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ORIGINAL ARTICLE

Abroma augusta Linn bark extract-mediated green synthesis of gold nanoparticles and its application in catalytic reduction

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Abstract The bark extract of *Abroma augusta* Linn is rich in medicinally important phytochemicals including antioxidants and polyphenols. First one step green synthesis of gold nanoparticles (AuNPs) has been described utilizing the bark extract of *Abroma augusta* L. and chloroauric acid under very mild reaction conditions. The phytochemicals present in the bark extract acted both as a reducing as well as a stabilizing agent, and no additional stabilizing and capping agents were needed. Detailed characterizations of the stabilized AuNPs were carried out by surface plasmon resonance spectroscopy, high resolution transmission electron microscopy, and X-ray diffraction studies. The catalytic activity of the freshly synthesized gold nanoparticles has been demonstrated for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol, and the kinetics of the reduction reaction have been studied spectrophotometrically.

Keywords Green synthesis · Gold nanoparticles · Catalytic reduction · Abroma augusta L. · Antioxidant

Introduction

Gold nanoparticles (AuNPs) with unique optical, electronic, and magnetic properties have drawn tremendous research interests during the last two decades (Alkilany et al. 2013, Zhang et al. 2012) because of their applications in diversified areas such as catalysis (Wittstock and Baumer 2014; Liu et al. 2014) drug delivery, biodiagnostics (Murphy et al. 2008), medical imaging (Huang et al. 2009), plasmonics (Pelton et al. 2008), and chemical sensing. AuNPs dispersed in water and stabilized with non-toxic biomolecules are required for many of such applications. Among various methods reported for the synthesis of AuNPs, the plant extract-mediated reductive method, involving the reduction of Au(III) to Au(0) by the phytochemicals, has gained profound significance in recent years due to the renewable and non-toxic nature of the phytochemicals, mild reaction conditions, eco-friendly aqueous medium, etc. The method is advantageous over other synthetic methods because the phytochemicals present in the plant extract act both as a reducing agent as well as a stabilizer, and no additional stabilizers or capping agents are needed. The green synthesis of AuNPs from the extracts of *Macrotyloma uniflorum* (Aromal et al. 2012), *Trigonella foenum-graecum* (Aromal and Philip 2012), *Aloe vera* (Chandran et al. 2006), *Acacia nilotica* leaf (Majumdar et al. 2013), *Saraca indica* bark (Dash et al. 2014), *Punica granatum* (Dash and Bag 2014), Green coconut shell (Paul et al. 2014), etc., has been reported (Mittal et al. 2013). Due to rapid emergence of newer applications of nanoparticles and nanomaterials, there is an ever growing need for the development of newer methods for the synthesis of metal nanoparticles utilizing plant resources as renewables.

Abroma augusta Linn is a small, ever-green plant growing up-to 3–4 m in height with velvety branches and found in

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tropical Asia, South and Eastern Africa, and Australia. Usually found as a wild plant or as an ornamental plant in garden, *Abroma augusta* L. belongs to the family of Sterculiaceae, and it is also an important Ayurvedic medicinal plant. Various parts of the plant have been used for the treatment of diabetes, rheumatic pain of joints, uterine disorders, headache with sinusitis, nervous dysmenorrhoea, amenorrhoea, sterility, menstrual disorder, etc. (Gupta et al. 2011). During our investigations on the utilization of triterpenoids (C30s) as renewable functional nano-entities (Bag and Dash 2011; Bag and Paul 2012; Bag et al. 2012, 2013; Bag and Majumdar 2012, 2014), it occurred to us that the medicinally important bark extract of *Abroma augusta* L., rich in polyphenolic compounds, can be utilized for the synthesis of AuNPs from $HAuCl_4$ (Mittal et al. 2013). Herein, we report the experimental evidence for the presence of antioxidants including polyphenols in the bark extract of *Abroma augusta* L. and the use of the bark extract for a very mild, environment friendly and efficient synthesis of AuNPs without any additional capping or stabilizing agents. The stabilized colloidal AuNPs were characterized by high resolution transmission electron microscopy (HRTEM), selected area electron diffraction (SAED), surface plasmon resonance (SPR) spectroscopy, and X-ray diffraction studies. Catalytic activity of the freshly synthesized colloidal AuNPs has been demonstrated for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol in water at room temperature as a model reaction, and the kinetics of the reduction reaction have been investigated spectrophotometrically.

