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Original Research Article

In-vitro cytotoxicity of biosynthesized gold nanoparticles against thyroid cancer cell lines

Yuan-Mei Li*, Yuan-Yuan Wang and Bo-Ning Cheng
Department of Endocrinology, Xixi Hospital of Hangzhou CITY, Hangzhou 310023, Zhejiang, China

For correspondence:* ***Email:* *liyuanmeiym@hotmail.com*

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Abstract

Purpose: *To undertake the biosynthesis of gold nanoparticles (AuNPs) using Shorea tumbuggaia bark extract and to study their in-vitro cytotoxicity in thyroid cancer (SW579) cell lines.*
Methods: *AuNPs were prepared by adding 10 mL of Shorea tumbuggaia extract to 5 mL of 2×10^{-3} M of chloroauric acid ($HAuCl_4$) and stirred at room temperature for about 20 min. The AuNPs were evaluated by x-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), ultraviolet-visible (UV–Vis) spectroscopy and transmission electron microscopy (TEM). They were also assessed for cytotoxicity against SW579 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.*
Results: *The reaction of Shorea tumbuggaia extract with $HAuCl_4$ led to a change in the color of the reaction solution to ruby red after 20 min, indicating the formation of AuNPs. The results of various instrumental tests, including XRD, TEM, UV–vis spectroscopy and FT-IR spectroscopy confirmed the formation of AuNPs. TEM images showed spherical NPs with a mean particle size of 20 nM. Further, in-vitro cytotoxicity results indicated concentration-dependent cytotoxicity against SW579 cell lines.*
Conclusions: *A simple, green and low-cost method for the preparation of AuNPs using Shorea tumbuggaia extract has been achieved. The AuNPs exert cytotoxic activity against SW579 cell lines.*

Keywords: *Shorea tumbuggaia, Polyphenols, Gold nanoparticles, Cytotoxicity, Thyroid cancer cell*

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INTRODUCTION

Nanoparticles (NPs) have been regarded as important materials in the fast growing field of nanoscience and nanotechnology. Due to their extraordinary size-dependent features, they have a wide scope of applications in many natural and human-related activities. Several physical and chemical methods are currently available for the effective production of NPs; however, due to their expensive and dangerous nature for bulk production, these synthetic approaches are less preferred for industrial scale production of NPs. Microbes, plant extracts and biomolecules could be considered alternative source materials for traditional chemical and physical methods for the biosynthesis of NPs [1-3].

Several studies have investigated the synthesis of gold (Au) and silver (Ag) NPs by green methods using plant extracts, yeast, bacteria, fungi and honey [4]. The larvicidal activity of noble metal NPs synthesized from *Eclipta prostrata leaf* extracts against vectors of malaria and filariasis was demonstrated by Rajakumar and Abdul Rahuman [5]. Similarly, antimicrobial AgNPs and AuNPs active against clinically isolated pathogens have also been reported [6].

In the present work, we demonstrated a simple

and green synthetic method for AuNP preparation in water using *Shorea tumbuggaia* bark extract polyphenols. Further, we have also studied the *in-vitro* cytotoxicity of the biosynthesized AuNPs against thyroid cancer cell lines (SW579).

EXPERIMENTAL

Materials

Chloroauric acid ($HAuCl_4$), dimethyl sulfoxide (DMSO), the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent and other solvents were purchased from Sigma-Aldrich Chemical Co. All experiments were performed using double distilled water as a medium.

Preparation of *Shorea tumbuggaia* leaf extract

The leaves of the Shorea tumbuggaia plant were collected from plants located nearby to Xixi Hospital in Hangzhou city in the month of August 2015 followed by drying under sunlight, and it was authenticated by Wei Zhang, a taxonomist at Institute of Botany, a herbarium in Beijing (PE). The voucher specimen of the plant leaf was kept for reference at the herbarium of Institute of Botany, Beijing (PE) with voucher no. 153.

The dried bark was then ground into a powder using a pestle and mortar. The bark extract was prepared by adding 5 g of fine dried powder of *Shorea tumbuggaia* bark to 50 mL of deionized water and then boiling for about 30 min in a temperature-controlled water bath. The mixture was cooled to room temperature and filtered to obtain a clear extract solution.

