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Synthesis and characterization of gold nanoparticles using Ficus religiosa extract

Kirtee Wani [(#, A)], Amit Choudhari [(#, A)], Rajeev Chikate [(B)] and Ruchika Kaul-Ghanekar [(*, A)]

(A) Interactive Research School for Health Affairs (IRSHA), Bharati Vidyapeeth University, Katraj-Dhankawadi, Pune-Satara Road, Pune-411043, Maharashtra, India.
(B) Nanoscience group, Department of Chemistry, Post-graduate & Research Center, MES Abasaheb Garware College, Karve Road, Pune-411004, Maharashtra, India.

[#] Contributed equally; ***Corresponding Author :** Ruchika Kaul-Ghanekar, Tel: +91-20-24366929/+91-20-24366931; Fax: +91-20-24366929/+91-20-24366931; E mail: ruchika.kaulghanekar@gmail.com, kaul_r@yahoo.com

We report a cost effective and eco-friendly biosynthesis of gold nanoparticles (F-AuNPs) using aqueous extract of *Ficus religiosa* as the reducing and stabilizing agent. These nanoparticles were characterized by various techniques such as UV-Vis, XRD, TEM and FTIR. The characteristic surface plasmon peak was observed at 540 nm while XRD analysis suggested it to be a face-centered cubic (fcc) structure with peaks at 38.06, 44.46, 64.75 and 77.56. FTIR studies indicated the capping of the nanoparticles with polyphenols, amines and carboxylates present in the extract of *Ficus religiosa* whereas TEM analysis showed spherical morphology with other shapes such as triangles and hexagons. The F-AuNPs were found to be non-toxic to HEK 293 cells, thereby suggesting their potential application in the field of nanobiotechnology.

Keywords : Biosynthesis, gold nanoparticles, *Ficus religiosa*, cytotoxicity.

Introduction : The field of nanotechnology has recently witnessed significant advances in the physical and chemical methods of synthesis of nanomaterials [1, 2]. Due to the use of toxic and aggressive chemicals as reducing and/or capping agents in such synthetic processes, large amounts of hazardous by-products are released in the environment, thereby raising an alarming concern [3, 4]. This has invited attention towards a clean, non-toxic and environment-friendly method of nanomaterial synthesis known as 'Green Nanotechnology' [3, 5]. Biological methods of synthesis of nanomaterials are considered to be environmentally safe and sound compared to the conventional synthetic methods [6, 7].

A number of studies have used biomolecules such as proteins, amino acids, carbohydrates, and sugars; whole cells of bacteria, fungi, and algae; or different plant parts such as roots, leaves, flowers, bark powders, seeds, roots and fruits for the synthesis of metal nanoparticles [8]. Since plants are rich in polyphenols, they serve as important materials for the production of metallic nanoparticles [9]. Such type of synthesis using phytochemicals minimizes or eliminates chemical interventions, thereby resulting into truly green and non-polluting eco-friendly industrial process. This type of synthesis of nanoparticles reduces the cytotoxicity as well as increases the bioavailability of the nanoparticles.

Currently, there has been a growing demand for the biogenic synthesis of gold nanoparticles to avoid toxicity problems, if such metal nanoparticles are intended for application to human beings [1]. Biosynthesis of gold nanoparticles has been reported with microbes such as bacteria [10], fungi [11] and actinomycetes [12]; as well as with phytochemicals present in hibiscus [13], geranium [14], lemon grass [15], cinnamon [16], neem [17], *Aloe vera* [18], tamarind [19], oat, wheat [20], alfalfa [21], bengal gram [22], tea [9], cumin [23] and onion [24]. Honey has also been reported to help in the synthesis of gold nanoparticles [25].

Ficus religiosa Linn. (Moraceae), has been known for medicinal properties due to presence of phenols, tannins, steroids, alkaloids and flavonoids, vitamin K, methyl oleanolate, n-octacosanol, β-sitosteryl-d-glucoside, lanosterol, stigmasterol, lupen-3-one [26]. Most of these phytochemicals are water soluble and, hence are easily extracted in water without losing their properties. Recently, we have reported the antioxidant activity of *Ficus religiosa* that may be due to the presence of the phytochemicals [26]. Such phytochemicals act as metal-reducing and capping agents, thereby resulting into one-step biosynthesis of metal nanoparticles.

