Biosynthesis of Cubic Gold nanoparticles

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Abstract
Green leafy vegetable (*Amaranthus dubius*) was used as reductant and capping agent for the synthesis of gold nanoparticles. The nanogold synthesis was carried out under various conditions to evaluate the rate of reduction of gold ions. The formation of gold nanoparticles was monitored by UV-visible spectroscopy. The shape of the nanogold was found to be spherical and the size was analyzed by XRD and SEM analysis. The FTIR measurements confirm that the protein molecule present in the extract aids the formation of gold nanoparticles. Hence green leafy vegetable mediated synthesis of gold nanoparticles may find applications in nutraceuticals.
Key words: *Amaranthus dubius*, gold nanoparticles, XRD, SEM.

1 INTRODUCTION

In the present scenario, the herbs have created a revolution symbolizing the importance and safety in contrast to the synthetic drugs all over the world. Of the world's population, 20% people are still using natural products as medicine. Green plants naturally synthesize various biochemical products which can be used as food, feed stocks and raw materials as drugs to provide scientific innovation [1]. Plants are good source of secondary metabolites which find use in the pharmaceutical research. In past years a large percentage of commercially available new drugs are from natural products. Hence biological synthesis of products has to be implemented for new findings [2].

Amaranthus plants (*Amaranthaceae*) grows under a wide range of climatic conditions and serves as useful feed, food products as cooked vegetables and in traditional medicine. The leaves of amaranth are a rich source of protein, carotenoids, vitamin C, dietary fiber and minerals. *Amaranthus dubius* is recommended for anaemia, kwashiorkor, insomnia, stomach ache and also for young children and nursing mothers due to its high protein content. The essential amino acids composition of the proteins isolated from the leaves of *A. dubius* are albumins (Asp, Glu and Arg), globulins (Lys, Arg and Leu) and glutelins (Lys, Arg and Glu). The biological value of the proteins present in *A.dubius* is higher compared to that of wheat proteins and soya beans [3, 4, 5].

In modern material science, nanotechnology field is the most attractive area of research. The improved properties such as optical, electronic, magnetic and catalytic depend on their size, distribution and morphology. Nanoparticles synthetic protocols involve toxic chemicals which can cause adverse effects in medical applications. Hence there is a need to develop eco-friendly and sustainable methods of synthesis. Gold nanoparticles have received attention due to its unique properties and surface Plasmon resonance and finds application in drug delivery, chemotherapeutics, catalyst, biosensors and biomedical fields [6, 7, 8, 9, 10, 11, 12, 13, 14].

Polyhedral gold nanocrystals like cubes, tetrahedrons, octahedrons exhibit high index facets and branched nanocrystals (stars, dendimers) have received more attention due to its applications in catalysis, plasmonics and SERS based sensors. But, most of the synthetic methods yield only gold nanoparticles of polyhedral with convex shapes of low index facets (111), (100) and (110). Efforts were put forward for the synthesis of gold nanoparticles with high index facets due to their higher catalytic activities than the low index facets [15].

Biosynthesis of gold nanoparticles is found to be non-toxic, cost effective and rapid synthesis. There are reports on the exploration of plant mediated synthesis of gold nanoparticles

using *Sargassum myriocystum* [16], *Cypress leaves* [17], *Chenopodium album* [18], *Barbated Skullcup (BS) herb* [19], *Phoenix dactylifera L. leaf* [20], *Maduca longifolia* [21], *Abelmoschus esculentus* [22], *Benincasa hispida* [23] and *Eichhornia crassipes* [24].

Plants from the genus Amaranth have received attention by the researchers due to their high nutritional value. In the present work, the synthesis of gold nanoparticles was achieved using the aqueous extract of *Amaranthus dubius*. The gold nanoparticles were characterized by UV-visible spectroscopy, XRD and SEM analysis.

2 MATERIALS AND METHODS

2.1 Preparation of the extract

Fresh plant (20 g) of *Amaranthus dubius* was weighed and sonicated with 100 ml of water. The extract was filtered through Whatmann filter paper and refrigerated.

2.2 Synthesis of gold nanoparticles

Gold chloride solution (1 ml, 2 ml, 3 ml, 4 ml and 5 ml) was treated with different volumes of plant extract (1 ml) and kept at i) room temperature ii) an elevated temperature (75-80 °C) iii) under sonication (Ultrasonics 1.5L (H)). A visible transition of colour from green to violet indicative of gold nanoparticles formation was noted. The nanogold solutions were centrifuged (Spectrofuge 7M) and characterized to arrive at the size and shape.

