RESEARCH ARTICLE

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Microwave-irradiated green synthesis of gold nanoparticles for catalytic and anti-bacterial activity

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Abstract

Background: Herein, we report a green, microwave irradiated synthesis of gold nanoparticles by using a simple, low cost and ecofriendly technique. The green technique has been developed for the synthesis of gold nanoparticles (AuNPs) from HAuCl₄ using papaya leaf extract. The papaya leaf extract served both as a reductant and a stabilizer.

Method: The synthesized AuNPs had been characterized by UV-Visible spectrophotometer, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Zeta potential and Transmission electron microscopy (TEM) techniques.

Results: The results indicated that the synthesized nanoparticles were crystalline, stable, well dispersed and spherical in shape and had an average diameter of 15 ± 2 nm. The AuNPs showed excellent catalytic activity for the reduction of p-nitrophenol (4-NP) to p-aminophenol (4-AP) in the presence of NaBH₄. The effects of concentration of NaBH₄, catalyst dose and temperature on the formation of nanoparticles were studied. The activation energy was found to be 28.15 ± 1.14 kJ/mol.

Conclusions: The papaya leaf extract capped AuNPs are highly stable, and have showed effective catalytic activity in the reduction of 4-NP to 4-AP and antimicrobial activity on both gram-positive and gram-negative bacteria.

Keywords: Gold nanoparticles, Papaya leaf extract, Antimicrobial activity, Catalysis

Background

In recent years, nano-sized materials have played a significant role in science and technology, due to their unique size, shape, and potential applications. Metal nanoparticles are unique building blocks with sophisticated electromagnetic, chemical and optical properties that differ dramatically from those of bulk metals (Murray 2008; Sardar et al. 2009). Among several metal nanoparticles, gold nanoparticles have excellent applications in diagnostics, imaging, sensing, catalytic, electrical, magnetic, anti-bacterial activity, and drug delivery (Cuenca et al. 2006; Ulvestad et al. 2015; Ojo et al. 2016; Deng et al. 2014; Reddy et al. 2015a; Dahl et al 2007; Prakash et al. 2013; Madhusudhan et al. 2014; Rodrı´guez-Sa´nchez et al 2000; Lateef et al. 2016).

Nanoparticles have been synthesized physically, chemically, electrochemically, and photochemically (Siavash 2011; Farkas et al. 2010). Among various methods, chemical reduction of Au3+ ions is considered to be the best way to synthesize AuNPs with controlled size and morphology. The conventional chemical reduction methods involve the usage of strong reducing agents like NaBH4, hydrazine etc. However, the usage of such chemical reagents, which are harsh, toxic, expensive, and ecologically injurious, are to be avoided in the synthesis of AuNPs. Hence, the above mentioned methods are non-ecofriendly in nature and have characteristic drawbacks. The replacement of harsh synthesis methods with environmentally friendly, clean, and non-toxic green synthetic methods are very much needed for the synthesis of AuNPs (Punuri et al. 2012; Sadeghi 2015). Several biological systems such as fruit extract, fungi, plants and bacteria can actively reduce metal ions to form metal nanoparticles in environmentally friendly conditions. Recently, green syntheses of gold

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nanoparticles by using *Zizyphus mauritiana* extract, palm oil mill effluent, enzymes, garlic extract, gum kondagogu and acacia nilotica are reported (Gan et al. 2012; WhiteII et al. 2012; Adelere and Lateef 2016; Reddy et al. 2015b; Majumdar et al. 2013).

In this communication, we investigated the use of papaya leaf extract as reducing and stabilizing agent for the synthesis of AuNPs. Papaya belongs to the family Caricaceae and commonly known as Paw Paw, Kates, and Papaw, and is a medicinal plant. The leaves of papaya have been shown to contain many active components such as papain, chymopapain, cystatin, α-tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides, and glucosinolates (Seigler et al. 2002). The papaya bark, leaf, and fruits are being used as medicines to treat several diseases such as amenorrhoea, general debility, constipation, corns, cutaneous tubercles, warts, sinuses, eczema, glandular tumours, blood pressure, diabetes, malaria, expel worms, gonorrhoea, and syphilis (Aravind et al. 2013; Sinhalagoda et al. 2013; Rashed et al. 2013). The previous work suggests that papaya fruit extract acted as an immunostimulant and possess antioxidant properties against acrylamide toxicity in rats. The extracts of papaya fruit and leaf are rich in phenols, proteolytic enzymes, vitamins, which can act as excellent antioxidants (Kadry 2012; Reddy et al. 2015c; Layek et al 2012).