Synthesis of silver nanoparticles

About 10 mL of *Shorea tumbuggaia* extract was added to 5 mL of 2×10^{-3} M $HAuCl_4$ and the subsequent reaction mixture was stirred at room temperature for about 20 min. The formation of AuNPs was indicated by the change in the color of the reaction solution to ruby red.

Cell culture

The SW579 cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplied with 100 µM streptomycin, 100 µM penicillin and 10 % fetal calf serum (FCS). The cells were then maintained at 37°C with 5 % CO_2 in a humidified cell incubator.

Cell viability by MTT assay

Cell viability studies were performed following a previously reported procedure [7]. Briefly, the cells were seeded in 96-well plates at a density of 1,500 cells per well and left overnight to attach to the walls. Then, the cells were exposed to various concentrations of AuNPs (0.05, 0.1 and 0.5 mM) for about 24 h at 37 °C under 5 % CO_2 atmospheric conditions. MTT solution was then added to the cells at a final concentration of 0.5 mg/mL and the cells were further incubated for 4 h. The optical absorbance of the cell suspensions was recorded at 560 nM using an Elisa reader. The IC_{50} value for AuNPs was also determined.

Characterization of AuNPs

The formation of the AuNPs was spectroscopically recorded using a Shimadzu 2400 ultraviolet-visible (UV–Vis) spectrophotometer at room temperature. The morphology and size of the *Shorea tumbuggaia* extract-mediated AuNPs was studied using a JEOL JEM 2100 high-resolution-transmission electron microscope (HR-TEM). Samples were prepared for analysis by placing a drop of Au nano colloid onto the copper grid surface and drying under vacuum.

A selected area electron diffraction (SAED) pattern was simultaneously recorded. The X-ray diffraction (XRD) pattern of the AuNPs was recorded using a Bruker D8 Advance diffractometer over 10º to 80º with a scan run of 4°/min, step size of 0.02° and Cu Kα radiation of λ = 1.54A˚. In addition, the dynamic light scattering (DLS) and zeta potential of the biosynthesized AuNPs was analyzed using a nanoparticle analyzer (SZ-100; Horiba Scientific Nanoparticci). The Ag nano colloid was diluted 10 times and used for DLS and surface charge measurements. The surface capping oxidized polyphenols of the prepared AuNPs was obtained by Fourier transform infrared (FT-IR) spectroscopy (JASCO). A purified AuNP powder was studied using FT-IR.

Statistical analysis

All experiments were performed in triplicate for each treatment. Absolute values of each assay were transformed into control percentages. The data are the mean of three separate experiments while error bars are used to represent standard error of the mean. Differences between groups were determined by

Student's t-test and statistical significance was set at $p < 0.05$.

RESULTS

The conversion of Au^{+3} to Au^{0} was confirmed initially by measuring the UV-visible spectrum of the Au nanocolloid by reaction time. The UV-visible optical absorption spectrum of the *Shorea tumbuggaia*-mediated AuNPs is represented in Figure 1. The existence of a well-defined absorption band at 535 nM, shown by the synthesized colloid, further indicated the formation of AuNPs.

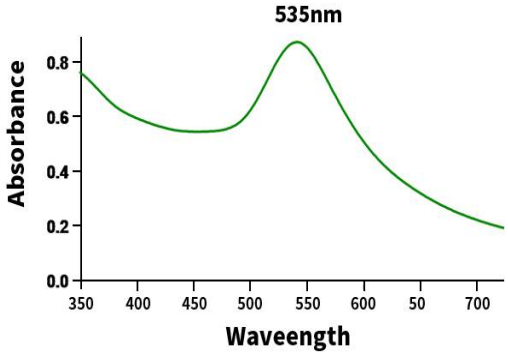

Figure 1: Ultraviolet-visible absorption spectrum of gold nanoparticles (AuNPs)

The crystal structure of the AuNPs synthesized using *Shorea tumbuggaia* extract was analyzed using XRD. The XRD analysis of the AuNPs is shown in Figure 2, and confirms that the synthesized NPs had a face cantered cubic (FCC) structure, with corresponding diffractions peaks at 2θ values of 38.2°, 44.48°, 64.7° and 77.7°. The characteristic indexing planes of AuNPs were (1 1 1), (2 0 0), (2 2 0) and (3 1 1) respectively; this further confirmed the crystalline structure of the AuNPs (JCPDS File No.87-0720).

