

Lantana camara Linn leaf extract mediated green synthesis of gold nanoparticles and study of its catalytic activity

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Abstract A facile one-step green synthesis of stable gold nanoparticles (AuNPs) has been described using chloroauric acid (HAuCl₄) and the leaf extract of *Lantana camara* Linn (Verbenaceae family) at room temperature. The leaf extract enriched in various types of plant secondary metabolites is highly efficient for the reduction of chloroaurate ions into metallic gold and stabilizes the synthesized AuNPs without any additional stabilizing or capping agents. Detailed characterizations of the synthesized gold nanoparticles were carried out by surface plasmon resonance spectroscopy, transmission electron microscopy, dynamic light scattering, Zeta potential, X-ray diffraction and Fourier transform-infrared spectroscopy studies. The synthesized AuNPs have been utilized as a catalyst for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol in water at room temperature under mild reaction condition. The kinetics of the reduction reaction has been studied spectrophotometrically.

Keywords *Lantana camara* Linn · Gold nanoparticles · Catalytic reduction · Green synthesis · Phytochemicals

Introduction

Creation of nanoscale objects having at least one of their dimensions in the size range of 1–100 nm and their

utilizations in various facets of science and technology have become an area of tremendous research interest in recent years because the physicochemical properties of the materials at this scale are significantly different compared with their bulk scale (Alkilany et al. 2013; Pan et al. 2013; Titoo et al. 2014). Among various noble metal nanoparticles, gold nanoparticles (AuNPs) having unique optical, electronic and magnetic properties have found applications in pharmacology, biodiagnostics, medicine, drug-delivery, catalysis, etc. (Gong and Mullins 2009; Zhang et al. 2012; Murphy et al. 2008; Laura and Alberto 2014; Thomas and Kamat 2003). Depending upon their size, shape and degree of aggregation, AuNPs exhibit different colors (Weisbecker et al. 1996; Fujiwara et al. 1999; Aslan and Perez-Luna 2002; Mie 1908). Though the colloidal gold particles have been used since fifth to fourth century B.C., the scientific method for the reductive synthesis of colloidal gold can be traced back to 1857 when Michael Faraday reported a reductive synthesis of gold hydrosols from an aqueous solution of chloroaurate using phosphorus dissolved in carbon disulfide (Daniel and Astruc 2004; Faraday 1857). AuNPs can be synthesized using a number of routinely used chemical and physical methods. But, most of these methods employ toxic chemicals and nonpolar solvents during synthesis followed by addition of synthetic additives or capping agents as stabilizers thereby limiting their applications in clinical and biomedical fields. Therefore, there is a growing need for the development of eco-friendly, benign, biocompatible, reliable and synthetic methods to avoid any undesired environmental and health effects (De et al. 2008). The plant extract-based reductive method, involving the reduction of Au(III) to Au(0) by the phytochemicals, has gained profound significance in recent years for the development of a clean, reliable, biocompatible, benign, cost-effective and eco-friendly process

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(Jain et al. 2011). As the phytochemicals present in the plant extracts act as stabilizers for the synthesized gold nanoparticles and no additional stabilizers or capping agents are needed, the method is advantageous over other synthetic methods. Syntheses of AuNPs from the extracts of *Acacia nilotica* leaves (Majumdar and Bag 2013), *Punica granatum* juice (Dash and Bag 2014), *Saraca indica* bark (Dash et al. 2014), *Ananas comosus* L. (Basavegowda et al. 2013), *Terminalia arjuna* bark (Majumdar and Bag 2012), *Ocimum sanctum* stem (Paul and Bag 2013), etc. have recently been reported. As newer applications of nanoparticles are emerging rapidly, there is an ever growing need for the development of newer methods for the synthesis of metal nanoparticles utilizing the rich diversity of plant resources as renewables.

