

Rapid green synthesis of silver and gold nanoparticles using peels of *Punica granatum*

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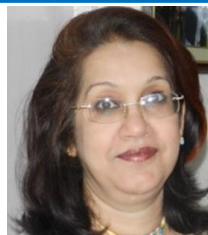
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ABSTRACT

We present a simple and eco-friendly biosynthesis of silver nanoparticles using Pomegranate peel extract as the reducing agent. Peel extract of Pomegranate was challenged with silver nitrate (AgNO_3) and chloroauric acid (HAuCl_4) solution for the production of silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs), respectively. The reaction process was simple for the formation of highly stable silver and gold nanoparticles at room temperature by using the biowaste of the fruit. The morphology and crystalline phase of the NPs were determined from UV-Vis spectroscopy, transmission electron microscopy (TEM), selected area electron diffraction (SAED) and X-ray diffraction (XRD) spectra. TEM studies showed that the average particle size of silver nanoparticles were 5 ± 1.5 nm whereas the gold nanoparticles were found to be 10 ± 1.5 nm. An effort has been also been made to understand the possible involved mechanism for the biosynthesis of the NPs. Presumably biosynthetic products or reduced cofactors play an important role in the reduction of respective salts to nanoparticles. Copyright © 2012 VBRI press.

Keywords: Phytodiversity; AgNPs; AuNPs; XRD; biowaste.



Naheed Ahmad is Associate Professor at Department of Botany, Patna University, India. This university is also her alma mater as she studied here for the Bachelors and master's degree. She also got her Ph.D. degree from here. She has been in the teaching field since the last 20 years. Her interests include Ecology, Molecular systematic, chemosystematics, biodiversity and bioprospecting of plants. She has guided a number of students for the Ph.D. degree.



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Radheshyam Rai did his PhD from Magadh University, Bodh Gaya, India in 2004 in Physics and worked on PLZT ferroelectric materials with different dopants. After the completion of PhD he worked on LPG and CNG gas sensor devices in National Physical Laboratory and Indian Institute of Technology, New Delhi. After that he joined as Young

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Introduction

The plant diversity is a product of thousands of years of evolution, in an attempt to survive across different realms and different ecological conditions, plants have evolved various mechanisms some in the form of secondary metabolites. These metabolites are important as they not only contribute to the attractive colour acting as fertilization attractants but also act as protective agent against UV light, heavy metal toxicity and against different pathogens. Various plant groups from algae to angiosperms possess different phyto-chemicals. Polyphenol compounds are widely distributed in the plant kingdom and are of more than 300 varieties. The structural variation in them is due to the change in number and position of hydroxyl constituents and variation in the number and position of glycosidic bonds, methyl, isoprenoid groups etc. Several physical and chemical processes [1–6] for synthesis of metal nanoparticles were developed considering the real life application of nanoparticles in the area of medicine [7], catalysis [8], detection [9], etc. Recently the studies started under green chemistry for the search of benign methods for

the development nanoparticles and searching antibacterial, antioxidant, and antitumor activity of natural products. Biosynthetic processes have received much attention as a viable alternative for the development of metal nanoparticles where plant extract is used for the synthesis of nanoparticles without any chemical ingredients [10–14]. There are several reports on the use of *Helianthus annuus*, *Basella alba*, *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolor*, *Zea mays* [1], *Azadirachta indica* (Neem) [4], *Medicago sativa* (Alfa alfa) [5,6], *Aloe vera* [7], *Embllica officinalis* (Amla) [8], *Capsicum annuum* [9], *Geranium* sp. [10,11], *Diopyros kaki* [12], *Magnolia kobus* [13], *Coriandrum* sp. [14] for the synthesis of nanoparticles to be used in pharmaceutical and biological applications. *Punica granatum*, the pomegranate is native in northern India and has been cultivated and naturalized over the whole Mediterranean region since ancient times. The fruit extract is a rich source of highly potent antioxidants and is widely used in several traditional medicinal systems for the treatment of arthritis and other diseases. Pomegranate extract is primarily composed of a variety of alkaloids and polyphenols. The term phenolic compound embraces a wide range of plant substances that bear in common an aromatic ring with one or more hydroxyl substituents. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Ellagic acid is a naturally occurring phenolic compound in Pomegranate with antioxidant properties. Antioxidants are substances which, when present in small quantities, prevent oxidation of free radicals. They may offer resistance against oxidative stress by scavenging the free radicals. Plant wastes including waste agricultural biomass often have composition, properties and structures that make it suitable for extraction of composites, fuel, chemical enzymes and food.

