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Acacia nilotica (Babool) leaf extract mediated size-controlled rapid synthesis of gold nanoparticles and study of its catalytic activity

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Abstract

The leaf extract of *Acacia nilotica* (Babool) is rich in different types of plant secondary metabolites such as flavanoids, tannins, triterpenoids, saponines, etc. We have demonstrated the use of the leaf extract for the synthesis of gold nanoparticles in water at room temperature under very mild conditions. The synthesis of the gold nanoparticles was complete in several minutes, and no extra stabilizing or capping agents were necessary. The size of the nanoparticles could be controlled by varying the concentration of the leaf extract. The gold nanoparticles were characterized by HRTEM, surface plasmon resonance spectroscopy, and X-ray diffraction studies. The synthesized gold nanoparticles have been used as an efficient catalyst for the reduction of 4-nitrophenol to 4-aminophenol in water at room temperature.

Keywords: Gold nanoparticle; Green synthesis; *Acacia nilotica*; Polyphenols; Catalytic reduction

Background

The synthesis of gold nanoparticles (AuNPs) and their utilization in diversified areas such as catalysis, drug delivery, biodiagnostics, medicine, and electronics have become an area of tremendous investigations during the last two decades because of their unique optical, physical, chemical, and magnetic properties compared to the bulk solids [1-4]. Among various synthetic methods, the solution phase synthesis involving the reduction of Au (III) to Au(0) by plant extracts has gained profound significance in recent years because of the renewable and nontoxic nature of the plant extracts, eco-friendly aqueous medium, and mild reaction condition [5]. Moreover, this method becomes more advantageous over other synthetic methods since the plant extract itself acts as a stabilizer, and no additional stabilizers or capping agents are needed [6]. The extracts of *Punica granatum* [7], *Breynia rhamnoides* [8], *Saraca indica* [9], *Piper betle* [10], etc. have been utilized for the synthesis of AuNPs. During our investigations on the utilization of triterpenoids (C30s) as renewable functional nanoentities [11-14], it occurred to us that the medicinally important

leaf extract of *Acacia nilotica*, rich in polyphenolic compounds, can be utilized for the synthesis of AuNPs from HAuCl₄. Herein, we report a very mild and environment-friendly method for the synthesis of AuNPs from the leaf extract of *A. nilotica* at room temperature without any additional capping or stabilizing agents. The AuNPs were characterized by surface plasmon resonance (SPR) spectroscopy, high-resolution transmission electron microscopy (HRTEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) studies. The synthesized AuNPs have been utilized as an efficient catalyst for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol at room temperature, and the reduction kinetics have been investigated spectrophotometrically.

Methods

Materials

Au(III) solution, HAuCl₄, was purchased from Sisco Research Laboratory, Mumbai, India, and used without further purification. HAuCl₄ (35.4 mg) was dissolved in distilled water (10 mL) to obtain a 10.42-mM Au(III) stock solution. The leaves of *A. nilotica* were collected from the Vidyasagar University, Midnapore, India campus. Doubled distilled water was used for experimental purposes.

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Preparation of the *A. nilotica* leaf extract

Dried and finely powdered *A. nilotica* leaf (2.9 g) was suspended in ethanol (30 mL) and stirred magnetically at room temperature for 1 h and then filtered. Volatiles of the greenish filtrate was removed under reduced pressure to afford a sticky solid (0.390 g) that was purified by column chromatography (Si gel, 100 to 200 mesh) using 30% methanol-ethyl acetate as the eluant affording a greenish solid (0.3 g). The column-purified leaf extract (0.005 g) was suspended in distilled water (10 mL) and sonicated in an ultrasonicator bath for 10 min to get a semitransparent solution (500 mgL^{-1}).

Synthesis of nanoparticles

Aliquots of Au(III) solution (0.16 mL, 10.42 mM each) were added dropwise to the leaf extract solution (containing polyhydroxy aromatic compounds) to prepare a series of stabilized AuNPs where the concentration of the leaf extract varied from 20 to 200 mg L^{-1} , and the concentration of Au(III) was fixed at 0.42 mM. UV-visible measurements of the solutions were carried out after 3 h of HAuCl_4 and *A. nilotica* leaf extract were mixed.