Lantana camara Linn (*L. camara*) is a popular ornamental and garden plant growing up to 2–4 meter in height, with a number of flower colors viz. yellow, red, pink and white. It also grows naturally at road or river sides up to elevations of 2,000 meters in tropical and subtropical temperature regions. The leaves of the plant are used in the treatment of tumors, tetanus, rheumatism, malaria, etc., and its antiseptic and carminative properties have also been reported (Raju 2000; Ganjewala et al. 2009; Ghisalberti 2000). During our investigations on the utilization of triterpenoids (C30 s) as renewable functional nanoentities (Bag and Dash 2011; Bag and Majumdar 2012; Bag and Paul 2012; Bag et al. 2012, 2013), it occurred to us that the medicinally important leaf extract of *L. camara*, rich in polyphenolic compounds, can be utilized for the synthesis of AuNPs from HAuCl_4 (Mittal et al. 2013). Herein, we report a very mild and environment friendly method for the synthesis of AuNPs from the leaf extract of *L. camara* 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, energy dispersive X-ray (EDX) and Fourier transform-infrared spectroscopy (FTIR) studies. Flower-like assemblies of AuNPs were observed at higher concentration of the leaf extract. Catalytic application of the synthesized AuNPs has been demonstrated for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol, and the reduction kinetics have been investigated spectrophotometrically.

Materials and method

Plant materials

The leaves of *L. camara* were collected from the campus of Vidyasagar University, Midnapore, West Bengal, India, identified at the Department of Botany and Forestry of this

University, and the specimen was deposited in our laboratory.

Chemicals

All chemicals used in the experiment were analytical reagent grade. Chloroauric acid ($\text{HAuCl}_4 \cdot \text{H}_2\text{O}$) was purchased from SRL and used without further purification. 4-Nitrophenol and sodium borohydride were purchased from Merck. Double distilled water was used for the experiments.

Synthesis of AuNPs

HAuCl_4 (36.5 mg) was dissolved in distilled water (10 mL) to obtain Au(III) (10.7 mM) stock solution. The purified *L. camara* leaf extract (see supporting information, 20 mg) was dissolved in 10 mL of distilled water and sonicated for 10 min to afford a yellowish stock solution ($2,000 \text{ mg L}^{-1}$). Aliquots of Au(III) solution (0.2 mL and 10.7 mM) were added drop wise to the leaf extract to prepare a series of stabilized AuNPs, where the concentration varied from 100 to 500 mg L^{-1} , keeping the concentration of Au(III) ion fixed at 0.54 mM. UV–visible spectroscopy of the gold colloids was carried out after 7 h of mixing HAuCl_4 , and the purified leaf extract and a band in the vicinity of 530 nm in the UV–visible spectrum confirmed the formation of AuNPs.

Reduction of 4-nitrophenol to 4-aminophenol

The sodium borohydride reduction of 4-nitrophenol to 4-aminophenol in the presence of AuNPs as catalyst was carried out as follows: an aliquot of 4-nitrophenol (0.2 mL and 1 mM) was treated with freshly prepared sodium borohydride solution (3.6 mL and 16.5 mM) and 0.2 mL freshly prepared colloidal AuNPs (synthesized with 100 mg L^{-1} plant extract) in a 10-mm quartz cuvette. Then, the reaction mixture was shaken thoroughly, and the UV–visible spectrum was recorded at room temperature (23–27 °C). The progress of the reaction was monitored by recording the absorption intensity of 4-nitrophenolate ion with certain time intervals, and the apparent rate constant was calculated. The experiment was repeated with 0.4-mL colloidal AuNPs (synthesized with 100 mg L^{-1} plant extract), and the apparent rate constant was measured.

Characterization

The morphology of the nanoparticles was analyzed from the high-resolution images obtained with a TECNAI G² 20 transmission electron microscope at an accelerating voltage of 200 kV. The X-ray diffraction (XRD) patterns of the

stabilized AuNPs were acquired by PANalytical X'Pert PRO diffractometer with Cu-K α radiation ($\lambda = 1.542 \text{ \AA}$). Mass spectra of the purified leaf extract were recorded in Shimadzu GCMS QP 2100 Plus instrument. UV–visible spectrum was carried out in Shimadzu 1601 spectrophotometer using 2-mm optical path quartz cuvette. FTIR spectra of the samples (with KBr pellet) were recorded using a Perkin Elmer spectrum 2 instrument where eight scans were accumulated to record the data. Particle size in the bulk and surface charge of gold nanoparticles were studied by dynamic light scattering (DLS) measurement and Zeta potential analysis using Malvern Nano-ZS90 instrument.

