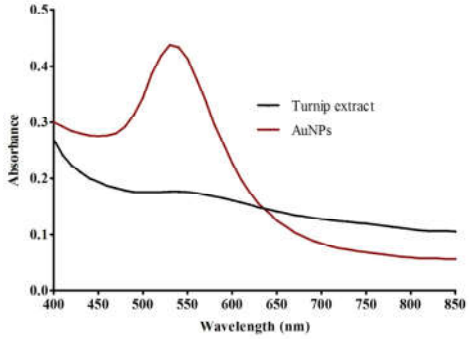

Figure 2 UV-Vis spectra of gold nanoparticles using turnip seed extract.

FTIR analysis of synthesized AuNPs

FTIR measurements were carried out to identify the major functional groups involved in the reduction and stabilization of biologically synthesized AuNPs. The FTIR analysis spectrum showed transmittance between4000 - 400 cm^{-1}as shown in Figure 3.

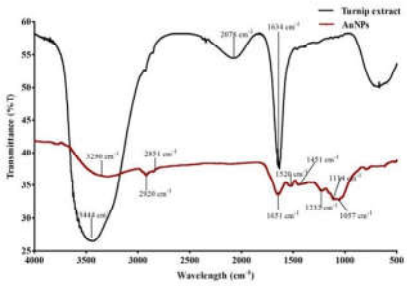

Figure 3 FTIR spectrum of synthesized AuNPs and seed extract confirming the presence of phenolic compounds.

Analysis was done for both the plant extract and synthesized AuNPs. The spectrum of plant extract showed major bands at 3444, 2078, 1658, 1651, 1644, and 1633cm^{-1}. The peak at 3444 corresponds to the hydroxyl functional group of alcohol and phenolic compounds (Sett *et al*, 2016). The bands at 1634 cm^{-1} correspond to amide I band. The AuNPs showed major bands at 3290, 2920, 1651, and 1232 cm^{-1} which confirmed the presence of alcohol or phenol, 2^{o} amines, amide I band, and amide III band respectively(Narayanan *et al*, 2008; Song *et al*, 2009). 1114 cm^{-1}band confirmed the presence of alcohol, carboxylic acid, esters and ethers (Mukherjee *et al*, 2012). The spectrum also showed the presence of some minor peaks at 2851, 1520, 1451, 1057cm^{-1}. These bands can be assigned to CH stretching in CH_3, ortho substituted aromatic compounds, C=C stretching, and C-N stretching vibrations of aliphatic amines or due to alcohols/phenols (Martinez *et al*, 2012). The presence of these functional groups indicates that the gold nanoparticles synthesized using turnip extract are surrounded by some proteins and metabolites such as amines, alcohols, phenols, and carboxylic acids. After bioreduction, shift in spectra at the alcohol, phenol and amide band confirms the involvement of these groups in reduction, capping and stabilization of the synthesized nanoparticles (Song *et al*, 2009).

XRD analysis

XRD patterns obtained for gold nanoparticles synthesized using turnip seed extract is shown in Fig 4. XRD analysis is mainly done to study the crystalline nature of the gold nanoparticles. The diffraction peaks were observed at 2θ values of 38.08, 44.16, 64.57 and 77.60 corresponding to Bragg's reflections of (111), (200),(220) and (311). In addition to Bragg peaks, additional peaks were also observed which were not assigned to the spectrum and may be present due to organic compounds present in plant extract, responsible for reduction and stabilization of synthesized gold nanoparticles (Narayanan *et al*, 2015). The XRD pattern thus clearly shows that the gold nanoparticles formed by the reduction of $HAuCl_4$ by turnip seed extract are crystalline in nature. The peak corresponding to (111) plane is more intense than the other planes suggesting that (111) is the predominant orientation as confirmed by the high resolution TEM measurement.

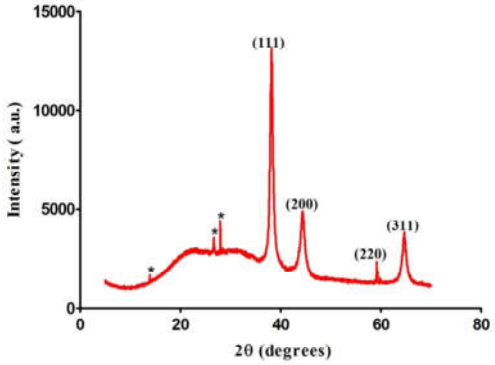

Figure 4 XRD pattern of synthesized AuNPs showing peaks of (111), (200), (220), and (311) confirming the crystallinity of the particles.

