Phoenix dactylifera (Date Palm) Seed Extract Mediated Green Synthesis of Gold Nanoparticles and its Application as a Catalyst for the Reduction of 4-nitrophenol to 4-aminophenol

Shib Shankar Dash, a Arun Kanti Sikder, b Braja Gopal Bag*, a Sujoy Bandyopadhyay a

a Department of Chemistry and Chemical Technology, Vidyasagar University, Midnapore, 721 102, West Bengal, India
Email: braja@mail.vidyasagar.ac.in
b High Energy Materials Research Laboratory (HEMRL), Defence Research and Development Organization (DRDO), Sutarwadi, Pune - 411 021, India

Received 29 April 2013; accepted 29 May 2013

Abstract
The seed extract of Phoenix dactylifera was utilized for the one step synthesis of gold nanoparticles at room temperature. The phytochemicals present in the seed extract act as the reducing agent for Au(III) and stabilizers for the synthesized gold nanoparticles. The synthesis of gold nano particles of 10-15 nm size was complete in several minutes and no photo irradiation or heat treatment was necessary. The stabilized gold nanoparticles were characterized by Surface Plasmon Resonance spectroscopy, HRTEM, X-Ray diffraction and FTIR studies. The synthesized gold nanoparticles were utilized as a catalyst for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol.

Keywords: Phoenix dactylifera, gold nanoparticle, green synthesis, Date palm, catalytic reduction

1. Introduction
Gold, the commonly used ornamental and coinage metal in the bulk scale, has become an area of intense research in the nano scale during last two decades because of their tremendous potential and realized applications in diversified areas of science and technology [1]. The gold nanoparticles (GNPs) exhibit unique optical resonance phenomena commonly known as surface plasmon resonance (SPR) arising due to collective oscillation of conduction electrons upon optical excitation [2]. Gold being biologically inert and resistant to oxidation, a great deal of research effort has been made towards the biological application of GNPs especially in biodiagnostics [3], drug delivery [4], medicine and biotechnology [5]. Because of the very high metallic surface area, the stabilized GNPs have also been utilized as a catalyst for different types of chemical transformations [6,7,8]. Various synthetic methods for GNPs broadly under the categories of ‘top-down’ and “bottom-up” approaches have appeared in the literature. Among these methods, the bio-based ‘bottom-up’ approach based upon plant metabolites as renewables, have recently drawn the attention of the scientific community because such methods will lead to a “green” and “sustainable” development [9,10]. During our investigations on plant based triterpenoids as renewable functional nano-entities [11,12,13,14,15], we have also demonstrated the eco-friendly green synthesis of GNPs from the extracts of Punica granatum juice [16], Saraca indica bark [17], Terminalia arjuna bark [18] and Ocimum sanctum stem [19]. Phoenix dactylifera, commonly known as “date palm”, is cultivated in various parts of Asia for its delicious fruits as well as medicinal importance. The seeds of the date fruits are rich in different types of phytochemicals including polyphenols. Herein we report a very mild and eco-friendly method for the synthesis of GNPs from the seed extract of Phoenix dactylifera without any extra stabilizing or capping agents. The synthesized GNPs have been characterized by Surface Plasmon Resonance spectroscopy, High Resolution Transmission Electron Microscopy (HRTEM), X-Ray diffraction and FTIR studies. Catalytic activities of the synthesized GNPs have also been demonstrated for the sodium borohydride reduction of 4-nitrophenol at room temperature.

2. Experimental
2.1 Materials
Chloroauric acid (HAuCl₄) was purchased from SRL (Sisco Research Laboratory) and used without further purification. Phoenix dactylifera fruits were collected from local market at Midnapore, West Bengal, India and its seeds were dried in air. Sodium borohydride and 4-nitrophenol...
were purchased from Merck. Double distilled water was used for the synthesis of GNPs.

2.2 Preparation of Au(III) solution
HAuCl₄ (36.5 mg) was dissolved in deionized water (10 mL) to obtain a 10.7 mM Au(III) stock solution.

