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Research Article

Synthesis of Gold Nanoanisotrops Using *Dioscorea bulbifera* Tuber Extract

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Biosynthesis of metal nanoparticles employing plant extracts and thereby development of an environmentally benign process is an important branch of nanotechnology. Here, the synthesis of gold nanoparticles using *Dioscorea bulbifera* tuber extract (DBTE) as the reducing agent is reported. Field emission scanning electron microscopy (FESEM), energy-dispersive spectroscopy (EDX), X-ray diffraction (XRD), and UV-visible absorption spectroscopy confirmed the reduction of gold ions to AuNPs. The anisotropic nanoparticles consist of a mixture of gold nanotriangles, nanoprisms, nanotrapezoid, and spheres. The kinetics of particle formation was time dependent and was enhanced by the increase of temperature from 6°C to 50°C, the optimum being 50°C. The optimum concentration of chloroauric acid was found to be 1 mM. Complete reduction of the metal ions within 5 hours by DBTE highlights the development of a novel ecofriendly route of biological synthesis of gold nanoparticles. This is the first paper on synthesis of gold nanoparticles using DBTE.

1. Introduction

The size and shape of the nanoscale matters have a considerable effect on its physicochemical properties. Among the recently studied shapes and structures of gold nanoparticles, the noteworthy includes nanorings, nanoplates, dendrimer-like shapes, nanocubes, and nanoprisms [1–5]. The nonspherical gold nanoparticles possess unusual optical and electronic properties, improved mechanical properties, and specific surface enhance spectroscopies that make them ideal structures for emerging applications in photonics, electronics, optical sensing and imaging, biomedical labeling, and catalysis [6–11]. Red colloidal gold has been used as medicine for revitalization in China and India [12]. Gold nanoparticles have found use in biomedicinal applications particularly diagnostic and drug delivery [13]. There are a number of chemical methods for the synthesis of Au nanoparticles [14–16] that may pose a risk due to adverse effects in medical applications rendered by presence of toxic chemical species adsorbed on the surface. Thus, currently there is a growing need to develop environmentally benign nanoparticle synthesis processes that are free from toxic chemicals in the synthesis protocols. Biosynthesis of nanoparticles employing microorganisms or plants can potentially eliminate this problem by making the nanoparticles more biocompatible. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems such as yeast [17], fungi [18–20], and bacteria [21–23]. In recent years plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and ecofriendliness. Biosynthesis of Au nanoparticles by plants such as *Magnolia kobus, Diospyros kaki* [24], *Azadirachta indica* [25], *Syzygium*

aromaticum [26], *Medicago sativa* [27], *Aloe vera* [28], and *Cinnamomum camphora* [29] have been reported.

Here in we report for the first time an environmentally friendly and rapid method for the aqueous synthesis and stabilization of gold nanoparticles by the reduction of aqueous $AuCl_4^-$ ions using the aqueous extract of *Dioscorea bulbifera* tubers (DBTE). *D. bulbifera* belongs to the family Dioscoreaceae and the bulbs are profusely used in Indian and Chinese traditional medicine. It is reported to have antidiabetic [30, 31], anti-inflammatory [32], antitumor [33], and antimicrobial properties [34]. DBTE is rich in flavonoids and catechin that belongs to a class of polyphenolic compounds [35] that have contributed to its enhanced antioxidant properties [36]. However, to date, there have been no reports on the synthesis of Au nanoparticles with DBTE. We have used tuber extract for the biogenic reduction of Au^{3+} ions. We also investigated the effects of reaction conditions such as time course, reaction temperature, and concentration of chloroauric acid on the rate of synthesis of the gold nanoparticles.