Characterization

Transmission electron microscopy of AuNPs was carried out in Tecnai G² 20 instrument (FEI, Hillsboro, OR, USA). XRD patterns of the stabilized AuNPs were studied in Panalytical X'pert Pro diffractometer (Almelo, The Netherlands) with Cu-K α radiation ($\lambda = 1.54 \text{ \AA}$). Mass spectral analysis of the leaf extract was carried out in Shimadzu GCMS QP 2100 plus (Kyoto, Japan). Shimadzu 1601 spectrophotometer was used for UV-visible spectroscopic measurements. The FTIR spectra of samples were recorded in Perkin Elmer FTIR Spectrum-II model (Waltham, MA, USA) using KBr pellet.

Results and discussion

A. nilotica (Babool) is a well-known medicinally important plant commonly found in the dry areas of Asia, Africa, and Australia. Various parts of the plant such as leaves, bark, roots, and fruits are used as medicine for the treatment of bronchitis, asthma, meningitis, hypoglycemia, pneumonia, sore throat, etc. Different types of plant secondary metabolites such as flavanoids, tannins, triterpenoids, saponines, etc. are present in the leaf extract of *A. nilotica* [15,16]. Au(III) having a high reduction potential can be reduced to Au(0) by the polyphenolic compounds present in the leaf extract with concomitant oxidation of the polyphenolic compounds to the corresponding quinones. The Au(0) atoms can collide with each other forming AuNPs, and the AuNPs can be stabilized by the resulting quinone derivatives and other coordinating ligands present in the plant

extract. The stabilizing ligands present on the surface of the AuNPs will not allow further aggregation of the AuNPs. To test this, we treated the increasing concentrations of the aqueous leaf extract contained in vials with HAuCl_4 solution (Figure 1). An appearance of pinkish red color within 5 min indicated the formation of AuNPs. On standing the solutions at room temperature for several hours, intensification of the color was observed and then the intensities remained constant for several months indicating the stabilities of the synthesized AuNPs.

UV-visible spectroscopy studies

Due to charge transfer interactions between metal and the chloro ligands of HAuCl_4 , the UV-visible spectrum showed two peaks at 220 and 288 nm (Figure 1a). On reaction with increasing concentration of the *A. nilotica* leaf extract, disappearance of these two peaks were observed with concomitant appearance of a SPR band between 533 to 529 nm region (Figure 1b,c,d,e,f,g). The variation of color of the AuNP colloids has been reported arising due to the change in size, shape, composition, crystallinity, etc. [1]. In our present studies, a shift of the SPR band was observed from 533 to 529 nm and the intensities increased

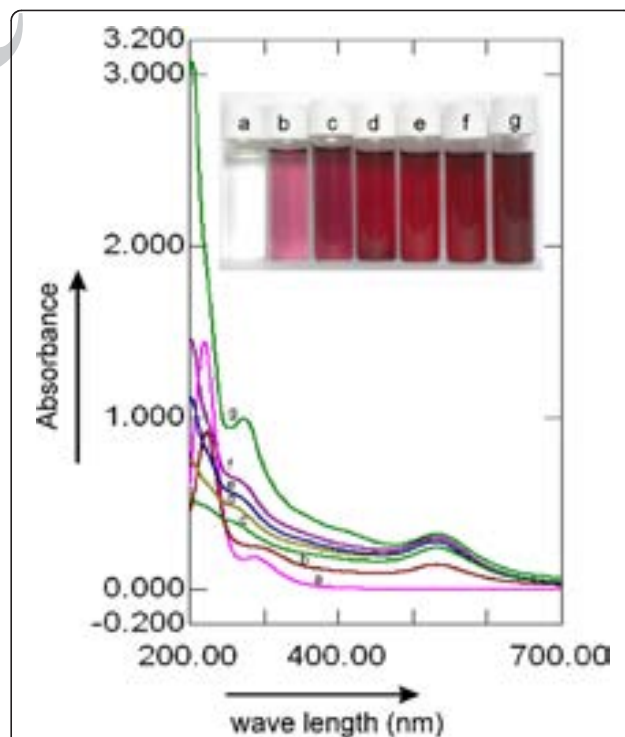


Figure 1 UV-visible spectra of HAuCl_4 and AuNPs synthesized with *Acacia nilotica* leaf extract. (a) HAuCl_4 (0.42 mM), (b-g) AuNPs at 20, 40, 60, 80, 100, and 200 mgL^{-1} concentrations of *Acacia nilotica* leaf extract, respectively. Inset: Photograph of the vials containing the stabilized colloidal AuNPs (after 1h of mixing).