Figure 3A and B show the TEM images of the biofabricated AuNPs. The TEM microscopic images showed that AuNPs are spherical in shape and polydispersed in nature, with an average particle size of 20 nm. Further, the SAED pattern of AuNPs, shown in Figure 3C, confirmed the crystalline structure of the synthesized AuNPs with well-distinguished diffraction spots.

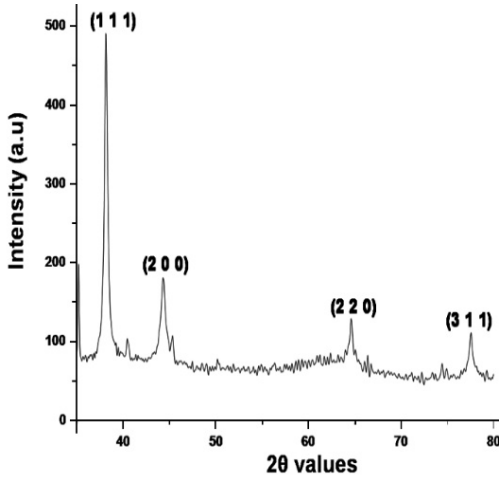

Figure 2: X-ray diffraction (XRD) patterns of biosynthesized AuNPs

Figure 4 shows the FTIR spectrum of the green synthesized AuNPs. Vibrational stretching bands at 3,395 and 1,383 cm^{-1} were observed, related to the stretching of hydroxyl and tertiary alcoholic groups. The bands present at 1,712, 1,615 and 1,055 cm^{-1} can be ascribed to ketonic, carboxylic –C=O and –C-O-C vibrational stretching, respectively. The size distribution and zeta potential of the synthesized AuNPs is shown in Figure 5A. The mean particle size of the NPs was found to be 20 nM. The zeta potential measurement on the AuNPs showed the negative surface charge of the synthesized NPs and was found to be -55 mV (Figure 5B).

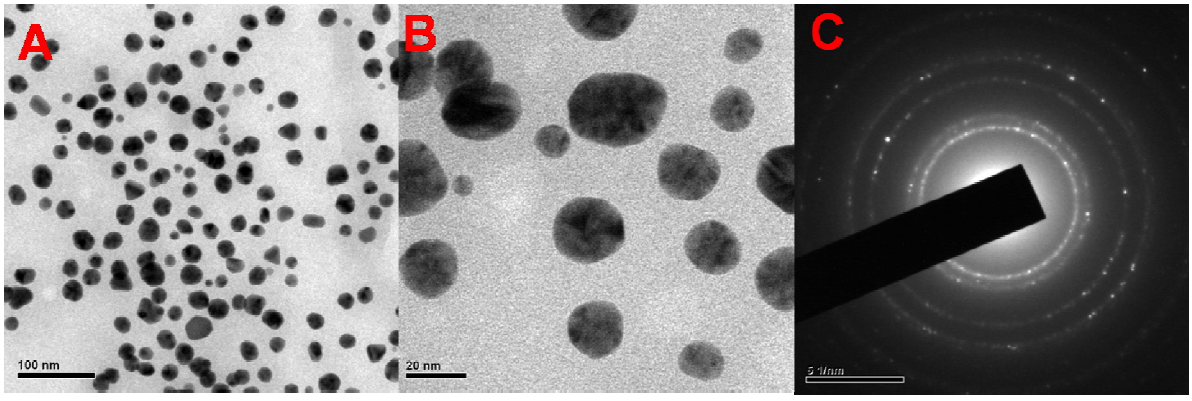

Figure 3: Transmission electron microscopy (TEM) images (A, B) and selected area electron diffraction (SAED) pattern of biosynthesized AuNPs

