

# Green Synthesis of Gold Nanoparticles using Leaves Extract of *Bauhinia tomentosa* Linn and *invitro* Anticancer Activity

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**Abstract** - Metal nanoparticles have several applications such as optics, biomedical sciences, drug delivery, catalysis and electronics. The present investigation deals with the green synthesis of gold nanoparticles (AuNP) using leaves extract of *Bauhinia tomentosa* Linn and its *invitro* anticancer activity. Qualitative phytochemical analysis of the leaves extract showed the presence of tannins, saponins, flavonoids, alkaloids, proteins, steroids and quinones. Aqueous extract (pH 7.4 - inherent pH of the extract) was reacted with 1mM Chloroauric acid (HAuCl<sub>4</sub>.3H<sub>2</sub>O) and kept at room temperature. The immediate change in color from pale yellow to ruby red indicated the reduction of Au<sup>3+</sup> ions to Au<sup>0</sup>. The synthesized AuNP's were monitored using UV-Visible spectrophotometer. Gold Surface Plasmon Resonance (SPR) occurred at 563 nm and steadily increased in intensity with longer duration of incubation time without shift in wavelength. FTIR spectra revealed the presence of reducing groups in the extract responsible for AuNP synthesis. FESEM analysis showed the presence of polydispersed spherical AuNP's. EDAX confirmed the presence of elemental gold. HR-TEM showed that the gold nanoparticles were near spherical with average diameter 31.32 nm. The XRD peaks at 38.04, 44.12, 64.52, 77.49 and 81.53 corresponding to [111], [200], [220], [311] and [222] showed that the AuNP's were nanocrystalline with fcc crystal structure. The *in-vitro* anticancer activity confirmed by MTT assay on the cell lines of laryngeal HEp-2 carcinoma cells showed IC<sub>50</sub> values of extract at 53.125 µg/mL and AuNP's at 34.375 µg/mL.

**Keywords:** *Bauhinia tomentosa* Linn; Gold Nanoparticles; Surface Plasmon Resonance; UV-Visible Spectrophotometer; FTIR; FESEM-EDAX; HR-TEM; XRD; MTT; HEp-2;

## I. INTRODUCTION

Metallic nanoparticles have several applications in many areas such as optics, biomedical sciences, drug delivery, catalysis and electronics. Chemical reducing agents such as hydrazine, sodium citrate and sodium borohydride were used to reduce the corresponding precursor salts to create uniformly suspended metallic nanoparticles [1]. Integration of green chemistry principles to nanotechnology is one of the key issues in nanoscience research. The inspiration for green chemistry and bioprocesses comes from nature through yeast, fungi, bacteria and plant extracts in the synthesis of biocompatible metal and semiconductor nanoparticles [2]. For the development of green chemistry, three main factors in nanoparticle preparation should be considered: solvent choice, the use of an environmentally benign reducing agent, and the use of a non-toxic material for nanoparticle stabilisation [1]. Green synthesis of nanoparticles uses water commonly as an environmentally benign solvent, replacing toxic organic solvents. Biological entities have been reported as serving as both reducing and stabilising agents for green synthesis of metallic nanoparticles [1]. Gold nanoparticles have been considered as important area of research due to their unique and tunable surface Plasmon resonance (SPR) and their applications in biomedical science including drug delivery, tissue/tumor imaging, photothermal therapy and immuno chromatographic identification of pathogens in clinical specimens [2].

*Bauhinia* is a genus with more than 200 species of flowering plants in the sub family Cesalpinioideae of the large flowering plant family Fabaceae, with a pantropical distribution. The species name "*tomentosa*" means hairy and it refers to the velvety/hairy Pods. These plants can be found along the coastal strip from southern Kwazulu-Natal to Maputoland, Mpumalanga as well as Mozambique, Zimbabwe, tropical Africa, India and Srilanka [3].

