

Synthesis of gold nanoparticles by various leaf fractions of *Semecarpus anacardium* L. tree

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Received: 15 April 2010/Revised: 18 August 2010/Accepted: 8 September 2010/Published online: 24 September 2010
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Abstract Gold nanoparticles (NPs) were synthesized using *Semecarpus anacardium* leaf extracts in water and the green biomass. Extract prepared at ambient condition by crushing the leaves in deionized water is identified as ‘green extract’, and that by boiling the leaf pieces as ‘boiled extract’. The mass remaining after separating the ‘green extract’ is identified as ‘green biomass’. These components triggered rapid reduction of Au(III) to Au (0) in H₂AuCl₄ solution indicating the natural ability of the leaves of *S. anacardium* to synthesize NPs in ambient conditions. Green extract produced more NPs compared to the boiled extract suggesting denaturation of some of the useful factors due to boiling. NPs were quantified using UV and ICP-AES analysis. These were characterized using Transmission electron microscopy, Fourier transform infrared spectroscopy and X-ray diffraction. TEM images of the particles formed with green extract, boiled extract and green biomass showed that the particles were of different shapes and sizes.

Keywords Gold nanoparticles · *Semecarpus* · Biosynthesis · TEM · Green extract

Introduction

The field of nanotechnology is one of the upcoming areas of modern science. Nanoparticles find various applications

in drug formulations and therapeutic medicine (Kumari et al. 2010a, b). Utilization of biological system has emerged as a novel route for the synthesis of nanoparticles (NPs) and nano wires. The advantages of using plant materials for the synthesis of nano materials against the use of microorganisms are emphasized (Shankar et al. 2004a). Microorganisms need maintenance of cultures and controlled conditions such as temperature, pH and other factors for growth. Plant extracts are continuously explored for the production of metal NPs (Shankar et al. 2003; Li et al. 2007). Use of freshly prepared untreated extracts, parts or whole plants in NPs synthesis is under-explored (Ankamwar et al. 2005). *Alfalfa* seedlings germinated on gold rich medium reduces the ionic form of gold and silver into NPs in leaf, stem and roots (Gardea-Torresdey et al. 2002, 2003). However, reports on synthesis of NPs by tissues of tree species are rather scarce. Production of NPs using tree tissues has additional advantages. The trees can serve as the perennial source of material for production of NPs from metal rich soil thereby supporting the concept of nanoparticles farming (Gardea-Torresdey et al. 2002, 2003). Although there are a few reports (Shankar et al. 2004a) on production of NPs using boiled extract of leaves from tree species (*Azadirachta indica*), there is no report on the synthesis of gold NPs using water extracts of green leaves or green leaf-derived biomass of trees growing in a natural environment. In this report we demonstrate the natural ability of a tree species for rapid synthesis of gold NPs using water extracts of *Semecarpus anacardium* leaves and leaf derived biomass to synthesize gold NPs from H₂AuCl₄. Both *Alfalfa* (Gardea-Torresdey et al. 2002) and *Brassica juncea* (Marshall et al. 2007) plants, in which synthesis of NPs have been demonstrated, are herbaceous species and are annual crops. Similar work is not done in tree species to demonstrate intracellular NP synthesis. There are few

Communicated by L. Gratani.

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reports on tree species *Boswellia ovalifoliolata* (Ankanna et al. 2010), *Jatropha curcas* L. (Rajasekharreddy et al. 2010), *Azadirachta indica* (Shankar et al. 2004a), describing synthesis of NPs using boiled extracts of bark and leaves. In *Syzygium cumini*, extracts of dry powders of leaf and seeds were used for the synthesis of silver NPs (Kumar et al. 2010). Some microorganisms (Shankar et al. 2003) have been identified for their ability to synthesize NPs isolated from plants. There are reports (Shankar et al. 2003, 2004a, b; Chandran et al. 2006; Song and Kim 2009; Song et al. 2009) describing methods for production of NPs using boiled leaf extracts of *Geranium*, *Aloe vera*, *Pine*, *Persimmon*, *Ginkgo*, *Mangnolia*, *Platanus*, *Magnolia kobus* and *Diopyros kaki*. The *Geranium* and *Aloe vera* leaves were boiled and used for reduction of NPs. The leaves of other plants were dried at room temperature, and then the leaves were boiled and used for the reduction of NPs. In the present investigation the boiled leaf extract of *S. anacardium* was tested as a control.