Materials and methods

Materials

Au(III) solution: $HAuCl_4$ was purchased from SRL and used without purification. $HAuCl_4$ (36.5 mg) was dissolved in distilled water (10 mL) to obtain a Au(III) stock solution (10.74 mM).

Preparation of the *Abroma augusta* bark extract

The bark of *Abroma augusta* L. was collected from the local area of Midnapore, West Bengal, India and identified at the Department of Botany and Forestry, Vidyasagar University, Midnapore. The bark was cut into small pieces, dried in air, and then powdered using a grinder. Finely powdered *Abroma augusta* bark (20 g) was suspended in ethanol (120 mL), refluxed for 5 h, and filtered. Volatiles of the filtrate were removed under reduced pressure to afford a greenish black sticky solid (0.44 g). The crude greenish black sticky solid was dissolved in ethanol (30 mL) and filtered through a Celite bed. The volatiles of the filtrate were removed under reduced pressure to afford a solid (0.33 g). Purified *Abroma augusta* bark extract (0.02 g) was suspended in a mixture of distilled water and ethanol (10 mL, 4:1) and sonicated using an ultrasonicator bath for 45 min to obtain a semi-transparent solution (2,000 mg L^{-1}).

DPPH assay

The most extensively used stable DPPH radical was employed for the study of antioxidant property of bark extract of *Abroma augusta*. Antioxidants present in the extract react with DPPH radical and convert it to 1,1-diphenyl-2-picryl-hydrazine. The color change from violet to yellowish within 30 min upon addition of the bark extract to DPPH solution indicated the antioxidant property of the extract. The reduction in the absorbance intensity at 517 nm was studied by UV–visible spectrophotometry.

Identification of polyphenolic compounds

The presence of phenolic compounds in the bark extract of *Abroma augusta* was examined qualitatively by ferric chloride test. The extract of the bark of *Abroma augusta* (1 mL) was mixed with an aliquot of freshly prepared concentrated $FeCl_3$ solution, and the mixtures was shaken vigorously. Appearance of greenish color almost instantly indicated the presence of phenolic compounds in the bark extract.

Synthesis of gold nanoparticles

Aliquots of Au(III) solution (0.2 mL, 10.74 mM) were added dropwise to the bark extract of *Abroma augusta* to prepare a series of stabilized AuNPs where concentration of bark extract varied from 100 to 800 mg L^{-1} (100, 200, 400, 600, and 800 mg L^{-1}), and the concentration of Au(III) was fixed at 0.43 mM. The appearance of reddish coloration (within few minutes) and the SPR band in the UV–visible spectroscopy of the gold colloids confirmed the formation of AuNPs.

Characterization

HRTEM images and SAED pattern of AuNPs were recorded JEOL JEM 2100 instrument. X-ray diffraction (XRD) analysis of the stabilized AuNPs were carried out in PANalytical X'pert Pro instrument with Co-Kα radiation ($\lambda = 1.789$ Å). Mass spectra of the purified samples were recorded in Shimadzu GCMS QP 2100 Plus instrument. UV–visible spectra of the gold colloids were recorded in Shimadzu 1601 spectrophotometer. FTIR spectra of

samples were analyzed using a Perkin Elmer FTIR spectrum two model using KBr pellet.