2. Experimental

2.1. Plant Material and Preparation of Extract. *Dioscorea bulbifera* tubers (DBTs) were collected from Pune, chopped into thin slices, and dried for 2 days at room temperature under shade. Tuber extract was prepared by taking 5 g of thoroughly washed and finely ground tuber powder in a 300 mL Erlenmeyer flask with 100 mL of sterile distilled water and then boiling the mixture for 5 min before finally decanting it. The extract obtained was filtered through Whatman filter paper No. 1. The filtrate was collected and stored at 4°C for further use.

2.2. Synthesis of Gold Nanoparticles. Reduction of Au^{3+} ions was initiated by addition of 5 mL of DBTE to 95 mL of 10^{-3} M aqueous $HAuCl_4 \cdot 3H_2O$ solution in a 500 mL Erlenmeyer flask. The pH of the extract was found to be neutral. Thereafter, the flasks were shaken at a rotation rate of 150 rpm in the dark at 40°C. Reduction of the Au^{3+} ions was monitored by measuring the UV-Vis spectra of the solution at regular intervals on a UV-1650CP Schimadzu spectrophotometer operated at resolution of 1 nm. Effects of temperature on the rate of synthesis and particle size of the synthesized gold nanoparticles were studied by carrying out the reaction in water bath at 4–50°C with reflux. Concentration of chloroauric acid in the solution was also varied from 0.3–5 mM.

2.3. Field Emission Scanning Electron Microscope (FESEM) and Dynamic Light Scattering (DLS) Measurements. Surface morphology and particle size of bioreduced gold nanoparticles were determined using a field emission scanning electron microscope (Hitachi S-4800) equipped with energy dispersive spectrometer (EDS). Energy dispersive spectra of gold nanoparticles were taken in the same instrument at an energy range 0–20 keV confirmed the synthesis of Au nanoparticles using DBTE. The size of particles in the 3 mL of reaction mixture was analyzed by using the dynamic light scattering equipment (Malvern: Zetasizer Nano-2590) in a polysterene cuvettes.

2.4. X-Rray Diffraction (XRD) Measurements. The phase formation of bioreduced gold nanoparticles was studied with the help of XRD. The diffraction data of thoroughly dried thin films of nanoparticles on glass slides was recorded on D 8 Advanced Brucker X-ray diffractometer with Cu Kα (1.54 Å) source.

2.5. Fourier Transform Infrared (FTIR) Spectroscopy. Dry powder of nanoparticle was obtained in the following manner. The Au nanoparticles synthesized after 90 min of reaction of 1 mM $HAuCl_4 \cdot 3H_2O$ solution with the DBTE were centrifuged at 10,000 rpm for 15 min at room temperature, following which the pellet was redispersed in sterile distilled water to remove any uncoordinated biological molecules. The process of centrifugation and redispersion in sterile distilled water was repeated three times to ensure better separation of free entities from the nanoparticles. The purified pellet was then dried and subjected to FTIR (Shimadzu IR Affinity) spectroscopy measurement using the potassium bromide (KBr) pellet technique in the diffused reflection mode at a resolution of 4 cm^{-1}. Au nanoparticle powder was mixed with KBr and subjected to IR source 500 cm^{-1}–4000 cm^{-1}. Similar process was used for the FTIR study of DBTE before and after bioreduction.

3. Results and Discussion

3.1. Visual Observation and UV-Vis Spectroscopy. It is reported that DBTE contains myrecetin and catechin as well as important terpenes like neoxanthin and lutein that may be responsible for reduction of metal ions by the oxidation of –OH groups in the molecules to carboxylic acid [32]. The stabilization may be due to the starch present in the extract which keeps the particles well separated. Colloidal solutions of Au nanoparticles showed a very intense ruby red color, which is absent in the bulk material as well as for individual atoms. Their origin is attributed to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field.