*Bauhinia tomentosa* (Linn) belongs to the Kingdom - Plantae, Family – Caesalpinaceae. It is commonly known as ‘Kanchini’ in Tamil and ‘Phalgu’ in Sanskrit. The dried leaf buds and flowers are prescribed in dysentery [4]. Leaves exhibited cytotoxic and antioxidant activity while flowers were found to possess anti-hyperglycemic and antilipidemic activity [5]. Pharmacognostical and phytochemical screening of *Bauhinia tomentosa* Linn leaves has been reported by S. Rhama and S. Madhavan [6]. Quantitative and qualitative phytochemical evaluation of solvent extracts of root and flowers of *Bauhinia tomentosa* Linn was reported by Gautam *et al.*, [7] and Sathya *et al.*, [8] respectively. The phytochemical evaluation of *Bauhinia tomentosa* Linn has been analysed to a certain extent, the synthesis of metal nanoparticles using various plant parts and the mechanism of reduction is yet to be explored. The present investigation deals with the green synthesis of gold nanoparticles (AgNP) using *Bauhinia tomentosa* Linn (Kanchini) leaves extract and its *invitro* anticancer activity.

## II. MATERIALS

Leaves of *Bauhinia tomentosa* Linn were collected from Madipakkam, Chennai and authenticated by Dr. S. Jayaraman, Director of Plant and Anatomy Research Centre, West Tambaram, Chennai. (Reg.No. PARC/2013/2189)

Chloroauric acid ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ) (99.99 %), Minimal Essential Media (MEM), Fetal Bovine Serum (FBS), Trypsin, Streptomycin, Methylthiazolyl Diphenyl- Tetrazolium Bromide (MTT), and Dimethyl Sulfoxide (DMSO) were procured from Hi Media and Sigma Aldrich, Mumbai.

**HEp-2** (Human Laryngeal epithelial carcinoma) cell lines were obtained from National Centre for Cell Sciences (NCCS), Pune.

## III. PREPARATION OF LEAVES EXTRACT

Leaves of *Bauhinia tomentosa* were washed thoroughly with deionized water and shade-dried for 10 days. The leaves were finely powdered and used for preparation of the extract. Five grams of powdered leaves was mixed with 100 mL of deionized water and kept under magnetic stirring for 24 hours at room temperature. Aqueous leaf extract was obtained by slow filtration process, stored at 4°C and used within 15 days.

## IV. SYNTHESIS OF GOLD NANOPARTICLES

Five milliliter of aqueous extract of *B.tomentosa* leaves was added to 95 mL of 1 mM aqueous  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  solution at room temperature for the reduction of  $\text{Au}^{3+}$  ions to  $\text{Au}^0$ . The immediate change in color of the solution from pale yellow to ruby red indicated the preliminary confirmation for the formation of plant extract mediated synthesis of gold nanoparticles [9].

## V. METHODS

Active phytoconstituents present in the aqueous extract were identified by qualitative chemical tests. Tests for terpenoids, flavonoids, saponins, tannins, sterols, carbohydrates, glycosides, proteins and aminoacids were carried out following the method of J.B. Harborne [10] and H.O. Edeoga [11]. UV-Visible spectrophotometer (Thermo Scientific – Evolution 201) was used for studying the spectral response of AuNP's. Fourier-Transform Infrared Spectroscopy (FTIR) results were obtained from Jasco 6300 spectrometer (ATR mode) in the range of 400 - 4000  $\text{cm}^{-1}$ . The surface morphology of gold nanoparticles and binding energy of the element were examined using FESEM (Hitachi SU6600, Japan) and EDAX (EMAX, Horiba 8121-H, Japan). The morphology of the synthesized gold nanoparticles was examined using a High Resolution Transmission Electron Microscope (Tecnai T-30 G<sup>2</sup>, D-905). Powder X-ray Diffraction (XRD) was performed using X-ray diffractometer - Cu K radiation. (Rigaku, Miniflex-600, Japan)

**HEp-2** cells were maintained in Minimal Essential Media supplemented with 10% FBS, Penicillin (100 U/ml), and Streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO<sub>2</sub> at 37 °C. The anticancer activity of samples on **HEp-2** was determined by MTT assay [12]. Cells (1 × 10<sup>5</sup>/well) were plated in 0.2 ml of medium/well in 96-well plates and incubated at 5 % CO<sub>2</sub> incubator for 72 hours. Various concentrations of the samples were added in 0.1% DMSO for 24 hours at 5 % CO<sub>2</sub> incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC<sub>50</sub>) was determined graphically. The effect of the samples on the proliferation of **HEp-2** cells was expressed as the % cell viability, using the following formula:

$$\text{Percentage (\%)} \text{ cell viability} = \text{A540 of treated cells} / \text{A540 of control cells} \times 100\%$$

## VI. RESULTS AND DISCUSSION

### A. Phytochemical analysis of *Bauhinia tomentosa* Linn leaves extract

Qualitative phytochemical analysis of aqueous extract of *Bauhinia tomentosa* Linn leaves is shown in Table 1. The analysis revealed the presence of terpenoids, flavonoids, tannins, carbohydrates, sterols and cardiac glycosides, saponins, proteins and amino acids.