In this study, we have reported an environmental friendly biosynthetic approach for the synthesis of gold nanoparticles (F-AuNPs) with *Ficus religiosa* extract. The F-AuNPs were synthesized by mixing an aqueous solution of *Ficus religiosa* extract with chloroauric acid solution. The as-obtained nanoparticles were characterized by UV/Vis spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR). Their stability was monitored and cytotoxicity was evaluated on human HEK-293 cells.

Materials and Methods :

Materials : All the chemicals including $HAuCl_4$, Analytical Reagent (AR) grade were procured from Sigma-Aldrich, USA. Bark of *Ficus religiosa* L. was collected from Pune District, Maharashtra, India. A voucher specimen (MPCC 2417) of authentic plant species has been deposited at the herbarium of Medicinal Plants Conservation Center (MPCC), Pune, Maharashtra, India. Triple distilled water was used throughout the experimental work. For cell culture, Dulbecco's Modified Eagle's Medium (DMEM) and Fetal Bovine Serum (FBS) were purchased from Sigma-Aldrich, USA. Penicillin / streptomycin and Trypsin were obtained from Gibco BRL, CA, USA, and L-glutamine was bought from Himedia. All other common reagents including 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylthiazolium bromide (MTT) were bought from Sigma Aldrich, USA. HEK-293 cell line was obtained from NCCS, India.

Methods :

1. Extract Preparation and Synthesis

1.1 Preparation of *Ficus religiosa* aqueous extract: The aqueous extract was prepared as described previously [26]. Briefly, the bark of *Ficus religiosa* was chopped into small pieces, shade dried at ambient temperature and ground into coarse powder in a grinder. Aqueous extracts was prepared as per standard Indian Pharmacopoeia [27]. The extract so obtained, was centrifuged at 13000 rpm for 15 min and the supernatant was used as reducing agent for synthesis of gold nanoparticles.

1.2 Synthesis of gold nanoparticles : Aqueous extract of *F. religiosa* was used for the biosynthesis of gold nanoparticles (F-AuNPs). Around 10 ml of 1mM auric chloride salt was heated to 80°C in a rotamantle and stirred continuously for 20 min to which was added 600 µl of 10 mg/ml concentration of *F. religiosa* aqueous extract. The reaction was carried out till the solution changed colour from yellow to wine red, indicating the formation of gold nanoparticles. The solution was further filtered through a 0.2 micron filter. The as-obtained gold nanopaticles (F-AuNPs) were characterized by UV-Vis absorption spectroscopy, XRD, TEM and FTIR.

2. Physical Characterization

2.1 UV-Vis Spectroscopy Studies : UV-Vis spectroscopy measurements of the F-AuNPs were carried out on BMG FLUOstar Omega

spectrophotometer. The spectra was recorded in the wavelength range of 450-1000 nm.

2.2 X-Ray Diffraction (XRD) Measurement : Determination of crystallinity, phase purity, lattice properties and identification of air-dried F-AuNPs was done by XRD studies using powder diffractometer with Cu-Kα radiation, operating at 40 kV and a current of 40 mA (X-Ray Diffractometer, Schimadzu, at Pune University, Pune). The crystallite size was calculated from the width of the XRD peaks by using Scherrer's formula :

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

where, D is the average crystalline size, λ is the X-ray wavelength used, β is full width at half-maximum intensity and θ is the Bragg's angle in degrees.

2.3 Transmission Electron Microscope (TEM) Measurements : TEM was performed to elucidate the morphology as well as size of the biosynthesised F-AuNPs on a JEOL model 1200EX instrument operated at an accelerating voltage at 80 kV. Colloidal solution F-AuNPs, in triple distilled water, was ultrasonicated for 15 min and then coated onto ultraclean carbon coated copper grid for analysis.

3. Fourier transform infrared spectroscopy (FTIR) Analysis : FTIR studies of as-synthesized F-AuNPs were performed on Schimadzu IR Infinity (at SP College, Pune) to analyze their capping/bonding with polyphenols present in the extract of *F. religiosa*. The spectra have been recorded between 400 to 4000 wavelength range by mixing the dried F-AuNPs with KBR powder and allowing the spectra to be run under controlled atmospheric conditions.