These surface plasmon resonance contribute to the development of an array of colors to the reaction mixtures. The progress of the reaction between metal ions and the DBTE was monitored by recording the absorption spectra as a function of time. In each case peak was observed in the range of 500 to 600 nm. Figure 1 shows the result of the reaction between the Au^{3+} ion-containing solutions and the DBTE extract with time. Upon shaking at 40°C for 5 min, no subsequent change was observed but at 10 min a small peak appears at 540 nm., the absorbance steadily builds up with time. The maximum production was found at 90 min. The absorption peak is assigned to the surface plasmon resonance (SPR) band of Au nanoparticles formed by the reduction of Au^{3+} ions.

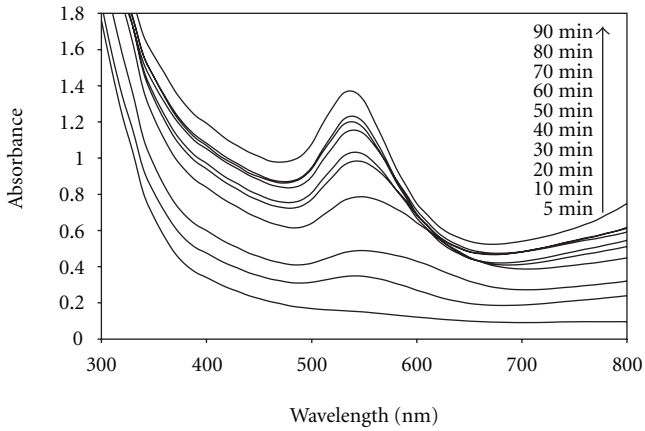

Figure 1: UV-Vis spectra recorded as a function of reaction time of 1 mM $HAuCl_4$ solution with DBTE at 40°C.

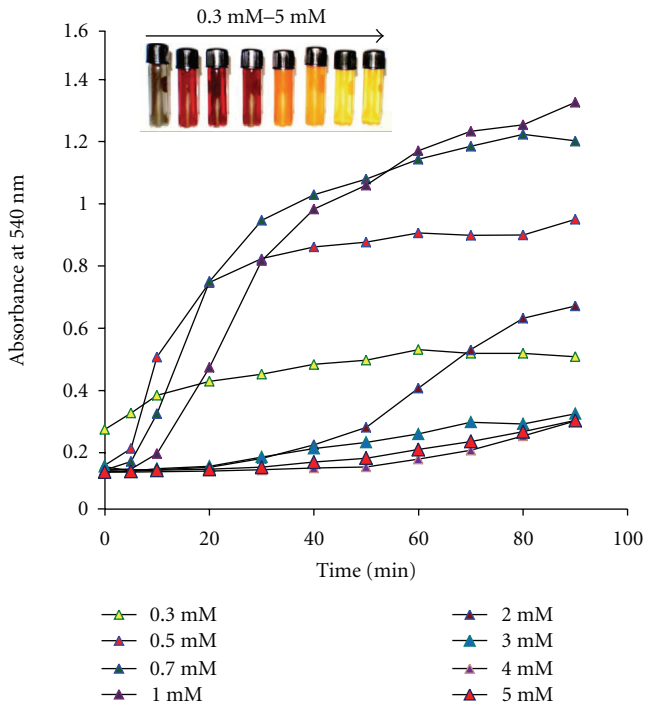

Figure 2: Time course of gold nanoparticles formation obtained with different concentrations of $HAuCl_4$ using DBTE at 40°C.