TABLE I  
PHYTOCHEMICAL SCREENING - AQUEOUS EXTRACT *Bauhinia tomentosa* LEAVES

S.No	Phytochemicals	Present (+) /Absent (-)
1.	Tannins	+
2.	Saponins	+
3.	Flavonoids	+
4.	Alkaloids	+
5.	Proteins	+
6.	Steroid	+
7.	Quinones	+
8.	Terpenoid	+
9.	Cardio glycosides	+

### B. UV-Visible Spectroscopy

Fig. 1 shows the UV-Visible spectra of synthesized AuNP's and increase in absorbance with respect to time. The absorbance maxima of Gold Surface Plasmon Resonance (SPR) occurred at 563 nm and the intensity increased steadily as a function of time without any shift in the peak wavelength. Similar behavior of AuNP's has been reported by Shiv Shankar *et al.*, [13].

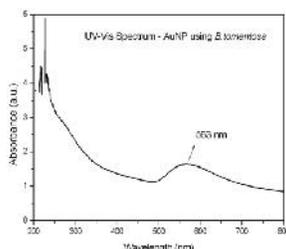


Fig. 1. UV-Vis Spectrum of AuNP

### C. Fourier Transform Infrared Spectroscopy (FTIR)

Fig. 2 shows the FTIR spectra of *B.tomentosa* leaf extract and AuNP. The spectrum of leaves extract showed peaks at 3340, 2343, 2300, 2177, 1968, 1635, 582, 539, 466 and 426  $\text{cm}^{-1}$ . Characteristic peaks of gold nanoparticles synthesized by reduction of  $\text{Au}^{3+}$  ions by *B.tomentosa* leaves extract were found to be 3342 (O-H stretch or H-bonded), 1737 (C=O), 1639 (N-H bend), 1436 (C-H bend), 1365 (C-H rock) and 1218  $\text{cm}^{-1}$  (C-O stretch or C-N stretch). Similar FTIR spectra for AuNP's have been reported by Shiv Shankar *et al.*, [13] and Ashwani Kumar Singh *et al.*, [14].

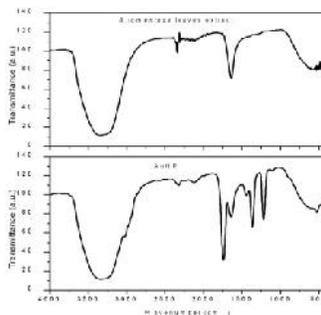


Fig. 2. FTIR spectra of *B.tomentosa* leaves extract and AuNP

### D. Field Emission Scanning Electron Microscopy – Energy Dispersive X-Rays (FESEM-EDAX)

Fig. 3 shows the FESEM analysis of synthesized AuNP's. The image showed the presence of polydispersed spherical AuNP's. The particle size ranged from 26 to 40 nm. EDAX analysis showed the presence of strong elemental gold peak (64.14 weight %) and weak oxygen peak (35.86 weight %) [Fig. 3(inset)]. The oxygen peak might be due to the presence of biomolecules bound to the surface of gold nanoparticles. J. Y. Song and B. S. Kim [15] reported spherical silver nanoparticles and silver peak using SEM-EDS for *Diopyros kaki* leaf broth.

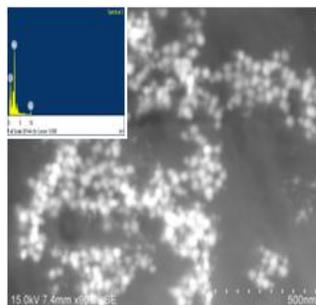


Fig. 3. FESEM-EDAX of AuNP

### E. High Resolution – Transmission Electron Microscopy (HR-TEM)

Fig. 4 shows the HR-TEM image for gold nanoparticles synthesized using aqueous extract of *B.tomentosa* leaves. The synthesized gold nanoparticles were near spherical and polydisperse with an average diameter of 31.32 nm. The AuNP's were encapsulated. Shiv Shankar *et al.*, [13] reported similar geometry of the synthesized gold nanoparticles using Neem leaves. Selected Area Electron Diffraction (SAED) pattern for the gold nanoparticles is shown in Fig. 4 [inset]. The ring like pattern indicated the crystalline structure of AuNP's.