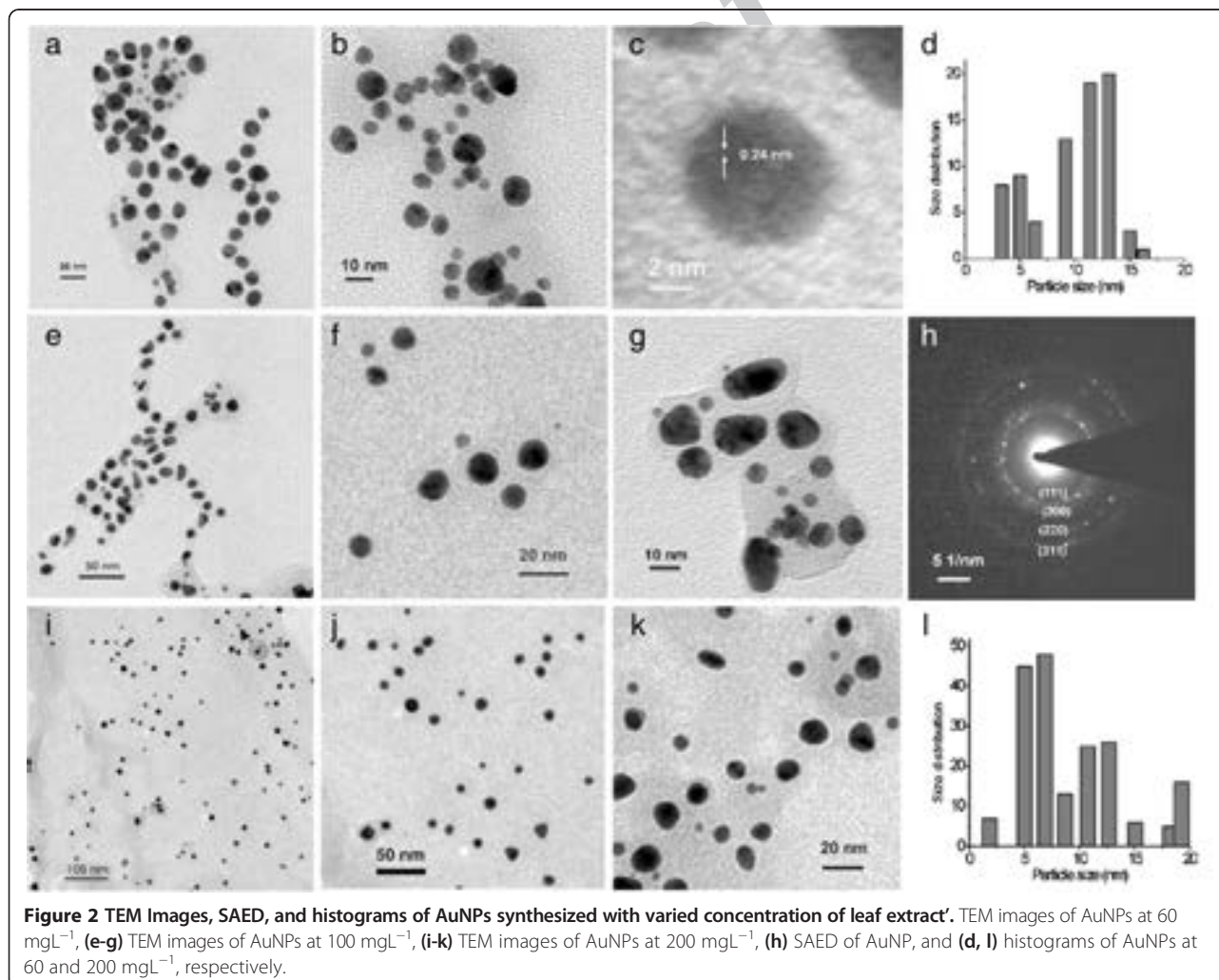
with the increasing concentration of the leaf extract from 20 to 200 mgL⁻¹. The strong peak at 272 nm at 200 mgL⁻¹ concentration is due to the formation of quinone moieties formed due to the oxidation of the phenolic compounds by Au(III) (Figure 1g).