2.3 Preparation of the Seed Extract of Phoenix dactylifera
Dried Phoenix dactylifera seeds were finely powdered (10.6 g) and refluxed with methanol (70 mL) for 2 h. The mixture was then cooled at room temperature and filtered to yield a deep brown colored filtrate. The volatiles of the filtrate were removed under reduced pressure to afford a reddish brown solid (1.16 g). The crude product was purified by column chromatography (Si-gel, 100–200 mesh) using 30% methanol/ethyl acetate as the eluant to afford the purified compound (containing a mixture of phytochemicals) as a reddish brown solid (0.4 g). The purified seed extract of Phoenix dactylifera (0.020 g) was dissolved in deionized water (10 mL) to obtain a reddish brown color solution (2000 mg/L).

2.4 Synthesis of gold nanoparticles
Aliquots of Au (III) solution (0.2 mL, 10.7 mM each) were added drop-wise to the seed extract solution to prepare a series of stabilized GNPs where concentration of the seed extract varied from 25 to 400 mg/L keeping the concentration of Au(III) fixed at 0.42 mM. UV-visible spectroscopic measurements of the solutions were carried out after seven hours of mixing HAuCl₄ and Phoenix dactylifera seed extract.

2.5 Procedure for the catalytic reduction of 4-nitrophenol to 4-aminophenol
An aliquot of 4-nitrophenol solution (1 mM, 0.2 mL) was diluted to 4 mL and its absorbance was measured (λmax = 318.5 nm, εmax = 9200 M⁻¹cm⁻¹). Another aliquot of 4-nitrophenol solution (1 mM, 0.2 mL) was treated with freshly prepared sodium borohydride solution (16.5 mM, 3.7 mL) and diluted to 4 mL (by the addition of 0.1 mL of distilled water) and its absorbance was measured (λmax = 401.5 nm, εmax = 19600 M⁻¹cm⁻¹). Subsequently, an aliquot of 4-nitrophenol solution (1 mM, 0.2 mL) was treated with freshly prepared sodium borohydride solution (16.5 mM, 3.7 mL) and treated with Phoenix dactylifera seed extract (100 mgL⁻¹) derived colloidal gold nanoparticle (0.1 mL). The mixture was thoroughly mixed and the progress of the catalytic reduction reaction was monitored by UV-visible spectroscopy.

2.6 Characterization
TEM images of GNPs were recorded from TECNAI G² 20 High Resolution Transmission Electron Microscope. X-ray diffraction (XRD) patterns of the stabilized GNPs were recorded in PANalytical X’Pert PRO diffractometer with Cu-kα radiation (λ= 1.54 Å). Mass spectra were recorded in Shimadzu GCMS QP 2100 Plus instrument. UV-visible spectra were recorded in Shimadzu 1601 spectrophotometer. FTIR spectra of the samples were recorded in Perkin Elmer Spectrum 2 instrument.

3. Results and Discussion
Phoenix dactylifera Linn., a member of the Arecaceae family, is used as an ayurvedic medicine in the treatment of digestive and urinary disorders, ulcer, tooth ache, etc [20]. Phytochemical screening of the methanolic extract of Phoenix dactylifera seed suggest that a large number of secondary plant metabolites such as alkaloids, steroids, triterpenoids, flavanoids and phenolic compounds are present in it [21]. Mass spectral analysis (supporting information Figure S2) of the purified product revealed the presence of flavanoids, terpenoids, steroids and hydroxy acids, etc. Additional evidence for the presence of phenolic compounds was obtained from ferrie chloride test (see supporting information). Hence, it occurred to us that the easily oxidizable phytochemicals present in the Phoenix dactylifera seed extract can be utilized for the green synthesis of gold nanoparticles. Indeed, on treatment of aqueous solutions of HAuCl₄ with increasing concentration of the seed extract of Phoenix dactylifera, pinkish red color appeared almost instantly indicating the formation of GNPs. The intensity of the color increased on standing the solutions at room temperature for several hours (Figure 1).