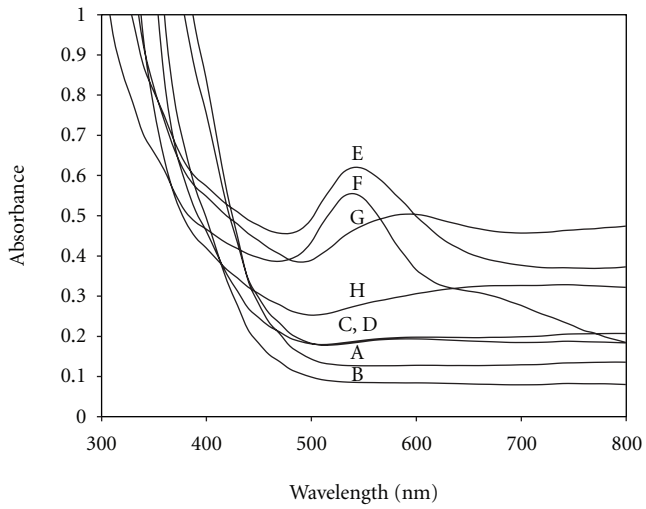

Figure 3: UV-Vis spectra of reaction mixtures with varying concentrations of $HAuCl_4$ (mM) [(A) 5, (B) 4, (C) 3, (D) 2, (E) 1, (F) 0.7, (G) 0.5, (H) 0.3].

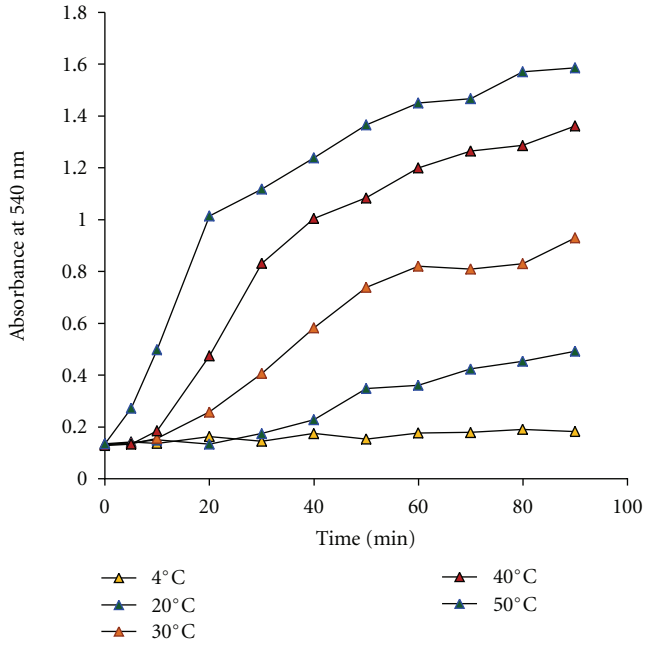

Figure 4: Time course of gold nanoparticles formation obtained with 1 mM $HAuCl_4$ using DBTE with different reaction temperature.

Optimization for concentration of chloroauric acid was carried on by plotting the peak absorbance at main peak wavelengths for Au nanoparticles against time. As shown in Figure 2, a variability in the reaction rate was observed with different concentrations of chloroauric acid. It is interesting to note that the absorbance increased steeply at early times, up to the first 90 min, after which the rate of change is reduced, and finally, a saturation was observed shortly after 100 min. A similar trend was followed by the concentrations ranging from 0.3 to 2 mM. However, in case of higher concentrations (3 mM to 5 mM) no increase in absorbance was observed. Optimization study showed a significant effect of concentration of chloroauric acid on the surface plasmon properties of the Au nanoparticles in Figure 2. Light grayish purple color was found to be developed in the reaction mixture after 5 min at 0.3 mM concentration. The yellow color of the gold chloride solution turned to be purplish pink at 0.5 mM followed by ruby red at 0.7 mM concentration. A dark brown color was obtained at 1 mM concentration. At higher concentration (2–5 mM) the property of the gold nanoparticles were found to be entirely different showing a shiny yellow color of metallic gold with deposition of the