In this work, we have explored an inventive contribution for synthesis of silver and gold nanoparticles using fruit peel of Pome. The procedure for the development of stable AgNPs and AuNPs is rapid, simple and viable. Synthesized nanoparticles were characterized by various methods, such as UV-Vis spectroscopy, TEM, XRD and EDX.

Experimental

Pome fruits were collected from the local market. Silver nitrate (AgNO_3 , >99.9%) and chloroauric acid (>99.9%) was obtained from Sigma-Aldrich. All glass-wares and Pome fruit were properly washed with de-ionized water and dried in oven. Fruit peel extract (FPE) of Pome was used as a reducing agent for the development of silver and gold nanoparticles. Properly washed 50 g of fresh peels of the fruit were added in 250 mL ultra-pure water in 500 mL Erlenmeyer flask and boiled for 10–15 min. Whatman filter paper (No. 40) was used for the filtration of boiled material to prepare the aqueous fruit peel extract, which was used as such for metal nanoparticle synthesis. Aqueous solution (1 mM) of silver nitrate and chloroauric acid solution was prepared and 50mL of the metal (Ag/Au) ion solution was reduced using 1.8 mL of FPE at room temperature for 5 min. Below than this FPE quantity, the solution takes more

than 10 min to get a significant surface plasmon resonance (SPR) for both the metal nanoparticles. As a result brown–yellow solution, indicating the formation of silver nanoparticles and pink colour solution, indicating the formation of gold nanoparticles were achieved after the addition of the FPE [11–15].

Spectral analysis for the development of nanoparticles at different reaction conditions were observed by UV–Vis spectroscopy using a Perkin-Elmer Lamda-45 spectrophotometer. Transmission Electron Microscope (TEM) JEM-1200EX, JEOL was used for the analysis of size and shape of developed nanoparticles. For TEM measurements, 3 μL of the sample solution was placed on copper grid making a thin film of sample on the grid and kept for drying at room temperature for 15 min, then extra sample was removed using the cone of a blotting paper and reserved in grid box. The presence of elemental silver was determined using energy dispersive X-ray analysis (EDX) with Zeiss Evo 50 instrument. The pH of the solution was maintained by 1N HCl and 1N NaOH. Resulting solutions of the developed nanoparticles of silver and gold was dried at 80°C for X-ray powder diffraction measurement. X-ray powder diffraction data was acquired by PAN analytical X'Pert PRO diffractometer in Bragg–Brentano geometry using step-scan technique and Johansson monochromator to produce pure $\text{CuK}\alpha$ radiation (1.5406 Å; 45 kV, 30 mA). The ICDD PDF2 powder diffraction database implemented in High Score Plus was used for the search-match phase identification analyses. To estimate crystallite sizes of the produced nanoparticles, most isolated diffraction peak positions were profile fitted using pseudo-Voigt function to establish the full-width at half-maximum values (FWHM) needed for the crystallite size evaluation made by Scherrer method.

Results and discussion

UV-Vis analysis

Equivalent amounts of the suspension (0.5 mL) were diluted in a constant volume of de-ionized water (5 mL) and subsequently analyzed at room temperature. The progress of the reaction between metal ions and the leaf extracts were monitored by UV–visible spectra of Ag and Au nanoparticles in aqueous solution with different reaction times (Fig. 1). The peak blue shifted in the absorption spectrum of AgNPs from 440 nm to 427 nm with increasing reaction time from 10 min to 30 min and the peak centered at 427 nm for 5, 10 and 15 minute whereas the peak in the absorption spectrum of AuNPs was observed ~550nm. The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within an hour of reaction, making it one of the fastest bio-reducing methods to produce Ag and Au nanostructures reported till date [16–18] by using biomass of an agricultural waste. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. As the peel extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from light yellow to tea brown and finally chocolate brown due to reduction of silver ion and pink-red colour in case of reduction of gold, (Fig. 2a and b) which indicated formation of silver

nanoparticles and gold nanoparticles respectively. Later these nanoparticles formed settled down at the base of the tube as shown in the leaving almost transparent fluid at the top.

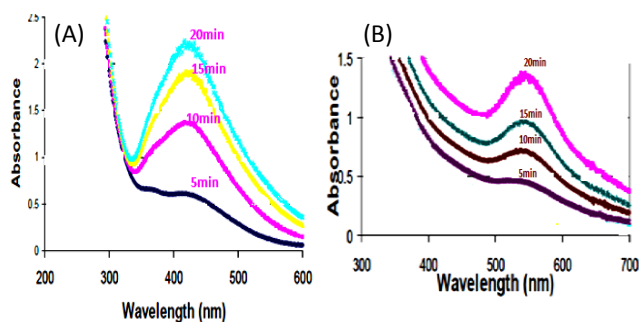


Fig. 1. UV-Vis spectra recorded at different time intervals from aqueous solutions of AgNO₃ (A) and HAuCl₃ (B) with FPE: 5, 10, 15 and 20 min.