Materials and methods

Preparation of water extracts and biomass

Intact leaves of *Semecarpus* were washed thoroughly with distilled water for few minutes and the water adhering to the tissue was air dried. The loss of biomolecules and soluble components was avoided using the intact leaves (with petioles). Thirty grams of leaves was weighed and homogenized in 100 ml of sterile deionized water and transferred to centrifuge tubes. These were centrifuged at 10,000 rpm for 15 min. The supernatant and the pellet were separated. Hereafter the liquid fraction is identified as “green extract” and the solid fraction as “green biomass”. Boiled extract of *Semecarpus* leaves was prepared by cutting 30 g of leaves into small pieces and boiling in 100 ml of sterile deionized water for 30 min. This extract was used as a control. The solution was cooled and filtered using filter paper. The extract prepared by boiling the leaves is identified as “boiled extract”. Boiled leaf extracts have been used for the synthesis of nanoparticles by other researchers (Chandran et al. 2006). The green extract, green biomass and boiled extract were used for the reduction experiments. The pH of the green extract was 5.37 whereas the boiled extract was 5.35. Both the liquid extracts were straw colored and the green extract was darker than the boiled extract.

In four graduated test tubes, 5 ml of 10^{-4} M solution of HAuCl_4 was taken. In two of these tubes 200 μl of green extract or boiled extract was added. In the third tube 500 mg of green mass was added. The volumes were made to 10 ml with 10^{-4} M HAuCl_4 solution.

After 4 h the mixture was filtered using Whatman filter paper and the filtrate was used for the detection, quantification and characterization of the NPs. In the fourth tube, the solution of 10^{-4} M HAuCl_4 , was taken as control.

The yield of particle synthesis was determined by UV and inductive coupled plasma atomic emission spectroscopy (ICP-AES). The particles were characterized using transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDAX), and X-ray diffraction (XRD). Distribution of the particles of various sizes was determined using the Gatan software. An attempt was made to identify the critical factors responsible for the reduction of Au using Fourier transform infrared spectroscopy (FT-IR) and the crystalline nature was verified both by XRD and TEM.

UV analysis

The reaction mixtures incubated at room temperature were scanned at 0 h from 300 to 800 nm wave lengths with UV Spectrophotometer (Perkin Elmer) using a dual beam operated at 1 nm resolution. The reaction mixtures were scanned under the same wavelengths after 1 and 4 h.

Quantification of gold by inductive coupled plasma spectrometry (ICP-AES)

Gold nanoparticles were separated from unreacted ions by centrifuging 3 ml each of green extract, boiled extract and biomass nanoparticle solutions at 15,000 rpm for 30 min. These separated gold nanoparticles were redispersed in 20 ml of deionized water, subsequently digested with 6 ml of aqua regia (3:1 v/v concentrated HCl and concentrated HNO_3) and the volume was made to 30 ml as reported by Orendorff and Murphy (2006). Gold nanoparticles in each reaction mixture were quantified using ICP-AES Spectro Arcos at 267.59 nm.

Transmission electron microscopy (TEM)

The images of Au NPs were recorded with TEM for determination of the shape, and size of the particles and diffraction. Two to three drops of the sample solutions and the control were placed individually on carbon coated copper grids and allowed the moisture to evaporate. TEM measurements were performed on JOEL model 1200EX instrument operated at an accelerating voltage of 120 kV. In green extract and in boiled extract 100 particles each and in biomass 73 particles were subjected to measurements of the sizes of the particles. Average particle sizes and distribution were determined using Gatan software.

Scanning electron microscopy (SEM)

One ml of Au NPs of green extract, green biomass and boiled extract were coated on the stub by placing small drops of nanoparticle suspension every time and eliminating the moisture before adding another drop. The NPs were analyzed with SEM, attached with phoenix EDAX to identify the elemental composition.

X-ray diffraction analysis (XRD)

The particles were isolated by centrifuging 20 ml of the suspension in deionized water containing Au NPs for 20 min at 10,000 rpm. These were washed three times to remove the unbound proteins and other metabolites. The pellet of NPs was re-suspended in deionized water and coated on a glass plate. This was dried in the oven at 50°C for removing water content and used for XRD studies.