Results and discussion

The bark extract of *Abroma augusta* Linn (L.) is extensively used for the treatment of gynecological disorders, dysmenorrhoea, wound healing, sterility, menstrual disorders, etc. (Gupta et al. 2011). The presence of a large number of plant secondary metabolites such as alkaloids, flavonoids, and tannins in the bark extract of *Abroma augusta* L. has been reported (Das et al. 2012). Mass spectral analysis carried out in our laboratory indicated the presence of polyphenolic compounds including flavonoids along with steroids (supporting information Figure S1). Evidence for the presence of phenolic compounds in the bark extract of *Abroma augusta* L. was also obtained from a positive ferric chloride test. Realizing the roles of free radicals and active oxygen species as the cause of various physiological disorders including cancer and tumors, we examined the presence of antioxidants in the bark extract of *Abroma augusta* L. against a long lived 2, 2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. Interestingly, on treatment of an alcoholic solution of DPPH with the plant extract, disappearance of the reddish brown color was observed and the intensity of the band at 517 nm decreased, indicating the presence of antioxidants in the bark extract (Fig. 1i). The easily oxidizable phytochemicals such as polyphenols present in the bark extract of *Abroma augusta* L. can reduce Au(III) to Au(0) with concomitant oxidation of the phytochemicals to a higher oxidation state such as quinones. The Au(0) atoms thus formed can collide with each other to form AuNPs which can be stabilized by phenolic compounds, benzoquinone derivatives and other coordinating phytochemicals. Indeed on treatment of the aqueous mixtures of the bark extract of *Abroma augusta* L. contained in vials (Fig. 1ii) with $HAuCl_4$ solution, reddish color appeared within few minutes indicating the formation of AuNPs. The intensities of the colors increased on standing the solutions at room temperature for several hours and then remained constant, and the AuNPs once formed were stable for several months at room temperature.

UV–visible spectroscopy studies

UV–visible spectroscopic studies of the gold colloids were carried out to confirm the formation of AuNPs (Fig. 1ii). In the UV–visible spectrum of $HAucl_4$, a strong peak at 220 nm and a shoulder peak at 288 nm appeared due to charge transfer interactions between the metal and the chloro ligands (Fig. 1iia). Interestingly, on addition of the bark extract (100 mg L^{-1}), the intensity of these two peaks decreased and concomitantly a new peak appeared at 549.5 nm due to surface plasmon resonance (SPR) characteristic of AuNPs. With increasing concentration of the bark extract to 200 and 400 mg L^{-1}, blue shift of the SPR band took place and appeared, respectively, at 543.5 and 540.5 nm. The blue shift of the SPR band was due to the formation of smaller-sized AuNPs at higher concentration of the bark extract. On increasing the concentration of the bark extract further to 600 and 800 mg L^{-1}, the SPR band redshifted to 541.5 nm. This is due to interparticle interactions taking place in the assembly of AuNPs (Ghosh and Pal 2007). The evidence for the formation of assembly of AuNPs was supported by HRTEM results (discussed below).