Gold in a bulk form has been observed to be a poor catalyst. But gold in the form of nanoparticles shows excellent catalytic activity towards the deoxygenation of epoxides into alkenes and reduction of ferrocyanate (III) and nitroarenes (Reddy et al. 2015a; Ju and Parales 2010). The 4-NP, along with other nitro phenols and derivatives, is a common by-product in the production of pesticides, synthetic dyes, drug industries and herbicides. Nitrophenols are refractory pollutants and are poisons due to their inhibitory nature and toxicity. In addition, nitrophenols have high stability and solubility in water. These nitrophenols tend to get accumulated in deep soil and stay indefinitely. 4-NP is considered as a diesel exhaust particle and it has an adverse impact on both male and female reproductive functions (Balakumar and Prakash 2016a). 4-NP is a well-known dangerous pollutant it causes damage to the central nervous system, erythema, kidney, liver, scaling, and cracking of the skin and causes headaches, eye irritation, infertility, and drowsiness (Balakumar and Prakash 2016b). Ecological contaminants such as 4-NP can adversely affect child development in various ways resulting in impaired fertility, fetal death, altered fetal growth, and birth defects some of which may not become apparent for years (Balakumar and Prakash 2015). Due to this reason, the reduction of 4-NP into 4-AP is an important one (Gangula et al. 2011). A variety of catalysts were reported in the past and recently, silver nanoparticles, gold nanoparticles and platinum nanoparticles have been used as catalysts for the reduction of 4-NP (Barad and Chakraborty 2014; Martı'nez et al. 2012).

In the present work, we report a rapid, green method for the synthesis of AuNPs using papaya leaf extract as reducing and stabilizing agent. Novelty of the present work lies in the usage of Papaya leaf extract, which is a cheap, readily available, and renewable resource and the use of microwave irradiation which results in the formation of AuNPs in less than 2 min of time. As synthesized AuNPs have been characterized by UV-Vis, FTIR, XRD, TEM. AuNPs were explored for their prospective catalytic and antibacterial applications.

Materials

Chloroauric acid was purchased from Sigma-Aldrich, sodium borohydride, nitric acid, hydrochloric acid, and p-nitrophenol; all reagents were purchased from S-D Fine chemicals and used without further purification.

Methods

Preparation of the plant extract

The fresh papaya leaves were collected from the papaya plant and were cut into fine pieces and dried under shade. After drying, it was powdered and used for further studies. One gram of leaf powder was taken in 100 ml Erlenmeyer flask containing double distilled water and heated at 55 °C for 30 min to facilitate the formation of aqueous extract. The obtained extract was filtered with Whatman No.1 filter paper and the filtrate was stored for further investigation.

Synthesis of gold nanoparticles

All glassware, used in the synthesis of AuNPs were washed with aquaregia (3:1HCl-HNO₃) and then thoroughly rinsed with double distilled water. All the solutions were prepared in double distilled water. Papaya leaf extract (4 ml) was mixed with 1.5 ml of chloroauric acid solution in a boiling tube and the mixture was subjected to microwave irradiation for 60–90 s at 750 W power. The resulting solution was of blushing red color, indicating the formation of gold nano particles (Chao et al. 2013). After formation, the AuNPs were centrifuged and dispersed in Milli-Q water. The centrifugation and dispersion process was repeated twice to remove any unreacted HAuCl₄ and leaf extract from the final product. These purified AuNPs were then used for further characterization.

Characterization

The spectra of AuNPs was recorded by UV-Vis (Shimadzu, Japan). Fourier transform infrared spectra of native papaya leaf extract and papaya leaf extract capped AuNPs was recorded with an instrument IR Affinity-1

(Shimadzu) in the scanning range of 500–4000 cm⁻¹. The morphology and size distribution of the papaya leaf extract capped AuNPs was investigated by transmission electron microscopy, by casting the nanoparticle dispersion on carbon-coated copper grids and allowed to dry at room temperature. Measurements were made on TECHNAI G2 F30 S-TWIN instrument. The crystallinity of the AuNPs was studied by X-ray diffraction equipment (Rigaku, MiniFlex) using Cu kα radiation.