2.3 Characterization of gold nanoparticles

The prepared nanogold was characterized by UV-visible spectroscopy, X-ray diffraction analysis, FTIR analysis and Scanning Electron Microscopy.

2.4 UV- Visible and FTIR spectroscopy

The formation of nanogold shows visible colour change and was monitored by UV-Visible spectroscopy (Double beam spectrophotometer – 2202, SYSTRONICS). The FTIR spectra were recorded in the 4000 to 400 cm^{-1} range on a Shimadzu FTIR–8400S spectrophotometer.

2.5 XRD analysis

The X-ray diffractometer analysis (X'pert Pro PANayltical) was used to analyze the size and shape of the gold nanoparticles. The crystalline size of the nanoparticles was determined using Debye – Scherrer's equation: $\mathbf{D = k\lambda / \beta \, Cos \, \theta}$

2.6 Scanning Electron Microscopy

The morphology of the synthesized nanogold was characterized by Scanning electron Microscopy (TESCAN) with Vega TC software for the synthesized AuNP's fabricated on a glass substrate.

3 RESULTS AND DISCUSSION

The green colour of the plant extract turns purple after the addition of gold chloride solution within 45 min at room temperature. The time taken for the formation of nanogold is only 15 min at higher temperature and sonication conditions. The rate of reduction of gold ions to nanogold takes place rapidly using the aqueous extract of *Amaranthus dubius* particularly at higher temperature and sonication. At higher temperature, the gold ions will be activated and quickens the formation of nanogold. Review of past work reveals that the sonochemical technique aids the formation of nanoparticles [25, 26]. The dispersion produced by the cavitational motion may facilitate the interaction of amino acids with the gold ions aiding nanogold formation.

The UV- Visible absorption spectra of gold nanoparticles shows an absorption band at 560 nm at room temperature and sonication conditions. Another band at 534 nm was observed with 560 nm for the nanogold at higher temperature. These SPR band are consistent with the gold nanoparticles and the variation may depend on the size and shape of the particles formed in aqueous medium. There is no change in the position of the bands with respect to intensity at room temperature. The variation in the intensity of bands was observed as the concentration of the gold chloride solution was increased in sonication and at higher temperature conditions.

The XRD patterns of the gold nanoparticles synthesized using the aqueous extract of *Amaranthus dubius* is given in figure 2. The diffraction angles at 38º, 44º, 64º corresponds to the (111), (200) and (220) Bragg's reflection of fcc lattice. The size of the nanogold was calculated using Debye Scherrer's equation and is given in table 1. The crystallite size of the gold nanoparticles varies with different conditions and less than 50 nm was established in sonication.