Results and discussions

The presence of a wide range of plant secondary metabolites such as triterpenoids, flavanoids as well as β -sitosterol-3-O- β -D-glucoside and a mixture of campesterol, stigmasterol and β -sitosterols have been reported in the leaf extract of *L. camara*. Mass spectral analysis of the leaf extract carried out by us has revealed the presence of most of the above compounds (supporting information Fig. S1 and S2). Presence of phenolic compounds was obtained by positive ferric chloride test (see supporting information). Hence, it occurred to us that the easily oxidizable phytochemicals present in the leaf extract of *L. camara* can be utilized for the reduction of chloroaurate ions having very high reduction potential to atomic gold. Collision of the gold atoms will lead to the formation of nano-sized particles and get stabilized by the polyphenols, quinones and other coordinating phytochemicals. To test this, we treated aqueous HAuCl₄ solutions with increasing concentration of the leaf extract of *L. camara*. Interestingly, we observed the appearance of pinkish red coloration within a 5 min, indicating the formation of AuNPs. The intensity of the color increased with time (Fig. 1).

UV–visible absorption and TEM Studies

On addition of HAuCl₄ solution to an aqueous leaf extract of *L. camara*, the color of the mixture changed from light yellow to reddish brown within five minutes, indicating the formation of AuNPs. UV–visible spectroscopy of HAuCl₄ solution and the mixtures containing HAuCl₄ and varied amounts of the leaf extract were carried out (Fig. 1) to find out the shifts in the absorption maxima. The strong absorption maximum at 222 nm and a shoulder peak at 296 nm appeared in the UV–visible spectrum of HAuCl₄ are due to charge transfer interactions between metal and chloro ligands (Fig. 1a). However, in the UV–visible spectrum of stabilized AuNPs, the intensities of these peaks

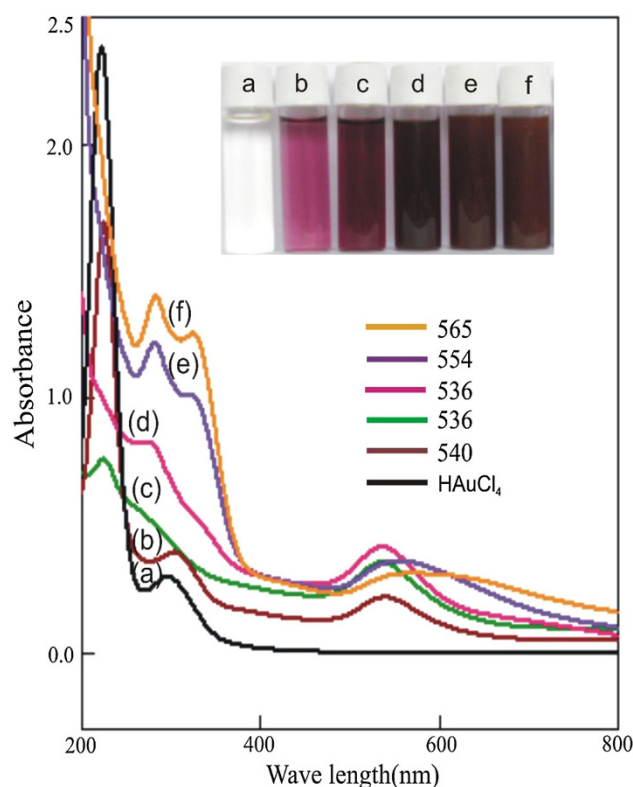


Fig. 1 UV–visible absorption spectra of gold alone and colloidal AuNPs at different concentrations of *L. camara* leaf extract: **a** solution of HAuCl₄ (0.54 mM); **b** 100 mg L⁻¹; **c** 200 mg L⁻¹; **d** 300 mg L⁻¹; **e** 400 mg L⁻¹ and **f** 500 mg L⁻¹. The wavelengths of the SPR bands of the respective AuNP colloids are shown in color. *Inset* Photograph of vials containing AuNPs of various concentrations

reduced, and a new peak in the region of 540–565 nm due to surface plasmon resonance phenomenon of AuNPs. At lower concentrations of plant extract (100 and 200 mg L⁻¹), the SPR bands appeared at 540 and 536 nm, respectively (Fig. 1b, c). Interestingly, on further increasing the concentration of the leaf extract (from 300 to 500 mg L⁻¹), broadening of SPR band took place with concomitant red shift, suggesting the formation of assembly of nanoparticles (Fig. 1d–f) (Ghosh and Pal 2007).