Fourier transform infrared analysis spectroscopy (FT-IR)

FT-IR measurements of the gold particles were carried out. The suspension was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and the pellet was washed with deionized water to remove the excess amount of

reducing agents and other compounds which were not bound to NPs. The pellet was dispensed in HPLC grade chloroform and the particles coated on NaCl cell substrate was scanned on Perkin-Elmer FT-IR spectrum in transmittance mode at a resolution of 2 cm^{-1} .

Results and discussion

The UV scan at 0 h reaction did not show peak formation at 530–550 nm in the reaction mixtures containing green extract, boil extract or green biomass (Fig. 1a). After a period of 15–20 min of the reaction at room temperature, there was a change in color of the reaction mixture to pink due to the reduction of HAuCl_4 to metallic Au (0) NPs (Fig. 2). There was no change in the color of the control mixture without extracts or green biomass. After 1 h these solutions were subjected to UV-vis spectroscopy scanning from 300 to 800 nm wavelength. The maximum intensity of the peaks for all leaf derived samples was noted at 530–550 nm (Fig. 1b, c) indicating the presence of gold particles (Henglein 1993). The peak intensities were different for each leaf derived sample. Most intense peak was noted in the mixture with green extract. This was followed by the boiled extract and the green biomass. Peak with higher intensity suggest increased gold NP concentration

Fig. 1 UV-vis spectra in ranges from 300 to 800 nm of 10^{-4} M HAuCl_4 solution with, 1 green extract, 2 boiled extract and 3 green mass. **a** 0 h reaction, **b** 1 h reaction, **c** 4 h reaction

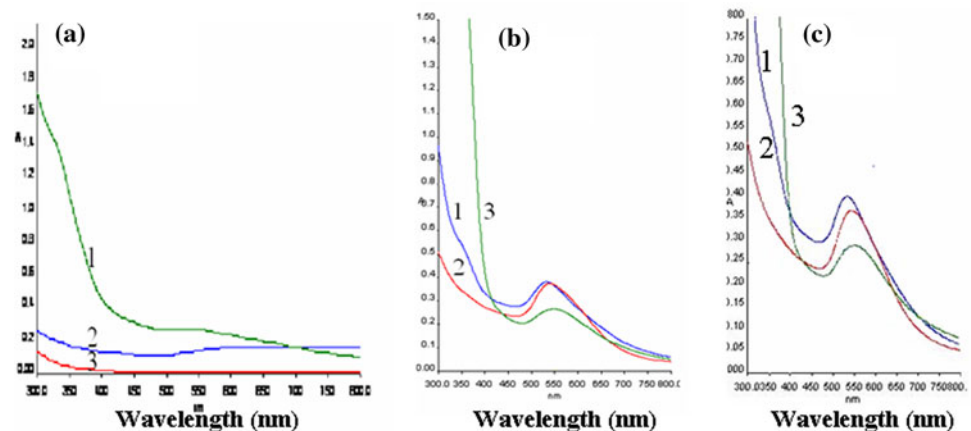


Fig. 2 Appearances of the green extract, boiled extract and green biomass used for reduction of 10^{-4} M HAuCl_4 and the change in color after the reaction and formation of gold NPs. No change in color in control with 10^{-4} M HAuCl_4



(Wang et al. 2007). NPs synthesized were quantified using ICP-AES Spectro Arcos at 267.59 nm. The amount of GNPs in green extract, boiled extract and biomass were 20.82, 17.99, and 8.29 ppm, respectively. The concentration was showing similar pattern as noted in UV spectroscopy. It is not clear if the increased response in the presence of the green extract could be due to the existence of active proteins and other bioactive molecules as the extract was prepared at room temperature. Due to the mutilation of the cells the proteins and other active biomolecules leached in the extract. Presumably these proteins or other molecules enhanced the reduction of HAuCl_4 . Production of NPs due to the presence of proteins was reported earlier (Shankar et al. 2003; Song et al. 2009; Song and Kim 2009). Alternately, the increased production of NPs by the unboiled extracts could be due to the presence of co-existing microorganisms in the leaves which enhanced the process of reduction and the yield of NPs. The microorganisms harbor the plants in the natural stands and there are reports on production of NPs by microorganisms (Shankar et al. 2003). Boiling of the extract may destroy the microorganisms or denaturize the proteins and other biomolecules causing reduced reduction of HAuCl_4 . The kinetics was studied to determine if the reaction has stopped or continued. The reaction mixtures were scanned with UV spectrophotometer at 0, 1 and 4 h. There was an increase in the intensity of peaks in UV absorbance (Fig. 1b, c), with the period of the reaction. With the extension of the reaction period the intensity of the peaks increased in all three reaction mixtures with *S. anacardium* leaf derived components suggesting the increase in gold NP concentration. The optimum reduction of HAuCl_4 in the presence of untreated water extract followed by boiled extract and minimum reduction by the untreated biomass indicates that (1) the factors responsible for the production