4. In vitro stability studies: In vitro stability studies of as-obtained F-AuNPs were performed by monitoring the UV-Vis absorbance over a period of 0 h, 24 h and 7 days **[8]**.

5. Cell viability determination by MTT Assay : The viability study was performed by using MTT dye in non-cancerous transformed human embryonic kidney cell line, HEK-293. The cells were grown in DMEM supplemented with 2 mM L-glutamine, 100 units/ml of penicillin / streptomycin, and 10% fetal bovine serum incubated in a humidified 5% CO_2 atmosphere at 37°C. The cells were seeded at 1×10^5cells/ml density in 96-well plates (BD Falcon, USA). After 24 h, the cells were incubated with fresh medium containing F-AuNPs added at concentrations ranging from 0-200 µM (each dose in triplicates) and the plates were incubated overnight at 37°C in 5% CO_2 incubator. The MTT solution (5 mg/ml) was added to each well and the cells were cultured for another 4 h at 37°C in 5% CO_2 incubator. The intensity of colored formazan derivative was determined by measuring optical density (OD) with the ELISA microplate reader (Biorad, Hercules, CA) at 570 nm ($OD_{570–630}$ nm). The mean OD value of three wells was used for assessing the cell viability expressed as percentage of control.

$$\%\text{Viability} = \frac{\text{Nanoparticle treated cells}}{\text{Control cells}} \times 100 \quad (2)$$

6. Statistical Analysis : All the experiments were performed in triplicates. The data has been represented as mean ±SD. Statistical analysis was conducted with the Graph Pad Prism 4 program using one-way ANOVA. The p value used for the comparison was <0.05.

Results and Discussion :

1. Synthesis of F-AuNPs : The production of gold nanoparticles was visually observed as the colour of gold salt was changed from yellow to wine red, as seen in Figure (1). The components in *Ficus religiosa* extract act as a trigger for the formation of gold nanoparticles (F-AuNPs). These components possess functional groups such as carboxylates, amides, hydroxylates and so on which reduce $HAuCl_4$ resulting in the formation of gold nanoparticles. F-AuNPs were formed by simply mixing $HAuCl_4$ with the aqueous extract of *Ficus religiosa* bark at 80°C. The as-synthesized nanoparticles possessed clear uniform consistency.

Figure (1) : F-AuNPs formation: A picture showing change in colour of 1mM $HAuCl_4$ gold precursor (yellow colour: left vial) after gold nanoparticles formation (wine red colour: right vial) due to the addition of *Ficus religiosa* extract.

2. Physical Characterization

2.1 UV-Vis Spectroscopy Studies : The UV-Visible spectra of F-AuNPs (Figure 2) showed prominent absorbance maxima at 540 nm, thereby confirming the production of gold nanoparticles. This peak was seen due to the excitation of surface plasmon vibrations in the gold nanoparticles.

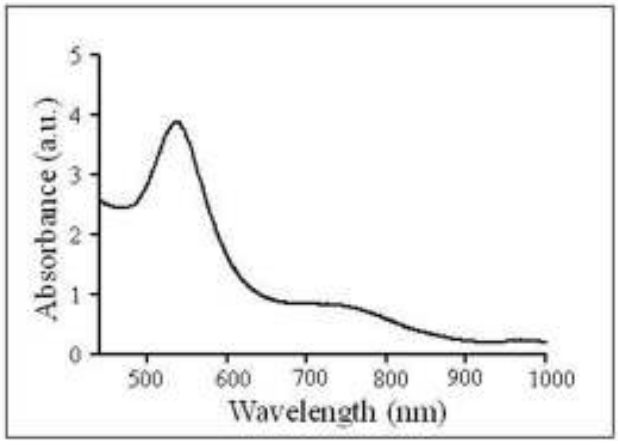

Figure (2) : UV-Vis spectra of as-obtained F-AuNPs: Graph of absorbance of F-AuNPs plotted against 450-1000nm wavelength range showing prominent absorbance maxima at 540 nm.

2.2 X-Ray Diffraction (XRD) Measurement : XRD spectra of F-AuNPs (Figure 3) exhibited diffraction peaks corresponding to (111), (200), (220) and (311) phases in the 2θ range of 30°-90°. This was in agreement with JCPDS 04-0784 data for gold nanoparticles. In the XRD pattern, diffraction peaks at angles of 38.1°, 40.4°, 65.3° and 77.4° could be assigned to face-centered cubic (fcc) metallic gold (111), (200), (220) and (311) facets of the gold crystals, respectively. The average crystallite size of the F-AuNPs was estimated from Scherrer's formula to be ~30 nm indicating that the dispersed nanoparticles have uniform growth along this plane. It also suggested a spherical morphology for F-AuNPs.