HRTEM, EDX, FTIR, and XRD studies

The size distribution, shape, and morphology of the AuNPs formed at different concentrations of *A. nilotica* leaf extract were studied by HRTEM (see Figure 2 and Additional file 1: Figure S2). AuNPs of mostly spherical shapes of 6 to 12 nm size were observed. The average size of the AuNPs formed at 60 mgL⁻¹ concentration of the leaf extract was 12.24 nm (calculated from 77 particles, Figure 2a,b,c,d). At a higher concentration of the leaf extract (200 mgL⁻¹), the average particle size was 5.99 nm (calculated from 217 particles, Figure 2i,j,k,l). Elongated AuNPs (Figure 2e,g) were observed along with spherical particles (Figure 2f) at 100 mgL⁻¹ concentration of the leaf extract. The presence of the AuNPs held inside

the organic matrix derived from the leaf extract was evident from Figure 2g.

With the increasing concentration of the leaf extract, smaller sized AuNPs can be effectively stabilized by the polyphenolic compounds and the oxidized quinones. As the steric bulk of the surrounding ligands of the AuNPs prevents aggregation, the size of the AuNPs were smaller at higher concentration of the leaf extract. Lattice fringes of the AuNPs with a d-spacing of 0.24 nm were observed from the HRTEM image (Figure 2c). The fringe spacing matched with the expected d-spacing of the [111] plane of face-centered cubic crystalline Au (JCPDS, no. 04-0784). Selected area electron diffraction (SAED) pattern obtained from a AuNP (Figure 2h) showed the diffraction rings from inner to outer associated with the [111], [200], [220], and [311] atomic planes of Au indicating the formation of crystalline gold nanoparticles. The elemental composition of the synthesized AuNPs was determined by energy dispersive X-ray analysis (EDX). Area profile analysis of the synthesized nanoparticles showed strong peaks of Au



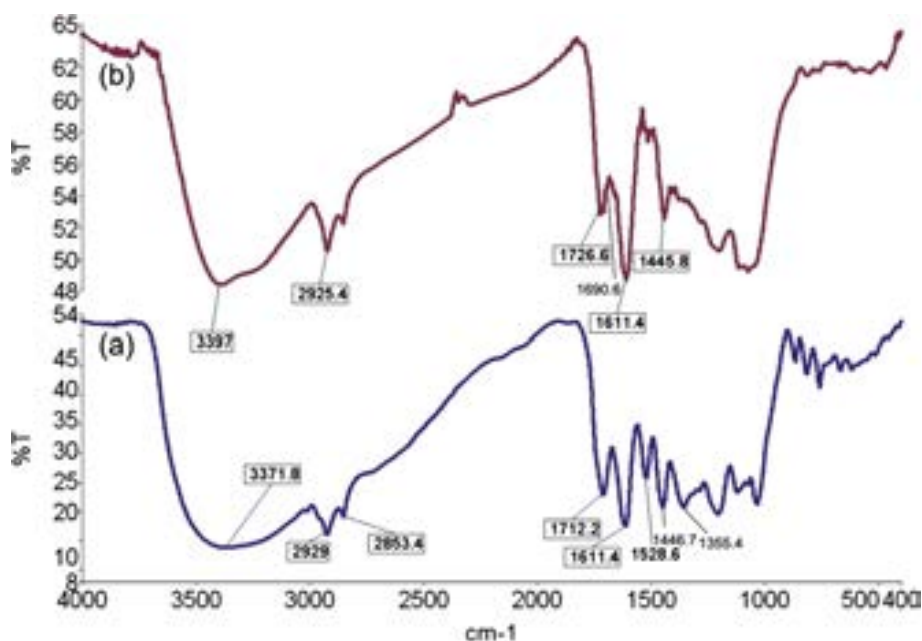


Figure 3 FTIR spectra of (curve a) *Acacia nilotica* leaf extract and (curve b) stabilized AuNPs.