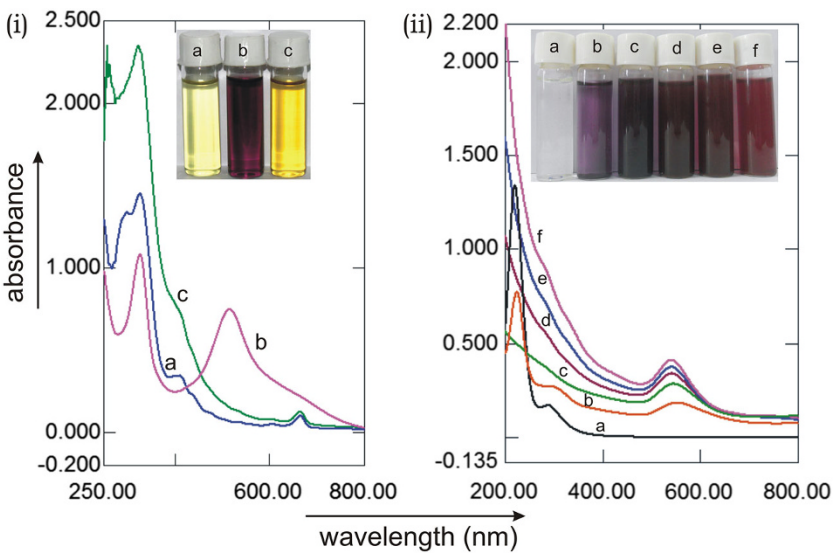

Fig. 1 **i** Antioxidant activity studies of the bark extract of *Abroma augusta*: UV–visible spectra of *a* extract, *b* DPPH, *c* DPPH + ethanol extract, inset: photographs of the vials containing the respective solutions; **ii** UV–visible spectra of *a* $HAuCl_4$ (0.43 mM), *b*–*f* AuNP's at 100, 200, 400, 600, and 800 mg L^{-1} concentrations of the extract of *Abroma augusta* bark, respectively. Inset: photograph of the vials containing *a* $HAuCl_4$ (0.43 mM) solution, *b*–*f* colloidal AuNP's at 100, 200, 400, 600, and 800 mg L^{-1}, respectively (after 24 h of mixing)

HRTEM, FTIR, and XRD studies

The size and morphology of AuNPs formed at various concentrations of the bark extract was investigated by HRTEM analysis. The analysis revealed the formation of mostly spherical-shaped particles along with a few triangular, pentagonal, and hexagonal particles at all the concentrations of phytochemicals studied (Fig. 2). The average particle size of AuNPs formed at 600 mg L^{-1} concentration of bark extract was 33.1 nm. At a higher concentration of bark extract (800 mg L^{-1}), the average particle size was 23.4 nm. Assemblies of AuNPs were observed at 800 mg L^{-1} concentration of the bark extract (Fig. 2b). This observation is also supported by the red-shift of the SPR band at a higher concentrations arising due to interparticle interactions. Lattice fringes were observed in the HRTEM images of AuNPs (Fig. 2k, l) with a d-spacing of 0.23 nm. The fringe spacing of the AuNPs matched with the expected d-spacing of the (111) plane of face-centered cubic (fcc) crystalline Au (JCPDS, No. 04-0784).

X-ray diffraction analysis of the *Abroma augusta* bark extract stabilized AuNPs is given in Fig. 3. The presence of sharp peaks at $2\theta = 44.7^{\circ}$, 52°, 77°, 93.7°, and 98.9° corresponding to the planes (111), (200), (220), (311), and (222), respectively, suggests the reduction of Au(III) to Au (0) and also confirms the crystalline behavior of gold atoms. The higher intensity of the peak corresponds to (111) plane indicated the predominant orientation of this plane. The values obtained here are in good agreement with the reported standards JCPDS file no. 04-0784.

The FTIR spectra of the bark extract of *Abroma augusta* and the stabilized AuNPs were compared (supporting information Figure S2). The presence of biomolecules in the stabilized AuNPs was evident from the FTIR stretching frequencies. The aliphatic and aromatic hydroxyl (O–H bond) groups appeared as broad peaks at 3,394 and