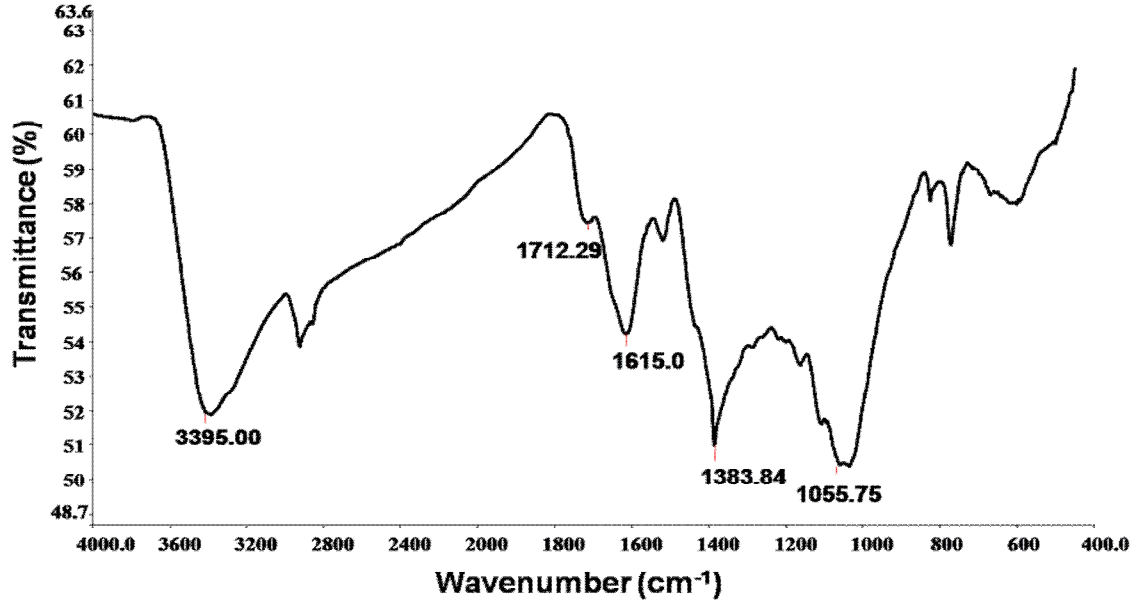

Figure 4: Fourier transform infrared spectroscopy (FT-IR) spectrum of biosynthesized AuNPs

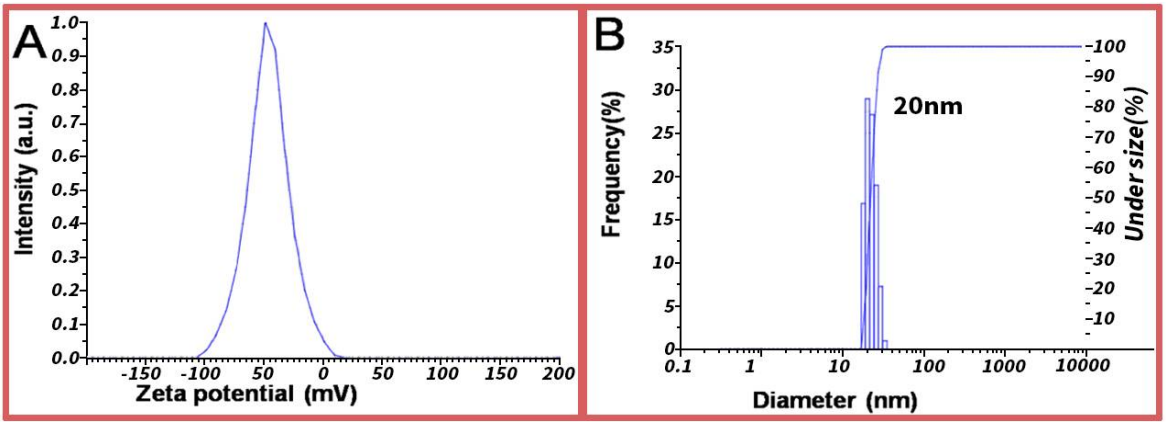

Figure 5: Dynamic light scattering (DLS) analysis (A) and zeta potential (B) of AuNPs synthesized using *Shorea tumbuggaia* extract

In-vitro cytotoxicity

The AuNPs were successfully prepared to investigate their cytotoxicity against thyroid cancer cell lines (SW579). The concentration effect of AuNPs on the cell viability of SW579 cell lines was studied by treatment with various concentrations of AuNPs (0.05, 0.1 and 0.5 mM). An *in-vitro* cytotoxicity assay on SW579 showed the toxic nature of AuNPs (Figure 5). The percentage cell viabilities were 88 % and 38 % when treated with AuNPs at concentrations of 0.05 and 0.5 mM, respectively. It is confirmed that the cell viability percentage is decreased with increase in the concentration of AuNPs.