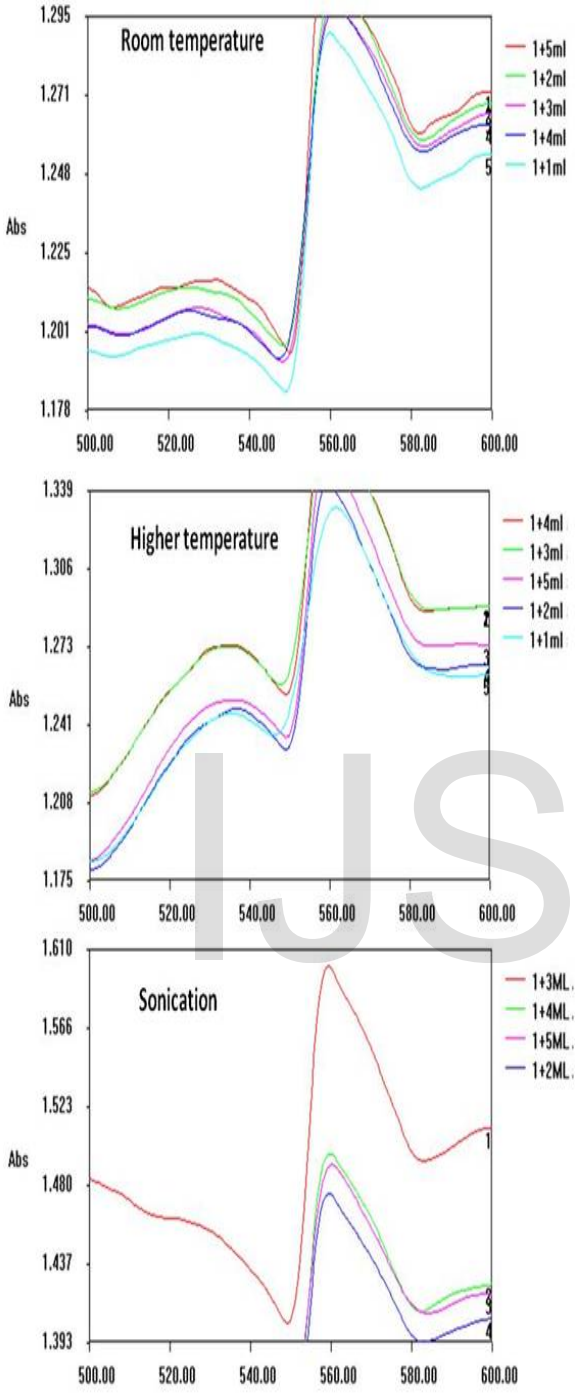

Fig. 1. UV-visible spectra of nanogold synthesized using the aqueous extract of *Amaranthus dubius* under room temperature, higher temperature and sonication

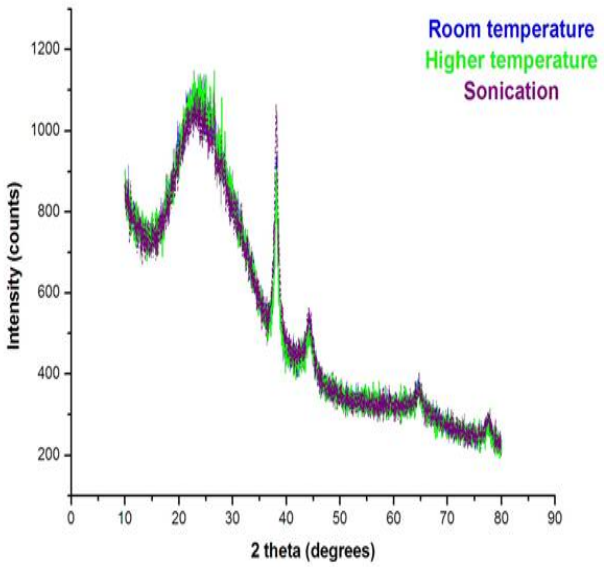

Fig. 2. XRD patterns of the nanogold synthesized using the aqueous extract of *Amaranthus dubius* under room temperature, higher temperature and sonication

Table 1. Determination of crystallite size of nanogold using Debye-Scherrer's equation

S.No.	Nanogold synthesized under various conditions	2θ (degrees)	FWHM (degrees)	$D = k\lambda / \beta . Cos\theta$ (nm)
1.	Room temperature	38.19	0.2676	31.49
		44.40	0.4015	21.39
		64.71	0.8029	117.27
		77.74	0.8029	127.25
2.	Sonication	38.21	0.2342	35.93
		44.30	0.6691	12.84
		64.59	0.6691	14.07
		77.65	0.8029	12.71
3.	Higher temperature	38.15	0.1338	62.96
		44.33	0.5353	16.04
		64.71	0.8029	11.72
		77.76	0.5353	19.09

Figure 3 (a, b, c & d) represents the SEM micrographs of the gold nanoparticles synthesized using the aqueous extract of *Amaranthus dubius* under three different conditions. It was noted that the shape of the gold nanoparticles is nanocube (figure 3d). The size of the nanogold was found to be greater than 100 nm in room temperature and higher temperature conditions. But in sonication, less than 100 nm size of nanocube was obtained (fig 3c). The capping nature of protein molecule in the extract may results in the formation of larger size gold nanoparticles. Hence these cubic gold nanoparticles with high index facets may find applications in catalysis and molecular sensors.