Catalytic reduction of p-nitrophenol

As a model reaction, we selected the reduction of 4-NP to 4-AP by NaBH₄. The reduction was followed with the aqueous solution in a standard quartz cell having a 1-cm path length. The reaction process was as follows: 1.5 of 0.15 mM 4-NP was mixed with 1.0 mL of 0.02 M NaBH₄ in the cell for UV-Vis measurements. Immediately, the colour change was observed from light yellow to deep yellow. 0.5 mL of AuNPs solution was added to the above mixture. The UV-vis spectra were recorded with a time interval of 1 min in a scanning range of 200–600 nm at ambient temperature (25 \pm 2 °C). The effects of varying the temperature, amount of catalyst and concentration of NaBH₄ were carried out for the conversion of 4-NP to 4-AP.

Antimicrobial activity

The agar disc diffusion method was employed for the determination of antimicrobial activity of the papaya leaf extract stabilized gold nanoparticles. 0.1 ml of 10^8 cfu/ml of different pathogenic bacterial suspension was spread on different plates nourished with LB media. Filter paper discs (5 mm in diameter) were placed on the plates and synthesized AuNPs and papaya leaf extract solutions were impregnated in different concentrations then onto the discs. Ampicillin ($10~\mu g/ml$ concentration), an antibiotic served as the standard for measuring the antibacterial activity. The plates were then incubated at $37~^{\circ}$ C for 24~h and the zones of inhibition were measured (Majumdar et al. 2013).

Results and discussion

UV-visible spectroscopy

UV-visible spectroscopy is one of the most widely used techniques for determining the formation, stability and size of AuNPs in aqueous colloidal solution (Saha et al. 2010). The formation of AuNPs was indicated with a change of colour from yellow to blushing red. The colour change is attributed to the surface plasmon resonance (SPR). A characteristic SPR band for AuNPs is obtained around 520–530 nm (Praveen et al. 2011). Figure 1 shows the role of papaya leaf extract concentration on the formation of AuNPs, studied by microwaving the different concentrations (0.1 to 1%) of papaya leaf extract containing

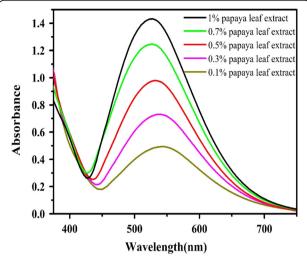


Fig. 1 The UV-vis absorption spectra of AuNPs synthesized by different concentration of papaya leaf extract solution at 1mM $HAuCl_4$ concentration

1 mM of HAuCl $_4$ for 2 min of time. With an increase in the concentration of papaya leaf extract, there is an enhancement in the concentration of AuNPs (Reddy et al. 2015b). Figure 2 shows the synthesis of nanoparticles by changing the concentration of HAuCl $_4$ and the reduction was studied with 1% papaya leaf extract. It reveals that the efficiency of nanoparticle synthesis increases with increasing the concentration of HAuCl $_4$.

FTIR

FTIR measurements were carried out to determine the possible functional groups in papaya leaf extract involved in the reduction and capping of synthesized gold

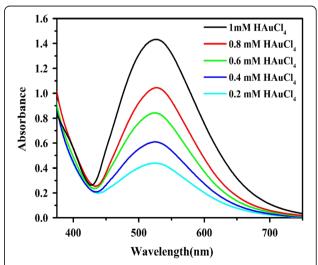


Fig. 2 The UV-vis absorption spectra of AuNPs synthesized by using different concentrations of HAuCl₄ and 1% papaya leaf extract solution

nano particles. Figure 3 shows the FTIR spectra of the papaya leaf extract and papaya leaf extract capped AuNPs. The major absorbance bonds present in the spectrum of papaya leaf extract were at 3245, 2862, 1810, 1679, 1491, 1354,1106, and 790 cm⁻¹. The broad band observed at 3245 cm⁻¹ could be assigned to stretching vibrations of -OH and -NH2 groups in the papaya leaf extract. The peak at 2862 cm⁻¹ is due to asymmetric stretching vibrations of methylene groups. The peak at 1679 cm^{-1} is due to the C = O stretching vibration of the carbonyl and carboxylic groups present in the amide linkage of the proteins. The symmetrical stretch of carboxylate group can be attributed to the bands present at 1491 and 1354 cm⁻¹. The bands at 1105 and 789 cm⁻¹ were due to the C-O stretching vibrations of polyols, ether and alcoholic groups, and bending vibration of C-N.