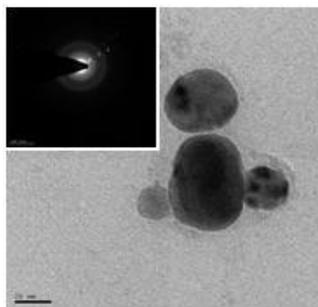


Fig. 4. HR-TEM image of Au nanoparticles

#### F. X-Ray Diffraction (XRD)

The XRD peaks at 38.04, 44.12, 64.52, 77.49 and 81.53 corresponding to the indices [111], [200], [220], [311], [222] are shown in Fig. 5. Their positions were in accordance with the reported pattern (JCPDS 04-0784) showed that the green synthesized AuNP's were nanocrystalline with fcc crystal structure. Similar pattern of XRD for gold nanoparticles has been reported by Shiv Shankar *et al.*, [13].

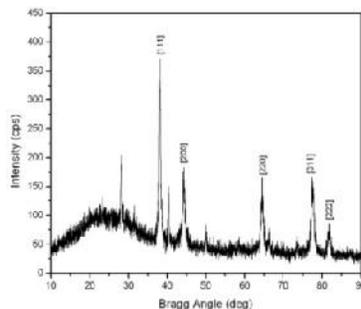


Fig. 5. XRD pattern of AuNP

#### G. *In vitro* anticancer activity of gold nanoparticles

The *in vitro* anticancer activity confirmed by MTT assay on the cell lines of laryngeal HEp-2 carcinoma cells showed IC<sub>50</sub> values of extract at 53.125  $\mu\text{g}/\text{mL}$  and AuNP's at 34.375  $\mu\text{g}/\text{mL}$ . (Fig. 6) The AuNP's inhibited the proliferation of HEp-2 cells in a dose and time dependent manner. AuNP's at eight different concentrations decreased the percentage (%) cell viability of HEp-2 cells to 78.5, 66.1, 51.2, 40.4, 28, 17.3, 13.2 and 6.61 respectively. The IC<sub>50</sub> value of AuNP showed that the concentration required to inhibit 50% of HEp-2 cells was less than that of *B.tomentosa* leaves extract. Similarly, Niranjana *et al.*, [16] reported the *in vitro* anticancer activity of AgNP's using *Cardiospermum halicacabum* extract against A-549 cells.

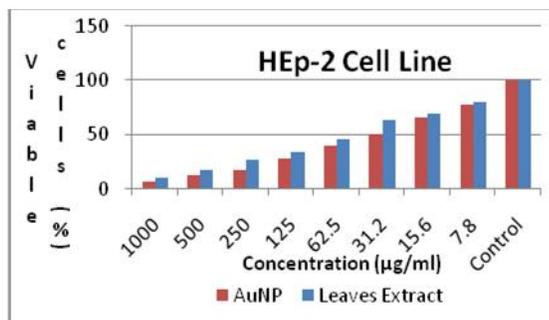


Fig. 6. *In vitro* cytotoxicity studies for AuNP's and *B.tomentosa* leaves extract

## VII. CONCLUSION

A simple, cost effective, non-toxic gold nanoparticles (AuNP) has been synthesized using leaves extract of *Bauhinia tomentosa* Linn and its *in vitro* anticancer activity has been studied. Qualitative tests confirmed the presence of bioactive phytoconstituents. FTIR spectra revealed the presence of reducing groups in the extract responsible for AuNP synthesis. The synthesized gold nanoparticles were near spherical with average diameter 31.32 nm. The *in-vitro* anticancer activity confirmed by MTT assay on the cell lines of laryngeal HEp-2 carcinoma cells showed IC<sub>50</sub> values of extract at 53.125 µg/mL and AuNP's at 34.375 µg/mL. The AuNP's inhibited the proliferation of HEp-2 cells in a dose and time dependent manner. The IC<sub>50</sub> value of AuNP showed that the concentration required to inhibit 50% of HEp-2 cells was less than that of *B.tomentosa* leaves extract.

The green synthesized AuNP's were found to be stable and the key ingredients responsible for reduction should be explored in detail. Further, this study suggests that green synthesized gold nanoparticles might be a potential agent in cancer therapy.

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