High-resolution transmission electron microscopy was carried out to investigate the size, shape and morphology of the synthesized AuNPs. Mostly spherical-shaped AuNPs were formed at 300 and 500 mg L⁻¹ concentration of the leaf extract having average size of 6–7 nm (Fig. 2a–f). Poly-shaped AuNPs were obtained at 100 mg L⁻¹ concentration of the leaf extract (Fig. 2g–i). Interestingly, at higher concentration of the leaf extract (500 mg L⁻¹), flower-like assembly of nanoparticles of nearly 100 nm diameter embedded in organic matrix was observed along with spherical-shaped particles (Fig. 2c). This result is in agreement with the pattern of SPR band obtained at this concentration (Fig. 1f). The fringe spacings measured to be 0.21 nm from the HRTEM

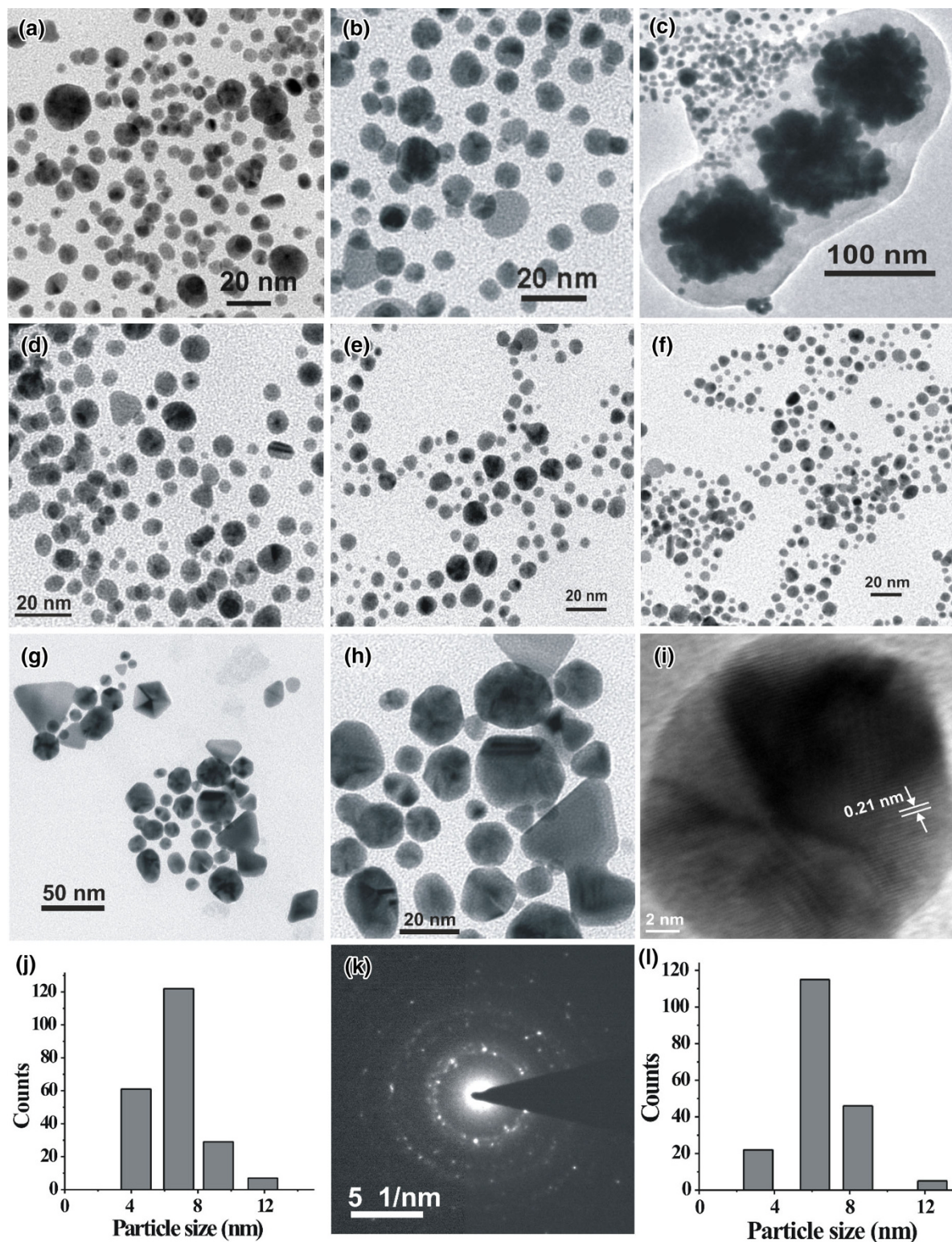


Fig. 2 HRTEM images of colloidal Au nanoparticles at various concentration of *L. camara* leaf extract: **a–c** at 500 mg L⁻¹; **d–f** 300 mg L⁻¹; **g–i** 100 mg L⁻¹; **j, l** histograms at 500 and 300 mg L⁻¹ concentration and **(k)** SAED pattern of AuNP