Figure 3 curve b shows the papaya leaf extract capped AuNPs characteristic absorbance bands at 3416, 2915, 1778, 1716, 1560, 1410, 1112, and 787 cm⁻¹. After formation of AuNPs, shifts in absorption peaks were observed from 3245 to 3416 cm⁻¹, 1810 to 1778 cm⁻¹, 1679 to 1716 cm⁻¹, 1491 to 1560 cm⁻¹ and 1354 to 1410 cm⁻¹. After the encapsulation of gold nanoparticles, the peaks become weaker and are shifted, suggesting that the carbonyl groups of proteins and hydroxyl groups of alcohol and phenolics may be involving in the synthesis and stabilizing of AuNPs (Balakumar and Prakash 2015).

As discussed above, the components of papaya leaf extract contain a large number of hydroxyl and carboxylic groups which facilitate the complexation of Au3+ ions. Subsequently, these gold ions oxidize the hydroxyl groups to carbonyl groups, during which the gold ions are reduced to elemental gold. Further, these nanoparticles are

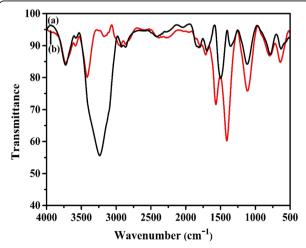


Fig. 3 FTIR spectra of (a) papaya leaf extract and (b) papaya leaf extract capped AuNPs

probably capped and stabilized by the polyphenolic compounds and proteins present in the leaf extract.

XRD

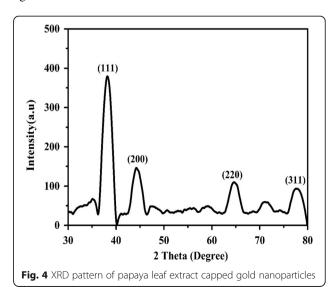
The XRD analysis was used to determine the crystalline structure of synthesized AuNPs. For XRD analysis, the prepared sample was lyophilized and precipitated under vacuum then used for XRD analysis. In Fig. 4, intense diffraction peaks were observed at 2θ values of 38.24°, 44.30°, 64.67°, and 77.66°, which were indexed to the (111), (200), (220), (311) reflections of crystalline metallic gold respectively. These values are similar to that given by Joint Committee on Powder Diffraction Standards (JCPDS no. 04-0784) revealing that synthesized AuNPs are of pure crystalline gold (Reddy et al. 2015b). The XRD pattern thus clearly shows that the AuNPs were crystalline in nature.

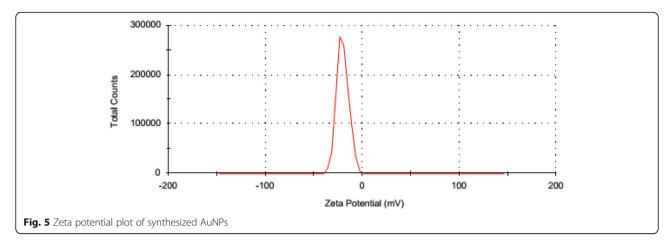
Zeta potential

Zeta potential values reveal details about the stability and surface charge of the synthesized AuNPs. Particles with highly negative or positive surface charge are considered to be stable. An absolute zeta potential value of \pm 30 mV is a general indication that the colloidal solution highly stable. Zeta potential (Fig. 5) of papaya leaf extract capped AuNPs is found to be -22.5 mV, which clear indicates that the nanoparticles are negatively charged and highly stable.

