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Rapid synthesis of gold nanoparticles with *Cissus quadrangularis* extract using microwave irradiation

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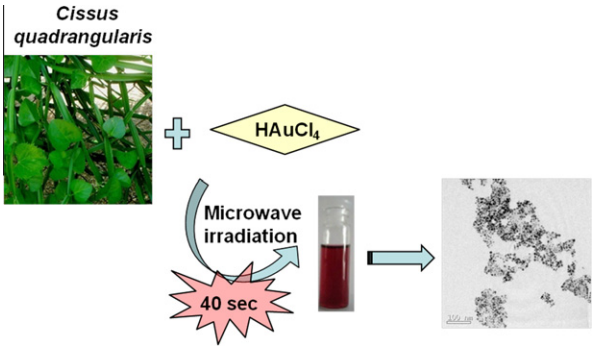
HIGHLIGHTS

- Synthesis of uniform sized AuNP using microwave irradiation.
- Formation of stable AuNP is concentration and pH dependent.
- Crystallinity revealed through XRD, HR-TEM and SAED.
- Average particle size was 12.0 ± 3.2 nm.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study focuses on the rapid synthesis of gold nanoparticles (AuNP) using the aqueous extract of *Cissus quadrangularis* (CQE) by microwave irradiation. The UV–Visible spectroscopy of the solution obtained from reduction of hydrogen tetrachloroaurate ($HAuCl_4$) by CQE revealed a sharp surface plasmon resonance (SPR) peak at 530 nm confirming the presence of AuNP. The formation of AuNP was optimal at a pH of 9. The AuNP was characterised by FT-IR, SEM, HR-TEM, SAED, XRD, TGA, DLS and Zeta potential measurements. The results indicated that microwave assisted synthesis produced well dispersed, small sized, uniform nanoparticles when compared to conventional room temperature synthesis. The spherical nanoparticle had an average size of 12.0 ± 3.2 nm as revealed through TEM. The crystalline nature of AuNP was confirmed through HR-TEM, SAED and XRD. The FT-IR and TGA data revealed the presence of the CQE components on the surface of the AuNP particles which serve as the capping agent. Upon incubation, the particles did not lyse the red blood corpuscles (RBCs) indicating that they are biocompatible. A possible mechanism for the formation of AuNP in the presence of CQE is proposed.

Introduction

Gold nanoparticles (AuNPs) have been widely investigated for their immense potential in various biomedical applications such as imaging, photo diagnostics and photo thermal therapy [1]. Several strategies have been developed for the effective synthesis of AuNP. Traditional method of reduction of Au^{3+} ions using citrate still remains the best method [2] but, residual citrate present in the product has been shown to be cytotoxic which may adversely affect its use in biomedical applications. Polymers like polyvinyl alcohol, chitosan have been used for synthesis of nanoparticles and AuNP in particular [3–6]. However they are time consuming or may require photo-irradiation. Reduction of Au^{3+} using plant extracts is advantageous since the phytochemicals have several medicinal properties which may aid in therapy and may be superior to polymer capped AuNP. Hence synthesis of AuNP using plant extracts is being attempted in many laboratories. Anisotropic AuNP

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with different shapes such as triangle, truncated triangles, hexagons, decahedral, marigold were formed in the presence of leaf extracts of coriander [7], *Coleus amboinicus* Lour [8], *Cinnamommum camphora* [9], guava [10], lemongrass [11], neem [12], geranium [13] and *Ocimum sanctum* [14]. *Lonicera japonica* flower extract formed AuNP which had the shapes of spheres and triangles [15]. Fruit extracts of pear, orange, peach and tamarind also yielded AuNP of various shapes [16–18]. Banana peel extracts were shown to form structured patterning of AuNP into microcubes and microwires [19]. Sesbania seedlings were used to form monodispersed AuNP within the plant tissues and were of spherical shape [20]. Synthesis of AuNP using *Trigonella foenum-graecum*, honey, *Macrotyloma uniflorum*, *Murraya koeginii* and *Benincasa hispida* seed produced spherical nanoparticles [21–25]. Using bayberry tannins, AuNP with a diameter as small as 2 nm were obtained which organised into a cluster of controlled particle size [26].

Microwave assisted route has been reported to be more beneficial than room temperature synthesis as it produces higher yield of nanoparticles and greater reproducibility with increased reaction kinetics. Microwave energy has been adopted for synthesis of many nanoparticles like Fe_2O_3 [27], SnO_2 [28], PbO [29], Pt supported on nanodiamond [30] and Pt supported on carbon [31]. Silver nanoparticles (AgNPs) of uniform size were formed within a short period of time using the extract of *Trachyspermum copticum* and marine micro-algae [32,33]. Microwave irradiation using citric acid and CTAB as reducing agents resulted in AuNP formation in short duration [34]. Further, gold nanorods were also prepared using microwave energy in the presence of citrate and tetra octyl ammonium bromide (TOAB) surfactant [35].