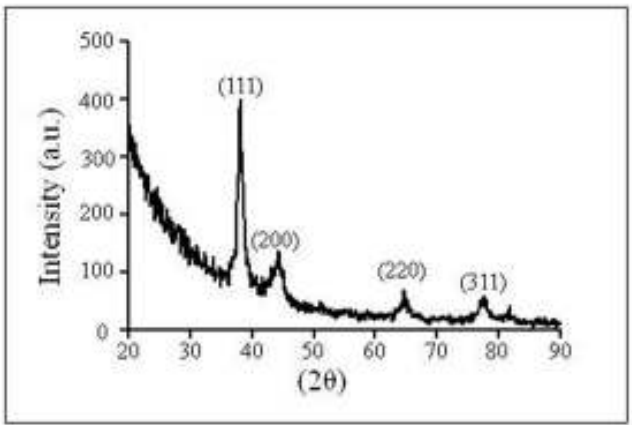

Figure (3) : XRD Spectra of F-AuNps: The XRD pattern showing characteristic peaks at specific angles that are the signature of gold nanoparticles.

2.3 TEM Measurements : The TEM image of F-AuNPs (Figure 4) showed mostly the presence of spherical nanoparticles in the range of 20-30 nm. As seen in Figure (4), some of the particles had diverse shapes such as triangular, pentagon or hexagonal indicating that their formation was dependent on the initial nucleation process governed by the spherical morphology. This size was also in accordance with UV-vis spectra and XRD analysis. Presence of polyshaped F-AuNPs predicts that different components present in the *Ficus religiosa* extract must be responsible for different shapes of the gold nanoparticles **[10, 28-31]**. Moreover, chloride ions (generated from gold chloride) also influence the shape and size of nanoparticles.

Figure (4) : TEM Image of F-AuNPs. The image shows mostly spherical F-AuNPs as well as presence of other shapes such as triangles, pentagons and hexagons. Scale bar is 200 nm.

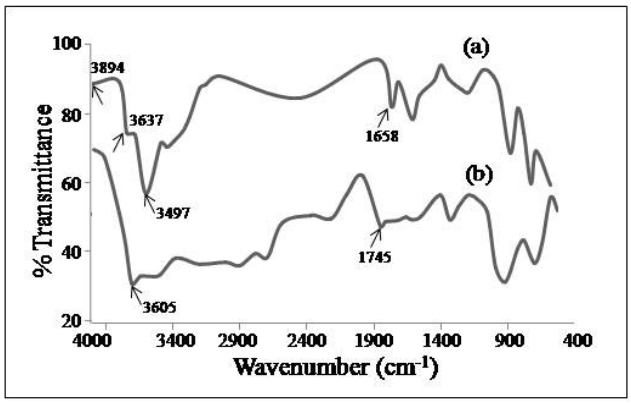

Figure (5) : FTIR spectra of F-AuNPs: Graph of % Transmittance plotted against wavelength range of 400-4000nm. FTIR spectra of (a) as-obtained F-AuNPs and (b) *Ficus religiosa* extract.

2.4 Fourier transform infrared spectroscopy (FTIR) Analysis : FITR spectra of F-AuNPs in the range 4000-400 cm^{-1} (Figure 5) exhibited characteristic finger printing regions of various functional groups such as –OH, amide, amine and carboxylates with –C=O stretching vibrations. For example, in Figure 5 (a) of F-AuNps, the peak at 3894 cm^{-1} indicated the bonded –OH groups while the other splitted peak at 3637 cm^{-1} corresponded to amine linkages from β-sitosteryl-d-glucoside that has come from the *F. religiosa* extract. The shift in the peak at 3605 cm^{-1} in *F. religiosa* extract (b) to 3497 cm^{-1} in (a) indicated that F-AuNPs were functionalized with amine groups. Other facet of the spectra indicated that the peak at 1745 cm^{-1} (Figure 5b), due to carboxylic acid functions present in the extract of *Ficus religiosa*, is shifted towards lower energy side i.e. 1658 cm^{-1} (Figure 5a) for F-AuNP's. This shift clearly reflects the capping of Au naoparticles with carboxylic acid groups which are responsible for their aqueous dispersion.

