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Spectroscopic investigations, antimicrobial, and cytotoxic activity of green synthesized gold nanoparticles

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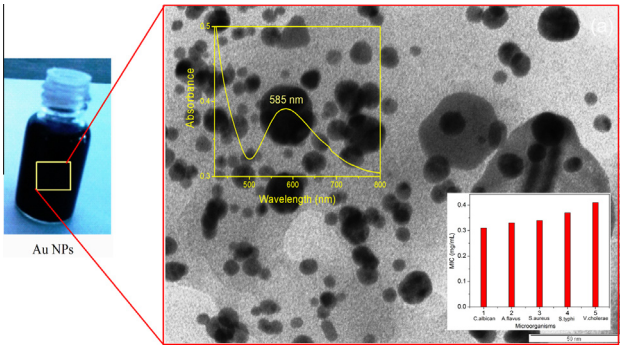
HIGHLIGHTS

- AuNP was synthesized using pomegranate extract – reducing and stabilizing agent.
- Stability of AuNP confirmed by UV–Vis and HRTEM analyses after one month.
- HRTEM images showed the spherical and triangular AuNP as small as 0.9 nm.
- AuNP showed excellent antimicrobial against various microorganisms and cytotoxic activities.

GRAPHICAL ABSTRACT



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ABSTRACT

The gold nanoparticles (AuNPs) were synthesized by using naturally available *Punica Granatum* fruit extract as reducing and stabilizing agent. The biosynthesized AuNPs was characterized by using UV–Vis, fluorescence, high resolution transmission electron microscopy (HRTEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR) and thermogravimetric (TGA) analysis. The surface plasmon resonance (SPR) band at 585 nm confirmed the reduction of auric chloride to AuNPs. The crystalline nature of the biosynthesized AuNPs was confirmed from the HRTEM images, XRD and selected area electron diffraction (SAED) pattern. The HRTEM images showed the mixture of triangular and spherical-like AuNPs having size between 5 and 20 nm. The weight loss of the AuNPs was measured by TGA as a function of temperature under a controlled atmosphere. The biomolecules are responsible for the reduction of $AuCl_4^-$ ions and the formation of stable AuNPs which was confirmed by FTIR measurement. The synthesized AuNPs showed an excellent antibacterial activity against *Candida albicans* (ATCC 90028), *Aspergillus flavus* (ATCC 10124), *Staphylococcus aureus* (ATCC 25175), *Salmonella typhi* (ATCC 14028) and *Vibrio cholerae* (ATCC 14033). The minimum inhibitory concentration (MIC) of AuNPs was recorded against various microorganisms. Further, the synthesized AuNPs shows an excellent cytotoxic result against HeLa cancer cell lines at different concentrations.

Introduction

The synthesis and development of metallic nanoparticles by biosynthetic processes has received much attention as an alternative tool in the field of nanotechnology. Therefore, chemical

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reduction method [1–3], heat evaporation [4,5], photochemical reduction [6] and electrochemical reduction [7] have been widely used for the synthesis of metal nanoparticles. For the past several years, algae, fungi, bacteria, viruses and plants have been used for energy-efficient, low-cost and nontoxic production of metallic nanoparticles [8–11]. Recently, it is reported that this green method can potentially eliminate the various problems faced by other methods. The AuNPs with different morphology like tube, sphere, wire, rod, plate, cubic, hexagonal, and triangular shape can be easily synthesized by economically cheap and reliable green method by changing the reaction conditions. Biosynthesis of AuNPs by various plants such as, *Aloe vera* [12], *Tamarindus indica* [13], *Cinnamomum camphora* [14], *Coriandrum sativum* [15], *Medicago sativa* [16] have been reported. Biocompatibility and non-toxic property of AuNPs have major role in antimicrobial, antitumour activities and drug delivery [17]. The biosynthesized AuNPs attracted the scientists for their unique physical, chemical properties, nanometer size and their conjugation with the drugs with a high degree of specificity [18,19]. *Punica granatum* which is commonly known as pomegranate played an important role for synthesizing AuNPs. The oxygen radical absorbance capacity (ORAC) of pomegranate juice was measured at 2860 units/100 g. Pomegranate is well known for a good source of vitamins (B_1, B_2, B_3, B_5, B_6 and B_9) and fiber. Water soluble organic materials present in the fruit extract were mainly responsible for the reduction of metal ions to metal nanoparticles. The most abundant polyphenol in pomegranate juice are the ellagitannins which formed when ellagic acid binds with carbohydrate. Ellagitannins account for 92% of its antioxidant activity. Recently, the efficacy of pomegranate juice against prostate cancer and other several heart risk factors has been reported [20]. Antimicrobial activity of pomegranate extract has also been reported [21]. The antimicrobial activity of the biosynthesized AuNPs was observed in various microorganisms such as *Candida albicans*, *Aspergillus flavus*, *Staphylococcus aureus*, *Salmonella typhi* and *Vibrio cholerae*. AuNPs, which can easily detect the cancer disease in a very small volume of tissue or cells has considered as an important biomedical device for cancer researchers [22].