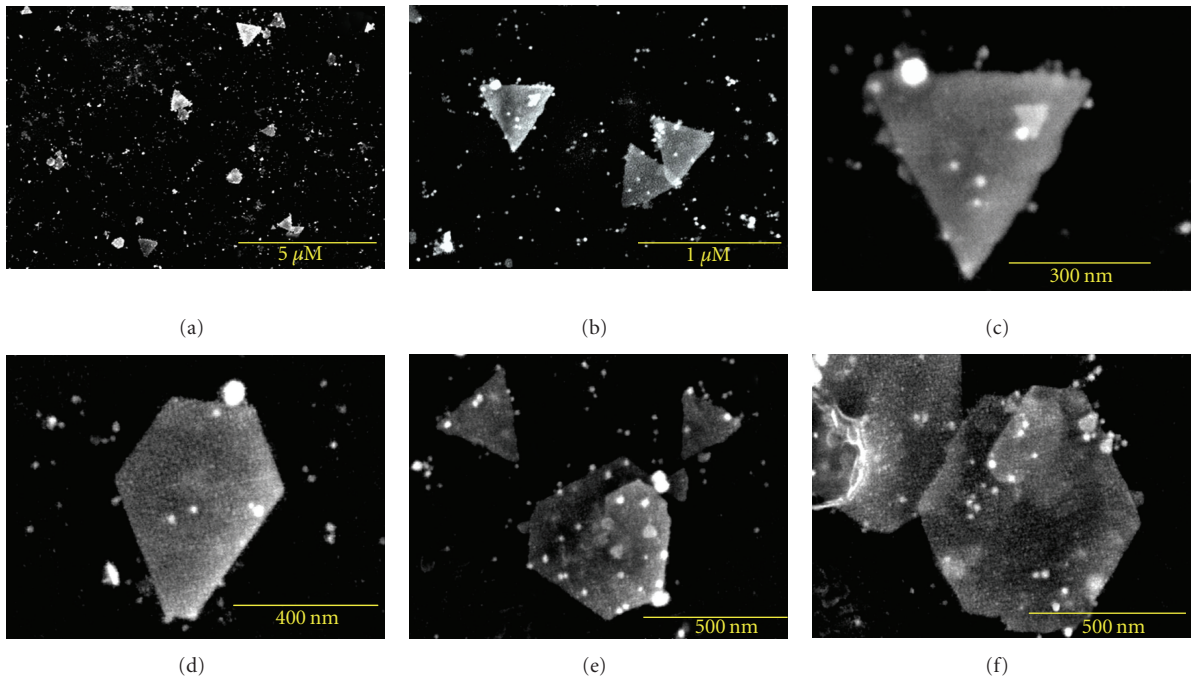

Figure 5: Characterization of gold nanoparticles formed with 1 mM $HAuCl_4$ and 5% DBTE extract at 40°C by field emission scanning electron micrographs (FESEM). (a) Gold nanoanisotrops; (b) gold nanotriangles; (c) gold nano-triangles with equilateral edges; (d) gold nanotrapezoids; (e) gold nanospheres adsorbed on nano-trapezoids and nano-triangles; (f) gold nanohexagons.

particles at the base. Our results thus demonstrate that a rapid biosynthesis of stable gold nanoparticles employing DBTE is brought about by the biogenic reduction of Au^{3+} ions the concentration optima of the gold chloride being 1 mM. The anisotropy was supported by the broadening of the peak in the UV-Vis scan of the reaction broths containing various concentrations of chloroaurate after 90 min of bioreduction by DBTE.

We further investigated the possibility of controlling the temperature conditions for maximal synthesis of Au nanoparticles by enhancement of the rate of the reaction. Figure 4 shows the effect of temperature on conversion of Au^{3+} to Au^0. It showed a steady rise in the absorbance with development of intense red color with increase in the temperature. Although the reaction was not static at 4°C, its absorbance recorded even after 90 min was not significantly high denoting a partial reduction of the gold nanoparticles at lower temperature. Similarly, lower reaction rate with faint coloration of the reaction mixture at 10, 20, and 30°C confirmed the temperature dependence nature of the bioreduction by DBTE. The maximum conversion was observed at higher temperatures, optimum being 50°C.