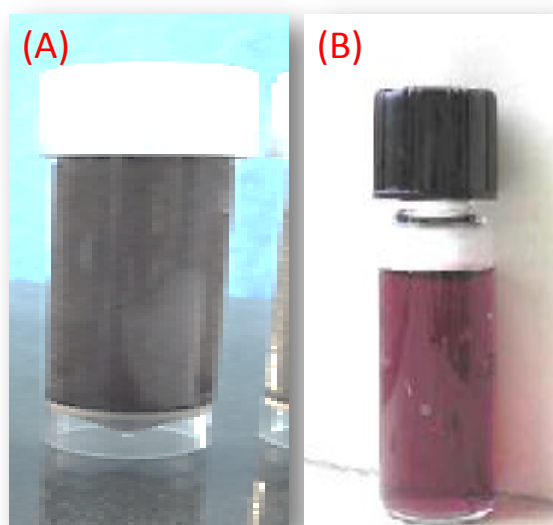


Fig. 2. Photographs of silver (A) and (B) gold solutions of NPs formed.

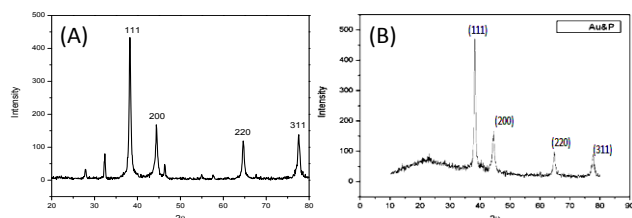


Fig. 3. XRD of (A) silver nanoparticles and (B) gold nanoparticles.

XRD analysis

The experimental powder diffraction (XRD) pattern of the prepared silver nanoparticles is shown in **Fig. 3**. In the XRD pattern of AgNPs, diffraction peaks at 38.13°, 44.21°, 64.47, 77.37 and 38.45, 65.77 and 82 can be assigned to face-centered cubic (fcc) metallic silver (identified by PDF-2 ref. 4-0783) corresponding to the (111), (200) and (220) facets of the silver crystal and (111), (200), (220), (311) and (222) facets of the gold crystals. Whereas any peaks originating from potential silver oxides cannot be observed. In addition to the Bragg peaks representative of fcc silver

and gold nanocrystals, additional, and yet unassigned, peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver. The broadening of the Bragg peaks indicates the formation of nanoparticles. The crystallite size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula.

$$D = 0.94 \lambda / \beta \cos \theta$$

where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained Si sample.

$$\beta_{\text{corrected}} = (\text{FWHM}_{\text{sample}}^2 - \text{FWHM}_{\text{si}}^2)^{1/2}$$

This modified formula is valid only when the crystallite size is smaller than 100 nm [19].

TEM analysis

Fig. 4a and **b** shows the typical bright-field TEM micrographs of the synthesised Ag and Au nanoparticles. These images suggest that the particles are polydisperse and are mostly spherical in shape. Hence it may be understood that the experimental conditions (viz., pH, temperature and the optimum concentration of Ag⁺ and Au⁺ etc.) will achieve the mono-dispersity and uniform shape. A few agglomerated nanoparticles were also observed in some places, thereby indicating possible sedimentation at a later time. It is evident that there is variation in particle sizes and the average size estimated was 5 nm and 10 nm for AgNPs and AuNPs respectively. It is reported earlier that proteins can bind to nanoparticles either through free amine groups and therefore, stabilization of the AgNPs and AuNPs by protein is a possibility [20-23]. The biological molecules could possibly perform dual functions of formation and stabilization of silver and gold nanoparticles in the aqueous medium. The crystalline nature of NPs is shown in **Fig. 5** as the characteristic rings of different crystal planes.

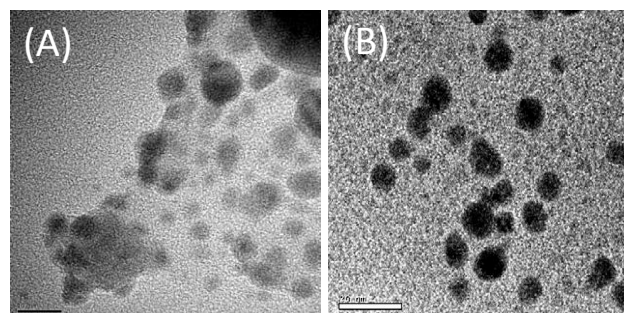


Fig. 4. TEM image of (A) AgNPs and (B) AuNPs.