3.1 UV-visible Spectroscopy Studies
The UV-visible spectroscopy studies of the samples were carried out in aqueous solution (Figure 1) at room temperature. A strong absorption peak at 220 nm and a shoulder peak at 290 nm were observed in the UV-visible spectrum of chloroaurate solution perhaps due to the interaction between metal and chloro ligands (Figure 1a). The absorption peaks in the range of 530 -570 nm (Figure 1b-f) appeared after mixing with the seed extract was due to surface plasmon resonance (SPR) [2]. This indicated the formation of GNPs from Au(III) ions upon reduction with

![Figure 1: UV-visible Spectra of (a) HAuCl₄ solution and (b-f) stabilized GNPs at 25, 50, 75, 100 and150 mgL⁻¹ concentration of the seed extract respectively. Inset: vials containing (a) HAuCl₄ (0.43 mM), (b-f) GNPs at 25, 50, 75, 100 and150 mgL⁻¹.](image-url)
the phytochemicals. On increasing the concentration of the plant extract from 25 mgL$^{-1}$ to 150 mgL$^{-1}$, a hypsochromic shift was observed which may be attributed to the formation of smaller sized, more stable GNPs. No increment of the intensity of SPR band was observed above 100 mgL$^{-1}$ concentration of the extract. This may be due to maximum reduction of the accessible Au(III) ion to metallic gold at this concentration.

3.2 HRTEM, EDX, XRD and FTIR studies

The morphologies and size distribution of the synthesized GNPs were studied by Transmission Electron Microscopy (TEM) analysis. The size of the nanoparticles formed at various concentrations of the Phoenix dactylifera seed extract are shown in Figure 2 (supporting information Figure S3). Nanoparticles formed were of 10-15 nm size and mostly spherical shaped. Crystalline nature of the GNPs were evident from the selected area electron diffraction (SAED) pattern (Figure 2e) taken from a single GNPs. The energy dispersive X-ray (EDX) (supporting information Figure S4) spectroscopy suggests that the nanoparticles are formed by metallic gold.

![Figure 2: TEM Images of Au nanoparticles obtained with Phoenix dactylifera extract at (a,b,c,d) 400 mgL$^{-1}$; (e) SAED image of stabilized GNPs and (f) histograms showing particle size distribution of the GNPs.](image)

![Figure 3: XRD pattern of stabilized gold nanoparticles (GNPs)](image)

The X-ray Diffraction (XRD) pattern obtained for the synthesized gold nanoparticles is shown in figure 3. The crystalline nature of the metallic face centered cubic (fcc) gold nanoparticles were confirmed from the intense peaks at $2 \theta = 38.3^\circ$, $44.3^\circ$, $64.8^\circ$, $77.9^\circ$ and $81.9^\circ$ corresponding to (111), (200), (220), (311) and (222) planes respectively. These values agreed well with the reported standards for crystalline gold (JCPDS file no. 04-0784).

FTIR spectroscopy studies were carried out to investigate the possible roles of biomolecules responsible for the reduction and capping of the GNPs synthesized by Phoenix dactylifera seed extract. Comparison of the FTIR spectra of Phoenix dactylifera seed extract and stabilized GNPs are given in Figure 4. The peak at 3391.9 cm$^{-1}$ in the FTIR spectrum of Phoenix dactylifera seed extract is due to saturated C-H stretching vibration. The presence of peaks at 1610.6, 1528.4 and 1443.7 cm$^{-1}$ region in the FTIR spectrum of seed extract might be due to aromatic C=C stretching vibration. However, in the FTIR spectrum of Phoenix dactylifera extract stabilized GNPs, the peak for O-H / N-H functionality becomes significantly narrowed and shifted to higher region (3403.5 cm$^{-1}$) suggesting the interaction of these groups with GNPs [16].

4. Mechanism of the formation of Stabilized AuNPs

The seed extract of Phoenix dactylifera is rich in different types of plant secondary metabolites. Mass spectral analysis carried out by us indicated the presence of different types of steroids, terpenoids and polyphenols (supporting information Figure S2). Evidence for the presence of the phenolic compounds present in the seed extract was obtained by ferric chloride test (supporting information). Phenolic compounds are known to be easily oxidized by transition metal ions at a higher oxidation state at room temperature. Moreover, the o-dihydroxy compounds present in the seed extract can easily form a five-membered chelate ring with the Au(III) ions. A schematic representation for the formation of GNPs is given in Figure 5. The redox reaction can take place in the chelate complex where the o-dihydroxy compounds can be oxidized to corresponding quinones with concomitant reduction of Au(III) ions to Au(0). The neighbouring Au(0) atoms can collide with each other forming the nano-sized gold particles and the GNPs can be stabilized by the quinones, as well as the phenolic compounds. The stabilizing ligands surrounding the GNPs prevent further metallic aggregation.
Mechanism of the formation and stabilization of GNPs by polyphenolic compounds present in Phoenix dactylifera seed extract: (i) treatment of Au(III) solution with the seed extract, (ii) reduction of Au(III) and stabilization of gold nanoparticles. ‘R’ indicating the remaining part of the o-dihydroxy compounds.