TEM

TEM analysis was done to examine the particle shape and particle size of the green synthesized AuNPs using papaya leaf extract. Figure 6a shows that the size of AuNPs ranges from 7 to 26 nm. TEM analysis suggests that the synthesized papaya leaf extract capped AuNPs were spherical in shape and the average size of the AuNPs was 15 ± 2 . The selected area





electron diffraction (Fig. 6b) pattern exhibited concentric rings with intermittent bright dots, indicating that these nano particles are highly crystalline nature. Histogram (Fig. 7) obtained by considering 120 nano particles suggests that there is a wide distribution of sizes from 11–18 nm.

Catalytic activity

The catalytic activity of AuNPs stabilized in papaya leaf extract was evaluated for the reduction of toxic pollutant 4-NP in the presence of excess NaBH₄. The catalytic reduction of 4-NP was studied in aqueous solution in a standard quartz cell with a 1-cm path length. The products and substrates of this reaction were easily monitored by UV-Vis spectroscopy. In a typical run, 1 ml of 0.02 M NaBH₄ was mixed with 1.7 ml of 0.15 mM 4-NP in a quartz cell. 4-NP solution is in light yellow color and exhibited a strong absorption peak at 317 nm. Upon the addition of NaBH₄ to the 4-NP the color changed to deep yellow color and a strong absorption peak at

400 nm (Reddy et al. 2015c), due to the formation of pnitrophenolate ion (Fig. 8) was observed.

Through the reduction of 4-NP to 4-AP using NaBH₄ is thermodynamically favorable (E° for $H_3BO_3/BH_4^- = -1.33$ V and 4-NP/4-AP = -0.76 V versus NHE). The presence of the kinetic barrier due to the large potential difference between donor and acceptor molecule decrease the feasibility of this reaction (Saha et al. 2010). The reaction was carried out at room temperature (30 °C) for 1–3 h without the addition of AuNPs. It was observed that there was no change in the peak position at 400 nm, which shows that no reaction could occur without the catalyst. This indicates that NaBH₄ itself was not able to reduce of 4-NP.

There is a fall in the intensity of the peak at 400 nm of the p-Nitrophenolate ion on the addition of AuNPs, although, there is a concomitant appearance of a new peak at 297 nm indicating the formation of product 4-AP (Fig. 8). The reduction reaction was complete within 16 min. The reaction follows pseudo-first order kinetics with respect to 4-NP (Balakumar and Prakash 2015).

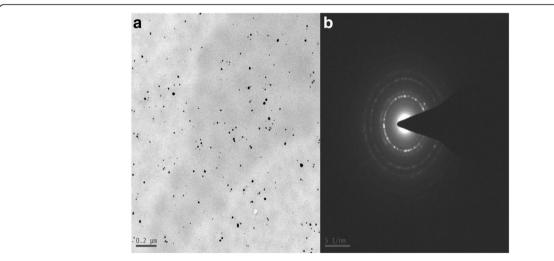


Fig. 6 (a) TEM image (b) corresponding SAED pattern of papaya leaf extract capped gold nanoparticles

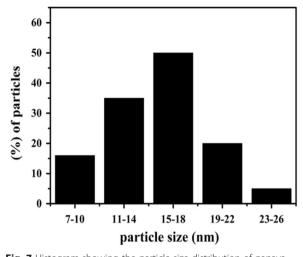


Fig. 7 Histogram showing the particle size distribution of papaya leaf extract capped gold nanoparticles

The concentration NaBH₄ was much higher compared to 4-NP. The rate constant of the reaction was independent of NaBH₄ concentration. The rate constant (k) was (Fig. 9) determined from the linear plot of ln (A_t/A_0) versus reduction time.

The effect of NaBH $_4$ concentration on the reduction of 4-NP was studied to select the optimum concentration of NaBH $_4$ required. As depicted in Fig. 10, in the case of 4-NP reduction using AuNPs catalyst, it was found that in the concentration range of 0.01–0.025 M of borohydride, the rate increased with the increase in concentration, whereas in the range of 0.15–0.25 M, it remained constant. Thus, the borohydride concentration selected for the entire range was 0.015 M, which was a large excess

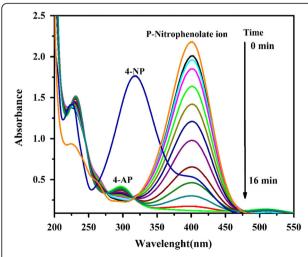
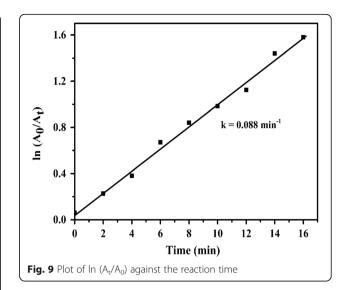


Fig. 8 Successive UV-vis absorption spectra of the reduction of 4-NP by NaBH₄ in the presence of papaya leaf extract capped gold nanoparticle as catalyst



compared to that of 4-NP, to make the reaction independent (zero-order) of borohydride concentration.