Transmission Electron Microscopy (TEM) and SAED pattern

The shape and size distribution of the AuNPs were characterized by TEM. Figure 5 represents the TEM micrographs obtained at different magnifications which revealed large population of monodispersed AuNPs of spherical shape. Sizes of AuNPs were in the range of 10-45 nm. A little aggregation is visible which may be due to the overlapping of the nanoparticles on each another. Images showed thin layer of some mucilaginous substances in which nanoparticles seems to be embedded.

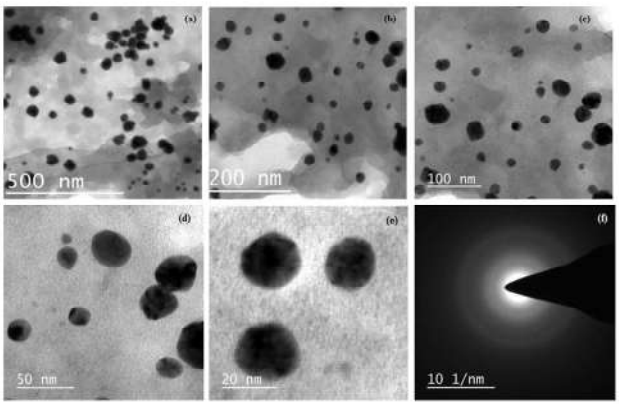

Figure 5 TEM images at different magnifications and SAED pattern of AuNPs

This coating is due to the layer of some capping organic material from the plant broth which remains stable in solution after synthesis as reported earlier in nanoparticle synthesis using plant extracts (Shankar *et al*, 2003; Shankar *et al*, 2004; Chandran *et al*, 2004).The SAED pattern confirms the crystalline nature of the AuNPs. Rings were observed corresponding to the (111), (200), (220) and (311) lattice planes of the face-centered cubic (fcc) structure, as observed in XRD pattern. The resultant histogram (Figure 6) represents the size distribution of the particles.

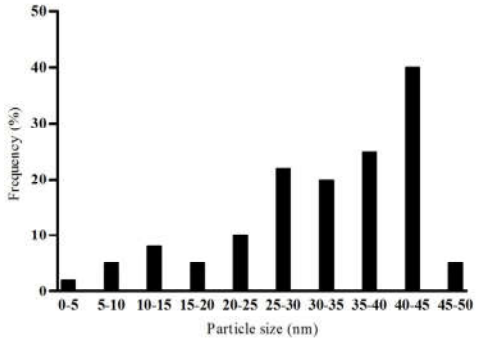

Figure 6 Histogram representing percent frequency distribution of gold nanoparticles.

EDX analysis

EDX analysis provided a qualitative and quantitative status of the elements present and involved in formation of nanoparticles. The elemental profile of synthesized nanoparticles of gold confirms the formation of gold nanoparticles. The elemental analysis as shown in Figure 7 reveals the highest proportion of gold (Au) followed by O, C and Si.

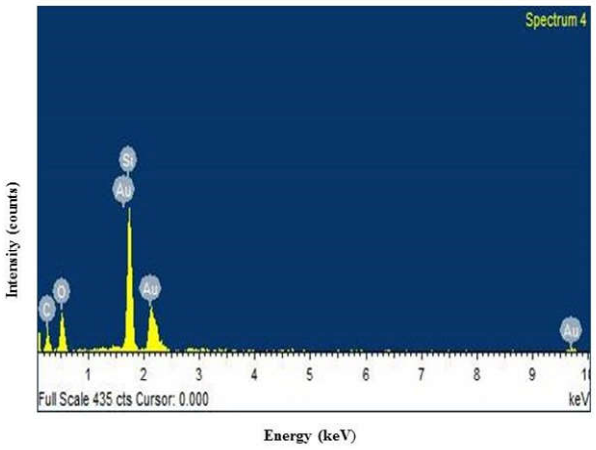

Figure 7 EDX spectrum of AuNPs.

CONCLUSION

In conclusion, we proposed a simple, inexpensive, and single-step green approach for synthesis of biocompatible gold nanoparticles using plant extracts.This study also signifies that turnip seed extract can be used as non-toxic, sustainable, and eco-friendly organic source, an important criterion for the green chemistry synthesis of nanoparticles.FTIR study revealed the presence of phenolic content of turnip extract having a strong anti-oxidant property which helped in the reduction of gold to AuNPs. Other phytochemicals present in extract acts as stabilizing and capping agent.TEM revealed the size of the synthesized nanoparticles in the range of 10-45 nm. The presence of different functional groups which make nanoparticles stable, size of the synthesized nanoparticles, and ecofriendly synthesis at large-scale make these synthesized AuNPs most promising in the field of catalysis, sensors, electronics, and biomedical applications.

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Conflict of interests

Authors have no conflict of interest affiliated with this article.

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