(Additional file 1: Figure S3) confirming the formation of AuNPs. The presence of organic matrix was evident from characteristic carbon peak. The AuNP samples were coated over a glass plate, and X-ray diffraction analysis of the AuNPs was carried out after removal of the volatiles. The characteristic reflections of the planes (111), (200), (220), and (311) at $2\theta = 38.3^\circ$, 44.3° , 64.8° , and 77.8° , respectively, (Additional file 1: Figure S4) indicated the crystallinity of the metallic face-centered cubic AuNPs. These values are in agreement with the reported standards JCPDS file no. 04-0784 for crystalline gold. The comparatively greater peak intensity of the (111) plane is indicative of the predominant orientation of the (111) plane.

The FTIR spectra of the leaf extract of *A. nilotica* and the stabilized AuNPs were compared (Figure 3). The broad peak around $3,371\text{ cm}^{-1}$ in the FTIR spectrum of the leaf extract is due to the stretching vibration of the aliphatic and aromatic hydroxyl groups. Intermolecular hydrogen bonding among the hydroxyl groups was evident from the broadness of the peak. The peaks in the $1,611$ to $1,400\text{ cm}^{-1}$ range ($1,611$, $1,528$, $1,447\text{ cm}^{-1}$) are due to the presence of aromatic rings in the extract. The peak at $1,355\text{ cm}^{-1}$ is due to the in-plane bending of the OH groups. In the FTIR spectrum of the stabilized AuNPs, the stretching vibration due to the aliphatic and aromatic OH groups became narrower and shifted to $3,397\text{ cm}^{-1}$ due to weakening of

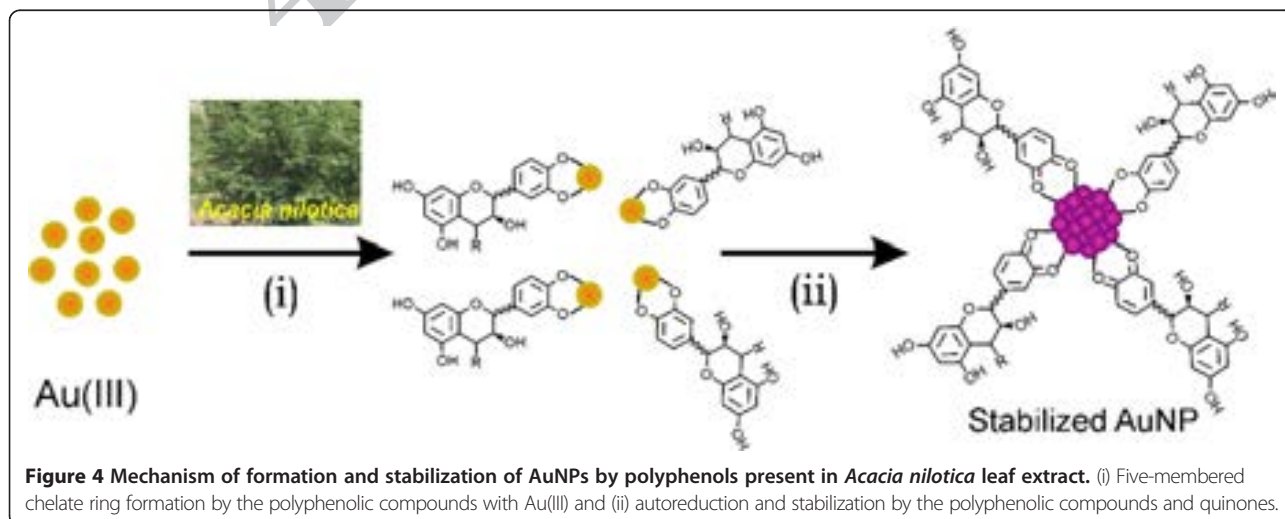


Figure 4 Mechanism of formation and stabilization of AuNPs by polyphenols present in *Acacia nilotica* leaf extract. (i) Five-membered chelate ring formation by the polyphenolic compounds with Au(III) and (ii) autoreduction and stabilization by the polyphenolic compounds and quinones.