Cissus quadrangularis L. (Veldt Grape) belongs to the family Vitaceae and its vernacular name is Perandai (Tamil). It has been used in Indian folk medicine for the treatment of several pathological conditions like osteoporosis, general inflammatory condition, ulcers, menstrual disorders, haemorrhoids and many more [36]. The bone healing property of the plant extract has been demonstrated [37,38]. A commercial preparation of CQE is available as dietary supplement [39]. The medicinal properties have been attributed to the phytochemicals present in the plant and some of the main constituents are ascorbic acid, flavanoids like quercetin, kaempferol and luteolin, stillbenes such as resveratrol, quadrangularin A and pallidol, terpenoids, gallic acid derivatives and glucosides like bergenin [40]. These are known to exhibit good antioxidant and reducing properties which is the reason for choosing the stem extract of this plant for AuNP preparation. Recently CQE has been used for preparation of AgNP and the anti-parasitic and antimicrobial activity have been demonstrated [41,42]. Whether AuNP can be formed using CQE extract is not known. This study describes a method of formation of uniform, well dispersed AuNP using aqueous extract of the medicinal plant *C. quadrangularis* with the application of microwave radiation. The potential of CQE for reducing $HAuCl_4$ and stabilizing the formed Au^0 is demonstrated. Further the AuNP capped with CQE is found to be biocompatible as revealed through the haemolytic assay.

Experimental details

Materials

Hydrogen tetrachloroaurate ($HAuCl_4{\cdot}3H_2O$, >99.9%), ascorbic acid and gallic acid were purchased from Sigma–Aldrich (USA). Sodium hydroxide (NaOH, >98%) was obtained from Merck. MilliQ water (conductivity of 18 mΩ cm^{-1}) was autoclaved and filter-sterilised using 0.22 μm filters. All chemicals were used as received without any further purification. *C. quadrangularis* plant material was collected from the local garden.

Preparation of CQE

Fresh, young aerial parts of the *C. quadrangularis* plant were collected, cleaned with water and washed with MilliQ water before being finely chopped into thin slices. 7.6 g were weighed and homogenised with 225 mL of MilliQ water in a Brinkmann homogeniser at 10,000 rpm for 5 min. The extract was filtered using a cheese cloth and centrifuged at 12,000 rpm for 30 min at 10 °C. The supernatant was further sterile filtered using 0.22 μm membrane filter and stored at 4 °C until use.

Estimation of ascorbic acid and total phenolic content of CQE

The ascorbic acid content of the aqueous extract of *C. quadrangularis* was determined by iodine titration method using starch as the indicator [43]. Iodine solution (0.005 mol L^{-1}) was prepared using iodine and potassium iodide. Appearance of pale blue black colour indicated the end point. The titration was repeated for concordant values.

The total phenolic content present in the extract was measured by Folin–Ciocalteau (FC) method using gallic acid as the standard [44]. Briefly, to 40 μl of the CQE, 520 μl of deionised water and 40 μl of FC reagent was added, mixed well and incubated for 5 min. 400 μl of 7% sodium carbonate solution was then added and incubated at room temperature for 90 min. The absorbance against the water blank was measured at 750 nm with a spectrophotometer (Tecan Infinite M 200). The total phenolic content was expressed in terms of mg of gallic acid/ml of extract.

Synthesis of gold nanoparticles (AuNPs)

Aqueous stock solution of $HAuCl_4$ was prepared using filtered MilliQ water. The AuNP was synthesised first at room temperature. For this, different volumes of $HAuCl_4$ from a stock solution were added to 7 mL of CQE and made up to 10 mL with water so that the final concentration of $HAuCl_4$ are 2 mM, 1 mM, 0.5 mM, 0.25 mM and 0.1 mM. The formation of AuNP was monitored using UV–Visible spectrophotometer (Tecan Infinite M 200). Different volumes of CQE were also used for the preparation of AuNP. The effect of pH was studied by adjusting the pH for $HAuCl_4$ solutions from 6 to 9. The AuNP synthesis was also studied using reflux heating with magnetic stirrer. The progress of the reaction was indicated by change of colour of the solution from pale yellow to deep red. The solution was allowed to cool, centrifuged at 10,000 rpm for 20 min, washed twice with MilliQ water and then used for further characterisation. AuNP was also synthesised using medium power microwave in a domestic microwave oven (2.4 GHz, 0.7 W). For this, the $HAuCl_4$ solution was first irradiated for 30 s and then CQE was added and irradiated for another 40 s. A deep red colour was formed indicating the reaction was completed. Centrifugation, washing and UV–Visible spectroscopy were carried out as indicated earlier. AuNP formation in the presence of ascorbic acid was also monitored using UV–Visible spectrophotometer.

Electron microscopy studies

The morphology and size distribution of the AuNP were determined using scanning electron microscopy (SEM) and high resolution-transmission electron microscopy (HR-TEM). For SEM analysis, a drop of the sample was placed on an adhesive carbon tape stuck to a stub, dried in a desiccator and observed using FEI Quanta 200 operating at a voltage of 25 kV. Samples for HR-TEM were deposited on carbon coated copper grid and observed using a TECNAI TEM operating at an accelerating voltage of 300 kV.