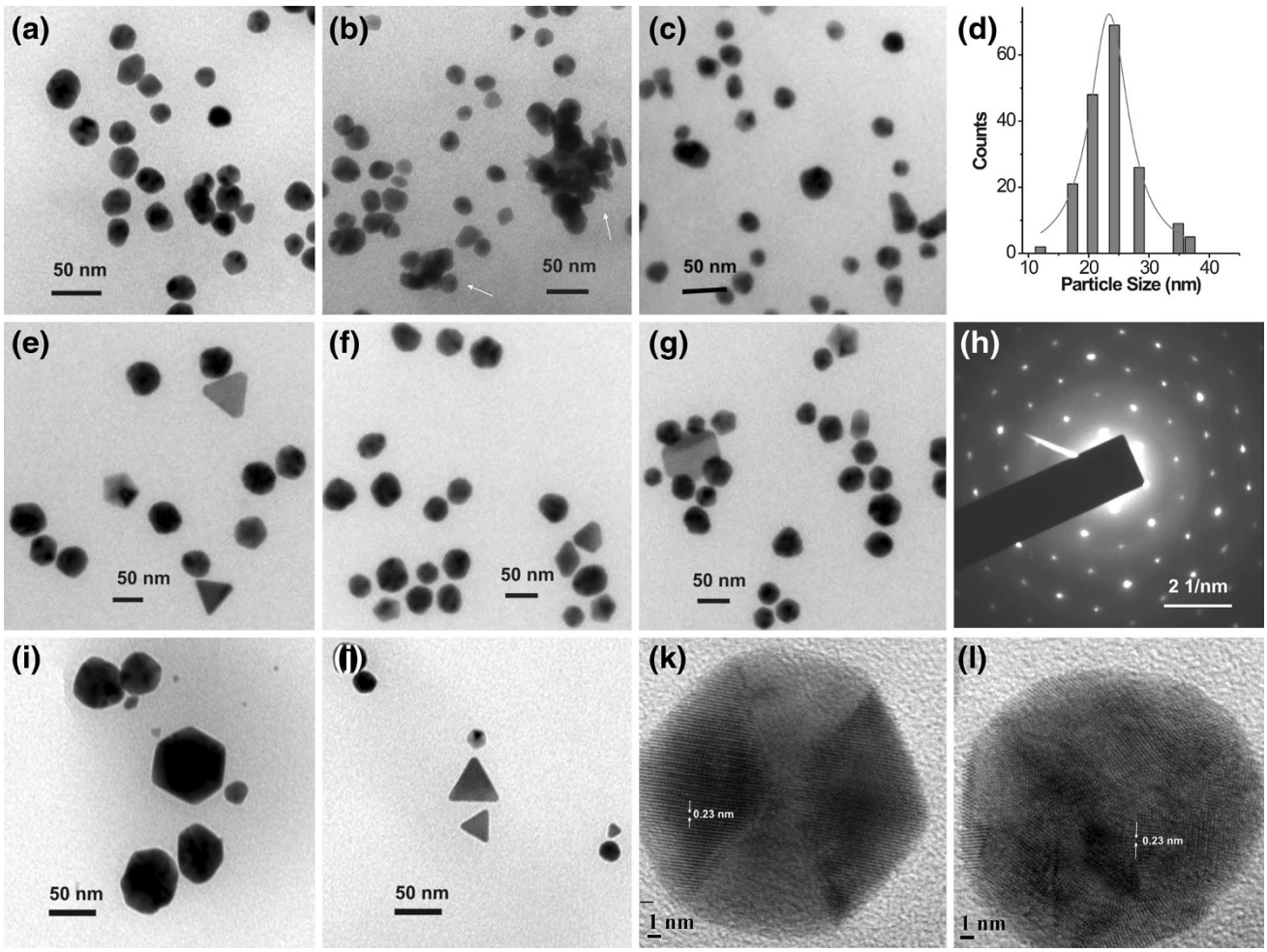

Fig. 2 HRTEM images of Au nanoparticles obtained with the bark extract of *Abroma augusta*: *a–c* at 800 mg L^{-1}; *e–g* at 600 mg L^{-1}; *i–l* and at 200 mg L^{-1} concentration, *d* histogram of AuNPs at 800 mg L^{-1} concentration, and *h* SAED of AuNP prepared at 200 mg L^{-1} concentration of plant extract