The effect of the amount of AuNPs on the reduction of 4-NP was studied using AuNPs from 50–300 μ L keeping the other parameters such as temperature, concentration of NaBH4 and concentration of 4-NP constant. The rate constant for 50, 100, 150, 200, 250 and, 300 μ L catalysts are 0.073, 0.124, 0.194, 0.224, and 0.310 min⁻¹, respectively. The rate constant values are plotted against varying amounts of catalyst and are shown in Fig. 11. As anticipated, the rate constant has increased with an increase in the amount of AuNPs which is evident from Fig. 11, due to an increase in the number of reaction sites.

The effect of temperature was studied for the reduction of 4-NP to 4-AP. The activation energy (E_a) of the reaction was determined from the Arrhenius equation $k = Ae^{-Ea/RT}$, where A is a constant and R is the

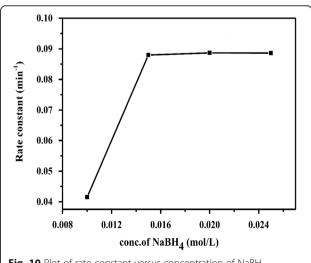
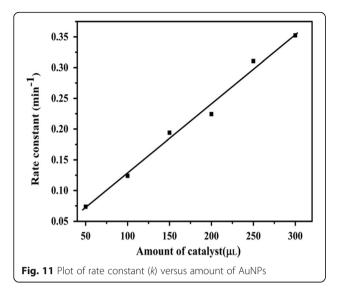


Fig. 10 Plot of rate constant versus concentration of NaBH₄



universal gas constant, k is rate constant of the reaction at temperature T (in kelvin). The reduction of 4-NP was studied at five different temperatures (30, 35, 45, 55, and 65 °C) using AuNPs as the catalyst. The rate constant of the reaction was calculated at five different temperatures. A plot of ln k versus 1/T, shown in Fig. 12, is linear for 4-NP reduction using AuNPs. It was noticed that the increase in temperature helps the rate of the reaction to increase. The catalytic studies were performed using the AuNPs, which exhibit higher catalytic performance compared to other earlier reported results (Reddy et al. 2015a; Tao et al. 2017). The activation energy was calculated from the slope (-Ea/R) of the straight line and was found to be 28.15 ± 1.14 kJ/mol.

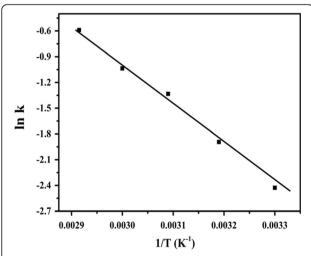


Fig. 12 Plot of rate constant against 1/T for the reduction of 4-NP by NaBH₄ in the presence of AuNPs as catalyst at different temperatures

Stability and reusability of the catalyst

Stability and reusability are important characteristics of a good catalyst. To investigate the reusability of papaya leaf extract capped AuNPs catalyst, recycling experiments were performed with the same catalyst. In this experiment, after completion of every reduction cycle, the catalyst was collected by centrifugation, dried and used for the next cycle of reduction. As shown in Fig. 13, the catalyst exhibited high activity even after four cycles of reaction. These results imply that the components of papaya leaf extract are effective in stabilizing the AuNPs during the catalytic reactions by preventing them from aggregation (Balakumar and Prakash 2015). Therefore, the papaya leaf extract capped AuNPs can serve as an effective catalyst.