5. Application of the Stabilized GNPs in Catalytic Reduction

In recent years one of the most important application of the GNPs are to catalyze some chemical reactions which are otherwise kinetically resistant [5]. In order to evaluate the catalytic activity of the synthesized GNPs from Phoenix dactylifera seed extract, the reduction of 4-nitrophenol (0.05 mM) to 4-aminophenol by sodium borohydride (15 mM) was tested. Treatment of an aqueous solution of 4-nitrophenol with freshly prepared aqueous solution of NaBH₄ shifted the absorption peak at 318.5 nm to 401.5 nm due to the formation of 4-nitrophenolate ion in the solution (figure 6). The peak positions remained unaltered even after a month due to large kinetic barrier for the reduction reaction. Interestingly, on addition of a freshly prepared GNP (0.1 mL, 100 mgL⁻¹), the intensity of the absorption peak at 401.5 nm started decreasing with a concomitant appearance of a new peak at 300 nm suggesting the catalytic reduction from 4-nitrophenol to 4-aminophenol. Thus, by lowering the activation energy, GNPs can act as a suitable catalyst for the reduction reaction. The progress of the reaction was monitored spectrophotometrically by recording the absorption spectra at various time intervals. As the concentration of sodium borohydride was 300 fold excess to that of 4-nitrophenol, the catalytic rate constant (k) can be calculated assuming a pseudo-first-order rate kinetics with respect to 4-nitrophenol concentration. Using the UV-visible data at different time intervals, the catalytic rate constant (k) for the reduction reaction was calculated to be 0.15 min⁻¹ (supporting information Figure S5) that was comparable to the rate constant obtained by us previously and by others [16].

![Figure 5](image5.png)

**Figure 5**: Mechanism of the formation and stabilization of GNPs by polyphenolic compounds present in Phoenix dactylifera seed extract: (i) treatment of Au(III) solution with the seed extract, (ii) reduction of Au(III) and stabilization of gold nanoparticles. ‘R’ indicating the remaining part of the o-dihydroxy compounds.

![Figure 6](image6.png)

**Figure 6**: (A) UV-visible spectrum of: (a) 4-nitrophenol, (b) mixture of 4-nitrophenol and NaBH₄ and (c) mixture of 4-nitrophenol and NaBH₄, 20 mins after the addition of GNPs; (B) Overlay of UV-visible spectra as a function of time during catalytic reduction of 4-nitrophenol to 4-aminophenol.

6. Conclusions

We have reported an environmentally benign method for the synthesis of stabilized GNPs using renewable Phoenix dactylifera seed extract under a very mild reaction condition. The phytochemicals present in the seed extract are highly efficient for the reduction of Au(III) ion. Moreover, the synthesized gold nanoparticles are stabilized by the phytochemicals present in the seed extract as well as the concomitantly formed oxidation products such as quinones formed during the reduction of Au(III). The morphologies of stabilized gold nanoparticles have been studied in detail using various techniques such as UV-visible spectroscopy, HRTEM, X-ray diffraction, FTIR, etc. The synthesized gold nanoparticles have been utilized as a catalyst for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol at room temperature. As the seeds of Phoenix dactylifera have medicinal significance, the results described here will be useful for its application in biomedicine as well as pharmaceuticals.

**Acknowledgments**

BGB thanks DRDO for funding. SSD thank CSIR, New Delhi for research fellowships.

**References**

2. (a) X. xie, W. xu, X. liu, Improving colorimetric assays through protein enzyme-assisted gold nanoparticle amplification, Accounts of Chemical Research, 45 (2012), 1511–1520.
15. B.G. Bag, S.S. Dash, First self-assembly study of betulinic acid, a renewable nano-sized, 6-6-6-6-5 pentacyclic monohydroxy triterpenic acid, Nanoscale, 3 (2011), 4564-4566.

Source of support: Nil; Conflict of interest: None declared