images (Fig. 2i) were in agreement with the spacing between two (111) planes of crystalline face-centered cubic (fcc) gold (0.235 nm) (Kanan and John 2008). The selected area electron

diffraction (SAED) pattern revealed that the nanoparticles were crystalline in nature (Fig. 2k). The nanoparticles were so stable that no further aggregation of the AuNPs took place on

keeping the colloids for several months at 23–27 °C. Elemental analysis of the AuNPs carried out using EDX analysis (supporting information Fig. S3) suggested that the nanoparticles were composed of pure crystalline gold. A strong band of carbon indicated the presence of biomolecules on the surface of AuNPs that might have played a role in the reduction of Au(III) and stabilization of AuNPs. This is in conformity with the HRTEM images (Fig. 2c).

Dynamic light scattering (DLS) and Zeta potential measurement studies

Dynamic light scattering studies of the colloidal AuNPs were carried out to investigate the average size of the particles in the bulk. The hydrodynamic diameters of the particles increased consistently as the concentration of leaf extract increased from 100 to 500 mg L⁻¹ (supporting information Fig. S4 and S5). At each concentration of the leaf extract, the particle size obtained by DLS study was larger than the particle size obtained by HRTEM study. This is due to the ligands attached to the surface of AuNPs (Gangula et al. 2011; Chandran et al. 2012). The surface charges (Zeta potential) of the AuNPs synthesized at 100 and 200 mg L⁻¹ concentration of the leaf extract were -27.8 and -37.4 mV, respectively (see supporting information Fig. S6). High negative value of Zeta potential indicated high electrical charge on the surface of the AuNPs. This can cause strong repulsive forces among the AuNPs and prevent their aggregation, resulting in higher stability of colloidal AuNPs.

X-ray diffraction (XRD) and FTIR studies

The XRD pattern of the synthesized AuNPs is shown in Fig. 3. Five sharp diffraction peaks at 38.3°, 44.5°, 64.8°, 77.7° and 81.6° can be indexed as (111), (200), (220), (311) and (222) Bragg reflections, respectively, confirming the fcc nature of colloidal AuNPs (JCPDS file no. 04-0784). The intensity of the peak due to (111) plane at 38.3° was much larger than the other peaks, indicating predominant orientation of the (111) plane.

Fourier transform-infrared spectroscopy studies of the leaf extract and the stabilized AuNPs were carried out to investigate the various functional groups involved in the reduction and capping of gold nanoparticles (Fig. 4). The appearance of a broad peak approximately around 3,314 cm⁻¹ region in the FTIR spectrum of *L. camara* leaf extract indicated the presence of -OH/N-H group containing compounds (Fig. 4a). The broadening of the peak was due to strong intermolecular H-bonding between these groups. The peak at 2,932.4 cm⁻¹ is due to the stretching vibration of aliphatic -CH- group containing compounds present in the leaf extract. The molecules present in the leaf