3.2. Field Emission Scanning Electron Microscope-Energy Dispersive Spectrum (FSEM-EDS) Analysis . FESEM analysis revealed that the DBTE leads to the synthesis of anisotropic gold nanoparticles. The biosynthesized gold nanoparticles presented variable shape, most of them being spherical in nature with a few having occasionally triangular shape. This is in agreement with earlier reports on biological synthesis employing lemon grass [25]. The gold nanospheres formed predominantly with diameter ranging from 11 to 30 nm where maximum number of gold nanoparticles size was around 18 nm. Still, the actual occurrence of the nanoprisms and nano-trapezoids is an intriguing phenomenon which has rarely been observed for Au nanoparticles synthesized with the help of natural reducing agents like plant extracts. Figure 5(a) shows the image of Au nanoparticles produced by the DBTE. Although the majority of the particles appeared more or less spherical, nevertheless nanoprisms were observed here too.

Growth process, resulting in elongation of the gold seeds rather than formation of new gold nuclei leads to anisotropic gold nanoparticles with larger dimensions [37–39]. Figure 5(b) shows distinct nanotriangle with equilateral edges, the length being approximately 270 nm. It has been reported that chloride ions are primarily responsible for the synthesis of gold triangles [40, 41]. The triangles were found to be well dispersed. Variation in the size of triangles was also observed which were in the range from 50 to 300 nm. The variation in size might be due to merging of large number of nuclei over the surface of the seed particles in the early stage of reaction. This kind of growth process is driven by very fast reduction rate of Au^+ ion, assembly and room-temperature sintering of spherical gold nanoparticles. Although the dried biomass fulfilled the reduction of chloroaurate ions, most of the quasispherical nascent nanocrystals synthesized at a later stage aggregated due to deficiency of biomolecules used up during the course of reduction. However, the nanospherical particles produced at the initial stage of reaction were stable owing to the shelter of the protecting biomolecules.

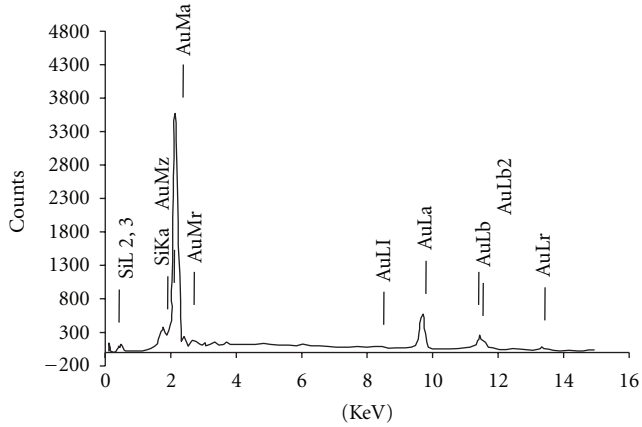

Figure 6: Representative spot EDS profile confirming the presence of gold nanoparticles.

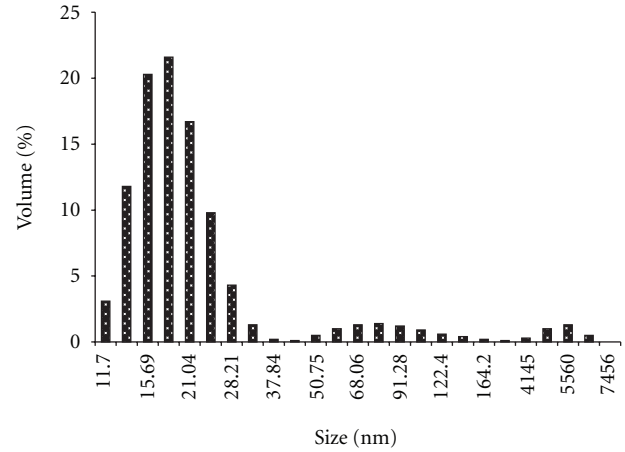

Figure 7: A histogram of size distribution of gold nanoparticles synthesized by DBTE.