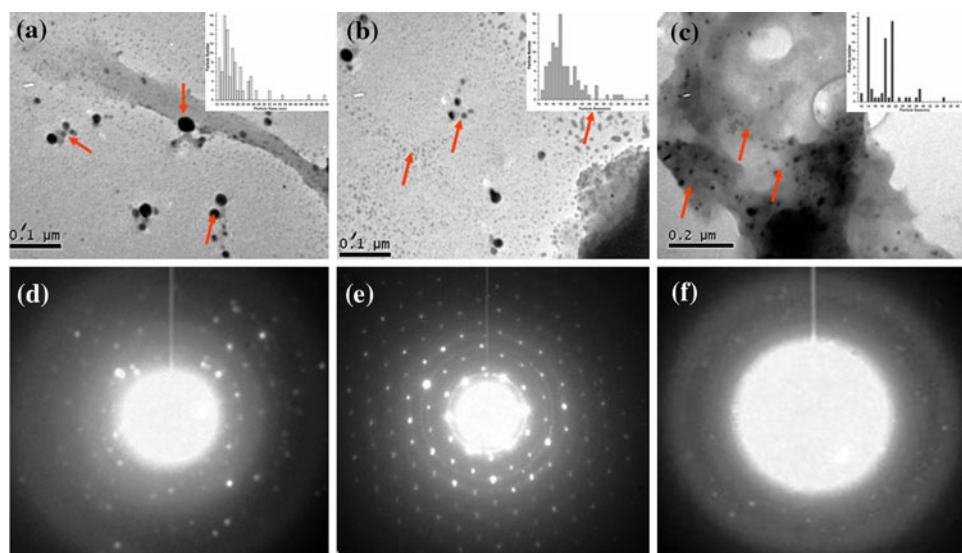
of NPs by the *S. anacardium* leaves have possibly moved out in the soluble fraction and (2) some of these factors are heat labile.

TEM images of particles formed with green extract, boiled extract and green biomass showed that the particles were of different shapes and sizes according to the results of surface plasma absorption. The particle sizes ranged from 13 to 55 nm (Fig. 3a–c) and were polydispersed. In green extract more number of particles were of 15 and 16 nm. In boiled extract more number of particles ranged between 16 and 18 nm. In biomass, 14 and 21 nm size particles were more (inserts in Fig. 3a–c). The average diameter of nanoparticles in green extract was 18.63 nm, in boiled extract 19.22 nm and in biomass was 18.12 nm. The particles of green extract, boiled extract and green biomass were in crystalline form as shown in diffraction (Fig. 3d–f) and XRD (Fig. 6a–c).

The EDAX analysis of the samples (Fig. 4a–c) shows a strong peak of Au in all the three reactions. Other peaks of Cu, Zn were from the stub used for EDAX analysis. The stubs were made up of the alloys of the metals. The weaker carbon peaks were possibly due to the biomolecules that are bound to the particles (Chandran et al. 2006). The intensity of Au NPs peaks was stronger in green extract, followed by boiled extract and green biomass. The trend was similar to the pattern noted in UV spectroscopy where equal volume of NPs solution was used for EDAX study.

The FT-IR spectra (Fig. 5a, 1, 2), show peaks at 771, 1,027, 1,377, 1,628 and 2,919 cm^{-1} . The peaks 1,027 and 1,377 cm^{-1} could be due to the bending vibration of C–O–C groups and the antisymmetric stretching bands of COH groups of polysaccharides or chlorophylls (Li et al. 2007). The peak at 1,628 cm^{-1} could be due to the amide-I protein. Similar peak at 1,658 cm^{-1} identified for amide-I protein was previously reported (Shankar et al. 2003).