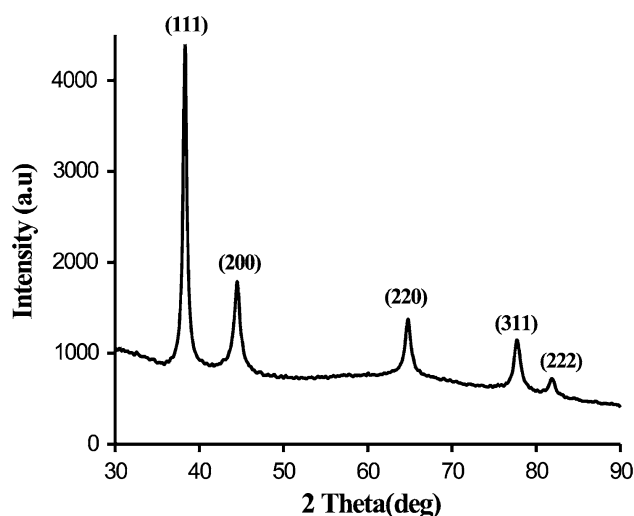


Fig. 3 X-ray diffraction pattern of stabilized AuNPs

extract containing -C=O group were evident from the peak at 1,704 cm⁻¹ region. The presence of aromatic rings containing compounds in the leaf extract was confirmed from the peaks at 1,611 and 1,522 cm⁻¹ arising due to the stretching vibration of C=C bond. The peaks at 1,270 and 1,374 cm⁻¹ can be assigned to stretching vibrations of C-N bond. In case of colloidal AuNPs, the peaks due to -OH/N-H groups became slightly narrower, suggesting the interaction between these groups and the AuNPs (Fig. 4b). The peak due to carbonyl group became significantly weaker in the FTIR spectrum of stabilized AuNPs, indicating the interaction of carbonyl groups with the AuNPs.

Reaction mechanism

The leaf extract of *L. camara* is rich in different types of plant secondary metabolites such as terpenoids, steroids and polyphenols including flavanoids (Ghisalberti 2000). Mass spectral analysis carried out by us indicated the presence of different types of polyphenols such as quercetin, gallic acid, caffeic acid and chlorogenic acid. (supporting information Fig. S1). A positive ferric chloride test (see supporting information) supported the presence of the phenolic compounds in the leaf extract. A schematic representation for the formation of AuNPs is given in Fig. 5. Phenolic compounds and other easily oxidizable phytochemicals can reduce Au(III) to Au (0). The electron-rich o-dihydroxy compounds present in the leaf extract can easily form a five-membered chelate ring with the Au(III) ions. In this case, the redox reaction can take place in the chelated complex where the o-dihydroxy compounds can be oxidized to corresponding quinones with concomitant reduction of Au(III) ions to Au (0). Collision of the neighboring Au (0) atoms with each other may lead to the

Fig. 4 FTIR spectrum of **a** *L. camara* leaf extract and **b** stable AuNPs

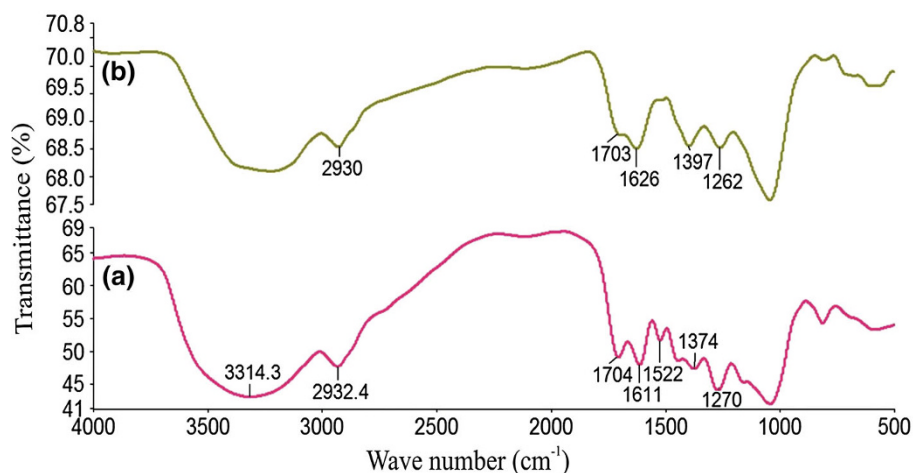
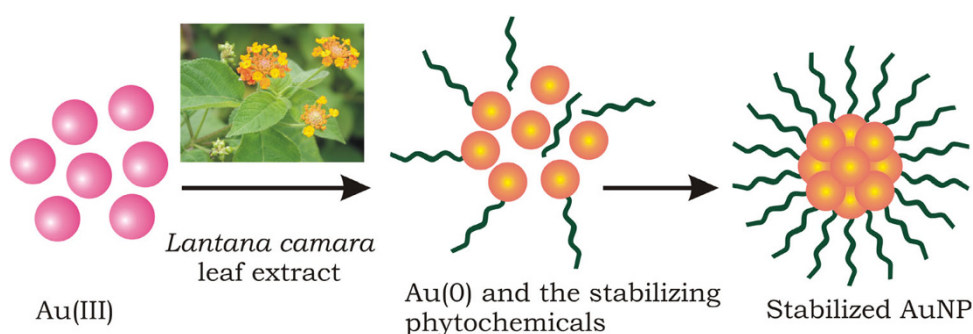


Fig. 5 Mechanism of the formation and stabilization of AuNPs by the phytochemicals present in the leaf extract of *L. camara*



formation of the nano-sized gold particles. The AuNPs thus formed can be stabilized by the quinones, phenolic compounds as well as other coordinating phytochemicals present in the leaf extract. Further aggregation of the AuNPs is prevented by the stabilizing ligands surrounding the AuNPs.