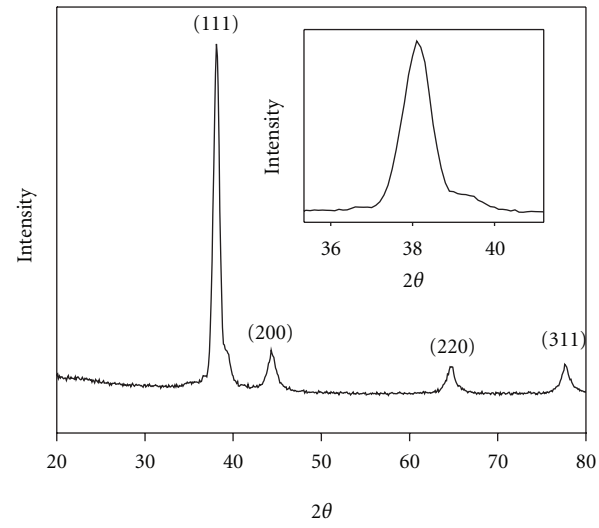

Figure 8: Representative XRD profile of thin film gold nanoparticles.

It is important to note that *D. bulbifera* contains saponin like spiroconazole A as one of its major phytoconstituent with surfactant property [42]. Surfactants are known to spontaneously organize into micelles in aqueous media [43, 44]. These spatially and dimensionalized water pools can be used as soft templates to promote the formation of gold nanoparticles with various shapes and sizes [45–48]. The anisotropy is evident in Figures 5(d), 5(e), and 5(f) that showed irregular shapes, especially Au nanotrapezoid and Au nanohexagons, respectively. Thus the saponins in *D. bulbifera* might play a major role as useful templates for generating nonspherical gold nanoparticles. Interestingly, nanospheres (18 nm) were found to adhere on the surface of the larger nanoparticles, namely, nanohexagons. It has been reported that as the solvent evaporates, capillary forces draw the nanospheres together, and the nanospheres crystallize into a hexagonally close packed pattern on the substrate [49]. Figure 5(d) shows the average size of the trapezoids to be 300 nm. Further study was carried out using EDX to confirm the element of the particle, and the EDX pattern obtained has been represented in Figure 6 that provided chemical analysis of the field view as well as spot analysis of the minute particles and confirmed the presence of specific elements.

The EDX analysis displayed signature spectra for gold convincingly evidenced the presence of gold in the polymorphic nanoparticles. These results are consistent with the EDS analysis data of gold structure synthesized by using extracts of *Scutellaria barbata* [50]. The particle size distribution of the gold nanoparticles determined by dynamic light scattering is shown in Figure 7 which was found to be in well agreement with FESEM analysis.

3.3. XRD Analysis. The bioreduced metal nanoparticle was confirmed as elemental Au using XRD. Figure 8 showed the XRD data of the bioreduced gold sample.

The obtained data matched with Joint Committee for Powder Diffraction Set (JCPDS) Data 04–0784 confirming the cubic phase of synthesized gold nanoparticles with lattice constant $a = 4.078$ Å. Since no other peaks than pure gold were observed in the diffraction data, the as-synthesized gold sample is pure. The inset shows peak broadening of the highest intensity peak corresponding to (111) plane, indicating the reduction in the crystallite size. The crystallite size was calculated using Scherrer's formula [51]:

$$d = \frac{0.9\lambda}{\beta \cos\theta}. \quad (1)$$

Where 0.9 is the shape factor, generally taken for a cubic system, λ is the X-ray source wavelength, typically 1.54 Å, β is the full width at half the maximum intensity (FWHM) in radians, and θ is the Bragg angle.

Using the above formula the crystallite size was calculated to be ~13 nm.