Anti-bacterial activity

The papaya leaf extract capped AuNPs has significant antibacterial activity against Gram-negative Pseudomonas putida and Gram-positive Staphylococcus aureus. It revealed that as the concentration of AuNPs was increased, bacterial growth decreased in both the cases. The antimicrobial efficiency of AuNPs increases because of their larger total surface area per unit volume (Lokina and Narayanan 2013). The zone of inhibition of AuNPs against P. putida and S. aureus bacteria is shown in Fig. 14a, b. The results suggested that AuNPs synthesized from papaya leaf extract showed effective antibacterial activity in gram positive than in Gram-negative bacteria. A small zone of inhibition was observed for the papaya leaf extract alone. Antibacterial activities of AuNPs have been reported but their exact mechanisms of action are poorly understood. Rai et al. (2010) suggested AuNPs make holes in the cell wall, resulting in the fact that leakage of cell contents leads to death, and in another way it can bind to the DNA of bacteria and

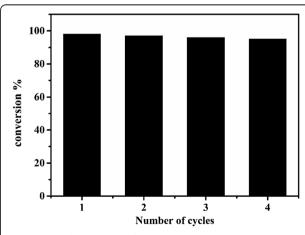


Fig. 13 Plot of percentage of conversion (4-NP to 4-AP) vs Number of cycles

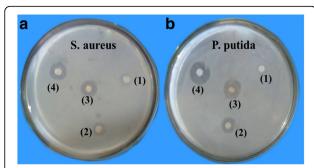


Fig. 14 Antibacterial activity of papaya leaf extract capped AuNPs using (a) *Staphylococcus aureus* (b) *Pseudomonas putida* (1 mg/ml solution: discs containing $1 = 10 \mu l$ of papaya leaf extract solution, $2 = 10 \mu l$ of AuNPs, $3 = 20 \mu l$ of AuNPs, $4 = 10 \mu l$ of ampicillin)

inhibit the DNA transcription. Because AuNPs closely bind to the surface of the microorganism causing visible damage to the cells, it can minimize the treatment duration and side effects of drugs (Zawrah et al. 2011). It has been also reported that gold nanoparticles can penetrate and can disrupt the membranes of bacteria. These AuNPs decreased the ATP levels. Moreover, the phospholipid portion of the bacterial membrane may also be the site of action for the AuNPs (Eom et al. 2012). Based on these results, it can be concluded that the green synthesized AuNPs had significant antibacterial action on both bacteria.

Conclusions

The papaya leaf extract is an effective source for the green synthesis of AuNPs. The papaya leaf extract acts both as a reducing and as a capping agent. The green synthesized AuNPs were characterized by UV-vis, FTIR, XRD, and TEM techniques. The XRD pattern revealed that the papaya leaf extract capped AuNPs were crystalline. FTIR suggest that both carbonyl groups of papaya leaf extract are involved in the synthesis and capping of AuNPs. TEM showed that the size and morphology of synthesized AuNPs were spherical in shape, with the average size distribution of 15 ± 2 nm. The catalytic activity of papaya leaf extract capped AuNPs was examined by the reduction of 4-NP. The detailed kinetic aspects for catalytic reduction of 4-NP were evaluated by changing parameters. It was observed that the concentration of NaBH4 does not affect much the reduction process. It was noticed that with an increasing in the amount of AuNPs and temperature, the total reaction time decreases and the rate of the reaction increases. The papaya leaf extract capped AuNPs showed effective antibacterial activity on both P. putida and S.aureus.

Abbreviations

4-AP: p-aminophenol; 4-NP: p-nitrophenol; AuNPs: Gold nanoparticles; cm: Centimeter; FTIR: Fourier transform infrared; g: Gram; GT: Gum tragacanth; min: Minute; mL: Milliliter; nm: Nanometer; rpm: Revolutions per

minute; TEM: Transmission electron microscopy; w/v: Weight/volume; XRD: x-ray diffraction

Acknowledgements

The authors are thankful to Centre for nanotechnology, University of Hyderabad, for providing the TEM facility and Department of Chemistry, Osmania University, for providing infrastructure.

Authors' contributions

SS, BRG, and RKD synthesized the AuNPs. RKB collected papaya leaf and prepared the aqueous leaf extract. DRK characterized the AuNPs using UV-Vis spectroscopy. GBR characterized the AuNPs using X-ray diffraction and TEM. MA conducted the catalytic studies on AuNPs. GVB and SS contributed in analyzing the data. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Received: 7 January 2017 Accepted: 26 April 2017 Published online: 03 June 2017

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