Application of AuNPs in catalytic reduction

Nanoscale materials have drawn considerable attention in recent years because of their unique application as catalyst for some chemical transformations, which are normally restricted due to the large kinetic barrier of the reaction (Kim et al. 2009). Whereas bulk gold at the macro scale is inactive as a catalyst, the AuNPs are useful as a catalyst for a number chemical transformation (Pan et al. 2013; Gong and Mullins 2009). The electrochemical potential (E_0) value suggests that the reduction of 4-nitrophenol to 4-aminophenol in the presence of sodium borohydride is a thermodynamically favorable reaction (E_0 for 4-nitrophenol/4-aminophenol -0.76 and for $\text{H}_3\text{BO}_3/\text{BH}_4^-$ -1.33 V). However, on treatment of an aqueous solution of 4-nitrophenol (0.05 mM) with a freshly prepared aqueous solution of sodium borohydride (16.5 mM), the absorption maxima at 319 nm shifted to 401.5 nm due to the formation of more

stable 4-nitrophenolate ion in alkaline medium (Fig. 6a). In the absence of AuNPs or in the presence of the leaf extract alone, no reduction of the nitro group to amino group took place on standing the reaction mixture for several days due to a large kinetic barrier for the reduction reaction. Interestingly, on addition of *L. camara* leaf extract stabilized AuNPs, the decolorization of yellowish color (of 4-nitrophenolate solution) was observed. The progress of the reduction reaction was monitored by UV–visible spectroscopy at various time intervals. After 30 s, the intensity of the peak at 401.5 nm reduced, and concomitantly a new peak appeared around 300 nm, suggesting the conversion of 4-nitrophenol to 4-aminophenol. The peak due to 4-nitrophenolate ion completely disappeared after 20 min, indicating the completion of reduction reaction. The absorption intensity (A) of 4-nitrophenolate ion at various intervals allowed us to calculate the apparent rate constant (k_{app}) for the reduction reaction. As the concentration of 4-nitrophenol largely exceeded (300 times) the concentration of sodium borohydride, the reaction was assumed to be pseudo-first order, and a good linear correlation was obtained between $\ln A$ vs time (t) plot. From the slope of the linear plot, the apparent rate constant (k_{app}) was measured to be 0.26 and 0.29 min^{-1} using 0.2 and 0.4 mL stabilized colloidal AuNPs, respectively (Table 1, supporting information TS1, TS2 and Fig. S7 and S8). The

small increase in the apparent catalytic rate constant (k_{app}) with higher volume of colloidal AuNPs (keeping other parameters constant) was perhaps due to increase in the number of reaction centers (Gangula et al. 2011). These rate constant values were comparable to the recently reported values on related systems (Gangula et al. 2011; Dash et al. 2014) (Fig. 6).

Table 1 Catalytic reduction of 4-nitrophenol (4-NP) at 25 °C using different amounts of colloidal AuNPs (synthesized with 100 mg L⁻¹ of leaf extract)

Entry	Conc. of 4-NP (mM)	Conc. of NaBH ₄ (mM)	Colloidal AuNPs used (mL)	Time for completion of reaction (min)	Apparent Rate constant (k_{app}/min^{-1})
1	0.05	16.5	0.2	7.5	0.26
2	0.05	16.5	0.4	6	0.29
3	0.05	0	0.2	No reduction	NA
4	0.05	16.5	0.0	No reduction	NA