DISCUSSION

Shorea tumbuggaia extract containing naturally occurring polyphenols was used as a reducing and capping agent for the preparation of AuNPs. The -OH groups of polyphenols of *Shorea tumbuggaia* extract play a significant role in the conversion of Au^{+3} to Au^{0} and subsequent stabilization of the readily formed Au nuclei [8]. The formation of AuNPs was confirmed visually by the change of color of the reaction solution from colorless to ruby red. The intense ruby red color of the synthesized AuNPs is due to the surface plasmon resonance (SPR) phenomenon of the noble metal NPs, caused by the interaction of surface conduction electrons of AuNPs with electromagnetic radiation [9]. The existence of an SPR band at 535 nM in the UV-visible optical absorption spectrum further confirmed the Au^{+3} reduction.

The formation of AuNPs was also confirmed by TEM and XRD analysis. The presence of XRD peaks characteristic of AuNPs and related indexing planes showed the crystalline structure of the synthesized NPs. The mean particle size obtained for the AuNPs was 20 nM, which is also supported by DLS analysis results. This result for the size of the AuNPs is in good agreement with the SPR absorption band of the AuNPs; however, it has been previously reported that the SPR absorption band of gold and silver NPs depends on their morphology and size [10].

The formation of spherical and smaller AuNPs is highly advantageous because of their applications in bioimaging and drug delivery. For instance, smaller spherical NPs can easily pass through the cell membrane, while larger NPs cannot. Moreover, the existence of biomolecular constituents on the surface of the synthesized NPs may increase the biocompatibility of the synthesized NPs [10].

The FT-IR spectral studies revealed stabilization of the synthesized AuNPs with plant biomolecules. The existence of vibrational bands characteristic of the hydroxyl and carboxylic groups in the FT-IR spectrum revealed stabilization of oxidized polyphenols onto the AuNPs surfaces. The presence of a vibrational stretching band characteristic of the ketonic group at 1,712 cm^{-1} further indicated the key role of –OH groups of *Shorea tumbuggaia* extract polyphenols in Au^{+3} reduction [8]. The consecutively formed oxidized polyphenols will cap the readily formed Au nuclei and thus stabilize them. It has been extensively reported that the –OH groups of plant polyphenols will donate electrons for Au^{+3} reduction and subsequently convert into their quinone forms. The resulting oxidized polyphenols will cap the formed Au nuclei. These results are also supported by the negative surface zeta potential of AuNPs, which later generates repulsive forces among the NPs resulting in the reduced agglomeration of NPs [11].

An MTT assay was conducted to investigate the cytotoxicity of the synthesized AuNPs. Several reports have already shown the cytotoxicity of metal NPs against different cancer cell lines [12]; however, there are no studies available on concentration-dependent toxicity against SW579 cell lines using AuNPs synthesized with *Shorea tumbuggaia* extract. The cytotoxicity results of the synthesized AuNPs indicated that the biosynthesized NPs showed considerable cytotoxicity towards SW579 cell lines, with cytotoxicity increasing with increasing NP concentration. From these results, it can be concluded that the toxicity of the green synthesized AuNPs is dose-dependent. The toxicity dependence on AuNP concentration against human lung cancer cell lines has been reported previously [10]; thus, AuNPs synthesized with the present method using *Shorea tumbuggaia* extract could act as anti-thyroid cancer agent through their cytotoxic mechanisms.

CONCLUSION

A low cost, green approach for the preparation of AuNPs using *Shorea tumbuggaia* extract is demonstrated in this work. Zeta potential and FT-IR results indicate that there is capping of oxidized polyphenols onto the AuNP surfaces. Furthermore, *Shorea tumbuggaia* extract polyphenol-coated AuNPs exhibit concentration-dependent cytotoxicity against SW579 cell lines. Hence, the synthesized AuNPs may find application in the synthesis of anti-cancer drugs in the future.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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