Green Synthesis of Gold Nanoparticles Using Gnetum Ula- An Endangered Medicinal Plant

Soni Das¹, Anuj Pundhir² and P. Sureshkumar¹,*

¹Department of Biotechnology, BIT-Campus, Anna University, Tiruchirappalli-620 024, INDIA.
²Department of Chemistry, IIT, Roorkee-247667, INDIA.

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ABSTRACT
Our present study, we have reported the novel use of the aqueous leaf extract of Gnetum ula to produce gold nanoparticles by reduction of AuCl₄⁻ ions. The phytochemicals present in the leaf extract served as effective reducing and capping agent. The gold nanoparticles obtained were characterized by UV–visible spectra, transmission electron microscopy (TEM) and X-ray diffraction (XRD). TEM studies showed the particles to be of various shapes and sizes. High-resolution TEM image confirmed a fcc phase and high crystallinity of the particles. The XRD patterns showed a (1 1 1) preferential orientation of the gold nanoparticles. Fourier transform infra-red spectroscopy (FTIR) measurements showed the GNP s having a coating of phenolic compounds indicating a possible role of biomolecules responsible for capping and efficient stabilization of the gold nanoparticles. AuNPs of Gnetum ula were having significant anti-arthritic activity.

Introduction
Gold nanoparticles (Au NPs) have attracted a great deal of attention of various scientific groups in recent years because of their unique catalytic, electronic, optical, and other structure dependent activities, and subsequent technological applications of Au NPs are being explored extensively [1]. Au NPs also find wide applications in medical science and technology as drug delivery vehicles [2] and fluorescent tags and also as image contrast agents for diagnosis and prevention of cancer [3,4]. Chemical stability and unique optical properties of AuNPs make them ideal candidates for living cell imaging [1,5]. Their performance and hence their applicability depend critically on their size, shape, surface morphology, composition, and fine structure, either as an alloy or core–shell [6,7]. Nanotechnology is making an impact in every field of life. Researchers are expending their interests towards synthesis of gold nanoparticles as they provide superior properties for different types of applications. Conventionally nanoparticles have been synthesised by various physical and chemical methods, having negative impact on environment. The production of nanoparticles using plant extract is alternative the conventional methods. The photosynthesis is a green and eco-friendly technology used for production of large scale nanoparticles.

Gnetum Ula is a rare endangered plant (IUCN List) belongs to the family Gnetaceae. It is found in Odisha, Andra pradesh, Goa, Karnataka, Maharashtra and Tamilnadu in India. It is a shrub occasionally found in evergreen (or) semi evergreen forest under shade. Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. The various phytochemicals present in plant extract used for the reducing and stabilisation of nanoparticles. Since ancient times, gold was highly regarded as the “elixir of life”. In 1925 gold complexes were being used in clinical trials to determine its efficacy to help alleviate rheumatoid arthritis.

Materials and Methods
Chloroauric acid (HAuCl₄) was purchased from Sigma (Bangalore, India). The plant was collected from Similipal Reserve Forest (Odisha).

Preparation of G. ula leaf extract and its application for GNP synthesis.
Leaves of G. ula were thoroughly washed with double distilled water (ddH₂O) and shade dried in dust free condition for one week at room temperature before being grinded to a fine powder. Finely powdered plant material (1 g) was extracted with ethanol (10 ml) under shaking incubation (100 rpm, 25 °C and 24 h). The extract was filtered and stored at 4 °C for further experiments. 2% (v/v) of the leaf extract was mixed with 1 mM HAuCl₄ aqueous solution and volumemade up to 2 ml with ddH₂O. The mixture solution was left on constant magnetic stirring (C-MAG-HS7, IKA®) at room temperature (25 °C) and observed for change in colour. The reaction was repeated for 4, 6, 8 and 10%, v/v of the leaf extract.

Characterization of GNP s
Synthesis of GNP s was confirmed by scanning the absorption maxima of the reacted mixture at the wavelength between 200–900 nm on a Carry 100 BIO UV–VIS spectrophotometer (Varian, CA, USA). Morphology, crystalline nature and size distribution of GNP s were analyzed by TEM (JEOL 2100 UHR TEM). X-ray diffraction (XRD) measurements of GNP s were analyzed with a Bruker D8 ADVANCE X-ray powder diffractometer (Bruker AXS Inc.) using CuKα (λ=1.54 Å) source in the region of 20 from 30° to 75°. FTIR spectra of dried leaf extract and GNP s were obtained with a Perkin-Elmer FTIR spectrophotometer (Norwalk, USA) in the range of 450–4000 cm⁻¹.
Result and Discussion

Reduction of \( \text{AuCl}_4^- \) was visually evident from the colour change and was completed within two and half hour with a stable ruby-red colour indicating the formation of GNPs. UV–Vis spectroscopic scanning of the coloured solution exhibited distinct surface Plasmon resonance (SPR) bands with an absorption peak centered at around 534 nm, characteristic of spherical GNPs (Fig. 1).

Representative TEM images and corresponding size distribution histogram of GNPs obtained with 2% leaf extract showed that nearly spherical GNPs were more dominant than triangular and hexagonal shapes with a wide spectrum of particle size distribution ranging from 10 to 40 nm. The ultra-high-resolution TEM (UHRTEM) images displayed clear lattice fringes on the particle surfaces. Selected area electron diffraction pattern (SAED) of a single spherical particle confirmed the single crystalline nature of GNPs with the fcc phase. We observed rings corresponding to (1 1 1), (2 0 0) and (2 2 0) planes of the fcc crystalline lattice of gold.

XRD patterns of the GNPs synthesized with varying leaf extract concentration (2–10%) displayed Bragg’s reflections representative of the fcc structure of gold. For all leaf extract concentrations (2–10%) the intensity of the (1 1 1) diffraction was much stronger than those of the (2 0 0) and (2 2 0). The broadening of Bragg’s peak indicated the formation of GNPs [8].

In the FTIR spectrum of GNPs distinct IR bands corresponding to alcohol, amines (both aromatic and aliphatic), phenols and carbonyl functional groups were noticed. High content of phenolic compounds with strong anti-arthritis activity of G. ula ethanolic leaf extract helped in the reduction of HAuCl4 to GNPs [9]. Signals for different functional groups found in the IR spectrum of GNPs indicated that some phenolic compounds were bound to the surfaces of GNPs that remained despite repeated washing. Through free amino (–NH2) or carboxylic (–COOH) groups these compounds might have interacted with gold surface making GNPs highly stable.

Conclusion

The high phenolic content of the ethanolic leaf extract of G. ula having strong anti-arthritis property helped in the reduction of gold cations to GNPs. Characterization of GNPs revealed an average size range of 10–40 nm and are capped by phytochemicals. This method for GNP synthesis do not use any toxic reagent and thus has the potential for use in biomedical applications.

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References