NA Not applicable

Conclusions

One-step synthesis of colloidal gold nanoparticles has been reported by utilizing the leaf extract of *L. camara* under very mild reaction condition. According to our knowledge, this is the first report for the synthesis of AuNPs utilizing the leaf extract of *L. camara*. The method described here is very simple, cost-effective and nontoxic in nature. Various phytochemicals present in the leaf extract are highly efficient to reduce Au(III) to Au (0) and simultaneously stabilize the synthesized AuNPs. The synthesized AuNPs were of 6–7 nm in size and mostly spherical shaped as evident from HRTEM studies. *Flower-like assemblies* of AuNPs of approximately 100 nm diameter were observed at a higher concentration of the leaf extract along with discrete AuNPs. The synthesized AuNPs were utilized for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol at room temperature, and the apparent catalytic rate constant for the reduction reaction was measured spectrophotometrically. As *L. camara* leaf extract has various medicinal applications, the results

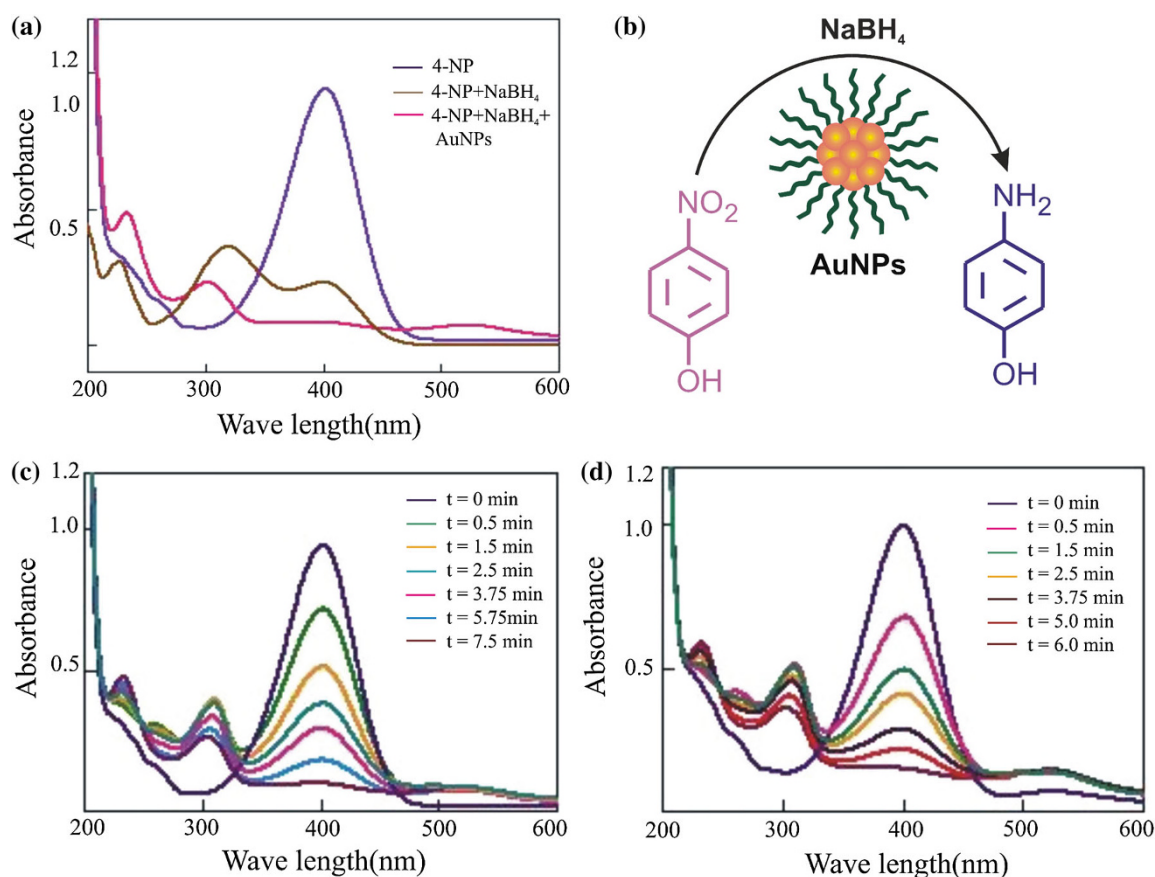


Fig. 6 **a** UV–visible spectrum of 4-nitrophenol, 4-nitrophenolate ion and 4-aminophenol; **b** a schematic representation of catalytic reduction of 4-nitrophenolate ion to 4-aminophenolate ion; **c** and

d overlay of UV–visible spectrum at various time intervals using 0.2 and 0.4 mL stabilized AuNPs, respectively, during catalytic reduction

described here open up its use in biomedical applications as well as nanoscience and nanotechnology.

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