FTIR data suggests that the polyphenols and aldehydes/ketones present in *Ficus religiosa* extract are not only responsible for the biosynthesis of F-AuNPs but also govern their size due to their binding ability resulting into capping of the nanoparticles. Moreover, binding of the functional groups present in the aqueous extract to the nanoparticles is directing their morphology, thus, resulting into synthesis of different shapes.

3. In vitro stability studies : The as-synthesized F-AuNPs were kept on the shelf for a period of 0 h, 24 h and 7 days and UV absorbance was taken during these time-periods (Figure 6). Besides slight changes in 600-700 nm region in 7-day period, there was no major change in the UV absorbance of F-AuNPs at 540 nm over different time-periods. Thus, the nanoparticles exhibited excellent stability over the time.

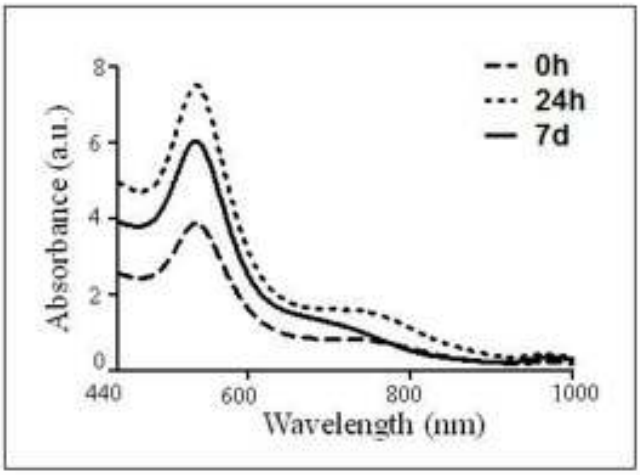

Figure (6) : UV-Vis absorbance spectra: Graph of absorbance of F-AuNPs in water at different time intervals (0h, 24h and 7 days) plotted against wavelength.

4. Cell Viability by MTT Assay : It was observed that the F-AuNPs were non-toxic to HEK293 cells (Figure 7). The cells exhibited

100% viability upto 80 µM concentration of F-AuNPs and around 87-79% viability at concentrations varying between 100-200 µM. The cells showed more than 70% viability up to 200 µM concentration of the nanoparticles, thereby confirming that the as-synthesized F-AuNPs were non-toxic and safe.

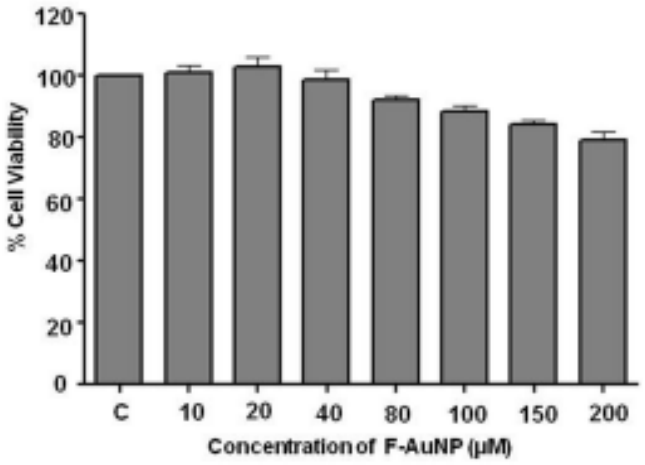

Figure (7) : Cell Viability Assay: Graph shows % viability of HEK 293 cells treated with different concentrations of F-AuNPs.

Conclusions : The present report demonstrates a rapid green synthesis of gold nanoparticles using *Ficus religiosa* extract. The nanoparticles showed excellent stability and uniform capping due to the presence of polyphenols, amines and carboxylates present in the extract. The effective capping and subsequent dispersiblility of these nanoparticles in water enhanced their bioavailability, thereby making them available for various biomedical applications. Thus, biological methods of nanoparticle synthesis have been suggested as ecofriendly alternatives to chemical and physical synthetic methods since the latter involve toxic chemicals that could be detrimental to human health.

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