Table 1 Catalytic activity of colloidal AuNPs (synthesized freshly with 60 mgL⁻¹ of leaf extract)

Sample number	Concentration of 4-nitrophenol (mM)	Concentration of sodium borohydride (mM)	Volume of colloidal AuNPs	Reaction completion time (min)	Catalytic rate constant (k) (min ⁻¹)
1	0.05	15.0	0.2	10	0.35
2	0.05	15.0	0.4	6	0.42
3	0.05	15.0	0.0	No reduction	Not applicable

the intermolecular H-bonding. The in-plane bending frequency of OH group became much weaker in the case of the stabilized AuNPs (Figure 3). The appearance of a new peak at 1,691 cm⁻¹ in the case of stabilized AuNPs is due to the formation of quinones.

Mechanism of the formation of stabilized AuNPs

The leaf extract of *A. nilotica* is rich in different types of plant secondary metabolites such as flavanoids, tannins, triterpenoids, saponines, etc. [15,16]. Evidence for the presence of polyphenolic compounds was obtained from the ferric chloride test (Additional file 1). Mass spectral analysis of the leaf extract carried out by us also supported the presence of the several polyphenolic compounds (Additional file 1: Figure S1) including quercetin (M⁺ 302), leucocyanidin (M⁺ 306), or their analogs. A schematic representation of the possible mechanism of the formation of AuNPs and their stabilization, based on leucocyanidin analogs as a representative framework, is shown in Figure 4. Ortho-dihydroxy compounds can form a five-membered chelate complex with Au(III). The chelated Au(III) can be reduced to Au(0) with concomitant oxidation of the ortho-dihydroxy compounds to quinones. Collision of the neighboring Au(0) atoms with each other lead to the formation of the AuNPs. The AuNPs can be stabilized by the polyphenolic compounds, quinones, as well as the other coordinating phytochemicals present in the leaf extract [17].

Study of the catalytic activity of stabilized AuNPs

To study the catalytic activity of the synthesized colloidal gold nanoparticles, the reduction of 4-nitrophenol (0.05 mM) to 4-aminophenol by sodium borohydride (15 mM) at room temperature was taken as a model system (Table 1) and the reaction was monitored by UV-visible spectroscopy (Figure 5) [17]. The absorption band of 4-nitrophenol at 318.5 nm shifted to 400 nm in the presence of sodium borohydride due to the formation of 4-nitrophenolate ion. No transformation was observed on standing the mixture at room temperature for several days. This is due to a very large kinetic barrier for the reduction reaction. Interestingly, on the addition of *A. nilotica* leaf extract-stabilized AuNPs (0.2 mL, synthesized with 60 mgL⁻¹ leaf extract) to the mixture, lowering of the intensity of the absorption peak at 400 nm was observed with concomitant formation of a new peak around 300 nm indicating the formation of 4-aminophenol. Complete disappearance of the 400-nm peak was observed within 10 min indicating completion of the reduction demonstrating the excellent catalytic activity of the synthesized colloidal AuNPs from the *A. nilotica* leaf extract. The change in intensity of the absorption maxima of 4-nitrophenol in alkaline medium (at 400 nm) with time provides a reasonable tool for the study of its reduction kinetics. As the concentration of BH₄⁻ was much higher (approximately 300-fold) than that of p-nitrophenol, its concentration remained practically constant during the reduction reaction and the reaction can be assumed as a