3.4. Fourier Transform Infrared Spectroscopy (FTIR) Analysis. *D. bulbifera* tubers are known to contain flavonoids [33], terpenoids [42, 52], phenanthrenes [53], amino acids [54], proteins [55, 56], and glycosides [57]. FTIR absorption

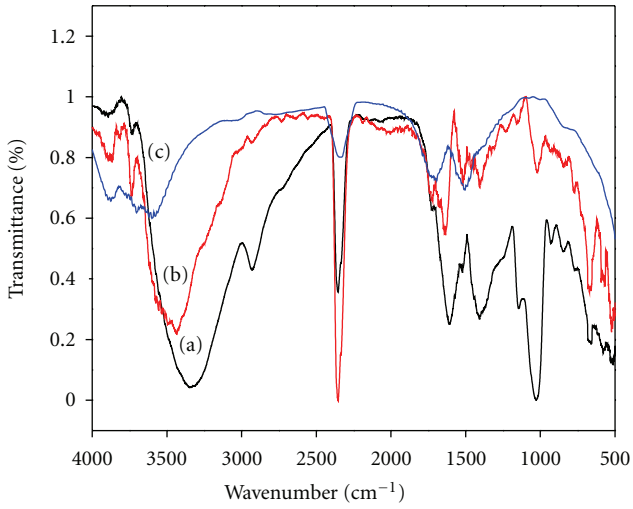

FIGURE 9: FTIR absorption spectra of dried DBTE before bioreduction (a), partial bioreduction at 4°C (b), after complete bioreduction at 50°C (c) of chloroaurate ions.

spectra of the dried biomass of *D.bulbifera* tuber before and after bioreduction, as shown in Figure 9, offered information regarding the chemical change of the functional groups involved in bioreduction. The plant extract showed strong peak at ~3300 cm^{-1} which is a characteristic of hydroxyl group in polyphenolic compounds. The intensity of the peak (3100–3400 cm^{-1}) corresponding to hydroxyl group in the plant extract was partially reduced during very slow reduction process at 4°C while significantly reduced (3580–3650 cm^{-1}) after the completion of bioreduction under optimum conditions. This was accompanied by appearance of a new C=O carbonyl stretch from carboxylic acid at 1718 cm^{-1}. Both the facts indicated involvement of hydroxyl group in the reduction of gold salt. The sharp peak at 1598 cm^{-1} represents N–H bond in –NH_2 group. The presence of peaks corresponding to 3749 and 1523 cm^{-1} indicate that the gold nanoparticles are surrounded by amines since the peaks indicate –NH_2 symmetric stretching and N–O bond in nitro compounds [58, 59]. Chen and Kimura had reported that the peaks 1540–1650 cm^{-1} and 1660–1450 cm^{-1} are characteristic of C=C [60]. The C–C is represented by the peak at 1022 cm^{-1}. One of the significant peaks is at 582 cm^{-1} which shows chloroalkane, that is, C–Cl bond, indicating the bonding of the salt $HAuCl_4$ to the plant extract through carbon-chlorine linkage/bond. As stated earlier, DBTE is mainly composed of flavonol glycosides and proteinaceous matter, which may play the key role in reduction, assembly, and stabilization of the gold nanoparticles

4. Conclusion

In conclusion, DBTE could be used as an efficient green material for the rapid and consistent synthesis of gold nanoparticles. The morphology of the particles formed consists of a mixture of gold nanoprisms, trapezoids, triangles, and spheres with face centered cubic (FCC) structure of gold (111). A variation in reaction conditions had pronounced effects on the reaction kinetics. This simple, low cost, nontoxic, and eco-friendly plant tuber could thus be used as an efficient alternative to the cost-intensive conventional methods. The gold nano-anisotrops generated by this non-conventional method could have variety of applications in the future. Moreover, this system could also be used as a model for understanding the mechanism of evolution of microstructure mediated by biological systems. The present study opens up a new possibility of very conveniently synthesizing Au nanoparticles using natural products which will be useful in optoelectronic and biomedical applications.

Acknowledgments

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