Mechanism analysis

Pomegranate extract is primarily composed of alkaloids and polyphenols. The active constituent that appears to be responsible for its multiple health benefits is Ellagic acid.

Ellagic acid is a naturally occurring phenolic compound found in several fruits and nuts. Pomegranate extract has demonstrated a variety of beneficial functions including antioxidant and anti-viral activity. Ellagic acid effectively protects cells from damaging free radicals. Additional phenolic compounds found in pomegranate known as anthocyanidins (also well-known scavengers of free radicals) combine synergistically with Ellagic acid to greatly augment pomegranate's potency as an antioxidant. Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups, able to bind to heavy metals. They may inactivate metal ions by chelating. When metal salts come in contact with the ellagic acid present in the peel extract the ester oxygen atom and the ortho-phenolic hydroxyl of the ellagic acid form a track conjugation effect. The esterification of the carboxyl and hydroxyl groups of ellagic acid make the ortho-phenolic hydroxyl lose the hydrogen much easily, forming a steadier semi-quinone structure. Thus, ellagic acid has an easy electron losing capacity which results in the formation of H^+ radical, which reduces the size of silver and gold particles to nanosize (Fig. 6).

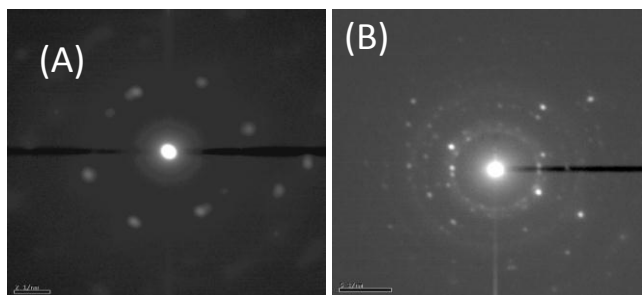


Fig. 5. Selected area electron diffraction showing the characteristic crystal planes of elemental (A) silver and (B) gold.

Punicalagins, tannin, along with Ellagic acid is a major antioxidant component of pomegranate and have free radical scavenging capacity. They are water soluble and have high bioavailability. It has shown remarkable pharmacological activities attributed in the presence of dissociable OH groups. The results are rationalized on the basis of the scavenging activity of punicalagin against various one-electron oxidizing radicals, namely, $^{\bullet}OH$, N_3^{\bullet} , and NO_2^{\bullet} . The formation of the transient species in these reactions and the rate constants of the scavenging reactions have been probed using a time-resolved kinetic spectrophotometric technique. The antioxidant action of punicalagin is expressed not only through its scavenging reactions but also due to the formation of H^+ radicals which in turn reduces the size of silver and gold particles to nanosize.

Conclusion

This spanning new and simple method for the biosynthesis of silver and gold nanoparticles offers a valuable contribution in the area of green synthesis and nanotechnology without adding different physical and chemical steps. Pomegranate fruit peel extract was prepared and successfully employed for the development of silver

and gold nanoparticles with spherical shapes. XRD study showed the face-centered cubic lattice of AgNPs and AuNPs. The average crystal size of AgNPs and AuNPs was found to be 5nm and 10 nm, respectively. The significant reduction in reaction time with fruit peel extract is an important result and will enable nanoparticle biosynthesis methods to compete with other routes for the formation of nanoparticles that are currently much more rapid and reproducible.

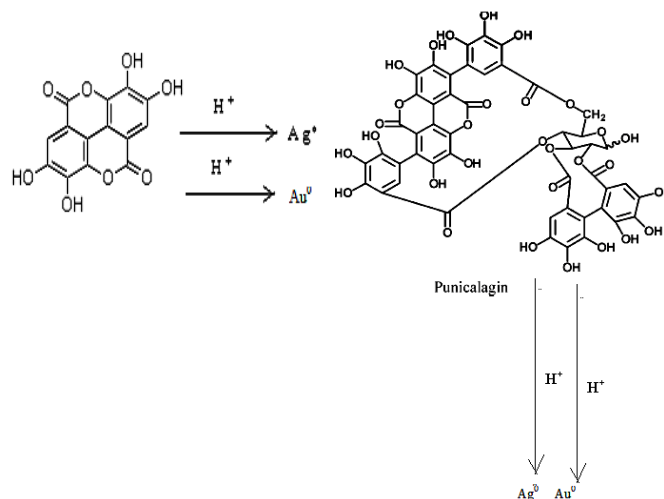


Fig. 6. Mechanism for the biosynthesis of AgNPs and AuNPs using Pomegranate fruit peel extract.

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