Fig. 3 TEM images of gold NPs synthesized by reducing 10^{-4} M HAuCl_4 ions using **a** green extract, **b** boiled extract, and **c** green biomass of *Semecarpus* leaf and diffractions (**d**, **e**, **f**) of corresponding NPs. *Insert images* show the particles size distribution



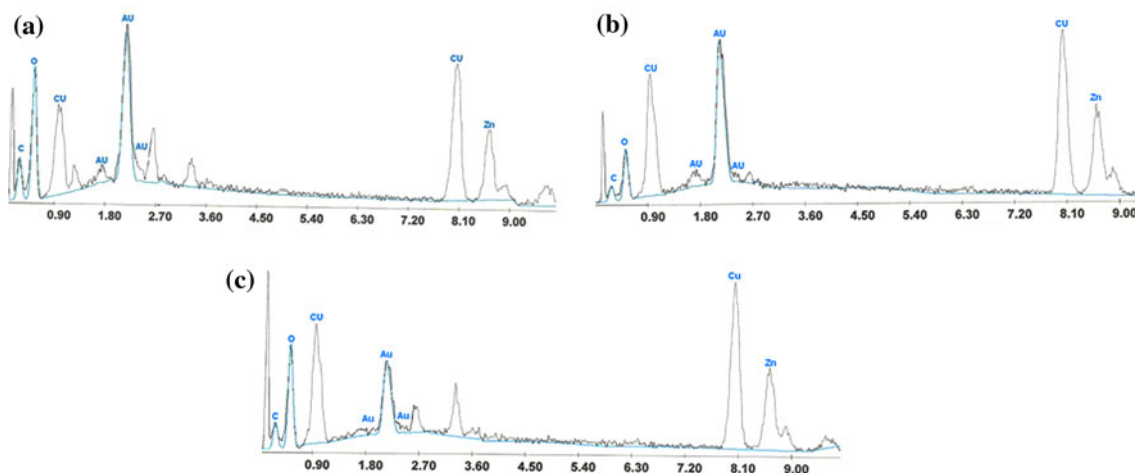
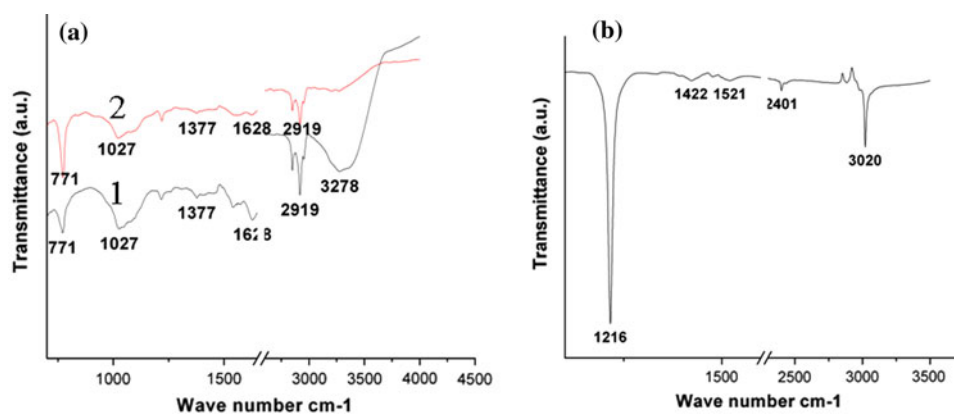


Fig. 4 EDAX of gold NPs. **a** green extract. **b** boiled extract. **c** green bio mass

Fig. 5 FT-IR spectra recorded from gold NPs solution on NaCl cell. **a** 1 green extract. 2 boiled extract and **b** green biomass



The peaks at $2,919\text{ cm}^{-1}$ could be due to the stretching vibrations of methylene groups (Ankamwar et al. 2005). Methylene compounds and Amide-I protein thus detected possibly supported the reduction of particles. The only extra peak in green extract is at $3,278\text{ cm}^{-1}$. This could be due to the hydrogen bonded NH group (<http://wwwchem.csustan.edu/Tutorials/INFRARED.HTM>). The spectrum for green biomass-reduced reaction (Fig. 5b) shows peaks at $1,216$, $1,425$, $1,521$, $2,401$ and $3,020\text{ cm}^{-1}$. The peak $1,425\text{ cm}^{-1}$ indicates bending vibration of COH groups and a strong peak at $1,216\text{ cm}^{-1}$ could be due to the amide III protein (Shankar et al. 2003). This indicates that some proteins had played a role in reduction of HAuCl_4 to generate particles. Peak $1,521\text{ cm}^{-1}$ is characteristic of stretching vibration of the NH groups (Li et al. 2007). The peak $3,020\text{ cm}^{-1}$ is O–H group of alcohols or phenols indicating phenols had also played a role in the reduction of NPs content (<http://wwwchem.csustan.edu/Tutorials/INFRARED.HTM>).