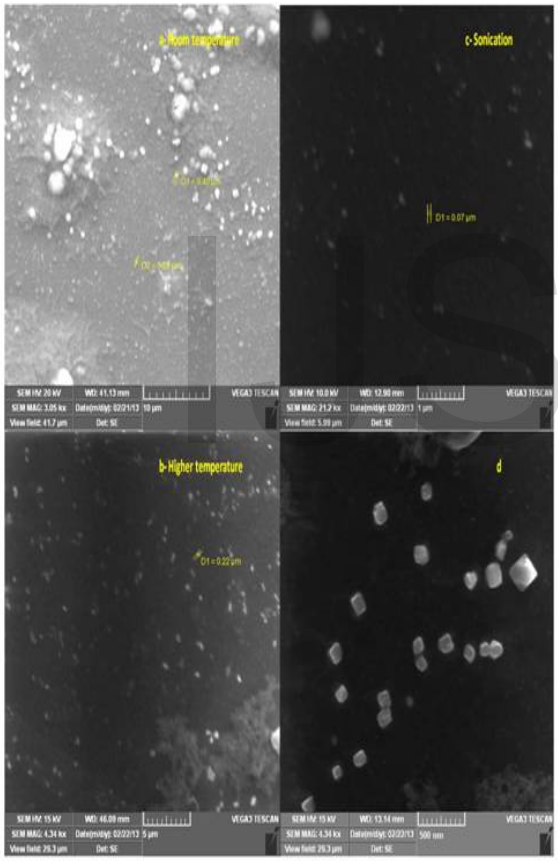

Fig. 3. SEM micrographs of the synthesized nanogold using the aqueous extract of *Amaranthus dubius* under room temperature, higher temperature and sonication

Figure 4 represents the FTIR spectra of synthesized gold nanoparticles using *Amaranthus dubius*. The peak located at 3342 and 3337 cm^{-1} may be due to the presence of –OH group. The peak at 1638 cm^{-1} revealed the presence of carbonyl stretching in proteins. The peak near 1220 cm^{-1} is assigned to C-O stretching vibrations. The FTIR measurements of gold nanoparticles have some deviations in the band compared to that of plant extract alone. This suggests the amino acids present in the *A. dubius* to possess strong ability to interact with the gold ions and hence facilitate the formation of gold nanoparticles.

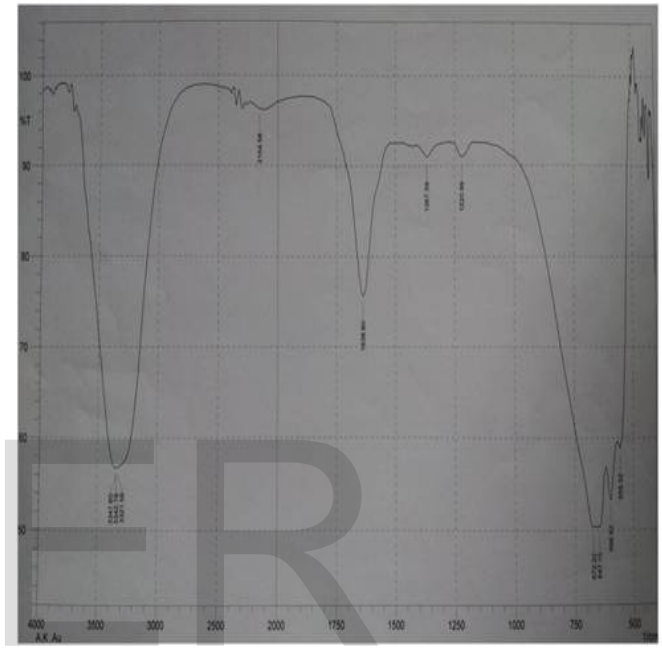

Fig. 4. FTIR spectra of gold nanoparticles synthesized using *Amaranthus dubius* extract

4 CONCLUSIONS

The reduction of gold ions to gold nanoparticles was achieved using the aqueous extract of green leafy vegetable (*Amaranthus dubius*) under different conditions. The amide acids were responsible for the rapid reduction as analyzed through FTIR measurements. The optimized concentration of the gold nanoparticles was determined and variation in size at different conditions was examined by XRD and SEM analysis. The crystalline size of less than 70 nm nanocube gold particles was obtained in sonication. The nanocube produces many actives sites which may be exploited in organic synthesis or in reactions for enhanced catalytic activity. Biological molecules present in the extract were found to serve as good reducing and capping agents. Thus the *Amaranthus dubius* mediated nanogold may be

utilized in nutraceuticals due to presence of high content of essential amino acids in the plant.

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