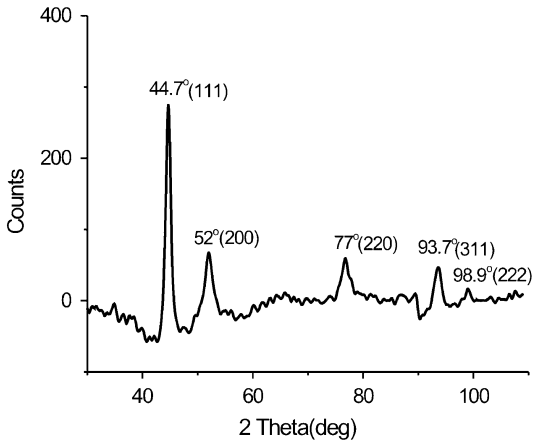

Fig. 3 XRD of stabilized AuNPs

3,392 cm^{-1} in the FTIR spectrum of the bark extract of *Abroma augusta* and the stabilized AuNPs. The broadness of the peaks was due to the intermolecular hydrogen bonding present among the OH groups. The peaks at 2,923 and 2,924 cm^{-1}, respectively, are due to the 'C–H' stretching frequencies. The peak at 1,721 and 1,723 cm^{-1} are due to the 'C=O' groups in the bark extract and the stabilized AuNPs.

Mechanism of the formation of stabilized AuNPs

The bark extract of *Abroma augusta* L. is rich in different types of phytochemicals including polyphenols, flavonoids, and steroids (supporting information Figure S1). The o-dihydroxy compounds along with other easily oxidizable compounds present in the bark extract can reduce Au(III) ions at room temperature (Fig. 4) to Au(0) with concomitant oxidation of the phytochemicals to a higher oxidation state. The freshly generated Au(0) atoms in the reaction mixture can collide with each other forming AuNPs which can be stabilized by the concomitantly formed quinones, unreacted polyphenols, and other coordinating phytochemicals present in the bark extract.

Application of AuNPs in catalysis

In recent years, there is a tremendous research interest in the use of AuNPs as a catalyst for various chemical transformations. To test whether the *Abroma augusta* bark extract derived stable colloidal AuNPs can be utilized as a catalyst, we chose the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol as a model reaction (Fig. 5). On treatment of an aqueous solution of 4-nitrophenol (0.05 mM) with sodium borohydride (15 mM) at room temperature, the absorption band of 4-nitrophenol at 317.5 nm shifted to 402.5 nm due to the formation of 4-nitrophenolate anion (Fig. 5ii). Although the reduction of 4-nitrophenol to 4-aminophenol by sodium borohydride is a thermodynamically favorable reaction (E_0 for 4-nitrophenol/4-aminophenol −0.76 and for H_3BO_3/BH_4^- −1.33 V), no reduction of the nitro group took place even on standing the mixture at room temperature for several days due to very high kinetic barrier of the reduction reaction. Interestingly, it was observed that the reduction was completed in few minutes in the presence of the freshly prepared *Abroma augusta* L. bark extract derived AuNPs. The progress of the reduction reaction was monitored spectrophotometrically. Using the UV–visible data at different time intervals, the catalytic rate constant (k) for the reduction reaction was calculated using different volumes of stabilized AuNPs. When 0.10 mL of the freshly prepared colloidal AuNPs was used for the reduction of 4-nitrophenol (0.05 mM) containing aqueous sodium borohydride (15 mM, with 4 mL as final volume of the mixture), the peak at 402.5 nm arising due to 4-nitrophenolate anion disappeared in 7 min and 30 s with concomitant appearance of a new peak at 298.5 nm indicating the formation of 4-aminophenol (Fig. 5iii). Evolution of bubbles was also observed during the reduction reaction. Interestingly, with 0.2 mL of the freshly prepared colloidal AuNPs (with 4 mL as final volume of the mixture),