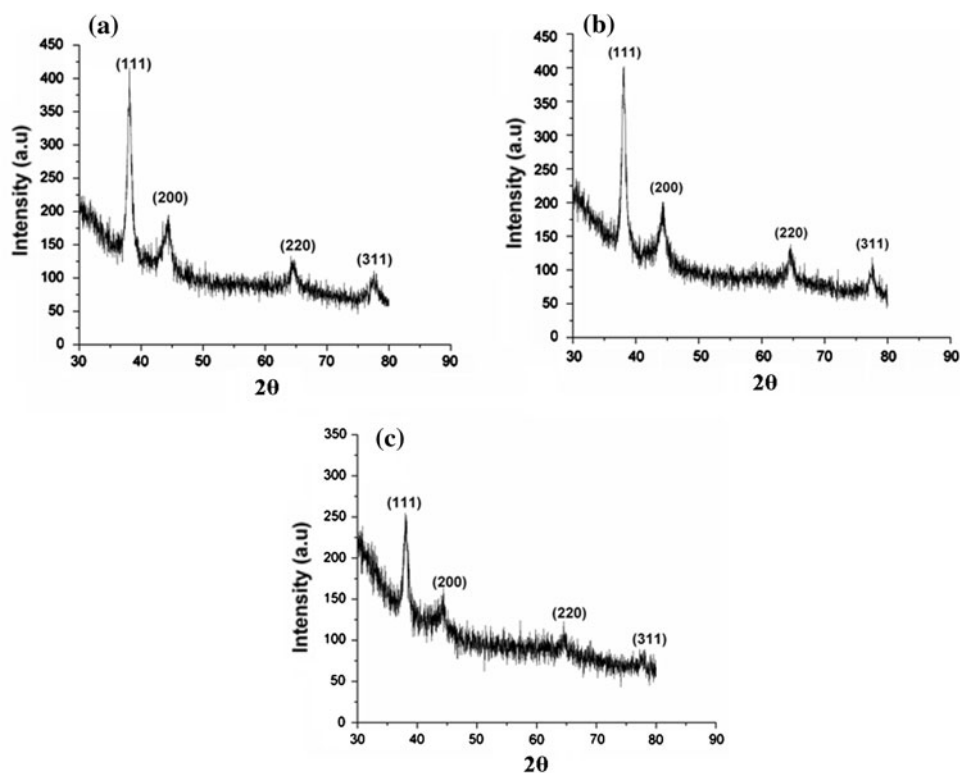
X-Ray diffraction (XRD) of particles of green extract, boiled extract and green biomass were carried out after

coating on a glass substrate. The prominent Bragg reflection could be seen. The reflections assigned to diffraction from (111), (200), (220), (311) and planes of face center cubic (fcc) at the 2θ of 38.109 , 44.36 , 64.17 , and 77.26 . All diffraction patterns of NPs of the substrate used in the reduction showed similar patterns despite different intensity of the peaks as illustrated in Fig. 6a–c.

Conclusion

In this report we demonstrated the natural ability of the *S. anacardium* leaf extract and leaf derived biomass to synthesize gold NPs from HAuCl_4 . Comparison of untreated extract, boiled extract and untreated biomass shows that reduction of HAuCl_4 was optimum in the reaction mixture with green extract. This suggests that the mechanism responsible for the reduction of HAuCl_4 to make NPs is naturally present in *S. anacardium* leaves and some of these factors are heat labile. Further experiments will demonstrate if the leaves or the *S. anacardium* plants

Fig. 6 XRD patterns of gold NPs synthesized by the reaction of 10^{-4} M HAuCl_4 solution with **a** green extract. **b** boiled extract. **c** green biomass



have the ability to reduce other metal ions and produce NPs. The present method for testing the green leaves of *S. anacardium* is simple and fast. This method can possibly be applied for testing the NP producing capability of other naturally growing plants.

Acknowledgments We acknowledge Dr. Vijaymohan for his careful reading of the manuscript and for providing helpful suggestions in preparation of manuscript, Kalpana Singh for helping in using ICP and Council of Scientific and Industrial Research (CSIR) Network programs P24-COR 0008 and NWP0019 for financial support.

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