In the present study, AuNPs were synthesized by green synthetic route using pomegranate fruit extract as reducing and stabilizing agent. It showed excellent antimicrobial activity towards various microorganisms. They showed an enormous potential to enhance the efficiency of cancer treatment against HeLa cell (cervical cancer, caused by Human Papilloma Virus) lines at different concentrations.

Materials and methods

Materials

A fresh and clean pomegranate was used for the synthesis of AuNPs. The hydrogen tetracholoro aureate (III) hydrate ($HAuCl_4 \cdot 3H_2O$) (99.9%) was purchased from Sigma Aldrich.

All the microorganisms were obtained from gene bank, Chandigarh. A HeLa cell line was obtained from King Institute, Guindy, Chennai. Methyl thiazolyldiphenyl-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and Trypsin were purchased from Sisco research laboratory chemicals, Mumbai. Fetal bovine serum (FBS) was purchased from Cistron laboratories, Dubai and Minimal Essential Media supplemented (MEM) was purchased from Hi Media Laboratories.

Synthesis of AuNPs

In a typical synthesis, 50 g of pomegranate fruit was thoroughly washed, crushed and filtered through Whatman No. 1 filter paper. The obtained 10 mL of fresh extract was diluted to 100 mL under magnetic stirring at room temperature. Later, the freshly prepared fruit extract was allowed to settle down the undissloved matter. 100 mL of 0.01 M $HAuCl_4$ aqueous solution was prepared and added with 100 mL of pomegranate extract. The slow color change in the reaction mixture indicated the reduction of $AuCl_4^-$. The lyophilization process was used to avoid the agglomeration for obtaining the stable AuNPs.

Characterization of AuNPs

A Perkin–Elmer UV–Vis spectrophotometer of model 1800 was used to record the bioreduction of $AuCl_4^-$. XRD measurement was done using a model 3000 from Rich Siefert, Germany to determine the phase and crystal structure of AuNPs. It was used with Cu $K_{\alpha 1}$ radiation using $\lambda = 1.54056$ Å. The morphology of AuNPs was measured by FEI-TECNAI-30 model of HRTEM with SAED pattern. Bruker FTIR spectrophotometer was used for analyzing the functional groups present in AuNPs. A model TGA Q50 V20.13 was employed to determine the moisture absorption content, degradation temperatures and solvent residues of AuNPs. The fluorescence measurement was carried out on a Cary fluorescence spectrophotometer of model FL1201M002.

Results and discussion

UV–Vis spectroscopic analysis

The formation of AuNPs was monitored by using UV–Vis spectroscopy. As the aqueous solution of $AuCl_4^-$ was mixed with the pomegranate extract, the color change from yellow to black was observed, which indicated the formation of AuNPs (Fig. 1a). There was no characteristic SPR (surface plasmon resonance) peak observed at the initial stage. After 2 h, SPR peak which was dependent on the shape, size, surrounding media and the interparticle distance of AuNPs, started to appear at the wavelength $\lambda_{max} = 585$ nm. This band became more prominent at infinite time (Fig. 1b). The observed λ_{max} value is characteristic for SPR band of AuNPs which is originated from the collective oscillation of free conduction electrons of AuNPs in resonance induced by interacting electromagnetic field [23–26]. Further, the absorption at 380 nm is due to the n–π^* transition of organic materials present in the pomegranate fruit extract. The polyphenols present in the pomegranate fruit extract act as a powerful reducing agent which may be responsible for the reduction of $AuCl_4^-$. The carboxylate group present in pomegranate fruit extract can act as a surfactant to AuNPs and it stabilizes them through electrostatic stabilization [27]. Hence it is found that pomegranate fruit extract has the ability to perform dual functions of reduction and stabilization of AuNPs. This is one of the most important aspects of biosynthesis of AuNPs which is in good agreement with the earlier reports [28]. The UV–Vis spectrum was recorded after 3 months of their synthesis to confirm the stability of AuNPs and it showed an absorption peak at the same wavelength. The synthesis of AuNPs by this method will find potential application in the field of bio-medical applications [29].

Fluorescence spectroscopy

Fluorescence spectroscopy is one of the important spectroscopic techniques used in the fields of nano-biotechnology. The fluorescence spectrum of AuNPs is presented in Fig. 2 which shows the peak at 548 nm with an excitation wavelength of 400 nm. Dulkeith et al. [30] observed similar behavior indicating plasmonic nature of the PL. Similar results have also been recently reported by Huang et al. [31].