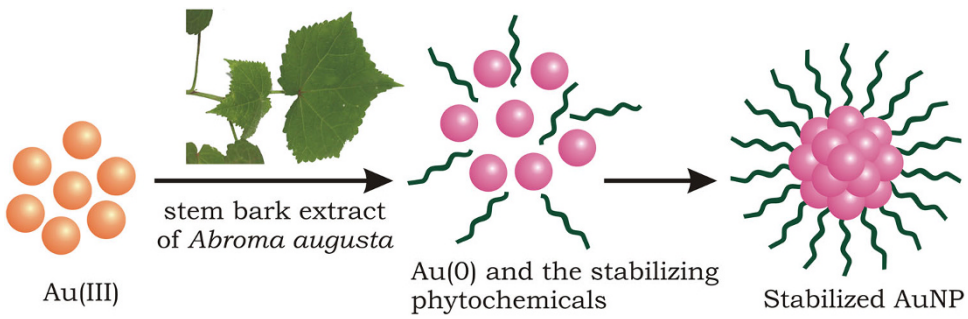

Fig. 4 Mechanism of the formation and stabilization of AuNPs by the phytochemicals present in the bark extract of *Abroma augusta*

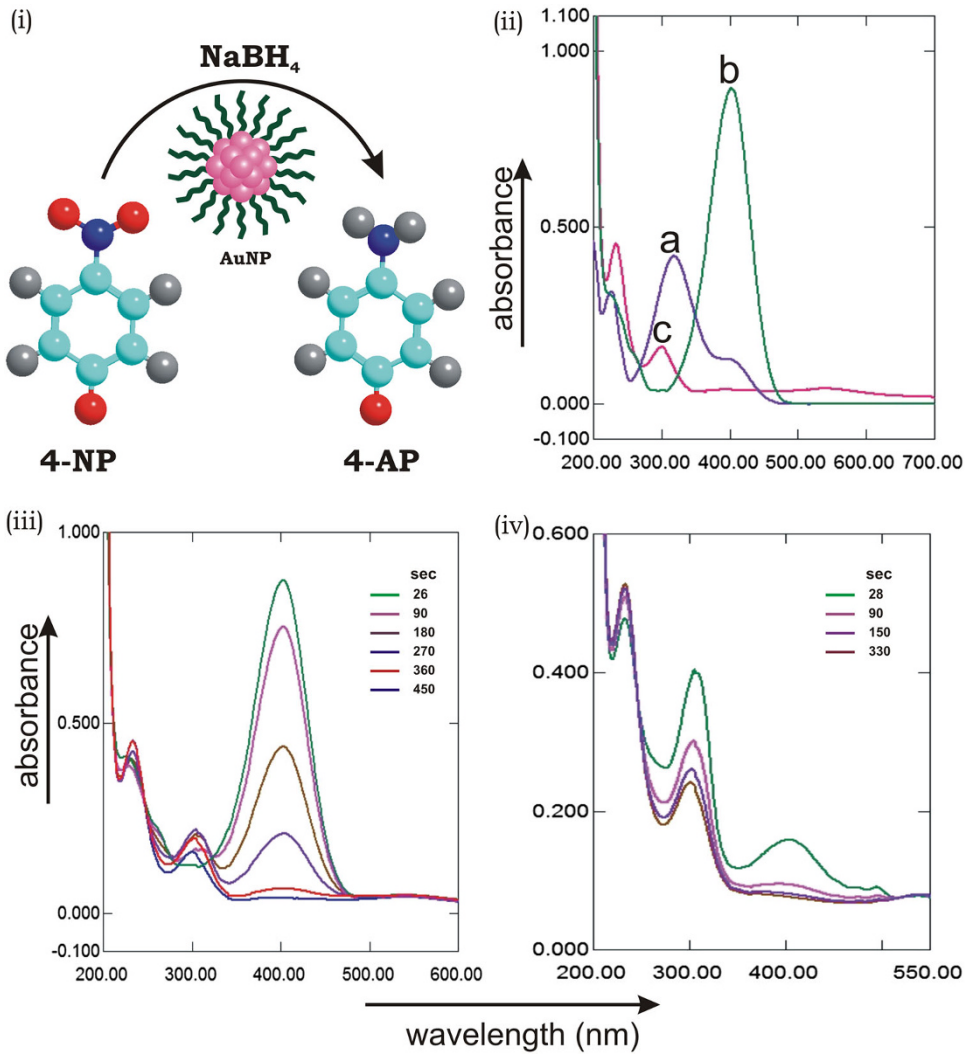

Fig. 5 **i** Schematic representation of $NaBH_4$ reduction of 4-nitrophenol to 4-amino phenol in the presence of *Abroma augusta* L. bark extract-stabilized colloidal AuNPs, **ii** UV–visible spectra of *a* 4-nitrophenol (0.05 mM), *b* 4-nitrophenol in the presence of added sodium borohydride (15 mM), and *c* after complete reduction using colloidal AuNPs as catalyst; **iii**, **iv** UV–visible absorption spectra of the reaction mixtures at various time intervals using 0.1 mL and 0.2 mL of freshly prepared colloidal AuNPs (synthesized with 200 mg L^{-1} of *Abroma augusta* L. bark extract), respectively

Table 1 Study of catalytic activities of stabilized AuNPs (synthesized with 800 mg L^{-1} concentration of *Abroma augusta* bark extract) for the borohydride reduction of 4-nitrophenol (4NP) at 25° C

Serial no.	Conc. of 4NP (mM)	Conc. of sodium borohydride (mM)	Volume of colloidal AuNPs (mL)	Time for completion of reaction (s)	Apparent catalytic rate constant (k) (sec^{-1})
1	0.05	15.0	0.1	450	7×10^{-3}
2	0.05	15.0	0.2	90	Could not be determined
3	0.05	0.0	0.1	No reduction	Not applicable
4	0.05	15.0	0.0	No reduction	Not applicable

the reaction was completed within 1 min and 30 s (Fig. 5iv). With a large excess of sodium borohydride (300 fold), we assumed a pseudo first-order rate constant for the reduction reaction and calculated the apparent rate constant (k) from the UV–visible data. From the plot of lnA vs time, the rate constant value for the reduction reaction was calculated to be 7×10^{-3} s^{-1} (Table TS1 and supporting information Figure S3). This rate constant value was comparable to the recently reported value on a related system (Paul et al. 2014). With 0.1 mL of colloidal AuNPs (synthesized with 200 mg L^{-1} concentration of *Abroma augusta* L. bark extract), the apparent rate constants were calculated to be 7×10^{-3} s^{-1}. Due to very fast reaction, the catalytic rate constant with 0.2 mL colloidal AuNPs could not be measured (Table 1). The increase in effective catalytic rate constant (k_{eff}) with