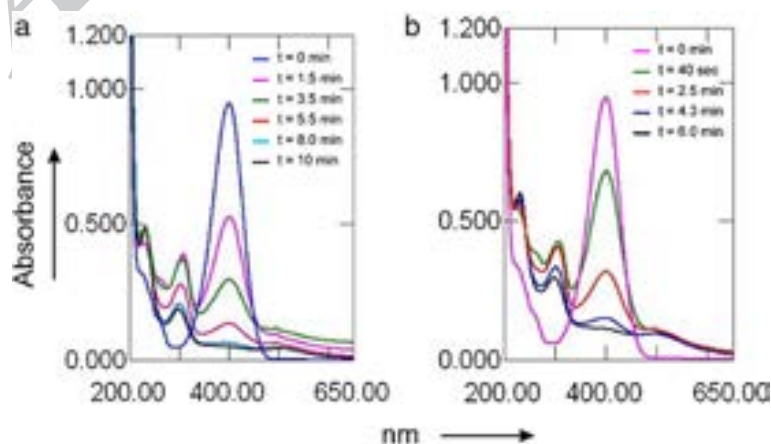


Figure 5 UV-visible spectra at different time intervals during the catalytic reduction of 4-nitrophenol to 4-aminophenol. Using (a) 0.2 and (b) 0.4 mL of stabilized AuNPs (synthesized with 60 mgL⁻¹ leaf extract).

pseudo first order reaction. A good linear correlation between $\ln A$ vs time was obtained (where A = absorbance of 4-nitrophenolate ion, Additional file 1: Figure S5), and the value of the catalytic rate constant (k) was calculated to be 0.35 min^{-1} . The observed rate constant was similar to that observed by us previously and others on similar systems [7,8]. Interestingly, on addition of double amount of stabilized AuNPs (0.4 mL, synthesized with 60 mgL^{-1} leaf extract) to the mixture, the rate of the reduction reaction became faster and the 400-nm peak completely disappeared within 6 min with concomitant formation of 4-aminophenol. Here also, a good linear correlation between $\ln A$ vs time was obtained and the value of the catalytic rate constant for this pseudo first order reaction was 0.42 min^{-1} . It is believed that both 4-nitrophenol and the reducing agent BH_4^- are adsorbed on the surface of the AuNPs, and the surface hydride ions are then transferred to 4-nitrophenol thereby facilitating the reduction reaction [18]. By comparing the rate constants of the two sets of reactions, it was obvious that with the larger volume of the catalyst, the rate of the reduction reaction increased due to the presence of larger number of reaction sites.

Conclusions

A very mild and efficient method for the green synthesis of colloidal gold nanoparticles has been demonstrated using the medicinally important *A. nilotica* leaf extract. According to our knowledge, this is the first report of the synthesis of AuNPs using *A. nilotica* leaf extract. The polyphenolic compounds present in the leaf extract act as an effective reducing agent as well as stabilizing agent, and AuNPs of 6 to 12 nm size were formed. Increasing the concentration of the leaf extract resulted in the formation of smaller sized nanoparticles as evident from HRTEM analysis. The synthesized colloidal AuNPs were highly efficient as catalyst for the reduction of p-nitrophenol to p-aminophenol at room temperature as evident from the spectrophotometric kinetic studies. As *A. nilotica* leaf extract has tremendous medicinal significance and AuNPs are biocompatible, the results described here will be useful in biomedical applications as well as nanoscience and nanotechnology.

Additional file

Additional file 1: Figures S1 to S5. Figure S1. Mass spectrum of *Acacia nilotica* leaf extract. **Figure S2.** High-resolution transmission electron microscopy images of AuNPs obtained with the leaf extract of *Acacia nilotica* at (a) 200 and (b) 60 mgL^{-1} concentration. **Figure S3.** Energy dispersive X-ray (EDX) spectra of AuNP derived from *Acacia nilotica* leaf extract (60 mgL^{-1}). **Figure S4.** X-ray diffraction pattern of AuNPs synthesized from HAuCl_4 solution (0.42 mM) using the leaf extract of *Acacia nilotica*. **Figure S5.** In A vs time plot (A = absorbance of 4-nitrophenolate), (a) with 0.2 mL AuNP colloid (60 mgL^{-1} leaf extract) and (b) with 0.4 mL AuNP colloid (60 mgL^{-1} leaf extract). From the slopes, the apparent catalytic rate constants (k) were calculated to be 0.35 min^{-1} in (a) and 0.42 min^{-1} in (b).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RM carried out the experiments, analyzed the data and participated in the manuscript preparation. BGB as the corresponding author participated throughout the investigations and manuscript writing. NM carried out preliminary studies.

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