increasing concentration of colloidal AuNPs is perhaps due to the increased number of active sites for the chemical transformations (Wunder et al. 2011).

Conclusion

The phytochemicals present in the bark extract of *Abroma augusta* L. has been utilized for the green synthesis of colloidal gold nanoparticles in water at room temperature. The phytochemicals present in the plant extract are very efficient to act both as reducing as well as stabilizing agents and gold nanoparticles of 23–33 nm size were obtained without any additional stabilizing or capping agents. Formation of flower-like assembly of AuNPs was obtained at a higher concentration of the bark extract. The presence of antioxidants including polyphenols in the bark extract has been studied against a long-lived radical DPPH. With the evidence for the presence of easily oxidizable phytochemicals including polyphenols, we have proposed a mechanism for the formation of gold nanoparticles. According to our knowledge, this is first report for the synthesis of gold nanoparticles using the bark extract of *Abroma augusta* L. The synthesized colloidal gold nanoparticles have also been utilized as a catalyst for the sodium borohydride reduction of 4-nitrophenol to 4-amino phenol in water at room temperature. Kinetic studies for the reduction reaction at different concentrations of the colloidal gold nanoparticles revealed that the rate of the reduction reaction increases with increasing concentration of gold nanoparticles. As *Abroma augusta* L. bark extract has tremendous medicinal significance, the results reported here will be useful for its application in nanobiotechnology and pharmaceuticals.

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